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George E. Barreto
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# Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health

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# About the Book

This book *Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health* presents chapters focused on natural products, in particular medicinal plants and their derived products, as an indispensable source of bioactive molecules that serve as either drug candidates or lead compounds for drug design and discovery purposes. There are several advantages for plant-derived therapeutics, including wide availability, diverse pharmacological actions, and a generally good profile of safety and tolerability. Over the recent years, there have been numerous reports from clinical studies testifying the efficacy and safety of medicinal plants and phytochemicals in ameliorating several human diseases. A plethora of basic studies has also unraveled molecular mechanisms underlying the health benefits of herbal medicines. Nevertheless, issues such as identification of bioactive ingredients, standardization of the products, and drug interactions remain to be further studied. In this book, we compiled 29 chapters on the medicinal properties and pharmacological action of natural products, mainly medicinal plants and phytochemicals, in different settings ranging from *in vitro* models to clinical trials. The goal is to present the reader a comprehensive collection on most of the therapeutic aspects of plant-derived natural products and molecular mechanisms thereof.

Most of the chapters are developed over the use of curcumin as a molecule with antioxidant and anti-inflammatory potential in various pathologies. In the chapter by Alidadi et al., the authors described the effect of curcumin on arterial stiffness, which correlates with lower body weight, and improved pulse wave velocity in patients. Curcumin has also been implicated in regulating long non-coding RNAs (Amini et al.), and modulated inflammatory mechanisms related to lipid metabolism in patients with non-alcoholic fatty liver disease (Mirhafez et al.), and atherosclerosis (Hatamipour et al. and Momtazi-Borojeni et al.). In addition, Naji et al. correlated curcumin with improvement in gastrointestinal cancers, while Shakour et al. highlighted the participation of curcumin in regulating c-reactive protein *in silico*. More interestingly, Zarrinfar et al. described the antifungal effects of curcumin, while Sohrevardi et al. discussed the role of this molecule in women with polycystic ovary, and finally its actions on functional dyspepsia (Panahi et al.). Due to the pandemic, and the search for nutraceuticals that may be able to reduce the symptoms associated with covid-19, Heidari et al. described how curcumin could be considered as a possible therapeutic alternative to drugs currently in use to treat the disease.

Other chapters discussed the pharmacology of plants, their medicinal properties, bioavailability, and metabolism in different tissues, in addition to describing how they can be used to treat different pathologies. In this sense, Ashrafizadeh et al. described the therapeutic properties of ginsenosides on endoplasmic reticulum stress and autophagy, while other authors focused on the effects of plants and their bioactive components on insulin resistance (Mahdavi et al.), plants with anti-addictive potential (Konrath et al.), sleep problems (Lelli et al.), candida albicans (Gharibpour et al.), cannabinoids and cardiovascular system (Liberale et al.), non-alcoholic fatty liver (Simental-Mendía et al.), diabetes and oxidative stress (Yaribeygi et al.), memory and cognitive functions (Yousefani et al), and alopecia (Boghrati et al.), apart from a detailed and insightful discussion on rheum species (Mohtashami et al.), genus rosa (Ayati et al.), Actaea racemosa L. (Salari et al.), Centella asiatica (Torbati et al.), Cichorium (Boghrati et al.), and genus Berberis (Sobhani et al.) plants. Finally, Panahi et al. showed us that magnesium, a molecule highly present in plants, has a protective effect after cerebral ischemia, while Cabezas et al. predicted using *in silico* tools that some bioactive components of plants can regulate fatty acid- binding protein 5 (FABP5), a protein involved in the dysregulation of lipid metabolism.

We hope this comprehensive book is of interest to researchers working in the field and serves as a source of inspiration for future proof-of-concept translational and clinical studies. We would like to acknowledge and thank all the authors for contributing with the presented chapters. Last but not the least, we would like to express our deep appreciation to Mr. Gonzalo Cordova who helped at every step of preparing this collection.

# Contents

# 1 The Effect of Curcumin Supplementation on Pulse Wave Velocity in Patients with Metabolic Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial

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**Abstract**

Cardiovascular disease is a leading cause of death in many societies. Arterial stiffness is an initial sign of structural and functional changes in the arterial wall. Pulse wave velocity (PWV) is the gold standard for non-invasive evaluation of aortic stiffness and a modifiable cardiovascular risk factor. Curcumin is a major component of turmeric with known anti-inflammatory and anti-oxidative effects. Since

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arterial stiffness is affected by inflammation and oxidative stress, it may be improved by curcumin supplementation. The purpose of this clinical trial was to investigate the potential effects of curcumin on improving arterial stiffness in patients with metabolic syndrome. This placebo-controlled, double-blind, randomized clinical trial was conducted among metabolic syndrome patients. Sixty-six eligible individuals were randomly assigned to active intervention or control groups. The active intervention group received curcumin supplement at a dose of 500 mg daily for 12 weeks, whereas the control group received placebo capsule. Physical activity, daily dietary energy intake, anthropometric body composition, and biochemical hemodynamic and arterial stiffness parameters were evaluated at baseline and at the end of the study. Body weight decreased significantly in the curcumin group compared to placebo. Also, curcumin intervention improved PWV, which remained significant after adjustment for potential confounding factors ($p = 0.011$). The current clinical trial demonstrated that daily intake of 500 mg of curcumin for 12 weeks can lead to the improvement of arterial stiffness and weight management among subjects with metabolic syndrome.

**Keywords**

Arterial stiffness · Vascular stiffness · Vascular aging · Arterial aging · Pulse wave velocity · Augmentation index · Curcuminoid · Curcumin · Turmeric · Metabolic syndrome · Obesity

## 1.1 Introduction

Cardiovascular disease (CVD) is the most prevalent cause of death in the world [1]. Arterial stiffness, specifically aortic stiffness, is a primary sign of structural and functional changes in arterial walls and is a predictor of cardiovascular events [2, 3]. Arterial stiffness explains the reduced ability of an artery in dilation and constriction in response to pressure alterations [4]. Collagen and elastin are two important proteins in the arterial wall and any imbalance between them, such as caused by inflammation or increased luminal pressure, results in increased collagen, reduced elastin and subsequently enhanced stiffness of arterial wall [5, 6].

Various methods, both invasive and noninvasive, have been accepted to assess arterial resilience. Pulse wave velocity (PWV) and wave reflection are two noninvasive methodologies for vascular stiffness assessment [7, 8]. Augmentation pressure (AP) and augmentation index (AIX) are measures of pulse wave reflection and evaluated using the pulse wave analysis (PWA) technique [9]. Large elastic artery stiffness and systemic arterial stiffness are evaluated through aortic PWV and wave reflection, respectively [10]. Aortic PWV, as the 'gold-standard' measurement of arterial stiffness, has been determined by carotid-femoral PWV (cf-PWV) [2, 11]. Also, AIX can demonstrate the CVD risks independently of peripheral pressures, as shown in a recent meta-analysis [12].

Several situations can reduce vascular elasticity such as aging, central obesity, smoking, diabetes, hypertension, inflammation disease, metabolic syndrome and genetic factors [8, 13]. Metabolic syndrome is one of the major causes of CVD, and has been described as one of the main public health global challenges [14, 15]. According to the International Diabetes Federation (IDF) definition, metabolic syndrome can be diagnosed with central obesity and the existence of two or more other clinical features that include elevated blood pressure, increased levels of triglyceride and fasting plasma glucose, and reduced HDL-cholesterol concentrations [16, 17]. Lifestyle modifications, such as improved dietary habits, can have a favorable effect on vascular stiffness [18]. Turmeric is a source of an orange-yellow pigment polyphenolic compound called curcumin [1,7-bis-(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione] [19]. Curcumin has been reported to have many beneficial effects on health [20–28]. It has been shown that curcumin can

induce nitric oxide production and reduce oxidative stress and inflammation in animal and in vitro models of vascular-related disorders [29–32]. A recent preclinical study in young and older male mice showed that 4 weeks of curcumin supplementation resulted in improved endothelial function and arterial stiffness by enhancement of nitric oxide bioavailability and reduced oxidative stress [33].

The purpose of this study was to test the hypothesis that 12 weeks of curcumin supplementation would lead to improved arterial stiffness indices in metabolic syndrome patients.

## 1.2 Methods

This randomized, double-blinded, placebo-controlled clinical trial with parallel design was conducted at the Persian cohort center of Imam Reza hospital, Mashhad, Iran. In this trial, 200 new cases of metabolic syndrome were assessed using inclusion and exclusion criteria. Of these cases, 66 individuals aged 30–60 years met the IDF criteria [17] and were incorporated into this 12 week study (Fig. 1.1). Exclusion criteria were the following: pregnancy; lactation; smoking; drug abuse; use of statins, contraceptive pills, analgesic, antidiabetic, antiplatelet, or anti-inflammatory drugs; consumption of antioxidants, multivitamins, multivitamin-mineral or herbal supplements 3 months before starting the study; and a history of diabetes, kidney failure, cancer, gallstones, calcium oxalate stones, autoimmune, biliary or obstructive diseases.

This investigation was approved by the Ethics Committee of Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran (serial no. IR.MUMS.MEDICAL.REC.1397.452), in accordance with the Declaration of Helsinki. In addition, this study was registered on Iranian Registry of Clinical Trials website (clinical trial registration no. IRCT20180619040151N2). At the beginning of the study, the nature, side effects, and advantages of the study were illustrated to volunteers and their written informed consent was obtained. All measurements were done at the Persian cohort center of Imam Reza hospital after 12 h fasting (water allowed) and > 24 h refrainment from physical activity.

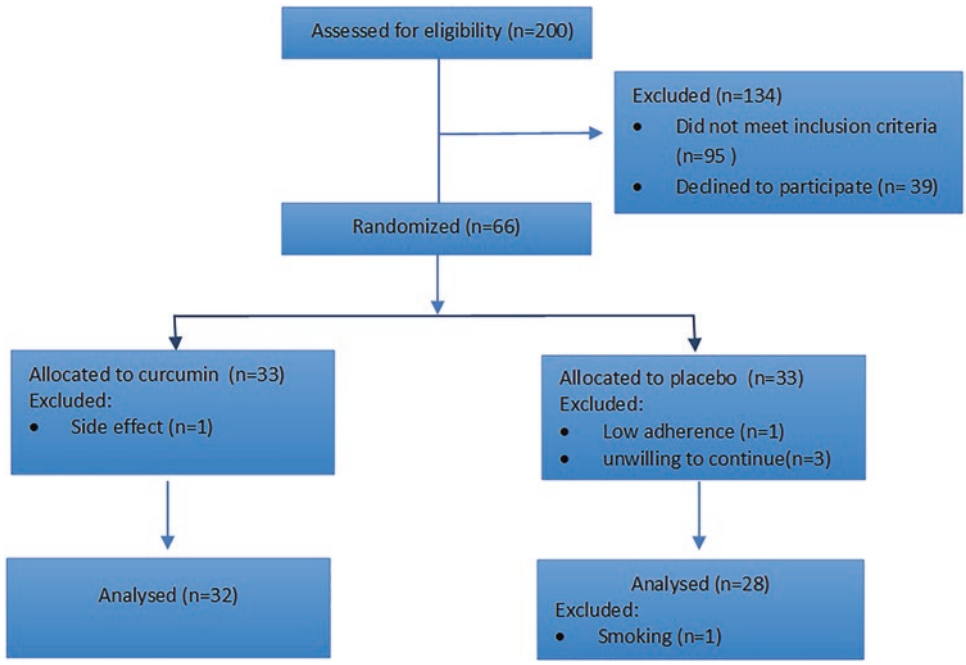


**Fig. 1.1** Flow diagram of study

### 1.2.1 Randomization Procedure

After conducting the screening and consent steps, the randomization procedure was performed using a stratified permuted block scheme, in which the stratification was based on age and gender. Subsequently, all participants were randomly allocated to either the curcumin or the placebo group.

### 1.2.2 Interventions

Curcumin [500 mg (95% total curcuminoids), provided by Karen Pharma and Food Supplement Company] or placebo capsules [500 mg of lactose, provided by the Faculty of Pharmacy, Mashhad University of Medical Sciences] were taken by the participants once per day with the midday meal. Every four weeks during the trial, in-person check-in visits were implemented to change the intervention capsules and evaluate participant compliance by survey and pill count. Additionally, tolerability and side effects of the interventions were assessed during these check-in visits.

### 1.2.3 Dietary and Physical Activity Assessment

Average daily dietary energy intake was evaluated by two-day dietary recall at baseline and at week 12. Dietary recall data were analyzed by Nutritionist IV software (N-Squared Computing, Salem, OR, USA). Also Physical activity was estimated by the long version of International Physical Activity Questionnaire (IPAQ) at baseline and week 12.

### 1.2.4 Anthropometric and Body Composition Assessment

Anthropometric and body composition measures were taken with subjects wearing light-weight clothing with no shoes on. Standing height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Waist, hip, wrist and neck circumferences were determined by a tension-gated tape at baseline and week 12. Waist circumference was measured to the nearest 0.5 cm at the midway of the distance between the lower rib margin and the iliac crest at the end of a gentle expiration and in the direction of the horizontal plane. Hip circumference was measured to the nearest 0.5 cm around the widest portion of the gluteal area in standing position [34]. Wrist circumference (WrC) was measured around the bony prominences of the radial and ulnar styloids [35]. Neck circumference (NC) was measured at the midpoint of the neck or just below the laryngeal prominence (‘Adam’s apple’) ‘in men with an obvious Adams apple, while the tape was placed vertically [34]. WrC and NC measures were taken to the nearest 0.1 cm. Also, weight and body composition parameters were determined via a bioelectrical impedance body composition analyzer (InBody 770, Biospace Co., Ltd. Seoul, South Korea). To decrease examiner-related errors, all the measurements executed by the same person.

### 1.2.5 Laboratory Parameters

Blood samples were collected from the antecubital vein after 12 h overnight fasting at baseline (0 weeks) and at the end of 12 weeks intervention. Serum concentrations of fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using the BT1500 chemistry analyser (Biotecnica Instruments S.p.A., Rome, Italy).

### 1.2.6 Blood Pressure and Arterial Stiffness Measurements

Brachial and aortic blood pressure, aortic pulse pressure (PP), mean arterial pressure (MAP,) heart rate (HR), AIX, AIX75, AP, and arterial age measurements were obtained after the participants had rested in a supine posture for at least 10 min in a calm, thermoneutral room. Measurements were obtained with the SphygmoCor XCEL System

(Sphygmocor; AtCor Medical, Sydney, Australia) by a trained physician. After PWA, cf-PWV was measured for assessment of aortic stiffness using the SphygmoCor XCEL System.

### 1.2.7 Statistical Analyses

The sample size was statistically calculated to achieve a power of 90 according to change in AIX75 in the Sugawara [36] investigation. Statistical analysis was performed using SPSS 16. Assessment of data normality was performed using the One-Sample Kolmogorov-Smirnov test. In addition, histogram plots were evaluated visually, and it was observed that data distribution for normality was acceptable. Finally, linear regression was used to confirm the final results.

## 1.3 Results

A total of 66 metabolic syndrome patients were initially enrolled in the study but five placebo group participants (one smoking, three unwilling to continue, one adherence less than 80%) and one curcumin group participant (reflux side effect) did not complete the study (Fig. 1.1). The baseline characteristics of the final 60 study participants, who were randomly assigned into the two treatment arms, are shown in Table 1.1. None of the participant characteristics were significantly different between the two groups at base-

**Table 1.1** Subject characteristics in curcumin and placebo intervention

| | Curcumin | | Placebo | |
|---|---|---|---|---|
| Variables | Week 0 | Week 12 | Week 0 | Week 12 |
| Sex[a] | | | | |
| Male (%) | 43.8 | – | 53.6 | – |
| Female (%) | 56.2 | | 46.4 | |
| Age (year) | 42.84 ± 6.25 | – | 44.43 ± 5.92 | – |
| Physical activity [b] (MET/min/wk) | 826.5(300–1829) | 573(316–1267) | 688.5(352–1160) | 495(233–1449) |
| Energy intake (kcal/day) | 2120.36 ± 501.91 | 2042.02 ± 549.22 | 2057.4 ± 583.15 | 2172.43 ± 575.44 |
| Weight (kg) | 80.09 ± 9.67 | 79.55 ± 9.71 | 79.34 ± 12.39 | 79.77 ± 12.81 |
| Waist circumference (cm) | 97.15 ± 7.46 | 96.25 ± 8.06 | 100.13 ± 11.46 | 100.16 ± 11.86 |
| Neck circumference (cm) | 37.63 ± 2.67 | 37.55 ± 2.67 | 38.3 ± 3.84 | 38.45 ± 3.88 |
| Waist to hip ratio | 0.89 ± 0.05[c] | 0.89 ± 0.05 | 0.94 ± 0.07 | 0.94 ± 0.07* |
| Waist to height ratio | 0.58 ± 0.05 | 0.57 ± 0.05 | 0.6 ± 0.06 | 0.6 ± 0.07 |
| Body mass index ($kg/m^2$) | 28.87 ± 3.65 | 28.7 ± 3.86 | 29.16 ± 4.06 | 29.3 ± 4.06 |
| A body shape index ($m^{11/6}\ kg^{-2/3}$) | 0.08 ± 0.003[c] | 0.079 ± 0.004 | 0.082 ± 0.004 | 0.082 ± 0.004* |
| Protein (kg) | 9.94 ± 1.71 | 9.98 ± 1.71 | 10.02 ± 1.96 | 10.11 ± 2.07 |
| Skeletal muscle mass (kg) | 28.06 ± 5.13 | 28.08 ± 5.16 | 28.26 ± 5.94 | 28.48 ± 6.27 |
| Fat mass (kg) | 36.68 ± 8.21 | 36.22 ± 8.55 | 35.9 ± 6.35 | 35.87 ± 6.74 |
| Visceral fat area($cm^2$) | 143.72 ± 45.23 | 140.7 ± 47.04 | 135.18 ± 37.43 | 135.41 ± 39.1 |
| FPG (mg/dl) | 101.13 ± 10.96 | 94.28 ± 12.8** | 99.04 ± 12.64 | 96.28 ± 17.25 |
| TC(mg/dl) | 180.56 ± 39.01 | 175.22 ± 41.93 | 181.71 ± 37.57 | 178.79 ± 42.92 |
| TG (mg/dl) | 163.25 ± 52.45 | 148.97 ± 59.87 | 189.46 ± 87.21 | 164.11 ± 77.01 |
| HDL-C (mg/dl) | 46.13 ± 10.66 | 44.16 ± 9.62 | 44.11 ± 8.82 | 43.89 ± 9.79 |
| LDL-C (mg/dl) | 101.79 ± 29.56 | 102.42 ± 32.28 | 99.71 ± 31.41 | 102.07 ± 35.89 |
| AST (U/l) | 22.25 ± 9.36 | 18.22 ± 9.73** | 22.57 ± 7.9 | 19.54 ± 8.68** |
| ALT (U/l) | 28.84 ± 13.69 | 23.69 ± 12.59** | 28.18 ± 13.91 | 24.54 ± 13.46** |

Values are means±SD. *FPG* fasting plasma glucose, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

*$P < 0.05$, between groups after 12 weeks of intervention

**$P < 0.05$, compared to baseline

[a]Chi-square

[b]Mann- Whitney test, values are medians ± interquartile range

[c] $P < 0.05$, compared to placebo at baseline

line (all $P > 0.5$), except for waist to hip (WHR) ratio and A Body Shape Index (ABSI), which were both higher in the control group ($P < 0.05$). In the current study, energy intake and physical activity did not change in either the curcumin and placebo groups compared to baseline.

### 1.3.1 The Effect of Curcumin on and Anthropometric, Body Composition, and Serological Tests

The effects of curcumin supplementation on anthropometric, body composition and biochemical tests are presented in Table 1.1 and Table 1.2. After 12 weeks of intervention, a statistically significant reduction in mean body weight was observed in the curcumin compared to the placebo group but body composition and other anthropometric parameters showed no significant changes. A decreasing trend was observed in anthropometric and body composition parameters, including waist circumference, neck circumference, body mass index (BMI), and visceral fat area after curcumin treatment relative to placebo. In both groups, liver enzymea (ALT and AST) were decreased significantly but there was no significant difference between the groups.

### 1.3.2 The Effect of Curcumin on Arterial stiffness and Hemodynamic Parameters

Table 1.3 indicates that there was no difference between the groups in vascular stiffness and hemodynamic parameters at the baseline of the study. As shown in Table 1.4, a significant reduction in PWV was observed following 12 weeks of curcumin intervention compared to placebo. Also, it was shown that after adjusting for confounding factors including age, gender, change in physical activity and energy intake by regression, the curcumin treatment significantly reduced aortic PWV, relative to placebo (Table 1.5). Finally, brachial and aortic systolic blood pressure (SBP) was reduced but totally hemodynamic parameters were not significantly improved with curcumin consumption compared to placebo.

**Table 1.2** Changes in anthropometric, body composition parameters, and serological tests during the intervention

| Variables | Curcumin | Placebo | P- value* |
|---|---|---|---|
| Weight (kg) | −0.53 ± 2.07 | 0.42 ± 1.5 | 0.04 |
| Waist circumference (cm) | −0.9 ± 2.51 | 0.02 ± 2.15 | 0.13 |
| Neck circumference (cm) | −0.07 ± 0.54 | 0.15 ± 0.64 | 0.14 |
| Waist to hip ratio | −0.003 ± 0.01 | −0.0007 ± 0.01 | 0.62 |
| Waist to height ratio | −0.005 ± 0.01 | 0.0005 ± 0.01 | 0.11 |
| Body mass index (kg/m$^2$) | −0.17 ± 0.75 | 0.13 ± 0.52 | 0.07 |
| A body shape index (m$^{11/6}$ kg$^{-2/3}$) | −0.0004 ± 0.001 | −0.0003 ± 0.001 | 0.72 |
| Protein (kg) | 0.03 ± 0.18 | 0.08 ± 0.33 | 0.49 |
| Skeletal muscle mass (kg) | 0.01 ± 0.5 | 0.21 ± 0.9 | 0.29 |
| Fat mass (kg) | −0.45 ± 1.77 | −0.02 ± 1.84 | 0.36 |
| Visceral fat area(cm$^2$) | −3.01 ± 12.39 | 0.23 ± 10.67 | 0.28 |
| FPG (mg/dl) | −6.84 ± 14.08 | −2.82 ± 33.53 | 0.29 |
| TC(mg/dl) | −5.34 ± 23.41 | −2.92 ± 6.33 | 0.74 |
| TG (mg/dl) | −14.28 ± 43.41 | −25.35 ± 65.66 | 0.43 |
| HDL-C (mg/dl) | −3.12 ± 6.11 | −0.21 ± 8.81 | 0.13 |
| LDL-C (mg/dl) | 0.63 ± 17.9 | 2.35 ± 29.08 | 0.78 |
| AST (U/l) | −4.03 ± 5.08 | −3.03 ± 6.51 | 0.5 |
| ALT (U/l) | −5.15 ± 7.26 | −3.64 ± 7.63 | 0.43 |

Values are means±SD
*Between groups

**Table 1.3** Cardiovascular parameters before and after intervention

| | Curcumin | | Placebo | |
|---|---|---|---|---|
| Variables | Week 0 | Week 12 | Week 0 | Week 12 |
| Aortic SBP (mmHg) | 108.44 ± 9.05 | 105.97 ± 7.22* | 109.36 ± 10.65 | 108.11 ± 11.25 |
| Aortic DBP (mmHg) | 74.5 ± 7.04 | 73.44 ± 5.66 | 76.57 ± 8.25 | 76.18 ± 10.22 |
| Aortic PP (mmHg) | 33.94 ± 5.29 | 32.53 ± 5.22 | 32.79 ± 5.1 | 31.93 ± 3.99 |
| Aortic MAP (mmHg) | 88 ± 8.23 | 86.47 ± 6.05 | 70.18 ± 7.66 | 69 ± 6.6 |
| HR (beats /min) | 69.69 ± 9.28 | 68.31 ± 5.64 | 70.18 ± 7.66 | 69 ± 6.6 |
| Aortic AP | 9.91 ± 5.14 | 9.78 ± 4.64 | 9.29 ± 3.75 | 8.86 ± 3.07 |
| Aortic AIX | 28.25 ± 11.75 | 29.38 ± 11.71 | 27.79 ± 9.35 | 27.32 ± 7.5 |
| Aortic AIX75 | 25.75 ± 12.68 | 26.19 ± 12.16 | 25.54 ± 9.98 | 24.36 ± 7.73 |
| Brachial SBP (mmHg) | 118.13 ± 9.54 | 114 ± 53 ± 7.83* | 118.75 ± 12.01 | 117.11 ± 12.23 |
| Brachial DBP (mmHg) | 73.75 ± 6.91 | 72.63 ± 5.71 | 75.86 ± 7.82 | 75.5 ± 9.92 |
| Arterial age(year) | 52.59 ± 18.82 | 51.53 ± 17.3 | 51.89 ± 16.45 | 48.54 ± 11.35 |
| Cf-PWV(m/s) | 7.60 ± 1.43 | 6.67 ± 0.97** | 7.43 ± 1.74 | 7.11 ± 2.03 |

Values are means±SD. SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, MAP: mean arterial pressure, HR: heart rate, AP; augmentation pressure, AIX; augmentation index, AIX75; augmentation index normalized to a HR of 75 bpm, cf-PWV; carotid to femoral pulse wave velocity

*$P < 0.05$, compared to baseline

**$P < 0.001$, compared to baseline

**Table 1.4** Differences of Cardiovascular parameters during the intervention

| Variables | Curcumin | Placebo | P-value* |
|---|---|---|---|
| Aortic SBP (mmHg) | −2.46 ± 6.81 | −1.25 ± 6.94 | 0.49 |
| Aortic DBP (mmHg) | −1.06 ± 6.04 | −0.30 ± 5.92 | 0.66 |
| Aortic PP (mmHg) | −1.4 ± 4.99 | −0.85 ± 4.36 | 0.65 |
| Aortic MAP (mmHg) | −1.53 ± 6.23 | −0.85 ± 6.24 | 0.67 |
| HR (beats /min) | −1.37 ± 9.07 | −1.17 ± 8 | 0.93 |
| Aortic AP | −0.12 ± 4.29 | −0.42 ± 3.24 | 0.76 |
| Aortic AIX | 1.12 ± 9.34 | −0.46 ± 7.2 | 0.46 |
| Aortic AIX75 | 0.43 ± 9.83 | −1.17 ± 7.89 | 0.49 |
| Brachial SBP (mmHg) | −3.59 ± 6.96 | −1.64 ± 7.69 | 0.3 |
| Brachial DBP (mmHg) | −1.12 ± 6 | −0.35 ± 5.78 | 0.61 |
| Arterial age(year) | −1.06 ± 18.31 | −3.35 ± 14.62 | 0.59 |
| Cf-PWV(m/s) | −1.09 ± 0.83 | −0.43 ± 1.24 | 0.03 |

Values are means±SD

*Between groups

**Table 1.5** Linear regression to adjust for confounding factors on PWV change

| Variables | B | Std. error | Beta | P value |
|---|---|---|---|---|
| Δ energy intake | −0.001 | 0.001 | −0.314 | 0.053 |
| Δ physical activity | 0.00006 | 0.00007 | 0.119 | 0.431 |
| Group | 0.84 | 0.319 | 0.39 | 0.011 |
| Gender | 0.547 | 0.307 | 0.251 | 0.082 |
| Age | 0.018 | 0.026 | 0.098 | 0.484 |

Δ after – before

## 1.4 Discussion

The present investigation demonstrated that 12 weeks of regular ingestion of curcumin supplement ameliorated aortic stiffness in metabolic syndrome patients. We assessed PWV as a principal marker of large arteries stiffness and observed that curcumin supplementation significantly reduced PWV. Analysis of potential sex differences did not show any significant improvement in arterial stiffness parameters in women who received curcumin intervention. Initial evidence in a preclinical study performed by Fleenor et al. [33] demonstrated that dietary curcumin supplementation improves age-related large elastic artery stiffness by nitric oxide bioavailability restoration, oxidative stress reduction and normalization of collagen I and advanced glycation end products (AGES) deposition in the arterial wall.

This finding is consistent with research showing that arterial stiffness (PWV or carotid arterial compliance) significantly improves after several weeks to months of curcumin treatment [20, 37, 38]. However, two studies reported that curcumin ingestion does not affect PWV [36, 39]. A recent study conducted by Campbell et al. demonstrated that only subjects with a higher baseline value of aortic PWV (arterial stiffness) responded to curcumin supplementation. In the present investigation, the mean age of patients receiving curcumin was 44 years old and the mean baseline cf-PWV value was 7.6 m/s. However, the mean cf-PWV for healthy 40–49 year-old subjects was found to be 7.2 m/s [40]. This suggests that there may be differences in the measured mean baseline cf-PWV across different studies.

We found that the reflection wave indices (aortic AP, AIX, and AIX75) were not affected by the curcumin intervention. In agreement with this finding, Sugawara et al. [36] found that curcumin significantly decreased aortic AIX75 only when combined with exercise training. AIX is a complicated variable that shows the stiffness of smaller muscular arteries as well as microvascular density, number and location of terminal arterioles that give rise to reflected waves, the velocity of the pressure wave, and the pattern of left ventricular ejection. In addition, in contrast to PWV, AIX is influenced by gender and anthropometric measurements [41].

Today lifestyle modification is the main strategy for prevention of CVD, and weight management is a key factor in this objective. For this objective, curcumin has been reported to have beneficial effects on obesity management [42–45]. A preclinical study suggested that curcumin has antiobesity effects through downregulating the expression of peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein α, which are key transcription factors in adipogenesis and lipogenesis [46]. This results in suppression of adipocyte differentiation, fatty acid esterification, and adipokine-induced angiogenesis in adipose tissue, and induction of fatty acid oxidation and increased apoptosis of adipocytes. In humans, 10 weeks of curcumin supplementation significantly decreased mean body weight in overweight type 2 diabetes patients [42]. consistent with this finding, we observed that 12 weeks of curcumin ingestion significantly decreased body weight compared to the placebo group. Although not statistically significant, the curcumin intervention also tended to decrease WC, NC, BMI, and visceral fat area, while these parameters had an increasing trend in the placebo group.

Metabolic syndrome is a serious health condition of impaired glucose tolerance and, consequently, elevation of fasting plasma glucose is one of its criteria. Many animal studies have demonstrated that curcumin anti-inflammatory and antioxidant activities may be responsible, at least in part, for its anti-hyperglycemic effects [47–49]. Studies in humans have been inconsistent as some have confirmed the curcumin anti-hyperglycemic effect [42, 43, 50], and others have found no effect [51, 52]. In our study, FPG did not significantly change with curcumin supplementation compared to placebo. Also, we did not observe any significant changes in lipid profiles between the groups. Many preclinical studies have found that curcumin reduces serum cholesterol levels via upregulating the expression of hepatic LDL receptors, inhibition of LDL oxidation, enhancement of cholesterol excretion by increasing bile acid secretion, and suppressing

the expression of genes involved in cholesterol biosynthesis [53, 54]. Furthermore, a recent animal study showed that curcumin reduced serum TG concentrations through inhibition of sterol regulatory element-binding protein 1 (SREBP-1c), liver X receptor alpha (LXR-α), and the target lipogenic enzymes fatty acid synthase and acetyl CoA carboxylase [55]. To our knowledge and according to literature review, curcumin should be consumed in higher doses or in a higher efficacy form (nano-formulation or in combination with an adjuvant) to influence FPG and lipid profiles.

Since the liver is the main organ for drug metabolism and elimination, it should be considered that hepatotoxic reactions may take place there in the present study. Such drug-induced hepatotoxicity manifestations are various, ranging from a mild elevation of liver enzymes to fatal hepatic failure [56]. During the present clinical trial, liver function was not affected by the interventions, as determined by the lack of effect on ALT and AST, which are commonly used as biomarkers of liver damage.

This study was limited by its short-term duration of follow-up that precluded the possibility of assessing hard cardiovascular endpoints. Furthermore, although the mean baseline cf-PWV values of participants were modestly elevated, not all participants had high PWV. The inability to present a mechanistic view for the beneficial effects of curcumin on vascular aging was another limitation of this study.

## 1.5 Conclusions

In the present study, we observed the favorable effects of 12 weeks of curcumin supplementation on arterial stiffness and weight control. We also demonstrated that curcumin intake for 12 weeks was well tolerated. Further trials are warranted to confirm the present findings in target populations with elevated arterial stiffness.

**Conflict of Interest** None of the authors had declarations of interest to publish.

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# 2 Role of Curcumin in Regulating Long Noncoding RNA Expression in Cancer

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**Abstract**

Phytochemicals are various compounds produced by plants. There is growing evidence on their potential health effects. Some of these compounds are considered as traditional medicines and used as painkillers, anti-inflammatory agents, and for other applications. One of these phytochemicals is curumin, a natural polyphenol derived from the turmeric plant (*Curcuma longa* L.). Curcumin is widely used as a food coloring, preservative and condiment. It has also been shown to have antioxidative and anti-inflammatory effects. Moreover, there is growing evidence that curcumin alters long noncoding RNAs (lncRNAs) in many kinds of cancer. These noncoding RNAs can cause epigenetic modulation in the expression of several genes. This study reviews reports of curcumin effects on lncRNAs in lung, prostate, colorectal, breast, pancreatic, renal, gastric, and ovarian cancers.

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**Keywords**

Cancer · Curcumin · Non-coding RNA · lncRNA · Epigenetic · Cancer · Therapeutics

## 2.1 Introduction

Cancer is one of the major human illnesses resulting in considerable mortality. The rate of morbidity due to cancer has been increased in recent years [1–4]. Chemotherapy is considered as one of the major therapeutic approaches for management of cancer by inducing apoptosis and inhibiting tumor growth [5–8]. However, chemotherapy affects both healthy and cancer cells resulting in considerable side effects [9]. Hence, there has been an increasing use of therapies which target cancer cells more specifically. Various methods of targeted therapies include monoclonal antibodies [10], small molecule inhibitors [11], immunotoxins [12], and the use of drug nanocarriers to deliver the chemotherapeutic agents to selected cancer cells [13–15]. Other therapeutic options for cancer treatment include various radiotherapies [16] and hormonal treatments [17–20].

Along with these standard methods of cancer treatment, a number of natural products [21–24], have been considered which target diverse signaling pathways in cancer cells [5, 25, 26]. These natural compounds are called phytochemicals and can be divided as as polyphenols, carotenoids, terpenoids, alkaloids, phytosterols, and lectins, to name a few The polyphenols are one of the most abundant secondary metabolites in plants with antioxidant protperties. One example of the polyphenol class which has received considerable attention is curcumin [(1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptane-3,5-dione], which is yellow pigmented polyphenol from the rhizome of *Curcuma longa* Linn with several health benefits (Fig. 2.1) [27–30]. Several studies have demonstrated the safety, pharmacological activity and possible therapeutic use of curcumin in the treatment of various diseases [31–45].

It has been shown that curcumin decreases the rapid growth of distinct cancer cells via inhibition of migration, growth and invasion. Additionally, curcumin can induce apoptosis and repress cancer cell development and progression, both in-vivo and in-vitro. One mechanism on how this is achieved occurs through noncoding

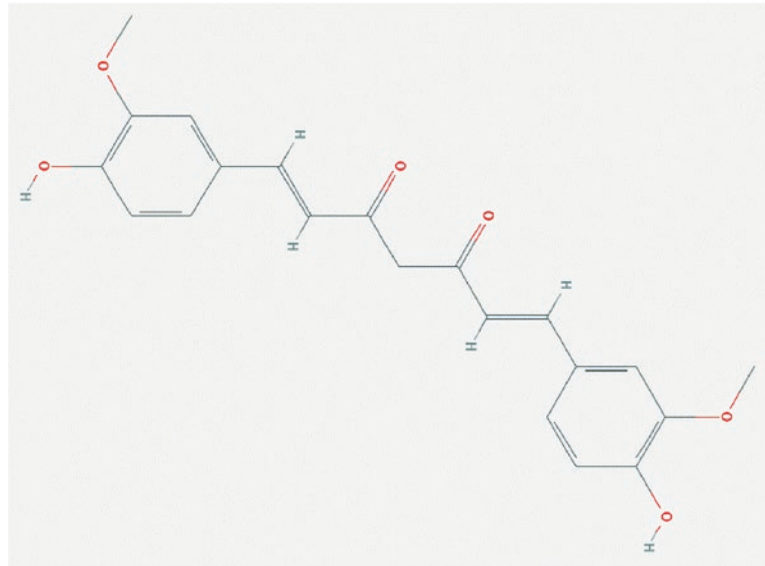

**Fig. 2.1** 2D structure of curcumin (PubChem CID: 969516)

RNA molecules, which regulate gene expression at the epigenetic level via complementary base-pairing with sequences within mRNA molecules. Curcumin has been shown exert epigenetic regulatory effects on noncoding RNAs in different types of cancers [46, 47]. Noncoding RNAs could be categorized as long noncoding (lnc) and short noncoding (snc) RNAs, based on length [48, 49]. Here, we review the effects of curumin on lncRNAs as a potential therapeutic approach in cancer treatment.

## 2.2 The Role of lncRNAs in the Development of Cancer

lncRNAs or long noncoding RNAs are noncoding sequences of ribonucleotides which are generally longer than 200 nucleotides without an open reading frame, and they are not translated into proteins. These lncRNAs are responsible for the expression of various genes associated with the development of diseases such as cancer [50]. These noncoding RNAs interact with RNA, DNA, and protein complexes, thereby, acting as chromatin organization, transcriptional, and post-transcriptional regulators. In the case of cancer, this could result in altered expression of genes associated with cell growth, metastasis, and tumor formation [51]. Many phytochemicals such as curcumin, can modulate lncRNAs and thereby dysregulate these processes in cancer [52].

There are three possible mechanisms by which lncRNAs are involved in cancer development and progression: i) translational regulation; ii) post-translational control; and iii) chromatin remodeling (Fig. 2.2). For example, HOTAIR is a lncRNA that upregulates the c-myc proto-oncogene in breast and ovarian cancer, which in turn could be down-regulated by curcumin at the transcriptional level [53, 54]. The influence of lncRNA regulation on chromatin remodeling occurs via effects on chromatin remodeling enzymes, which alters chromatin structure and thereby changes susceptibility to genetic reprogramming mechanisms.

## 2.3 Biogenesis and Function of lncRNAs

It is commonly recognized that lncRNAs are by-products of transcription and usually consist of more than 200 bases [55]. This occurs mostly through the actions of RNA polymerases II and III [49, 56]. Similar to the microRNAs (miRNAs), lncRNAs have the capability of binding to specific proteins, RNA, as well as nucleating RNA compartments, which generate the ribonucleoprotein complexes. It should be noted that lncRNAs could operate directly following their synthesis, and function as scaffolds for promoting dynamic gene control [57]. In this way, lncRNAs can have both normal and pathological functions [48]. In cancer, the expression of thousands of lncRNAs can vary based on the kind of tumor [49, 58]. Most of the well-studied lncRNAs play a crucial role in controlling critical cellular processes such as growth and apoptosis to maintain homeostasis while others take part in cancer development via promoting uncontrolled cell proliferation, metastasis, inducing genetic instability, developing drug resistance and invasion capacity [59].

Two studies registered 7258 sncRNAs and 15,767 annotated lncRNAs in the GENCODE database, consisting of the greatest popular compilation of transcripts [48, 60]. Based on the outcomes of the tissue microarray analyses and next-generation sequencing, it is understood that gene expression can be modulated by the lncRNAs at various stages, including epigenetic, transcriptional and post-transcriptional levels. Therefore, lncRNAs have considerable scope as drug targets in diverse medical areas [61]. Notably, lncRNAs may function as a molecular decoy or sponge of miRNAs, which influences miRNA activity and the level of expression [62]. Likewise, lncRNAs directly or indirectly target the miRNAs. However, an active crosstalk has been observed between miRNAs and lncRNAs via a double-negative feedback circle [58]. Ye et al. demonstrated an example of such cooperation between micoRNAs and lncRNAs which results in the development of cancer [63]. In this study, 5 miRNA nodes, 13 lncRNA nodes, and 45

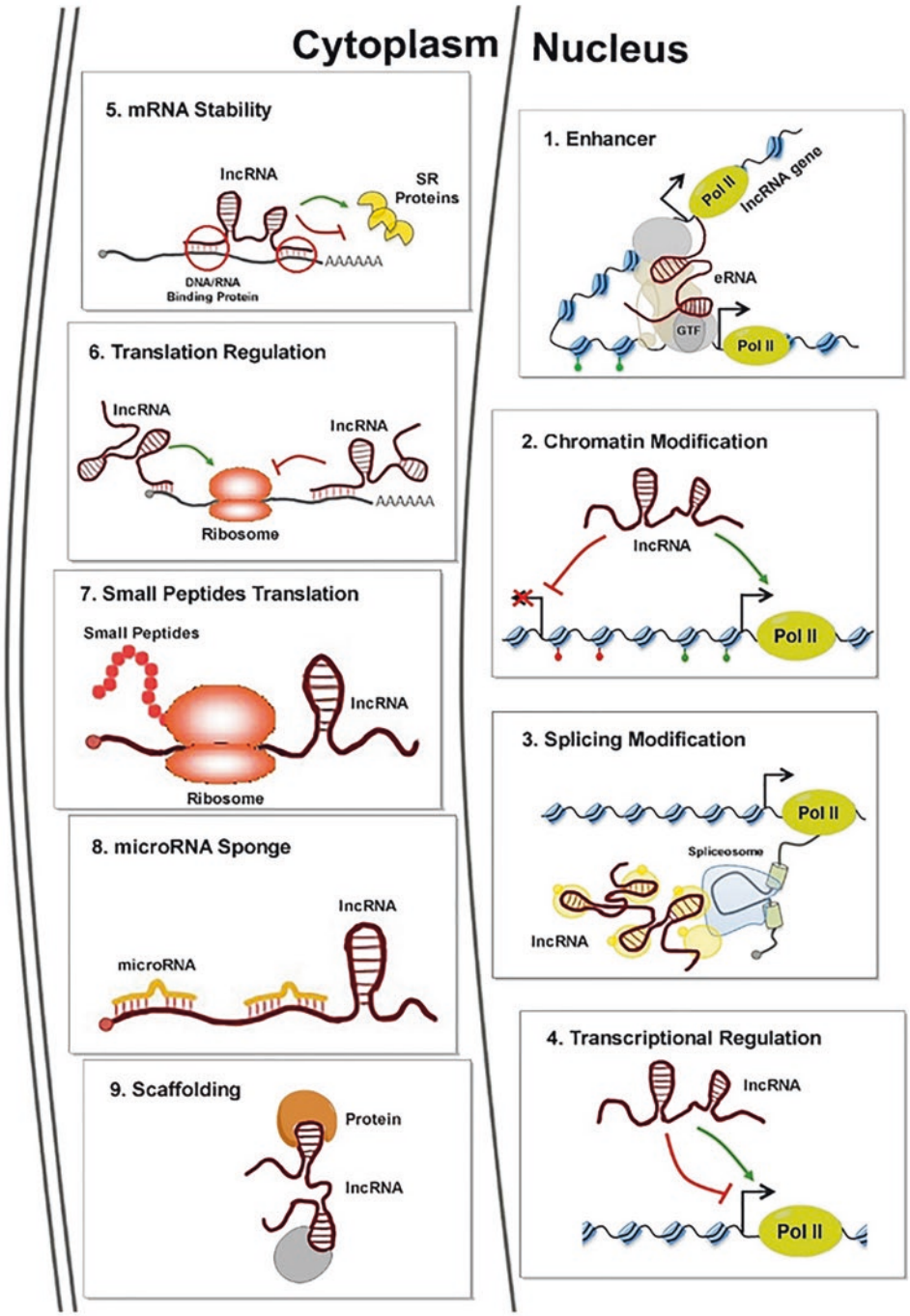


**Fig. 2.2** Long non-coding RNAs' (lncRNAs) main mechanisms of action. 1: enhancing DNA transcription, 2: employing chromatin-modifying complexes (e.g. histone methylases, acetylases, and deacetylases) to target sites in the genome, 3: regulating pre-mRNA splicing, 4: binding to transcription factors and changing their function, 5: binding to mRNAs to increase stability and regulate trafficking, 6: binding to mRNAs to induce translation activation or suppression, 7: some lncRNAs encode biological small peptides, 8: competing with the regulatory activity of miRNAs, 9: LncRNAs can alter protein function by scaffolding roles and providing docking site in the same biological pathway

mRNA nodes participate in phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling pathway regulation and dysregulation of important oncogenes related to prostate cancer. In this role, the lncRNAs contribute to the epigenetic, post-transcriptional, and transcriptional modulation of gene expression. Moreover, two primary groups of lncRNAs have been reported with either oncogene [e.g., metastasis-associated lung adenocarcinoma transcript 1(MALAT1), SOX2 overlapping transcript (SOX2-OT), HOX transcript antisense RNA (HOTAIR) and H19], or tumor suppressor [e.g., maternally expressed 3 (MEG3), taurine upregulated gene 1 (TUG1), growth arrest specific 5 (GAS5), and promoter of CDKN1A antisense DNA damage-activated RNA (PANDAR)] roles, based on the respective pathological characteristics [64].

## 2.4 Effects of Curcumin on lncRNA Expression in Cancer

The following sections highlight examples in which the ability of curcumin treatment to alter lncRNA expression is tested in various cancers.

### 2.4.1 Colorectal Cancer (CRC)

Evidence suggests the contribution of lncRNAs in the metastasis, invasion, chemotherapy and radiotherapy resistance in CRC via interaction with distinct signaling pathways like Wnt, epithelial to mesenchymal transition (EMT), transforming growth factor-β (TGF-β), and miRNAs [65]. In particular, many studies have shown the ability of lncRNAs for direct regulation of the metastatic paths in CRC. As a result, 28 CRC-associated oncogene lncRNAs (including UCA1, HOTAIR, MEG3 and H19) have been recognized in one of the studies and 13 tumor suppressor lncRNAs (such as GAS5 and MEG3) have found in the other [66].

However, in-vitro experiments showed that curcumin treatment led to increased lncRNA PANDAR in CRC cells and this attenuated senescence and increased apoptosis [67]. In addition, silencing PANDAR in curcumin treated cells enhanced the apoptosis rate potentially by an increased level of p53 upregulated modulator of apoptosis (PUMA). Curcumin increases the expression of the neighbor of BRCA1 gene NBR2 lncRNA, which inhibits proliferation, clone formation, and decreases the percentage of S-phase cells in colorectal cancer via the 5′ AMP-activated protein kinase (AMPK) pathway [68]. Plasmacytoma variant translocation 1 (PVT1) is another lncRNA which is also expressed in colorectal cancer and its effects on tumor formation, expansion, and drug resistance are well-established. The finding that curcumin prevents PVT1 over-expression in tumor cells [69] adds further weight to the idea that it may be considered as a potential novel adjuvant treatment in CRC.

### 2.4.2 Pancreatic Cancer

We identified increased expression levels of H19, lncRNAs, regulator of reprogramming (ROR), nuclear-enriched abundant transcript-1 (NEAT1), MIR31 host gene, and nuclear transport factor 2 pseudogene 3 (NUTF2P3) in pancreatic cancer using various systems [70]. Another study reported curcuminn treatment in BxPC3-GemR pancreatic ductal adenocarcinoma cells augmented reversal of gemcitabine resistance via suppression of the expression of the polycomb repressive complex 2 (PRC2) subunit, enhancer of zeste homolog-2 (EZH2), and the respective lncRNA, PVT1 [69] (Table 2.1).

### 2.4.3 Lung Cancer

A study by Wang et al. showed that curcumin induces apoptosis in A549 lung cancer cells by down-regulation of urothelial cancer associated-1 (UCA1) lncRNA [72]. This occurs via suppression of Wnt and mTOR pathways. Curcumin has also been found to regulate other lncRNAs which influence oncogene expressions in lung cancer. Another study has shown that there are multiple

**Table 2.1** Alterations of long noncoding RNAs in cancer in response to curcumin

| Cancer type | lncRNA* | Cell line | References |
|---|---|---|---|
| Colorectal | PANDAR (UP)<br>NBR2 (UP)<br>PVT1 (DOWN) | CRC | [67, 68] |
| Pancreatic | ROR, H19, NEAT1 (UP)<br>Nuclear-enriched abundant transcript-1 (NEAT1) (UP)<br>MIR31HG (UP)<br>Nuclear transport factor 2 pseudogene 3 (NUTF2P3) (UP)<br>PVT1(DOWN) | BxPC3-GemR | [70–72] |
| Lung | Urothelial cancer associated-1 (UCA1) (DOWN)<br>GAS5, PANDAR, MEG3 (DOWN)<br>HOTAIR, MALAT1, H19 (UP)<br>PVT1(DOWN) | A549 | [72–78] |
| Breast | MALAT1, HOTAIR, H19<br>GAS5 (DOWN)<br>Tusc7, ATB (DOWN) | MDA-MB231, SKBR3, and MCF7 | [76–78] |
| Ovarian | MEG3 (UP) | A2780cp | [80] |
| Prostate | HOTAIR, SOCS2-AS1, PVT1<br>MEG3, GAS5, and H19<br>ROR<br>CCAT1 (DOWN) | CRPC<br>HuPCaSCs<br>PC3-TXR /DU145-TXR | [5, 23, 82–85] |
| Renal | HOTAIR (DOWN) | 769-P, 769-P-vector, 769-P-HOTAIR, 786–0, and Kert-3 | [86, 87] |
| | XIST (DOWN) | ACHN and Caki-2 | |
| Hepatocellular | MEG3 (UP) | HepG2 and HuH-7 | [88] |
| Gastric | H19 (DOWN) | SGC-7901 | [89] |
| Nasopharyngeal | GUCY2GP (UP)<br>H2BFXP (UP)<br>LINC00623 (UP)<br>ZRANB2-AS2 (DOWN)<br>LOC100506835 (DOWN)<br>FLJ36000 (DOWN) | CNE-2 | [90] |

dysregulated lncRNAs in non-small cell lung cancer (NSCLC), which are potential candidates for new biomarkers are drug targets [73]. They found downregulation of 9 lncRNAs (e.g., GAS5, PANDAR, MEG3) and upregulation of 24 lncRNAs (e.g., HOTAIR, MALAT1, H19) in NSCLC. The PVT1 lncRNA contributes to a number of cancers including NSCLC. This lncRNA promotes lung cancer cell proliferation, invasion, metastasis, and drug resistance. The molecular process underlying its effects appears to involve interaction with the c-Myc oncogene, modulation of miRNAs, and regulation of gene transcription and protein expression. In addition, over-expression of the PVT1 gene has been observed in patients suffering from NSCLC [74, 75]. Since curcumin has been found to downregulate the expression of this lncRNA, further studies should assess its utility as a novel treatment approach in lung cancer.

### 2.4.4 Breast Cancer

In the case of breast cancer, the association of multiple lncRNAs (e.g., MALAT1, HOTAIR, H19) has been described [76]. In addition, a study showed that treatment of MDA-MB231, SKBR3 and MCF7 breast cancer cells with dendrosomal curcuminin (DNC) led to increased expression of

growth arrest-specific 5 (GAS5) and tumor suppressor candidate 7 (Tusc7) lncRNAs [77]. Conversely, down-regulation of GAS5 decreased the anti-cancer effects of the DNC treatment. The activated by TGF-β (ATB) lncRNA is overexpressed in breast cancer cells and exacerbates the metastasis of these cells via the ROR pathway. Interestingly, curcumin treatment suppresses this effect, again supporting its potential use as a breast cancer treatment [78].

### 2.4.5 Ovarian Cancer

A meta-analysis has identified lncRNA clusters with distinctive metastatic capacities in ovarian cancer cells [79]. One of these, lncRNAs, maternally expressed 3 (MEG3), is known to be decreased in ovarian cancer. This is important as another study showed that curcumin was able to suppress resistance of ovarian cancer cells to the chemotherapeutic cisplatin via a change in gene methylation leading to reduction of miR-214 and restoration of MEG3 levels [80].

### 2.4.6 Prostate Cancer

Prostate cancer antigen-3 (PCA3) was one of the first highly up-regulated lncRNAs to be indentified for prostate cancer and it appears to be specific for this form of cancer [81]. Seventeen lncRNAs (HOTAIR, SOCS2-AS1, PVT1, & so on) were found to be involved in the progression of prostate cancer. Further, lower expression level of three lncRNAs (MEG3, GAS5, & H19) was also found in prostate cancer [82]. In-vitro, studies in pancreatic cancer cell line (HuPCaSCs) revealed an overexpression of miR-145, cell cycle arrest, suppression of the cell rapid growth, and invasion following pre-treatment with curcumin. Consequently, luciferase activity assays reflected that lncRNA-ROR and Oct4 could relatively attach to the miRNAs as a result of the respective popular binding sites of miR-145. Altogether, downregulating the endogenous lncRNA-ROR increases the expression level of miR-145 in HuPCaSC and thus miR-145 suppresses the rapid cell proliferation via the declined level of Oct4 expression [5, 80, 83]. In a research conducted in the year 2020, CCAT1 or colon cancer-associated transcript 1 found in colon cancer has a decisive role in the progression of prostate cancer as well as reducing the sensitivity to paclitaxel (PTX) a chemotherapeutic agent in prostate cancer. The expression of this lncRNA is accompanied by microRNA-24-3p (miR-24-3p) and fascin1 (FSCN1) synthesis in malignant cells. Curcumin is found to reduce the level of CCAT1 and inactive PI3K/Akt/mTOR pathways [84, 85].

### 2.4.7 Renal Cancer

During renal cell carcinoma (RCC), kidney cancer cells originate from proximal convoluted tubule. According to Pei et al., a direct correlation exists between HOTAIR mRNA expression and cell migration and metastasis of renal cancer cells. In the mentioned study, curcumin dose-dependently inhibited cell migration [86]. Another lncRNA called XIST (x inactive specific protein) plays a pivotal role in renal cell carcinoma progression. The underlying molecular mechanism is still unclear. However, documents show the possibility of the interaction between miR-106b-5p and increased expression of P21. Curcumin regulates XIST/miR-106b-5p/P21 axis in RCC cells [87].

### 2.4.8 Hepatocellular Cancer

Hepatocellular carcinoma is a prevalent type of cancer that is associated with high rates of chemotherapy resistance. On the other hand, MEG3 is a common tumor suppresser lncRNA expressed in healthy cells. This lncRNA is downregulated in hepatocarcinoma cells, and the reason underlies the specific methylation pattern of MEG3 promoter by DNMT1, DNMT3A and 3B. Curcumin overexpresses MEG3 *via* downregulating DNMT1, DNMT3A and 3B, thereby altering methylation process and assisting in hepatocellular cancer treatment [88].

### 2.4.9 Gastric Cancer

Long noncoding RNA H19 is overexpressed in gastric cancer cells and can directly inhibit p53 expression, thereby promoting gastric cancer progression. Turmeric extract has been reported to reduce overexpression of H19 and protect against gastric cancer [89].

### 2.4.10 Curcumin in Nasopharyngeal Cancer

In nasopharyngeal CNE-2 carcinoma cells, curcumin was found to radiosensitize the cells. Moreover, curcumin significantly up-regulated the expression of lncRNAs such as GUCY2GP, H2BFXP, and LINC00623, while the expression of ZRANB2-AS2, LOC100506835, and FLJ36000 were down-regulated [90]. In another study, curcumin modulated the lncRNAs such as AF086415, AK056098, AK095147, AK294004, MUDENG, and RP1-179 N16.3, thereby radiosensitizing these cells [91].

## 2.5 Conclusions and Perspectives

Noncoding RNAs contributes to the regulation of the biology of cancer cells so that these RNAs could be recognized as the promising approaches to the management of various cancers. The ability of curcumin to modulate lncRNA expression has provided a new molecular basis for its biological activities Moreover, it has been found that natural products such as curcumin and other plant derivatives would apply considerable antiproliferative impacts on different types of cancer cells. Inhibiting the proliferation of the cancer cells via regulation of specific noncoding RNAs by phytochemicals could be a potential turning point in cancer treatment. Thus, it could be concluded that the above results would help elucidate the mechanisms underpinning the effectiveness of phytochemicals, which would present worthwhile insight into the assessment of the novel cancer treatments. However, most of these studies have been carried out in cell culture models. Future studies should also elucidate if curcumin can effectively regulate lncRNA expression in human subjects.

**Competing Interests** Muhammed Majeed is the founder of Sabinsa Corporation and Sami Labs Ltd. The authors have no other conflicting interests to disclose.

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# 3 The Effect of Curcumin Phytosome on the Treatment of Patients with Non-alcoholic Fatty Liver Disease: A Double-Blind, Randomized, Placebo-Controlled Trial

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**Abstract**

Non-alcoholic fatty liver disease (NAFLD) is a global health problem with increasing prevalence among overweight and obese patients. It is strongly associated with conditions of insulin resistance including type 2 diabetes mellitus (T2DM) and obesity. It has detrimental consequences ranged from simple steatosis to irreversible hepatic fibrosis and cirrhosis. Curcumin is a dietary polyphenol with potential effect in improving NAFLD. Therefore, the aim of this trial was to examine the effect of curcumin supplementation on various aspects of NAFLD. In this trial, a total number of 80 patients were randomised to receive either curcumin at 250 mg daily or placebo for 2 months. Lipid profiles, hepatic enzymes, anthropometric indices and hepatic fat mass were assessed at the baseline and the end of the trial, and compared within the groups. The

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grade of hepatic steatosis, and serum aspartate aminotransferase (AST) levels were significantly reduced in the curcumin group ($p = 0.015$ and $p = 0.007$, respectively) compared to the placebo. There was also a significant reduction in high density lipoprotein (HDL) levels and anthropometric indices in both groups with no significant differences between the two groups. Low dose phospholipid curcumin supplementation each day for 2 months showed significant reduction in hepatic steatosis and enzymes in patients with NAFLD compared to placebo. Further studies of longer duration and higher dosages are needed to assess its effect on other parameters of NAFLD including cardiovascular risk.

**Keywords**

NAFLD · Non-alcoholic fatty liver disease · Curcumin · Phytosome · Turmeric · NASH

## 3.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is a global health problem with increasing prevalence worldwide in parallel with obesity. It is a condition of excess hepatic fat accumulation in non-alcoholic subjects [1], associated with conditions of insulin resistance such as type 2 diabetes mellitus (T2DM) and obesity. Therefore, it is regarded as a hepatic manifestation of metabolic syndrome [2]. NAFLD has a wide spectrum of manifestations from simple steatosis with benign hepatic features to non-alcoholic steatohepatitis (NASH) and irreversible hepatic fibrosis [3]. NASH is a necroinflammatory process of the hepatic cells with a tendency to progress to liver cirrhosis and hepatocellular carcinoma [4]. The pathophysiology of NAFLD is associated with metabolic diseases such as insulin resistance and obesity [5]. It was initially hypothesised that the hepatic triglyceride accumulation is mediated by inflammatory reactions (cytokine/adipokines, oxidative stress and mitochondrial dysfunction) as the main driver for the underlying pathogenesis of steatohepatitis and fibrosis [6]. However, more recent hypotheses have proposed that the pathophysiology of NAFLD is driven by a combination of genetic, epigenetic, environmental and nutritional factors, as well as by obesity, hormone secretion from adipose tissue and insulin resistance [7].

Despite the recent advances in understanding of the pathological mechanism of NAFLD, effective therapeutic options are still limited. Currently, treatment options are primarily focused on improving metabolic parameters such as body weight, physical activity, insulin sensitivity, as well as lipid profiles and glycaemic control. Thus, insulin sensitising agents (e.g., metformin or pioglitazone), lipid lowering compounds (e.g. statins), weight loss medications (e.g., orlistat or sibutramine) and even bariatric surgery have been introduced as potential means for managing NAFLD [8]. There are also numerous new and emerging potential NASH therapeutic approaches including anti-oxidants such as vitamin C, vitamin E and anti-inflammatory agents [9, 10]. However, the challenge still remains to gain approval of these as a treatment approach in NAFLD patients. Within the past few years, curcumin popularity as a potential therapeutic option for treatment of NAFLD has increased. Traditionally, curcumin is in common use in Asian cooking, but also used as household triage for various diseases [11]. Its safety and therapeutic activities, including anti-inflammatory and antioxidant properties, have been reported in several previous studies [12–21]. It has been shown that curcumin prevents liver fibrosis and subsequent liver cirrhosis through its anti-inflammatory effects and suppression of the hepatic satellite cell (HSC) activity [22]. Furthermore, short term supplementation with curcumin has been demonstrated to improve anthropometric measures, hepatic enzymes and liver fat mass, as assessed by ultrasonography [23].

Given the limited number of clinical studies investigating the potential therapeutic effects of curcumin supplementation on various metabolic parameters in patients with NAFLD, we aimed to examine the therapeutic effect of low dose phospholipid curcumin on lipid profiles, hepatic enzymes and hepatic fat mass in patients with NAFLD in a randomised controlled clinical

study. Previous studies have shown that this curcumin formulation drives higher systemic levels of curcumin compared to the non-formulated version, thereby increasing its bioavailability [24, 25].

## 3.2 Methods

### 3.2.1 Trial Design

This study was an 8-week, double-blind, placebo-controlled, parallel-group conducted in Neyshabur City in the northeast of Iran. The allocation ratio was 1:1 for two groups. The study was approved by the Institutional Review Board and the Ethical Committee of Neyshabur University of Medical Sciences (Code: IR.NUMS.REC.1394.18). Also, the study was registered in the Iranian Registry of Clinical Trials (http://www.irct.ir; IRCT registration number: IRCT2015052322381N1). All participants who were recruited signed a consent form before any trial-related procedures occurs.

### 3.2.2 Participants

Eligible patients were all adults aged 18 to 65 years who met the eligibility criteria for NAFLD according to ultrasound examination and laboratory results. NAFLD was defined based on higher echogenicity of the liver compared with that of the renal parenchyma due to fatty infiltration. A normal liver was defined if the echogenicity of the liver parenchyma was equal to or only slightly higher than that of the renal parenchyma [26]. Eighty patients with NAFLD were

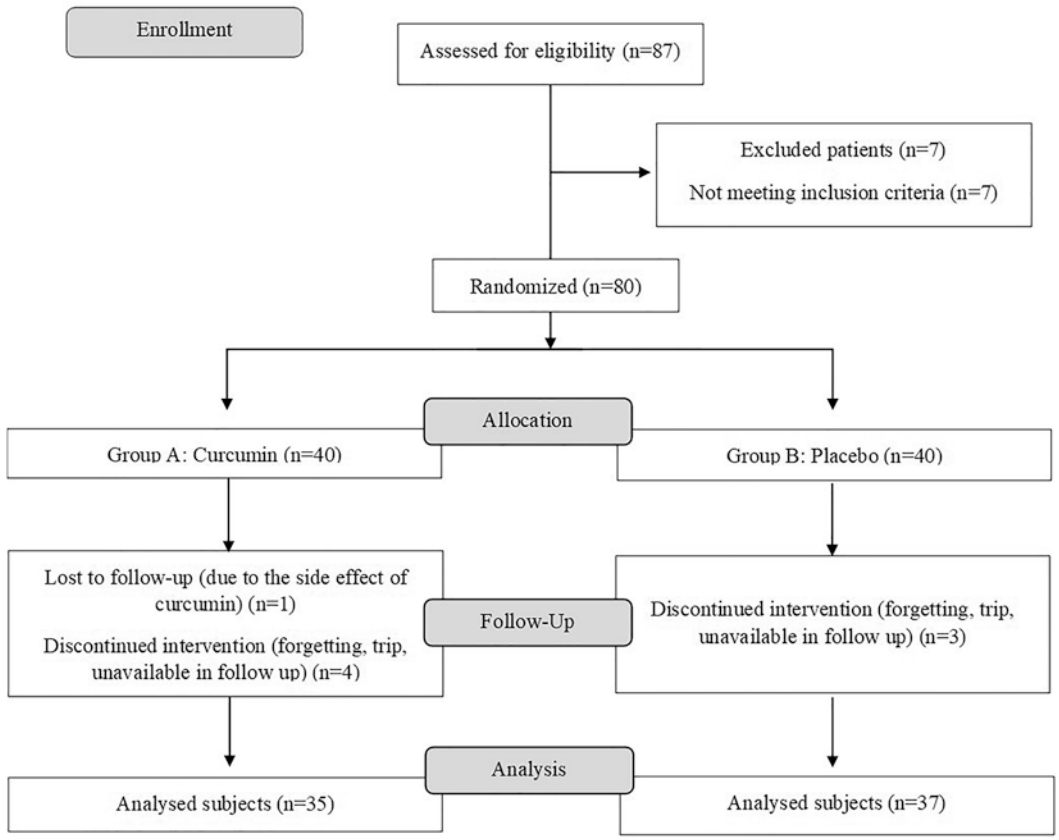


**Fig. 3.1** Flow diagram of study participation

recruited for the study and 8 of these dropped out (Fig. 3.1). Referred patients of the 22 Bahman Hospital (Neyshabur, Iran) from January 2017 to August 2017 were recruited. The exclusion criteria included females with pregnancy/lactation, the presence of alcoholic liver disease, severe heart or lung disease, or the taking of anti-inflammatory drugs such as corticosteroids and liver enzyme inducer drugs, acute or chronic liver disorders such as viral and autoimmune hepatitis, metabolic liver disorders including hemochromatosis and Wilson's disease, Budd–Chiari syndrome, or other medical disorders such as hyper/hypothyroidism, alpha-1 antitrypsin deficiency, celiac disease or cancer.

### 3.2.3 Randomization

The subjects were randomly allocated to the curcumin or control group using a balanced block randomization technique. Accordingly, two letters were prepared and written on two sheets of paper with "A" for "curcumin" and "B" for "control." The following quad blocks were possible: AABB, ABAB, ABBA, BBAA, BABA and BAAB. After this, the number was selected randomly using a table of random numbers. To ensure that implementation of the random allocation sequence occured without the knowledge of which patient will receive which treatment, the entire randomization process was concealed. To achieve this, the drugs had already been put in envelopes labelled a serial number from 1 to 80 and no one knew the nature of the envelopes apart from the coordinator of the trial.

### 3.2.4 Intervention

The patients in the treatment group received capsules of phospholipidated curcumin (250 mg/day, Meriva curcumin phytosome; Indena SpA, Milan, Italy). Each capsule was composed of 250 mg curcumin phytosome powder, which was equivalent to 50 mg/day pure curcuminoids. The control group received matched placebo capsules at the same dose. The drug consumption route was oral for a period of 2 months. To keep track of the medication, the bottles of the drug were given to the subjects at the beginning and in the middle (after 1 month) of the interventions period.

### 3.2.5 Assessment of Outcomes

The primary and secondary outcomes were ultrasound examination and the anthropometric and clinical measurements, respectively.

#### 3.2.5.1 Biochemical and Anthropometric Measurement

Venous blood samples were taken from each patient after an overnight fasting period before and after the intervention on days 0 and 60. This was carried out since the levels of biochemical measurements can be influenced by food intake and diurnal rhythms. For separation of serum, blood samples were centrifuged at 1000 x g for 10 min. Biochemical and lab measurements such as lipid variables, fasting blood glucose (FBG) and liver function tests were performed immediately after serum preparation via the BT-2000 Auto Analyzer machine (Biotechnica; Rome, Italy) using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran).

Bodyweight and body mass were measured using the BPM040S12FXX 770 device (In Body; Seoul, South Korea), with an accuracy of 0.1 kg. According to the protocol of the device, all patients were barefoot with lightweight clothing during the measurements. Body mass index (BMI; $kg/m^2$) and other anthropometric measurements were calculated using the device. Body height was measured by a BSM 370 digital stadiometer (InBody), with accuracy to the nearest 0.1 cm.

Due to the difference in the diet of patients and its possible impact on outcomes, the subjects reported a favourable response to the diet. All

patients were asked to have an energy balanced diet according to the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults from the National Institutes of Health and the North American Association for the study of obesity. According to the guideline, the recommended diet consists of ≤30% fat (one-third saturated and two-thirds unsaturated fatty acids), 52–53% carbohydrates, 20–30 g/day fibre, < 300 mg/dL cholesterol and 15–18% protein (all percentages related to the total energy value). Also, all patients were advised to exercise three times each week for at least 30 min.

#### 3.2.5.2 Statistics Analysis

Normal and non-normal distribution variables were presented as mean ± standard deviation (SD) and median (interquartile range (IQR)), respectively. The Kolmogorov-Smirnov test was used for assessing normality of the variables. To compare two related samples (before, after) for parametric and non-parametric variables, the dependent t-test and the Wilcoxon signed-rank test were used, respectively. For comparing characteristics of patients in the treatment and placebo groups, the independent T-test and the Mann-Whitney U test were performed for normal and non-normal distribution variables, respectively. Furthermore, categorical data such as sex and smoking were analyzed using chi-square and Fisher's exact test.

## 3.3 Results

Out of 87 patients recruited with NAFLD, 7 subjects did not meet the inclusion criteria (Fig. 3.1). Thus, 80 patients were randomly allocated to the two groups (curcumin and control). After enrolment of all patients, 8 were lost during the follow up period due to curcumin side effects, discontinuation or forgetfulness regarding the intervention, or unavailability for other reasons.

### 3.3.1 Characteristics of Patients

The demographics and medical history of the patients with NAFLD are shown in Table 3.1. As can be seen, there was no significant difference between the curcumin and placebo groups apart for history of hypertension.

### 3.3.2 Anthropometric, Biochemical and Sonography Analyses

Anthropometric, biochemical and sonography data before and after intervention are given in Table 3.2. This showed that high-density lipoprotein cholesterol (HDL-C) was significantly increased in the placebo group, while this was decreased in the curcumin group ($p < 0.05$). Also, aspartate aminotransferase (AST) levels and NAFLD grade (based on sonography) were significantly decreased after the curcumin treatment ($p < 0.05$). However, there were no significant differences in these parameters in the placebo group. No other variables showed significant differences due to treatment.

### 3.3.3 Comparison of the Changes of Anthropometric, Biochemical and Sonography Data of Patients with NAFLD Between the Curcumin and Placebo Groups

The changes of anthropometric, biochemical and-sonography data before and after intervention are represented in Table 3.3. The changes in each variable were obtained through data differences before and after the intervention. As can be seen in Table 3.3, AST and NAFLD grade were decreased significantly following treatment in the curcumin group compared to the effects on these same parameters in the placebo group ($p < 0.05$). The comparison of changes of other variables such as anthropometric, blood pressure and other biochem-

**Table 3.1** Characteristics of demographic, medical history, biochemical, anthropometric and sonography of all patients with NAFLD at baseline

| Characteristics | | NAFLD patients | | |
|---|---|---|---|---|
| | | Placebo (n = 37) | Curcumin (n = 35) | P-value[a] |
| Age (year) | | 43.1 ± 11.6 | 45.0 ± 11.1 | 0.459 |
| Sex (male) | | 60 | 55 | 0.821 |
| Smoker | | 17.5 | 7.9 | 0.312 |
| Ex-smoker | | 60 | 67.6 | 0.163 |
| Taking medication | | 2.7 | 2.8 | 0.996 |
| History of hypertension | | 12.5 | 36.8 | 0.021 |
| History of diabetes | | 15 | 13.2 | 0.815 |
| History of hyperlipidemia | | 37.5 | 52.6 | 0.277 |
| History of heart disease | | 12.5 | 10.5 | 0.730 |
| History of weight loss | | 22.5 | 23.7 | 0.514 |
| History of kidney disease | | 27.5 | 18.4 | 0.424 |
| History of liver disease | | 15 | 10.5 | 0.738 |
| Weight (kg) | | 80.0 ± 11.9 | 85.3 ± 18.6 | 0.152 |
| BMI (kg/m$^2$) | | 29.2 ± 4.2 | 30.8 ± 5.1 | 0.153 |
| Body fat mass | | 28.3 ± 9.5 | 33.0 ± 11.6 | 0.064 |
| HC (cm) | | 103.1 ± 5.4 | 105.4 ± 8.1 | 0.168 |
| AC (cm) | | 99.5 ± 11.1 | 104.9 ± 12.9 | 0.060 |
| WHR | | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.043 |
| NC (cm) | | 39.2 ± 2.8 | 39.9 ± 3.4 | 0.358 |
| FBG (mg/dL) | | 107.8 ± 43.9 | 103.1 ± 46.3 | 0.645 |
| TC (mg/ dL) | | 194.0 ± 36.2 | 202.8 ± 37.2 | 0.289 |
| TG (mg/ dL) | | 135.5(108.0–166.0) | 132.0(114.5–180.0) | 0.446 |
| HDL-C (mg/ dL) | | 45.6 ± 10.6 | 44.8 ± 9.6 | 0.731 |
| LDL-C (mg/ dL) | | 105.6 ± 25.2 | 111.2 ± 29.5 | 0.369 |
| SBP (mm hg) | | 112.5 ± 14.7 | 117.3 ± 14.1 | 0.150 |
| DBP (mm hg) | | 79.9 ± 10.2 | 84.1 ± 14.3 | 0.141 |
| NAFLD grade | (1) | 47.5 | 30 | 0.266 |
| | (2) | 47.5 | 62.5 | |
| | (3) | 5 | 7.5 | |

The continuous and categorical variables were presented as mean ± SD and percentage, respectively

[a]The continuous and categorical variables were analysed using independent student t test and chi square/ Fisher's exact, respectively

*BMI* body mass index, *HC* measured circumference of hip, *NC* measured circumference of neck, *AC* measured circumference of abdomen, *WHR* waist-hip ratio, *FBG* fasting blood glucose, *TC* total Cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *NAFLD* non-alcoholic fatty liver disease

ical data were not significantly different between the curcumin and placebo groups ($p > 0.05$).

## 3.4 Discussion

The present clinical study investigated the significant impact of low dose phospholipid curcumin supplementation on biochemical markers of NAFLD. In addition to abnormal liver enzymes and lipid profile, the sonographic features of hepatic steatosis (grades 1–3) were improved by the treatment. Consistent with current research findings, Rahmani et al. also showed the reduction of serum levels of AST and ALT as well as hepatic fat mass using bioavailability-enhanced curcumin in patients with NAFLD compared to the placebo group. The therapeutic properties of curcumin in improving liver steatosis and fibrosis have been previously reported [27, 28].

**Table 3.2** Comparison of anthropometric, biochemical and sonography data of patients with NAFLD within groups

| | | Placebo (n = 37) | | | Curcumin (n = 35) | | |
|---|---|---|---|---|---|---|---|
| Characteristics | | Before | After | P-value | Before | After | P-value |
| Weight (kg) | | 80.0 ± 11.9 | 76.4 ± 11.0 | 0.021 | 85.3 ± 18.6 | 79.1 ± 12.1 | 0.102 |
| BMI (kg/m2) | | 29.2 ± 4.2 | 28.6 ± 3.8 | 0.023 | 30.8 ± 5.1 | 29.1 ± 3.8 | 0.005 |
| Body fat mass | | 28.3 ± 9.5 | 26.8 ± 7.9 | 0.009 | 33.0 ± 11.6 | 28.8 ± 7.7 | 0.001 |
| HC (cm) | | 103.1 ± 5.4 | 101.6 ± 5.0 | 0.002 | 105.4 ± 8.1 | 102.5 ± 5.9 | 0.004 |
| AC (cm) | | 99.5 ± 11.1 | 97.0 ± 9.7 | 0.001 | 104.9 ± 12.9 | 100.0 ± 9.1 | 0.001 |
| WHR | | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.040 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.002 |
| NC (cm) | | 39.2 ± 2.8 | 38.5 ± 2.5 | 0.070 | 39.9 ± 3.4 | 38.8 ± 2.4 | 0.158 |
| FBG (mg/dl) | | 107.8 ± 43.9 | 107.1 ± 46.5 | 0.810 | 103.1 ± 46.3 | 95.2 ± 12.7 | 0.413 |
| TC (mg/dl) | | 194.0 ± 36.2 | 188.7 ± 36.0 | 0.304 | 202.8 ± 37.2 | 199.4 ± 44.5 | 0.466 |
| TG (mg/dl) | | 135.5(108.0–166.0) | 130.5(100.0–177.7) | 0.678 | 132.0(114.5–180.0) | 161.0(113.5–211.0) | 0.301 |
| HDL-C (mg/dl) | | 45.6 ± 10.6 | 43.5 ± 8.9 | 0.033 | 44.8 ± 9.6 | 42.3 ± 7.8 | 0.022 |
| LDL-C (mg/dl) | | 105.6 ± 25.2 | 107.5 ± 32.1 | 0.696 | 111.2 ± 29.5 | 118.0 ± 34.4 | 0.232 |
| SBP (mmHg) | | 112.5 ± 14.7 | 116.6 ± 15.3 | 0.194 | 117.3 ± 14.1 | 115.7 ± 13.4 | 0.823 |
| DBP (mmHg) | | 79.9 ± 10.2 | 83.0 ± 9.7 | 0.157 | 84.1 ± 14.3 | 81.1 ± 8.6 | 0.671 |
| AST (mg/dl) | | 25.5 ± 9.6 | 28.8 ± 9.7 | 0.139 | 32.1 ± 17.4 | 26.9 ± 8.5 | 0.028 |
| ALT (mg/dl) | | 40.2 ± 28.1 | 38.9 ± 17.6 | 0.753 | 46.2 ± 33.3 | 41.6 ± 23.4 | 0.171 |
| ALP (mg/dl) | | 185.8 ± 51.1 | 181.3 ± 48.0 | 0.116 | 200.0 ± 69.2 | 194.3 ± 67.8 | 0.147 |
| NAFLD grade, % | (0) | 0 | 5.4 | 0.796[a] | 0 | 11.4 | 0.001[a] |
| | (1) | 47.5 | 40.5 | | 30 | 45.7 | |
| | (2) | 47.5 | 45.9 | | 62.5 | 40 | |
| | (3) | 5 | 8.1 | | 7.5 | 2.9 | |

Dependent student t and Wilcoxon test were performed for comparing normal and non-normal variables before and after intervention, respectively
Values are expressed as mean ± SD and median (interquartile range (IQR)) for normal and non-normal distribution variables, respectively
*BMI* body mass index, *HC* measured circumference of hip, *NC* measured circumference of neck, *AC* measured circumference of abdomen, *WHR* waist-hip ratio, *FBG* fasting blood glucose, *TC* total Cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *NAFLD* non-alcoholic fatty liver disease
[a]Ordinary data was analysed by Wilcoxon test

**Table 3.3** Changes of anthropometric, biochemical and sonography data of patients with NAFLD between groups

| | | Placebo (n = 37) | Curcumin (n = 35) | P-value |
|---|---|---|---|---|
| Weight (kg) | | −1.9 ± 4.3 | −0.8 ± 2.6 | 0.260 |
| BMI (kg/m$^2$) | | −0.3 ± 0.9 | −0.6 ± 1.1 | 0.338 |
| Body fat mass | | −1.0 ± 2.1 | −1.8 ± 2.7 | 0.240 |
| HC (cm) | | −0.9 ± 1.4 | −0.7 ± 1.2 | 0.670 |
| AC (cm) | | −1.8 ± 2.4 | −1.7 ± 2.1 | 0.944 |
| WHR | | −0.01 ± 0.02 | −0.01 ± 0.01 | 0.746 |
| NC (cm) | | −0.3 ± 0.9 | −0.1 ± 0.6 | 0.451 |
| FBG (mg/dL) | | −1.0 ± 26.8 | −6.1 ± 45.0 | 0.554 |
| TC (mg/ dL) | | −5.7 ± 40.0 | −4.8 ± 40.0 | 0.914 |
| TG (mg/ dL) | | −0.5(−22.5–28.2) | 8.0(−28.0–55.5) | 0.707 |
| HDL-C (mg/dL) | | −2.7 ± 7.7 | −3.0 ± 7.7 | 0.861 |
| LDL-C (mg/dL) | | 2.0 ± 30.5 | 5.5 ± 27.0 | 0.603 |
| SBP (mmHg) | | 3.5 ± 13.0 | −0.5 ± 11.0 | 0.264 |
| DBP (mmHg) | | 4.0 ± 13.5 | −1.2 ± 13.0 | 0.179 |
| AST (mg/ dL) | | 2.5 ± 10.5 | −5.5 ± 14.5 | 0.007 |
| ALT (mg/ dL) | | −1.3 ± 25.5 | −5.7 ± 25.0 | 0.449 |
| ALP (mg/ dL) | | −6.0 ± 22.5 | −8.5 ± 35.0 | 0.701 |
| NAFLD grade, % | (−2) | 0 | 2.9 | 0.015[a] |
| | (−1) | 18.9 | 48.6 | |
| | (0) | 67.6 | 45.7 | |
| | (1) | 10.8 | 2.9 | |
| | (2) | 2.7 | 0 | |

Independent student t and Mann Whitney U test were performed for comparing normal and non-normal distribution variables, respectively. Values are expressed as mean ± SD and median (interquartile range (IQR)) for normal and non-normal distribution variables, respectively

*BMI* body mass index, *HC* measured circumference of hip, *NC* measured circumference of neck, *AC* measured circumference of abdomen, *WHR* waist-hip ratio, *FBG* fasting blood glucose, *TC* total Cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *NAFLD* non-alcoholic fatty liver disease

[a]Mann Whitney U test was performed

The current findings are consistent with those of a previous trial with a high bioavailability curcumin-phosphatidylcholine complex that was administered at a higher dose (1000 mg/day) [23]. Most of the previous studies which used higher doses of curcumin used concentrations ranging from 500 mg to 1000 mg per day [29, 30], as a means of maximizing therapeutic effects. However, in this trial we managed to demonstrate the efficacy of curcumin in improving metabolic parameters at an even lower dose (250 mg per day for 8 weeks). Similar results were reported in previous animal studies where curcumin consumption dosages ranged from 50 mg to 200 mg a day, and these demonstrated significant improvement in insulin resistance, hepatic fat levels and it attenuated liver injury [21, 32].

These results can be explained by hormetic effect of curcumin. For example, low-dose curcumin administration could have antioxidant characteristics and high dose may induce autophagy and apoptosis. The observed biphasic dose–response potential of curcumin on cells showed the stronger effect of low dose administration than at higher dosages [33].

Curcumin modulates some metabolic risk factors such as inflammation, along with lipid, glycemic and oxidative pathways in NAFLD [34]. Regarding these positive effects and the lack of approved medications for NAFLD, it would be valuable to investigate the hepatoprotective effect of phospholipid-curcumin in NAFLD patients. Thus, curcumin may be able to slow down the initiation of the "first hit" in development of hepatic steatosis as well as significantly reduce the pro-inflammatory cytokines triggering the "second hit" of NAFLD pathogenesis [23].

Lifestyle changes through increasing the adherence to a well-established diet and optimal physical activity are considered as an initial step in the prevention and treatment of NAFLD [35, 36]. In this trial, all participants were instructed to follow energy balanced diets according to the current clinical guidelines for management of overweight and obesity. This is the likely reason why we did not find significant differences in terms of weight reduction and glycaemic control between the groups. The enhancement of physical performance and physiological fatigue reduction following curcumin supplementation might contribute to BMI reduction and other indices of NAFLD [37].

This study has several strengths. These include the balanced block randomisation design, rigorous inclusion and exclusion criteria, a lengthy (8 week) follow up period, and the direct comparison of curcumin and placebo effects. Moreover, we used phospholipid-curcumin which has optimal bioavailability unlike the natural form of curcumin used in previous studies which has a lower bioavailability [38].

There are also limitations of this trial that should be considered in interpretation of the results. First, we used ultrasonography to assess hepatic steatosis instead of other modalities such as elastography or histopathology. In addition, it was a single centre trial which could jeopardise its generalizability.

## 3.5 Conclusions and Future Perspectives

In conclusion, the findings of the present trial suggest a hepatoprotective effect of low dose phospholipid-curcumin supplementation associated with disease severity alterations in patients with NAFLD. While no pharmacological therapy has yet been approved for NAFLD, supplementation with curcumin may provide a safe and viable approach for patients and suppress the progression of NAFLD. However, further trials over longer durations and which assess various dosages of curcumin and its effects on the metabolic parameters in patients with NAFLD are needed.

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**Conflict of Interests** None.

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# 4 Protective Effects of Curcumin Phytosomes Against High-Fat Diet-Induced Atherosclerosis

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**Abstract**

Curcumin has been shown to have beneficial effects on pathogenic factors involved in the development of atherosclerosis. The aim of this study was to assess the effects of curcumin phytosomes on atherosclerosis induced by high-fat diet in rabbits. A total of 16 adult male New Zealand white rabbits (1.8–2 kg) were fed with a diet containing 0.5% cholesterol for 4 weeks. The rabbits were randomly divided into four groups of four animals each. Group I orally received PBS for 4 weeks. Group II animals were treated with curcumin–phosphatidylcholine solid state dispersion (Meriva®, Indena, Italy) suspended in normal saline at doses equivalent to 100 mg/kg of cur-

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cuminoids per day p.o., for 4 weeks. Groups III and IV were treated with curcumin–phosphatidylserine solid state dispersion (Meriserin®, Indena, Italy) suspended in normal saline at doses equivalent to 10 and 100 mg/kg of curcuminoids, respectively, per day p.o., for 4 weeks. For atherosclerosis evaluation, histological examinations on aortic arch section were performed. Blood samples were obtained to determine lipid profile and high-sensitivity C-reactive protein (hs-CRP) levels. Curcumin-phosphatidylserine (100 mg/kg) therapy resulted in a significant reduction in grading of atherosclerotic plaque and intima/media thickness ratio ($P < 0.05$) and decreased presence of inflammatory cells in the atherosclerotic lesions compared to the control group. However, no significant differences were observed between the curcumin–phospholipid preparations and the control group regarding lipid profile and hs-CRP levels. Results of the present study revealed an atheroprotective effect of curcumin-phosphatidylserine (100 mg/kg) solid dispersion as revealed by a reduction in the development of atherosclerotic lesions.

**Keywords**

Curcumin · Atherosclerosis · Phytosome · Rabbit

## Abbreviations

| | |
|---|---|
| H&E | Hematoxylin and eosin |
| HDL-C | High-density lipoprotein cholesterol |
| HMG-CoA | 3-hydroxy-3-methyl-glutaryl-coenzyme A |
| hs-CRP | High-sensitivity C-reactive protein |
| JNK | c-Jun N-terminal kinase |
| LDL-C | Low-density lipoprotein cholesterol |
| LXRα | Liver X receptor-α |
| MMP-9 | Matrix metalloproteinase-9 |
| NF-κB | Nuclear factor-kappaB |
| PPARα | Peroxisome proliferator-activated receptors-α |
| TNF-α | Tumor necrosis factor-α |
| VLDL | Very low-density lipoprotein |

## 4.1 Introduction

Atherosclerosis is a chronic immunoinflammatory disease associated with pathophysiological changes in the vascular wall [1, 2]. This process is triggered by endothelial dysfunction, which is accompanied by high plasma cholesterol and oxidized low-density lipoprotein (LDL) levels and oxidative stress [3] resulting in plaques rich lipids and inflammatory cells such as monocytes and macrophages [2]. After monocyte adhesion and migration through the endothelium, these cells differentiate into macrophages which incorporate oxidized lipoproteins forming foam cells. Also, the production of plaques is characterized by smooth muscle cell proliferation and differentiation and extracellular matrix deposition [4]. The lesion continues to grow due to the migration of new mononuclear cells, connective tissue production by fibroblasts, accumulation of extracellular lipid, and calcium deposition leading to sclerosis of the arteries [5].

Evidence emerged from experimental, epidemiological, and clinical studies have suggested that consumption of fruits and vegetables rich in polyphenols may decrease the risk of cardiovascular disease [6–8]. Curcumin is a polyphenol contained in turmeric and it is often used as spice mostly in Asian countries [9]. Consistently, this bioactive compound is recognized for its safety as well as antioxidant, anti-inflammatory, anti-tumor, antiatherosclerotic, cardioprotective, lipid-modifying, and hepatoprotective properties [10–17]. Furthermore, previous studies have reported that curcumin exerts an anti-atherogenic effect via inhibition of LDL oxidation, lowering lipid levels, and regulating gene expression [18, 19]. Also, curcumin may attenuates inflammatory responses of tumor necrosis factor (TNF)-α-stimulated human endothelial

cells through the modulation of nuclear factor-kappaB (NF-κB) and c-Jun N-terminal kinase (JNK) [20] and inhibits platelet-derived growth factor-stimulated vascular smooth muscle cell function [21]. Since curcumin has shown positive effects on pathogenic factors involved in the development of atherosclerosis, we aimed to further explore the effects of curcumin-phospholipid solid state dispersions on atherosclerosis induced by a high-fat diet in rabbits.

## 4.2 Materials and Methods

### 4.2.1 Animal Models and Drug Administration

A total of 16 adult male New Zealand white rabbits (1.8–2 kg) were used in this animal study to induce atherosclerosis. All rabbits were fed with a diet containing 0.5% cholesterol for 4 weeks. All rabbits were singly caged and maintained in a strict temperature (22 ± 2 °C) and humidity control (50 ± 5%), and a 12-hr light/dark cycle. After 8 weeks, the rabbits were subjected to cholesterol-free diet. The rabbits were randomly divided into four groups of four animals each. Group I orally received PBS for 4 weeks and served as control. Group II animals were treated with curcumin–phosphatidylcholine solid dispersion (Meriva®, Indena, Italy) suspended in normal saline at doses equivalent to 100 mg/kg of curcuminoids per day p.o., for 4 weeks. Groups III and IV animals were treated with curcumin–phosphatidylserine solid dispersion (Meriserin®, Indena, Italy) suspended in normal saline at doses equivalent to 10 and 100 mg/kg of curcuminoids per day p.o., for 4 weeks, respectively. The study protocol was approved by the Institutional Ethics Committee and Research Advisory Committee of Mashhad University of Medical Sciences in accordance with the Animal Welfare guideline.

### 4.2.2 Evaluation of Atherosclerosis

At the end of the experimental period, all rabbits were anesthetized with thiopental sodium (Panpharma, Paris, France) and the blood collected from the auricular artery. For atherosclerosis evaluation, aorta was rapidly dissected out and the excess tissue sticking to the aorta was removed and fixed by immersion at room temperature in 4% formalin for 1 day. For the histological examinations such as changes in carotid intima and elastic layer, the fixed aortic arch section was placed in paraffin, and the tissue blocks were cut to obtain 5 μm thick sections. The paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E) and photos were obtained subsequently. The prepared photo analyzed quantitatively using ImageJ (version 1.41; National Institutes of Health, Bethesda, MD) by measuring the thickness of intima and media and intima/media thickness ratio. The presence of fatty streak, medial calcifications and development of fibrous plaque in atherosclerotic plaques were evaluated by experienced pathologists. Lesions were scored using a four point intensively quantitative scale of one (mild fatty streak) to four (advanced occlusive plaque). Lesions were graded into four classes as follows: grade 1 intima less than half as thick as the media with macrophages and isolated foam cells underneath the endothelium; grade 2 intima at least half as thick as media with lipid and macrophages accumulation; grade 3 intima thickness equally half thickness of media with an abundance of macrophages, smooth muscle cells and connective tissue; and grade 4 intima thickness greater than half thickness media with large plaque including macrophages, foam cells and calcification in the fatty core.

### 4.2.3 Lipid Profile Measurement and Biochemical Analyses

Obtained blood samples were allowed to clot at room temperature for about 1 h, centrifuged at 4500 g for 10 min to obtain serum which was separated and kept at −20 °C prior to analysis. The separated serum was used to determine total cholesterol, LDL-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides by enzymatic colorimetric methods using commercial kits (Pars Azmoon Co, Tehran, Iran). In order to evaluate the inflammatory

response, simultaneous measurements of high-sensitivity Creactive protein (hs-CRP) were performed.

### 4.2.4 Statistical Analysis

All data are presented as means ± SEM. Between-group comparisons were performed using independent-sample t-test. Atherosclerotic plaque grades were compared using Mann-Whitney U test. A *p*-value of less than 0.05 was considered significant. Data were analyzed using SPSS statistical software package (IBM Corp, NY).

## 4.3 Results

### 4.3.1 Changes in Blood Lipids and Biochemical Parameters

At the end of 8 weeks, the control group and curcumin-phosphatidylcholine had a higher concentration of cholesterol compared to groups fed with curcumin-phosphatidylserine (10 and 100 mg/Kg). With respect to lipid profile, rabbits fed on the curcumin-phosphatidylserine (100 mg/kg) had a lower value of total cholesterol, LDL, triglycerides and very low-density lipoprotein (VLDL) than those fed 10 mg/kg curcumin-phosphatidylserine or curcumin-phosphatidylcholine and control groups. However, no significant differences were observed between the control group and curcumin-phospholipid groups for total cholesterol, LDL-C, HDL-C, triglycerides and VLDL (p = 0.42, 0.12, 0.56 and 0.17) (Fig. 4.1). The CRP levels are presented in Fig. 4.2. The curcumin-phospholipid groups had reduced levels of CRP in comparison with the control group, which was higher in rabbits fed with curcumin-PS 100 mg/Kg but without statistical significance ($p = 0.19$).

### 4.3.2 Effects of Curcumin-Phospholipid Solid Dispersions on Atherosclerotic Lesions

The histopathological assessment revealed that curcumin-phosphatidylserine (100 mg/kg) treatment significantly reduced the intima/media ratio ($p < 0.05$) (Fig. 4.3). Figure 4.3a showed that a 4-week 0.5% cholesterol diet induced an increase in atherosclerotic plaque in control group.

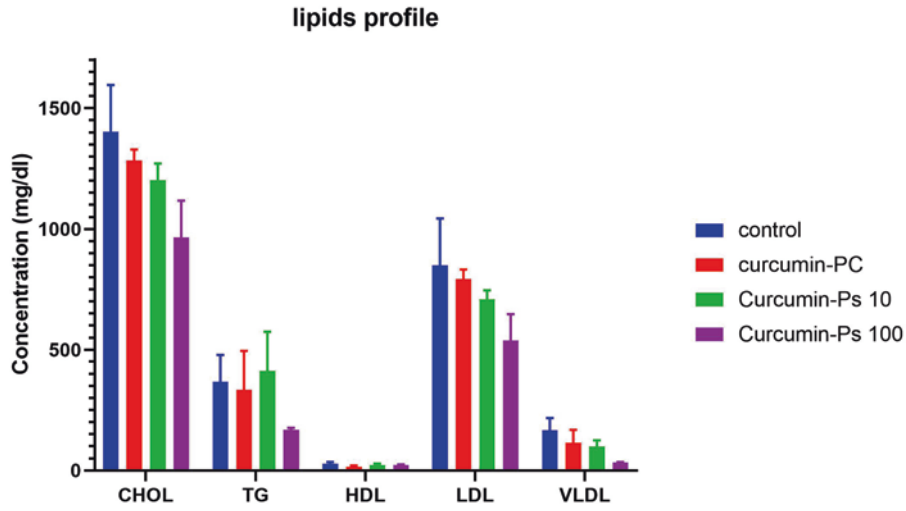


**Fig. 4.1** Comprison of fasting serum lipids between the study groups. *CHOL* total cholesterol, *TG* triglycerides, *LDL* low-density lipoprotein cholesterol, *HDL* high-density lipoprotein choelsterol, *VLDL* very low-density lipoperotein cholesterol

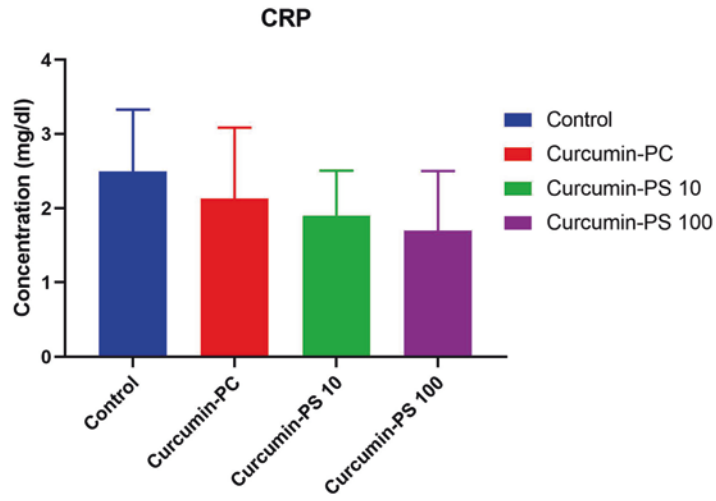


**Fig. 4.2** Comprison of serum C-reactive protein (CRP) concentrations between the study groups

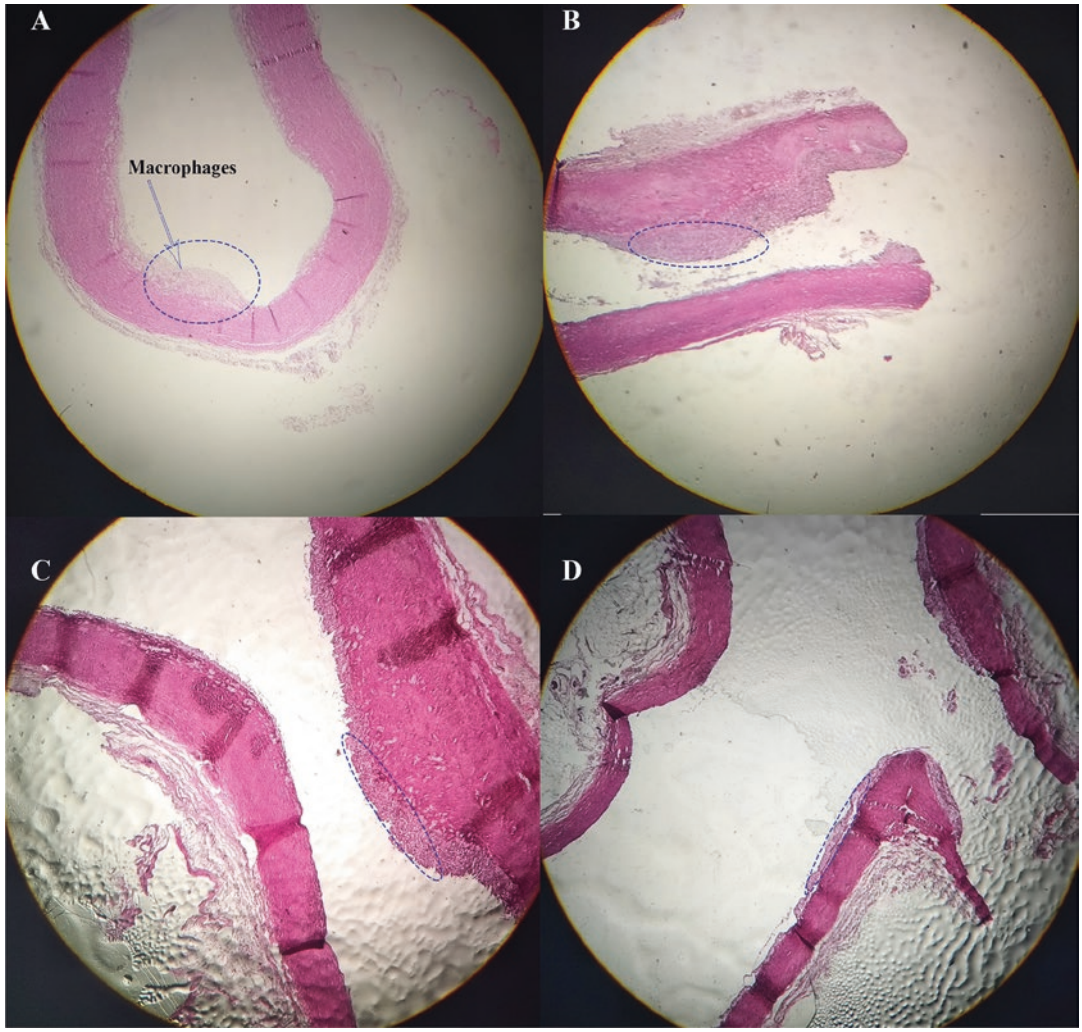


**Fig. 4.3** Hematoxylin and eosin staining of aortic arch atherosclerotic plaques from control (**a**), curcumin-phosphatidylcholine (100 mg/kg) (**b**), curcumin-phosphatidylserine (10 mg/kg) (**c**) and curcumin-phosphatidylserine (10 mg/kg) (**d**) groups

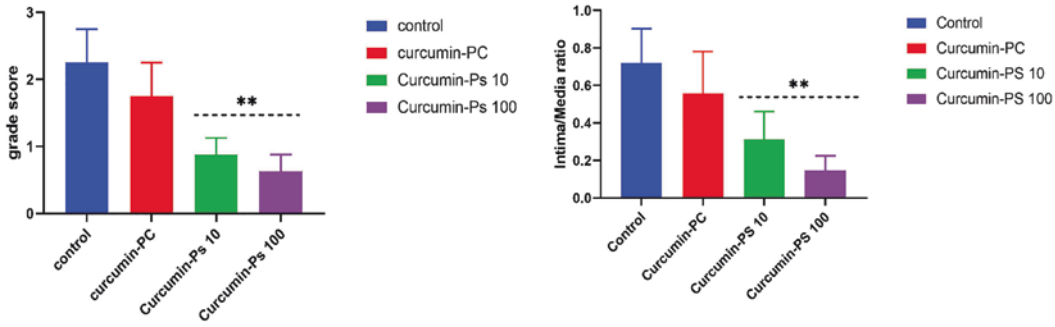


**Fig. 4.4** Comparsion of atherosclerotic plaque grading and intima-to-media ratio between the study groups

Additionally, control and curcumin-phosphatidylcholine-treated animals had a thick plaque with greater fibrosis and a distinctive atherosclerotic plaque (Fig. 4.3). The media of all aorta showed no calcifications. Curcumin-phosphatidylserine (100 mg/kg) therapy resulted in a significant grade reduction of the plaque and intima/media thickness ratio ($P < 0.05$) compared with the control group (Fig. 4.4). In the control group, atherosclerotic plaque was characterized by the presence of macrophages and foam cells in the lipid core at the site of arterial plaque while the curcumin-phosphatidylserine (100 mg/kg) group had few and sporadic plaque foam cells.

## 4.4 Discussion

Findings of the present study showed that curcumin-phospholipid solid dispersions have no beneficial effect on lipid profile but exert anti-atherosclerotic action through reduction of plaque grade and intima/media thickness ratio. In line with our results, a previous experimental study reported that curcumin treatment prevents atherosclerosis and lipid infiltration via hepatic regulation of lipoprotein cholesterol metabolism [19]. In this regard, curcumin inhibits the transcription of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and upregulates hepatic peroxisome proliferator-activated receptors-α (PPARα) and liver X receptor-α (LXRα) expression. In contrast with Shin et al. [19], we found no significant effect of curcumin solid dispersions on serum levels of total cholesterol, LDL, HDL, and triglycerides. This inconsistency might be due to the short duration of treatment in our study (4 weeks) which is insufficient to induce changes in lipid profile.

Interestingly, we observed a protective effect of curcumin-phosphatidylserine (100 mg/kg) on early atherosclerotic lesions by significant reduction of both plaque formation and intima/media thickness ratio which is in agreement with previous studies [22, 23]. Additionally, this phytochemical decreased macrophage migration and differentiation into foam cells. In this context, an experimental study described similar results including decrease in monocyte adhesion to the endothelium and migration through the vascular wall without plasma cholesterol lowering effect [24]. Thus, it has been suggested that anti-atherogenic activity of curcumin may involve a direct effect on the arterial wall and/or monocyte subsets. Some molecular mechanisms have been described to elucidate the beneficial impact of curcumin at the vascular level. In this regard, this natural agent induces changes in expression of genes involved in cell adhesion and transendothelial migration resulting in the inhibition these atherosclerotic processes. Profile expression of these genes could be associated with the increased expression of IκB protein and decreased TNF-α-induced NF-κB/DNA binding and NF-κB-transcriptional activity after curcumin therapy [24]. Also, curcumin may attenuates the proinflammatory response in endothelial cells by regulating p38 and STAT-3 in addition to NF-κB and JNK [20]. Moreover, this phytochemical protects against the development of atherosclerosis via inhibition of platelet-derived growth factor-stimulated migration of vascular smooth muscle cells through suppression of matrix metallopro-

teinase-9 (MMP-9) expression by down-regulation of NF-κB [25].

Although both experimental and clinical studies have reported that curcumin exhibits anti-inflammatory activities by lowering CRP levels [19, 26], our findings did not show significant reduction of this inflammatory protein after curcumin treatment. The lack of efficacy of this polyphenol on CRP concentrations could also be explained by the short intervention period; hence, further research is needed to assess the anti-inflammatory effect of curcumin-phospholipid solid dispersions following long-term administration.

The main limitation of our study was the short treatment period which may be ineffective to lower the circulating lipid concentrations and CRP levels. Besides, it will be interesting to explore the anti-atherosclerotic effects of curcumin-phosphatidylserine phytosomes in combination with conventional lipid-lowering drugs, e.g. statins. Finally, it remains to be established if higher doses (> 100 mg/kg) of the curcumin-phosphatidylserine preparation could lead to augmented effects on atherosclerotic plaque.

In conclusion, results of the present study revealed an atheroprotective effect of curcumin-phosphatidylserine (100 mg/kg) solid dispersion through a decrease in plaque formation and also the presence of inflammatory cells in the atherosclerotic lesions. Given the safety of this preparation, further studies are warranted to confirm these findings in the clinical setting.

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**Ethics Approval and Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

**Availability of Data and Material** This is a review article and there is no raw data.

**Competing Interests** Dr. Banach has served on speaker's bureau and as an advisory board member for Amgen, Sanofi-Aventis and Lilly. Other authors have no conflict of interests to disclose.

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# 5 Intravenous Curcumin Mitigates Atherosclerosis Progression in Cholesterol-Fed Rabbits

Amir Abbas Momtazi-Borojeni, Narges Amel Zabihi, Ramin Khameneh Bagheri, Muhammed Majeed, Tannaz Jamialahmadi, and Amirhossein Sahebkar

**Abstract**

Orally administered curcumin has been found to have a moderate therapeutic effect on dyslipidemia and atherosclerosis. The present study was conducted to determine lipid-modulating and antiatherosclerosis effects of injectable curcumin in the rabbit model of atherosclerosis induced by a high cholesterol diet (HCD). New Zealand white male rabbits were fed on a normal chow enriched with 0.5% (w/w) cholesterol for 8 weeks. Atherosclerotic rabbits were randomly divided into three groups, including a control group receiving intravenous (IV) injection of the saline buffer, two treatment groups receiving IV administration of the injectable curcumin at low (1 mg/kg/week) and high (10 mg/kg/week) over 4 weeks. Plasma lipid parameters, including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and total cholesterol (TC) were measured. Aortic arch atherosclerotic lesions were assessed using hematoxylin and eosin (H&E) staining. The low dose of curcumin significantly reduced plasma levels of TC, LDL-C, and TG by −14.19 ± 5.19%, −6.22 ± 1.77%, and − 29.84 ± 10.14%, respectively, and increased HDL-C by

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14.05 ± 6.39% ($p < 0.05$). High dose of curcumin exerted greater lipid-modifying effects, in which plasma levels of TC, LDL-C, and TG were significantly ($p < 0.05$) decreased by −56.59 ± 10.22%, −44.36 ± 3.24%, and − 25.92 ± 5.57%, respectively, and HDL-C was significantly increased by 36.24 ± 12.5%. H&E staining showed that the lesion severity was lowered significantly in the high dose ($p = 0.03$) but not significantly ($p > 0.05$) in the low-dose curcumin groups, compared to control rabbits. The median (interquartile range) of plaque grades in the high dose and low dose, and control groups was found to be 2 [2-3], 3 [2-3], and 4 [3-4], respectively. The injectable curcumin could significantly improve dyslipidemia and alleviate atherosclerotic lesion in HCD-induced atherosclerotic rabbits.

**Keywords**

High cholesterol diet · Atherosclerosis · Rabbit dyslipidemia · Curcumin

## 5.1 Introduction

Atherogenic dyslipidemia, characterized by dysregulation of plasma lipid profile, is known to be one of the major risk factors and the dominant cause of atherosclerotic cardiovascular-mediated mortality. There is a significant association between increased plasma levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) with an elevated risk of atherosclerotic cardiovascular disease (ACVD). On the other hand, increased plasma levels of high-density lipoprotein cholesterol (HDL-C) are associated with a decreased risk of ACVD [1, 2].

Currently, there are several approved-classes of lipid-lowering/modulating agents for the treatment of dyslipidemia [3]. Among them, statins are the most effective and the most widely used drug category despite important drawbacks such as their limited effectiveness on some lipid parameters such as HDL-C and TG [4] as well as serious side effects, including myopathies and hepatotoxicity [5, 6]. Regarding these concerns, there has been a growing attempt to discover functional natural products as alternatives to the current lipid-modulating therapies.

Curcumin is a polyphenolic natural ingredient that is mainly present in the rhizomes of turmeric (Curcuma longa L.). This phytochemical is an active compound that provides the yellowish color as well as most of the medicinal effects of turmeric. Curcumin is known to be one of the most widely studied bioactive compounds from nature [7]. Several in vitro, animal and clinical studies have demonstrated anti-inflammatory and immunomodulatory activities [8–10] of curcumin and its safety as well as protective effects against a wide and diverse range of chronic disorders, including various cancers, Alzheimer's disease, Parkinson's disease, inflammatory bowel syndrome, systemic lupus erythematosus, non-alcoholic fatty liver disease, rheumatoid arthritis, and diabetic complications [8, 11–28]. The cardioprotective impact is suggested to be another important medicinal property of curcumin owing, but not limited, to modulation of atherogenic lipid profiles [29–35]. However, some contradictory results have also been reported from in vitro, animal and human studies, which generally show inefficient lipid-lowering effects of orally administered curcumin [36–43]. This can be due to the low-bioavailability of active curcumin in the body following oral administration.

Overall, the systemic bioavailability of curcumin after oral dosing is low due to the low aqueous solubility, relatively poor intestinal absorption as well as extensive first-pass intestinal and hepatic metabolism, followed by rapid excretion through the gallbladder [44]. In mammals, curcumin occurs in three main forms, including free, a reduced state and as a glucuronide- or sulphate-conjugated form, in which the conjugated forms are biologically inactive or at least less potent than the free compound [44–47]. Following oral administration, the majority of curcumin is excreted unchanged through the feces, and negligible amounts are detectable in

urine and plasma [44, 47]. The remaining undergoes biotransformation during absorption in the intestinal tract and enterohepatic recirculation. Curcumin is rapidly metabolized through conjugation and reduction in enterocytes and hepatocytes. When orally supplemented, curcumin undergoes metabolic O-conjugation to curcumin glucuronide and curcumin sulfate in human gastrointestinal mucosal tissues and liver [48]. Consequently, minor free and intact curcumin can be detected in plasma after the administration [49, 50]. Therefore, modifications of curcumin and its delivery systems that are successful in improving curcumin's bioavailability are supposed to elevate its pharmaceutical effects. In the current study, we proposed that intravenous injection of curcumin, due to trapping intestinal/hepatic barriers, can enhance the bioavailability of the intact molecule and thereby increase its lipid-modulating and anti-atherosclerosis effects. To verify this, we evaluated the effects of injectable curcumin in a rabbit model of atherosclerosis induced by a high cholesterol diet (HCD).

## 5.2 Materials and Methods

### 5.2.1 Animal Study

Fifteen 4–6-month-old New Zealand white male rabbits (2 ± 0.15 kg) were purchased from the laboratory animal facility of the Razi Vaccine and Serum Research Institute of Mashhad, Iran. All animal handling procedures were carried out in strict accordance with the Animal Welfare guidelines approved by the Institutional Ethics Committee and Research Advisory Committee of the Mashhad University of Medical Sciences, Mashhad, Iran. The rabbits were housed in an air-conditioned room at a constant temperature of 22 ± 2 °C with a 12:12 h light/dark cycle. Animals were fed on an HCD containing 15% fat and 0.5% cholesterol for 8 weeks, after which they were equally divided into three groups. Curcumin at a dose of 1 mg/kg (low-dose curcumin group) and 10 mg/kg (high-dose curcumin group) was injected once per week via intravenous administration over 4 weeks. Control animals received a saline injection at the same interval. At the end of the study, all animals were euthanized by intraperitoneal injection (i.p.) (30 mg/kg) of thiopental sodium [51, 52]. Curcumin material (C3 Complex®) was obtained from Sami Labs Ltd. (Bangalore, India).

### 5.2.2 Measuring Plasma Lipid Parameters

Peripheral blood was collected, and plasma was prepared, and total cholesterol (**TC**), direct LDL-C, direct HDL-C, and TG were measured using BioVision kits (BioVision, Inc., San Francisco, CA, USA). The atherogenic index was calculated using two routinely used equations i.e. LDL/HDL ($AI_1$) and log (triglycerides/HDL-C) ($AI_2$).

### 5.2.3 Histological Assessment of Atherosclerosis

At the end of the study, rabbits were sacrificed, and the aortic tissues were isolated to evaluate atherosclerotic development and lesion severity. Briefly, the aortic arch, identified by the appearance of aortic valve leaflets, was cut and immersion-fixed in 10% buffered formalin. The formalin preserved tissue was gradually dehydrated, embedded in paraffin, and serially cut into sections with 5 μm thickness and intervals of 50 μm. The slides were deparaffinized in p-xylene and rehydrated in decreasing concentrations of ethanol (100, 80, 70, and 50%) and rinsed in water. The slides were stained in hematoxylin for 5 min, rinsed with water and counterstained in eosin, and mounted in distyrene, tricresyl phosphate, and xylene (DPX). For assessment of atherosclerotic lesion thickness and severity, the lesions were classified into an arbitrary scale of trace and 1–4 as follows [53]: trace, minimal thickening of subintima with little injury to arterial endothelium (early fatty streak); grade 1, plaque containing foam cells less than half as thick as the media with some form of endothelial dysfunction (regular fatty streak); grade 2,

plaque at least half as thick as the media with accumulation of intracellular lipid, macrophages, and smooth muscle cells (mild plaque); grade 3, plaque as thick as the media with an abundance of macrophages, smooth muscle cells, and connective tissue (moderate plaque); and grade 4, plaque thicker than the media with evidence of large extracellular intimal lipid core, foam cells, and calcification in the lipid core (severe plaque). The lesions were croos-checked using light microscopy (Olympus BX 51 microscope) supplied with a digital camera at magnifications of 100X and 400X. The lesion area was analyzed with ImageJ open source software (https://imagej.net/Welcome) [54].

### 5.2.4 Statistical Analysis

Statistical analysis was performed using GraphPad Prism software s (version 7, San Diego, CA, USA). Unpaired 2-tailed Student's t-test and one-way ANOVA followed by Tukey's post-hoc multiple comparison test, were used to assess the significance of any differences among various groups. Results with $p < 0.05$ were regarded as statistically significant. Values were expressed as mean ± SD.

## 5.3 Results

### 5.3.1 Lipid-Modifying Effects of Injectable Curcumin in Hyperlipidemic Rabbits

Injection of low-dose (1 mg/kg) curcumin for 4 weeks resulted in significant ($p < 0.05$) lowering of plasma levels of TC, LDL-C, and TG by −14.19 ± 5.19%, −6.22 ± 1.77%, and − 25.92 ± 5.57%, respectively, and an increase in HDL-C by 14.05 ± 6.39%. Greater lipid-modifying effects were observed with high-dose curcumin (10 mg/kg) with a reduction in plasma levels of TC, LDL-C, and TG by −56.59 ± 10.22%, −44.36 ± 3.24%, and − 29.84 ± 10.14%, respectively, and an increase in HDL-C by 36.24 ± 12.5% (Fig. 5.1).

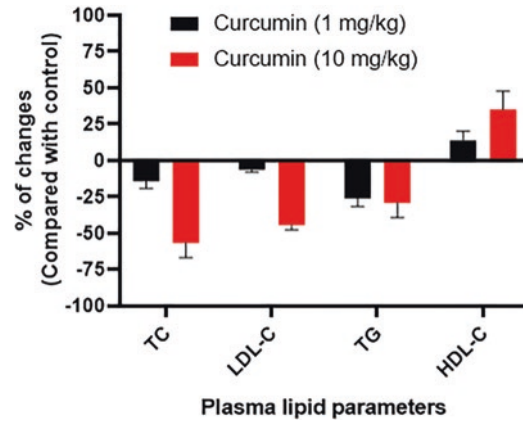


**Fig. 5.1** Plasma level changes of lipid parameters in treated compared to control rabbits at the end of study. Low-dose (1 mg/kg) and high-dose (10 mg/kg) injectable curcumin significantly decreased plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), and increased plasma levels of high-density lipoprotein cholesterol (HDL-C). Data are expressed as mean ± SD

### 5.3.2 Atherogenic Indexes

To evaluate the overall effect of injectable curcumin on the cardiovascular risk, two routinely used atherogenic indexes, $AI_1$ [LDL-C/HDL-C] and $AI_2$ [Log(TG/HDL-C)], were calculated. Both low- and high-dose injectable curcumin resulted in significantly decreased atherogenic indexes $AI_1$ and $AI_2$, when compared with saline buffer. High-dose curcumin showed significantly lower atherogenic indexes than low-dose curcumin (Fig. 5.2).

### 5.3.3 Inhibitory Effect of Injectable Curcumin on Atherosclerotic Lesion Progression

To assess the effect of injectable curcumin on atherosclerotic lesion progression in the HCD fed-rabbit model of atherosclerosis, the aortic arch was isolated and lesion size, intima to media thickness (IMT), and lesion grade, were analyzed using H&E staining. Representative images of aortic arch lesions of the treated and control rabbits are depicted in Fig. 5.3.

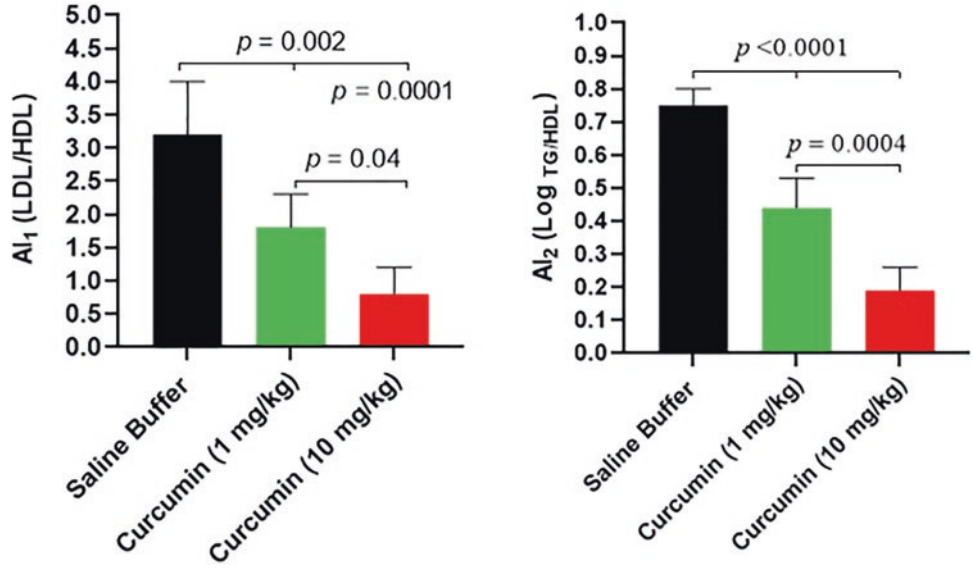


**Fig. 5.2** In vivo effects of injectable curcumin on $AI_1$ (calculated as LDL-C/HDL-C) and $AI_2$ (calculated as log triglycerides/HDL-C). Values are expressed as mean ± SD. The results were analyzed using one-way ANOVA followed by Dunnett's post-hoc multiple comparison test to evaluate significant differences between groups. $p < 0.05$ was considered significant. *AI* atherogenic index

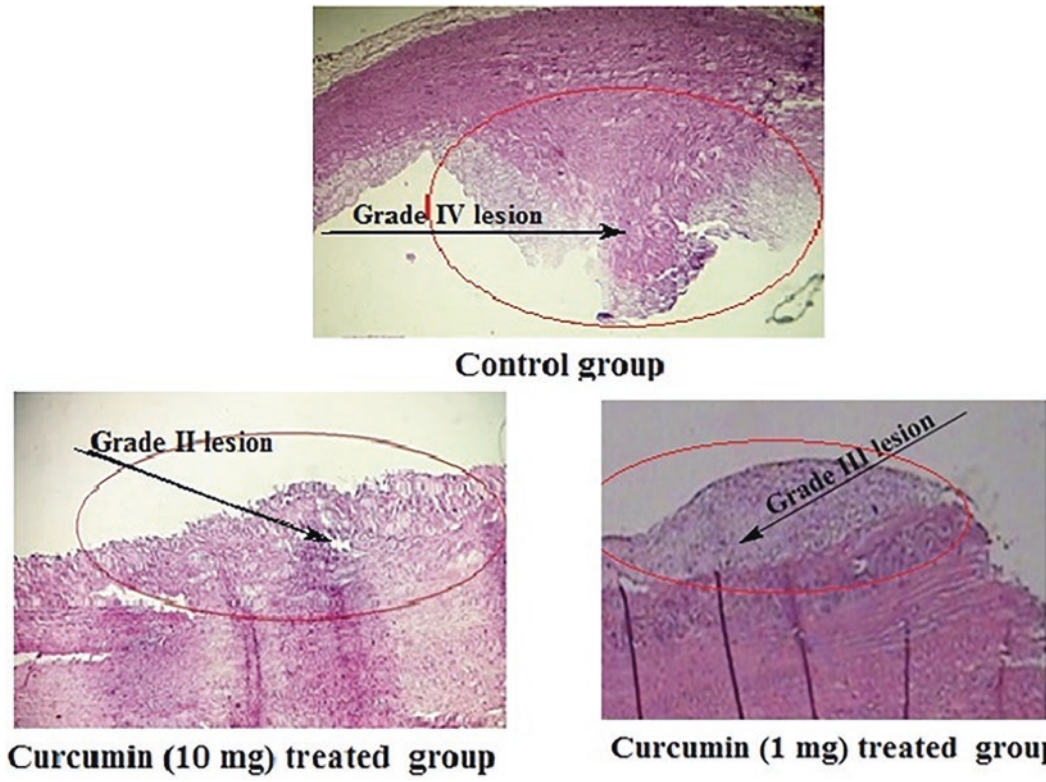


**Fig. 5.3** H & E staining showing grades IV, III, and II of atherosclerosis lesions in control, low-dose curcumin (1 mg/kg), and high-dose curcumin (10 mg/kg) groups, respectively

The mean ± SD of plaque size in the low-dose curcumin (139 ± 7.5 μm; 95% CI: 130–149 μm) and high-dose curcumin (97 ± 6 μm; 95% CI: 90–104 μm) groups were found to be significantly ($p < 0.0001$) lower than those in the control group (182 ± 11.5 μm, 95% CI: 167–196 μm) (Fig. 5.4a).

The mean ± SD of IMT in the low-dose curcumin (1.1 ± 0.1 fold; 95% CI: 1–1.3 fold) and high-dose curcumin (0.9 ± 0.08 fold; 95% CI: 0.8–one fold) groups were shown to be significantly ($p < 0.05$) smaller than those in control group (1.7 ± 0.15 fold; 95% CI: 1.5–1.9 fold) (Figs. 5.4b). The lesion severity was classified based on an arbitrary scale of 1–4 grade [53]. As interpreted by analyzing IMT ratios, the liaison severity in the high-dose and low-dose curcumin groups was found to be significantly ($p = 0.03$) and non-significantly ($p > 0.05$) lower, respectively, compared to control rabbits, in which medians (interquartile range) of plaque grades in the high-dose and low-dose, and control groups were indicated to be 2 [2-3], 3 [2-3], and 4 [3-4], respectively (Fig. 5.4c).

## 5.4 Discussion

This is the first study to show that injectable curcumin significantly ameliorates dyslipidemia and atherosclerosis progression in HCD-fed rabbits. Although mechanistic studies have confirmed lipid-modulating effects of curcumin [55], animal studies [56] and human trials [43, 57] have only shown a moderate therapeutic effect on dyslipidemia and atherosclerosis. This is likely due to the fact that the majority of orally administered curcumin undergoes metabolism during absorption in the intestinal tract and enterohepatic recirculation, and curcumin conjugates are known to be biologically inactive or at least less potent than the free compound [44–47].

LDL-C is an independent risk factor of ASCVD. Reducing the increased levels of plasma LDL-C (below 70–100 mg/dL) has been shown to have a beneficial effect on the primary prevention of patients at risk of ASCVD [58]. However, preclinical and clinical studies have shown that orally administrated curcumin can decrease plasma levels of LDL-C only up to 15% in dyslipidemic conditions [55]. Interestingly, the present animal study showed that IV injection of curcumin could dose-dependently decrease plasma levels of TC and LDL-C by up to 56% and 44%, respectively, in the HCD-fed rabbit

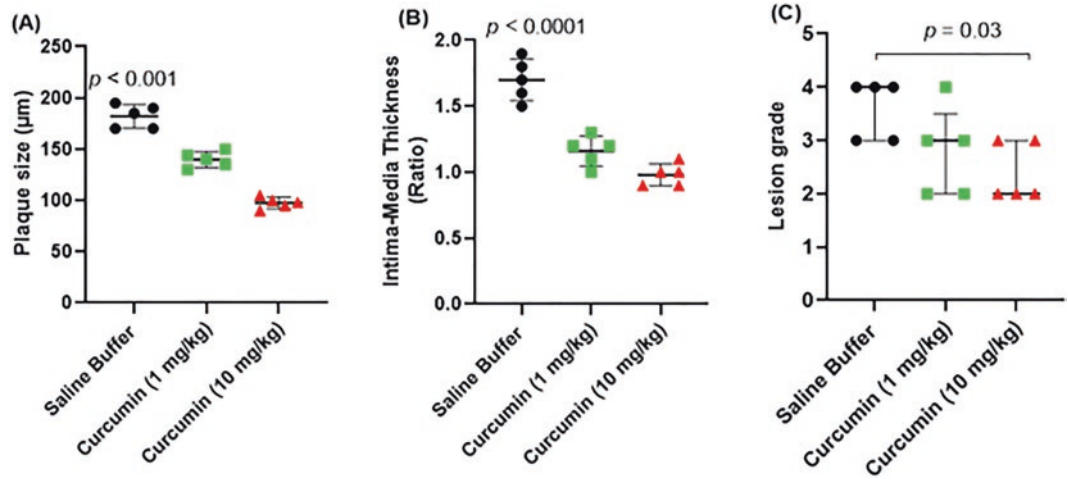


**Fig. 5.4** Analysis of plaque size (**a**), IMT (**b**) and plaque grade (**c**) in the treated and control groups. Data are expressed as group medians (25% percentile – 75% percentile). Significance compared to control values was analyzed by Mann–Whitney U test. $p < 0.05$ was considered significant between control group and treatment groups

model of atherosclerosis. This might be due to high-bioavailability of free curcumin that can more efficiently modulate targets implicated in lipid metabolism. Curcumin has been found to inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase as the main enzyme in the biosynthesis of cholesterol, causing an overall decrease of whole-body cholesterol [33, 59]. Curcumin can also decrease plasma LDL-C through increasing the clearance of plasma LDL-C via increasing expression of hepatic LDLR [59–61] and through down-regulating the expression of the major apolipoprotein of LDL particles, apoB100 [29, 33, 38].

Furthermore, dyslipidemia characterized by increased TG and low HDL-C levels is another ASCVD risk factor. HDL-C acts as an anti-atherogenic lipoprotein as its plasma levels show an inverse association with the risk of ASCVD [62–66]. Our results showed that IV injection of curcumin, in a dose-dependent manner, could efficiently increase plasma levels of HDL-C by 36%. However, its oral administration in clinical studies have shown no significant effect on HDL-C plasma levels [57]. Hypertriglyceridemia is an atherogenic condition that, even in the statin-treated patients with normal LDL-C levels, can lead to a substantial residual risk of ASCVD [62, 63]. Here, injectable curcumin resulted in a dose-dependent decrease in plasma TG levels by 26% in HCD-fed rabbits. Curcumin has been shown to modulate the expression and activity of several factors involved in TG hemostasis, such as lipoprotein lipase, cholesteryl ester transfer protein (CETP) and the peroxisome proliferator-activated receptors α and γ [67]. Compared to the statins that have slight effects on plasma levels of HDL-C and TG, injectable curcumin can be considered as a potential lipid-modulating agent that can simultaneously regulateplasma LDL-C, TG and HDL-C levels in dyslipidemia.

Another important finding of the present study was the remarkable effect of injectable curcumin on the progression of atherosclerosis lesions in the aortic arc of HCD-fed rabbits. Elevated levels of plasma LDL-C can be deposited in macrophages that are eventually transformed to cholesterol-rich foam cells, leading to initiation and development of atherosclerotic lesions [68]. Therefore, the inhibitory effect of curcumin on lesion progression may partly be due to its LDL-lowering effect. On the other hand, the extra amount of cholesterol can be transferred from the peripheral tissues, such as the aorta to liver route via a process termed “reverse cholesterol transport” (RCT). This is known to be impaired in dyslipidemic patients, leading to accumulation of foam cells in sub-endothelial layer of artery wall and consequently progression of atheroma [69]. Curcumin was reported to improve RCT activity by inducing the PPARγ-LXR-ABCA1 pathway involved in cellular cholesterol efflux [70–72] and enhancing HDL activity via inducing Apo-A1 expression that mediates cholesterol transfer from cells to HDL particles [29, 33, 38, 72, 73].

In conclusion, intravenous injection of curcumin can provide more active curcumin that traps intestinal and liver metabolism and recapitulates the defined effects in vitro. Therefore, injectable curcumin shows higher anti-atherogenic activity compared with orally administrated curcumin. Further studies on the effects of injectable curcumin may reveal further beneficial applications in other disease or medical areas.

**Conflict of Interest** Muhammed Majeed is the founder of Sabinsa Corp. and Sami Labs Ltd. The other authors declare no competing interests.

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# 6 Updated Review on the Role of Curcumin in Gastrointestinal Cancers

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**Abstract**

Malignant conditions of the gastrointestinal tract and accessory organs of digestion, including the oral cavity, esophagus, stomach, biliary system, pancreas, small intestine, large intestine, rectum and anus, are referred to as gastrointestinal cancers. Curcumin is a natural compound derived from turmeric with a wide range of biological activities. Several *in vitro* and *in vivo* studies have investigated the effects of curcumin on gastrointestinal cancers. In the current review, we aimed to provide an updated summary on the recent findings regarding the beneficial effects of curcumin on different gastrointestinal cancers in the recent decade. For this purpose, ScienceDirect," "Google Scholar," "PubMed," "ISI Web of Knowledge," and "Wiley Online Library" databases were searched using "curcumin", "cancer", and "gastrointestinal organs" as keywords. *In vitro* studies performed on different gastrointestinal cancerous cell lines have shown that curcumin can inhibit cell growth through cycle arrest at the G2/M and G1 phases, as well as stimulated apoptosis and autophagy by interacting with multiple molecular targets. *In vivo* studies performed in various animal models have confirmed mainly the chemopreventive effects of curcumin. Several nano-formulations have been proposed to improve the bioavailability of curcumin and increase its absorption. Moreover, curcumin has been used in combinations with many anti-tumor drugs to increase their anticarcinogenic properties. Taken together, curcumin falls within the category of plant-derived substances capable of preventing or treating gastrointestinal cancers. Further studies, particularly clinical trials, on the effi-

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cacy and safety of curcumin are suggested in this regard.

**Keywords**

Gastrointestinal · Cancer · Curcumin · Esophagus · Colon · Oral cavity

## 6.1 Introduction

Cancer is the most challenging disease leading to deaths around the world [1]. Although a wide variety of medical researches and different approaches to treatment have been studied and applied such as surgery, chemotherapy, hormonal therapy, radiotherapy, the incidence of cancer is expected to raise 15.5 million cancer patients in the world and 11.5 million of them lead to death by 2030 [2, 3]. Cancer is one of the chronic inflammatory diseases attributed to dysregulation in cellular signaling pathways [4]. Chronic inflammation is a significant trigger factor for several malignancies, including the gastrointestinal tract [5, 6]. Gastrointestinal (GI) cancer mainly occurs in the entire of the tract from the proximal oral cavity to the distal anus. By the end of 2020, approximately 1,806,590 new cases and 606,520 cases-related deaths in both sexes in the USA are estimated [7]. Reducing apoptosis and increasing cell proliferation maytrigger the pathogenesis of GI malignancy [8]. Obesity, *Helicobacter pylori* infection, Barrett's esophagus, alcohol, Tobacco, and inappropriate diet are certain risk factors contributing to the development of GI cancer [9, 10].

Nutraceuticals, plant-derived dietary agents, are secondary or tertiary metabolites of plants with a wide range of bioactivities [11]. Herbal phytochemicals are categorized according to their structure and function into various groups such as polyphenols, alkaloids, and terpenoids. These herb-extract constituents, including curcumin, are non-toxic and safe, less expensive, and potent to act as anticancer and antioxidant agents [8, 11, 12]. Curcumin, derived from *Curcuma longa* L., belongs to one of the most important phytochemical classes in this plant known as curcuminoids [13, 14]. Curcuminoids, responsible for the yellow color of turmeric, includes curcumin (~85%), demethoxycurcumin (~17%), bis-demethoxycurcumin, (~3%), and cyclocurcumin [15, 16]. Besides safety, Curcumin has been reported to have biological activities and therapeutic effects in chronic diseases, for instance, inflammations, cardiovascular diseases, chronic kidney diseases, diabetes, liver diseases, metabolic syndrome, neurodegenerative diseases, and several cancers [17–25]. The critical factor for preventing and treating cancer is investigating molecular alterations in cellular pathways contributing to the development and progression of this malignant disease. A large number of studies have indicated that curcumin has a leading role in many cellular signaling pathways [26, 27]. Curcumin can regulate various genes, cell signaling, molecular pathways, and cell cycle involved in the proliferation, viability, metastasis, and angiogenesis of cancerous cells [28]. So far, studies have shown that curcumin as an anti-carcinogenic agent has a significant role in the downregulation of the Bcl-2 expression, upregulating pro-apoptotic proteins (Bax, caspase 3, 8, 9) expressions, and autophagy factors (such as P53) activities. Curcumin exerts potent antioxidant activity by inhibiting reactive oxygen species (ROS) and anti-proliferative effects via modulation of some proteins, including cyclin D1, cyclin B, PAK1 kinase, activator protein-1 (AP-1) molecule, and epidermal growth factor receptor (EGFR). Also, downregulation of JAK/STAT, PI3K/Akt/mTOR, nuclear factor-kappa B (NF-κB), and Wnt/ β catenin signaling pathways are some important targets of curcumin [30–32]. The main aim of this updated review is to focus on the therapeutic effects of curcumin and its derivatives in gastrointestinal malignancies, including oral cavity, esophagus, gastric, colorectal, and pancreatic cancers at the end of this decade.

## 6.2 Search Strategy

The keywords "Curcumin" and "Cancer" in combination with "gastrointestinal," "gastric," "colorectal," "esophagus," "oral cavity," "pancreatic" were searched through scientific databases

such as "ScienceDirect," "Google Scholar," "PubMed," "ISI Web of Knowledge," and "Wiley Online Library" to find the relevant works in the last decade.

## 6.3 Oral Cavity Cancers

Oral cancer is a part of head and neck cancers, the eighth most common cancer worldwide. The incident of cancer within the mouth and oropharynx mostly occurs due to the use of alcohol, tobacco, and many chronic irritations, HPV, and diet. Oral cancer mostly begins at squamous cells at the oral site and mask floor of mouth, lips, on the lateral and ventral surfaces of the tongue and hypo-pharynx, termed oral squamous cell carcinomas (OSCC). OSCC is the most common type of oral cavity cancers [33]. Many studies have reported that curcumin administration is associated with many beneficial effects against cancerous cells, mainly via inducing apoptosis and autophagy against oral cavity cancers (Table 6.1). The apoptotic effects of curcumin against oral cancer have been asserted in various *in vitro* and *in vivo* studies. In several studies, curcumin has been used in combinations with other medications such as metformin, 5-fluorouracil (5-FU), and gefitinib, showing the potential synergistic effects against oral cancer cells [34]. Besides, due to the low bioavailability of curcumin, different nanoparticles and various delivery systems have been suggested. Mazzarino et al. have designed curcumin-loaded chitosan-coated nanoparticles as a new approach for the local treatment of oral cavity cancer [35].

## 6.4 Esophageal Cancers

Esophageal cancer is a disorder in which cancerous cells form in the tissues of the esophagus. Smoking, heavy alcohol use, and Barrett's esophagus can increase the risk of esophageal cancer. *In vitro* studies have shown the protective and anti-inflammatory effects of curcumin in various pathological conditions, including Barrett's esophagusin which there is an increased risk for esophageal adenocarcinoma formation and acid reflux esophagitis [36]. According to *in vitro* studies, curcumin can decrease cell viability, proliferation, and invasion by increasing mitotic disturbance, apoptosis, and autophagy, decreasing the number or size of cells, the cell resistance, and cell cycle arrest at sub-G1 and G2/M phase. For example, curcumin could decrease the acid-induced expression of IL-6 and IL-8 in human esophageal epithelial cells (HET-1A) by regulating PKC, MAPKs, and NF-kappaB signaling pathways [37]. Curcumin has also been shown to effectively prevent the esophageal mucosal damage induced by acute reflux esophagitis *in vivo* [38]. To increase the bioavailability of curcumin on esophageal squamous cell carcinoma (ESCC), combination treatment with highly bioavailable curcumin, and NQO1 inhibitor named theracurmin (THC) exhibited potent antitumor effects using xenograft models [39]. THC increased ESCC cell death and suppressed the growth of xenografted tumors more efficiently than curcumin. ROS levels were increased, and the NRF2–NMRAL2P–NQO1 expressions were activated by curcumin combination therapy. Inhibition of NQO1 in ESCC cells by shRNA or NQO1 inhibitor resulted in an increased sensitivity of cells to THC, whereas overexpression of NQO1 antagonized it. Notably, the NQO1 inhibitor significantly enhanced the antitumor effects of THC in ESCC PDX tumors. Moreover, curcumin exerts its esophagus-protective activity against acidic reflux injury through maintaining the mitochondrial function by the preservation of both the MnSOD expression and activity and is able to inhibit the STAT3 signaling pathway in ESCC cells [40] (Table 6.2).

## 6.5 Gastric Cancers

Gastric cancer is one of the most cancer-related death in men and women worldwide [41]. In many cases, a gastric cancer diagnosis in the primary stage remains challenging due to the silence of the symptoms, but 5 years of survival is 90%

**Table 6.1** The therapeutic effects of curcumin on oral cavity cancers

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/ pathway | Biological response | Ref. |
|---|---|---|---|---|---|---|
| Cell line | Human oral cancer cell line SAS | PGA-Gef/cur NPs[a] | Gef = 20 μM Curcumin = 10 μM | Modulation of caspase- and mitochondria-dependent pathways | A potential therapy for oral cancer by increasing apoptosis and cell death | [49] |
| | Different cell lines of OSCC[2] | Curcumin in combination with copper | The best $IC_{50}$ was observed in H314 cells after 48 h = 5.3 μM treated with curcumin at $IC_{50}$ 50 μM for 24 h and 25 μM for 48 h | ↑ intracellular ROS[3] ↑ Nrf2 level | Copper enhances the inhibitory effect of curcumin treatment on migration and viability of oral cancer cells | [50] |
| | Human oral cancer cell line SCC-9 | Curcumin loaded into nanoparticles coated with chitosan | Cur-np with chitosan (0,10,25,50,75 and 100 μM of cur)17.46–271.5 μM | ↑ selectivity index | Decrease cell viability in a concentration and time-dependent manner | [51] |
| | HPV[4]16-positive oral carcinoma cell line | Curcumin | 50–100 μM | ↑ Bax ↓ Bcl-2 and cIAP ↓ transcription of oncogene E6 ↓ AP-1 and NF-kB expression and activation | Decrease cell viability and induce morphological changes in cells Induce apoptosis in HPV-positive cells Decrease HPV transcription through cellular transcription factors AP-1 and NF-kB in HPV-16-infected oral cancer cells | [52] |
| Animal model | Male albino rats | Curcumin | 30 or 100 mg/kg/d (oral gavage) | ↓ PCNA, Bcl-2, SOCS1 e − 3, STAT3 expression ↓ genes associated with epithelial-mesenchymal transition (EMT) ↓ cellular atypia under microscopic analysis | Chemopreventive effects during oral carcinogenesis | [53] |
| | Xenograft mice model (athymic BALB/c nude mice) | PGA-Gef/cur NPs | 10.5/6 mg/kg | _ | Suppress tumor size | [49] |

(continued)

**Table 6.1** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/ pathway | Biological response | Ref. |
|---|---|---|---|---|---|---|
| | 4NQO[6] induced mice oral carcinogenesis model | Curcumin and metformin | Curcumin (0.5, 1 μM) + metformin (2.5, 5 μM) | ↑ CD44 and CD133expression | The combination of curcumin and metformin might improve chemo-preventive effects against oral squamous cell carcinoma via a CSC[5]-associated mechanism<br>Decrease tumor volume<br>Decrease migratory and self-renewal properties | [54] |
| Clinical | Oral leukoplakia, a randomized double-blind placebo-controlled phase IIB trial (n = 223) | Curcumin | 3.6 g/d for 6 months | _ | Combined clinical and histologic response assessments indicate a significantly better response with curcumin | [55] |

[a]γ-polyglutamic acid-coated nanoparticles loaded with Gefitinib and Curcumin; [2]Oral squamous cell carcinoma; [3]Reactive oxygen species; [4]Human papilloma virus; [5]Cancer stem cell; [6]4-nitro-quinoline-oxide

[42]. Common risk factors of gastric cancer are age, sex, races with the highest incidence in East Asia, and the lowest incidence in North America and Africa. Moreover, tobacco smoking, obesity, gastro-esophageal reflux disease (GERD), *Helicobacter pylori* infection, and unhealthy diets have significant roles in the incidence of this type of cancer [42, 43]. According to the studies reviewed in this paper (Table 6.3), curcumin can suppress multiple signaling pathways and inhibit cancer cell proliferation by apoptosis and autophagy, invasion, metastasis, and angiogenesis mainly through decreasing ROS and pro-inflammatory factors. Moreover, curcumin has shown a potent effect on drug-resistance cell lines by increasing the cytotoxicity and the anti-carcinogenic effects of some chemotherapy medications such as cisplatin, doxorubicin, etoposide, methotrexate, oxaliplatin, Paclitaxel, Vincristine, and 5-FU through the promotion of apoptosis and inhibition of NF-κB signaling pathway. *In vivo* studies have shown the anti-tumor effect of curcumin through suppression of lymphatic vessel density (LVD) and gastrin-mediated acid secretion.

## 6.6 Colorectal Cancers

Colorectal cancer (CRC), as the second cause of cancer death worldwide, is diagnosed in both males and females equally. The incidence of cancer arises as a family history of a polyp-noncancerous growth-from the inner rectum or

**Table 6.2** The therapeutic effects of curcumin on esophagus cancers

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| Cell lines | Esophageal cancer EC-109 cells | Curcumin | 20–80 mmol/L | ↑ cytochrome C release<br>↑ Caspase-3 activation | Increase the apoptosis of cancerous cells<br>Increase mitochondrial damage<br>Cell cycle arrest at sub-G1 phase | [56] |
| | Human esophageal squamous and adenocarcinoma cell lines OE19, OE21, OE33 and KYSE450 | Curcumin | 5–50 μM | ↑ accumulation of poly-ubiquitination<br>↑ cyclin B1 expression | Decrease the viability of all cell lines through increasing mitotic disturbance, apoptosis, and autophagy<br>Cell cycle arrest at G2/M phase | [57] |
| | TE-7, TE-10 (esophageal adenocarcinoma cancer cells in human) and ESO-1 (esophageal adenocarcinoma cancer cells in mouse) | Curcumin | 0–50 μM | ↑ caspase 3 activation<br>↑ Bax/Bcl2 ratio<br>↓ cyclin D1<br>↓ Notch-1 activation<br>↓ Jagged-1 and Hes-1 expression<br>↓γ-secretase complex proteins such as Presenilin 1 and Nicastrin<br>↓ Notch-1 specific microRNAs miR-21 and miR-34a expressions<br>↑ tumor suppressor let-7a miRNA | Cell death in esophageal cancer cells through modulating notch signaling<br>Decrease the number and size of esophageal cancer cells | [58] |
| | Human esophageal cancer cell lines SKGT-4 and TE-8 | Curcumin, (−)-epigallocatechin-3-gallate (EGCG) and lovastatin | Curcumin = 20 or 40 μmol/L<br>EGCG = 20 or 40 μmol/L<br>Lovastatin = 2 or 4 μmol/L | ↓ phosphorylated extracellular-signal-regulated kinases (Erk1/2), Ki67, c-Jun and cyclooxygenase-2 (COX-2) expression<br>↑ caspase 3 activation | Decrease the viability and invasion capacity of esophageal cancer cells | [59] |
| | Human esophageal cancer cell line (Eca-109 cells) | Curcumin | 5, 20 and 50 μM | ↓ intracellular ROS levels<br>↑ SOD[a] activity<br>↑ Total GSH<br>↓ p-JAK, p-SATA3 and total STAT3 | Increase cell adhesion via inhibiting JAK/STAT3 | [60] |
| | Human esophageal cancer vincristine (VCR)- resistant Eca-109/VCR cells | Curcumin and VCR | Curcumin (20 μmol/L) + VCR (2 μg/mL) | ↓Notch1, Jagged1 and Hes1 mRNAs expression | Reverse the resistance of esophageal cancer Eca-109/VCR cells to VCR via inhibiting Notch1 signaling pathway, down-regulating P-gp expression, and increasing apoptosis | [61] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Human esophageal cancer VCR – resistant Eca-109/ VCR cells | Curcumin and VCR | Curcumin (20 μmol/L) + VCR (2 or 3 μg/mL) | ↓ P-glycoprotein<br>↓ multidrug resistance-related protein (MRP)<br>↓ MDR1 mRNA | Reverse multidrug resistance of esophageal cancer Eca-109/ VCR cells via down-regulating P-gp and MRP expressions | [62] |
| | Human esophageal carcinoma Eca-109/ VCR cells | Curcumin and VCR | Cur (20 μmol/L) + VCR (2. 0 mg/L) | ↓ P-gp the protein expression<br>↓ Wnt2 level and p-catenin mRNA<br>↓ Wnt2, β-catenin, MMP-2 and HMGB1 relative protein levels | Increase the apoptosis ratios of cells | [63] |
| | Esophageal squamous-cell carcinoma Eca109 and EC9706 cells | Curcumin and irradiation (IR) | Curcumin (10 μM) + IR (2.16 Gy/min for 14 consecutive days) | ↓ NF-κB signaling pathway<br>↓ cyclin D1 and Bcl-2 expressions | Enhanced the pro-apoptotic effect of IR | [64] |
| | Human esophageal squamous cell carcinoma Eca109 and EC9706 cells | Curcumin analogs 2-pyridyl cyclohexanone | $IC_{50}$ = 1.40 and 0.77 mM against the Eca109 cells, and 2.10 and 0.65 mM against the EC9706 cells | Inhibits the JAK2-STAT3 pathway | Inhibited the proliferation of the cells by inducing apoptosis | [65] |
| | Esophageal squamous cell carcinoma (ESCC) | SinaCurcumin®, a novel Nano-micelle product | 1.87 mg/mL | ↓ cyclin D1 expression | Nano-curcumin increased cell cytotoxicity and decreased $IC_{50}$ | [66] |
| Animal model | Nu/nu nude mice xenografts | Curcumin, (−)-epigallocatechin-3-gallate (EGCG) and lovastatin | Curcumin = 50 μg/kg/day<br>EGCG = 50 μg/kg/day<br>Lovastatin = 50 μg/kg/day<br>(and their combinations the same doses used individually) | ↓ expression of Ki67, p-Erk1/2 and COX-2 expression | Inhibit tumor growth in combination | [59] |
| | Immunodeficiency NPG mice (xenograft tumor models) | Curcumin and IR | Curcumin (10 lM daily for 12 consecutive days) + IR (20 Gy in three fractions every 4 days) | – | Prolong the median survival time by IR<br>Decrease of xenograft tumor weight and size<br>Maximum antitumor activity<br>Curcumin treatment cells sensitized to IR | [64] |

[a]Superoxide dismutase

**Table 6.3** The therapeutic effects of curcumin and its analogs on gastric cancers

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| Cell line | Human gastric adenocarcinoma (AGS) | Curcumin and 2,6-bis benzylidine cyclohexanone derivatives | 0.83–5.42 μg/mL | ↑ Bax and caspase-3 mRNA<br>↓ cyclin-D1, VEGFA[a], Bcl-2, c-myc, and survivin | Increase apoptosis<br>Cell cycle arrest at G1 phase | [67] |
| | Gastric cancer cell lines BGC-823, MKN-45 and SCG-7901 | Curcumin | 1, 5, 10 and 30 μM | _ | Induce cell-growth inhibition and apoptosis in gastric cancer cells | [69] |
| | Gastric cancer cell lines BGC-823, SGC7901, MKN28 and MKN45 cells | Curcumin | 0–100 μM | ↓ HER2 expression<br>↓ PAK1 kinase activity<br>↓ cyclin D1 mRNA and protein expression | Inhibit the proliferation and invasion of gastric cancer cell lines<br>Suppress transition of the cells from G1 to S phase | [69] |
| | Human gastric cancer cell lines BGC-823 and SGC-7901 | Curcumin and WZ35 (an analog of curcumin) | Curcumin = 1, 5, 10,20 μg/mL<br>WZ35 = 1, 5, 10,20 μg/mL | WZ35 =<br>↓ glycolysis<br>↑ reactive oxygen species (ROS) generation<br>↑ Jun N-terminal kinase (JNK) activation<br>↓ YAP[2] | WZ35 = anti-gastric cancer by inhibiting glycolysis through the ROS-YAP-JNK pathway<br>WZ35 exhibits more potent antitumor activities than curcumin in gastric cancer cells | [70] |
| | AGS and SGC-7901 cell lines | Curcumin | 0–50 μg/mL | ↓ HMGB1[3] mRNA and protein expression<br>↓ VEGF-D[4]<br>↑ caspase-3activation | Decrease cell viability and cause dose-dependent cell apoptosis via the activation of caspase-3<br>Perhaps anti-lymphangiogenesis in gastric cancer by inhibition of HMGB1/VEGF-D signaling | [71] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Gastric cancer<br>Cell line AGS | Curcumin<br>Paclitaxel<br>Methotrexate<br>VCR | Curcumin = 15–50 μM<br>Paclitaxel = 300 nM<br>Methotrexate = 100 μM<br>Vincristine = 5 nM | _ | Enhance cytotoxicity effects of paclitaxel, methotrexate, and VCR in gastric cancer cells | [72] |
| | Gastric cancer cell lines SGC-7901 and BGC-823 | Curcumin | 10–40 μM | ↑ P53 signaling pathway activation<br>↑ P53 and P21<br>↓PI3K[5] pathway<br>↓PI3K, p-Akt, and p-mTOR[6] | Inhibit cell proliferation<br>Induce apoptosis and autophagy | [73] |
| | Human gastric cancer cell line MGC 803 | Curcumin and 5-FU plus cisplatin (FP) | Curcumin = 15 μmol/L<br>FP (low-dose) = 25 μmol/L<br>5-FU + 1 μmol/L DDP[7]<br>Medium dose = 50 μmol/L<br>5-FU + 2 μmol/L DDP<br>High dose = 100 μmol/L<br>5-FU + 4 μmol/L DDP | ↑ Caspase-3/caspase-8 activity<br>↑ Bax expression<br>↓ Bcl-2 expression | Increase cell viability, cell migration, and colony formation<br>Enhance the anticancer effects of FP chemotherapy via the promotion of apoptosis | [74] |
| | Human gastric cancer cell line SGC-7901 (SGC) | Curcumin and 5-FU | $IC_{50}$ (5-FU) = 17.98 μM<br>$IC_{50}$ (SGCR/5-FU) = 341.25 μM | ↓ NFκB-signaling pathway<br>↓ TNFα messenger RNAexpression level | A combination of 5-FU and curcumin cause synergistic inhibition of growth and induction of apoptosis in the resistant cancer cell lines | [75] |
| | Gastric cancer cell lines BGC-823, SGC-7901 and MKN-28 | Curcumin | $IC_{50}$ (MKN-28) = 16.17 μM<br>$IC_{50}$ (SGC-7901) = 50.45 μM<br>$IC_{50}$ (BGC-823) = 37.58 μM | ↑ acidic vesicular organelles (AVOs)<br>↑ autophagy-related proteins<br>↓ PI3K/Akt/mTOR signaling pathway | Induce apoptotic cell death and protective autophagy | [76] |
| | Gastric cancer cell lines BGC-823 and MKN-28 | Curcumin | $IC_{50}$ (BGC-823)= 15.18 μmol/L<br>$IC_{50}$ (MKN-28)= 15.84 μmol/L | ↑ Bax, caspase-3 and caspase-9<br>↓Bcl-2<br>↑ formation of acidic vesicular organelles in cytoplasm<br>↑ autophagy-related proteins Beclin1, Atg7 and Atg5-Atg12<br>↓ PI3K/Akt/mTOR signaling pathway | Induce apoptosis and protective autophagy in human gastric cancer cellsin a dose-dependent manner | [77] |

(continued)

**Table 6.3** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| | Gastric cancer cell line SGC-7901 | Curcumin | 25 and 50 μM | ↓ H19 expression<br>↑ p53 expression<br>↑ Bax/Bcl-2 ratio<br>↓ c-Myc oncogene<br>↓ exogenous c-Myc protein | Suppresses the proliferation of cells by downregulating H19 | [78] |
| | Gastric cancer cell lines SGC7901, MKN45 and NCI N87 | Curcumin | $IC_{50}$ = 21. 93 μmol/L | ↓ N-cadherin, snail1, Wnt3 a, p-β-catenin, p-LRP6 and Bcl-2<br>↑ E-cadherin and Bax<br>↑ Caspase-3, caspase-8, caspase-9 activation | Inhibit proliferation, migration, and invasion of gastric cancer cells via Wnt3a/β-catenin/ EMT signaling pathway | [79] |
| | Gastric cancer cell line SGC-7901 | Curcumin | 15–60 μmol/L | ↑ loss of MMP[8] | Inhibit proliferation of gastric cancer cells through impairing ATP-sensitive potassium Channel opening Increase apoptosis | [80] |
| | Gastric cancer cell line BGC-823 | Curcumin derivative L6H4 (curcumin L6H4) | 0, 5, 10, 15, 20 μmol/L | ↑ p53, p21, Bax, and Bcl-2 | L6H4 has potent of antitumor properties | [81] |
| | Gastric adenocarcinoma cell line AGS | Curcumin, berberine, quercetin and 5-FU | $IC_{50}$ (curcumin) = 40.3 μM<br>$IC_{50}$ (Berberine) = 29.2 μM<br>$IC_{50}$ (quercetin) = 37.5 μM<br>5-FU: 0–100 μM | ↓ pSTAT3 levels,<br>↓ Survivin expression | 5-FU and berberine or curcumin shows a synergistic effect on decreasing cell proliferation via inhibiting survivin and STAT3 expression | [82] |
| | Human gastric cancer cell line MGC-803 | Curcumin and PD98059[9] | Curcumin = 10–40 μM<br>PD98059 = 10 μM | ↓ p-Akt protein expression<br>↑ PTEN[10] expression<br>↓ miR-21 levels<br>↑ cell apoptosis after curcumin treatment | Curcuminand PD98059 may be a possible strategy for managing gastric cancer via regulating the miR-21/ PTEN/Akt pathway | [83] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Human gastric cancer cell lines BGC-823 and SGC-7901 | Curcumin and allylated monocarbonyl analogs of curcumin (CA6) | Curcumin = 20 μM<br>CA6 = 5–15 μM<br>2.5–40 μM | ↑ ROS levels<br>↓ Thioredoxin reductase R1 (TrxR1)<br>↓ Akt<br>↑ Forkhead O3A (FoxO3a) | CA6 inhibits gastric cancer growth through inhibiting TrxR1 and increasing ROS | [84] |
| | Human gastric cancer cell line HGC-27 | A curcumin derivative (CH-5) | 2.5–40 μM | ↓ matrix metalloproteinase 2 expression and activity | Induce apoptosis<br>Suppress migration and invasion in HGC-27 cells | [85] |
| | Human gastric cancer cell line SGC-7901 | Curcumin and miR-34a agomir (miR-34a) | 50 μM | ↑ miR-34a microRNA (mRNA) content<br>↓Bcl-2, CDK4, and cyclin D1 protein expression | Promote miR-34a expression and suppress the proliferation of gastric cancer cells<br>Cell cycle arrest at G0/G1-S phase<br>Inhibit migration, proliferation, and invasion of cancer cells | [86] |
| | Gastric cancer cell lines SGC-7901 and BGC-823 | Curcumin | 5–40 μmol/L | ↑ miR-33b expression<br>↓ XIAP[11] mRNA expression | Curcumin inhibits cell growth and induces cell apoptosis through upregulation of miR-33b in gastric cancer | [87] |
| | Human gastric cancer cell linesMKN-1 and KATOIII<br>Primary gastric culture from endoscopic biopsy | Curcumin | $IC_{50}$ (MKN-1) = 18.5 μM<br>$IC_{50}$ (KATOIII) = 11.5 μM | ↓ TNF-α, IL-6 and IL-8<br>↓ NF-kB | Curcumin could use as a chemopreventive agent for gastric cancer in H. pylori-infected patients | [88] |
| | SGC 7901 and MKN28 cell lines | Curcumin | 5–30 μM | ↓ zinc finger protein-139 mRNA and protein expression<br>↓ Bcl 2<br>↓ Survivin | Inhibit gastric cancer cell proliferation through down-regulation of zinc finger protein-139 and suppressing tumor growth<br>Decrease cell viability<br>Increase apoptosis | [89] |

(continued)

**Table 6.3** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| | Human gastric cancer cells MKN45 and AGS and normal gastric mucosal cells GES-1 | Curcumin and 5-FU | Curcumin = 3.125, 6.25, 12.5, 25.0 and 50.0 μmol/L<br>5-FU = 6.25, 12.5, 25.0, 50.0, and 100.0 μmol/L<br>$IC_{50}$ (MNK45) =5.38 ± 1.05 after 72 h<br>$IC_{50}$ (AGS) = 12.97 ± 1.36 after 72 h<br>$IC_{50}$ (GES-1) = 38.44 ± 1.89 after 72 h | ↓ COX-2 and NF-κB protein expressions | Enhance the anticancer effect of 5-FU against gastric cancer | [90] |
| | Human AGS and MGC-803 cell lines | CL-6 (a curcumin derivative) | CL-6 =<br>2.5, 5.0, and 7.5 μM<br>$IC_{50}$ (AGS) = 13.72 ± 1.80, 4.23 ± 0.05, and 1.82 ± 0.04 μM after 24, 48, and 72 h<br>$IC_{50}$ (MGC-803) = 12.64 ± 7.6, 3.50 ± 0.09, and 2.85 ± 0.26 μM after 24, 48, and 72 h | ↑ Bax,<br>↓ Bcl-2, and<br>↑ Bax/Bcl-2 ratio<br>↓ YAP protein and mRNA expression<br>↑Lats and p-YAP (Ser127)expression | Apoptosis of GC cells by CL-6 administration decrease proliferation Decrease migration and invasion | [91] |
| | Human gastric cancer cells SGC-7901 | Curcumin and chemotherapeutics (etoposide and doxorubicin) | Curcumin = 10–160 μmol/L<br>Doxorubicin = 0.03–3 μmol/L<br>Etoposid = 2–200 μmol/L | ↓ NF-κB activation<br>↓ Bcl-2 and Bcl-xL | Curcumin potentiates the antitumor effects of chemotherapeutics in gastric cancer through suppressing NF-κB and NF-κB-regulated anti-apoptotic genes Increase apoptosis Decrease cell growth | [92] |
| | Human gastric cancer cell line MGC-803 | Curcumin and quercetin | $IC_{50}$ (curcumin) = 9.32 ± 1.06 μM<br>$IC_{50}$ (quercetin) = 23.35 ± 2.14 μM | ↓ potential (ΔΨm)<br>↑ cytochrome C release<br>↓ phosphorylation of AKT and ERK[12] | Curcumin and quercetin induce apoptosis via the mitochondrial pathway and decrease cell proliferation | [93] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Human gastric cancer cell line SGC7901 | Curcumin | 25 μM | ↑ Caspase-3,<br>↓ gastrin secretion,<br>↓ tumor growth<br>↑ gastric pH | Curcumin suppresses gastrin-mediated acid secretion, which inhibits gastric cancer progression, proliferation and increases cell apoptosis | [94] |
| | Gastric cancer cell line BGC-823 | Curcumin and 5-FU and oxaliplatin (OXL) | $IC_{50}$ (curcumin) = 10 μM<br>$IC_{50}$ (5-FU) = 0.1 mM<br>$IC_{50}$ (OXL) = 5 μM | ↓ Bcl-2 protein and mRNA expression<br>↑ Bax expression<br>↑ caspase 3, 8, and 9 | Curcumin enhances the effect of 5-FU and oxaliplatin via inducing apoptosis through the Bcl/Bax-caspase 8,9-caspase 3 pathway | [95] |
| | Gastric cancer cell line SGC7901 | Curcumin | – | ↓ p-AKT and FoxM1[13] expression | Inhibit cell proliferation and induce apoptosis in gastric cancer stem cells through the ATK/FoxM1 signaling pathway | [96] |
| | Human gastric cancer cell line BGC-823 | Curcumin | 5–40 μM | ↑ reactive oxygen species (ROS) production<br>↑ ASK1[14]<br>↑ phosphorylation of JNK,<br>↑ JNK, MKK4[15], and phosphorylated JNK protein expression | Curcumin induces apoptosis by ROS-mediated ASK1-MKK4-JNK stress signaling pathway | [97] |
| | Human gastric cancer cell lines: SNU-1, SNU-5, and AGS | Curcumin | 8–32 μM | ↓ cell viability<br>↑ apoptosis<br>↓ Wnt3a, LRP6, phospho-LRP6, b-catenin, phospho-β-catenin, C-myc, and survivin | Inhibit gastric carcinoma cell growth and induce apoptosis by suppressing the Wnt/β-catenin signaling pathway | [98] |
| | Human gastric cancer cell line AGS | Curcumin loaded PMMA (poly methyl methacrylate)-PEG/ ZnO nanocomposite | 0.0001–1 μg/mL | ↓ cyclin D1 expression<br>↑ cell cycle arrest at the S – phase | Biocompatible curcumin loaded PMMA-PEG/ ZnO nanocomposite induce apoptosis and cytotoxicity in human gastric cancer cells | [99] |

(continued)

**Table 6.3** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| | GC-MSCs[16] HUVECs[17] and HGC-27 cells | Curcumin | 30 μM | ↓ fibroblast proteins (α-SMA[18]& vimentin) ↓ human umbilical vein endothelial cells (HUVEC) tube formation, migration and colony formation ↓ NF-κB signaling activity and VEGF production | Inhibit gastric cancer-derived mesenchymal stem cells mediated angiogenesis by regulating NF-κB/VEGF signaling | [100] |
| Animal model | Gastric tumor-bearing nude mice | Curcumin | 40, 80 and 160 mg/kg/d | ↓ Prox-1, podoplanin, and VEGFR-3 expression level ↓ lymphatic vessel endothelial receptor 1 (LYVE-1), Prox-1, podoplanin, and VEGFR-3 mRNA expression ↓ lymphatic vessel density (LVD) | Curcumin suppresses lymphatic vessel density | [101] |
| | Xenograft tumor model (athymic BALB/c nu/nu female mice) | Curcumin and WZ35 | Curcumin or WZ35 = 0.2 mL, 25 mg/kg | _ | WZ35 suppresses BGC-823 tumor proliferation | [70] |
| | Gastric cancer xenograft model (BALB/c nu/nu female mice) | Curcumin and CA6 | Curcumin = 50 mg/kg CA6 = 10, 20 mg/kg | ↓ TrxR1 activity ↑ ROS | Decrease the growth of gastric cancer xenografts in tumor-bearing mice | [84] |
| | Male Wistar rats | Curcumin | Curcumin = 200 mg/kgdaily for 3 and 20 weeks | ↓ phospho-IκB$\alpha$[19] ↓8-OHdG[20] | Attenuate cancer through a reduction of phospho-IκB$\alpha$ and 8-OHdG expressions | [102] |
| | Male BALB nude mice | Curcumin | 10–30 mg/kg | _ | Inhibit tumor growth and development markedly | [89] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Nude mouse model of tumor xenograft (female athymic nude mice) | Curcumin and 5-FU | Curcumin = 74 mg/kg<br>5-FU = 52 mg/kg | _ | Enhance the anticancer effect of 5-FU against gastric cancerwithout increasing toxicity in nude mice bearing MKN45 tumor xenografts | [90] |
| | Gastric cancer xenograft model (Balc/c nude mice) | Curcumin | 100 mg/kg | _ | Curcumin suppresses gastrin-mediated acid secretion, which inhibits gastric cancer progression | [94] |
| | Nude mouse model of tumor xenograft (male BALB/c athymic nude mice) | Curcumin and 5-FU and oxaliplatin (OXL) | Curcumin = 10 mg/kg<br>5-FU = 33 mg/kg<br>OXL = 10 mg/kg<br>FOLFOX[21] = (0.1 mM 5-FU + 5 μM OXL) | _ | The combination of curcumin and 5-FU/ oxaliplatin exhibit potent growth inhibition of BGC-823 xenograft tumors | [95] |
| | Male BALB/c nude mice | Curcumin | 1 mg/kg/d | _ | Reduce tumor volumes and weight | [98] |

[a]Vascular endothelial growth factor A; [2]Yes-associated protein 1; [3]High mobility group box 1; [4]Vascular endothelial growth factor D; [5]Phosphoinositide 3-kinase; [6]Phosphorylated mammalian target of rapamycin; [7]5-fluorouracil (5-FU) plus cisplatin; [8]Mitochondrial membrane potential; [9]A selective inhibitor of mitogen-activated protein kinase; [10]phosphatase and tensin homolog;;[11]X-linked inhibitor of opoptosis protein; [12]Extracellular signal-regulated kinase; [13]Forkhead box protein M1; [14]Apoptosis signal-regulating kinase 1; [15]Mitogen-Activated Protein Kinase Kinase 4; [16]Gastric cancer mesenchymal stem cells; [17] Human umbilical vein endothelial cells; alpha-smooth muscle actin;[19]Phosphorylated inhibitor kappaB alpha; [20]8-hydroxy-2-deoxyguanosine; [21]Folinic acid/5-FU/oxaliplatin chemotherapy

colon tissues and grows a tumor in the colorectal region [44]. It is vital to perceive the pre-cancerous polyps and prevent significant progress in CRC. Like other organs of the gastrointestinal system, the environmental factors may increase the development of cancer such as sedentary lifestyle, high-fat, high-calorie, low-fiber diet, and obesity [45, 46]. *In vitro* studies have indicated that curcumin can inhibit cell growth through cycle arrest at the G2/M and G1 phases, and stimulate apoptosis by interacting with multiple molecular targets. Some studies have reported the higher efficacy of some curcumin derivatives such as EF31 and UBS109 than curcumin. The combination of curcumin and the other compounds or other medications can be extended the synergistic activity of curcumin and cells' chemo-sensitivity. Additionally, *in vivo* studies have demonstrated that curcumin can reduce tumor growth and relative tumor volume (RTV) and acts as a chemopreventive agent. Clinical studies have proven the increasing endurance of patients in combination therapy with curcumin and chemotherapy regime (Table 6.4). Accordingly, curcumin can be considered a plant origin substance able to prevent CRC. However, more clinical studies are necessary to determine its efficacy and safety in humans.

## 6.7 Pancreatic Cancers

Pancreatic adenocarcinoma is the fourth of the deadliest acute clinical disease throughout the world.It is estimated that the malignancy will arise the second cause of cancer-related death by 2030 [47]. Pancreatic cancer is diagnosed in the advanced stage due to the unspecific and late symptoms. Additionally, metastasis, tumor microenvironment, the chemo-resistant cancer stem cells can cause to prolong the duration of treatment [48]. *In vitro* and *in vivo* studies have demonstrated that curcumin can reduce the inflammatory cytokines and the severity of the malignancy in pancreas cancers. Moreover, curcumin can decrease the amylase activity, blood glucose level, and increase insulin secretion, the number of islands, and gland weight. Clinical studies have demonstrated that curcumin, combined with gemcitabine-based chemotherapy, is safe and tolerable in cancerous patients and reduces adverse events (Table 6.5). However, more clinical researches are essential to investigate the efficacy and the ability of other curcumin formulation to enhance the gemcitabine or other chemo-medications efficacies [155].

## 6.8 Conclusion

Accumulating studies have demonstrated the anti-cancer effect of curcumin on gastrointestinal cancer cells, preventing proliferation, elevating apoptosis, and reducing associated genes mostly in a dose-dependent manner. Curcumin, as a natural compound, can suppress multiple biological pathways and various protein expression profiles. Moreover, it can arrest the cell cycle and decrease tumor size and growth. This review implies that curcumin has therapeutic importance and no serious adverse effects in gastrointestinal cancers. Furthermore, curcumin can boost the efficacy of other medications in a synergic manner. Clinical studies have proven the safety and tolerability of curcumin. Hence patients with gastrointestinal cancers might gain a benefit from curcumin supplementation.

**Table 6.4** The therapeutic effects of curcumin and its analogs on colorectal cancers

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| Cell line | Human colon carcinoma HCT116 (CCL-247) | Curcumin- and piperine-loaded emulsomes | Curcumin (7 μM) + piperine (25 μM) $IC_{50}$ (CurcuEmulsome) =19.69 ± 3.27 μM | ↑ caspase 3gene expression | Enhance the anti-cancer activity via increasing apoptosis and decreasing cell viability<br>Cell cycle arrest at G2/M phase | [103] |
| | Human colorectal carcinoma HCT116<br>Human metastatic colorectal adenocarcinoma LoVo | Curcumin | $IC_{50}$ (HCT-116) = 10 ± 0.03<br>$IC_{50}$ (LoVo) =20 ± 0.05 μM | – | A promising anti-cancer agent by decreasing cell proliferation, angiogenesis, and metastasis | [104] |
| | Colon cancer cell line HT-29 and SW480 | Dimethoxy curcumin (DMC) | $IC_{50}$ (HT-29) =43.4 μM<br>$IC_{50}$ (SW480) = 28.2 μM | ↑ pro-caspases-3 and PARP cleavage<br>↓ Survivin expression<br>↑ E-cadherin | Induce apoptosis by suppressing survivin and inhibit invasion by enhancing E-cadherin | [105] |
| | Human colorectal carcinoma cell line HT29 | DDP and curcumin | DDP (5 μmol/L) + Curcumin (1.25–40 μmol/L) | ↑ Bcl-2-associated X protein (Bax) expression<br>↓ Bcl-2 expression<br>↓ Notch1,<br>↓ NICD1[a]<br>↓ Hes-1[2] | Promote apoptosis synergistically | [106] |
| | Colon cancer cell line HCT116 and DLD1 | Nitrogen-containing curcumin analog | $IC_{50}$ (HCT116) = 2.13 μM<br>$IC_{50}$ (DLD1) = 10 μM | ↓ STAT3 and NF-κB<br>↓ CD44, Oct-4 and ALDHA1 expression<br>↑ caspase 3, catalase, clusterin and cytochrome C | Inhibit canonical and non-canonical functions of telomerase via STAT3 and NF-κB inactivation | [107] |
| | Colon cancer SW480 | Si-MALAT1[3] and curcumin | $IC_{50}$ (curcumin) = 77.69 μmol/L<br>$IC_{50}$ (curcumin+si-MALAT1) = 30.02 μmol/L | ↓ β-catenin, c-myc and cyclinD1expression | Decrease cell viability, migration, and invasion<br>Increase the wound healing rate | [108] |

(continued)

**Table 6.4** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| | Human colon cancer cell line HT-29 | 5-FU and curcumin loaded chitosan/reduced graphene oxide (CS/rGO) nanocomposite | $IC_{50}$ = 23.80 μg/mL | _ | Decrease the cell growth | [109] |
| Cell line | Human colorectal carcinoma HCT116 | Curcumin and 3′,4′-didemethylnobiletin (DDMN) | Curcumin (2 to 10 μM) + DDMN (3 to 15 μM) | ↑ p53, p21, HO-1, c-poly (ADP-ribose) polymerase, Cdc2, and Cdc25c | Decrease the cell growth<br>Cell cycle arrest at G2/M phase<br>Increase apoptosis | [110] |
| | Colon cancer SW480 | Curcumin | 40 μM | ↓ Wnt/β-catenin signaling pathways via inhibiting miR-130a | Suppress the colon cancer proliferation | [111] |
| | Human colorectal cancer HCT-8 (drug-sensitive) and HCT-8/5-FU (drug-resistant) | Curcumin | $IC_{50}$ (HCT-8) = 10.384 μg/mL<br>$IC_{50}$ (HCT-8/5-FU) = 12.962 μg/mL | ↓ Bcl-2, survivin, P-gp, and HSP-27 expression | ↓ the $IC_{50}$ of 5-FU for HCT-8/5-FU cells | [112] |
| Cell line | DLD-1 and Caco-2 colorectal cancer | Curcumin and resveratrol | $IC_{50}$ (DLD-1) = 71.8 μM<br>Curcumin (20.5 μM) + resveratrol (51.3 μM)<br>$IC_{50}$ (Caco-2) = 66.21 μM<br>Curcumin (18.9 μM) + resveratrol (47.3 μM) | ↑ PMAIP1, BID, ZMAT3, Caspase3, Caspase7, FAS and BAXgene expression | Decrease the proliferation<br>Increase apoptosis | [113] |
| | Human colon cancer cell lines HCT-116, SW-620, and HT-29 | A mono-carbonyl analog of curcumin EF24 | 2.5, 5, 7.5 μM | ↑ caspases 9 and 3<br>↑ mitochondrial cytochrome c<br>↓ Bcl-2 protein expression<br>↓Bcl-2/Bax ratio | Cell cycle arrest at G2/M phase<br>Increase apoptosis<br>Increase ROS accumulation in colon cancer cells | [114] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Human colon cancer cell lines HCT-8 and HCT-8/5-FU | Curcumin | $IC_{50}$ (HCT-8) = 10.38 μg/mL<br>$IC_{50}$ (HCT-8/5-FU) = 12.96 μg/mL | ↓ P-gp and HSP-27 expression | Decrease the $IC_{50}$ of 5-FU for HCT-8/5-FU cells<br>Decrease the proliferation<br>Cell cycle arrest at G0/G1 phase<br>Increase apoptosis | [115] |
| | CT26 cells | FA/Nano-cur (folate nano curcumin) | $IC_{50}$ = 5.777 μg/mL at 24 h<br>$IC_{50}$ = 1.373 μg/mL at 48 h | _ | Increase apoptosis | [116] |
| | Human colon cancer cell lines HT-29, HCT116, SW480 and SW620<br>Colorectal cancer stem cells (CCSCs) | Curcumin | 50 μM | ↑ glyceraldehyde<br>↑ Hydroxypropionic acid<br>↓ glutamine content | Increase apoptosis | [117] |
| | Human colon cancer cell line HCT116 | Curcumin | 40 μM | ↑ sodium dependent monocarboxylate transporter-1 (SMCT1) mRNA | Increase cytotoxicity | [118] |
| | Human colon SW480 | Nanoencapsulation of curcumin with whey protein (70:30 (NEC-1), 50:50 (NEC-2), and 35:65 (NEC-3)) | 1–50 μM | ↑ p53 and bax/Bcl2 ratio (NEC3)<br>↑ cytochrome-c in cytosol (NEC-2) | Increase cytotoxicity (equivalent to 30.91, 20.70 and 16.86 μM of NEC-1, 2 and 3 respectively) | [119] |

(continued)

**Table 6.4** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| Cell line | SW480 | Folic acid-curcumin micelles | > 0.5 μg/mL | ↓ cyclin D1<br>↓ C-myc | Decrease cell viability | [120] |
| | Human colon carcinoma SW480 and HCT-8 | A mono-carbonyl curcumin analog (MC37) | $IC_{50}$ (SW480) = 0.33 ± 0.02 μM<br>$IC_{50}$ (HCT-8) = 0.34 ± 0.05 μM | ↑ Bax/Bcl-2 ratio<br>↑ caspase-9/3 cascade<br>↓ CDK1 protein | Decrease intracellular microtubule assembly<br>Cell cycle arrest at G2/Mphase<br>Increase apoptosis<br>Collapse the mitochondrial membrane potential (MMP) | [121] |
| | Human epithelial colorectal adenocarcinoma Caco-2 | Curcumin and Chrysin polymeric nanoparticles | Curcumin (10–60 μM) + Chrysin (30–180 μM)<br>$IC_{50}$ = 13.15 mM | ↓ hTERT expression | Arrest the growth of cancer cells | [122] |
| | CT26 | Phytosomal Curcumin and5-FU | Phytosomal Curcumin (0–1000 μM) + 5-FU (1–500 nM) | Modulation of Wnt-pathway and E-cadherin<br>↓ cyclin D1 | Decrease cell growth and invasion | [123] |
| Cell line | DLD-1, LoVo, HCT116 | Curcumin and silymarin | Curcumin (12.5 μM) + silymarin (1.56–100 μM) | ↑ Caspase3/7 | Decrease cell proliferation<br>Increase apoptosis | [124] |
| | HCT116 and HT-29 | Curcumin and its analogs EF31 and UBS109 | $IC_{50}$ (HCT116) = 25 μM, 7.5 μM, 0.620 μM, curcumin, EF31 and UBS109 respectively<br>$IC_{50}$ (HT-29) = 20 μM, 2.5 μM, and 2.5 μM; respectively | ↓ EGFR, pAkt and pERK expression<br>↓ cyclin D1, CDK4, and pRb levels<br>↑ p16 and p21 levels<br>↓ E2F-1 and TS protein levels<br>↓ NF- κB activation | Decrease colony counts<br>Cell cycle arrest at the G0/G1 phase | [125] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Colorectal cancer cell lines HCT116 and HT29 | Curcumin and its analogs EF31 and UBS109 | $IC_{50}$ (HCT116) = 25 mM, 7.5 mM, 620 nM, curcumin, EF31, and UBS109 respectively<br>$IC_{50}$ (HT-29) = 20, 2.5, and 2.5 mM, respectively | ↓ VEGF-A synthesis and secretion<br>↓ HIF-1α, COX-2, and p-STAT-3 expression<br>↓ nuclear NF-kB expression<br>↓ transfection of p65-NF-kB | Decrease cell proliferation<br>Decrease angiogenesis | [126] |
| | HCT116, SW480, SW620, HT29, RKO and LoVo | Curcumin and oligomeric proanthocyanidins (OPCs) | Curcumin = 0.5 ng/μL<br>OPCs = 25 ng/μL | ↓ PCNA and cyclin D1 levels<br>↓ transcription factor E2F1<br>↓ HSPA5, G6PD, GCLC, IHH and PDE3B expression<br>↑ Sec61b mRNA | Decrease cellular growth | [127] |
| | HT29, LoVo, and DLD1 | Curcumin and Oxaliplatin (OXA) | $IC_{50}$ = 9.5–14.6 μM | ↓ NF-κB signaling cascade<br>↓ CXCL8 (Interleukin-8), CXCL1 (Gro-α) and CXCL2 (Gro-β)<br>Modulation of CXC-chemokine/ NF-κB signalling pathway | The combination of Curcumin and OXA is more effective and synergistic in cell lines with acquired resistance to OXA | [128] |
| | HCT116 and HT29 | Tolfenamic acid and curcumin | Tolfenamic acid (50 μM) + curcumin (7.5 μM) | ↓ Sp1, survivin expression<br>↓ NF-kB translocation<br>↑ caspase 3/7 activity and PARP cleavage | Increase apoptosis<br>Increase ROS activity | [129] |
| | Human colon cancer cell lines SW480 and SW620 | Curcumin | $IC_{50}$ = 20 μM | ↓ mTORC1<br>AMPK/mTOR pathway modulation | Decrease cell growth<br>Reduce cell proliferation | [130] |
| | C26 murine colon carcinoma cells | PEGylated long-circulating liposomes co-delivering curcumin and doxorubicin (DOX) | Curcumin = 20 μM<br>DOX = 0.15 μM | ↓ pNF-κB p65 expression | Decrease cell proliferation<br>Increase cytotoxicity | [131] |

(continued)

**Table 6.4** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| | Irinotecan (CPT-11)-resistant colon cancer cell line LoVo/CPT-11 | CPT-11 and curcumin | CPT-11 = 5–200 μM<br>Curcumin = 2.5–40 μM | ↓ CSC expression<br>↓ CD44, CD133, EpCAM and CD24<br>↓ Bcl-2<br>↑ Caspase-3, 8, 9<br>↑ Bax | Ameliorate resistance of colon cancer cells to CPT-11<br>Decrease $IC_{50}$ of CPT-11<br>Increase apoptosis | [132] |
| | SW480 and HCT116 | Curcumin | 5–30 μM | ↓ miR-17-5p expression<br>↑ cleaved caspase-3,9 expressions<br>↑ cleaved PARP | Decrease cell viability<br>Increase apoptosis | [133] |
| | SW480 and LoVo | Curcumin | 0.01–50 μM | ↓ MMP9, uPA and p65 NF-κB the expression<br>↑ AMPK phosphorylation | Decrease cell proliferation and invasion | [134] |
| | HT29 and HCT116 | Curcumin-loaded CS-GA nanoparticles | 1–50 mg/mL | _ | Decrease cell viability<br>Increase cellular uptake<br>Increase apoptosis<br>Increase number of cells at sub G1 phase | [135] |
| | HT-29 | Curcumin and IR | Curcumin (2.5 μM) + IR (10 Gy) | ↑ DNA repair-related genes CCNH and XRCC5<br>↓ LIG4 and PNKP genes | Increase apoptosis | [136] |
| | OXA-resistant cell HCT116/OXA | Curcumin and OXA | Curcumin (1–64 μM) + OXA (0.5–32 μM) | ↓ expressions of p-p65 and Bcl-2<br>↑ the level of active-caspase3<br>↓ TGF-β/Smad2/3 signaling pathway | Decrease cell migration | [137] |
| | HCT116, HCT8, SW480, and SW620 | Curcumin | 10 μM | ↑ NBR2 expression<br>↑ AMPK pathway<br>↓ mTOR | Decrease proliferation, clone formation, and the percentage of cells at S-phase | [138] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Multidrug resistancehuman colorectal cancer HCT-8/5-FU | Curcumin and 5-FU | Curcumin (10 μM) + 5-FU (10 mM) | ↑ Bax/Bcl-2 ratio<br>↓ Bcl-2 and Nrf2mRNA and protein expression | Increase the chemosensitivity of cells to 5-FU<br>Increase apoptosis | [73] |
| | HCT116, CT26 and SW620 | Curcumin and WZ35 | $IC_{50}$ = 3.3 μM (HCT116), 2.7 μM (SW620), and 1.6 μM (CT26) | ↓ MDM2, Cdc2, and cyclin B1expression<br>↑ cleaved-PARP<br>↑ cleaved-caspase 3 expression | Increase apoptosis<br>Cycle cell arrest at G2/M phase | [139] |
| | HT29 | Curcumin and 5-FU | Curcumin (10, 20 μM) + 5-FU (10, 20 μM) | ↓ phospho-Akt and phospho-mTOR expressions<br>↓ phospho-AMPK and phospho-ULK1 levels | Increase the chemosensitivity of cells to 5-FU | [140] |
| | SW620 | Curcumin | 5–40 μmol/L | ↑ NKD2 and E-cadherin the expression<br>↓ Wnt signaling pathway and EMT<br>↓ CXCR4 and vimentin expression | Decrease cell proliferation and cell viability | [141] |
| | HCT116 | Curcumin | 5–30 μM | ↑ miR-206 expression<br>↓ SNAI2 and EMT | Decrease cell metastasis | [142] |
| | HCT116 and SW620 | Curcumin | 2, 4, 8, and 16 μM | ↓ YAP expression | ↓ proliferation of cells<br>↑ cell autophagy | [143] |
| Animal model | Female BALB/cA-nu/nu mice | DMC | 50 mg/kg, p.o.) 5 d/week for 3 weeks | _ | ↓ the relative tumor volume (RTV) | [74] |
| | Xenograft tumor model | Curcumin | 200 mg/kg for 5 days by intraperitoneal (i.p.) | _ | ↓ the cells growth<br>↓ proliferation rather than promoting apoptosis | [111] |
| | Heterotopic CRC model | FA/Nano-cur (folate nano curcumin) | 50 mg/kg | _ | ↑ tumor proliferation and anti-tumor activity<br>↑ tumor cell apoptosis<br>↑ anti-angiogenesis | [116] |

(continued)

**Table 6.4** (continued)

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | References |
|---|---|---|---|---|---|---|
| | Female nude mice xenograft models of colorectal cancer | Curcumin and its analogs EF31 and UBS109 | EF31 and UBS109 (25 mg/kg,every 3 days for 3 weeks) | ↑ p21 mRNA<br>↓ cyclin D1, E2F-1, Rb and TS mRNA | Decrease tumor index | [125] |
| | Male athymic nude mice | Curcumin and oligomeric proanthocyanidins | 50 and 100 mg/kg each | ↓ IHH, PDE3B, cyclin D1 and HSAP5 the expression<br>↑ HMOX1 and SEC61B | Decrease tumor-growth | [127] |
| | Female athymic (immunodeficient) nude mice | Curcumin and its analogs EF31 and UBS109 | EF31and UBS109 (25 mg/kg I.V. twice weekly for 3 weeks) | ↓ HIF-1α, COX-2, p-STAT-3, and VEGF expression | Decrease subcutaneous tumor growth | [126] |
| | Male BALB/c nude mice were injected with HCT116 cells | Curcumin | 30 mg/kg/d for 8 weeks | _ | Reduce tumor volume and weight | [133] |
| | BALB/c nude mice xenograft mouse model of HCT116/OXA | Curcumin and OXA | Curcumin (60 mg/kg) + OXA (10 mg/kg) for 3 weeks | ↓ Smad2/3 pathway | Reduce the tumor volumes and weights | [137] |
| | HT29 tumor xenograft-bearing mice | Turmeric ethanolic extract (curcumin and turmerones) and bevacizumab | Turmeric ethanolic extract (75 mg/kg) + bevacizumab (0.4, 1.2 and 2.5 mg/kg) | _ | Decrease the tumor growth | [144] |
| | Xenograft colorectal cancer rat model of 1,2-dimethylhydrazine (DMH) | Curcumin analogs = hexagamavunone-0 (HGV-0) and gamavutone-0 (GVT-0) | Curcumin (20–80 mg/kg), GVT-0 (20–80 mg/kg), and HGV-0 (20–80 mg/kg) orally twice a week for 15 weeks | ↓ APC mutation and COX-2 expression | Superior chemoprevention of HGV-0 than others Reduce the number and volume of colorectal nodules at a dose of 40 mg/kg | [145] |
| | Xenograft mouse model of HT-29 colon cancer | Curcumin and IR | Curcumin (20 mg/kg/dose ip.) + IR (10 Gy, twice weekly) | _ | Sensitize the HT-29 tumor to IR | [136] |
| | CT26 xenograft mouse model | Curcumin and WZ35 | Curcumin (50 mg/kg oral) + WZ35 (25,50 mg/kg oral) | ↑ reactive oxygen species (ROS) generation and endoplasmic reticulum (ER) stress | Decrease tumor growth | [139] |
| | HCT116 xenograft mouse model | Curcumin and 5-FU | Curcumin (40 mg/kg) + 5-FU (30 mg/kg) | ↓ P-AMPK and P-ULK1 | Reduce tumor growth | [140] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | AOM -DSS colon cancer model | Phytosomal Curcumin (and 5-FU) | Phytosomal Curcumin (25 mg/kg/d by oral gavage) + 5-FU (35 mg/kg once weekly) | ↓ disease-activity-index (DAI)<br>↑ Malondialdehyde (MDA)<br>↓ Total-thiols (T-SH) level<br>↓ catalase (CAT) activity | Decrease tumor-number and tumor-size in both distal and middle parts of the colon<br>Decrease colonic inflammation | [123] |
| Clinicalphase IIa | 28 patients with metastatic colorectal cancer and FOLFOX regime | Curcumincombined with FOLFOX | 2 gonce every two weeks. For ≤12 cycles or until patient progression, Unacceptable toxicity, death, or withdrawal | _ | Curcumin is a safe and tolerable adjunct to FOLFOX chemotherapy | [146] |
| Clinical | 20 males and 20 females with Colon cancer | Curcumin | 3 g oral capsulestwice a day before mealsfor 1 month | ↓ Forkhead box protein (Foxp3)<br>↑ IFN-γ production | Curcumin can convert Tregs[4] to Th1[5] cells | [147] |

[a]Notch1 intracellular domain; [2]hairy and enhancer of split 1; [3]Small interfering RNA targeting metastasis-associated lung adenocarcinoma transcript1; [4]Regulatory T cells; [5]T helper 1

**Table 6.5** The therapeutic effects of curcumin and its analogs on pancreatic cancer

| | Experimental model | Cmpd. | $IC_{50}$/Dose | Involved genes/pathway | Biological response | Ref. |
|---|---|---|---|---|---|---|
| Cell line | AR42J rat pancreatic cancer cells | Curcumin and Cerulein | Curcumin (2.5, 5, 10 mg/l) + Cerulein (0.5 nM) | ↓ amylase activity<br>↓ phosphorylation of p38 | ↓ cell viability | [148] |
| Animal model | Female Sprague Dawley rats | Curcumin (dissolved in DMSO) | Curcumin = 200 mg/kg of body weight (intraperitoneal) | ↓ TNF-α serum expression levels<br>↓ CRP[a] | ↓ the severity of acute pancreatitis via the MAPK signal pathway<br>↓ inflammatory response | [148] |
| | Male Swiss albino mice & Sprague Dawley rats | CuMPs[2]<br>Curcumin<br>Cerulein | CuMPs = equivalent to 7.5 mg/kg cur (subcutaneously)<br>Curcumin =<br>1) orally = 100 mg/kg<br>2) i.p. (7.5 mg/kg)<br>Cerulean = six serial injections at 50 mg/kg | ↓ serum amylase<br>↓ lipase levels | ↓ inflammatory cytokines<br>↓ Nitrosative & oxidative stress in CuMPs administration | [149] |
| | Male Sprague-Dawley rats | Curcumin | Curcumin = 20 mg/kg | ↓ inflammatory, nitrosative and oxidative responses<br>↓ iNOS[3] and TNFα expressions | ↑ antioxidant and anti-inflammatory effects<br>↓ airway hyperreactivity induced by pancreatic ischemia-reperfusion | [150] |
| | Wistar rats | Curcumin<br>MLD-STZ[4] | Curcumin = 7.5 mg/kg body weight at 24-h intervals for 60 days | ↓ $\alpha_2$-adrenergic receptor expression,<br>↑ β-adrenergic receptor expression in pretreatment with curcumin<br>↑ insulin receptor, CREB, glucose transporter-2, and phospholipase C | Enhance insulin gene expression, pancreatic glucose sensing, and insulin secretion | [151] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Male albino mice | Curcumin<br>Alloxan | Curcumin = 100 mg/kg body weight per day for 15 days (intraperitoneal)<br>Alloxan = 150 mg/kg body weight (subcutaneous) | _ | ↑ bodyweight<br>↓ blood glucose level<br>↑ pancreatic gland weight<br>↑ size and number of islets after curcumin administration | [152] |
| Clinical phase I | 16 patients with pancreatic or biliary tract cancer and failed gemcitabine Chemotherapy | Theracurmin® and gemcitabine | Theracurmin® (200 mg of curcumin (level 1) and 400 mg of curcumin (Level2)) + gemcitabine | _ | High concentrations of curcumin did not increase the incidence of adverse reactions | [103, 153] |
| Clinical phase I/II | 21 gemcitabine-resistant patients with pancreatic cancer | Curcumin and gemcitabine | Curcumin (8 g oral daily) + gemcitabine | _ | Safe in combination therapy | [154] |
| Clinical phase II | 17 patients | Curcumin and gemcitabine | Curcumin (8000 mg oral daily) + Gemcitabine (1000 mg/m 2IV weekly for 3 of 4 weeks) | _ | A combination of gemcitabine and oral curcumin is impossible, as 29% of the patients had to stop curcumin due to gastrointestinal toxicity | [155] |
| | 44 patients (13 locally advanced and 31 metastatic) | Meriva® and gemcitabine | Meriva® (2000 mg/d) + gemcitabine (10 mg/m2/min infused over 100 min on days 1, 8, 15) for 28 days | _ | A combination of curcumin and gemcitabine is a safe and efficient first-line therapy of advanced stage | [156] |

[a]C-reactive protein, [2]Cur microparticles, [3]Inducible nitric oxide synthase, [4]Multiple low-dose streptozotocin

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# Curcumin Can Bind and Interact with CRP: An *in silico* Study

7

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**Abstract**

Curcuminis a polyphenol with anti-inflammatory and antioxidative properties, found primarily in turmeric, a flowering plant of the ginger family. Among its numerous medical uses, curcumin has been used in the management of metabolic syndrome, and inflammatory conditions such as artrhritis, anxiety and hyperlipidemia. In this paper, we used molecular docking tools to assess the affinity of four curcumin derivatives (Curcumin, Cyclocurcumin, Demethoxycurcumin, Bisdemethoxycurcumin) as well as the endogenous ligand phosphorylcholine to C-reactive protein (CRP), a sensitive marker of systemic inflammation. Our results showed that curcumin interacts through H bond with CRP at GLN 150 and ASP 140. Similar H bond inter-

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actions were found for each of the four curcumin derivatives with CRP. Moreover, a molecular dynamic simulation were performed to further establish the interaction between CRP and the ligands in atomic details using the Nanoscale Molecular Dynamics (NAMD) and CHARMM27 force field. Importantly, our results suggest the possible interaction between curcumin and curcumin related molecules with CRP, thus showing an important regulatory function with plausible applications in inflammatory and oxidative processes in diseases.

**Keywords**

Curcumin · Curcumin derivatives · C-reactive protein · Molecular docking · Molecular dynamic simulation

## 7.1 Introduction

The polyphenol curcumin has consistently been shown to exert numerousanti-inflammatory and antioxidative effects in different diseases such as diabetes, rheumatoid related problems, atherosclerosis, cancers and infectious diseases [1–10]. Curcumin is the main curcuminoid of turmeric (*Curcuma longa*), a flowering plant of the ginger family (Zingiberaceae) with a long story of use in the traditional medicine of India, China and Iran [11, 12]. Curcumin (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a diarylheptanoid with two aromatic ring systems containing o-methoxy phenolic groups, connected by a seven carbon linker consisting of an α,β-unsaturated β-diketone moiety [12]. Additionally, curcumin molecule is a tautomeric compound which exists in equilibrium with its enol tautomer [13].

Among a myriad of salutary effects [14–22], different studies have shown that curcurmin is able to downregulate the expression of proinflammatory molecules such as COX-2, Lox-5 and inflammatory cytokines such as interferon-γ, TNF-α, IL-1, IL-6, IL-8 and CRP (C-reactive protein) in animal models and human studies [11, 23–30], through the inhibition of nuclear factor kappa B (NF-κB) and IκB kinase signaling pathway [31].

C-reactive protein (CRP) is a calcium ion dependent homopentameric acute-phase inflammatory protein highly conserved in plasma which exhibits an increased expression during inflammatory conditions such as cardiovascular diseases, rheumatoid arthritis, and infection [32, 33]. CRP binds to polysaccharides like phosphocoline present in microorganisms and dying cells and triggers the classical complement pathway of innate immunity by activating C1q, and thus promoting phagocytosis by macrophages [33]. In this aspect, recent studies have shown that both curcurmin and related molecules, including different types of cucurminoids, are able to decrease the circulating levels of CRP (C-reactive protein) in blood [26, 34, 35]. Despite these first evidences on the actions of curcumin in CRP, it is unclear whether both interact to each other, as a potential mechanism to explain curcumin's lowering effects on this proinflammatory protein. Therefore, in the present study, using in silico tools we assessed the potential binding of curcumin, and related curcuminoids, on CRP.

## 7.2 Materials and Methods

### 7.2.1 Molecular Docking

The orientation of the respective ligands and phosphorylcholine in the CRP active site was examined by Molecular Operating Environment (MOE, Chemical Computing Group Inc. Montreal, http://www.chemcomp/com) docking experiment (Table 7.3). Phosphorylcholine bound to CRP crystal structures were downloaded from the RCSB Protein Data Bank (PDB entry: 1B09). All the computational procedures were carried outwith MOE.The molecular structures of the Curcumin, Cyclocurcumin, Demethoxycurcumin and Bisdemethoxycurcumin, as well as the molecular structures of the phosphorylcholine was prepared by MOE Builder and energy minimized using Hamiltonian-Force Field-MMFF94x by MOE. Docking procedure was performed with

the default settings of the MOE-DOCK. The final docking scores was evaluated using theGBVI/WSA dG scoring function with the Generalized Born solvation model (GBVI) [36]. The GBVI/WSA dG is a forcefield-based scoring function, which estimates the free energy of binding of the ligand from a given pose. The dissociation constant (Ki) was computed through the binding free energy estimated with the GBVI/WSA dG scoring function according to the following equation:

$$\Delta G = RTLn\left(K_i\right)$$

where R represented the gas constant and T the temperature in kelvin. The Ki was computed starting from the binding free energy values at a fixed temperature (300 K).

### 7.2.2 Molecular Dynamic Simulation

Molecular Dynamic (MD) simulation analyses were performed to explore protein-ligand interactions in atomic details at a real physiological condition, i.e., aqueous solution at T = 37 °C, P = 1 atm. Calculations were performed using the Nanoscale Molecular Dynamics NAMD Git-2018-04-24 for Linux-x86_64-multicore-CUDA program (www.ks.uiuc. edu/Research/namd) with CHARMM27 force field. Visual Molecular Dynamics (VMD) (www.ks.uiuc.edu/ Research/vmd) was used to analyze the results. The force field parameters of the Curcumin were obtained from SwissParam (http://swissparam.chr). The protein structure was explored for missing atoms and bonds, and the whole system was immersed in the center of a TIPS water box with dimensions of 6 × 6 × 6 nm using the VMD program, and the systems were neutralized by adding sodium and chloride ions and energy minimization with the steepest descent method for 5000 steps. The Particle-Mesh Ewald (PME) algorithm with a grid spacing of 1A and periodic boundary conditions were applied. A cut-off 15 (Å) was applied to the short-range Lennard-Jones interactions. Finally, MD simulations were conducted with a time step of 2 fs for 70 ns. The trajectory of the system was stored at every 1 ps and analyzed using VMD analyzer Tools. The system was complex and required significant computation. We used resources at the High-Performance Computing Center Mashhad University of Medical Sciences. AMD Ryzen 16-core processors (2-GHz) were employed.

## 7.3 Results

### 7.3.1 Molecular Docking Studies

In this paper, we studied the affinity of four curcumin derivatives (Curcumin, Cyclocurcumin, Demethoxycurcumin, Bisdemethoxycurcumin) as well as endogenous ligand phosphorylcholine to CRP. Docking experiments showed that Curcumin had interaction with CRP (pki = 13.12) through one H bonding of its carbonyl group to GLN_150 residue and one H bonding of its hydroxyl group to ASP_140 residue, Cyclocurcumin had interaction with CRP (pki = 12.40) through one H bonding of its hydroxyl group to LYS_28 residue, Demethoxycurcumin had interaction with CRP (pki = 9.40) through one H bonding of Oxygen from methoxy group to SER_68 residue, Bisdemethoxycurcumin had interaction with CRP (pki = 8.85) through one H bonding of its carbonyl group to GLN_150 residue, X-ray Phosphorylcholine had interaction with CRP (pki = 14.63) through one H bonding of its hydroxyl group to ASN_61 residue. Docking results indicated that each of the ligands Curcumin and Bisdemethoxycurcumin had an H-bonding between GLN_150 and ligand. (Table 7.1:Right column). As a result, according to the above contents Curcumin with pki values of 13.12 was found to bind to CRP firmer than other derivatives (Table 7.2).

### 7.3.2 Molecular Dynamic Simulation

The apo-form and ligand-bound protein were compared in terms of dynamic stability of secondary structure elements and conformational

**Table 7.1** Data from the interaction the Curcumin at the active sites of C-reactive protein (CRP) molecular were obtained after stimulation of 70 ns

| | Hydrogen bonds | | | Hydrophobic interaction | | |
|---|---|---|---|---|---|---|
| Index | Residue | AA | Distance D-A | Residue | AA | Distance |
| 1 | 138U | GLU | 2.87 | 60U | ASP | 3.55 |
| 2 | 150U | GLN | 3.08 | 147U | GLU | 3.77 |

**Table 7.2** Data for the docking interactions of Phosphorylcholin and Curcumin and its derivatives at the active sites of C-reactive protein (CRP) molecular target

| IIo. Target protein (PDB-ID) | Ligand | Docking score | $pK_1$ | H-bonding interactions with aninoacid residues | Hyrogen bond number |
|---|---|---|---|---|---|
| 1B09 | Phosphorylcholin | −20.0800 | 14.63775 | ASII_61 | 1 |
| | Cucumin | −18.0033 | 13.12754 | GLII_150 ASP_140 | 2 |
| | Cyclocurcumin | −17.0233 | 12.4095 | LYS_28 | 1 |
| | Demethoxycurcumin | −12.9029 | 9.405847 | SER_68 | 1 |
| | Bisdemethoxycurcumin | −12.1474 | 8.855109 | GLII_150 | 1 |

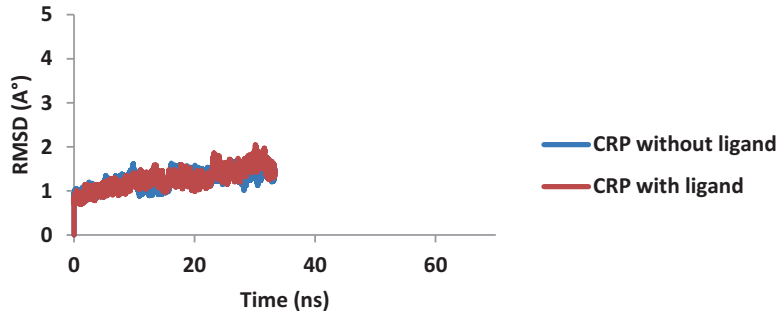



**Fig. 7.1** RMSD between Curcumin with ligand and without ligand

changes using root-mean-square derivations (RMSD) and root mean square fluctuation (RMSF) plots following 70 ns simulations. RMSD is a measure of the stability of the structures. (Fig. 7.1) showed that the RMSD of backbone of apo-form and Curcumin-bound protein reached stability after about 20 ns of simulation. The average RMSD of apo-form and the ligand-bound protein were close to eachother with average RMSD values of 1.26 ± 0.17A° and 1.31 ± 0.19A°, respectively. RMSF reflects the macromolecular flexibility and local changes in the protein structure [37]. The RMSF plots of apo-form and ligand-bound protein are illustrated in (Fig. 7.2), suggesting similar RMSF distribution and same trends of dynamic features. The average RMSF of ligand-bound protein and apo-form were 2.60 ± 1.05 and 3.50 ± 0.66A°, respectively. The relatively lower RMSF of ligand-bound protein was found for Asp60, Glu138, Asp140, Glu147 and Gln150 residues that are located in the binding site of the protein and have stronger and less flexible binding to the ligand. The residues with higher fluctuation were located either in the loops or terminal parts of the protein, or were distant from the active site. As shown in Fig. 7.3 (Right column), radius of gyration of the apo-form and Curcumin-bound protein were constant and equal to 15.84 ± 0.04 and 15.83 ± 0.047 A° (mean ± SD), respectively. Electrostatic, Van der Waals (VdW) and potential interaction energies of ligand and Curcumin complex were calculated and shown in Fig. 7.3 (Left column). The stability of the hydrogen-bond network formed in the active site of the protein due to interaction with the ligand is demonstrated in Fig. 7.4 (Right column). The mean number of intramolecular hydrogen bonds

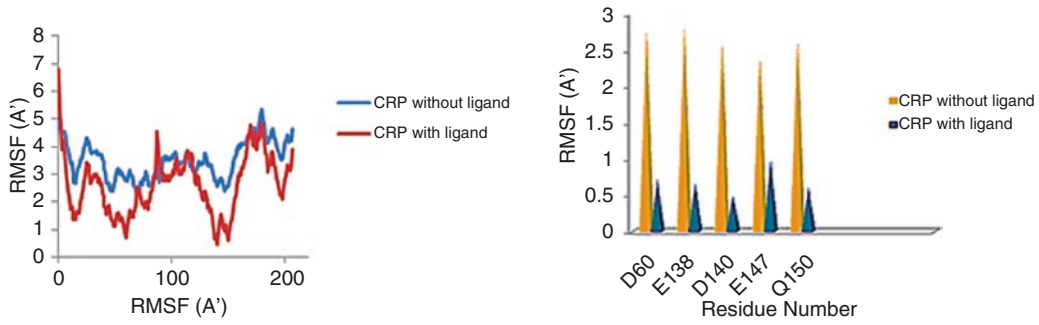



**Fig. 7.2** Right column: Per residue RMSF of CRP with ligand and without ligand during 70 ns simulation, Left column: RMSF for residues in active site CRP with ligand and without ligand

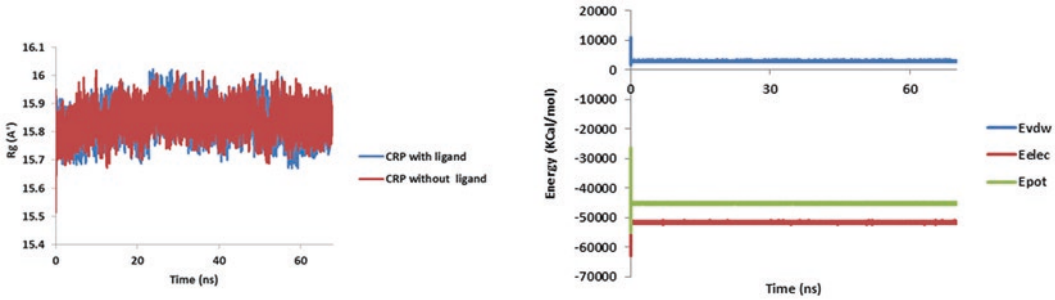



**Fig. 7.3** Right column: Rg of CRP with ligand and without ligand, Left column: Plot of electrostatic (ele), Van der Waals (vdw) and potential (pot) energies of the bound complex

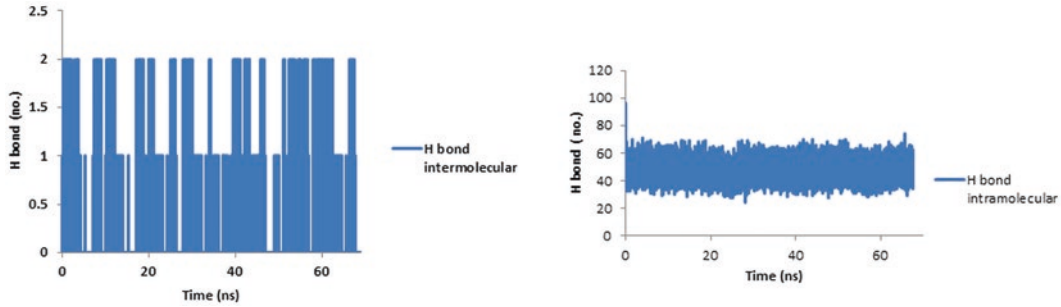



**Fig. 7.4** Right column: Number of hydrogen bonds between CRP and ligand in time scale, Left column: Number of intramolecular hydrogen bonds CRP in time scale

of the protein was 53 ± 5.67. These hydrogen bonds can contribute to the stabilization of the structure and interaction with the ligand (Fig. 7.4, Left column). The Protein-Ligand Interaction Profiler (PLIP, http://plip.biotec.tu-dresden.de/plip web/plip/index) was used to depict the 3D schematic of the curcumin-CRP interaction following 70 ns simulation (Fig. 7.5). To present the 2D schematic of the interaction (following 70 ns simulation), LigPlot [38] was used (Fig. 7.6). The results showed that ligand formed two hydrogen bonds (with GLU-138 and GLN-150, distance was 2.87 and 3.08, respectively) and two hydrophobic bonds (with ASP-60 and GLU-147, distance was 3.55 and 3.55, respectively) with protein (Table 7.1).

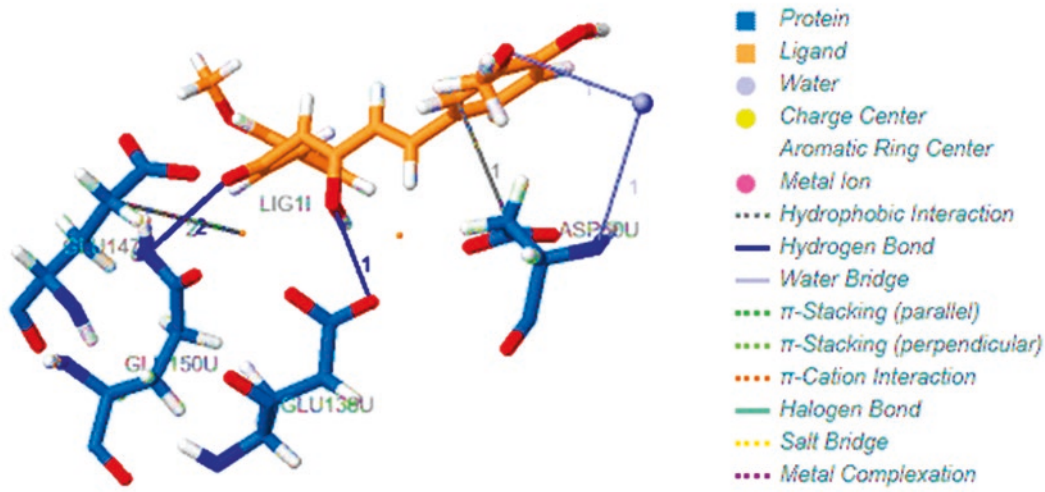



**Fig. 7.5** The 3D representation of the interaction between compound Curcumin in the crystal structure of CRP (last frame) after 70 ns simulation by Protein-Ligand Interaction Profiler (PLIP), two $Ca2^{+}$ ion (pink) is seen at the center, water (light blue) is seen at right

## 7.4 Discussion

In the present study, we performed a molecular docking interaction and molecular dynamic simulation for 4 curcumin derivatives (Curcumin, Cyclocurcumin, Demethoxycurcumin and Bisdemethoxycurcumin) and the endogenous ligand phosphorylcholine to CRP. Our results showed that curcumin interacted through two H bonds with CRP at the carbonyl group of GLN 150 and the hydroxyl group of ASP 140. Similar H bond interactions where observed for each of the four curcumin derivatives with CRP. In this aspect, it was found that cyclocurcumin had an H bond interaction with CRP, the hydroxyl group of its LYS_28 residue, while demethoxycurcumin had an H bond interaction with CRP through the oxygen the methoxy group of SER_68 residue, and Bisdemethoxycurcumin had an H bond interaction with CRP through the carbonyl group of its GLN_150 residue. Furthermore, our results suggest that both curcumin and related curcuminoids exhibited high affinity interactions with CRP active site (pki =13.12, 12.40, 9.40 and 8.85, respectively). A molecular dynamic simulation was performed to further stablish the interaction between CRP and the studied ligands using the Nanoscale Molecular Dynamics (NAMD) software and the CHARMM27 force field. The results showed that the ligand formed two hydrogen bonds (GLU-138, GLN-150, distance is 2.87 and 3.08 respectively) and two hydrophobic bonds (ASP-60, GLU-147, distance is 3.55 and 3.55 respectively) with CRP (Table 7.3).

Previous evidences around the actions of curcumin on CRP have been reported. For example, in an initial meta-analysis of six human trials with 172 subjects supplemented with cucurminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) and 170 placebo subjects, Sahebkar (2013) reported a significant reduction in circulating CRP levels [35]. Similarly, Tabrizi et al. (2018) conducted a meta-analysis of 15 randomized controlled trials (RTCs) to assess the effect of curcumin-containing supplements on inflammation and oxidative stress biomarkers [26]. Their results showed that curcumin supplements significantly reduced serum concentrations of IL-6, malondialdehyde (MDA) and high sensitivity C-reactive protein (hs-CRP), suggesting the importance of this compound in the modulation of inflammation and oxidative processes. Similarly, in a double-blind randomized study, Alvarenga et al. (2020) evaluated oral curcumin

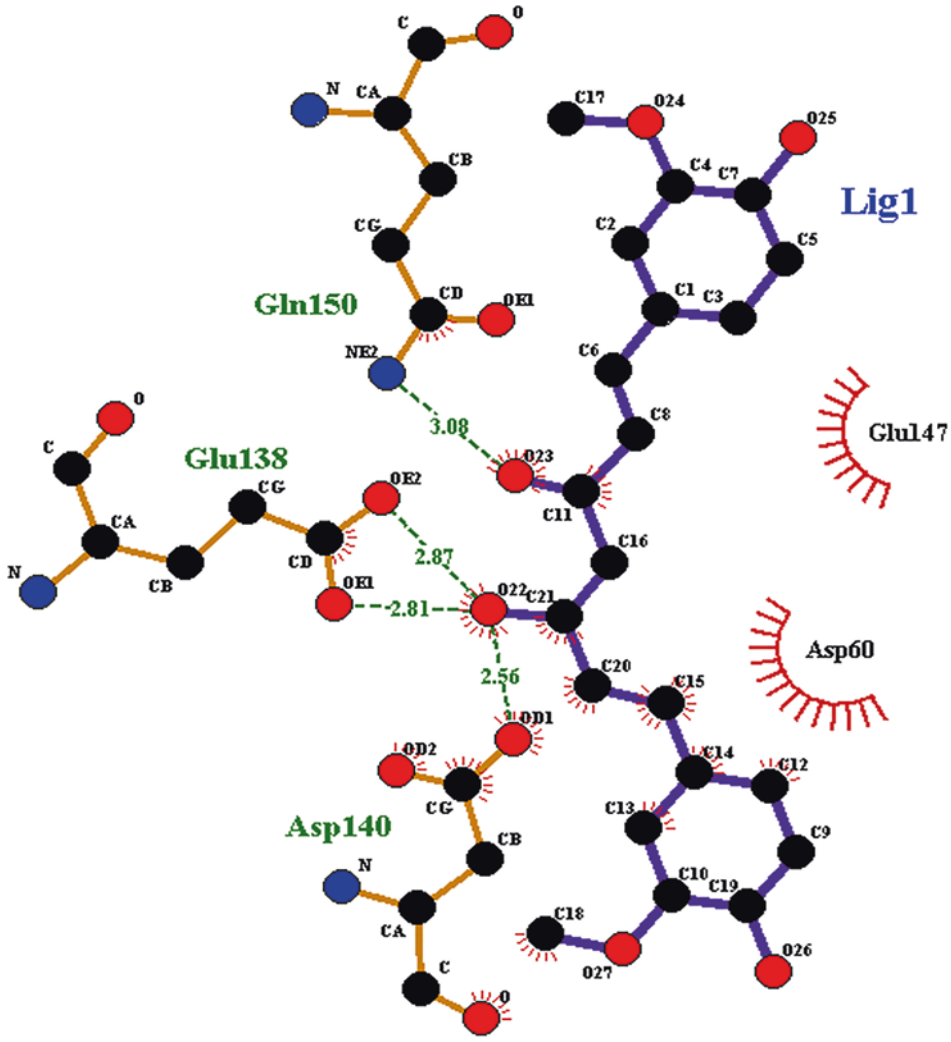
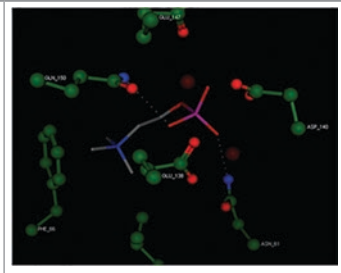
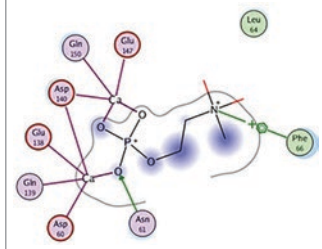
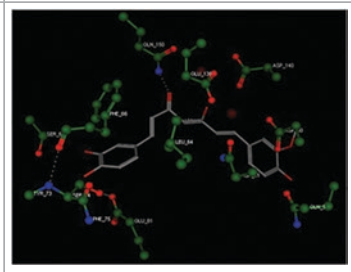
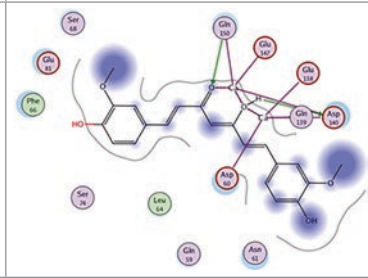
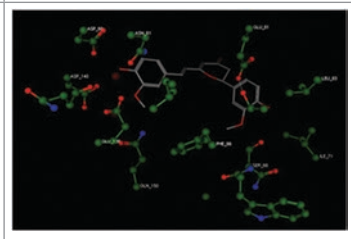
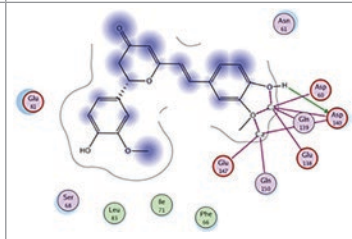
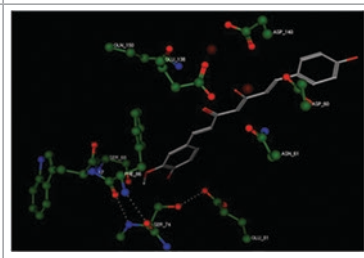
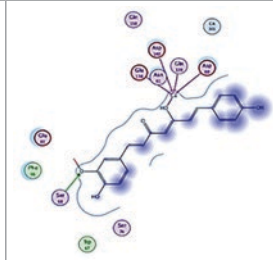
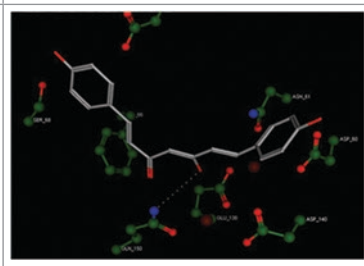
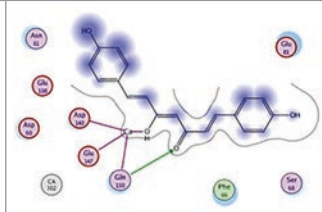
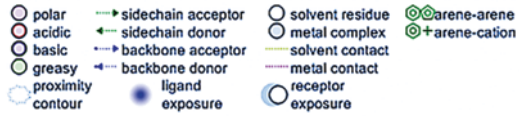



**Fig. 7.6** A diagrammatic 2D representation of interactions between Curcumin and CRP at the active site after 70 ns molecular dynamic simulation. The green dotted lines represent hydrogen bond interaction and red arcs with radiating spokes represents the amino acids showing hydrophobic interaction with protein. Carbon, nitrogen and oxygen atoms have been shown in black, blue and red, respectively. The violet lines represent the ligand bond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

(2.5 g turmeric) administration in chronic kidney disease patients and assessed the blood levels of NF-kB, and hs-CRP [39]. Remarkably, after 3 months of curcumin administration, patients had decreased both NF-kB and hs-CRP, demonstrating anti-inflammatory actions in chronic disease patients. Using a nutraceutical combination of curcumin (200 mg) with other antioxidant compounds has proven to show protective effects in lowering CRP levels and lipoproteins in patients with metabolic diseases [40]. Altogether, these results indicate curcumin as a potential regulator of CRP in various diseases.

The effect of curcumin and curcuminoids on inflammation and oxidative stress is growing in interest, particularly after different studies have

**Table 7.3** Right column: 3D-docking of Curcumin and its derivatives with the C-reactive protein (CRP) molecular target, $Ca^{2+}$ (brown) is seen at the center, Left column: Docking of C-reactive protein with a-e compounds in 2D diagram. (a) Phosphorylcholine; (b) Curcumin; (c) Cyclocurcumin; (d) Demethoxycurcumin; E: Bisdemethoxycurcumin

| Ligands | Docking 3D representation | Docking 2D representation |
|---|---|---|
| (a) |  |  |
| (b) |  |  |
| (c) |  |  |
| (d) |  |  |
| (e) |  |  |
| |  | |

shown that this compound exerts a negative regulation on proinflammatory and oxidative molecules such as malendionate IL-6, COX-2, TNF, IL-1, IL-6, IL-8 and CPR [11, 23–26]. The present study, based on *in silico* approaches by molecular docking and molecular dynamic simulations, on the interaction between CRP and different curcuminoids on the binding site of CRP is thus of potential clinical interest and may lead to the development of novel regulatory agents for inflammatory processes.

Previous docking studies have shown a possible interaction between CRP and five phytochemicals present in *C. Procera* with anti-inflammatory properties [41]. Importantly, they found two phytochemicals (methyl myrisate and methyl behenate) with close energy values (kcal/mol) and similar binding sites to those present in paracetamol and ibobrufen. Moreover, Sohilait et al. (2017) performed a molecular docking study between a set of 15 curcumin analogues and COX-2 in order to establish their inhibitory activities on this cytokine. From this set, three curcumin analogues had a significant inhibition in COX-2 active site [42]. These combined results show the potential of cucurmin and other similar molecules on the regulation of inflammatory cytokines. In conclusion, our study suggests a positive interaction of curcumin with CRP, in which might have potential implications in attenuating inflammatory mechanisms; however, additional experimental studies are needed in order to establish the *in vivo* and *in vitro* interactions of curcumin and curcuminoids with CRP and its regulatory mechanisms in inflammatory and antioxidative processes.

**Conflict of Interests** None of the authors has a competing interest directly related to the content of this study.

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# 8

# Antifungal Activities of Curcuminoids and Difluorinated Curcumin Against Clinical Dermatophyte Isolates

Hossein Zarrinfar, Mahsa Behnam, Mahdi Hatamipour, and Amirhossein Sahebkar

**Abstract**

Dermatophytes are a group of fungal agents that can invade humans' keratinized tissues such as skin, nail, and hair, thereby causing dermatophyte infection (dermatophytosis) or ringworm. Some natural products have been reported to possess fungicidal effects. Hence, the present study investigated the effect of curcuminoids (CUR) and difluorinated curcumin (CDF) against clinical isolates of dermatophytes. CUR and CDF powders were evaluated against dermatophyte species including *Trichophyton tonsurans* (n = 21), *T. mentagrophytes* (n = 19), *T. interdigitale* (n = 18), *Microsporum canis* (n = 4), *T. benhamiae* (n = 1), and *T. verrucosum* (n = 1), based on the CLSI M38-A2 guideline. The minimum inhibitory concentration (MIC) ranges of CUR were 4–16, 8–16, 4–16, 8, 8, and 16 μg/ml for *T. tonsurans*, *T. mentagrophytes*, *T. interdigitale*, *M. canis*, *T. benhamiae*, and *T. verrucosum*, respectively. In addition, MIC ranges of CDF were obtained as 2–32, 4–16, 0.125–16, 8–16, 8, and 16 μg/ml, for *T. tonsurans*, *T. mentagrophytes*, *T. interdigitale*, *M. canis*, *T. benhamiae*, and *T. verrucosum*, respectively. CUR and CDF showed an inhibitory effect against dermatophyte isolates. CDF showed a stronger effect than CUR, especially against *T. interdigitale*. CUR and CDF have the capacity to be developed for use in dermatophytosis to augment existing preventative/therapeutic strategies.

**Keywords**

Curcumin · Difluorinated curcumin · Dermatophyte · Antifungal

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## 8.1 Introduction

Dermatophytes as keratinophilic fungi can invade to keratinized tissues of vertebrates. They can cause dermatophytosis (tinea or ringworm), as various disorders in the skin, nails, and hair, and also deep tissue in some cases [1]. More than 50 species of dermatophytes have been introduced as causative agents of dermatophytosis in different parts of the world [2]. The dermatophytosis prevalence and causative agents varies depending on the geographical area and its climate, as well as the lifestyle of the people [3]. However, the dermatophyte species express various susceptibility to antifungal drugs [4]. Some of species may cause chronic and do not respond well to the usual therapeutic procedure [5]. There are reports regarding drug resistance among various dermatophyte species in vitro and in vivo results [6]. Thus, it can lead to fail or requires long-term treatment, and cause anxiety with serious complications for this group of patients [7]. As resistance to antifungal drugs has been increased, natural products and nanoparticles can being widely screened as the potential sources of novel antifungal agents [8, 9]. In many cases and in recent years, herbal extracts and their derivatives have been used to cure infectious diseases, especially fungal infections [10, 11]. These plants can usually produce aromatic chemicals and secondary metabolites against microbial pathogens [12]. Many studies have been conducted to determine antifungal susceptibility by testing the clinical isolates [9]. However, these studies have been limited to some specific types of fungal agents and the antifungal patterns [13]. Nonetheless, there is restricted information regarding the in vitro activity of herbal plants against various species of dermatophytes. Therefore, the determination of antifungal susceptibility of herbal plants on dominant dermatophytes seems necessary. Curcuminoids (CUR) are natural polyphenols extracted from turmeric, which are known to have numerous pharmacological effects [14–20]. A fluorinated analog of CUR, named difluorinated curcumin (CDF), has been introduced with improved metabolic stability and pharmacological activity [21, 22]. The fungicidal activity of CUR against some fungi has been shown [10]. However, no information has been provided on the antifungal effect of this plant and its synthetic derivatives against different species of dermatophytes. Concerning this, the aim of present study was to investigate the effect of CUR and CDF against clinical isolates of dermatophytes obtained from patients with dermatophytosis.

## 8.2 Materials and Methods

The clinical isolates of dermatophytes were previously identified based on molecular method (ITS sequencing) [23, 24]. They included six species of *Trichophyton tonsurans* (n = 21), *T. mentagrophytes* (n = 19), *T. interdigitale* (n = 18), *Microsporum canis* (n = 4), *T. benhamiae* (n = 1), and *T. verrucosum* (n = 1).

Difluorinated curcumin was prepared using the synthesis method previously described [25]. Briefly, a solution comprising curcumin (1 mmol) and piperidine (0.05 mmol) was added to difluorobenzaldehyde (1 mmol) in methanol. At room temperature under a nitrogen stream, the reaction mixture was stirred for 48 h. The chemical structure of difluorinated curcumin was confirmed with the use of nuclear magnetic resonance (NMR).

The CLSI M38A2 guideline was used to antifungal susceptibility testing procedure [4]. Briefly, the dermatophytes were cultured on potato dextrose agar (Sigma, Germany) and incubated at 30 °C for 2 weeks. Fungal suspensions containing harvested conidia and hyphal fragments were prepared using sterile saline solution along with Tween 20. They were evaluated using a spectrophotometer at a wavelength of 530 nm to reach a 65–70% transmittance. The suspension was diluted 1:50 in RPMI 1640 medium to achieve the final concentrations (1–3 × $10^3$ CFU/ml). The inocula along with the indicated concentrations of CUR and CDF were added to 96-well plates, and incubated at 35 °C for 3–5 days. The final concentrations of CUR and CDF were 0.0625–16 (0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16) μg/ml, Finally, the minimum inhibitory concentration (MIC) ranges were

determined visually as the lowest concentration of CUR/ CDF that resulted in at least 80% growth inhibition, compared to the growth of the control well. The definition of $MIC_{50}$ and $MIC_{90}$ was as inhibition the minimum concentration at which 50% and 90% of the isolates, respectively. The average geometric mean (GM) of the MICs of the antifungal compounds, and differences between the mean values were determined using the SPSS software (version 16).

## 8.3 Results

The detailed information on the results of antifungal effects of CUR and CDF against the dermatophyte clinical isolates is shown in Table 8.1.

According to the results, generally, CDF had a more effect than CUR, and *T. interdigitale* showed the lowest MIC with 0.125 μg/ml than other species. Followed by *T. tonsurans* at concentration of 2 μg/ml. However, CUR had a more effect on *T. interdigitale* and *T. tonsurans* than other species with 4–16 μg/ml. Accordingly, CDF had also the lowest $MIC_{50}$ for *T. interdigitale* at concentration of 4 μg/ml. However, *T. mentagrophytes* showed the highest $MIC_{50}$ and $MIC_{90}$ at concentration of 16 μg/ml.

The GM of CUR for *T. tonsurans*, *T. mentagrophytes*, *T. interdigitale*, and *M. canis* were estimated at 9.75, 11.52, 9.33, and 8, respectively. Moreover, CDF of GM for *T. tonsurans*, *T. mentagrophytes*, *T. interdigitale*, and *M. canis* were estimated at 6.56, 8.92, 2.61, and 9.51, respectively. Due to small number, GM MIC values of *T. benhamiae* and *T. verrucosum* species could not be achieved. Generally, the dermatophyte isolates showed GM MIC values of 5.78 and 10.04 for CUR and CDF, respectively. Furthermore, according to the GM MIC, CDF was the most effective agent against species of *T. interdigitale*, *T. tonsurans*, and *T. mentagrophytes*, respectively.

## 8.4 Discussion

Although few reports of resistance to antifungal agents have been presented on dermatophytes as causative agents of dermatophytosis; the development of resistance to the current clinically used antifungal drugs, has emerged as a growing problem [26, 27]. Several dermatophyte species,

**Table 8.1** The antifungal susceptibility profiles for curcuminoids and difluorinated-curcumin among dermatophyte clinical isolates

| Dermatophyte species | No. (%) | Antifungal compounds (CUR/ CDF) | MIC range (μg/ mL) | $MIC_{50}$ (μg/ mL) | $MIC_{90}$ (μg/ mL) |
|---|---|---|---|---|---|
| *Trichophyton tonsurans* | 21 (32.8%) | CUR | 4–16 | 8 | 16 |
| | | CDF | 2–32 | 8 | 16 |
| *T. mentagrophytes* | 19 (29.7%) | CUR | 8-16 | 16 | 16 |
| | | CDF | 4-16 | 8 | 16 |
| *T. interdigitale* | 18 (28%) | CUR | 4-16 | 8 | 16 |
| | | CDF | 0.125-16 | 4 | 16 |
| *Microsporum canis* | 4 (6.3%) | CUR | 8 | 8 | 8 |
| | | CDF | 8-16 | 8 | 8 |
| *T. benhamiae* | 1 (1.6%) | CUR | 8 | – | – |
| | | CDF | 8 | – | – |
| *T. verrucosum* | 1 (1.6%) | CUR | 16 | – | – |
| | | CDF | 16 | – | – |
| Dermatophyte isolates | 64 (100%) | CUR | 4–16 | 8 | 16 |
| | | CDF | 0.125–32 | 8 | 16 |

*MIC* minimum inhibitory concentration, Due to small number, $MIC_{50}$ and $MIC_{90}$ of *T. benhamiae* and *T. verrucosum* species could not be achieved

in particular, *T. interdigitale* and *T. rubrum* described are resistant or less susceptible to several classes of antifungals [28]. It appears the developed acquisition of resistance mechanisms to antifungal agents be following a prior exposure [28]. The present study was aimed at evaluating the activity of CUR and CDF against the clinical isolates of dermatophytes obtained from patients affected by dermatophytosis. In general, both tested compounds exhibited good activity against all tested isolates. Collectively, these data demonstrated that CUR/CDF have the therapeutic potential to be used in dermatophytosis. Natural products can be used as the potential sources of novel antifungal agents, particularly in the case of acquired or high antifungal resistance [8, 11]. Traditionally, medicinal plants have been investigated to prevent and cure infectious diseases [29]. They can produce aromatic chemicals and secondary metabolites as defense mechanisms and antimicrobial effects [12]. Polyphenols, found in various edible plants, may serve as an alternative source of antimicrobials [10]. CUR is a bioactive polyphenol that is extracted from the rhizomes of the *Curcuma longa* plant [30]. Curcumin constitutes typically about less than 5% of turmeric composition [31, 32]. Interestingly, it displays broad-spectrum antimicrobial activity, particularly antibacterial and antifungal properties [33–35]. Also, it is able to influence the adhesion ability of bacteria, whereby less fimbriae leads to reduced adhesion ability against planktonic and biofilm cells [36, 37]. Possibly, CUR can directly impact cell wall permeability through signaling of the MAP kinase and calcineurin-mediated signaling, pathways which play significant role in cell wall integrity [38]. Interestingly, some studies showed that CUR can reduce the production of aflatoxin B1 by *Aspergillus flavus* too [39].

There is little information about the use of CUR as a natural antifungal compound for fungal pathogens so far. Unfortunately, up until now, there is no significant report about CDF antifungal effect, as a CUR analogue, against fungal pathogens too. However, most of studies investigated CUR effect again *Candida* and *Aspergillus* species [10, 35]. To our knowledge, there have been no studies that report on pathogenic dermatophytes. In a study, Martins et al. (2009) investigated the antifungal effects of CUR and fluconazole against *Candida* spp., *Cryptococcus neoformans*, *Sporothrix schenckii*, *Paracoccidioides brasiliensis* and *Aspergillus* species [35]. They reported *P. brasiliensis* isolates were the most susceptible to CUR while the growth of *Aspergillus* isolates was not affected. Moreover, CUR was much more efficient than fluconazole in inhibiting the adhesion of *Candida* species to human buccal epithelial cells. They obtained that a MIC range of 0.5–32 μg/ml against *P. brasiliensis* isolates, but in our study the MIC range were 4–16, 0.125–32 μg/ml for CUR and CDF, respectively. Our results indicate that CDF may be a promising compound for the design of new antifungal agents capable of inhibiting of *P. brasiliensis* with more impact. To the best of our knowledge, this study reports for the first time the effect of CUR and CDF on the growth of dermatophytes of clinical interest. Our *in vitro* results highlight the potential of CUR and CDF as the effective antifungals against various dermatophytes strains including *T. tonsurans*, *T. mentagrophytes*, *T. interdigitale*, *M. canis*, *T. benhamiae*, and *T. verrucosum*. Among the dermatophyte isolates examined in the present study, the widest MIC range of CDF was observed for *T. interdigitale* (0.125–16 μg/ml) that is higher than those obtained for CUR. This concentration show that CDF could act against dermatophyte species more effectively, particularly on *T. interdigitale*. This is important since the *T. interdigitale* species is one of the most common species among patients with dermatophytosis in the world. Therefore, it can play an effective role in the prevention and treatment of dermatophytosis. The widest MIC range of CUR was observed for *T. tonsurans T. tonsurans* with 4–16 μg/ml. However, CUR showed more effect against them than other species. The $MIC_{50}$ of CDF against *T. interdigitale* (with 4 μg/ml concentration) was lower than CUR, thus showing a stronger effect against this species. However, the $MIC_{50}$ (8 μg/ml) of CDF was equal to CUR for the 64 dermatophyte isolates in general. Nevertheless, several studies have shown that the different types of

fungal agents have different sensitivities to CUR [35, 40]. In a study performed by Alalwan et al. (2017) investigating the antifungal effects of CUR, was reported to be efficient in 50 μg/ml concentration to inhibit and kill *C. albicans* [10]. This concentration is different from the results obtained against dermatophyte isolates in the present study. It appears that different fungal pathogens could show various susceptibility to CUR. In the case of clinical pathogenic fungi, this difference can be even due to different geographical locations. In this regard, general and specific relevance of environmental conditions can affect specific secretion of enzymes and the expression of certain genes in pathogenic fungi [41]. Furthermore, the number of fungi examined and their diversity can fundamentally influence the results. Thus, one of the limitations of the present study was the low number of clinical isolates of dermatophytes.

In another study by Garcia-Gomes et al., the synergistic effect of curcumin and fluconazole was evaluated on the *C. albicans* isolate resistant to fluconazole [42]. They showed that CUR has a great capability to inhibit fluconazole resistance of the isolate of *C. albicans*, and it could restore its sensitivity to this azole. Therefore, interestingly, azole-resistant isolates can be further investigated by azole-CUR whether such combinations can be beneficial. Oglah et al. in their review article stated that analogues of CUR had antifungal activities against the genera of *Aspergillus*, *Penicillium* and *Alternaria* [43]. Therefore, the use of these analogues can be evaluated among the clinical isolates too. However, there are no reports of possible effects of CUR and its analogues on dermatophytes.

The present study was the first attempt investigating the antifungal effect of CUR and its analogue (CDF) against clinical dermatophyte isolates obtained from dermatophytosis. Based on the results of the present study, and according to GM definition, CUR and CDF, particularly CDF had an effective impact against the dermatophyte isolates. Furthermore, among dermatophyte species, *T. interdigitale* showed a greater sensitivity to CDF and CUR, respectively.

## 8.5 Conclusion

As the results of the present study indicated, CUR and CDF had respectively the antifungal effect against clinical isolates of dermatophyte. Furthermore, none of the dermatophyte isolates showed too high MIC to these compounds. Additionally, *T. interdigitale*, as a common agent of dermatophytosis in the world, showed the lowest GM (the highest sensitivity) to CDF and CUR, respectively.

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**Conflicts of Interest** The authors declare that they have no conflicts of interest.

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# 9 Therapeutic Effect of Curcumin in Women with Polycystic Ovary Syndrome Receiving Metformin: A Randomized Controlled Trial

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**Abstract**

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility, for which the insulin sensitizer metformin has been used therapeutically. It has been shown that curcumin also exhibits insulin-sensitizing properties. Given that metformin acts as an ovulation inducing agent and both curcumin and metformin can reduce insulin resistance, the aim of the current study was to evaluate the effect of metformin with and without curcumin nanomicelles in the treatment of women with polycystic ovary syndrome. This clinical trial was conducted on 100 women with PCOS, diagnosed according to the Rotterdam criteria, who were sequentially recruited and randomly divided into two groups (n = 50 each). Group 1 received 500 mg metformin three times daily and group 2 received 80 mg/day capsule of curcumin nanomicelle and 500 mg metformin three times a day for

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3 months. After collecting fasting blood samples, biochemical parameters including triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, plasma glucose, alanine amino transferase (ALT) and aspartate aminotransferase (AST) were evaluated based on enzymatic methods. Hormonal parameters were assessed using immunoassay kits. Insulin resistance (HOMA-IR) and insulin-sensitivity check index (QUICKI) were also assessed. After treatment, fasting insulin, HOMA-IR, and total testosterone in group 2 were significantly lower than those in group 1 ($p < 0.05$). Post-treatment LDL-C levels in groups 1 and 2 were 117.9 ± 24 and 91.12 ± 19.46 mg/dL, respectively ($p < 0.01$). In addition, HDL-C levels were increased with curcumin (group 1: 38.1 ± 4.36 mg/dL; group 2: 44.12 ± 7.3 mg/dL, $p < 0.05$). Total cholesterol was decreased with curcumin level (group 1: 207.9 ± 39.84 mg/dL; group 2; 159.7 ± 48.43 mg/dL, $p < 0.05$), with a decrease in triglycerides levels (group 1: 141.6 ± 9.57; group 2: 97.5 ± 8.8 mg/dL, $p < 0.01$). This study showed that curcumin has a synergistic effect with metformin in the improvement of insulin resistance and lipid profile in patients with PCOS. Therefore, the combined use of metformin and curcumin may have therapeutic utility in patients with PCOS.

**Keywords**

Nanomicelle curcumin · Metformin · Polycystic ovary syndrome

## 9.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous and complex disease [1, 2] characterized by chronic anovulation, menstrual irregularity and hyperandrogenism [1], with associated infertility and metabolic disturbances including insulin resistance, glucose intolerance, hypertension, obesity [3], and dyslipidemia [1] in women of reproductive age. PCOS is a polygenic disorder influenced by genetic susceptibility and environmental risk factors. Among environmental parameters, lifestyle aspects including sedentary behavior and improper diet contribute to the pathogenesis of PCOS [3, 4].

Metformin is the most commonly used drug in the treatment of type 2 diabetes that improves the sensitivity of peripheral tissues to insulin, prevents hepatic gluconeogenesis and decreases oxidative stress. Moreover, the use of this drug decreases androgen levels and increases the levels of sex hormone binding globulin (SHBG) [1]. In addition, it improves metabolic syndrome in patients with PCOS. Therefore, the use of metformin in women with PCOS is common. However, it is associated with the side effects of abdominal pain, nausea, diarrhea, anorexia, flatulence and bloating [1].

Curcumin is an extracted polyphenol from turmeric, which is safe and endowed with numerous biological effects [5–12]. Anti-inflammatory, anti-obesity and anti-diabetic properties of curcumin are reported in some studies [13]. Curcumin exerts anti-inflammatory effects by modulating cytokines and chemokines [14–16], and it shows antioxidant activities by inhibiting reactive oxygen species and inducing an antioxidant response [17]. In addition, favorable effects of curcumin on cancers may be due to direct anti-inflammatory and anti-oxidative effects as well as its ability to modulate the immune system. Recently, some studies have reported that curcumin through reducing luteinizing hormone (LH) can induce ovulation and modulate ovarian responsiveness [18]. The effect of curcumin in diabetes is through decreasing death of pancreatic islet beta cells, preventing insulin resistance and improving beta cell function. Current research on curcumin has concentrated on nanocarrier delivery systems including nanostructured lipid carrier and nanoparticles to enhance aqueous stability, solubility and bioavailability [19]. Therefore, it seems that nanocarrier and nanomicelles can be considered as promising delivery systems for curcumin [14].

Given that insulin resistance plays an important role in the pathogenesis of PCOS [20], we hypothesized that insulin resistance would be

reduced to a greater degree with nanomicelle curcumin plus metformin in comparison to metformin alone in the treatment of women with PCOS.

## 9.2 Materials and Methods

### 9.2.1 Sample Selection

This clinical trial study was conducted on 100 women with PCOS presented sequentially to the Shahid Sadoughi Hospital- Yazd- Iran from March 2017 to March 2018 and were randomized either into one or the other clinical trial arms. All patients fulfilled the Rotterdam criteria for diagnosis with 2 out of 3 features of oligomenorrhea/amenorrhoea, clinical or biochemical hyperandrogenism (Ferriman-Gallwey score >8; free androgen index >4 respectively), and polycystic ovaries on transvaginal ultrasound (≥ 12 antral follicles in at least one ovary or ovarian volume of ≥10 $cm^3$) [21]. Study participants had no concurrent illness, were not on any medication for the preceding 9 months and were not planning to conceive. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumors were excluded by appropriate tests. Written informed consent was given by each participant and the study was approved by ethics committee (IR.SSU.MEDICINE.REC.1396.195) of Shahid Sadoughi University. The study was recorded in the Iranian Registry of Clinical Trial system with number IRCT20090422001836N11. The patient flowchart in shown in Fig. 9.1.

### 9.2.2 Classification of Patients

Patients were randomly divided into two groups (n = 50), using a random number table. The first group received 500 mg metformin three times daily and the second group received 80 mg/day capsule of curcumin nanomicelle (Exir Nano Sina Co, Tehran, Iran) and 500 mg metformin three times daily. In each group, treatment was undertaken for 12 weeks.

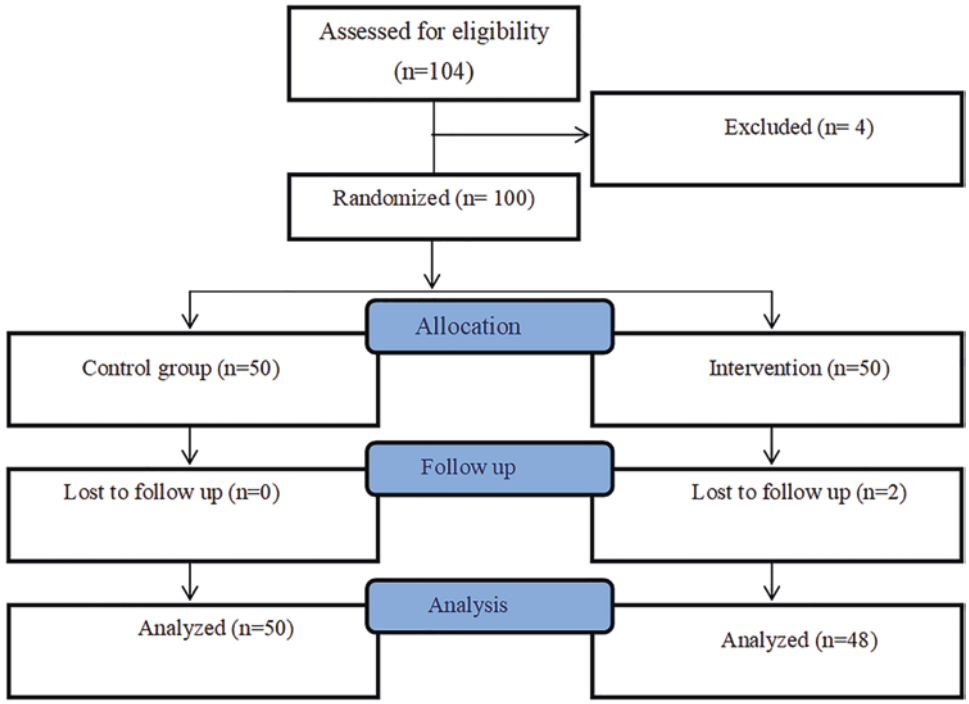


**Fig. 9.1** Patient flowchart

### 9.2.3 Analysis of Biochemical and Hormonal Parameters

Blood samples were taken from patients after 8 h fasting. After collecting blood samples, serum was separated by centrifugation at 1000 x g for 1 min and frozen at −20 °C for subsequent analyses. Biochemical parameters including fast blood sugar, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, alanine aminotransferase (ALT), and aspartate aminotransferase (AAT) were assessed based on an enzymatic method (Pars Azmoon kit, Tehran, Iran) using a BT-3000 PLUS analyzer. Fasting serum insulin was evaluated by an enzyme-linked immunoadsorbent assay (ELISA) kit (Insulin AccuLite CLIA Kit, Monobind Inc., Lake Forest, CA, USA). TSH was measured based on immunoassay method using an ELISA kit (Padtan Gostar kit, Iran) and Prolactin (PRL) was measured using a PRL assay kit (Padyab Teb kit, Tehran, Iran). Dehydroepiandrosterone (DHEAS) was also measured using an ELISA method *(DEMEDITEC Diagnostics* GmbH, Kiel, Germany). An ELISA kit was used for measuring serum level of LH and testosterone according to Pidgin Teb kit protocol, and follicle stimulating hormone (FSH) was measured according to the Padyab kit protocol. The homeostatic model assessment (HOMA) for quantify insulin resistance (IR) was calculated using the formula:

$$\text{HOMA}-\text{IR}=\begin{bmatrix}\text{fasting glucose}(\text{nmol}/\text{L})\times\\ \text{fasting insulin}(\mu U/\text{mL})/22.5\end{bmatrix}$$

The quantitative insulin-sensitivity check index (QUICKI) was assessed based on following formula:

$$1/\begin{pmatrix}\log(\text{fasting insulin}\,\mu U/\text{mL})+\\ \log(\text{fasting glucose}\,\text{mg}/\text{dL})\end{pmatrix}$$

Data including weight, body mass index (BMI), age and gender were extracted from the medical records.

### 9.2.4 Statistical Analysis

Sample size was calculated using a statistical power of 80%, an α value of 0.05, and a difference in means of insulin levels between intervention and control groups (11μU/mL) [22]. Data trends were visually evaluated for each androgen and non-parametric tests were applied on data that violated the assumptions of normality when analyzed using the Kolmogorov-Smirnov Test. Significance was defined at $\alpha = 0.05$. All analyses were conducted using IBM-SPSS version 17.0.

## 9.3 Results

In this study, the age range of patients was 18–40 years old. The mean ages of patients in the two groups did not differ (28.8 ± 2.44 and 29.2 ± 2 years old, respectively). Comparison of biochemical parameters in two groups before treatment is shown in Table 9.1. This revealed no significant difference between the two groups for any of the biochemical parameters before treatment. Table 9.2 shows the between group effect of metformin and metformin/curcumin on biochemical parameters after 3 months treatment. This shows that significant differences were seen between two groups for fasting insulin, HOMA-IR, LDL, HDL, total cholesterol, triglycerides and testosterone ($p < 0.05$).

Table 9.3 shows the within group comparison of biochemical parameters before and after treatment with metformin alone or curcumin with metformin. Significant differences were seen before and after treatment with metformin, in terms of fasting glucose, fasting insulin, HOMA-IR, QUICKI, fasting glucose/insulin (G/I) ratio, ALT, AST, testosterone, LH and the LH/FSH ratio ($p < 0.05$). In addition, significant differences were seen before and after treatment of metformin/curcumin, regarding fasting glucose, fasting insulin, HOMA-IR, QUICKI, fasting G/I ratio, cholesterol, triglyceride, LDL, HDL, total testosterone, LH, LH/FSH ratio, AST and ALT ($p < 0.05$).

**Table 9.1** Comparison of biochemical parameters in two groups before treatment

| Characteristics | Metformin (group 1) N = 50 | Metformin/curcumin (group 2) N = 48 | p-value (ANOVA) |
|---|---|---|---|
| Age | 28.8 ± 2.46 | 29 ± 2 | 0.36 |
| Weight (kg) | 73 ± 5 | 73.7 ± 4.6 | 0.48 |
| BMI (kg/m$^2$) | 27.01 ± 1.65 | 27.2 ± 2.2 | 0.26 |
| Fasting glucose (mg/dL) | 107.86 ± 7 | 110.36 ± 5.6 | 0.054 |
| Fasting insulin (μU/mL) | 18.1 ± 2.7 | 18 ± 2.8 | 0.86 |
| HOMA-IR | 4.16 ± 1.42 | 4.36 ± 0.72 | 0.52 |
| QUICKI | 0.31 ± 0.01 | 0.3 ± 0.01 | 0.91 |
| Fasting G/I (mg/dL per μIU/mL) | 8.4 ± 5.56 | 8.6 ± 5. 1 | 0.11 |
| LDL cholesterol (mg/dL) | 119.28 ± 23.5 | 114.04 ± 24.6 | 0.28 |
| HDL cholesterol (mg/dL) | 37.2 ± 5.2 | 33.1 ± 6.1 | 0.76 |
| Serum cholesterol (mg/dL) | 215.1 ± 37.2 | 200.3 ± 31.4 | 0.83 |
| Serum triglyceride (mg/dL) | 143 ± 8.3 | 129.5 ± 5.7 | 0.43 |
| DHEAS (μg/dL) | 180 ± 4.4 | 191 ± 11.1 | 0.69 |
| Total testosterone (μg/L) | 0.7 ± 0.14 | 0.74 ± 0.18 | 0.4 |
| FSH (IU/L) | 5.9 ± 3.2 | 5.4 ± 4.2 | 0.34 |
| LH (IU/L) | 7.6 ± 2.9 | 7.9 ± 1.9 | 0.39 |
| LH/FSH | 1.7 ± 0.92 | 1.7 ± 1.5 | 0.72 |

*BMI* body mass index, *HOMA-IR* homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *G/I* glucose/insulin, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *DHEAS* dehydroepiandrosterone sulfate, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone

**Table 9.2** The effect of metformin and metformin/curcumin on biochemical parameters between groups after treatment

| Characteristics | Metformin group N = 50 Post treatment | Metformin/curcumin N = 48 Post treatment | p- value (ANOVA) |
|---|---|---|---|
| Weight (kg) | 72.6 ± 4.7 | 73.3 ± 4 | NS |
| BMI (kg/m$^2$) | 26.81 ± 1.82 | 26.37 ± 3.7 | NS |
| FPG (mg/dL) | 94.26 ± 8.4 | 95.32 ± 9 | NS |
| Fasting insulin (μU/mL) | 13.75 ± 3 | 12.5 ± 3.4 | <0.05 |
| HOMA-IR | 2.83 ± 0.65 | 2.57 ± 0.76 | <0.05 |
| Testosterone (μg/L) | 0.62 ± 0.12 | 0.4 ± 0.16 | 0.035 |
| DHEAS (μg/dL) | 175 ± 43 | 189.3 ± 10.67 | NS |
| QUICKI | 0.32 ± 0.02 | 0.32 ± 0.03 | NS |
| Fasting G/I ratio (mg/dL per μIU / mL) | 13.9 ± 10.32 | 14.73 ± 6.4 | 0.058 |
| LDL cholesterol (mg/dL) | 117.9 ± 24 | 91.12 ± 19.46 | <0.01 |
| HDL cholesterol (mg/dL) | 38.1 ± 4.36 | 44.12 ± 7.3 | <0.05 |
| Serum cholesterol (mg/dL) | 207.9 ± 39.84 | 159.7 ± 48.43 | <0.01 |
| Serum triglyceride (mg/dL) | 141.6 ± 9.57 | 97.5 ± 8.8 | <0.01 |
| FSH (IU/L) | 6.2 ± 2.8 | 5.8 ± 3.8 | NS |
| LH(IU/L) | 6.4 ± 2.6 | 6.3 ± 1.7 | NS |
| LH/FSH | 1.1 ± 0.78 | 1.1 ± 0.65 | NS |

*BMI* body mass index, *FPG* fasting plasma glucose, *HOMA-IR* homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *LDL* low density lipoprotein, *HDL* high density lipoprotein, *DHEAS* dehydroepiandrosterone sulfate, *FSH* follicle-Stimulating hormone, *LH* luteinizing hormone

**Table 9.3** Comparison of biochemical parameters before and after treatment in two groups

| Variable | Metformin treatment (group 1) | | | Metformin/curcumin treatment (group 2) | | |
|---|---|---|---|---|---|---|
| | Pretreatment | Post treatment | p- value | Pretreatment | Post treatment | p- value |
| Weight (kg) | 73 ± 5 | 72.6 ± 4.7 | NS | 73.7 ± 4.6 | 73.3 ± 4 | NS |
| BMI (kg/m$^2$) | 27.01 ± 1.65 | 26.81 ± 1.82 | NS | 27.2 ± 2.2 | 26.37 ± 3.7 | NS |
| FPG (mg/dl) | 107.86 ± 7 | 94.26 ± 8.4 | 0.008 | 110.36 ± 5.6 | 95.32 ± 9 | 0.003 |
| Fasting insulin (μU/mL) | 18.1 ± 2.7 | 13.75 ± 3 | 0.004 | 18 ± 2.8 | 12.5 ± 3.4 | <0.001 |
| HOMA-IR | 4.16 ± 1.42 | 2.83 ± 0.65 | 0.001 | 4.36 ± 0.72 | 2.57 ± 0.76 | 0.0013 |
| QUICKI | 0.31 ± 0.01 | 0.32 ± 0.02 | 0.009 | 0.3 ± 0.01 | 0.32 ± 0.03 | 0.006 |
| Fasting G/I (mg/dL per μIU/mL) | 8.4 ± 5.56 | 13.9 ± 10.32 | <0.05 | 8.6 ± 5. 1 | 14.73 ± 6.4 | <0.01 |
| LDL cholesterol (mg/dL) | 119.28 ± 23.5 | 117.9 ± 24 | NS | 114.04 ± 24.6 | 91.12 ± 19.46 | <0.001 |
| HDL cholesterol (mg/dL) | 37.2 ± 5.2 | 38.1 ± 4.36 | NS | 33.1 ± 6.1 | 44.12 ± 7.3 | <0.01 |
| Serum cholesterol (mg/dL) | 215.1 ± 37.2 | 207.9 ± 39.84 | NS | 200.3 ± 31.4 | 159.7 ± 48.43 | >0.001 |
| Serum triglyceride (mg/dL) | 143 ± 8.3 | 141.6 ± 9.57 | NS | 129.5 ± 5.7 | 97.5 ± 8.8 | >0.01 |
| DHEAS (μg/dL) | 180 ± 44 | 175 ± 43 | NS | 191 ± 11.1 | 189.3 ± 10.67 | NS |
| Total testosterone (μg/L) | 0.7 ± 0.14 | 0.62 ± 0.12 | 0.003 | 0.74 ± 0.18 | 0.4 ± 0.16 | >0.001 |
| FSH (IU/L) | 5.9 ± 3.2 | 6.2 ± 2.8 | NS | 5.4 ± 4.2 | 5.8 ± 3.8 | NS |
| LH(IU/L) | 7.6 ± 2.9 | 6.4 ± 2.6 | >0.01 | 7.9 ± 1.9 | 6.3 ± 1.7 | >0.01 |
| LH/FSH | 1.7 ± 0.92 | 1.1 ± 0.78 | >0.01 | 1.7 ± 1.5 | 1.1 ± 0.65 | >0.01 |
| AST | 31 ± 7.48 | 27.54 ± 6.57 | 0.017 | 29.5 ± 6.84 | 21 ± 6.0 | >0.01 |
| ALT | 37.16 ± 10.8 | 32.98 ± 10.3 | 0.049 | 38.26 ± 9.45 | 23.28 ± 7.89 | <0.01 |

*FPG* fasting plasma glucose, *HOMA-IR* Homeostatic model assessment of insulin resistance, *QUICKI* quantitative insulin check index, *HDL* High density lipoprotein, *DHEAS* Dehydroepiandrosterone sulfate, *LDL* low density lipoprotein, *FSH* follicle-stimulating hormone, *AST* aspartate aminotransferase, *LH* luteinizing hormone, *ALT* alanine aminotransferase, *NS* non- significant

## 9.4 Discussion

This study showed a significant reduction in fasting insulin and HOMA-IR after metformin plus curcumin treatment in women with PCOS. Aguilar et al. evaluated the effect of curcumin supplementation on insulin sensitivity and observed that curcumin consumption decreased insulin resistance and improved glucose tolerance [23], which is consistent with our findings. Additionally, Rahimi et al. observed decreased fasting plasma glucose following the administration of nano-curcumin in diabetic patients [24]. Ameli et al. reported that curcumin improves insulin secretion via reducing plasma glucose in diabetic rats [25]. Another study showed that curcumin improved glucose metabolism by activation of adenosine monophosphate (AMP) kinase in the liver and induced glucose transporter-4 (GLUT-4) expression leading to increased peripheral glucose uptake [26]. Furthermore, it was revealed that curcumin reduced glucose levels and increased insulin secretion through peroxisome proliferator-activated receptor (PPAR)-γ activation [27, 28], explaining the hypoglycemic effects of this natural agent.

In our study, curcumin therapy increased FSH levels and decreased LH, DHEAS, and testosterone concentrations in patients with PCOS. Mohammadi et al. found increased levels of FSH and decreased LH and testosterone concentrations following curcumin treatment in a model of PCOS [1], which is consistent with our results. These effects may be because curcumin inhibits tumor necrosis factor (TNF)-α, interleukin (IL)-6, and C-reactive protein expression, improving ovulation and the corpus luteum [1]. This suggests that this polyphenol could improve the reproductive endocrine function and induce follicular development. However, this study was not designed to look at this specifically. Nabiuni et al. demonstrated that curcumin treatment resulted in improvement of PCOS symptoms and initiation of ovulation via antioxidant and anti-inflammatory effects [18]. Other studies have shown that curcumin induces apoptosis, inhibits

pituitary tumor cell proliferation, and reduces the production and release of LH [29].

Yan et al. found that curcumin treatment mitigates oxidative stress, apoptosis, and ovarian injury through regulation of nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) and phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathways in mice with induced ovarian failure [30]. Consistent with this, Melekoglu et al. observed that curcumin administration ameliorated ovarian failure by decreasing oxidative stress markers, FSH and LH levels, and improving histophatological parameters [31], suggesting a protective effect of this natural compound against ovarian failure. It is important to note that although the intervention period of our study was too short to demonstrate the regularization of menstrual cycle, the benefits of curcumin on PCOS symptoms and hormonal levels may reflect an amelioration in menstrual and ovulatory cycles. Therefore, longer clinical trials are needed in order to corroborate the potential clinical impact of curcumin on menstrual disorders and endocrine dysfunction.

Beneficial effects of curcumin plus metformin were seen on lipid parameters by a reduction in total cholesterol, LDL, triglycerides, and increased HDL levels. In accordance with our findings, others have reported a significant decrease in total cholesterol, LDL, and triglyceride concentrations after curcumin therapy [32, 38]. These lipid-lowering effects of curcumin could be explained by a number of possible mechanisms, such as increased activity of lipoprotein lipase and fatty acid β-oxidation, and inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, fatty acid synthase, and acyl coenzyme A cholesterol acyltransferase [33]. Moreover, a previous meta-analysis revealed that curcumin supplementation significantly decreased plasma concentrations of triglycerides and increased HDL levels [34], which is also consistent with our study. This potential cardioprotective action of curcumin could be explained through an increase in lipoprotein lipase activity and hydrolysis of triglyceride-rich lipoproteins, causing reduced circulating triglyceride levels [35]. Also, curcumin may ameliorate HDL function by regulating apolipoprotein-AI, cholesteryl ester transfer protein (CETP), lecithin–cholesterol acyltransferase (LCAT), serum paraoxonase/arylesterase 1 (PON1), and myeloperoxidase (MPO) activities [36]. It is well-known that atherogenic dyslipidemia, characterized by elevated triglycerides and low HDL concentrations, is a central therapeutic target in patients with cardiovascular disease due to the high residual risk of cardiovascular events and microvascular complications [37]. Because curcumin showed a combined effect by raising HDL levels and decreasing triglycerides, this natural compound might be considered as an additional therapeutic option in the treatment of atherosclerotic cardiovascular disease.

Limitations of this study include that the treatment period was short to assess menstrual frequency and ovulatory cycles. However, 3 months were sufficient to found significant effects of curcumin administration on metabolic parameters. Secondly, although our study was not blinded and did not use a placebo, randomization would have minimized this potential source of bias.

The main strength of our study was the sample size which conferred adequate statistical power to support the efficacy of curcumin treatment in PCOS.

## 9.5 Conclusions

This study suggests that the nanomicellar curcumin exerts a synergistic effect with metformin in the improvement of insulin resistance and the lipid profile in patients with PCOS. Therefore, combined use of metformin and nanomicelle curcumin may be a promising alternative therapy for the treatment of PCOS. However, the clinical benefits of curcumin on menstrual disorders remain to be determined in clinical trials of longer duration.

**Conflict of Interests** None.

**Funding** Iran University of Medical Sciences, Tehran, Iran.

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# 10 Effect of Curcumin on Severity of Functional Dyspepsia: a Triple Blinded Clinical Trial

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**Abstract**

**Background**

Functional dyspepsia is the main cause of upper abdominal discomfort affecting 5–10% of the world population. Despite various therapeutic approaches, up to 50% of patients with functional dyspepsia seek alternative treatments. In the present study we evaluated the effect of curcumin supplementation along with famotidine therapy on severity of functional dyspepsia. A total of 75 patients with functional dyspepsia according to Rome III criteria were allocated into intervention (N = 39) or control (N = 36) groups. The intervention group was treated with a combination of 500 mg curcumin and 40 mg famotidine daily for 1 month. The control group received placebo and 40 mg famotidine. Severity of dyspepsia symptoms was determined using the Hong Kong questionnaire at baseline, after the 1 month treatment and after a 1 month follow-up. The presence of *H. pylori* antigens in the stool samples was also investigated in all subjects. No significant difference was

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observed between intervention and control groups in biochemical indices, severity of dyspepsia and rate of *H. pylori* infection. A significant decrease was observed in severity of dyspepsia ($p < 0.001$) and rate of H. *pylori* infection ($p = 0.004$) immediately after the treatment and follow-up in the curcumin intervention group. This study indicated that curcumin therapy could be a favorable supplementation in the symptom management of functional dyspepsia. Moreover, curcumin could help efficient eradication of *H. pylori* in these patients.

**Keywords**

Functional dyspepsia · Curcumin · *H. pylori*

## 10.1 Introduction

Functional dyspepsia is the main cause of upper gastrointestinal tract discomfort manifested by abdominal fullness, heartburn, nausea, belching, acid taste and upper abdominal pain [1, 2]. It is a relapsing and remitting condition that affects 5–11% of population worldwide [1]. It is estimated that the financial burden of drugs in dyspepsia is about £450 million in the United Kingdom [3]. Based on Rome III criteria, functional dyspepsia is defined as chronic presence of early satiation, postprandial fullness, epigastric pain or epigastric burning, in endoscopy-negative patients [2, 4]. Genetic background and psychological distress are the most important factors related to this condition that evoke an inflammatory response and further clinical symptoms [1]. Some proposed treatments for functional dyspepsia are *H. pylori* eradication [5], acid-suppression [3], and administration of pro-kinetic agents [3], antidepressants [6–9] and herbal supplements [10, 11]. Due to increasing dissatisfaction with conventional therapeutic approaches in functional dyspepsia, almost 50% of patients seek alternative treatments [12]. Hence, more effective and yet safer agents are required to treat functional dyspepsia.

Curcumin is a yellow pigment derived from turmeric. A wide range of its pharmacological effects have so far been been reported for this dietary safe phytochemical [13–21]. Furthermore, clinical studies have indicated that curcumin supplementation could positively affect symptoms related to the gastrointestinal tract including epigastric pain, post-prandial fullness, bloating, belching and nausea. However, no improvement in *H.pylori* eradication has been shown thus far [22]. Similarly, curcumin treatment could efficiently improve some gastrointestinal diseases such as peptic ulcer, Barrett's esophagus, non-alcoholic fatty liver disease, irritable bowel syndrome (IBS), pancreatitis, ulcerative colitis and gastrointestinal cancers [23–33].

Evidence of curcumin efficacy on treatment of functional dyspepsia is scarce. Here we aim to investigate the effect of curcumin on improving of functional dyspepsia according to Hong Kong score and *H. pylori* eradication rate.

## 10.2 Materials and Methods

### 10.2.1 Subjects

Adult subjects were selected from the outpatient unit of Gastroenterology clinic of Baqiyatallah Hospital (Tehran, Iran) with symptoms of dyspepsia and qualified as candidates for upper gastrointestinal endoscopy. Eligible patients diagnosed with functional dyspepsia according to Rome III criteria [2] were enrolled in the study. Gastrointestinal structural abnormalities were ruled out by upper gastrointestinal endoscopy. Exclusion criteria were liver and renal dysfunction, chronic disease, previous anti-helicobacter therapy, malignancies, deterioration of dyspepsia and other related complications during the study period. The study design was approved by the institutional ethics committee and was conducted as per the Helsinki declaration. Written informed consent was taken from all of participants. The Hong Kong index of dyspepsia severity was recorded prior to intervention, at the end of the study and after a follow-up for 1 month. Blood samples were obtained in order to measure the fasting blood sugar (FBS), hemoglobin (Hb), triglycerides (TG), cholesterol (Chol), low density lipoprotein (LDL), high density lipoprotein

(HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and *alkaline phosphatase* (ALP). Stool samples were collected for assessment of *H. pylori* antigen prior to treatment, after the 1 month treatment and 1 month of follow-up periods. The patient flow chart is shown in Fig. 10.1.

### 10.2.2 Study Design

This study was performed as a randomized triple-blind placebo-controlled trial. Patients were randomly allocated into intervention or control groups. The intervention group received 40 mg famotidine and one capsule containing 500 mg curcuminoids (C3 Complex®, Sami Labs Ltd., Bangalore, India) after lunch for 30 days (n = 39). For the control group (n = 36), 40 mg famotidine and a placebo capsule were administered daily for the same period. The curcuminoid and placebo capsules were identical in size and shape. Each curcuminoid capsule also contained piperine (5 mg; Bioperine®, Sami Labs Ltd.), added for the purpose of enhancing bioavailability. The trial protocol was registered in the Iranian Registry of Clinical Trials (IRCT20080901001165N45).

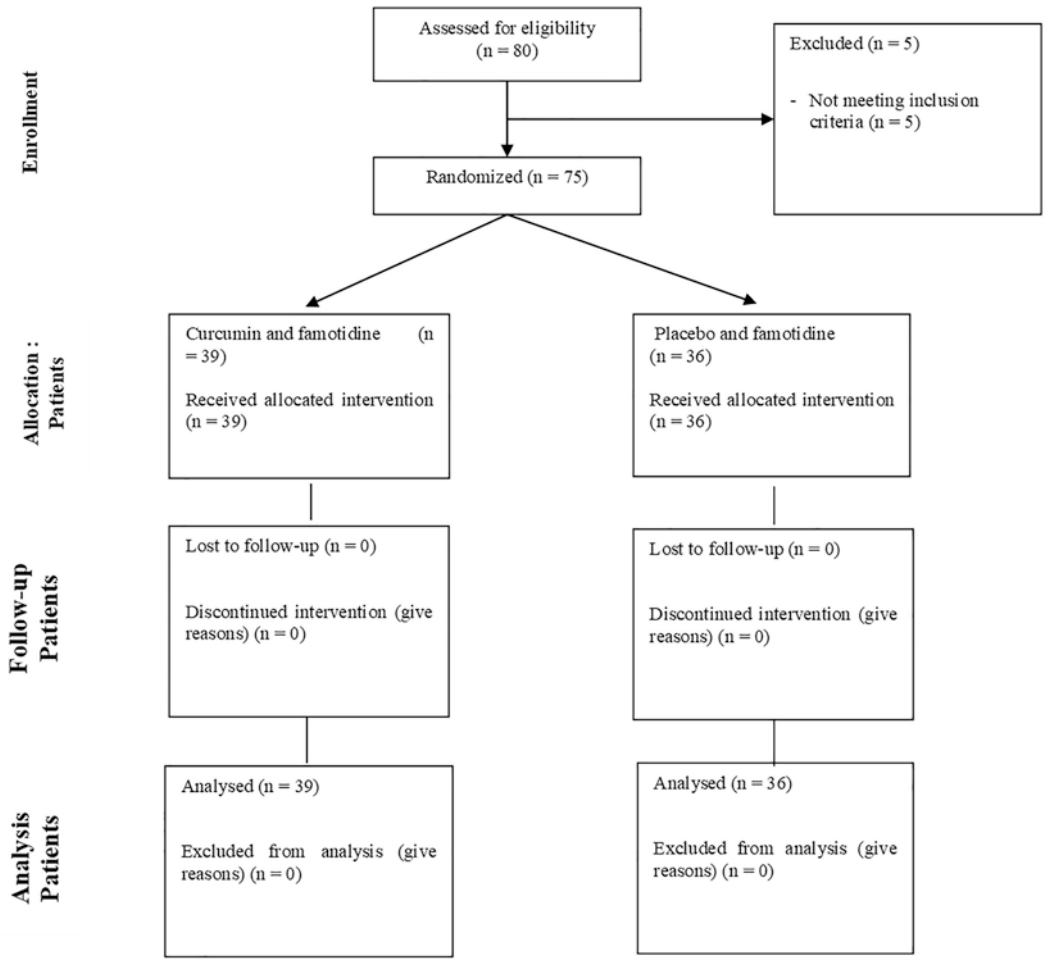


**Fig. 10.1** Patient flow chart

### 10.2.3 Hong Kong Dyspepsia Index

Symptom severity of functional dyspepsia was determined using the Hong Kong dyspepsia index. The questionnaire consists of 12 items including stomach pain, upper abdominal dull pain, stomach pain before meals, stomach pain during anxiety, heartburn, upper abdominal bloating, vomiting, nausea, belching, acid regurgitation, feeling of acidity in the stomach and loss of appetite. Each item was scored from 1 (asymptomatic), 2 (mild symptoms that can be easily ignored), 3 (awareness of symptoms but easily tolerated), 4 (severe symptoms sufficient to interfere with normal daily activities) and 5 (incapacitating symptoms causing inability to perform daily activities and/or require days off work). Subsequently, all the above-mentioned scores were added together (from 12 to 60) to calculate the severity of functional dyspepsia [34].

### 10.2.4 Statistical Analysis

Data was analyzed using SPSS version 18 software. Data were reported as number and percent or median ± standard deviation. For analyzing categorical variables, the Chi-square test was used. Comparisons between groups were performed using independent Student's T-tests. For comparison of quantitative variables prior to treatment, immediately after and 30 days later, repeated measures analysis of variance (ANOVA) was used. P-values <0.05 were considered significant.

## 10.3 Results

Total of 75 eligible subjects completed the study as shown in the patient flow diagram (Fig. 10.1). Baseline characteristics of participants are indicated in Table 10.1. Duration of functional dyspepsia as well as endoscopic findings, were not significantly different in the study groups. As shown in Table 10.2, none of the laboratory indices were different between two groups before or after intervention. The rate of *H. pylori* infection was decreased significantly in the intervention group (p = 0.004) in comparison to placebo, whereas no significant change in *H. pylori* was observed (p = 0.126) (Table 10.3). According to the Hong Kong questionnaire, dyspepsia severity index was decreased significantly in both groups (Table 10.4).

Comparison between different items of Hong Kong questionnaire is summarized in Fig. 10.2. Abdominal pain, belching and acid regurgitation were decreased in both the intervention ($p < 0.001$ for all three symptom scores) and control ($p = 0.033$, $<0.001$ and $0.022$, for the respective symptom scores) groups. Upper abdominal dull pain ($p < 0.001$), stomach pain before meals ($p < 0.001$), stomach pain when anxious ($p < 0.001$), heartburn ($p < 0.001$), upper abdominal bloating ($p < 0.001$), nausea ($p = 0.033$) and feeling of acidity in stomach ($p < 0.001$) were decreased significantly in the curcumin group. Vomiting and loss of appetite were the only symptoms that did not change after treatment in both groups.

**Table 10.1** Baseline characteristics of study groups

| Characteristics | | Case (N = 39) | Controls (N = 36) | P value |
|---|---|---|---|---|
| Age (years) | | 39.9 ± 8.6 | 37.2 ± 9.5 | 0.189 |
| Sex (n%) | *Male* | 19 (48.7%) | 20 (55.6%) | 0.359 |
| | *Female* | 20 (51.3%) | 16 (44.4%) | |
| Functional dyspepsia duration (years) | | 4.21 ± 1.02 | 3.94 ± 0.88 | 0.140 |
| Endoscopic findings | Esophagitis (%) | 4 (10.3%) | 1 (2.8%) | 0.289 |
| | Antral gastritis (%) | 18 (46.2%) | 22 (61.1%) | |
| | Pan gastritis (%) | 7 (17.9%) | 4 (11.1%) | |
| | Gastro-duodenitis (%) | 10 (25.6%) | 9 (25%) | |

Results are expressed as mean ± standard deviation or number (percentage)

**Table 10.2** Changes in laboratory indices before and after intervention

| Laboratory indices | | Case (N = 39) | Controls (N = 36) | P value |
|---|---|---|---|---|
| FBS (mg/dL) | Before intervention | 92.68 ± 14.08 | 94.32 ± 12.1 | 0.260 |
| | After intervention | 93.51 ± 14.02 | 91.45 ± 13.7 | 0.177 |
| Hb (mg/dL) | Before intervention | 13.24 ± 1.04 | 13.09 ± 1.12 | 0.482 |
| | After intervention | 13.78 ± 1.6 | 13.28 ± 1.8 | 0.550 |
| TG (mg/dL) | Before intervention | 124.4 ± 34.1 | 130.7 ± 44.2 | 0.178 |
| | After intervention | 118.3 ± 31.6 | 129.2 ± 38.9 | 0.099 |
| Chol (mg/dL) | Before intervention | 174.9 ± 37.1 | 183.1 ± 39.7 | 0.385 |
| | After intervention | 172.4 ± 31.2 | 183 ± 38.5 | 0.609 |
| LDL (mg/dL) | Before intervention | 116.2 ± 28.2 | 113.2 ± 33.4 | 0.595 |
| | After intervention | 109.3 ± 32.2 | 110.9 ± 28.7 | 0.761 |
| HDL (mg/dL) | Before intervention | 47.31 ± 11.42 | 46.14 ± 9.3 | 0.215 |
| | After intervention | 48.52 ± 10.5 | 46.99 ± 9.6 | 0.195 |
| AST (mg/dL) | Before intervention | 24.12 ± 13.2 | 23.58 ± 14.9 | 0.318 |
| | After intervention | 23.10 ± 11.9 | 23.46 ± 11.6 | 0.450 |
| ALT (mg/dL) | Before intervention | 21.65 ± 12.4 | 22.17 ± 11.3 | 0.351 |
| | After intervention | 21.19 ± 9.5 | 22.31 ± 11.9 | 0.182 |
| ALP (mg/dL) | Before intervention | 149.2 ± 68.3 | 168.9 ± 78.2 | 0.107 |
| | After intervention | 167.3 ± 53.5 | 192.2 ± 63.8 | 0.112 |

**Table 10.3** Rate of positivity of *H. pylori* antigen prior to treatment, immediately after and 1 month later

| *H.pylori* antigen | Case (N = 39) | Control (N = 36) | P value |
|---|---|---|---|
| Before intervention | 29 (69.2%) | 27 (75.0%) | 0.580 |
| After intervention | 20 (51.3%) | 24 (66.7%) | 0.132 |
| One month after intervention | 18 (46.2%) | 23 (63.9%) | 0.095 |
| P-value | 0.004 | 0.126 | – |

**Table 10.4** Changes in severity of dyspepsia prior to treatment, immediately after and 1 month later

| Severity of dyspepsia | Case (N = 39) | Control (N = 36) |
|---|---|---|
| Before intervention | 27.38 ± 2.63 | 28.19 ± 2.14 |
| After intervention | 19.46 ± 2.29 | 24.97 ± 2.29 |
| 1 month after intervention | 15.77 ± 2.56 | 23.94 ± 2.82 |
| P-value | <0.001 | <0.033 |

## 10.4 Discussion

This study was designed to evaluate the efficacy of curcumin supplementation on improving the severity of symptoms in patients with functional dyspepsia. This study indicated that 500 mg of curcumin as adjunct therapy per day could improve the severity of functional dyspepsia according to Hong Kong score over a 30 day period and after 30 days of follow-up. Curcumin administration could efficiently ameliorate upper abdominal dull pain, stomach pain before meals, stomach pain when anxious, heartburn, upper abdominal bloating, nausea and feeling of acidity in the stomach. Furthermore, the current study indicated that curcumin administration could efficiently eradicate the *H. pylori* infection rate.

Curcumin is a natural polyphenol with anti-inflammatory properties comparable with some steroidal and non-steroidal compounds. It modulates inflammation through inhibition of cyclooxygenase-2 (COX-2), lipoxygenase (LOX), inducible nitric oxide synthase (iNOS), production of cytokines such as interleukin (IL)-6, IL-8, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), and through inactivation of transcription factors like nuclear factor kappa B (NF-κB), and activator protein 1 (AP-1) [35]. Experimental studies have supported the concept of curcumin effectiveness in *H. pylori* eradication [36, 37]. *H. pylori* adheres to the gastric epithelium and secretes virulence factors which, in turn, activate the pro-inflammatory pathways

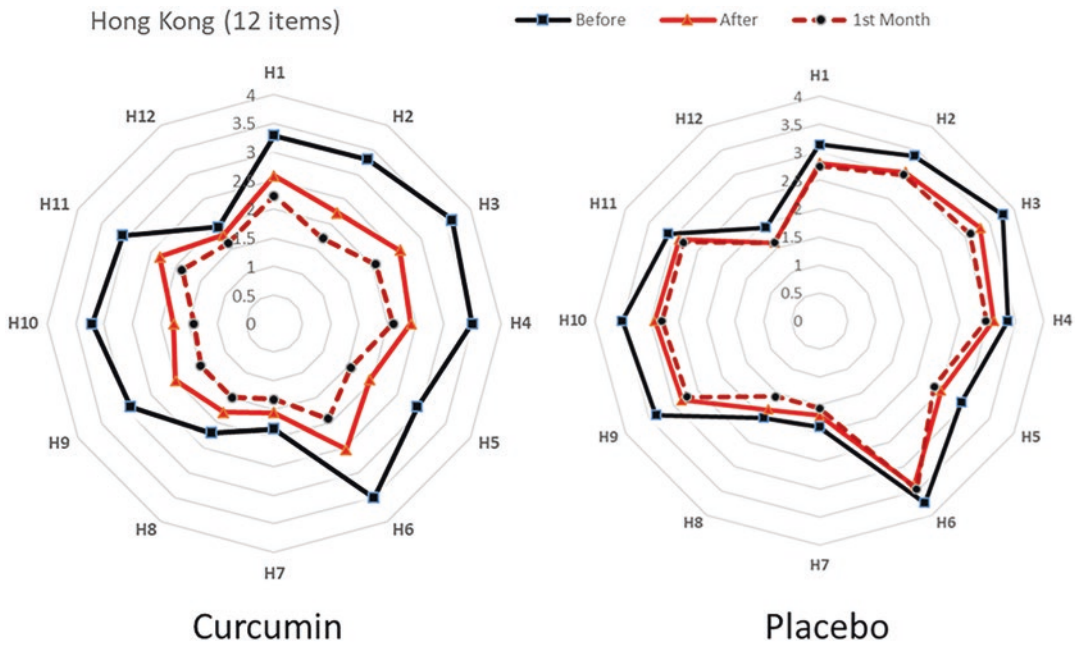


**Fig. 10.2** Comparison between different items of hong kong questionarre in case and control group (H1: abdominal pain, H2: upper abdominal dull pain, H3: stomach pain before meals, H4: stomach pain when anxious, H5: heartburn, H6: upper abdominal bloating, H7: vomiting, H8: nausea, H9: belching, H10: acid regurgitation, H11: feeling of acidity in stomach, H12: loss of appetite)

causing gastric mucosal damage. Curcumin supplementation could significantly downregulate NF-κB and matrix metallopeptidase (MMP)-3 and MMP-9 activities which play roles in *H. pylori* infection [38–40]. Furthermore, an experimental study indicated that curcumin could suppress gastric inflammation caused by *H. pylori*-infection in mouse mucosa [31]. A comprehensive review by Sarkar et al. introduced curcumin as a potential treatment for *H. pylori* and its associated diseases [35]. On the contrary, some studies have found inconsistent results. Di Mario et al. studied 25 *H. pylori*-positive subjects with functional dyspepsia. The patients were treated with a combination of pantoprazol, N-acetylcystein, lactoferrin and 30 mg curcumin two times a day for 7 days. These therapies were not effective for *H. pylori* eradication [22]. In another study, the efficacy of curcumin was evaluated on *H. pylori* eradication in patients with *H. pylori*-positive peptic ulcers through a parallel-group design. Subjects were treated with 500 mg of curcumin daily plus a standard *H. pylori* medication (clarithromycin, amoxicillin, pantoprazole) for four weeks. In contrast with the current study, no significant effect was observed for *H. pylori* eradication [23]. These inconsistencies may be due to differences in curcumin dosage, duration of intervention, combination therapies and comorbidities across the studies. In addition, the source of curcumin also plays a role in its antibacterial activity. As shown by Vetvica et al. only some curcumin preparations have shown a good activity against *H. pylori* infection [40].

In contrast to the conflicting results related to the effects of curcumin on the rate of *H. pylori* infection, to our knowledge all available studies have found that curcumin attenuates the symptoms of dyspepsia. Di Mario et al. reported that curcumin decreased the overall severity of dyspepsia and some symptoms of dyspepsia including stomach pain, post-prandial fullness, bloating, belching and nausea up to 2 months after intervention [22]. Khonche et al. also reported significant alleviation of symptoms including upper abdominal dull pain, stomach pain before meals

and belching following curcuminoid supplementation [23].

## 10.5 Conclusions

The findings of this study revealed that adding curcumin as an adjunct therapy not only improves clinical symptoms of dyspepsia but also eradicates the rate of *H. pylori* infection. Considering the good safety profile of curcumin and its pleiotropic actions, it could be used as an efficacious agent in functional dyspepsia. However, additional larger and long-term investigations are needed to confirm these promising results and the potential role of adjunctive curcumin therapy in the management of functional dyspepsia. Finally, it seems that the presence of *H. pylori* infection is a confounding factor in functional dyspepsia and the efficacy of curcumin on *H. pylori* eradication was not fully established. The impact of the presence *H. pylori* infection on the efficacy of curcumin in ameliorating the symptoms of functional dyspepsia needs to be further scrutinized.

**Conflict of Interest** Muhammed Majeed is the founder of Sabinsa Corp. and Sami Labs Ltd. The other authors declare no competing interests.

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# 11 Possible Mechanisms and Special Clinical Considerations of Curcumin Supplementation in Patients with COVID-19

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**Abstract**

The novel coronavirus outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was recognized in late 2019 in Wuhan, China. Subsequently, the World Health Organization declared coronavirus disease 2019 (COVID-19) as a pandemic on 11 March 2020. The proportion of potentially fatal coronavirus infections may vary by location, age, and underlying risk factors. However, acute respiratory distress syndrome (ARDS) is the most frequent complication and leading cause of death in critically ill patients. Immunomodulatory and anti-inflammatory agents have received great attention as therapeutic strategies against COVID-19. Here, we review potential mechanisms and special clinical considerations of supplementation with curcumin as an anti-inflammatory and antioxidant compound in the setting of COVID-19 clinical research.

**Keywords**

Curcuminoids · Inflammation · COVID-19 · ARDS

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## 11.1 Introduction

The novel coronavirus outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was recognized in late 2019, following announcement of a cluster of pneumonia cases in Wuhan, China [1]. World Health Organization declare coronavirus disease 2019 (COVID-19) as a pandemic on 11 March 2020 [2]. According to published reports, the proportion of potentially fatal infections may vary by location, age, and underlying risk factors [3]. Acute respiratory distress syndrome (ARDS) is the most frequent complication and leading cause of death in severely ill



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patients. Cytokine release syndrome characterized by elevated serum levels of proinflammatory cytokines including interleukin (IL)-6, IL-1β, IL-2, IL-8, IL-17, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), and tumor necrosis factor α (TNF-α) was detected in severe cases of COVID-19 [1, 4, 5]. Unrestrained immune activation and subsequent systemic inflammation result in diffuse alveolar damage and pulmonary capillary endothelial injury that recognized as ARDS [6]. As a consequence, immunomodulatory and anti-inflammatory agents have received great attention in therapeutic strategies for COVID-19[5].

Turmeric, as a precious spice obtained from *Curcuma longa* rhizomes, has a remarkable history in traditional herbal medicine. Curcumin (diferuloylmethane) is a bioactive polyphenolic ingredient of turmeric with manifold pharmacological effects including potent anti-inflammatory and antioxidant properties [7–14]. Studies demonstrated that curcumin shows anti-inflammatory, antioxidant and antineoplastic properties through regulation of cytokines, transcription factors, adhesion molecules and enzymes. Due to such properties, some studies are being conducted to evaluate the probable clinical profit of curcumin for the treatment of COVID-19. In the current study, we focused on probable mechanisms of curcumin against ARDS and acute lung inflammation (ALI) and discussed the special clinical considerations of curcumin supplementation in patients with COVID-19.

## 11.2 COVID-19-Associated ARDS and Related Cytokines

ARDS is a serious lung inflammatory disorder which mortality rate is estimated to be 30–50% [15]. It was described as a syndrome accompanied by inflammation and enhanced permeability of pulmonary capillary endothelial cells followed by fluid leakage into lung parenchyma and activation of inflammatory responses leading to ALI [16]. It was demonstrated that the severity of ARDS directly depends on the magnitude of induced inflammatory responses [17].

Recent studies reported that the cytokine profile of COVID-19 is similar to ARDS and sepsis. Besides, studies confirmed the relevance of elevated levels of inflammatory cytokines with poor prognosis of COVID-19 patients [18]. The inflammatory cytokines that playing key roles in development and progression of ARDS, are introduced here to better understand the relying mechanisms of curcumin in Table 11.1.

**Table 11.1** Role of different mediators playing key roles in ARDS

| Mediators affecting ARDS | Function | References |
|---|---|---|
| TNF-α | Pro-inflammatory cytokine, neutrophil recruitment, activation of ROS generation | [20, 71] |
| IL-1β | Pro-inflammatory, neutrophil activation on ARDS | [17] |
| IL-6 | Pro-inflammatory cytokine, leukocytes recruitment and activation, early biomarkers of lung injury | [72] |
| IL-10 | Anti-inflammatory cytokine, suppresses the release of proinflammatory cytokines | [31] |
| ICAM-1 | Neutrophil adhesion and trafficking to the lung tissue | [73] |
| SP-D | Regulates surfactant hemostasis synthesized in alveolar type II cells and Clara cells of lungs | [38] |
| Reactive oxygen and nitrogen species | Increased endothelial permeability, promoting the migration of PMNs | [17, 26] |
| Chemokines | Activate leukocyte integrins, causing firm adhesion and extravasation | [74] |
| IL-8 | Potent neutrophil attractant and activator | [75] |

*TNF-α* tumor necrosis factor α, *IL-1β* interleukin 1β, *IL-6* interleukin 6, *IL-10* interleukin 10, *ICAM-1* intercellular adhesion molecule 1, *SP-D* surfactant protein D, *IL-8* interleukin 8

TNF-α and IL-1β are among the first proinflammatory cytokines released into the systemic circulation in response to an infectious stimulus. It was demonstrated that TNF-α and IL-1β both activate neutrophils and in sepsis, they are released within 30–90 min from exposure to lipopolysaccharides (LPS) which stimulate the release of the second inflammatory cascade including cytokines, reactive oxygen species (ROS), and upregulation of cell adhesion molecules. Thereupon, inflammatory cells adhere to vascular endothelial cells and migrate into tissues [19]. Moreover, it was shown that TNF-α caused multi-organ damage through the recruitment of neutrophils [20].

The nuclear factor kappa B (NF-**κ**ß) plays a critical modulatory role in the transcription of adhesion molecules, cytokines and other mediators involved in the function of immune system, inflammatory and acute responses, recruitment of leucocytes to extravascular tissues [21]. The NF-**κ**ß is mainly composed of two subunits which are sequestered in the cytoplasm through integration with I-**κ**ß [22]. When activation mediators such as TNF-α or IL-1β are bound to their receptors, I-**κ**ß becomes phosphorylated resulting in activation of NF-**κ**ß. Afterwards, the complex translocate to the nucleus where enhancing the transcription of its target genes. Rather than TNF-α and IL1 β, viral and bacterial products such as double stranded RNA, LPS, and free radicals are potent inducers of NF-**κ**ß [23]. Studies demonstrated that enhanced activation of NF-**κ**ß pathway results in increased cell viability and decreased apoptosis. In fact, enhanced viability of activated neutrophils in the lung tissue of patients with ARDS, leads to more production of ROS and proinflammatory cytokines which might preserve and prolong pulmonary inflammatory process [24]. Actually, neutrophils play a fundamental role in the development and progression of ARDS. Previous studies confirmed that accumulation of alveolar neutrophils correlated with enhanced lung permeability, hypoxemia, and low survival rates. Increased amount of neutrophils in airspaces led to microvascular and lung tissue damage. IL-8 is also a potent neutrophil attractant which plays a significant role in ALI/ARDS [25]. Reactive oxygen and nitrogen species (ROS and RNS, respectively) are produced by lung endothelial cells, alveolar cells and airway epithelial cells, neutrophils, and macrophages which lead to increased endothelial permeability promoting the migration of polymorphonuclear leukocytes (PMNs) and fluid into the alveolar lumen. This process finally stimulates the release of proinflammatory agents, expression of adhesion molecules needed for leukocyte recruitment and neutrophil migration promoting the lung injury [17, 26].

IL-6 is secreted by almost all stromal cells and immune cells such as macrophages, monocytes and T/B lymphocytes [27]. The presence of IL-6 and other inflammatory agents is essential for host defense against infections. However, elevated levels of IL-6 can lead to severe acute systemic inflammatory responses named cytokine release syndrome (CRS) [28]. Studies indicated that sustained release of IL-6 is correlated with serum viral RNA load in COVID-19 patients which is correlated to disease severity [29]. Additionally, high serum levels of IL-6 in acute phase of disease were associated with lung lesions in coronavirus infected patients [30]. As a result, ongoing clinical trials of tocilizumab (a humanized monoclonal antibody against IL-6 receptor) in severe COVID-19 cases are being conducted worldwide. In contrast, IL-10 is an anti-inflammatory cytokine which suppresses the release of proinflammatory cytokines such as TNF-α, IL-1β and IL-6, thereby limiting the damage to the host tissues [31].

Macrophages play a dual role in ALI and ARDS. In the acute phase, resident alveolar macrophages' main phenotype is M2 which shift into M1 phenotype and release potent proinflammatory cytokines such as interferon gamma (IFN-γ), TNF-α, and IL-1β that demonstrate inflammatory effects in the early stage of disease. Besides, blood monocytes are also recruited which finally differentiate to M1 macrophages. In the later stage of ALI/ARDS, macrophages differentiate into M2 phenotype which is regulated through the release of IL-4, IL-10, IL-13, signal transducer and activator of transcription 6 (STAT6), and interferon regulatory factor 4 (IRF4). This

process finally leads to elimination of debris, pathogens and apoptotic cells exhibiting an anti-inflammatory effect [32].

The results of a clinical trial in critically ill patients with ALI/ARDS demonstrated that the levels of inflammatory biomarkers including inflammatory cytokines (IL-6 and IL-8), protein C, surfactant protein D (SP-D), intercellular adhesion molecule 1 (ICAM-1), plasminogen activator inhibitor (PAI), tumor necrosis factor receptor (TNFR), and von Willebrand factor (VWF) were significantly lower in surviving patients when compared to non-survivors. However, among all these, the best prognostic biomarkers were IL-8 which is a potent neutrophil attractant and SP-D [33].

Immune system possesses a complex structure and describing all the details of known mechanisms involved in ARDS pathophysiology is so intricate. The main purpose of this part was to classify inflammatory and anti-inflammatory biomarkers and to briefly describe the role of each cytokine in the process to better understanding the potential pathways that curcumin involves and modulates the immune system responses.

## 11.3 Potential Anti-Inflammatory Mechanisms of curcumin Against ARDS

Studies have indicated that curcumin shows anti-inflammatory, antioxidant and anti-neoplastic activities [34, 35] which are regulated through several molecular targets such as cytokines (e.g. TNF-α, IL-10, IL-6), transcription factors (e.g. NF-κß), enzymes (e.g. matrix metalloproteinases [MMPs]) and adhesion molecules (e.g. ICAM-1) playing key roles in inflammation and carcinogenesis [36]. In this part we are going to study the effects and anti-inflammatory mechanisms of curcumin in the setting of ALI/ARDS resulted by different underlying causes.

Sepsis, severe pneumonia, aspiration, toxic inhalation and trauma are the major underlying conditions leading to ARDS [17]. Xiao et al. prepared rat models of sepsis-induced ALI using cecal ligation puncture (CLP) model. Then, they studied the effect of different doses of curcumin on various cytokines' concentrations and the final survival rate. The results revealed that the use of curcumin downregulated the pro-inflammatory cytokines such as TNF-α and IL-8. Besides, the results showed that the treatment with curcumin led to improvement of the survival rate by 40–50% in CLP induced ALI model [37]. Additionally, it reduced the oxidative stress in the lung tissue through reduction of myeloperoxidase (MPO), malondialdehyde (MDA) and enhancement of superoxide dismutase (SOD) activity. As demonstrated above, ROS react with macromolecules, produce lipid peroxidases and mutate DNA leading to host tissues toxicity. SOD is an anti-oxidant enzyme which scavenges superoxide substrate and studies demonstrated that its concentration is decreased in sepsis induced ARDS.

In a rat model of intestinal ischemia and reperfusion induced ALI, oral treatment with curcumin further confirmed the antioxidant activity of curcumin. The results indicated the reduction of elevated tissue MDA levels, enhancement of SOD and glutathione peroxidase. Also there was a significant reduction in inducible nitric oxide synthase activity and enhanced the expression of SP-D in lung tissue [38]. SP-D plays vital roles in innate host defense of the lung tissue and regulates surfactant homeostasis [39].

Another interesting mechanism was highlighted in a study by chai et al. They showed that curcumin promoted T regulatory (Treg) cells differentiation and enhanced Treg-derived IL-10 in serum and broncho-alveolar lavage fluid (BALF). Enhancement of Treg-derived IL-10 is the main factor affecting macrophage polarization and conversion of M1 macrophages to M2 [40].

Madathilparambil et al. demonstrated that the administration of cyclodextrin-curcumin complex in LPS induced ALI in mice led to reduced pulmonary edema and neutrophil accumulation in BALF and lung tissue. Besides, the proinflammatory transcription factor, NF-κß was decreased causing the reduction of severe inflammation and lung injury [41]. Qingquan et al. showed that the levels of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in rat model of LPS induced

ALI was remarkably increased. They demonstrated that curcumin pretreatment resulted in inhibition of lung CINC-1 expression leading to suppression of neutrophil recruitment and activity in the lung tissue [42].

High-mobility group box 1 (HMGB1) is one of the important inflammatory inducers which is produced by activated monocytes and macrophages. Binding of HMGB1 to receptor for advanced glycation end products (RAGE) stimulates NF-κß signaling pathway, promoting the expression of pro-inflammatory cytokines. Studies indicated that the administration of curcumin to the rat model of LPS-induced ALI led to upregulation of peroxisome proliferator-activated receptor γ (PPAR γ) pathway, further inhibiting the HMGB/RAGE pro-inflammatory pathway [43].

Avasarala and collogues also demonstrated that the use of curcumin affects both pro-inflammatory and anti-inflammatory biomarkers causing a remarkable decrease in development of ARDS and lung injury [44]. They showed that the mechanism was regulated through downregulation of NF-κß and reduction of transforming growth factor beta (TGF-ß) receptor II in virus-induced ARDS resulting in inhibition of inflammatory responses and further lung fibrosis.

Taken together, the precise modulatory mechanisms of curcumin in ARDS has not been defined yet. However, in the current section, we pointed out some of the prominent pathways by which curcumin affects the inflammatory cascade in ARDS.

## 11.4 Curcumin Formulations

Multiple drug delivery systems such as micelles, liposomes, phospholipid complexes, emulsions, micro-emulsions, nano-emulsions, solid lipid nanoparticles, nanostructured lipid carriers, biopolymer nanoparticles, and micro-gels have been formulated to enhance oral absorption, bioavailability, and therapeutic outcomes of curcumin. Compared with unformulated curcumin, significant enhancement of absorption and bioavailability have been obtained with the micellar and micronized formulations (>100-fold) [45–47].

## 11.5 Curcumin Dosage

Although the results from both in-vivo and in-vitro studies on curcumin have been promising, clinical trials in patients with viral pneumonia and/or ARDS have not yet been reported. Therefore, in case of curcumin supplementation in COVID-19 patients with or without ARDS, it should be used in typical doses for other medical conditions ranging from 500–4000 mg per day [7, 48]. However, dose adjustment should be made with respect to bioavailability-enhanced preparations as these formulae may cause systemic concentrations in the order of hundreds to thousands of times higher those obtained with unformulated curcumin.

## 11.6 Common Adverse Effects Between COVID-19 and Curcumin

Previous studies have been investigated the safety and clinical benefits of curcumin in a broad range of diseases including gastrointestinal diseases, rheumatic diseases, pulmonary diseases, diabetes, cardiovascular diseases, liver diseases, pancreatic diseases, neurologic and neurodegenerative diseases, infectious disease and malignancies [7, 8, 49–52]. In accordance with the results of clinical trials, curcumin has a long-established record of tolerability and safety in human studies. An acceptable daily intake (ADI) of 0–3 mg/kg of body weight per day for curcumin was established by JECFA (The Joint United Nations and World Health Organization Expert Committee on Food Additives) and EFSA (European Food Safety Authority) [7, 53]. Although generally well tolerated, curcumin may cause mild nausea, dyspepsia, diarrhea, yellow stool, headache, and rash in some patients [54–56]. Nausea, diarrhea, rash, and headache occur in patients with COVID-19 as well. Therefore, initiation of curcumin supplementa-

tion in patients with COVID-19 may increase the risk or intensity of mentioned side effects. Furthermore, curcumin supplementation causes increased bile formation and demonstrates cholekinetic effects [57, 58]. Moreover, curcumin presents antiplatelet, anticoagulation, and fibrinolysis activities [59, 60]. In addition, curcumin supplementation increases insulin sensitivity and subsequently leads to lower blood glucose levels among diabetic patients who were taking diabetes medications [61, 62]. As a result, curcumin supplementation should be avoided in COVID-19 patients with gallbladder diseases, bleeding disorders, and diabetes.

## 11.7 Clinically Significant Drug Interaction Between Curcumin and Conventional Medications

Natural products including herbal medications and dietary supplements can interact with co-administered conventional medications, potentially lead to unexpected side effects, toxicity, and/or suboptimal therapeutic responses [63, 64]. Also curcumin is a safe natural product, clinically significant drug interactions must be avoided in the setting of clinical trials especially in critically ill patients with polypharmacy. Curcumin supplementation can lead to reduced activities of cytochrome p450 monooxygenase (CYP1A1, 2A6, 1B1, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4), P-glycoprotein (P-gp), organic anion-transporting polypeptide (OATP), glutathione-S-transferase (GST), and uridine dinucleotide phosphate glucuronosyltransferases (UDPG) [65–68]. Clinically important drug interactions of curcumin are summarized in Table 11.2. Although, limited trials have investigated the pharmacokinetic and/or pharmacodynamic interactions between curcumin and conventional medications. We recommend to exclude the COVID-19 patients who take listed medications from curcumin supplementation trials. However, included patients must be investigated and monitored closely due to the lack of adequate and well-designed clinical trials that investigate the pharmacokinetic and pharmacodynamic drug interactions between curcumin and other conventional medications. Furthermore, there is possible drug interactions between curcumin and antiviral agents for the treatment of COVID-19. For example, remdesivir is an adenosine nucleotide prodrug that converted to the pharmacologically active nucleoside triphosphate form (GS-443902) into cells and subsequently intervene in the viral RNA-dependent RNA polymerase action. Remdesivir is metabolized by CYP (2C8, 2D6, and 3A4), OATP1B1/1B3, and P-glycoprotein/ABCB1 enzyme systems [69–71]. So, pharmacokinetic drug interactions are possible in patients who administrated curcumin and COVID-19 specific therapies. Although, clinical data are not available in this area.

## 11.8 Conclusion

Ongoing COVID-19 pandemic is the pressing global health challenge of our time and our information about COVID-19 is being updated almost on a daily basis. Immunomodulatory and anti-inflammatory agents have received great attention during this period of time based on the inflammatory nature of disease. However, one should minimize any unnecessary co-medication in the setting of COVID-19 due to the lack of clinical and/or experimental information and potential risk of toxicity. We suggest not using curcumin supplementation outside of the setting of clinical trials given the lack of clear clinical evidences on the benefit of curcumin in COVID-19 patients. Further clinical investigations should be performed to clarify the role of curcumin supplementation in the setting of COVID-19. It is also unknown if curcumin can help the patients during the initial phase of the disease or might be more effective in later phases to ameliorate complications.

**Competing Interests** none.

**Funding** none.

**Table 11.2** Curcumin-conventional drug interactions

| Concomitant medication | Outcome | Mechanism | References |
|---|---|---|---|
| **Cardiovascular medications** | | | |
| Losartan | Increased $C_{max}$ and total AUC of losartan | Decreased P-gp activity | [77] |
| Talinolol | Decreased $C_{max}$ and total AUC, increased total clearance of talinolol | MRP2 upregulation | [78] |
| Celiprolol | Increased $C_{max}$ and total AUC, decreased clearance of celiprolol | Decreased P-gp level | [79] |
| Rosuvastatin | Increased $C_{max}$ and total AUC of rosuvastatin | Decreased OATP activity | [80] |
| **Anticoagulants** | | | |
| Warfarin | Increased $C_{max}$ and total AUC, decreased clearance of warfarin<br>No effect on pharmacodynamic parameters such as anticoagulation rate and INR | Decreased P-gp activity | [81, 82] |
| Clopidogrel | Increased $C_{max}$ and total AUC, decreased clearance of clopidogrel<br>No effect on pharmacodynamic parameters such as platelet aggregation and in-vivo bleeding time. | Decreased P-gp activity | [81–83] |
| **Antibiotics** | | | |
| Norfloxacin | Increased total AUC and absorption rate constant, decreased overall elimination rate constant of norfloxacin. | Decreased UDPG levels<br>Decreased CYP3A4 activity<br>Decreased P-gp activity | [84] |
| **Antihistamines** | | | |
| Loratadine | Increased $C_{max}$ and total AUC of loratadine | Decreased CYP3A4 activity<br>Decreased P-gp activity | [85] |
| **Antineoplastic agents** | | | |
| Paclitaxel | Increased total AUC and bioavailability of paclitaxel | Decreased P-gp level<br>Decreased CYP3A2 level | [86] |
| Docetaxel | Increased $C_{max}$, total AUC, half-life, and bioavailability, decreased clearance of docetaxel | Decreased OATP1B1 and OATP1B3 activity | [87, 88] |
| Etoposide | Increased $C_{max}$, total AUC, and bioavailability of etoposide | Decreased CYP3A4 activity<br>Decreased P-gp activity | [89] |
| Tamoxifen | Increased $C_{max}$ and total AUC of tamoxifen | Decreased CYP3A4 activity<br>Decreased P-gp activity | [90] |
| Everolimus | Decreased $C_{max}$ and AUC of everolimus | Increased CYP3A4 activity<br>Decreased P-gp activity | [91] |
| Phospho-sulindac | Increased $C_{max}$ and total AUC of phospho-sulindac | Decreased P-gp activity | [92] |

$C_{max}$ maximum serum concentration, *AUC* area under the curve, *P-gp* P-glycoprotein, *MRP2* multi-drug resistance protein 2, *OATP* organic anion-transporting polypeptide, *UDPG* uridine dinucleotide phosphate glucuronosyltransferases, *CYP* cytochrome p450 monooxygenase

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# 12 Paving the Road Toward Exploiting the Therapeutic Effects of Ginsenosides: An Emphasis on Autophagy and Endoplasmic Reticulum Stress

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**Abstract**

Programmed cell death processes such as apoptosis and autophagy strongly contribute to the onset and progression of cancer. Along with these lines, modulation of cell death mechanisms to combat cancer cells and elimination of resistance to apoptosis is of great interest. It appears that modulation of autophagy and endoplasmic reticulum (ER) stress with specific agents would be beneficial in the treatment of several disorders. Interestingly, it has been suggested that herbal natural products may be suitable candidates for the modulation of these processes due to few side

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effects and significant therapeutic potential. Ginsenosides are derivatives of ginseng and exert modulatory effects on the molecular mechanisms associated with autophagy and ER stress. Ginsenosides act as smart phytochemicals that confer their effects by up-regulating ATG proteins and converting LC3-I to -II, which results in maturation of autophagosomes. Not only do ginsenosides promote autophagy but they also possess protective and therapeutic properties due to their capacity to modulate ER stress and up- and down-regulate and/or dephosphorylate UPR transducers such as IRE1, PERK, and ATF6. Thus, it would appear that ginsenosides are promising agents to potentially restore tissue malfunction and possibly eliminate cancer.

**Keywords**

Ginsenoside · Endoplasmic reticulum stress · Autophagy · Apoptosis · Cancer therapy

## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| AMPK | AMP-activated protein kinase |
| AS | atherosclerosis |
| ASK1 | apoptosis signal-regulating kinase-1 |
| ATF-6 | activating transcription factor-6 |
| ATG | autophagy-related gene |
| Aβ | amyloid-β |
| BAX | Bcl2-associated x protein |
| Beclin1 | Bcl2-interacting protein 1 |
| CHOP | C/EBP homologous protein |
| CMA | chaperone-mediated autophagy |
| DAPK1 | death-associated protein kinase 1 |
| DDIT3 | DNA damage-inducible transcript 3 |
| DM | diabetes mellitus |
| DR5 | death receptor 5 |
| eIF2α | eukaryotic translocation factor 2α |
| ER | endoplasmic reticulum |
| ERAD | ER-associated degradation |
| ERK | extracellular signal-regulated kinase |
| FDA | Food and Drug Administration |
| GFB | glomerular filtration barrier |
| GSK-3β | glycogen synthase kinase-3β |
| I/R | ischemic/reperfusion |
| IL | interleukin |
| IRE1 | inositol-requiring enzyme-1 |
| JNK | c-Jun N-terminal kinase |
| LC3 | light chain-3 |
| LPS | lipopolysaccharide |
| miR | microRNA |
| mTOR | mechanistic target of rapamycin |
| NAFLD | non-alcoholic fatty liver disease |
| NDs | neurological disorders |
| NLRP3 | nucleotide-binding domain and leucine-rich repeat containing protein 3 |
| Nrf2 | nuclear factor erythroid 2-related factor 2 |
| PD | Parkinson's disease |
| PERK | PRK-like ER kinase |
| PI3K | phosphoinositide 3-kinase |
| SCI | spinal cord injury |
| SGLT1 | sodium-dependent glucose co-transporter 1 |
| SHP | small heterodimer protein |
| Sirt1 | sirtuin1 |
| t-BHP | tert-Butyl hydroperoxide |
| TRAF2 | tumor necrosis factor receptor-associated factor 2 |
| TRAIL | tumor necrosis factor-related apoptosis-inducing ligand |
| ULK1/2 | unc51-like autophagy activating kinase 1/2 |
| UPR | unfolded protein response |
| XBP1 | X box-binding protein 1 |

## 12.1 Introduction

Using herbal-based pharmacologic agents to treat a variety of diseases is gaining more recognition [1, 2]. These biologically-active agents can often produce effective therapeutic outcomes with fewer side effects [3–5]. Ginseng is a valuable plant, which has traditionally been used to treat many complications of various diseases. This medicinal plant has been used for its therapeutic effects and as a dietary supplement. There is ample evidence suggesting that it has protective effects in various pathophysiologic processes and improves neurological disorders [6], complications from renal disease [7], cardiovascular disor-

ders [8], hepatic diseases [9], metabolic disorders, and immune system dysfunction [10]. However, the exact molecular mechanisms associated with its beneficial effects are not completely understood.

Autophagy and endoplasmic reticulum (ER) stress are two primary mechanisms involved in many cellular events, as well as the numerous complications arising from abnormal cellular function. In recent decades, studies have focused on clarifying the molecular mechanisms involved in autophagy-dependent cellular death and its disturbance in a number of diseases [11, 12]. The long-term goal is to control a disease by re-adjusting cell survival and restoring cellular homeostasis toward a normal physiologic state using herbal-based products [13–15]. ER stress is closely associated with a number of disorders such as arthritis [16]. Therefore, identifying biomolecules derived from natural sources that promote normal cell survival and repair, if damaged, is of great interest to the scientific and medical communities [17]. As mentioned above, natural, herbal products continue to be investigated and used as alternative therapeutic agents for various health problems, including cancer and diabetes mellitus, due to their low toxicity and infrequent side-effects [3, 15, 18]. Consequently, in this review, we discuss the major interactions between ginsenosides and the molecular pathways and signaling cascades involved with autophagy and ER stress.

"Regulated" or "accidental" are phrases that describe various forms of cell death [19]. Commonly, there are three main types of cellular death; namely, apoptosis (type I), autophagy (type II), and necrosis (type III) [20, 21]. It must be noted that cell death is crucial for preserving tissue homeostasis, and its balance with cell survival produces a physiological healthy condition in both eukaryotes and prokaryotes [20, 21].

Apoptosis is a programmed cell death, which is induced by various physicochemical changes that result in cellular stress such as acidic conditions, DNA damage, sustained cell starvation, and interaction with certain growth factors and pro-inflammatory cytokines [13, 22, 23]. Membrane blubbing, cell shrinking, chromatin condensation, and formation of apoptotic bodies represent the main hallmarks of apoptosis [24, 25]. Another programmed mode of cell death includes autophagy, which is a catabolic process that is stimulated under conditions of cellular stress (*e.g.*, cell starvation) and leads to the degradation of damaged or aged organelles and molecules [26]. Necrosis is the third form of cell death, which is an accidental process and characterized by cell degeneration and the appearance of a 'cloudy' swelling [27].

## 12.2 Molecular Mechanisms Associated with Autophagy

Autophagy, as type II programmed cell death, is a self-digestion cellular mechanism, which has been evolutionary conserved [28, 29]. This process is responsible for recycling and degradation of cellular compartments and damaged organelles, as well as denatured proteins, and results in the enhancement of cell survival under acute conditions [30, 31]. As mentioned above, autophagy is another type of programmed cell death that has several differences with apoptosis and plays remarkable role in preserving cell homeostasis, cell growth, and self-renewal [21]. Based on the targets and the delivery method of the 'cargo', autophagy can be categorized into three main forms, including microautophagy, macroautophagy (autophagy), and chaperone-mediated autophagy (CMA) [3]. Microautophagy is related to the invagination of cytoplasmic materials (cargo) into the lysosome membrane [32]. In CMA, chaperones mediate the transportation of proteins towards the lysosome [33]. Lastly, autophagosomes are responsible for the translocation of cellular material (cargo) to the lysosomes during macroautophagy [34]. In the remaining portion of this review, we discuss macroautophagy (autophagy).

During the initial stage of autophagy, the formation of a double-membraned vesicle around the cargo occurs, which is referred to as an "autophagosome" and is located between the ER and mitochondria [34]. Subsequently, elongation, autophagosome maturation, fusion with the lyso-

some, and degradation occur in this order [21]. A number of signaling pathways are involved in the regulation of autophagy [34, 35]. Under stressful cellular conditions, such as cell starvation, ULK1/2 (unc51-like autophagy activating kinase 1/2) complex promotes the process of autophagy [36]. When cellular energy is sufficient, mTORC1 (mechanistic target of rapamycin complex-1) phosphorylates and inactivates the ULK1/2 complex [25]. Under conditions of cell starvation, AMPK (AMP-activated protein kinase) is activated and, in turn, it inactivates mTORC1, resulting in activation of the ULK1/2 complex [34]. ULK1/2 activation is accompanied with increased levels of autophagosome formation [34]. Subsequently, ULK1/2 targets and phosphorylates ATG13 (Autophagy-related protein 13), FIP2000 (a family integrating protein), and Bcl-2-interacting protein 1 (Beclin1) to form a phagophore [34, 37]. The group of genes known as autophagy-related genes (ATGs) play an active role in the expansion of phagophore and autophagosome formation [37]. Beclin-1 also plays a significant role in the stimulation of ATG5/ATG7-independent autophagy [37, 38]. Furthermore, ATG12, ATG5, and ATG16, along with the light chain-3 (LC3)-II, promote autophagosome maturation [38, 39]. The final step is the fusion of the autophagosome with the lysosome and degradation of cargo within the lysosome [21, 38]. Importantly, the level of LC3II/I is a key indicator of autophagosome formation [21].

## 12.3 Endoplasmic Reticulum Stress

ER is a double-membraned organelle that is involved in the synthesis and export of lipids, proteins, and carbohydrates, and is also responsible for the processing and maturation of biomolecules *via* folding, glycosylation, and disulfide bond formation [40]. This vital organelle shuttles biomolecules to their correct destination to preserve cellular homeostasis [41]. ER-resident chaperones, including GRP78/BiP and GRP94, play a significant role in protein folding [42]. Quality control mechanisms within the ER guarantee that proper folding of proteins occurs before they are translocated to their intracellular or extracellular destinations. Therefore, it is crucial that ER homeostasis be maintained. It has been demonstrated that impairment in ER homeostasis is associated with the aggregation of unfolded or misfolded proteins in the ER lumen, resulting in a condition called “ER stress” [43].

During ER stress, the unfolded protein response (UPR) is activated in an effort to maintain cellular homeostasis [40]. The primary goal of the UPR is to induce ER homeostasis by down-regulation of global proteins, up-regulation of proteins involved in folding, enhancing the level of chaperones, and increasing the extent of degradation of misfolded or unfolded proteins *via* the activity of proteasomes and the process of autophagy [40]. The UPR has three major mechanisms, which include adaptation, alarming, and pro-apoptosis [44]. It has been reported that during prolonged ER stress, which exceeds the capacity of the UPR, the cell activates a number of intracellular signaling pathways that may lead to cell suicide through apoptosis [45]. In fact, under physiological conditions, the UPR mainly contributes to the folding of proteins and also improves the activity of chaperones. However, the UPR has a certain capacity or limit in protein folding, and if the loading of unfolded proteins is greater than the capacity of the UPR, it leads to the induction of ER stress. Subsequently, ER stress stimulates autophagy to diminish the load of unfolded proteins so as to cause their degradation. The failure of autophagy to restore the homeostasis of the ER results in induction of apoptotic cell death [46, 221].

Three distinct transmembrane signal transducers are involved in the stimulation of the UPR including inositol-requiring enzyme (IRE) 1, activating transcription factor (ATF)-6, and PRK-like ER kinase (PERK) [40]. Under normal cellular conditions, these transducers exist in an inactive form *via* binding of their luminal domains to BiP [40]. However, during ER stress, Bip is disassociated from the transducers and leads to the transducer’s oligomerization and activation [40]. IRE1 has endoribonuclease activity and primarily affects mRNA [40]. The phos-

phorylated form of IRE1 accelerates the splicing of mRNA of X box-binding protein 1 (XBP1) [40]. It has been shown that spliced XBP1, known as XBP1s, translocates to the nucleus, where it enhances the expression of a variety of ER chaperones that assist with ER homeostasis [40]. This process is called 'adaptation'. IRE1 also has another vital role that leads to 'alarming' *via* a pro-inflammatory response, and it is this response that alarms the extracellular environment about the stressful conditions within the cell [40, 44]. Only IRE1 possesses the alarming function among the various UPR transducers [40].

PERK primarily targets eukaryotic translation factor-2α (elF2α) [47]. In turn, elF2α decreases the accumulation of misfolded and/or unfolded proteins by two main pathways, which include (1) inhibition of global mRNA translation, and (2) upregulation of ATF4 [44]. ATF4 positively affects the expression of genes, which are associated with protein folding, autophagy, apoptosis, and cellular homeostasis. For instance, DNA damage-inducible transcript 3 (DDIT3) and C/EBP homologous protein (CHOP) are two major targets of ATF4. Multiple studies have determined the antioxidant sensing potential of PERK [44, 48, 49].

The third transducer is ATF6. It has been reported that ATF6 is cleaved by proteases within the Golgi apparatus. Subsequently, cleaved ATF6 is transferred to the nucleus where it stimulates, and consequently increases the transcription of different chaperone proteins such as BiP and calreticulin [50]. It is noteworthy that XBP1 is a key target of ATF6 [44, 51].

## 12.4 Ginsenosides

Ginseng is a valuable Chinese herb that has been extensively used in the treatment of various diseases and for the improvement of several pathophysiological conditions [52] such as cancer [53], fatigue, infection, and inadequate immune response [54]. Panax ginseng Meyer (Korean red ginseng), Panax quinquifolium (American ginseng), and Panax notoginseng (Burk) F.H Chen (notoginseng) are important commercial forms of ginsengs that have been extensively used as a traditional herbal remedies [55]. To date, various therapeutic strategies have investigated to treat neurological diseases [56] and bone repair [57]. However, ginsenosides, as the active ingredients of ginseng, have also been shown to possess neuroprotective [58] and osteogenic potential [59]. Additionally, the ginsenosides exhibit therapeutic anti-inflammatory [54], anti-oxidant [60], anti-tumor [53, 61], immunomodulatory [62], cardiovascular [63], anti-stress [64], hepatoprotective [65], anti-obesity [66], and antimicrobial [67] properties.

More than 100 ginsenosides have been recognized as either the predominant or major ginsenosides (due to their high content in ginseng) such as Rb1, Rb2, Rc, Rd., Re, Rg1, or the minor ginsenosides (due to their lower content in ginseng) such as Rg3 Rh1, F2, C-k, Rg2, Rh1, Rg5, and F1 [68, 69]. Experimental evidence has demonstrated various pharmacologic benefits of these isoforms [70, 71]. Fan et al. in 2019 investigated the effects of ginsenoside-Rg1 on insulin resistance in a HepG2 cell line [71]. Their results showed that ginsenoside-Rg1 enhanced the uptake of glucose in human liver cells by reducing the production of reactive oxygen species, as well as eliciting a decrease in the phosphorylation of P38 MAPK [71]. Moreover, ginsenoside-Rg3 prevented lung injury in diabetic rats by exerting anti-inflammatory effects and stimulating the PI3K and MAPK signaling pathways [70]. Li et al. demonstrated that ginsenoside-Rg1 decreases hypoxia/reoxygenation injury in cardiomyocytes by suppressing apoptosis and free radical production, activating caspase-3, and maintaining the membrane integrity of mitochondria [73]. Although minor ginsenosides have higher biological activity compared to the major forms, the quantity of natural minor ginsenosides contained in ginseng are rather low [74]. Minor ginsenosides are extracted from the major ginsenosides using hydrolysis [74]. It must be noted that the bioavailability of ginsenosides following oral ingestion are significantly low [75].

Gut microbiota, or the microbe population residing in the intestine, is responsible for the metabolism of ginsenosides and accelerates their

absorption across the epithelial membrane, as well as the conversion of major ginsenosides to minor ones [76]. However, the density of the gut microbiota and the pH of the intestinal milieu influence the metabolism of ginsenosides [77]. The translocation of ginsenosides across the gastrointestinal membrane is energy-dependent [78]. Although it is still not known which transporter is engaged in the process of ginsenoside transport, it has been suggested that the sodium-dependent glucose co-transporter 1 (SGLT1) may be involved [78]. Gut microbiota plays a significant role in the biotransformation of compounds [2] and, in the case of ginsenosides, intestinal microflora produce ginsenoside compound K from protopanaxadiol (PPD)-type ginsenosides (Fig. 12.1) [79].

However, there are a number of adverse effects associated with the use of ginseng and its derivatives, which include diarrhea, insomnia, headache, rapid heartbeat, alteration in blood pressure, and breast tenderness, as well as vaginal bleeding. To date, the use of ginseng and its derivatives have been confined to the treatment of diabetes. However, it is suggested that their beneficial effects be explored for the treatment of other pathological conditions such as cancer and kidney disorders.

## 12.5 Induction of Autophagy and Apoptosis via ER Stress

It has been well confirmed that ER stress and autophagy are involved in the elimination of extra unfolded or misfolded proteins [40, 80]. To further explain this relationship, elF2α must be considered, because elF2α stimulates autophagy in a number of diseases [41]. Moreover, PERK-induced ATF4 and DDIT3 stimulate autophagy gene transcription including ATG5, ATG1, and ATG12 [81]. ATF4 is involved in the up-regulation of DDIT4 and CHOP [80]. DDIT4 suppresses the mTORC1 signaling pathway, which leads to activation of autophagy. Additionally, PERK is related to the stimulation of nuclear factor erythroid 2-related factor-2 (Nrf2), which results in up-regulation of genes associated with oxidative stress [82]. Among the UPR modulators, CHOP has a remarkable role in the induction of apoptosis during sustained ER stress. CHOP initiates the release of calcium into the mitochondria, which subsequently leads to apoptosis. Briefly, CHOP stimulates Bim and death receptor 5 (DR5) and induces the down-regulation of Bcl-2 anti-apoptotic protein [83].

ER-associated degradation (ERAD) is another mechanism that is activated during ER stress [80, 84]. ERAD and autophagy cooperate to eliminate

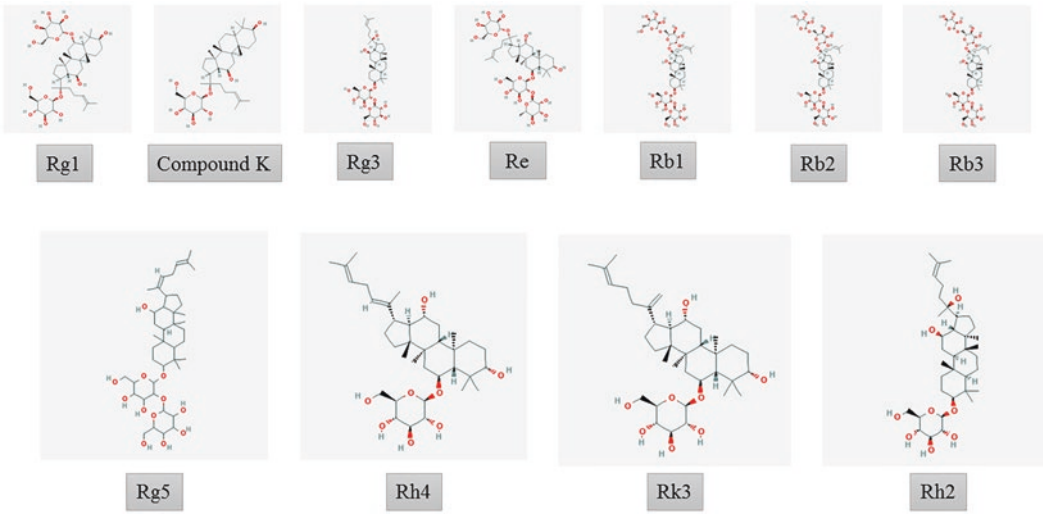


**Fig. 12.1** The chemical structure of some of the ginenosides

extra unfolded or misfolded proteins [85]. Also, ER stress enhances the expression of death-associated protein kinase 1 (DAPK1) which, in turn, stimulates autophagy [86]. Evidence has suggested that ER stress is an inducer of autophagy during moderately stressful cellular conditions [44]. IRE-1 (serine/threonine-protein kinase/endoribonuclease inositol-requiring enzyme-1) indirectly triggers apoptosis by forming a link with TRAF2 (tumor necrosis factor receptor-associated factor 2) and ASK1 (apoptosis signal-regulating kinase-1), which results in the activation of caspase-12 [87, 88]. Furthermore, Bcl-associated x protein (Bax) and Bcl2-antagonist/killer 1 (Bcl-2) modulate IRE1 signaling pathways [44].

## 12.6 Ginsenoside and Autophagy

### 12.6.1 Protective Effects of Ginsenosides

Statistics demonstrate the high prevalence of renal disorders around the world [89]. Notably, podocytes play a significant role in glomerular injury and generation of renal disorders. It has been demonstrated that podocyte injury is associated with the development of a number of renal disorders due to their remarkable role in the glomerular filtration barrier (GFB) [90]. Excessive autophagy negatively affects podocytes and leads to renal injury [90]. Ginsenoside Rg1 effectively down-regulates Beclin-1 and autophagosome formation by inhibition of AMPK and subsequently, activation of mTOR. These effects suppress autophagy to prevent damage in podocytes [91]. It appears that aldosterone trigger podocyte injury by the induction of autophagy. Again, Rg1 suppresses the adverse effects of aldosterone on podocytes by inhibition of autophagy *via* down-regulation of Beclin-1 and LC3-II [92]. Although chemotherapy is still one of the common treatment modalities for cancer, there are a number of harmful effects as they relate to the kidney. For example, as a negative modulator of mTOR, AMPK phosphorylation undergoes inhibition during cisplatin therapy. Consequently, mTOR upregulation leads to the inhibition of autophagy and adverse effects on the kidney [93].

On the other hand, autophagy adversely influences cells during ischemic/reperfusion (I/R) injury. Hence, pharmacological targeting of autophagy is beneficial with regard to the prevention of harmful effects of some drugs. Ginsenoside Rg1 reduces neuron cell death by inhibition of autophagy *via* down-regulation of LC3-II and upregulation of p62. It appears that ginsenoside Rg1 affects two major signaling pathways. Rg1 administration remarkably decreases the expression of the PI3K/Akt/mTOR and PI3K/Beclin-1/Bcl-2 signaling pathways, leading to the inhibition of autophagy [94]. Furthermore, ginsenoside Rg1 inhibits AMPK phosphorylation to stimulate mTOR, which leads to a decrease in autophagy-mediated cell death and protection of cardiomyocytes against I/R injury [95]. Ginsenosides are able to suppress autophagy and attenuate I/R injury by down-regulation of Beclin-1 and upregulation of the PI3K/Akt/mTOR signaling pathway [96–99].

A number of studies have investigated the neuroprotective effects of ginsenosides. In the present review, we discuss how ginsenosides affect autophagy to exert their neuroprotective properties. Exposure to toxic chemicals is associated with neurotoxicity and tert-butyl hydroperoxide (t-BHP) is an example of a chemical that results in neurotoxicity [100]. It is believed that autophagy induction by t-BHP facilitates its adverse effects [101]. Ginsenoside Rg1 supplementation remarkably decreases autophagy by inhibition of LC3-II and Beclin-1, leading to protection against t-BHP-mediated neurotoxicity [102]. The same result occurs with ginsenoside Rb1, and it has been shown that this compound effectively suppresses glutamate-mediated neurotoxicity by inhibiting autophagy *via* down-regulation of Beclin-1 [103]. Following spinal cord injury (SCI), an increase in autophagy occurs, which often leads to the deterioration of the specific neurological condition. Hence, studies have focused on inhibition of autophagy during SCI [104, 105]. Ginsenoside Rb1 exerts protective effects after SCI by reducing autophagy. It appears that ginsenoside Rb1 enhances the expression of p62, while it dimin-

ishes autophagosome formation to suppress autophagy [70]. However, it is noteworthy that stimulation of autophagy is suggested to be beneficial in the treatment of some neurological disorders (NDs). For instance, high aggregation of amyloid-β (Aβ) predisposes an individual to Alzheimer's disease (AD) [106] and the administration of ginsenoside compound K, as a potential inducer of autophagy, is associated with enhanced elimination of Aβ and a decrease in AD symptoms [107]. Importantly, it has also been reported that impairment in autophagy triggers prion disease, a lethal neurodegenerative condition [108]. Pharmacological induction of autophagy is of importance in the treatment of prion disease. Ginsenoside Rg3 restores the autophagic flux that, in turn, suppresses apoptotic cell death in neurons by inhibition of mitochondrial dysfunction [109].

Accumulating data has demonstrated that autophagy primarily functions as a cell survival mechanism and protects cardiomyocytes against oxidative damage and apoptotic cell death [110–112]. As it was previously mentioned, autophagy undergoes upregulation under a physiological stressful condition like starvation. It appears that potentiating autophagy is beneficial in the inhibition of apoptotic cell death in cardiomyocytes. Ginsenoside Rg1 exerts protective effects on H9C2 cells during starvation by enhancing autophagy *via* induction of Bcl-2-Beclin-1 complex dissociation [113]. With respect to the potential role of autophagy in the inhibition of oxidative stress and subsequent apoptosis, its modulation can be advantageous in the treatment of cardiovascular disorders. Ginsenoside Rg3 is able to ameliorate oxidative damage by targeting mitochondria, as well as targeting the AMPK/mTOR signaling pathway. This effect leads to the upregulation of mitochondrial autophagy by enhancing the expression of ATG7 and Beclin-1. Consequently, attenuation of oxidative stress restores mitochondrial function and suppresses apoptotic cell death [114]. Atherosclerosis (AS) is well-documented to be a dangerous cardiovascular disorder that can lead to heart disease (*e.g.*, myocardial infarction) and stroke [115]. Based on the critical role of autophagy in lipid metabolism, it has been reported that impairment of autophagy in macrophages is associated with the accumulation of lipids in atherosclerotic plaques [116]. Ginsenoside Rb1 significantly enhances LC3-II and p62 expressions to induce autophagy. Stimulation of autophagy restores lipid metabolism in macrophages, leading to the stabilization of atherosclerotic plaques [117]. Autophagy induction not only improves lipid metabolism, but also inhibits apoptotic cell death during AS [118]. However, excessive stimulation of autophagy negatively affects the viability of cardiomyocytes. In order to minimize the cardiotoxicity caused by autophagy induction, ginsenosides exert inhibitory effects on autophagy through inhibition of the PI3K/mTOR signaling pathway and relevant genes controlling autophagy (Table 12.1, Fig. 12.2) [119].

### 12.6.2 Anti-tumor Effects of Ginsenosides

Autophagy functions as an anti-tumor process to significantly reduce the viability and proliferation of tumor cells. Ginsenosides are potential modulators of autophagy in cancer therapy. The MAPK signaling pathway family has three major subsets including p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK) [120]. It appears that elevated concentrations of ROS stimulates MAPK [121]. This important signaling pathway plays a significant role in cancer malignancy [122, 123]. Data acquired to date has shown that the MAPK pathway has a connection with autophagy and apoptosis in cancer cells [6]. In addition, the MAPK pathway can regulate the cell cycle [124, 125]. Ginsenoside Rg5 is capable of suppressing the invasion and malignancy of gastric cancer cells. Specifically, ginsenoside Rg5 stimulates the generation of ROS to activate the MAPK signaling pathway. MAPK activation by Rg5 leads to an increase in the expression of LC3B-II, Beclin-1, ATG5, and ATG12, and a decrease in the expression of p62. Consequently, the induction of autophagy triggers apoptotic cell death and G2/M cell cycle arrest [126]. The same mechanism is elicited by ginsenoside Rh4, which elevates the concentra-

**Table 12.1** Protective effects of ginsenosides mediated by autophagy modulation

| Drug | In vitro/ In vivo | Cell line/animal model | Major outcomes | References |
|---|---|---|---|---|
| Ginsenoside Rg1 | In vitro | Podocytes | Inhibition of autophagy suppresses the harmful effects of angiotensin II on podocytes and development of renal disorders | [91] |
| | In vitro | Podocytes | Down-regulation of autophagy is associated with decreased toxic effects of aldesterone of podocytes | [92] |
| | In vitro | Cardiomyocytes | Stimulation of PI3K/Akt/mTOR signaling pathway attenuates autophagy and protects against I/R injury | [99] |
| | In vitro | PC12 cells | Administration of Rg1 reduced autophagy injury upon I/R | [94] |
| | In vitro | H9c2 cardiomyocytes | Inhibition of autophagy alleviates cardiac injury | [95] |
| | In vitro | Mouse microglial BV2 cells | Inhibition of autophagy protects neurons against cell death | [102] |
| | In vitro | H9c2 cells | Protection against starvation by dissociation of Bcl-2-Beclin1 complex | [113] |
| | In vivo | Mice | Down-regulation of autophagy and ER stress decreases adverse impacts of doxorubicin on heart | [190] |
| | In vitro | Lung epithelial cells | Inhibition of LPS-induced apoptosis by induction of autophagy | [191] |
| | In vitro | Serum deprivation macrophages | Upregulation of autophagy by activation of AMPK/mTOR signaling pathway and subsequently, decreasing apoptosis | [129] |
| | In vivo | Mice | Inhibition of autophagy by down-regulation of LC3-II and p62, leading to the protection against sepsis-associated encephalopathy | [192] |
| Ginsenoside compound K | In vitro | Primary astrocytes | Induction of autophagy enhances Aβ clearance and inhibits AD | [107] |
| Ginsenoside Rg3 | In vitro | Primary neurons and human neuroblastoma cell line SK-N-SH cells | Stimulation of autophagy flux inhibits apoptosis in neurons by suppressing mitochondrial dysfunction | [193] |
| | In vivo | Sprague-Dawley rats | Suppressing oxidative damage by induction of mitochondrial autophagy | [114] |
| | In vitro In vivo | LPS-induced sepsis hepatocytes Sepsis model | Inhibition of sepsis-mediated injury and improving mitochondrial dysfunction by autophagy induction through AMPK activation | [194] |
| Ginsenoside Re | In vitro | H9c2 cardiac muscle cells | Increasing the survival of cardiac cells by inhibition of autophagy | [195] |
| | In vitro | Human CD4+ T cells | Suppressing IFN-γ-dependent autophagy induction and improving T cell viability | [196] |
| Ginsenoside Rb1 | In vivo | Animal model of I/R injury | Restoring the elevation of Beclin-1 and LC3 decreased autophagy injury | [96] |
| | In vitro | Cardiomyocytes | Amelioration of autophagy diminishes cardiac injury | [97] |
| | In vitro | SH-SY5Y cells | Protection against neuron cell death by induction of PI3K/Akt pathway and subsequently, inhibition of autophagy | [98] |
| | In vitro | Cortical neurons | Alleviation of glutamate-induced neurotoxicity by inhibition of autophagy | [103] |
| | In vivo | Animal model of SCI | Down-regulation of autophagy is associated with attenuation of SCI injury | [70] |

(continued)

**Table 12.1** (continued)

| Drug | In vitro/ In vivo | Cell line/animal model | Major outcomes | References |
|---|---|---|---|---|
| | In vitro In vivo | Primary peritoneal macrophages AS model | Improving atherosclerotic plaque by induction of autophagy flux and promoting lipid metabolism | [117] |
| | In vivo | Apo E−/− mice | Inhibition of apoptotic cell death by elevating autophagy | [118] |
| | In vivo | Pressure-overload heart failure rat model | Inhibition of autophagy by down-regulation of PI3K/mTOR improves cardiac function | [119] |
| | In vitro | H9c2 cells | Inhibition of doxorubicin-mediated autophagy promotes cell viability | [197] |
| Ginsenoside Rb2 | In vitro In vivo | HepG2 cells Male db/db mice | Stimulation of Sirt1 and AMPK activates autophagy, resulting in attenuation of hepatic lipid accumulation | [198] |
| Ginsenoside Rb3 | In vitro In vivo | HEK293 cells ICR mouse model | Regulation of AMPK/mTOR-mediated autophagy suppressed cisplatin-induced nephrotoxicity | [93] |
| Ginsenoside PPD | In vitro | Endometrial stromal cells | Exerting anti-endometriosis effect by induction of autophagy | [127] |

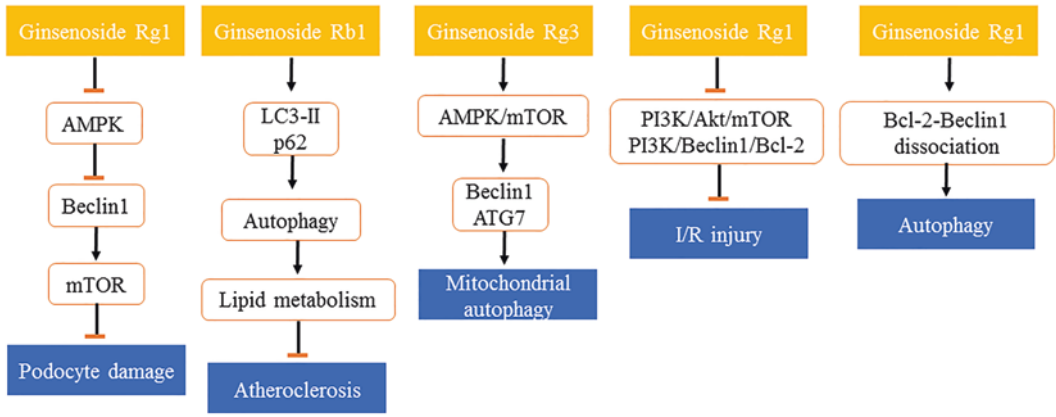


**Fig. 12.2** Protective effects of ginsenosides mediated through autophagy regulation. *AMPK* AMP-activated protein kinase, *mTOR* mechanistic target of rapamycin, *ATG* autophagy-related gene, *PI3K* phosphoinositide 3-kinase, *I/R* ischemic/reperfusion

tion of ROS. The elevation in the concentration of ROS, in turn, stimulates the JNK signaling pathway, leading to the induction of autophagy and apoptosis. It is noteworthy that upregulation of ATG7 and Beclin-1 are involved in the induction of autophagy [127]. It should also be emphasized that AMPK functions as a sensor of metabolism and inhibits mTOR signaling [123]. Thus, AMPK plays a significant role in the stimulation of autophagic cell death in tumor cells [128]. Ginsenoside 20(S) and compound K regulate the AMPK/mTOR and JNK signaling pathways to trigger autophagy-mediated cell death [123, 129]. In addition to AMPK, PI3K/Akt can also affect mTOR signaling. It has been demonstrated that stimulation of the PI3K/Akt pathway occurs in a number of cancer cells to guarantee their proliferation and malignancy [130, 131]. Importantly, the PI3K/Akt/mTOR signaling pathway is associated with enhanced malignancy of tumor cells due to an inhibition of autophagy [132–135]. Ginsenoside Rk3 significantly dimin-

ishes the viability and proliferation of tumor cells by down-regulation of the PI3K/Akt/mTOR signaling pathway [136]. Similarly, ginsenoside Rg5 enhances the expression of ATG5, ATG7, and ATG12 by down-regulation of the PI3K/Akt signaling pathway, leading to the stimulation of autophagic cell death in breast cancer cells [132].

MicroRNAs (miRs) are small non-coding RNAs with the capacity to regulate genes at the post-transcriptional level [137]. Various studies have shown the role of miRs in cancer progression and it appears that ginsenoside Rh2 has modulatory effects on miRs in cancer therapy [138]. MiRs are able to function as upstream mediators of autophagy [139]. Administration of ginsenoside Rh2 decreases the expression of miR-638, leading to the induction of autophagic cell death in human retinoblastoma cells [140].

Another key player in apoptosis is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which is a member of the cytokine family with few adverse effects on normal cells [141–143]. TRAIL is able to induce both intrinsic and extrinsic apoptosis pathways and a variety of drugs have been designed to target TRAIL in cancer therapy [144]. However, some tumor cells develop resistance to TRAIL-induced apoptosis by down-regulation of DR5 [145], which supports the premise that novel drugs need to be developed for cancer therapy. Ginsenoside compound k is suggested to be beneficial in improving TRAIL-mediated apoptosis by autophagy-mediated DR5 upregulation [105]. In view of the above discussion, it is clear that different ginsenosides affect autophagy at various stages, which range from the initiation stage to the fusion stage, in order to induce autophagic cell death and significantly diminish the viability and proliferation of cancer cells. Moreover, they can increase apoptotic cell death by induction of autophagy. It should be noted, however, that autophagy may function as a survival mechanism and enhance the viability and proliferation of cancer cells. In fact, in the case of hepatocellular carcinoma cells (HepG2) in cell culture, it has been shown that the inclusion of an autophagy inhibitor together with ginsenoside Rk1 (which induces autophagy and subsequent apoptosis) is an effective anti-cancer regimen (Table 12.2, Fig. 12.3) [146, 147].

**Table 12.2** The anti-tumor activity of ginsenosides mediated by autophagy modulation

| Drug | In vitro/In vivo | Cell line/animal model | major outcomes | References |
|---|---|---|---|---|
| Ginsenoside Rg5 | In vitro | Human gastric cancer cell lines SGC-7901 and BGC-823 | Induction of autophagic cell death through ROS-mediated MAPK | [126] |
| | In vivo | Mouse model of breast cancer | By inhibition of PI3K/Akt pathway, Rg5 potentiates autophagic cell death | [199] |
| Ginsenoside Rh4 | In vitro<br>In vivo | Human colorectal cancer cells (Caco-2 and HCT116)<br>Human colorectal cancer xenograft mouse model | Suppressing the viability and proliferation of cancer cells by autophagy induction via ROS/JNK/p53 signaling pathway | [127] |
| Compound K | In vitro | A549 and H1975 cells | Stimulation of autophagy-mediated apoptosis through AMPK/mTOR and JNK pathways | [129] |
| | In vitro | Human colon cancer cells | Sensitizing cancer cells to TRAIL-mediated apoptosis through autophagy-dependent DR5 upregulation | [200] |
| Ginsenoside 20(S) | In vitro | SK-MEL-28 cells | Reducing the viability and proliferation of melanoma cells by autophagy induction via AMPK/JNK phosphorylation | [123] |

(continued)

**Table 12.2** (continued)

| Drug | In vitro/In vivo | Cell line/animal model | major outcomes | References |
|---|---|---|---|---|
| Ginsenoside Rk3 | In vitro<br>In vivo | Eca109 and KYSE150 cells<br>Human esophageal cancer xenograft nude mouse model | Inhibition of PI3K/Akt/mTOR signaling pathway decreases the proliferation of cancer cells by induction of autophagic cell death | [136] |
| Ginsenoside Rh2 | In vitro | A375 and G361 melanoma cells | Improving the anti-tumor activity of SMI-4a by stimulation of autophagic cell death | [201] |
| | In vitro | Human retinoblastoma cells | Down-regulation of miR-638 enhances autophagic cell death in cancer cells | [202] |
| | In vitro | K562 cells | Activation of p38 triggers autophagy in cancer cells | [198] |
| | In vitro | U937 and K562 cells | Induction of autophagic cell death | [203] |
| | In vivo | Human leukemia K562 cells allograft tumor model | Inhibition of histone deacetylase 6 activates autophagic cell death | [204] |
| | In vitro | Hepatocellular carcinoma cells | Suppressing invasion by autophagy | [205] |
| | In vitro | HCT-116 human colorectal carcinoma cells | Induction of autophagy and improving the potential of chemotherapy | [206] |
| | In vitro | Human skin squamous cell carcinoma | Inhibition of cancer stem like cells through autophagy induction | [207] |
| Ginsenoside Rh2 and Rg3 | In vitro | HepG2 cells | As well as apoptosis, the autophagic cell death was induced | [208] |
| Ginsenoside Rg3 | In vitro | Human lung cancer cells | Sensitizing cancer cells to icotinib by autophagy stimulation | [209] |
| | In vitro<br>In vivo | Hepatocellular carcinoma cells<br>Xenograft model | Inhibition of autophagy sensitizes cancer cells to chemotherapy | [210] |
| | In vitro | Ovarian cancer cell line SKOV3 | Inhibiting the migration and invasion of cancer cells | [211] |
| | In vivo | Breast tumor-bearing mice | Promoting cell autophagy decreases cancer growth | [212] |
| Ginsenoside Rg2 | In vitro | MCF-7 cells | Enhancement of autophagy reduced the viability and proliferation of cancer cells | [213] |
| Ginsenoside F2 | In vitro | Breast cancer stem cells | Autophagy induction plays a survival role in cancer and its inhibition is associated with decreased survival of cancer cells | [147] |
| Ginsenoside Rk1 | In vitro | Hepatocellular carcinoma cells | Inhibition of autophagy enhances the apoptotic cell death in cancer cells | [146] |
| Ginsenoside M1 | In vitro | MCF-7 and MDA-MB-231 cells | Induction of autophagy and apoptotic cell death in cancer cells | [214] |
| Ginsenoside Ro | In vitro | ECA-109, TE-1, and H460 cells | Sensitizing esophageal cancer cells to 5-fluorouracil treatment by inhibition of autophagy through ESR2-NCF1-ROS pathway and stimulation of DNA damage | [215] |

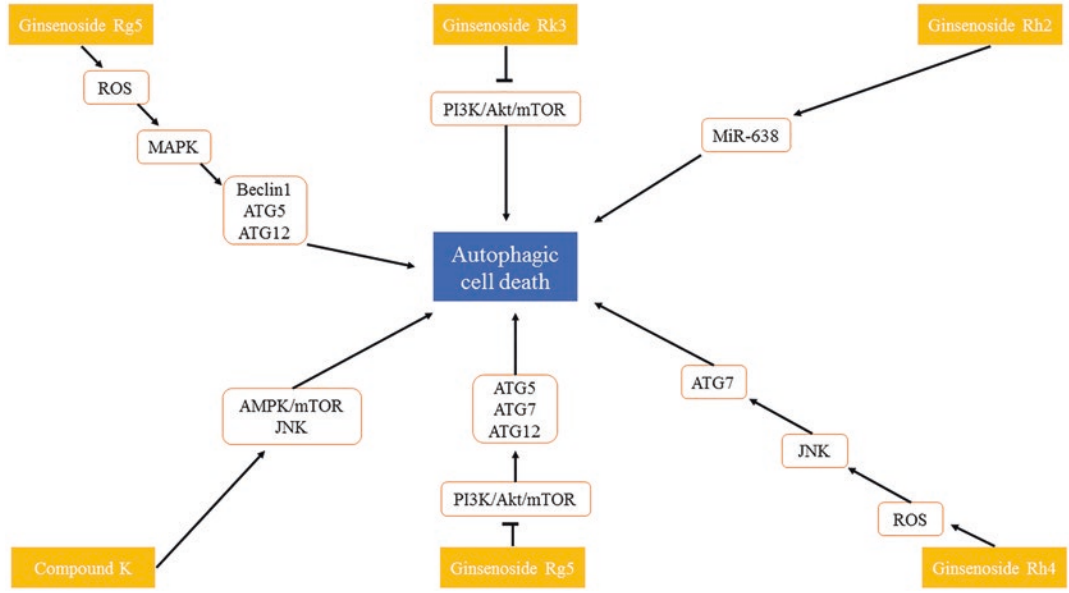


**Fig. 12.3** Anti-tumor activity of ginsenosides mediated through autophagy modulation. *ROS* reactive oxygen species, *MAPK* mitogen-activated protein kinase, *ATG* autophagy-related gene, *JNK* c-Jun N-terminal kinase, *AMPK* AMP-activated protein kinase, *mTOR* mechanistic target of rapamycin, *PI3K* phosphoinositide 3-kinase, *miR* microRNA

## 12.7 Ginsenoside and ER Stress

During sepsis-induced inflammation, cells undergo stress leading to apoptotic cell death. Sirtuin 1 (Sirt1) is one of the important signaling pathways involved in the regulation of protein function. As a $NAD^+$-dependent deacetylase, Sirt1 plays a remarkable role in attenuation of a number of pathological conditions such as atherosclerosis and cardiomyopathy [148–150]. Multiple studies have demonstrated that Sirt1 can be considered a potential target in reducing ER stress and, consequently, prevent various pathological conditions [132, 151]. Notably, ginsenoside Rg1 is capable of targeting the Sirt1 signaling pathway in inhibiting sepsis-induced lung injury. Exposure of cells to LPS leads to the generation of ROS. Enhanced ROS production negatively affects cells by induction of ER stress. Administration of ginsenoside Rg1 significantly suppresses LPS-mediated ER stress and apoptotic cell death by elevating the expression of Sirt1 [152].

A similar mechanism occurs with non-alcoholic fatty liver disease (NAFLD). As a metabolic syndrome, the stimulation of ER stress occurs during NAFLD, which, in turn, diminishes the viability of cells and triggers apoptotic cell death [153–155]. Induction of GRP78 and, subsequently, CHOP, plays a significant role in apoptosis during NAFLD. Dietary supplementation with ginsenoside Rg1 is associated with down-regulation of GRP78 and CHOP, and, subsequently, alleviation of apoptosis [99].

From 1991 onward, there has been as much as a 27% reduction in deaths caused by cancer. However, during recent years, the medical community has witnessed an increase in the incidence of cancer due primarily to an unhealthy life style [156]. As mentioned above, naturally-occurring compounds have demonstrated great potential in cancer therapy. It appears that induction of apoptotic cell death is the most common method by which plant-derived chemicals reduce the viability and invasion of cancer cells [72]. Ginsenoside Rg3 applies this same mechanism in combating

gallbladder cancer cells; namely, it enhances the expression of elF2α, ATF4, and CHOP to trigger apoptosis in cancer cells [157]. It has been demonstrated that ginsenoside Rh2 primarily induces ROS generation. Accordingly, increased production of ROS leads to stimulation of ER stress and upregulation of ATF4 and CHOP [158].

It should be mentioned that diabetes mellitus (DM) is an important pathological condition due to its widespread prevalence [159]. This metabolic disorder results in a high socioeconomic cost and demands more medical attention for its management and treatment [160]. Importantly, DM affects a variety of molecular signaling pathways. It has been determined that DM is associated with adverse effects on various parts of body due to the stimulation of apoptosis [28]. Thus, pharmacologically targeting apoptotic cell death is important with regard to inhibiting diabetic complications. Administration of ginsenoside Rg1 significantly reduces diabetic cardiomyopathy by inhibiting ER stress-mediated apoptosis *via* down-regulation of GRP78 and CHOP [161]. Based on these findings, AMPK can be considered a promising target in the treatment of DM. AMPK functions as an inhibitor of hepatic gluconeogenesis and, during DM, AMPK undergoes down-regulation [162]. Moreover, a small heterodimer partner (SHP) contributes to glucose and cholesterol metabolism and is a downstream target of AMPK [163, 164]. By attenuation of ER stress, ginsenoside Rb2 stimulates AMPK and, subsequently, SHP, which leads to the inhibition of gluconeogenesis [165]. One of the challenges in the treatment of DM is insulin resistance, which promotes an increase in the plasma concentrations of free fatty acids (FFAs) [166, 167]. Succinate accumulation is associated with insulin resistance. It has been reported that induction of hypoxia in adipose tissue triggers adipose dysfunction. ER stress and relevant signaling pathways such as ARF6, PERK, and IRE1α play a significant role in this case [168–170]. With respect to the capacity of ginsenosides to regulate ER stress, it would appear that they can be advantageous in inhibiting insulin resistance and adipose dysfunction. Ginsenoside Rg5 decreases the phosphorylation of PERK and IRE1α to prevent ER stress and succinate accumulation, while it has no effect on the expression of ATF6 [171]. The important point, however, is the potential role of nucleotide-binding domain and the leucine-rich repeat containing protein 3 (NLRP3) inflammasome in insulin resistance, since the NLRP3 inflammasome is involved in immunological reactions and affects the secretion of a variety of cytokines such as interleukin-1β (IL-1β). An elevated concentration of plasma glucose induces the release of IL-1β through the NLRP3 inflammasome. It has been demonstrated that inhibition of the NLRP3 inflammasome is considered a 'smart' strategy in the prevention of insulin resistance. Notably, ER stress is capable of stimulation of the NLRP3 inflammasome [172–177]. A combination of ginsenoside Rb1 and compound K has been suggested to be beneficial in improving insulin resistance by inhibition of the ER stress-associated NLRP3 inflammasome *via* dephosphorylation of IRE1α and PERK [178]. It is noteworthy that ginsenosides can also exert neuroprotective activity through modulation of ER stress. Several studies have revealed that ER stress can be considered a predisposing factor for a number of neurodegenerative diseases (NDs) such as AD and Parkinson's disease (PD) [179, 180]. Furthermore, ER stress has been documented to contribute to the induction of cognitive impairment [181, 182]. It appears that elevated levels of blood glucose (i.e., a condition like DM) induces ER stress-mediated apoptosis through CHOP upregulation, and this, in turn, leads to the cognitive impairment [183]. Ginsenoside Rb1 alleviates high glucose-mediated neurotoxicity by down-regulation in the expression of PERK and CHOP. Inhibition of ER stress has been shown to be associated with upregulation of anti-apoptotic factor Bcl-2 [184]. Importantly, glycogen synthase kinase-3β (GSK-3β) is one of the key components of the Wnt signaling pathway and accounts for modulation in ER stress-mediated CHOP expression [185]. Fortunately, ginsenoside Rb1 provides an inhibition of CHOP by down-regulation of GSK-3β, which results in protection of neurons against glucose-induced neurotoxicity (Table 12.3, Fig. 12.4) [186].

**Table 12.3** Studies supporting the relationship between ginsenoside and endoplasmic reticulum stress

| Drug | In vitro/in vivo | Cell line/animal model | Major outcomes | References |
|---|---|---|---|---|
| Compound K | In vitro | HepG2 and SMMC-7721 (human liver cancer cells) | Induction of ER stress and decreased viability of cancer cells | [216] |
| Ginsenoside Rb1 and compound K | In vitro | 3 T3-L1 adipocyte cells | Down-regulation of ER stress-mediated NLRP3 inflammasome and improving insulin resistance | [178] |
| Ginsenoside Rg1 | In vitro | Mouse model of (NAFLD) | Inhibition of CHOP and subsequently, ER stress, leading to the protection against NAFLD | [217] |
| | In vivo | Rats with nephropathy | Inhibition of ER stress and protective effects against nephropathy | [218] |
| | In vivo | Sprague-Dawley rats | Suppression of ER stress-mediated apoptosis after unilateral ureteral obstruction | [219] |
| | In vitro | Cultured human pulmonary epithelial cell line A549 | Sirt1 upregulation results in alleviation of ER stress and subsequently, inhibition of apoptosis | [31] |
| | In vivo | Streptozotocin-induced diabetes rat model | Down-regulation of GRP78 and CHOP is associated with decreased ER stress-mediated apoptosis and alleviation of adverse effects of DM | [161] |
| Ginsenoside Rb1 | In vitro | Rat PC12 rats | Protection against oxidative stress-induced ER stress | [220] |
| | In vitro | Primary hippocampal neuronal cells | Inhibition of ER stress via suppressing CHOP and protection of hippocampal neurons from high glucose-induced neurotoxicity | [186] |
| | In vitro | Primary rat hippocampal neurons | Neuroprotective effects via inhibition of ER stress-mediated PERK and CHOP | [184] |
| Ginsenoside Rg3 | In vitro | Human gallbladder cancer cell line GBC-SD | Upregulation of elF2α, ATF4 and C/EBP homologous protein results in ER stress-mediated apoptosis | [157] |
| Ginsenoside Rh2 | In vitro<br>In vivo | H1299 cells<br>Mice bearing lung cancer tumor | Induction of ER stress-mediated apoptosis | [158] |

(continued)

**Table 12.3** (continued)

| Drug | In vitro/in vivo | Cell line/animal model | Major outcomes | References |
|---|---|---|---|---|
| Ginsenoside Rb2 | In vitro | H4IIE cell line (rat hepatocytes) | Inhibition of gluconeogenesis by down-regulation of AMPK-mediated SHP through suppressing ER stress | [165] |
| Ginsenoside Rg5 | In vitro | 3 T3-L1 (a cell line of preadipocyte) | Improving insulin resistance by down-regulation of PREK and IRE1α and subsequently, inhibition of adipose dysfunction | [171] |

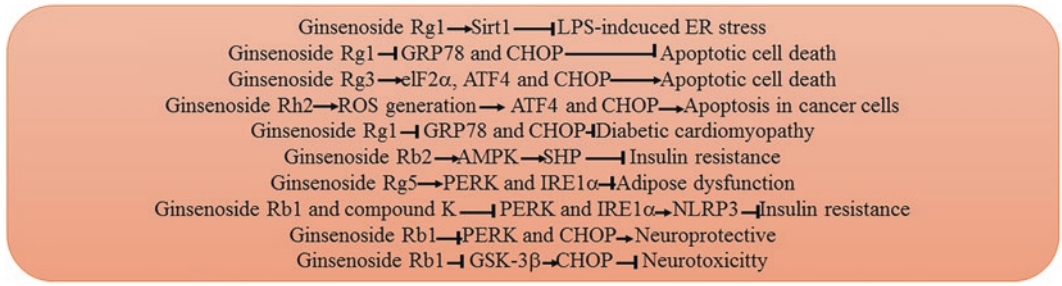


**Fig. 12.4** Involvement signaling pathways in the modulation of ER stress by ginsenosides. *Sirt1* Sirtuin 1, *LPS* lipopolysaccharide, *ER* endoplasmic reticulum, *CHOP* CCAAT-enhancer-binding protein homologous protein, *GRP78* glucose-regulated protein 78, *elF2α* eukaryotic translation factor 2α, *ATF-4* activating transcription factor-4, *ROS* reactive oxygen species, *AMPK* AMP-activated protein kinase, *SHP* small heterodimer protein, *IRE1α* inositol-requiring enzyme-1α, *PERK* PRK-like ER kinase, *NLRP3* nucleotide-binding domain and leucine-rich repeat containing protein 3, *GSK-3β* glycogen synthase kinase-3β

## 12.8 Conclusion

Armed with an advanced and more complete understanding of cancer biology, it has recently been demonstrated that cancer cells exhibit a resistance to apoptosis. Along these lines, modulation of cell death mechanisms to combat cancer cells and troublesome diseases, as well as methods aimed at eliminating resistance to apoptosis have garnered intense interest in the scientific and medical communities. Clearly, specific agents that could modulate biochemical/physiological pathways that involve autophagy and ER stress would be beneficial in the treatment of a number of disorders/diseases. In this regard, it has been suggested that natural herbal products, or their many pharmacologically-active constituents, may be ideal candidates due to their infrequent side effects. Ginsenosides are compounds derived from the ginseng plant and exert modulatory effects on autophagy and ER stress. The protective potential of ginsenosides is related to the up-regulation of Atg proteins and the conversion of LC3I to II, which results in autophagosome maturation. Not only do ginsenosides promote autophagy but they also confer protective and therapeutic activity by modulating ER stress and the up- and down-regulation and/or dephosphorylation of UPR transducers such as IRE1, PERK and ATF6. Taking everything into account, it would appear that ginsenosides hold promise for the recovery of various functional impairments of cells/tissues and could potentially be useful for the treatment of cancer. However, more studies are needed to more fully elucidate the effects of ginsenosides on autophagy and ER stress, as well

as validate the beneficial properties of ginsenosides in carefully-controlled clinical trials. Finally, it has been shown that an enhanced level of oxidative stress and mitochondrial dysfunction are involved in ER stress-mediated apoptosis. Accumulating data suggests that there is a relationship between the UPR signaling pathway, oxidative damage, and mitochondrial dysfunction during ER stress [187–189]. Importantly, ginsenosides are able to suppress mitochondrial dysfunction and apoptosis by induction of mitochondrial autophagy. Lastly, although a large number of studies have investigated the effects of ginsenosides on autophagy and ER stress, there is still a lack of understanding concerning improving their bioavailability (*e.g.*, the use of nanocarriers) to optimally exploit their therapeutic effects on autophagy and ER stress.

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# 13

# Medicinal Plants and Phytochemicals Regulating Insulin Resistance and Glucose Homeostasis in Type 2 Diabetic Patients: A Clinical Review

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**Abstract**

Diabetes is a major health problem affecting more than four hundred million adults worldwide. The transition from normal glucose tolerance to type 2 diabetes (T2D) is preceded by increased Insulin resistance (IR), an independent predictor of the development of T2D in high risk (e.g. obese populations, prediabetes) individuals. Insulin deficiency resulting from increased IR results in progressive glucose homeostasis dysfunction. Data has shown that IR is affected by many different factors such as genetics, age, exercise, dietary nutrients, obesity, and body fat distribution. One of the most important factors is diet, which plays an essential role in addressing T2D and metabolic syndrome. Nutraceuticals and medicinal plants have been shown to have efficacy in preventing chronic diseases like cancer, non-alcoholic fatty liver disease (NAFLD), cardiovascular disease, diabetes mellitus and metabolic syndrome, likely through the anti-inflammatory properties found in nutraceuticals. However, the effect of these compounds, including traditional plant medicines, herbal formulations or their extracts on IR have not been systematically investigated. The objective of this review was to assess the reported effects of medicinal plants and bioactive natural com-

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pounds on IR. The findings confirm that most of the herbal bioactive compounds including resveratrol, garlic, curcumin, cinnamon, ginger, nuts, berberine, anthocyanin, soybean, flaxseed, vegetable oils, and soluble fibers have benefit in their efficacy for decreasing IR, fasting blood sugar (FBS), fasting insulin and HbA1c.

**Keywords**

Type 2 diabetes · Metabolic syndrome · Insulin resistance · Medicinal plants

## 13.1 Introduction

Diabetes is a major health problem affecting more than four hundred million adults worldwide [1]. The prevalence of T2D has been increasing steadily and it is thought that nearly 600 million people will be affected by 2035 [2]. More than 80% of diabetic patients suffer from T2D with both macrovascular and microvascular complications leading to an increasing burden on healthcare systems [3]. In addition, T2D is also affecting an increasing number of children, adolescents and young adults [4]. Evidence indicates that the development of T2D is a result of genetics and the environment [5] (Fig. 13.1). The transition from normal glucose tolerance to type 2 diabetes (T2D) is preceded by increased insulin resistance (IR), an independent predictor of the development of T2D in high risk (e.g. obese populations, pre-diabetes) individuals [6, 7]. Insulin deficiency resulting from increased IR results in progressive glucose homeostasis dysfunction. Mutations within the peroxisome proliferator activating receptor gamma (PPAR-γ) receptor gene contribute a key role in T2D [8] and hyperglycemia alters the functional phenotype of monocytes, macrophages, neutrophils, NK cells, and CD8+ T cells [9]. Results from the British Whitehall II study showed that IR precedes diabetes development indicating lowered insulin sensitivity (IS) and reduced β-cell function in the pre-diabetic stage [10]. IR can increase the progression and development of diabetic cardiomyopathy that is a specific form of cardiomyopathy [11]. Diabetic cardiomyopathy may be distinguished by the presence of impaired myocardial insulin signaling and mitochondrial dysfunction [12] leading to systolic heart failure [13]. Hyperglycemia is one of the main causes of T2D [14] as hyperglycemia promotes reactive oxygen species (ROS) production [15, 16] that in turn increases oxidative stress leading to injured cellular organelles and increased lipid peroxidation [17]. These pathways can affect insulin activity, function and secretion contributing to the progression to T2D [18]. In experimental models it has been shown that α-lipoic acid (LA), an anti-

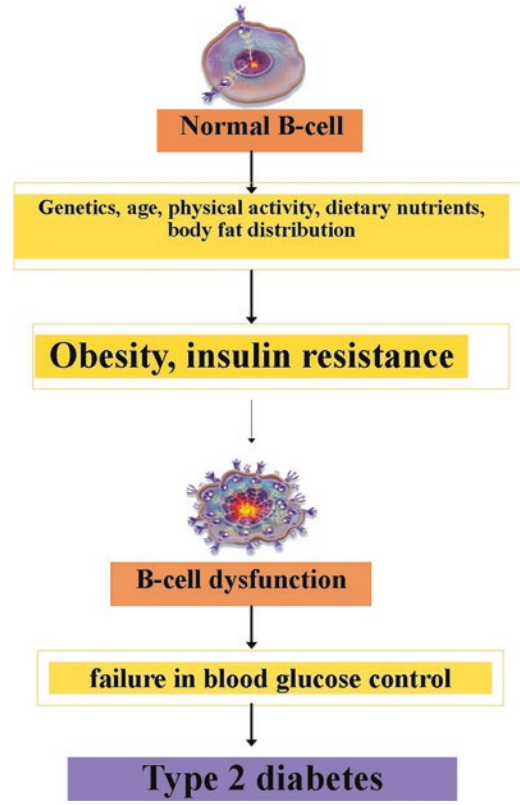


**Fig. 13.1 Effect gene and environmental factors on B-cell dysfunction and progression of Type 2 diabetes.** Genetics, age and environmental factors (physical activity, dietary nutrients, body fat distribution) are responsible for obesity and insulin resistance. Impairment in the function of pancreatic B cells-cells producing insulin causes failure in blood glucose control and development of type 2 diabetes

oxidant, may increase insulin sensitivity [19]. Obesity is another major cause of increased IR and T2D [20] as adipose tissue releases increased amounts of non-esterified fatty acids, glycerol, hormones and adipokines contributing to increased IR [21]. Impairment in the function of pancreatic B cells, responsible for producing insulin, leads to increased glucose dysregulation c [20, 22]. Dysfunction in β-cells is therefore important in determining the risk and development of T2D [20]. Current studies have identified various T lymphocyte subtypes in obese adipose tissue of humans and mice [23, 24]. In obesity, adipose tissue TH1 lymphocytes may help to attract macrophages into adipose tissue leading to increased tissue inflammation and enhancing IR [25].

The mechanism(s) that underlies the progress of IR in humans remains unclear. What has become clear is that IR is controlled by many different factors such as genetics [25], age [26, 27], exercise [28], dietary nutrients [29], obesity [30, 31], and body fat distribution [32, 33]. Aging is correlated with a reduction in the body's responsiveness to carbohydrate (19, 20). Exercise effects are more complex because exercise has both acute and chronic effects (21). However, in one study, exercise reduced the intra-abdominal fat area by 25% and improved in IR by 36% [5, 34].

The development and treatment of T2D could be addressed in part with lifestyle changes, including maintaining a healthy body weight, having a healthy diet, staying physically active, not smoking and not drinking alcohol [35]. The most important factor is a healthy diet, which plays an essential role in reducing T2D and metabolic syndrome [36]. Among the dietary components, nutraceuticals and medicinal plants have an important role in preventing chronic diseases like cancer, non-alcoholic fatty liver disease (NAFLD), cardiovascular disease, and T2DM and metabolic syndrome [25, 37–42]. There is a need for therapeutic agents that can decrease the risk of IR, and the number of nutraceuticals compounds with potential therapeutic properties to treat T2D patients continues to increase [25]. The expressed anti-inflammatory properties of nutraceuticals may be very important for the treatment of such diseases [25]. For instance, some of these natural agents have been found to repress the expression of PAI-1 by inhibiting the transcription factor early growth response, which has been associated with IR and obesity [43]. However, the effect of these compounds, including traditional plant medicines or herbal formulations or their extracts on IR have not been systematically reviewed. Therefore, the objective of this review is to detail the effects of medicinal plants and bioactive natural compounds on IR. The main findings of previous studies are summarized in Table 13.1.

## 13.2 Resveratrol

Resveratrol has the properties of being an antioxidant and anti-inflammatory factor that may decrease or limit the progress of many diseases including cancer, hypertension, cardiovascular diseases (CVD), T2DM and other metabolic diseases [98–100]. In a meta-analysis study of 11 randomized control trials, a total of 388 subjects were included and results showed that resveratrol significantly decreased fasting blood sugar (FBS), hemoglobin A1C (HbA1c) and IR by evaluation of homeostasis model of assessment for insulin resistance (HOMA-IR) in participants with T2DM though it had no significant effect on subjects without diabetes [101]. In a double-blind clinical trial study, 21 patients with T2DM were asked to take 480 mg/day resveratrol (intervention group) for 4 weeks and 22 individuals with diabetes without any treatment were considered as a control group. At the end of the study there was a significant reduction in fasting insulin and HOMA-IR levels observed in the intervention group compared with the control group. There was no significant difference in fasting blood glucose and TG (triglyceride) between intervention and control groups [44]. In a double-blind study, 19 male patients with T2D were recruited into two groups: patients in the resveratrol group to take oral resveratrol 10 mg/day and nine patients to placebo as a control group, and the intervention was conducted for 4 weeks. At the end of the

**Table 13.1** The effects of medicinal plants and bioactive natural compounds on Insulin resistance and glucose hemostasis in type 2 diabetic patients

| Author, year | Agent | Dose per day | Treatment duration | Subjects | Main outcomes |
|---|---|---|---|---|---|
| Zare Javid A et al. 2017 [44] | Resveratrol | 480 mg/day | 4 weeks | Patients with diabetes | Significant decreases in fasting insulin and HOMA-IR levels weere observed in intervention group compared with control group |
| Brasnyó P et al. 2011 [45] | Resveratrol | 10 mg/day | 4 weeks | Patients with T2D | No effect |
| Movahed A et al. 2013 [46] | Resveratrol | 1 g/day | 45 days | Patients with diabetes | There were significant reductions in fasting blood glucose, HbA1c, insulin, and HOMA-IR in resveratrol supplementation. |
| Bhatt JK et al. 2012 [47] | Resveratrol | 250 mg/day | 3 months | Patients with diabetes | HbA1c had a significant decrease in the group supplemented with resveratrol. |
| Bo S et al. 2016 [48] | Resveratrol | 500 and 40 mg/day | 6 months | Patients with diabetes | No effect for both doses of resveratrol. |
| Talaei B et al. 2017 [49] | Cinnamon | 3 g/day | 8 weeks | Patients with diabetes | No effect |
| Solomon TP et al. 2009 [50] | Cinnamon | 3 g/day | 14 days | Healthy male | Cinnamon diminished the glucose, and also decreased insulin and developing insulin responsiveness |
| Akilen R et al. 2010 [51] | Cinnamon | 2 g/day | 12 weeks | Patients with diabetes | Cinnamon significantly reduced HbA1c compared to placebo. There was no significant effect on fasting plasma glucose in the cinnamon group |
| Vanschoonbeek K et al. 2006 [52] | Cinnamon | 1.5 g | 6 weeks | Postmenopausal women with T2D | No effect |

(continued)

**Table 13.1** (continued)

| Author, year | Agent | Dose per day | Treatment duration | Subjects | Main outcomes |
|---|---|---|---|---|---|
| Mozaffari-Khosravi Hello et al. 2014 [53] | Ginger | 3 g/day | 8 weeks | Patients with diabetes | Fasting blood sugar, fasting insulin concentration and HOMA-decreased significantly between 2 groups. The QUICKI rose significantly in two groups, but differences of this index were significantly higher in ginger group |
| Shidfar F et al. 2015 [54] | Ginger | 3 g/day | 3 months | Patients with diabetes | The levels of glucose, fasting insulin, HOMA-IR, Hb1AC were significantly lower in the ginger group compared with the placebo group |
| Mahluji S et al. 2013 [55] | Ginger | 2 g/day | 2 months | Patients with diabetes | There were significant reductions in the level of insulin, HOMA-IR raised the QUICKI index in the ginger group compared with the control group |
| Arablou T et al. 2014 [56] | Ginger | 1600 mg | 12 weeks | Patients with diabetes | Ginger significantly lowered the levels of insulin, fasting plasma glucose, HbA1c, HOMA-IR in comparison to the control group |
| Yin J et al. 2008 [57] | Berberine | 1500 mg/day | 3 months | Patients with diabetes | There were significant reductions in HbA1c, fasting blood glucose and postprandial blood glucose in the berberine group in two levels A and B, and fasting plasma insulin and HOMA-IR were diminished only in level B |

(continued)

**Table 13.1** (continued)

| Author, year | Agent | Dose per day | Treatment duration | Subjects | Main outcomes |
|---|---|---|---|---|---|
| Zhang Y et al. 2008 [58] | Berberine | 1.0 g/day | 3 months | Patients with diabetes and dyslipidemia | Berberine had significant improvements in fasting plasma glucose and 2-h OGTT plasma glucose, HbA1c, and HOMA-IR decreased |
| Shidfar F et al. 2012 [59] | Berberis vulgaris fruit extract | 3 g/day | 3 months | Patients with diabetes | There were significant reductions in serum glucose and insulin and HOMA-IR between two groups |
| Atkin M et al. 2016 [60] | Aged garlic extract | 1200 mg/day | 4 weeks | Patients with diabetes | No effect |
| Ghorbani A et al. 2019 [61] | Garlic | 300 mg/day | 12 weeks | Patients with diabetes and dyslipidemia | The levels of HbA1c decreased significantly in the intervention group though no effect on fasting blood glucose |
| Li D et al. 2015 [62] | Anthocyanin | 320 mg/day | 24 weeks | Patients with diabetes | Significant reductions in plasma fasting plasma glucose and HOMA-IR levels were observed in the anthocyanins group compared with the placebo group |
| Moazen S et al. 2013 [63] | Freeze-dried strawberry | 50 g/day | 6 weeks | Patients with diabetes | The level of HbA1c reduced significantly in the intervention group and there was no significant difference in serum glucose concentrations between two groups |
| Banihani S et al. 2014 [64] | Fresh pomegranate juice | 1.5 mL/kg body weight | Blood samples were obtained after 12 h of fasting, 1 and 3 h after the ingestion of the juice. | Patients with diabetes | HOMA-IR reduced between diabetic patients after 3 h of pomegranate juice ingestion |

(continued)

**Table 13.1** (continued)

| Author, year | Agent | Dose per day | Treatment duration | Subjects | Main outcomes |
|---|---|---|---|---|---|
| Liu C-Y et al. 2014 [65] | Green tea extract | 1500 mg/day | 16 weeks | Patients with diabetes and dyslipidemia | There was a significant reduction in triglyceride and HOMA-IR |
| Hua C et al. 2011 [66] | Decaffeinated green tea extract | 500 mg/day | 16 weeks | Patients with diabetes | No effect |
| Fukino Y et al. 2005 [67] | Green tea extracts/ powder | 544 mg/day | 2 months | Patients with diabetes | No effect |
| Ryu O et al. 2006 [68] | Green tea | 9 g/day | 4 weeks | Patients with diabetes | No effect |
| MacKenzie T et al. 2007 [69] | *Camellia sinensis* (eg, green, oolong, and black tea) | 0, 375, or 750 mg of 40% catechins from green tea (150 mg) | 3 months | Patients with diabetes | No effect |
| Ahn HY et al. 2018 [70] | Fermented soybean powder mixture | 19.45 g/day | 12 weeks | Patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or newly diagnosed T2D | The level of fasting glucose, glucose at 60 min, HOMA-IR decreased in intervention group |
| Kim J-I et al. 2005 [71] | Soybean-derived pinitol | 600 mg/day | 13 weeks | Patients with diabetes | The levels of fasting plasma glucose, insulin, fructosamine, HbA1c, and HOMA-IR diminished significantly |
| T Sathyapalan et al. 2017 [72] | Soy protein | 15 g/day | 3 months | Male patients with diabetes | A significant linear correlation between the decrease of βCTX in the SPI group with a decrease of HbA1c and HOMA-IR. |
| J Konya et al. 2019 [73] | Soy protein | 15 g/day | 8 weeks | Patients with diabetes | The level of HbA1c improved in the soy protein group compared with the placebo group. |
| V Jayagopal et al. 2002 [74] | Soy protein | 30 g/day | 12 weeks | Postmenopausal women with T2D | There were significant reductions in the levels of insulin resistance, fasting insulin, HbA1c, HOMA-IR. |
| S González et al. 2007 [75] | Soy protein | Soy that included 132 mg isoflavone capsules | 12 weeks | Postmenopausal women with T2D | No effect |

(continued)

**Table 13.1** (continued)

| Author, year | Agent | Dose per day | Treatment duration | Subjects | Main outcomes |
|---|---|---|---|---|---|
| Soleimani Z et al. 2017 [76] | Omega-3 fatty acids from flaxseed oil | 1000 mg/day | 12 weeks | Patients with diabetic foot ulcer grade 3 | Omega-3 fatty acids reduced significantly serum insulin, HOMA-IR, HbA1c and increased significantly QUICKI compared with the placebo group |
| Zheng JS et al. 2016 [77] | Flaxseed oil, fish oil, corn oil | 2.5 g/day of alpha-linolenic acid | 180 days | Patients with diabetes | No effect |
| Soleimani A et al. 2017 [78] | Omega-3 fatty acids from flaxseed oil | 1000 mg/day | 12 weeks | Patients with diabetic nephropathy | Omega-3 fatty acids significantly reduced the level of insulin, HOMA-IR and increased QUICKI compared with the placebo group |
| Foster M et al. 2014 [79] | Zinc and flaxseed oil | Flaxseed oil (2 g/day) | 12 weeks | Women with diabetes | No effect |
| Panahi Y et al. 2018 [80] | Curcuminoids | 500 mg/day with piperine 5 mg/day | 3 months | Patients with diabetes | Serum levels of insulin, HbA1c, and HOMA-IR reduced significantly in both groups, whereas serum levels of glucose and HbA1c reduced significantly after curcuminoids group compared with the placebo group |
| Na LX et al. 2013 [81] | Curcuminoids | 300 mg/day | 3 months | Patients with T2D that was overweight or obese | Fasting blood glucose, HbA1c, HOMA-IR diminished significantly after curcuminoids supplementation |
| H Hodaei et al. 2019 [82] | Curcumin | 1500 mg three times daily | 10 weeks | Patients with diabetes | Curcumin had a significant reduction in FBS but did not affect HOMA-IR, HbA1c and insulin. |

(continued)

**Table 13.1** (continued)

| Author, year | Agent | Dose per day | Treatment duration | Subjects | Main outcomes |
|---|---|---|---|---|---|
| RN Thota et al. 2019 [83] | Curcumin | 2 × 500 mg tablets | 12 weeks | Patients with diabetes | There was no difference in levels of HbA1c and fasting glucose between all groups. Insulin sensitivity increased significantly in CC group compared with PL. |
| S Asadi et al. 2019 [84] | Nano-curcumin | 80 mg | 8 weeks | Patients with diabetes | HbA1c and FBC reduced significantly in nano curcumin group compared with placebo group. |
| LX Na et al. 2013 [81] | Curcumin | 300 mg | 3 months | Patients with diabetes | There were significant reductions in FBS, HbA1c and HOMA-IR in curcumin group. |
| Rabiei K et al. 2018 [85] | Extract of *Juglans regia* (walnut) leaves | 100 mg/day | 8 weeks | Patients with diabetes | No effect |
| Hosseini S et al. 2014 [86] | *Juglans regia* leaf extract | 200 mg/day | 3 months | Patients with diabetes | Juglans regia reduced significantly levels of fasting blood glucose and HbA1c: There was no effect on insulin levels. |
| Parham M et al. 2014 [87] | Pistachio nuts | 50 g/day | 12 weeks | Patients with diabetes | Fasting blood glucose and HbA1c decreased in the pistachio group but there was no effect on HOMA-IR |
| Hernández-Alonso P et al. 2014 [88] | Pistachio diet | 57 g/day | 4 months | Prediabetic patients | Pistachio diet diminished fasting glucose, insulin, and HOMA-IR. |
| Li S-C et al. 2011 [89] | Almond diet | 20% of calorie intake were almonds | 4 weeks | Patients with diabetes and mild hyperlipidemia | Levels of fasting insulin, fasting glucose, and HOMA-IR were lower in the almond diet |
| Jenkins DJ et al. 2014 [90] | A bread that enriched with canola oil | 31 g canola oil per 2000 kcal | 3 months | Patients with diabetic and hyperlipidemic | The level of Hb1Ac decreased in both groups but the reduction was greater in the test diet |

(continued)

**Table 13.1** (continued)

| Author, year | Agent | Dose per day | Treatment duration | Subjects | Main outcomes |
|---|---|---|---|---|---|
| Sarbolouki S et al. 2013 [91] | Eicosapentaenoic acid (EPA) in intervention group and corn oil in control group. | (EPA) (2 g/day) and corn oil (2 g/day) | 3 months | Patients with diabetes | The levels of fasting plasma glucose, HbA1c, HOMA-IR reduced significantly in the EPA group compared with control group |
| Mostad IL et al. 2006 [92] | Fish oil in the intervention group and corn oil in the control group. | 17.6 mL/day of fish oil and 17.8 ml/day of corn oil | Short-term (1 week) and longer-term (9 week) | Patients with diabetes | The mean blood glucose concentrations and fasting blood glucose concentrations were significantly greater after 8 week in the fish oil group compared with the corn oil group, but at baseline, 1 week, and 9 week there was no changes |
| Jacobo-Cejudo MG et al. 2017 [93] | Docosahexaenoic acid plus eicosapentaenoic acid-enriched fish-oil (FOG) | 520 mg/day | 24 weeks | Patients with diabetes | Hb1Ac decreased and insulin, HOMA-IR raised significantly in both groups |
| Ogawa S et al. 2013 [94] | Liquid diet with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) | Liquid diet with EPA (25 mg/100 kcal) and DHA (17 mg/100 kcal) | 3 months | Patients with diabetes | There were significant decreases in the levels of fasting plasma glucose and HbA1c in the diet with EPA/DHA compared with a diet without EPA/DHA |
| Kamalpour M et al. 2018 [95] | Psyllium powder | 7 g | 2 weeks | Patients with diabetes | There was a significant reduction and increase in fasting plasma insulin and HOMA-IR, respectively |
| Dall'Alba V et al. 2013 [96] | Partially hydrolyzed guar gum (PHGG) | 10 g/day | 4 and 6 weeks | Diabetic patients with metabolic syndrome | HbA1c decreased in the intervention group after baseline, 4 and 6 weeks |
| Abutair AS et al. 2016 [97] | Soluble fiber from psyllium | 10.5 g/day | 8 weeks | Patients with diabetes | Soluble fiber supplementation improved fasting blood sugar, HbA1c, insulin, HOMA.IR and HOMA-β % |

study, there was no significant difference in serum insulin levels and HOMA assessment of b-cell function (HOMA-b) values between the resveratrol and the placebo groups; however, HOMA-IR values significantly reduced in the intervention group compared with placebo [45]. In another clinical trial study, 66 T2DM patients were divided into two groups to receive resveratrol (1 g/day) or placebo group for 45 days. At the end of the study there was a significant reduction in fasting blood glucose, HbA1c, insulin, and HOMA-IR in the resveratrol group compared to their baseline levels [46]. In another study, a total of 62 subjects with T2D were asked to consume resveratrol (250 mg/day) or placebo (control group) whilst using an oral hypoglycemic agent in both groups. After 3 months of intervention, only HbA1c was significantly decreased in the resveratrol group [47]. In a further control trial, 192 patients with T2D were assigned to receive resveratrol 500 mg/day, resveratrol 40 mg/day or placebo for 6-months. Results showed that no significant changes were observed in plasma FBS, HbA1c or insulin between groups for both resveratrol 500 and resveratrol 40 compared with the placebo group [48].

## 13.3 Cinnamon

Cinnamon has been used for many years as a herbal Medicine [102]. It has been shown that cinnamon may stimulate insulin secretion, increase insulin sensitivity, and insulin signaling resulting in a decrease in blood glucose and improvement in the lipid profile [103–107]. In one double-blind, randomized, placebo-controlled clinical trial study, 44 patients with T2D were randomly recruited into two groups, placebo or 3 g/day cinnamon supplement daily. After 8 weeks, results showed that there was no significant difference in the level of fasting blood glucose, insulin, HbA1c, HOMA-IR between two groups [49]. In a single-blind randomized cross-over design, 8 healthy males were entered to study, each subject performed two 20-day interventions including control intervention and a cinnamon (3 g/day). Oral glucose tolerance tests (OGTT) were completed on days 0, 1, 14, 16, 18, and 20. Cinnamon diminished the glucose on day 1 and day 14 and also decreased insulin and enhanced insulin responsiveness on day 14 [50]. In a clinical trial, a total of 58 T2DM subjects were asked to take cinnamon (2 g/day) or placebo for 12 weeks. At the end of the study, cinnamon capsules significantly reduced HbA1c compared to placebo, but there was no significant effect on fasting plasma glucose between groups [51]. In another study, 55 postmenopausal women with T2D were enrolled to take 1.5 g of cinnamon or a placebo daily for a period of 6 weeks. At the end of the study, that cinnamon supplementation had no effect on HbA1c, HOMA-IR or oral glucose tolerance [52].

## 13.4 Ginger

Ginger is a pharmaceutical plant that has been utilized for many years as a food spice [108]. Ginger is one of the functional foods that includes essential compounds applicable to gingerol, shogaol, paradol and zingerone [54]. Several health benefits have been ascribed to ginger including immunomodulatory, anti-inflammatory anti-cancer, anti-thrombotic, anti-hyperglycemic and hypolipidemic actions [109, 110].

In a double-blind randomized controlled study, 88 patients with T2DM were randomly recruited into two groups: ginger (GG) and placebo (PG) groups. The GG took 3 g/day for 8 weeks. After the intervention, the results indicated that the median fasting blood sugar, fasting insulin concentration and HOMA-decreased significantly between 2 groups. The QUICKI (quantitative insulin sensitivity check index) as an insulin resistance index rose significantly in both groups, but differences of this index were significantly higher in GG than PG [53]. In another double-blind, placebo-controlled, randomized clinical trial study, 50 T2D patients were asked to consume 3 g of powdered ginger or placebo daily. After 3 months, the level of glucose, fasting insulin, HOMA-IR, Hb1AC was significantly lower in the ginger group compared with the placebo group [54]. In a previous clinical trial study, 64

participants with T2DM were randomised to receive 2 g/day of ginger or placebo for 2 months. At the end of the study there was a significant reduction in the level of insulin, HOMA-IR raised the QUICKI index in the ginger group compared with the control group, and no significant changes were observed in plasma fasting blood glucose [55]. In another study, 70 patients with T2D were assigned to receive 1600 mg ginger or 1600 mg wheat flour placebo every day. After 12 weeks of intervention, ginger supplementation significantly lowered the levels of insulin, fasting plasma glucose, HbA1c, HOMA-IR in comparison to the control group [56].

## 13.5 Berberine

Berberine has several natural activities that may have positive effects on various metabolic disorders including reducing hyperglycemia and dyslipidemia [111–113]. Berberine is an ancient Chinese herb that has been used to treat T2DM and to treat gastrointestinal infections for thousands of years [57]. In a pilot study, 36 individuals with recently diagnosed T2D were asked to take berberine or metformin (500 mg three times daily) in a 3-month trial. After 3 months, there was a significant reduction in HbA1c, fasting blood glucose and postprandial blood glucose in the berberine group in comparison with the metformin group. In another study, 48 T2D patients with poor glycemic control received 500 mg berberine three times daily added to their current medication for a 3 month period. At the end of the study the level of HbA1c, fasting blood glucose and postprandial blood glucose, fasting plasma insulin and HOMA-IR were all decreased [57]. In another study, 116 randomized participants with T2D and dyslipidemia were recruited to consume berberine (1.0 g/day) or placebo for a 3 month period, following which taking berberine had a significant improvement in fasting plasma glucose, the 2-h OGTT plasma glucose and HbA1c. HOMA-IR was decreased in the berberine group, whereas no difference was found in the placebo group [58]. In another double-blind randomized clinical trial study, 31 patients with diabetes were enrolled to receive 3 g/day BVFE (berberis vulgaris fruit extract) or placebo. Comparison of the glycemic indices after 3 months showed that there were a significant reductions in serum glucose, insulin and HOMA-IR between the BVFE and placebo groups [59].

## 13.6 Garlic

Garlic has several active herbal and phytochemicals that may elicit antioxidant activities and has been used for hundreds of years [114]. Garlic has a high amount of organosulfur compounds, such as allicin and flavonoids that may prevent oxidative injury and decrease blood pressure and hyperglycemia, and may be beneficial in the reduction and prevention of CVD and some types of cancers [115, 116]. Preclinical studies showed that garlic has anti-diabetic, anti-obesity, anti-atherosclerotic, anti-carcinogenic and anti-thrombotic properties [117–119]. In a crossover pilot study, 26 participants with T2DM were asked to take 1200 mg of Aged Garlic Extract (AGE) or a placebo daily; however, after 4 weeks, there was no significant difference in HOMA-IR and HbA1c between the two groups [60]. In another recent clinical trial study, 50 diabetic T2DM subjects with dyslipidemia were recruited into two groups: a control group taking a traditional therapy with hypolipidemic and hypoglycemic drugs and intervention group taking traditional therapy and the herbal compound (300 mg garlic). After 12 weeks treatment, the levels of HbA1c decreased significantly in the intervention group, but had no effect on fasting blood glucose [61].

## 13.7 Anthocyanin

Anthocyanin has many bioactive compounds including the flavonoid category of polyphenols seen in berry fruits, including cherry, blueberries, and strawberries [120]. It has been suggested that anthocyanin has multi- health benefits for obesity

and diabetes control, CVD prevention, and recovery of optical and brain functions [120–122]. Anthocyanin, a normal antioxidant, has been considered to lower oxidative stress and to decrease IR and diabetes [62]. In a study, 58 individuals with T2D were assigned to receive 320 mg/day of anthocyanins or placebo for 24 weeks. At the end of the study, a significant reduction in plasma fasting plasma glucose and HOMA-IR levels were observed in the anthocyanins group compared with the placebo group [62]. In a previous clinical trial, 36 patients with T2D were assigned to receive 2 cups of freeze-dried strawberry (FDS) drink (50 g of FDS is equivalent to 500 g of fresh strawberries) or placebo powder with strawberry flavor daily for 6 weeks. The level of HbA1c reduced significantly in the FDS group compared with the placebo group and there was no difference in serum glucose concentrations between two groups [63]. In another study, 85 subjects with T2D were entered to study. The subjects were given fresh pomegranate juice at a dose of 1.5 mL/kg body weight and then blood samples were obtained after 12 h of fasting, 1 and 3 h after the ingestion of the juice. At the end of the study, HOMA-IR reduced in the T2DM patients after 3 h of pomegranate juice ingestion. Response to fasting serum glucose (FSG) was different, patients with lower FSG showed a larger hypoglycemic effect than those with higher FSG [64].

## 13.8 Green Tea

Green tea has a contains many flavonoids and has been reported to have several potential health benefits including anti-cancer, anti-arteriosclerotic, antioxidant and anti-thrombotic effects, as well as beneficial effects to reduce hyperlipidemia, hypertension and hyperglycemia. The main flavonoids of green tea are catechins that constitute almost 22% of green tea [123–130]. Recent animal studies indicate that green tea has a protective effect on glucose homeostasis, high fat-induced hepatic steatosis, IR and inflammation, and the underlying mechanism may involve the AMPK pathway [131, 132]. In a clinical trial, 92 participants with T2D and dyslipidemia were randomized into 2 groups to receive placebo or 1500 mg green tea extract. After 16 weeks, there was a significant reduction in triglyceride and HOMA-IR [65]. In another study, 68 patients with type 2 diabetes were asked to take 1500 mg of a decaffeinated green tea extract (GTE) or placebo for 16 weeks. At the end of the study, there were no significant difference between the two groups [66]. In a randomized controlled trial study, 66 participants with T2DM were assigned to receive a packet of green tea extracts/powder containing 544 mg polyphenols (456 mg catechins) daily for a period of 2 months at the end of which there were not differences between the green tea and placebo groups, but the level of insulin was related to polyphenol intake in the intervention group [67]. In a clinical trial of 55 T2D participants were asked to take 900 ml water containing 9 g of green tea every day for 4 weeks; at the end of the study, there was no effect on insulin resistance [68]. In a randomized controlled trial, a total of forty-nine T2DM participants were assigned to receive 0, 375, or 750 mg of 40% catechins from green tea (150 mg) and 20% aflavins from black tea for 3 months. At the end of the study, there were no differences in HbA1c or a hypoglycemic effect between the 3 groups [69].

## 13.9 Soybean

Soybeans have bioactive ingredients, including isoflavones, saponins, soy protein and flavonoids that have potential benefits for the prevention and treatment of chronic diseases, such as CVD and T2DM [133–135]. In a study, 60 patients with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or newly diagnosed T2D were asked to consume 40 g of a Jerusalem artichoke and fermented soybean powder mixture (19.45 g each) every day or placebo. After 12 weeks, the level of fasting glucose, glucose at 60 min, HOMA-IR decreased in those receiving the Jerusalem artichoke and fermented soybean powder mixture [136]. In another study, 30 participants with T2D were asked to consume 600 mg

soybean-derived pinitol or placebo twice daily. After 13 weeks the results showed that the level of fasting plasma glucose, insulin, fructosamine, HbA1c, and HOMA-IR diminished significantly [71]. In another study, 200 male patients with T2D were asked to consume only 15 g soy protein (SP) every day or 15 g soy protein with 66 mg isoflavones (SPI) every day. After 3 months, results showed a significant linear correlation between the decrease of type I collagen crosslinked beta C-telopeptide in the SPI group with a decrease of HbA1c and HOMA-IR [72]. In a recent randomized controlled trial, 84 diabetic patients were assigned to receive only soy protein (SP) 15 g/day, soy protein (15 g) plus isoflavones (32 mg) (SPI), soy protein (15 g) plus cocoa(400 mg) (SPC), soy protein plus isoflavones with cocoa (SPIC) or placebo given twice daily for 8 weeks. At the end of the study, the level of HbA1c improved in the soy protein group compared with the placebo group [73]. In a previous randomized controlled trial, 32 postmenopausal women with T2D were recruited to two groups to consume soy protein (30 g/day) and isoflavones (132 mg/day) or placebo (30 g/day) for 12 weeks, considering 2-weeks washout periods to separate interventions. Results showed that there was a significant reduction in the levels of IR, fasting insulin, HbA1c, HOMA-IR [74]. In one clinical trial study, 32 postmenopausal women with T2D were asked to receive placebo or soy that included 132 mg isoflavones with 4-weeks washout periods to separate the placebo and active phases (12 weeks each). At the end of the study, there was no significant effect on glucose, HbA1c, and HOMA-IR [75].

## 13.10 Flaxseed

Flaxseed is rich in lignans that have both antioxidant and estrogen-like functions [137]. Flaxseed is rich in α-linolenic acid that may have benefits on CVD risk factors, atherosclerosis, diabetes, metabolic syndrome and dyslipidemia [137–141]. In a randomized controlled trial, 60 patients with diabetic foot ulcer grade 3 were recruited to two groups to consume 1000 mg omega-3 fatty acids from flaxseed oil or placebo twice per day. After 12 weeks, omega-3 fatty acids reduced significantly serum insulin, HOMA-IR, HbA1c and increased significantly the QUICKI compared with the placebo group [76]. In another study, 185 Chinese T2D subjects were assigned to receive fish oil (2 g/day of eicosapentaenoic acid + docosahexaenoic acid), flaxseed oil (2.5 g/day of alpha-linolenic acid), or corn oil (control group) for 180 days. At the end of the study, there was no difference between groups for HOMA-IR, fasting insulin, or glucose [77]. In a recent study on 60 subjects with diabetic nephropathy, participants were randomized into two groups with 1000 mg/day omega-3 fatty acids from flaxseed oil or placebo for 12 weeks. At the end of the study, omega-3 fatty acids significantly reduced the level of insulin, HOMA-IR and increased QUICKI compared with the placebo group [78]. In a another randomized, double-blind, placebo-controlled trial, 48 postmenopausal women with T2DM were asked to consume zinc (40 mg/day) and flaxseed oil (2 g/day). After 12 weeks of treatment, Zinc or flaxseed oil had no significant effects on either glycemia or HOMA-IR [79].

## 13.11 Curcumin

Curcumin a brilliant yellow chemical derived from Curcuma longa L. (turmeric) that has been utilized as a food ingredient for flavor and an old herbal medicine [142]. Many studies have shown that curcumin, as a natural polyphenol, is safe and has beneficial health effects including the reduction of hyperlipidemia, anti-cancer, antioxidant, decrease inflammation, lowering IR, antihepatic, anti-atherosclerotic and cardioprotective antithrombotic, antidepressant and antirheumatic activities [143–155]. In a recent study, 100 individuals with T2D were recruited to receive dietary advice with curcuminoids (500 mg/day with piperine 5 mg/day) or placebo. After 3 months of treatment, serum levels of insulin, HbA1c, and HOMA-IR decreased significantly in both groups, whereas serum levels of glucose and HbA1c reduced significantly after curcuminoids group compared with the placebo group

[80]. In a study, a total of 100 patients with T2D who were overweight or obese were asked to consume curcuminoids (300 mg/day) or placebo for 3 months. At the end of the study, fasting blood glucose, HbA1c, HOMA-IR diminished significantly after curcuminoids supplementation versus the placebo group [81]. In another study, 53 diabetic subjects were assigned to consume 1500 mg curcumin or placebo three times daily. After 10 weeks, curcumin had a significant reduction in FBS but did not affect HOMA-IR, HbA1c and insulin [82]. In a recent double-blind randomized controlled study,64 patients with T2D were randomly recruited to four group: (i) placebo, (ii) curcumin (2 × 500 mg) and placebo matching for long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA), (iii) LCn-3PUFA with placebo matching for curcumin,(iv) curcumin with LCn-3PUFA for 12 weeks. At the end of the study, there was no effect on HbA1c and fasting glucose, but in the curcumin plus placebo matched for LCn-3PUFA (CC) group sensitivity had a significant improvement in triglycerides [83]. In one clinical trial study, 80 patients with T2D were randomized into 2 groups to receive placebo or 80 mg of nano-curcumin for 8 weeks. Results showed that HbA1c and FBC reduced significantly in the nano curcumin compared with placebo group [84]. In another study,100 diabetic patients were asked to consume curcuminoids (300 mg/day) or placebo. After 3 months, the levels of HbA1c, fasting blood glucose and HOMA-IR decreased significantly in the curcumin group compared with the placebo group.

## 13.12 Nuts

It has been shown that nuts contain high amounts of unsaturated fats, soluble fiber, antioxidants and phytosterols that have an effect on serum lipids, blood pressure, blood glucose and inflammation [156]. In a clinical trial, 50 T2DM subjects were randomized into 2 groups to receive 100 mg extract of *Juglans regia* (walnut) leaves or control group for 8 weeks. At post-intervention, there were a significant reductions in the level of postprandial glucose and HbA1c with walnut leaves though there was effect on blood glucose level or HOMA-IR [85]. In another study, 61 participants with T2D were randomized into 2 groups to receive a placebo or 200 mg/day *Juglans regia* leaf extract. After 3 months, Juglans regia reduced significantly the fasting blood glucose level and HbA1c at the end of the study, though there was no significant effect on insulin levels [86]. In a randomized crossover trial, a total of forty-eight T2DM subjects were recruited two groups to consume 50 g pistachio nuts daily or control that did not consume any nuts for a 12 week period, with a 8-week washout periods between interventions. In the first and second phases, fasting blood glucose and HbA1c decreased in the pistachio group, but there was no significant effect on HOMA-IR [87]. In a randomized clinical trial, a total of 54 prediabetic participants randomly recruited into two groups: diet including a supplement of pistachio diet (PD) and a control diet (CD) for 4 months, with a 2-week washout period. The diet for PD included 50% calories from carbohydrate and 35% from fat and containing 57 g/day pistachios, although these percentages for CD were 55% and 30% respectively. At the end of the study, there was a marked reduction in fasting glucose, insulin, and HOMA-IR in the PD compared with the CD [88]. In a crossover clinical trial, 20 Chinese subjects with T2D and mild hyperlipidemia were divided into two groups: an almond diet or control diet for 4 weeks, with a 2-week washout period. At the end of the study, levels of fasting insulin, fasting glucose, and HOMA-IR were lower in the almond diet compared with the control diet [89].

## 13.13 Vegetable Oil

Vegetable oils such as olive oil and canola oil contain a high quantity of Monounsaturated fatty acids (MUFAs) shown to have beneficial effects on blood lipids and inhibition of coronary heart disease, and improvement in insulin sensitivity, lipid peroxidation, and inflammation [157, 158]. In a study, a low-glycemic-load with α-linolenic acid (ALA), MUFA taken as bread that was enriched with 31 g canola oil per 2000 kcal, or a

whole-wheat bread supplement were administrated to 141 T2DM and hyperlipidemic adults for 3 months. Results showed that Hb1Ac decreased in both groups but the reduction was greater in the test diet with canola oil than the control group [90]. In another study, 67 subjects with T2D were asked to take purified eicosapentaenoic acid (EPA) (2 g/day) and corn oil (2 g/day) in the control group. After 3 months of treatment, a significant reduction in plasma fasting plasma glucose, HbA1c, HOMA-IR levels were observed in the EPA group compared with control group [91]. In a double-blind controlled study, 26 T2DM patients were recruited to consume 17.6 mL of fish oil/day in the intervention group and 17.8 ml corn oil/day in the control group. The study examined short-term (1 week) and longer-term (9 week). The mean blood glucose concentrations and fasting blood glucose concentrations were significantly greater after 8 week in the fish oil group compared with the corn oil group. No significant changes were observed in fasting insulin concentrations at baseline, 1 week, and 9 week in both groups [92]. In another study, 54 participants with T2D were asked to consume docosahexaenoic acid (DHA) plus EPA-enriched fish-oil (FOG) (520 mg/day) or placebo for 24 weeks. The result showed that FOG reduced Hb1Ac and insulin; however, HOMA-IR was raised significantly in both groups after the end of the study [93]. In a further study, a total of 30 adults with T2D were randomized into 2 groups to consume a liquid diet enriched with EPA (25 mg/100 kcal) and DHA (17 mg/100 kcal) or liquid diet without EPA and DHA for 3 months. At post-intervention, levels of fasting plasma glucose and HbA1c decreased significantly in the diet with EPA/DHA compared with a diet without EPA/DHA [94].

## 13.14 Soluble Fibers

Soluble fibers have been reported to have several health benefits particularly in obesity, hypertension, diabetes, coronary heart disease, stroke and certain gastrointestinal diseases [159, 160]. It is clear that fiber consumption plays a beneficial role in lessening blood lipids and blood pressure, increasing insulin sensitivity and lowering the prevalence of CVD [161]. In a recent study, thirty-seven T2DM subjects were asked to take diet with medium carbohydrate and low-energy plus 7 g of psyllium powder or a diet with low carbohydrate and low energy plus placebo powder. After 2 weeks, serum fasting plasma glucose and insulin did not change significantly; however, there was a significant reduction in fasting plasma insulin and an increase in HOMA-IR in the intervention group [95]. In another study, 44 T2DM participants with metabolic syndrome were assigned to receive a usual diet plus partially hydrolyzed guar gum (PHGG) in the intervention group or control group with usual diet. The HbA1c decreased significantly in the intervention group after baseline, 4 and 6 weeks; however, no significant changes were observed in fasting plasma glucose in both groups [96]. In a randomized control trial, 40 patients with T2D were randomly recruited into two groups: 10.5 g/day soluble fiber from psyllium in the intervention group and a regular diet in the control group for 8 weeks. At the end of the study, fasting blood sugar, HbA1c, insulin, HOMA.IR and HOMA-β % improved after soluble fiber supplementation versus the control group [97].

## 13.15 Conclusion

This review has comprehensively evaluated the effects of nutraceuticals and some herbal-based bioactive compounds on insulin resistance as well as FBS, HOMA-IR, HbA1c, QUICKI and lipid profiles in human clinical studies. The findings confirm that most of these agents such as resveratrol, garlic, curcumin, cinnamon**,** ginger, nuts, berberine, anthocyanin, soybean, flaxseed, vegetable oils, soluble fibers have beneficial effects on IR and decrease FBS, fasting insulin and HbA1c (Fig. 13.2). However, few studies have shown that green tea has a positive effect on

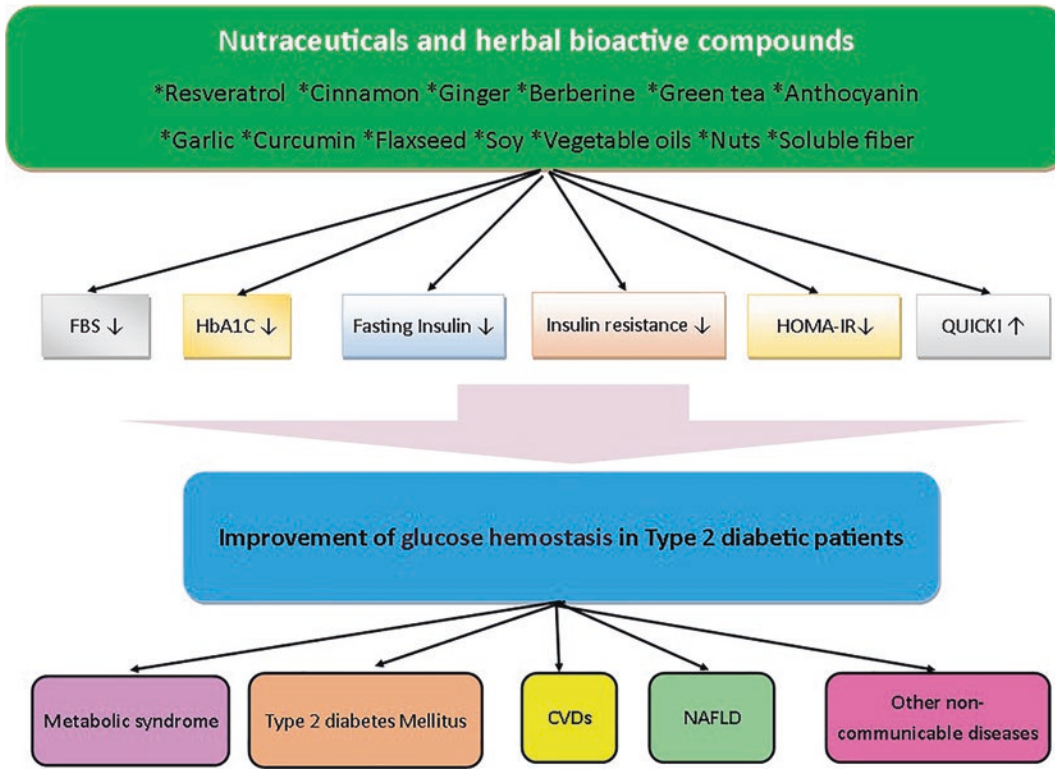


**Fig. 13.2 Schematic summary of pathways depicting the possible effects of nutraceuticals and herbal bioactive compounds on glucose hemostasis in Type 2 diabetic patients and its potential outcomes on non-communicable diseases.** *FBS* fasting blood sugar, *HbA1c* hemoglobin A1C, *HOMA-IR* homeostasis model of assessment for insulin resistance. *QUICKI* quantitative insulin sensitivity check index, *CVD* cardiovascular diseases, *NAFLD* non alcoholic fatty liver disease

IR. However, the data is limited by the number of studies, duration of the intervention and the different dosages and preparations used for each group reviewed. Many of these studies should also be undertaken in those subjects newly diagnosed with T2DM who may have a greater therapeutic response than those with established long standing disease where the response of IR is likely to be less. Therefore, further clinical trials will focus on evaluating the efficiency of other dietary ingredients and nutraceuticals in patients with T2DM with IR, and more definitive studies are needed for the investigation of optimal doses of each the products for their therapeutic effect.

**Conflict of Interests** None

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# 14 Plants with Anti-Addictive Potential

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**Abstract**

Drug addiction is prevalent among individuals of modern society, being a major cause of disability and premature loss of life. Although the drug addiction have profound social, economical and health impact in the world population, its management remains a challenge as available pharmacological treatments remains ineffective for most people. The limited efficacy and adverse effects have led to a search for alternative therapies to treat drug addiction. In this context, natural products are an important source for new chemical substances with a potential therapeutic applicability. Therefore, this chapter will present data obtained after an extensive literature search regarding the use of medicinal plants as a pharmacological alternative for drug addiction treatment.

**Keywords**

Addiction · Drug dependence · Natural products · Substance abuse · Opioid dependence

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## 14.1 Introduction

Drug addiction is one of the leading causes of disability and premature death worldwide, being accompanied by high costs for the world economy, mainly on health care, law enforcement, lost work productivity and other direct and indirect costs [140]. According to the World Drug Report, published by United Nations Office on Drugs and Crime in 2019, 35 million people suffer from drug use disorders and require treatment services in the world. This number increased in comparison with the previous estimate, which was of 30.5 million. In 2017, 585,000 people died as a result of drug use. The most commonly used drug is Cannabis, corresponding to 3.8% of



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the global population aged 15–64, followed by opioids, amphetamines, ecstasy and cocaine [175].

Drug addiction is understood as a chronic relapsing mental disorder characterized by compulsive drug-seeking, loss of control in limiting drug-intake and a negative emotional state when the access to the drug is not allowed. This process is strongly associated with genetic, neurodevelopmental and sociocultural factors [92]. Clinically, the diagnosis criteria for drug addiction/substance-use disorder is established by the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5). In overall, the diagnosis is based on a pathological pattern of behaviors related to the use of the substance [199]. According to DSM-5, the substance use disorder presents on a range from mild to moderate to severe, with severity of an addiction depending on how many of the established criteria are applied [9, 92].

It is widely accepted that most abuse drugs produce their initial reinforcing effects through the activation of brain reward circuits. Chronic drug use impairs brain function, interfering with the ability of self-control over drug-taking behaviors and making the brain more sensitive to stress and negative moods [184]. Dopaminergic neurons located in the midbrain ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc) play a key role in the processing of reward-related stimuli. Drugs with addiction potential, through their action in different molecular targets, increase the dopamine release in the NAc and thus the mechanism through which the brain signals reward [133, 184, 185].

Substance use disorders are chronic conditions that often require long-term management (National Institute on Drug Abuse). At present, the pharmacological treatment for drug addiction primarily targets the specific action site of each drug. For example, the most recommended treatment for opioid-use disorder is opioid partial or full agonists, buprenorphine and methadone, respectively. Another example is the treatment of nicotine addiction by modulating the activity of nicotine receptors [105]. However, to date, there are no medications to specifically treat many drug use disorders, such as those caused by cannabis and stimulants [175]. Furthermore, it is important to mention that many current treatments produce serious side effects [105]. Although drug addiction have profound social, economical and health impacts in the world community, available pharmacological treatments are ineffective for most people [133]. The limited efficacy and adverse effects calls for the search for innovative drug addiction therapies, as recommended by the National Institute on Drug Abuse (NIDA). There is a clear need to develop better treatment strategies that find the biological substrates of addiction across stages, including detoxification, recovery maintenance, and relapse prevention [132].

In this context, natural products can be an important source for new chemical substances with potential therapeutic applicability [28, 108]. About 35% of the 1.1 trillion US dollars annual global medicine market originated directly or indirectly from natural products including plants (25%). With the development of cutting edge technology for chemical analysis and plant propagation natural-derived products constitute an extremely important resource for research and development strategies among global pharmaceutical companies. There are many examples of plant-derived extracts and/or compounds isolated from plants that have been widely used in the treatment of many significant diseases [19]. In addition, most classes of drugs have a substance of plant origin as a prototype molecule, demonstrating the importance and usefulness of studying natural compounds [52].

Among the strategies for the search for new pharmacologically active molecules from plant origin is ethnopharmacology, in which the therapeutic properties alleged by users and specialists of traditional medicine systems for homemade medicinal practices are examined, especially herbal preparations [49]. The herbal medicine, that sometimes is also called as traditional or natural medicine, existed in one way or another in different cultures/civilizations, such as Egyptians, Western, Chinese, Kampo (Japan) and

Greco-Arab or Unani/Tibb (south Asia) [52]. It is believed that about 80% of the population worldwide, especially Asian and African countries use plants and herbal medicines as a source for medicinal agents and primary health care. Traditional medicine is an important form of health care for many people and covers a wide variety of therapies and practices, which vary from country to country [35]. Some traditional medicine systems use plant treatments in the management of drug addiction, as for instance documented in traditional Chinese medicine [125, 166]. Although less common, in the west reports of popular use of plants to treat addiction to alcohol and other drugs are also available and scientific publications indicating plants or its products as potentially useful to cope with drug addiction are increasing.

The ethnopharmacology approach includes a valuable shortcut in drug development, since the traditional use by human communities may be regarded not only as indication of effectiveness, but also of bioavailability and acceptable acute toxicity. Ethnopharmacology seems to be particularly useful in diseases without a clear understanding of the pathophysiological substrates when rational drug design is impossible due to lack of defined drug target, as in the case of drug addiction. Another strategy that can be used to investigate products of plan origin is chemotaxonomy, based on the observation that the occurrence of certain chemical compounds is restricted to certain groups of plants. Chemotaxonomy can represent a set of data of great validity in the medical and pharmaceutical fields, which combined with ethnopharmacology, can facilitated the discovery of new drugs of plant origin [57]. The search for derivatives or structurally similar substances due to the chemotaxonomic proximity of a species traditionally used for a given purpose is an example of the combination of ethnopharmacology and chemotaxonomy [47]. Therefore, giving this context, this chapter will present and discuss data obtained after an extensive literature search regarding the use of medicinal plants as a pharmacological alternative for drug addiction treatment.

## 14.2 Plants Containing Alkaloids as Active Constituents

Alkaloids constitute a large and diverse group of nitrogen-containing secondary metabolites, displaying numerous important pharmacological and physiological effects on vertebrates. Typically, these low molecular weight, heterocyclic compounds exhibit a limited distribution in nature and are called plant toxins due to fact they exert protective effects in plants, acting as a protective chemical strategy against predators and pathogens. Due to their potent biological activities, a number of alkaloids are medicinal agents of inestimable therapeutic status in current clinical use, such as the hypnoanalgesics morphine and codeine, obtained from opium poppy plants (*Papaver somniferum*), the anti-cancer vincristine from periwinkle (*Catharanthus roseus*) and the sedative scopolamine isolated from Solanaceae species. Other well-known alkaloids include the psychostimulant agent caffeine, present in daily beverages, nicotine in tobacco, and cocaine, used for both medicinal and recreational purposes, obtained from leaves of *Erythroxylum* species growing in South America. Although a number of medicinal plants containing promising bioactive alkaloids are still used only in folk medicine, many species are supplied to pharmaceutical industries to produce extracts and derived herbal medicines end-products. Important examples are goldenseal (*Hydratis canadensis*), source of berberine and used to reduce symptoms of cold, flu and sore throat, and boldo (*Peumus boldus*), frequently used in phytomedicines to relieve digestive complaints. Considering their modulatory effects on CNS, many alkaloids are also called neuroactive molecules, binding and interacting to specific receptors due to the presence of a nitrogen-containing core, which resembles the structure of neurotransmitters (for further readings, see [118]).

### 14.2.1 *Mitragyna speciosa (Korth.)* Havil (Rubiaceae)

Kratom is the common name of a medicinal plant native to Southeast Asia (*Mitragyna speciosa*) and cultivated in Myanmar, traditionally used by

local communities in Thailand and Malaysia as a mood enhancer to increase work performance. The fresh leaves of kratom tree are typically chewed or smoked, used in herbal decoctions or even swallowed when dried and ground leaves are incorporated into beverages and food to masker their bitter taste [124]. Cultural and recreational uses are associated to their putative opioid and non-opioid effects, producing stimulant effects similar to classical opioids. Early reports claimed that heavy users of kratom could undergo intense fatigue situations even under extreme hot weather. Side effects in kratom consumers are reportedly less severe compared to the effects of poppy opioids, undergoing less mental and physical impairment disconnected to the stigma associated to opioid abuse. Several monoterpene indole alkaloids, sharing a corynanthe-type core are described in *Mitragyna speciosa*, being mitragynine (Fig. 14.1a) and 7-hydroxymitragynine (Fig. 14.1b) the main bioactive compounds [4]. In terms of concentration, mitragynine is the major alkaloid in kratom, present in higher quantities in leaves collected in Thailand (66%) than in those collected in Malaysia (12%). Although the kratom alkaloids do not share the structure of morphine and other opioid analgesics, mitragynine and their analogues produce euphoria, induce sexual desire and stimulation effects, being a sedative-narcotic at higher concentrations [11, 177, 188]. Prolonged use of kratom preparations is associated with tremors, convulsions and psychosis symptoms. Such effects are associated to its potent full agonist activity on μ and κ opioid receptors, 13 times more potent than morphine [117]. Additionally, 5-$HT_{2a}$ and postsynaptic α2-adrenergic receptors as well as neuronal calcium-channels blocking are associated to the pharmacological activity of mitragynine [116]. Due to their alleged addictive properties, kratom is tested in non-medically supervised treatment of opioid abstinence syndrome [187]. Currently, kratom use is prohibited in Malaysia and criminalized in Thailand. In U.S., it is considered a legal opioid replacement of easy obtainment in some states, despite the fact that its use remains unregulated by FDA. Preclinical investigations conducted with *Mytragina speciosa* aqueous extracts demonstrated a remission on withdrawal symptoms following the cessation of chronic alcohol consumption in mice and the reduction of jumping behavior induced by naloxone in rodents on a morphine withdrawal syndrome model [29, 183, 196]. However, a number of case reports detail abuse potential and important side effects including seizures and hepatotoxicity in kratom users. In 2017, the U.S. Centers for Disease Control and Protection (CDC) notified 152 deaths involving the use of kratom, especially prevalent in heroin and fentanyl users who co-administer this drug along with other opioids [182]. To date, no scientific evidences demonstrating the safe use of kratom in opioid-dependent patients to reduce morphine intake have been found in scientific literature. As such, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) controls *Mitragyna speciosa* and mitragynine both sell and usage in a number of E.U. countries. Neither *Mitragyna speciosa* nor mitragynine or other kratom alkaloids are currently listed in any of the

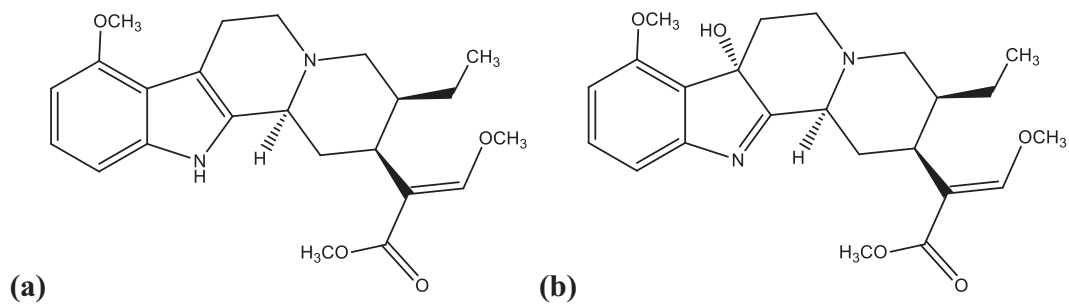



**Fig. 14.1** Structures of the bioactive kratom alkaloids: (**a**) mitragynine and (**b**) 7-hydroxymitragynine

Schedules of the United Nations Drug Conventions [4].

### 14.2.2 *Tabernanthe iboga* Baill. (Apocynaceae)

The iboga plant (*Tabernanthe iboga*) is a shrub native to West Africa, found in Gabon and Cameroon, traditionally used in rituals of the Bwiti religion by shamans who are said to detain the knowledge about the psychoactive properties of the plant. Their ceremonies involve the preparation of a potion made with root barks of *Tabernanthe iboga*, which can be used in infusions or also be chewed [76]. In a religious context, the ingestion of the preparations in higher doses produce the characteristic psychoactive effects, including hallucinations and the power to connect with spirits of the ancestors in rites of passage [34]. The root barks of iboga contain a number of different indole monoterpene alkaloids, which includes ibogaine, ibogamine and tabernanthine. Among these, ibogaine (Fig. 14.2a) is the major alkaloid in plant material and herbal preparations, found in concentrations up to 80% and being considered the bioactive compound of iboga. The pharmacological properties of ibogaine include psychedelic, hallucinogen and oneirogenic effects, leading to a waking or lucid dream state of consciousness. The mechanism of action for both ibogaine and its metabolite noribogaine (*O*-desmethylibogaine) is complex, acting as antagonist on NMDA glutamate and α3β4 nicotinic receptors, agonist on κ- opioid receptor and 5-$HT_2$, 5-$HT_3$ [97, 111]. On μ-opioid receptors, ibogaine behaves as a weak or partial agonist [200]. The modulation of opioids receptors is connected to the psychoactive effects, while the hallucinogenic effects seem to be connected to the serotonin $5HT_{2A}$ receptor weak agonist modulation [64]. Ibogaine has been used since 1960s in non-medical detoxification settings as an anti-craving agent, reducing the nicotine, cocaine, alcohol, methamphetamine and opioids dependence. This alkaloid was found to decrease accumbal dopamine release and morphine-induced increases of dopamine extracellular levels in the nucleus accumbens, consistent with a putative anti-addictive action [55]. Several preclinical trials conducted in animal models showed reductions in withdrawal signs in morphine-dependent animals, on drug-induced conditioned place preference (CPP) paradigms and on self-drug administration for morphine, alcohol, amphetamines, nicotine and cocaine [8, 25, 54, 154]. Additionally, ibogaine seems to cause a potential reduction in the amount of drug self-administration in animals in a strong and long-lasting manner. Noteworthy, the metabolite noribogaine has about a ten-fold higher affinity for the serotonin transporter than the precursor ibogaine and consistently, noribogaine is more potent than ibogaine in raising extracellular levels of serotonin in the nucleus accumbens [13, 115]. The negative effects connected to ibogaine administration in rodents include impaired motor function and cerebellar Purkinje cell loss in high dosages intraparenterally administered [190]. The efficacy of ibogaine as an alternative for the treatment of opioid addiction has been extensively discussed due to the morbidity and mortality observed with the exponential current consume of illicit drugs worldwide. The administration of low ibogaine doses associated with narcotic painkillers was already proposed for pain management, in order to reduce the use of opioids such as oxycodone. Due to the illegality of ibogaine as a medi-

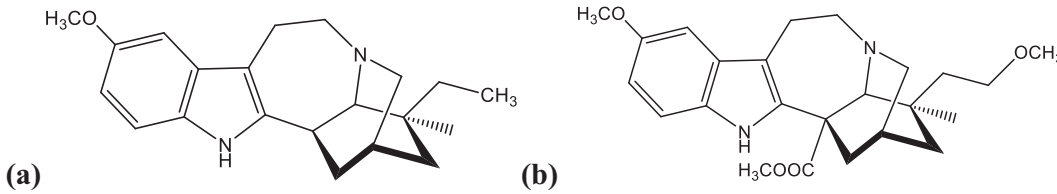



**Fig. 14.2** Structures of the iboga-related compounds: (**a**) ibogaine, (**b**) 18-methoxycoronaridine

cal treatment, clinics offering non-regulated applications of the compound for the treatment of opioids craving are spread in many countries, offered in clandestine scene and causing many fatalities. The main reasons rely on the potentially fatal cardiac arrhythmias, tremors, toxicity and acute depressant effect on responding for water observed in pre-clinical assays following ibogaine administration [91]. As such, synthetic 18-methoxycoronaridine (18-MC, Fig. 14.2b) emerged as a derivative to ibogaine with a similar activity on opioid withdrawal signs, decreasing the intravenous self-administration of morphine and cocaine and the oral self-administration of ethanol and nicotine in rats [122, 138, 156]. These alkaloids have similar affinities for μ-and κ-opioid receptors and for 5-$HT_3$ receptors, attenuating the amphetamine-induced euphoria in humans and cocaine-induced locomotion, alcohol intake, and morphine withdrawal signs in rodents. Conversely, due to the lower affinity for sigma-receptors, fewer side effects are associated to the use of 18-MC, including the neurotoxicity evoked by ibogaine use.

Iboga and ibogaine use for drug addiction remains illegal in several countries, where these substances are used without quality control or a close monitoring of a qualified medical staff. In U.S., FDA declined additional studies with ibogaine due to death reports following an approved clinical trial in humans in 1993. Because of the banned status for ibogaine, 18-MC is supported as an alternative for opioids addiction, with several controlled clinical trial conducted worldwide. Additionally, *Tabernanthe iboga* and ibogaine were added to the list of controlled substances in France and is scheduled in nine countries in the European Union. In other countries, ibogaine is unregulated, except for Brazil, New Zealand, and South Africa where it is regulated as a medicinal substance for use by licensed medical practitioners [34]. Some clinical trials supervised by physicians and accompanied by psychotherapy suggest ibogaine as a safe and effective treatment for dependence on non-opiate users, including alcohol, cannabis, cocaine and crack. Prolonged periods of abstinence without complications or fatalities were observed, and some authors suggest oral and low dosages of ibogaine to minimize heart arrhythmias and deaths.

### 14.2.3 *Areca catechu* L. (Arecaceae)

Areca nuts are used in the composition of betel quid, a popular herbal masticatory in Asia Southeastern countries, mainly due to its psychoactive properties. Chewing betel nuts is also considered a more socially accepted habit than the use of other types of drugs, causing a perception of well-being, hot sensation in the body and an increased capacity for work [143]. Currently, areca nuts are considered the fourth most popular mind-altering product used worldwide, after nicotine, alcohol and caffeine. However, the prolonged consume may cause addiction and is associated to a number of systemic effects, such as an increased risk of oral cancers, heart attack and other conditions [58]. Arecoline, arecaidine, guvacoline and guvacine are pyridine-type alkaloids found in betel nuts, for which the addictive and carcinogenic effects are attributed. Arecoline is the major alkaloid, able to rapidly cross the blood-brain barrier and exert cholinomimetic effects. In brain, both arecoline and guvacoline behave as muscarinic agonists on acetylcholine receptors, but only arecoline active nicotinic receptors, acting as a competitive inhibitor of gamma-aminobutyric acid (GABA) neurotransmitter, thus causing parasympathetic symptoms as euphoria, salivation and agitation [68]. Withdrawal symptoms are also observed in Areca chewers, including mood swings, anxiety and insomnia [104]. Additionally, hydrolysis of arecoline (Fig. 14.3a) and guvacoline (Fig. 14.3b) produces arecaidine (Fig. 14.3c) and guvacine (Fig. 14.3d), respectively, which are inhibitors of GABA uptake [53].

Some previous studies conducted with a dichloromethane fraction of Areca nuts indicated a reduction on withdrawal symptoms and decrease the number of jumping of naloxone-precipitated morphine withdrawal in mice [94]. The mechanism of action proposed *in vivo* and *in vitro* was a MAO-A inhibitory effect similarly to moclobemide, thus promoting antidepressant

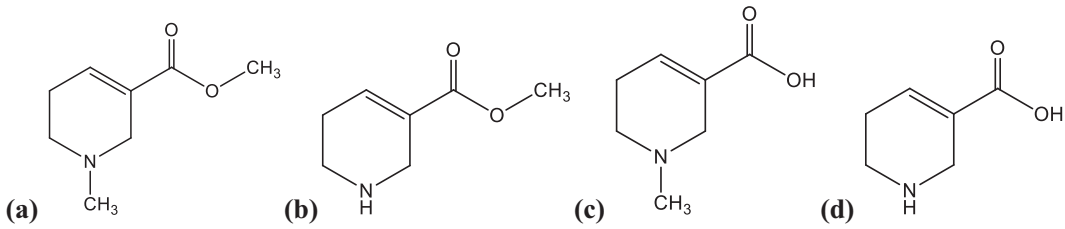



**Fig. 14.3** Structures of the Areca compounds: (**a**) arecoline, (**b**) guvacoline, (**c**) arecaidine, and (**d**) guvacine

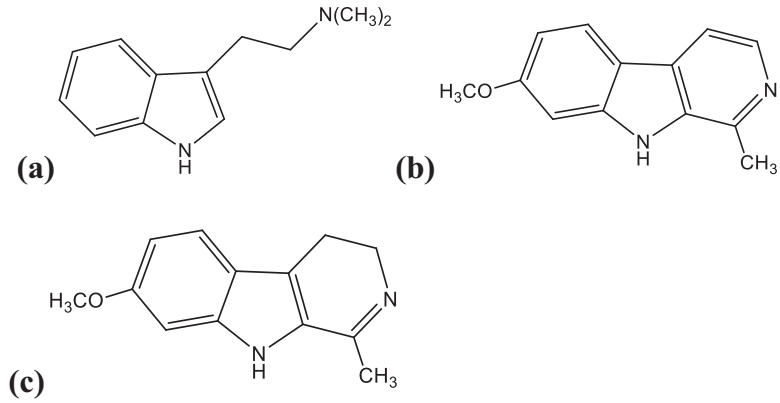



**Fig. 14.4** Structures of the ahayuasca alkaloids: (**a**) DMT, (**b**) harmine and (**c**) harmaline

activity. Because Areca alkaloids were not found to inhibit MAO-A, some authors attribute this effect to unknown phenolic compounds present in plant extract.

### 14.2.4 Ayahuasca - *Psychotria viridis* Ruiz & Pav. (Rubiaceae) and *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton (Malpighiaceae)

The ayahuasca consists of a hallucinogenic brew prepared as a decoction of a mix containing several medicinal plant species, traditionally consumed by Northwestern Amazonian tribes from Brazil, Colombia, Ecuador and Peru due to their healing and spiritual purposes. After oral ingestion, religious leaders and shamans are said to facilitate healing, prophesy, and divination, which spread the use of ayahuasca in ritual ceremonies from syncretic religions as Santo Daime. Among the plant species used for the preparation, stems of *Banisteriopsis caapi* and leaves of *Psychotria viridis* are the main components, which may also include Solanaceae plants as *Brugmansia* sp. and tobacco [123]. *P. viridis* leaves are rich in indole alkaloids, including the psychoactive *N,N*-dimethyltryptamine (DMT, Fig.14.4a), a tryptamine derivative that induces a rapidly altered state of consciousness when smoked or snorted. *B. caapi* is a jungle vine containing β-carboline alkaloids such as harmine (Fig. 14.4b) and harmaline (Fig. 14.4c), all of them reversible monoamine oxidase-A inhibitors (IMAO-A) [102]. DMT is a serotonergic agonist, especially for 5-$HT_{1A/2A/2C}$ receptors, but is inactivate when orally administered due to peripheral (gastrointestinal and liver) metabolism by MAO-A. However, the combination of both species produces pharmacological responses due to the synergic effect, caused by the enzymatic inhibition of MAO by harmane alkaloids, thus preserving the DMT structure. Following the ingestion, the psychoactive effects of ayahuasca typically last six to 12 h, promoting symptoms that may include nausea and diarrhea, intensification of emotions, introspection, positive mood and sense

of well-being [157]. Long-term consumption of ayahuasca may induce alteration in brain cortex thickness, but cognitive or psychiatric disorders were not described. Culturally, members from religious groups incorporating ayahuasca in their rituals report loss of interest in typical use of addictive drugs, including cocaine, barbiturates, amphetamines, solvents, tobacco and alcohol, suggesting the intake of the brew and its alkaloids as anti-addictive agents [28, 48]. Animal models and preliminary clinical evidences suggest the use of these alkaloids for major depression, anxiety and addiction treatment. Results confirmed that DMT and β-carbolines present in ayahuasca showed antidepressive and anxiolytic effects in both humans and animals, due to the serotonergic receptor agonist effects, besides the MAO inhibitory activity [62]. After ingestion in healthy volunteers, ayahuasca also promoted an increase of plasma cortisol levels, which are altered in patients with major depression [45].

A number of anecdotal reports from psychotherapeutic centers using both the brew or ayahuasca alkaloids in the treatment of drug dependence indicate promising beneficial effects, but controlled studies are still needed to confirm such results [135]. As stimulation of 5-$HT_{2A}$ receptors can reduce dopamine release in the mesolimbic, nigrostriatal, and mesocortical pathways, it is presumed that binding of DMT at serotonergic receptors is able to decrease the release of dopamine, reducing activity in the reward or pleasure center of the brain. Result from a preliminary observational study of ayahuasca-assisted treatment for problematic substance use and stress conducted in Canada highlights ayahuasca as a potential treatment for cocaine dependence, with a statistically significant reduction in use (by self-report) that is greater than the reduction in either tobacco or alcohol use [176]. Ayahuasca is also reported to reduce also addiction in alcoholic patients, but results remain inconclusive. Studies reveal that ayahuasca prevented significantly the development of alcohol-induced behavioral sensitization in mice, reversing long-term drug effects expression and inhibiting the reinstatement of alcohol-induced behavioral [136]. The effect of the beverage on alcohol intake in rats was evaluated after 8 weeks of intermittent access to alcohol, during which animals received an administration of ayahuasca extract in the last 5 days [134]. Ethanol intake remained unchanged compared to the baseline level, but ayahusca reduced c-fos expression increased due to drug stimulus.

The use of ayahuasca is legal in Brazil in the context of religious use, since therapeutic use needs further evidences. Out of South America it is possible to use it legally also in the Netherlands, but syncretic churches exist in many European countries. Ayahuasca community is well-established internationally, enabling commercialization and availability almost worldwide [67].

### 14.2.5 *Coptis japonica* Makino (Ranunculaceae)

*Coptis japonica* is a medicinal plant native from Asia used as an anti-bacterial and anxiolytic agent due to the presence of benzylisoquinoline alkaloids in roots such as berberine and palmitine. Among them, berberine (Fig. 14.5a) is the main product, associated to a wide range of pharmacological properties including anti-inflammatory and anti-amnesic actions [59]. This compound caused a decrease in dopamine content in neuronal cells by inhibiting tyrosine hydroxylase activity, suggesting a possible modulation on morphine-induced adverse effects *in vivo*. Studies revealed that pre-treatment with a methanolic extract of *Coptis japonica* significantly reduced morphine-induced CPP in mice, through a mechanism that may involve the modulation of c-Fos proteins, p-CREB expression, dopaminergic and glutamatergic systems [96, 98]. Berberine administration blocked the depression and anxiety behavior of opioid abstinence in mice, blocking the increase in hypothalamic corticotropin-releasing factor expression, the tyrosine hydroxylase expression in locus coeruleus and decreasing the hippocampal brain-derived neurotrophic factor (BDNF) [100]. Berberine is also found to reduce the ethanol-induced CPP, modulating induced rewarding effects in mice. Coptisine (Fig. 14.5b) is other

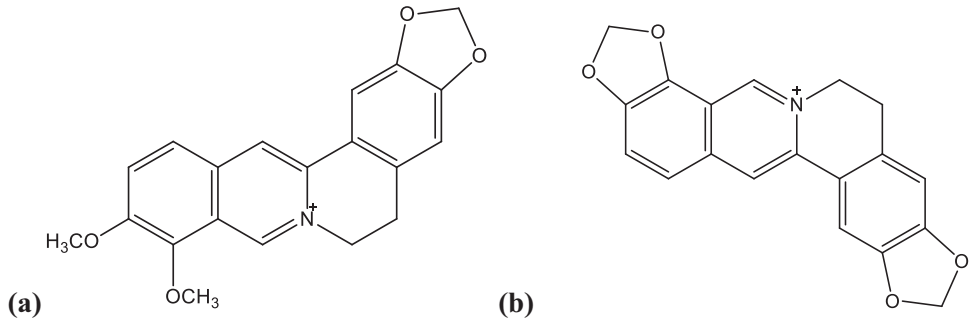



**Fig. 14.5** Structures of the *Coptis* alkaloids: (**a**) berberine and (**b**) coptisine

alkaloid found in *Coptis japonica* and associated with the reduction of morphine withdrawal symptoms in mice, due to the *in vitro* MAO-A inhibitory activity described.

### 14.2.6 *Papaver rhoeas* L. (Papaveraceae)

Aerial parts of corn poppy are commonly used in preparations as a pain reliever and for cough and sleep disorders. Among the compounds described for the species, (−)-tetrahydropalmatine, and a number of benzylisoquinoline and papaverine-type alkaloids were found, contributing for the pharmacological activities described. The administration of a hydroalcoholic extract of *Papaver rhoeas* before morphine injection in opioid-dependent mice demonstrated a reduction on jumping and diarrhea, due to a mild opioid effect and antagonism in dopaminergic and cholinergic systems [146]. The chronic intraperitoneal administration of the same extract also reduced the acquisition and expression of morphine-induced behavioral sensitization in mice [162].

### 14.2.7 Isoquinoline Alkaloids

Tetrahydropalmatine (THP, Fig.14.6a) is a bioactive alkaloid present as a racemic mixture in herbal preparations with analgesic and sedative activities, consisting of the species *Corydalis ambigua* Cham. & Schltdl. (Papaveraceae) and *Stephania tetrandra* S. Moore (Menispermaceae) [172]. The levo isomer (−)-THP, known as rotundin, has an important role in the therapeutic effects of these preparations and its medicinal use as a non-opioid analgesic and anxiolytic agent is approved by the Chinese government [172, 186]. This isomer is a selective dopaminergic antagonist, with a strong affinity for D1 receptors and modulating both D2 and D3 receptors [114], suggesting that it can be useful for chemical dependence treatment. Additionally, interaction with α-adrenergic and GABAergic systems are reported for this alkaloid [61]. On the other side, the dextro isomer (+)-THP causes a selective dopamine depletion, associated with the toxicological profile described for the administered preparations [106]. Pre-clinical evidences support the use of (−)-THP in the treatment of cocaine addiction, due to an attenuation in cocaine reinforcing and rewarding effects in rat models. The administration of this alkaloid also reduced the hyperlocomotion and climbing behavior induced by methamphetamine in rodents, associated with the regulation of 5-HT neuronal activity and dopamine D3 receptor expression [194]. A number of clinical trials conducted in China with (−)-THP showed promising results due to its ability in reducing heroin craving and promoting detoxification in addicts [191]. The clinical examination for this compound as effective in human cocaine addict populations is still required. Of note, preparations containing this compound have illegal status in U.S. and therefore have poor quality and some toxic effects associated.

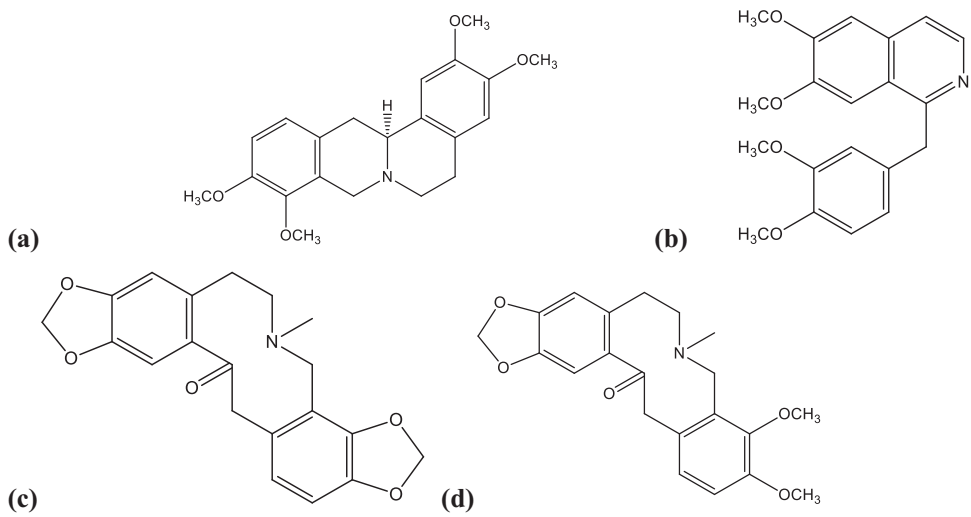



**Fig. 14.6** Structures of the isoquinoline alkaloids tetrahydropalmatine, THP (**a**), papaverine (**b**), protopine (**c**) and allocryptopine (**d**)

The opium alkaloid papaverine (Fig. 14.6b), a smooth muscle relaxant, demonstrated positive effects due to prevention or reversal of naloxone-precipitated withdrawal contractures in an *in vitro* acute morphine-dependent guinea-pig ileum model [22]. Such an effect was attributed to a putative effect on μ and κ opioid receptors and interaction with cholinergic system.

Some alkaloids isolated from the South American medicinal species *Argemone mexicana* L. (Papaveraceae) and *Aristolochia constricta* (Aristolichiaceae) produced a significant influence in the guinea-pig ileum contraction induced by opiate withdrawal [24]. In this study, the isoquinoline alkaloids exerted their effects as agonists on both μ and k opioid receptors, while the protopine alkaloids reduced morphine withdrawal due to their anticholinergic properties. The major compounds found in *Argemone mexicana* are protopine (Fig. 14.6c) and allocryptopine (Fig. 14.6d), so this effect can be associated with these isoquinoline alkaloids [23].

### 14.2.8 *Camellia sinensis* L. Kuntze (Sapindaceae)

The aminoacid L-theanine (Fig. 14.7a) is found in green tea leaves and marketed as a dietary supplement in U.S. to reduce stress and improve cognition. Using a model for spontaneous opioid withdrawal in human opioid addicts, the administration of this compound attenuated opioid-withdrawal signs in morphine-dependent rhesus monkeys and produced anxiolytic-like effect in mice without affecting the motor behavior, with a quick onset and duration of action persisting for 2.5 hours. [189]. Moreover, a number of clinical studies conducted with L-theanine confirmed its positive effects on stress-related conditions, depression and anxiety [65, 66]. The mechanism of action purposed involves antagonistic effects on NMDA receptors and increase on levels of GABA, dopamine and serotonin [78, 90, 193]. On the other hand, caffeine (Fig. 14.7b), a methylxanthine component of green tea, increased withdrawal signs in morphine-dependent rats producing opioid-like symptoms [83], precipitated withdrawal signs in opioid-addicted monkeys and induced opioid withdrawal signs in some normal monkeys [3]. Theophylline (Fig.14.7c) is known to antagonize morphine antinociceptive effects and produced quasi-abstinence opioid signs, intensified by naloxone and suppressed by heroin [32], proving that adenosine antagonists influence opioid withdrawal, especially at A1 receptors [21]. L-theanine and methilxantines occur concomitantly in green tea

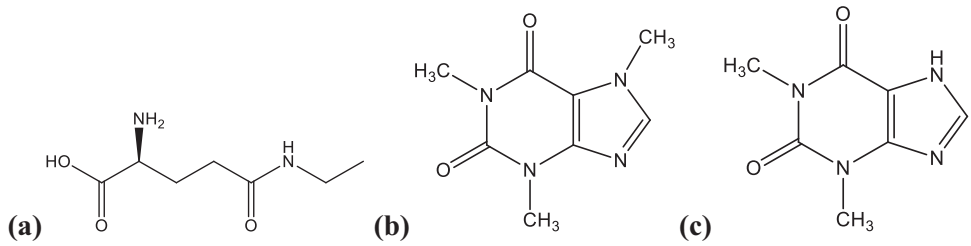



**Fig. 14.7** Structures of the green tea compounds L-theanine (**a**), caffeine (**b**), theophylline (**c**)

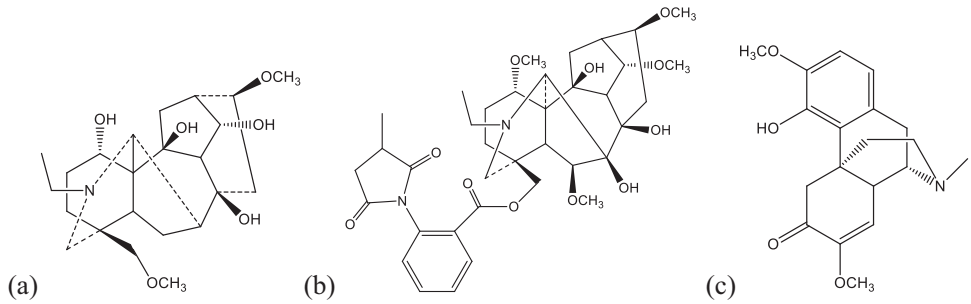



**Fig. 14.8** Structures of jadwarine-A (**a**), methyllycaconitine (**b**) and sinomenine (**c**)

preparations, and L-theanine has been shown to inhibit caffeine's excitatory effects at the concentration regularly associated with drinking tea [77]. However, studies related to the effects of *Camellia sinensis* effects on opioid withdrawal signs in rodents and humans remain to be performed.

### 14.2.9 Other Species

*Brugmansia arborea* (L.) Lagerh. (Solanaceae) is a species used in folk South American medicine as an analgesic and antispasmodic, which contains a number of tropane alkaloids as atropine, scopolamine and nor-hyoscine. All of the compounds significantly reduced *in vitro* morphine withdrawal on guinea-pig ileum in a dose-dependent manner, possibly due to their anticholinergic activity [20]. Extract, fractions and isolated alkaloids were *in vivo* assayed in opioid-dependent mice, attenuating the development and expression of dependence with no effects on acquisition of morphine tolerance [121]. A methanol extract of *Brugmansia arborea* attenuated in part the morphine-induced motor activity and blocked the CPP induced by morphine in mice [16]. The same study reported that cocaine-induced hyperactivity was also abolished by the extract, with no effects on cocaine-induced CPP, demonstrating a complex mechanism of action for *B. arborea*, possibly by modulation of the dopaminergic and cholinergic systems.

The dried roots of *Delphinium denudatum* Wall. ex Hook. f. & Thomson (Ranunculaceae), known as Jadwar, have several medicinal uses in Asia as a pain reliever, anticonvulsant, sedative and anti-fatigue agent [36]. The concomitant oral administration of an aqueous *Delphinium denudatum* extract with morphine caused a dose-dependent attenuation on the naloxone-precipitated jumping in mice, therefore reducing withdrawal symptoms [197, 198]. Some norditerpenoids alkaloids were described for the species, such as jadwarine-A (Fig. 14.8a), showing a competitive inhibitory effect for acetyl and butyrylcholinesterase activities [5]. The putative mechanism of action seems to be independent to opioid receptors and associated with cholinergic and blocking activity at α7-type neuronal nico-

tinic receptor, as reported for the main alkaloid, methyllycaconitine (Fig. 14.8b) [149].

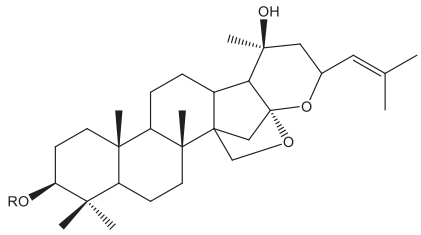
*Sinomenium acutum* (Thunb.) Rehder & E.H. Wilson (Menispermaceae) dried stems (Caulis sinomenii) are used for thousands of years in Tradicional Chinese Medicine for the treatment of rheumatic diseases. Extracts of Calis sinomenii and its main alkaloid, sinomenine (Fig. 14.8c), were evaluated on morphine-induced-CPP in mice [126]. The results showed a suppression on morphine place preference and the modulation of histamine brain levels in morphine-dependent mice.

## 14.3 Plants Containing Triterpenes and Steroids as Active Constituents

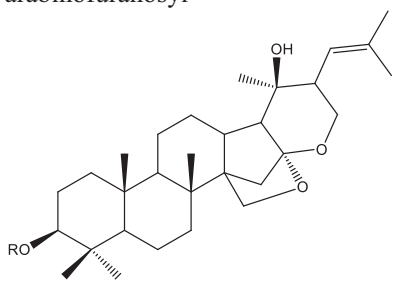
Terpenoids constitute a large and diverse group of secondary metabolites derived from isoprene units ($C_5$) linked in a head-to-tail manner. Usually, isoprene units are synthetized from mevalonic acid pathway, but an alternative biosynthetic pathway derived from 1-deoxy-D-xylulose-5-phosphate is also described. The extension of isoprene units generates structures multiples of C5, which can be later cyclized or chemically modified. The squalene (C30) is a precursor of triterpenoids and steroids, produced after a series of cyclization and additional reactions. Among the plant-derived compounds of pharmacological importance belonging to this class, saponins and cardioactive glycosides are the main metabolites with current clinical use. Some examples of medicinal plants containing triterpenes and steroids as active components are liquorice roots (*Glycyrrhiza glabra* L., Fabaceae), source of glycyrrhizin, a saponine with reported corticosteroid-like activity and sweet taste and used as demulcent, mild expectorant and for inflammatory conditions and *Digitalis* spp., source of digitoxin and digoxin administrated for congestive heart failure. For further reading, see Devick [40].

### 14.3.1 *Bacopa monnieri* (L.) Wettst (Plantaginaceae)

In the Indian subcontinent, *Bacopa monnieri* is a common medicinal species used in Ayurvedic medicine as a brain tonic, for memory loss, insomnia and a number of neuropsychiatric disorders. Sterol glycosides were described for the plant, but the pharmacological effects are associated to the presence of dammarane triterpenoid glycosides, mainly bacoside A, the bioactive compound. Further studies characterized this compound as a mixture of four saponins (bacoside A3, bacopaside II, bacopaside X and bacopasaponin C, Fig. 14.9). The administration in mice of a polar extract of *Bacopa monnieri* containing bacoside A3 significantly reduced both expression and development of tolerance to morphine analgesia [151]. The extract blocked the effect of calcium channels, resulting in the modulation of adenyl-cyclase activation by opioid receptors. The same extract demonstrated a decrease in the morphine withdrawal-induced hyperactivity in mice. Samples of mice striatal tissue were also analyzed for dopamine, serotonin and their metabolites, and the result was significant lowered levels of 3,4-Dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), dopamine and 5-Hydroxyindoleacetic acid (5-H1AA) induced by morphine, therefore demonstrating an antidopaminergic/serotonergic activity [152]. Chronic administration of a standardized *Bacopa monnieri* extract (4 μg bacoside A3/mg extract) significantly inhibited opioid withdrawal induced depression in mice [153]. A potent antioxidant activity was also demonstrated for the extract, exerting a protective effect against morphine-induced cerebellar toxicity and attenuating histopathological changes [165]. The beneficial effects of *Bacopa monnieri* extracts on morphine-withdrawal symptoms seem to be associated with the antioxidant, anticholinergic and calcium-channel antagonist activities of the saponins.




Bacoside A3 R= α-L-arabinofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]- β-D-glucopyranosyl
Bacopaside X R= α-L-arabinofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]- α-L-arabinofuranosyl




Bacopaside II R= α-L-arabinofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]- β-D-glucopyranosyl

Bacopasaponin C = R=α-L-arabinofuranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]- α-L-arabinofuranosyl

**Fig. 14.9** Structure of bacoside A

### 14.3.2 *Panax ginseng* C.A. Meyer (Araliaceae)

The roots of wild ginseng have been used in Chinese Traditional Medicine preparations over centuries as a stimulant or an "elixir of life". The bioactive products found in ginseng are the triterpene saponins known as ginsenosides, containing tetracyclic and pentacyclic cores. Standardized ginseng extracts are used in a number of phytomedicines used with alleged therapeutic effects as adaptogens, reducing the stress-related symptoms. Studies suggest that ginseng saponins prevent behavioral hyperactivity induced by psychomotor stimulants, including nicotine, morphine and cocaine. The chronic administration of ginseng extract was able to inhibit the analgesic and hyperthermic effects of morphine [15]. A standard ginseng extract also mitigated the morphine-induced dopamine receptor supersensitivity, the development of morphine-induced tolerance and physical dependence in mice [87]. Moreover, the morphine-induced antinociception was prevented by pretreatment with ginseng total saponins due to their non-opioid interactions. In fact, catecholaminergic and serotonergic mechanisms are involved in the antagonism of morphine-induced antinociception by ginseng in mice. Administration of wild ginseng extract proved to be effective at inhibiting the anxiety and depression behaviors associated to morphine withdrawal, in response to a modulation of corticotrophin-releasing factor and neuropeptide Y systems on hypothalamus [99]. The mechanism of action for ginseng saponins on morphine withdrawal symptoms seems to be associated to multiple pharmacological interactions between dopamine receptors and a serotonergic/adenosine $A_{2A}$/δ-opioid receptor complex [88]. The ginseng metabolites also seem to modulate the GABA

receptor complex, ameliorating the morphine dependence. Studies revealed that the ginsenosides inhibit the morphine-6-dehydrogenase, responsible for the conversion of morphine into its toxic metabolite, morphinone, playing an important role in the development of both morphine-induced analgesic tolerance and dependence. The effect is therefore associated with morphine detoxification process, increasing the hepatic glutathione levels. Wild ginseng also showed significant inhibition on morphine-induced hyperactivity, increasing the c-Fos expression in nucleus accumbens and the expression of tyrosine hydroxylase enzyme in ventral tegmental area. Studies with both crude extracts and isolated ginsenosides showed significant results on morphine withdrawal symptoms [192]. The chronic administration of ginsenoside Rg1 (Fig. 14.10) significantly improved the spatial learning capacity impaired by chronic morphine administration and reversed the long-term potentiation impaired by morphine, restoring neural plasticity due a mechanism dependent to NMDA receptors [147].

Studies described that the administration of ginseng total saponins during nicotine treatment in mice prevented not only nicotine-induced hyperactivity and CPP but also postsynaptic dopamine receptor supersensitivity [86]. Further investigations analyzed the effect of ginseng saponins on dopamine release in the striatum of freely moving rats induced by nicotine administration, using the microdialysis technique [168]. Results showed that the extract inhibited the striatal DA release stimulated by local infusion of nicotine, possibly due to a modulation on presynaptic nicotinic acetylcholine receptors or receptor-operated $Na^+$ channels in dopaminergic nerve terminals. The total saponins also decreased the nicotine-induced Fos protein expression in the nucleus accumbens and striatum of mice, reflecting the attenuation of nicotine-induced effects by *Panax ginseng* related with the inhibition of the dopaminergic transmission [89].

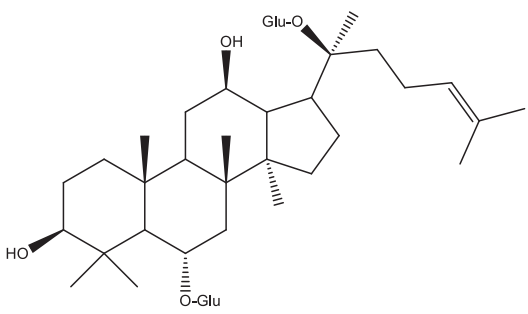



**Fig. 14.10** Structure of ginsenoside Rg1

The bioactive fraction of saponins also inhibited the cocaine-induced reverse tolerance and CPP, promoting the development of postsynaptic dopamine receptor supersensitivity in mice). The extract administration caused a suppression in the development of reverse tolerance and the reappearance of sensitization to methamphetamine and cocaine in mice [180]. The neurochemical basis for the prevention of withdrawal symptoms in mice is related to the attenuation of the cocaine-induced release of dopamine, preventing the rebound increase during acute withdrawal [130].

### 14.3.3 *Withania somnifera* (L.) Dunal (Solanaceae)

The whole plant has medicinal uses, but freshly roots of Indian ginseng have therapeutic purposes in Ayurvedic medicine for the treatment of anxiety, neurosis and sexual debility [37]. Chemically, the species is characterized by the presence of alkaloids, steroids and unusual steroidal lactones, known as withanolides, considered the main bioactive compounds. Chronic treatment with *Withania somnifera* root extract in mice attenuated the development of tolerance to the analgesic effect of morphine and the naloxone- induced jumping, proving to be useful to mitigate symptoms of morphine withdrawal [93]. The administration of the extract also prevented the spine density reduction in the nucleus accumbens shell in spontaneous and pharmacologically precipitated morphine withdrawal [80]. Another study showed that *Withania somnifera* extract injection (100 mg/kg) prevented the acquisition and expression of morphine elicited CPP. The authors also characterized the effect using receptor-binding assays, pointing out to an affinity for

GABA-B receptors and to a less extent for μ-opioid receptors [159]. The *in vitro* incubation of the plant extract alone or concomitantly with morphine in neuroblastoma SH-SY5Y cells prevented opioid receptors down-regulation elicited by morphine, relating to the *in vivo* modulation of morphine-mediated analgesia by the plant extract [26]. Recently, a PPARγ-mediated mechanism in the effects of *Withania somnifera* extract on morphine-mediated nociception was also demonstrated [27].

Studies also showed that roots extract impaired the ethanol self-administration in rats by blocking GABA-B receptors, altering the alcohol-elicited CPP and conditioned place-aversion (CPA) [141]. Additionally, it was demonstrated that the same extract significantly reduced the spontaneous neuronal firing of rat dopaminergic neurons stimulated by morphine and alcohol in the ventral tegmental area, via GABA-A mechanism. The morphine- and alcohol-elicited increases of dopamine in the shell of the nucleus accumbens was also significantly prevented by the extract, as measured by *in vivo* brain microdialysis [12].

### 14.3.4 *Crocus sativus* L. (Iridaceae)

Saffron is a golden-colored spice of strong flavor, commonly used in the Mediterranean cuisine and extracted from the dried stigma present in *Crocus sativus* flowers. The saffron characteristic color is due to the presence of crocin (Fig. 14.11a), a water-soluble carotenoid with a number of pharmacological activities, such as antidepressant, anti-inflammatory, neuroprotective and anticarcinogenic [128]. Safranal (Fig. 14.11b) is a terpene aldehyde and the major constituent present in the essential oil, responsible for the distinct aroma of this spice, whilst its β-D-glycoside picrocrocin (Fig. 14.11c) is associated with the strong flavor described.

The antinociceptive activity reported for an aqueous extract of saffron was partially blocked by naloxone, demonstrating an interaction with the opioid system [72]. Saffron also inhibited the acquisition and expression of morphine-induced behavioral sensitization in mice, reducing the morphine-induced hyperactivity. Aqueous and ethanolic extracts of saffron stigma and isolated crocin were investigated in the morphine-withdrawal model in mice after intraperitoneal injection concomitantly with morphine [69]. Results showed that both extracts and crocin attenuated the severity of jumping in mice precipitated by morphine withdrawal, but safranal exaggerated withdrawal symptoms, possibly due to a partial opioid agonist effect. Crocin also decreased the acquisition of the morphine-induced CPP and the reinstatement of morphine-induced CPP, when administered before a single dose of morphine in animals after extinction of morphine-induced CPP. In rat model of neuropathic pain, preemptive administration of crocin during chronic constriction injury maintained morphine analgesia throughout time, preventing the development of morphine tolerance and suppressing BDNF levels increase induced by neuropathic pain [160]. Clinical trials conducted with

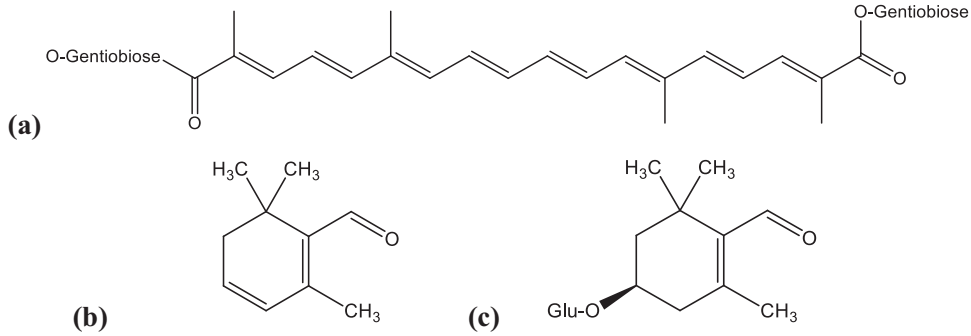



**Fig. 14.11** Structure of saffron bioactive compounds: (**a**) crocin, (**b**) safranal and (**c**) picrocrocin

patients under methadone maintenance treatment receiving crocin showed beneficial effects on their mental health and improved their metabolic profiles [50, 82]. Findings indicate that crocin can be recommended as an adjunct to methadone in opioid withdrawal protocols because of the ability to improve the quality of life, reducing opioids side effects in these patients.

### 14.3.5 *Salvia* spp. (Lamiaceae)

Some species of *Salvia* are used worldwide in folk medicine due to their multiple pharmacological actions on CNS, as anti-inflammatory, analgesic, anticonvulsant and sedative agents. Among the compounds found in these species, diterpenes known as tanshinones are allegedly the bioactive metabolites, highlighting miltirone, carnosic acid and carnosol [75]. The neoclerodane diterpene salvinorin A is the major active compound in the hallucinogenic species *Salvia divinorum*, and one of the most potent naturally occurring hallucinogen thus far isolated. This compound is considered as an emerging target for next-generation non-nitrogenous analgesic drugs due to its potent and selective kappa-opioid receptor binding affinity [158]. Moreover, several *Salvia* species demonstrated antiaddictive properties.

The administration of the ethanol extract of *Salvia leriifolia* leaves reduced in a dose-dependent manner the jumping behavior in morphine withdrawal symptoms induced by naloxone in mice. At a 500 mg/kg dose, the extract was as effective as diazepam at 5 mg/kg, but the effect was antagonized by aminophylline, indicating a possible effect of the extract on the adenosine system [70]. A *Salvia limbata* methanol extract obtained from macerated aerial parts demonstrated a reduction of withdrawal signs of morphine when administered before naloxone challenge in mice. The extract also displayed a central antinociceptive on hot-plate test, reversed by the administration of naloxone [7]. The putative mechanism of action for *Salvia limbata* involves interaction with opioid and adenosine receptors, since a number of neoclerodanes are reported as opioid receptor ligands [158]. Other studies showed that *Salvia hypoleuca* aerial parts extract produced a significant inhibition of pain and the development of the incidence of escape jumps observed in morphine dependence mice [79] and *Salvia officinalis* leaves extract promoted antinociceptive effects and the decrease of both tolerance and dependence induced by repeated morphine administration in rats [63].

Several experiments demonstrated the efficacy of the extract of dried roots of *Salvia miltiorrhiza*, a species native to Japan and China, in reducing voluntary alcohol intake in alcohol-preferring rats. Further experiments showed that a standardized extract of the species delayed dose-dependently the acquisition of alcohol drinking behavior in alcohol-preferring rats that had never experienced alcohol before the study, supporting other pre-clinical studies [18, 181]. A *S. miltiorrhiza* extract, containing 21% total tanshinones and 4.3% miltirone, reduced the reinforcing and motivational properties of alcohol in rats [110]. The same effect was observed when alcohol-naive and alcohol-experienced rats were treated with miltirone and exposed to the 2-bottle choice regimen, confirming the compound as the responsible for the anti-addictive activity [33]. The mechanism of action is connected to the presence of diterpenes in the roots of Salvia *miltiorrhiza* such as miltirone (Fig. 14.12a), a low-

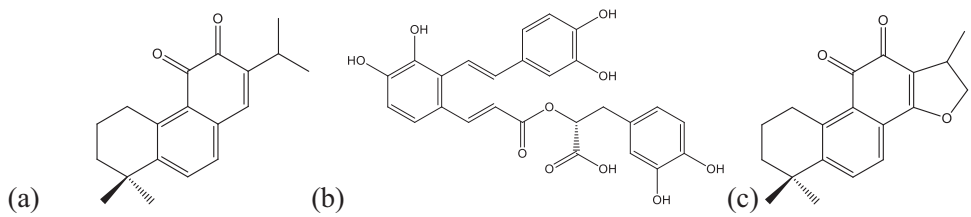



**Fig. 14.12** Structure of bioactive compounds from *Salvia* spp.: (**a**) miltirone, (**b**) salvianolic acid A and (**c**) cryptotanshinone

affinity ligand for benzodiazepine site in $GABA_A$ receptors that is able to mitigate the withdrawal symptoms associated with the long-term administration of alcohol [127]. Also, salvianolic acid A (Fig. 14.12b) is able to protect the liver of rats against chronic alcohol-induced injury, reducing the presence of lipid droplets and the plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), alcohol and ammonia levels in blood by a SIRT1/β-catenin pathway [167]. The compound cryptotanshinone (Fig. 14.12c) activated the phosphorylation of AMP-activated protein kinase (*AMPK*), sirtuin 1 (*SIRT1*), and nuclear factor E2-related factor 2 (*Nrf2*) proteins countering ethanol-promoted hepatic steatosis in mice [129].

### 14.3.6 *Polygala telephioides* Willd. (Polygalaceae)

This is a species widely distributed in southern China and it has been used as a detoxification agent for heroin poisoning. In mice, the methanolic extract of the plant was capable of antagonizing the analgesic effect induced by morphine and reduced the plasmatic levels of the drug. When coadministered with chronic use of morphine, the extract significantly decreased the nalaxone-induced jumping behavior [46]. The extract of the plant roots also attenuated cocaine induced hyperlocomotion and conditioned place preference, possibly via the activation of the adenosine $A_{2A}$ receptors [169]

## 14.4 Plants Containing Essential Oils as Active Constituents

Essential oils are widely used in the flavor, food, fragrance, and cosmetic industries in many applications. They are complex mixtures containing mostly monoterpenoids and sesquiterpenoids. (−)-α-Thujone, obtained from the oil of wormwood (*Artemisia absinthum*), is one of the most notorious monoterpenes. It is the major bioactive ingredient of the hallucinogenic liquor absinthe, a favorite of artists and writers in the nineteenth and early twentieth centuries. It is widely held to have been responsible for psychoses and suicides, possibly including that of Vincent van Gogh. Matricin and chamazulene, the major components of the extract of German chamomile (*Matricaria chamomilla*) inflorescences, which have anti-inflammatory properties, are examples of known sesquiterpenoids.

Cumin, *Cuminum cyminum* Linn., Apiaceae, is native from Mediterranean region but, due to its largely used in culinary, it is widely cultivated in Asian countries. Its essential oil has depressant CNS effects, bounding to $GABA_A$ receptors [163]. Animal studies have shown that the *C. cyminum* essential oil was able to decrease behaviors related to morphine withdrawal and the tolerance to morphine-induced analgesia [60]. Another plant with similar characteristics is *Pimpinella anisum* L. Apiaceae, whose essential oil reduced the morphine-induced conditioned place preference (CPP) in mice, probably through a GABAergic mechanism [161]. *Zhumeria majdae* Rech. f. & Wendelbo Lamiaceae presents an essential oil cointaing monoterpenes such as linalool, camphor and borneol, which inhibited the jumping behavior during morphine withdrawal syndrome in mice [73].

*Kelussia odoratissima* Mozaff., Apiaceae is a flavor species used in Iranian traditional medicine to treat hypertension and inflammation. The main constituent of the essential oil of this plant is the phthalide z-ligustilide (Fig. 14.13), which has voltage-dependent calcium channel blocking properties. Chronic treatment with the essential oil relieved symptoms of morphine withdrawal in mice [148]. Another endemic aromatic medicinal plant of Iran is *Thymus daenensis* Celak, Lamiaceae, whose extract and essential oil atten-

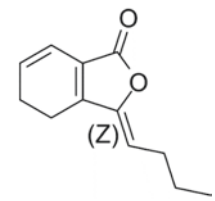



**Fig. 14.13** Chemical structure of z-ligustilide

uated morphine withdrawal behaviors in mice [85]. Similarly, the oil of *Zingiber officinale* Roscoe Zingiberaceae, a well-known condiment and used worldwide that completely prevented the tolerance for morphine in mice. The effect is related to the $Ca^{+2}$ L type channel blocker [38].

*Nigella sativa* L., Ranunculaceae has been traditionally used for the treatment of several disorders. Medicinal properties of *N. sativa* have been attributed to its seeds extracts and/or oil. The oil attenuated tolerance and dependence induced by morphine and tramadol in mice [1, 2] and the seed extract reduced the morphine-induced conditioned place preference in rats [10]. The major constituent of *N. sativa* is the quinonic compound thymoquinone, which also attenuated the morphine tolerance and dependence in mice [74].

## 14.5 Plants Containing Flavonoids as Active Constituents

Flavonoids are polyphenolic compounds comprising 15 carbons, with two aromatic rings connected by a three-carbon bridge. In plants, flavonoids are involved in such diverse processes as UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance. The main subclasses of flavonoids are flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins. Examples of flavonoids with pharmacological activity are the isoflavones (genistein and daidzein) and the coumestan (coumestrol) from lucerne and clovers (*Trifolium* spp), which have sufficient oestrogenic activity to seriously affect the reproduction of grazing animals such as cows and sheep and are termed phyto-oestrogens.

### 14.5.1 *Morinda citrifolia* L (Rubiaceae)

*Morinda citrifolia*, commonly known as "noni," is a small tropical tree that grows widely in Southeast Asia. The different parts of this plant (e.g., fruit, leaf, bark, root, flower, and seed) have long been employed in folklore medicine to treat a broad range of diseases including diabetes, hypertension, arthritis, depression, senility, menstrual difficulties, headaches, and drug addiction. The methanolic extract of *M. citrifolia* unripe fruits, containing the coumarin scopoletin (Fig. 14.14a) and the flavonoid rutin (Fig. 14.14b), effectively reversed the ethanol [84], heroin [131] and methamphetamine-induced conditioned place preference [139] in mice and rats. The mechanism is not completely understood but it is related to the antagonism activity in dopaminergic $D_2$ receptor and MAO inhibition.

### 14.5.2 *Pueraria lobata* (Willd.) Sanjappa & Pradeep (Fabaceae)

Kudzu (*Pueraria lobata*) is a vine indigenous to eastern Asia. It has been used as an herbal remedy in China to treat a variety of disorders, including neck pain, eye pain, fever, and measles. More recently, kudzu flower root is reported to be used in China for the treatment of alcohol addiction. Puerarin (Fig. 14.15a), daidzin (Fig. 14.15b), and daidzein (Fig. 14.15c) are three of the major

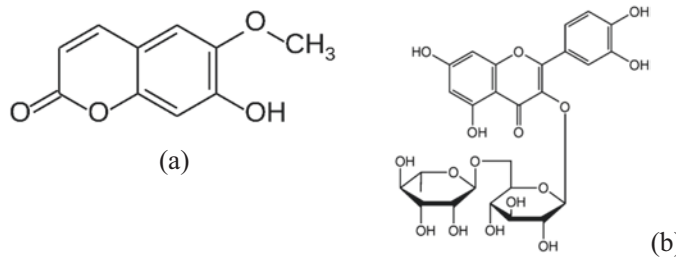



**Fig. 14.14** Chemical structure of (**a**) scopoletin and (**b**) rutin

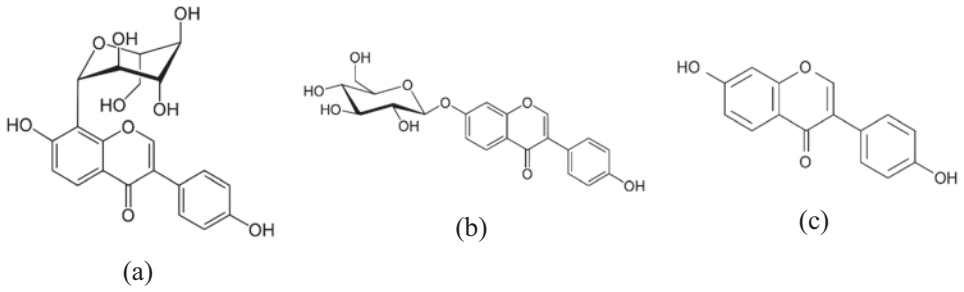



**Fig. 14.15** Chemical structure of (**a**) puerarin, (**b**) daidzin, and (**c**) daidzein

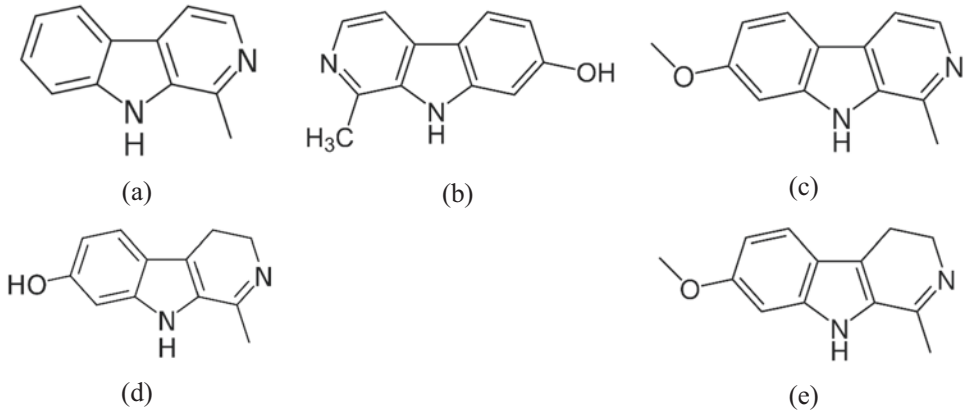



**Fig. 14.16** Chemical structures of the indole beta-carboline type alkaloids (**a**) harmane, (**b**) harmol, (**c**) harmine, (**d**) harmalol, and (**e**) harmaline present in *Passiflora incarnata*

isoflavonoid compounds identified in the extract of *P. lobata*. These flavonoids are effective in the reduction of alcohol consumption in rats [109]. In humans, a standardized kudzu extract (NPI-031) reduced alcohol drinking in non treatment-seeking male heavy drinkers with no adverse events, changes in vital signs, blood chemistry, renal or liver function [109]; and in binge drinking paradigm [142]. Both puararin and daidzin seem to have inhibitory action in benzodiazepinic receptors [137]. Moreover, daidzin inhibits ALDH-2, making the decreased drinking be attributed to aversive properties of acetaldehyde accumulated during alcohol consumption in a disulfiram-like mechanism [81, 95, 107, 155].

Studies have compared the effects of daidzin, daidzein and pueranin on the suppression of ethanol intake. Daidzine is more effective than the others in all comparative studies performed, while pueranine had a lower, but still significant suppressor effect. Daidzeine was ineffective [103, 155].

### 14.5.3 *Passiflora incarnata* L. (Passifloraceae)

*Passiflora incarnata* is used in phytomedicine, with alleged therapeutic properties such as anxiety, nervousness, and insomnia treatments. Interestingly, in India, it is the species traditionally used to treat morphine's dependence. The chemistry of *P. incarnata* is complex, and the active constituents include flavonoids and alkaloids as the bioactive compounds. The indole beta-carboline alkaloids, namely harmane, harmol, harmine, harmalol, and harmaline (Fig. 14.16), are minoritary constituents of the

plant and act as monoamine oxidase inhibitors. The flavonoids represent 2.5% of the plant compounds, which include vitexin, isovitexin, orientin, isoorientin, kaempferol, apigenin, and chrysin.

Preclinical studies evidence beneficial properties of *P. incarnata* as a treatment for addictive behaviors linked to substances such as nicotine [14, 17, 41], and benzodiazepines [44]. A standardized extract of *P. incarnata*, containing only flavonoids (Fig. 14.17), reversed the analgesia resulted from alcohol withdrawal syndrome in rats [164]. In mice, the benzoflavone moiety of *P. incarnata* decreased the anxiety induced by chronic ethanol abuse, as well as exhibited lower dependence level and fewer withdrawal signs compared with the ethanol treated mice [42]. Additionally, the benzoflavone moiety also prevented the development of tolerance and dependence of cannabinoids in mice [43].

In humans, a double-blind randomized controlled trial including 65 opiates addicts pointed that *P. incarnata*, alone or in combination with clonidine, was effective in the treatment of physical withdrawal symptoms of opioids. The combination *Passiflora*-clonidine, used during the withdrawal, might have a faster onset of action, whereas *Passiflora* extract can have therapeutic benefits in the management of opioids psychological withdrawal symptoms, suggesting that the plant extract might be an effective adjuvant agent in the management of opiate withdrawal [6].

### 14.5.4 Other Species

*Matricaria chamomilla* L. Asteraceae is an ancient European herb with several uses including anti-inflammatory, spasmolytic, and sedative. The plant contains flavonoids with benzodiazepine-like activity and inhibitory action on phosphodiesterase, leading to the increase cAMP levels. The repeated coadministration of the extract of *M. chamomilla* containing 0.3% apigenin, with morphine significantly attenuated the severity of the withdrawal syndrome probably due to the same mechanism reported for other phosphodiesterases' inhibitors

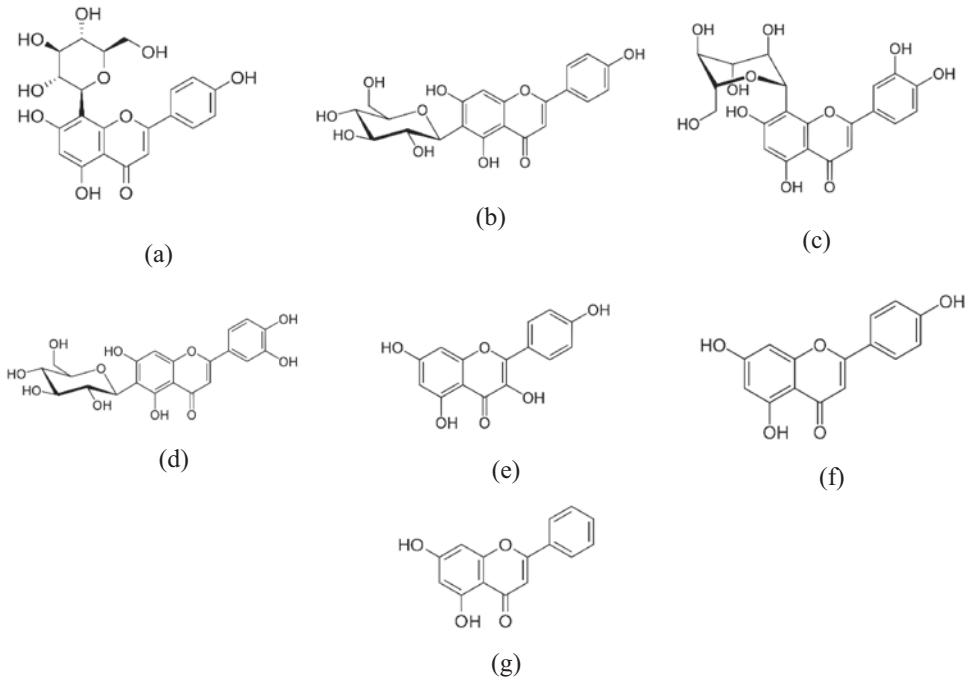



**Fig. 14.17** Chemical structures of the flavonoids (**a**) vitexin, (**b**) isovitexin, (**c**) orientin, (**d**) isoorientin, (**e**) kaempferol, (**f**) apigenin, and (**g**) chrysin present in *Passiflora incarnata*

(e.g. 3-isobutyl-methylxanthine, nefiracetam or rolipram) [56].

The aqueous and ethanolic extract of aerial parts of *Rosmarinus officinalis* L., Labiatae reduced the signs of morphine withdrawal in rodents. Phytochemical characterization of the extracts indicated the presence of flavonoids, tannins, saponins and. Alkaloids, the latter detected only in the aqueous extract. Such constituents have opioid-like analgesic effect, which is reinforced by the fact that the antinociceptive activity of these extracts were inhibited by naloxone. Therefore, the morphine withdrawal relief could be attributed to an opioid-like action [71]. Similarly, total and polyphenolic extracts of fruits from *Carum copticum* (L.) C. B. Clarke C., Apiaceae, a plant endemic to India, Iran and Egypt, reduced morphine withdrawal syndrome [51].

Herbal preparations of *Scutellaria baicalensis* Georgi Lamiaceae are used in oriental traditional medicine for the treatment of many neuropsychiatric diseases for centuries. The aqueous extract of the roots of *S. baicalensis* and one of its main flavonoids, baicalin, have shown efficacy in reducing the morphine-induced conditioned place preference, probably due to the modulation of dopaminergic receptors [195].

Korean pear (*Pyrus pyrifolia* (Burm.) Nak.Rosaceae) has been used as a traditional medicine for alcohol hangover in Korea. The plant contains phenols, mainly flavonoids, including catechin, rutin, quercetin and kaempferol. In mice, the pear extract decreased the alcohol level in blood and increased the acetaldehyde levels by stimulation of the two-key alcohol-metabolizing enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) [101].

The leaves of *Jodina rhombifolia* (Hook. & Arn.) Reissek (Santalaceae) are commonly utilized as anti-alcoholic in Argentine folk medicine. Among the major chemical constituents found are phenolic compounds, tannins, C-glycosyl flavonoids, steroids, gums and mucilage. The lyophilized aqueous extract decreased ethanol intake and preference in adolescent male Wistar rats [173, 174].

## 14.6 Plants Containing Polysaccharides as Active Constituents

Polysaccharides refers to a class of structurally complicated and bulky carbohydrate synthesized from the condensation and dehydration of multiple single sugar molecules, considered as one of the four fundamental substances that constitute life. Currently, polysaccharides have been extracted from hundreds of species of plants and important and special biological activities have been associated to them.

*Millettia pulchra* Kurz var. *typica* Dunn, Fabaceae, also known as Yulangsan, is a traditional Chinese medicinal herb. The Yulangsan polysaccharide (YLSP) is the major active component of the roots and attenuated naloxone-induced morphine withdrawal signs in morphine dependent rats. Additionally, the polysaccharide presented modulatory effects in the expression of nitric oxide (NO) and NO synthase and also modulated the levels of monoaminergic neurotransmitters in the ventral tegmental area (VTA), hippocampus and *nucleus accumbens* [30]. YLSP also inhibited the reinstatement of morphine-induced conditioned place preference [31].

## 14.7 Plants Containing Multiple Compounds

### 14.7.1 *Nepeta menthoides* Boiss. & Buhse (Lamiaceae)

*N. menthoides* is an endemic specie of Iran, commonly known as "Ustukhuddoos" in folk medicine and prescribed for a number of nervous disorders such as epilepsy, anxiety, and depression, chronic pain and restlessness, gastrodynia, high blood pressure, bone pain, and rheumatism. Chemical analysis of the essential oil showed that the main terpenes are 1,8-cineol, α-terpineol, α-linalool, β-pinene, and α-pinene. Additionally, plant extract contains flavonoids (rosmarinic acid, salvianolic acids A and B, and caffeic acid), tannins, saponins and cardiac glycosides. A hydroalcoholic plant extract prevented the development of morphine

dependence and tolerance, and potentiated morphine antinociception in mice [150].

### 14.7.2 *Rhodiola rosea* L., Crassulaceae

*Rhodiola rosea*, known as golden root or rosenroot, grows in arctic regions of Europe and Asia, and due to the anxiolytic, antidepressive, and anti-stress properties associated, its derived phytomedicines are indicated as adaptogens. The rhizomes of the plant contain flavonoids, monoterpenes, triterpenes, phenolic acids, phenylethanol derivatives (salidroside and tyrosol), and phenylpropanoid glycosides such as rosin and rosavin (Fig. 14.18a). Among these, salidroside (Fig. 14.18b) is one of the most active constituents. In mice, *R. rosea* extract (containing 3% total rosavins, expressed as rosavin and 1% salidroside) abolished affective and somatic signs induced by nicotine withdrawal [119] and reduced the rewarding properties of nicotine [179]. The same extract also attenuated morphine tolerance and dependence [120] and cocaine-induced conditioned place preference [178]. Investigating the mechanism of action of *R. rosea* extract, it was found out an increase in 5-$HT_{1A}$ receptors in the thalamic nucleus, which might promote the benefits observed in animals treated with this extract [113].

### 14.7.3 *Hypericum perforatum* L. Hypericaceae (St. John's Wort)

*H. perforatum* standardized extracts are extensively investigated and widely consumed due to their therapeutic effects on mood disorders, and action in mild to moderate depression. Several groups of bioactive natural products were identified in this plant species, among them naphtodianthrones (i.e., hypericin (Fig. 14.19a) and

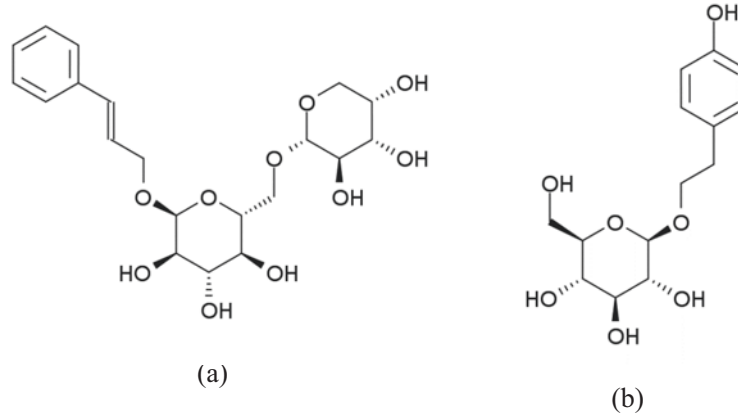



**Fig. 14.18** Chemical structure of (**a**) rosavin and (**b**) salidroside

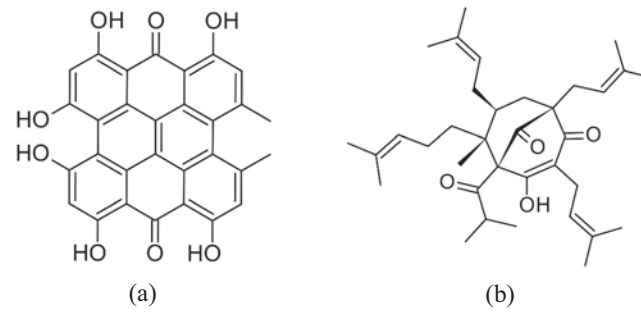



**Fig. 14.19** Chemical structure of (**a**) hypericin and (**b**) hyperforin

pseudohypericin), phloroglucinols (i.e., hyperforin (Fig. 14.19b) and adhyperforin), flavonol glycosides (including quercetin, hyperoside or hyperin, rutin, isoquercitrin and isoquercitrin), biflavonoids (i.e., amentoflavone and biapigenin), phenylpropanes (including chlorogenic acid and caffeic acid), proanthocyanidins, tannins, xanthones, and certain amino acids (i.e., GABA). These constituents are present in different amounts in *Hypericum* extracts and therapeutic effects of extracts are associated to a possible synergism among the different constituents. In an alcohol-preferring genetic rats, intraperitoneal acute administration of an ethanolic extract of *H. perforatum* dose-dependently reduced alcohol intake in a two-bottle choice procedure [39]. Similarly, a *H. perforatum* $CO_2$ extract reduced ethanol self-administration in rats [145], and the reduction was more pronounced when the extract was associated with naloxone and naltrexone, providing evidence that extract and opiate receptor antagonists could act synergistically [144]. Additionally, a *H. perforatum* extract containing 50% flavonoids, 0.3% hypericin, and 4.5% hyperforin reduced nicotine withdrawal signs in mice after oral administration, The effect was linked to a serotonergic mechanism, in which the extract increased cortical 5-HT content in nicotine treated mice, with a concomitant increase of $5\text{-HT}_{1A}$ receptor [112]. Aqueous *H. perforatum* extract also attenuated abdominal constrictions both acutely and chronically when orally administered to heroin dependent rats [171]. In spite of all of this pre-clinical evidence, in a randomized, blinded, placebo-controlled clinical trial with 40 subjects, a hydroalcoholic *H. perforatum* extract (standardized in 0.3% hypericin) did not increase smoking abstinence [170].

## 14.8 Final Considerations and Future Directions

Drug abuse is one of the leading problems in human health nowadays, leading to tolerance and dependence as a main problem associated. Alcohol, opioids, nicotine, cocaine and amphetamines abuse are a main concern and the treatment of the psychosomatic syndrome induced by the withdrawal among users remains complicated. Due to the lack of pharmacological therapeutics to the treatment of drug addiction, plants and their active ingredients are promising choices to ameliorate drug-induced pharmacological changes such as tolerance, dependence, and withdrawal syndrome. Some plant families showed great potential in drug addiction therapy because of their bioactive compounds, mainly indole, isoquinoline and tropane alkaloids (Menispermaceae, Papaveraceae, Ranunculaceae, Rubiaceae and Solanaceae families), terpenes (Araliaceae, Lamiaceae, Plantaginaceae and Solanaceae families), essential oils (Apiaceae and Lamiaceae families), flavonoids (Rubiaceae and Asteraceae), and polysaccharides. Some families are distinguished for producing compounds belonging to different classes, such as Lamiaceae (essential oils and diterpenes) and Solanaceae (alkaloids and terpenes), while some species contain distinct bioactive compounds, such as *Passiflora incarnata* (alkaloids and flavonoids), *Hypericum perforatum* (polyphenols, phloroglucinol and naphthodianthrone derivatives) and *Rhodiola rosea* (flavonoids and phenylpropanoid derivatives).

Considering the mechanisms of action, the mentioned species offer a great variety of actions including symptomatic improvement (i.e. spasmolytic and sedative), opioid partial agonism (similar to replacement medications), neurotransmitter (cholinergic, adrenergic, dopaminergic, glutamatergic, and GABAergic) modulation, and interference with signaling pathways such as the cAMP and NO pathways. Although most of the literature in this issue is confined to animal studies, the results seem to be promising. Clinical studies, however, are needed to confirm the safety and efficacy of many of these herbal extracts and preparations. They could be used experimentally in detoxification centers along with standard pharmacological and psychological therapy. Traditional compound herbal formulae have been effective in a holistic approach, however, certain classes of compounds such as alkaloids and flavonoids have been demonstrated to be effective.

Therefore, screening for other potentially effective plants and natural products in these classes should be continued, and further research should be carried out to identify specific fractions and active components of the plants already tested.

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# Use of Plant-Derived Natural Products in Sleep Disturbances

15

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**Abstract**

Sleep disorders have a high prevalence both in the general population and especially in specific populations such older adults and oncologic patients. Impacting on quality of life, they often translate in drug prescription, with consequent increased risk of drug-drug interactions and adverse drug reactions. In the last years several products derived from plants have been developed with the aim of treating insomnia with lower risk of side effects. Despite several studies have been performed with this aim, the available evidence is inconclusive, and reviews summarizing the most recent evidences on the effectiveness of plant-derived products in treating insomnia are lacking.

This narrative review aims at summarizing the evidences of the mechanism of action, effectiveness and safety of the most commonly used plant-derived products for the treatment of sleep disorders (Valerian, Lemon balm, Passionflower, Chamomile, Hops, and Jujube).

**Keywords**

Insomnia · Phytotherapy · Valerian · Lemon balm · Passionflower · Chamomile · Hops · Jujube

## 15.1 Introduction

The term "sleep disorders" refers to a wide variety of disorders including difficulty in falling asleep, insufficient sleep duration, frequent or early awakenings, obstructive sleep apnea, restless legs syndrome, which are often generically referred by patients as "insomnia".

Estimates of prevalence of insomnia are variable due to difference in the definitions used and, in the population, studied. A study published in 2011 reported that from 20% to 41.7% of the general population is dissatisfied with the amount of sleep and reports its sleep as insufficient, with higher prevalence in specific groups, such as older people or shift workers [1].

Several comorbidities are associated with insomnia, and it has been estimated that up to 90% of people with this condition also have conditions causing hypoxemia and dyspnea, gastroesophageal reflux disease, pain conditions, and neurodegenerative [2].

The impact of insomnia on patients' quality of life translates into frequent medical prescrip-

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tions, with a consequent increase in the risk of adverse drug events, drugs/drug interactions, and addiction to some drug classes, such as benzodiazepines [3]. Given these risks, the use of traditional herbal medicine products is appealing, and in the last years several products derived from plants have been developed with the aim of treating insomnia with lower risk of side effects [4].

Despite several studies have been performed to evaluate the effectiveness of herbal products for the treatment of insomnia, the available evidence is inconclusive because of poor methodological quality of the studies included, and the 2017 European guidelines for the diagnosis and treatment of insomnia and the American Academy of Sleep Medicine did not recommend the use of valerian and other phytotherapeutics for the treatment of insomnia [5, 6]. However, several authors support the use of phytotherapy as the first line of treatment or as an adjuvant therapy in association with conventional therapies [7], and about 5% of people with insomnia report to use an alternative medicine medication to treat the disorder [8].

Given these conflicting reports and the lack of recent reviews on the effectiveness of plant-derived products in treating insomnia, in this study we summarized the recent evidences on the use of plant-derived medications for insomnia.

## 15.2 Methods

This is a narrative review of the current evidences on plant-derived products used for the treatment of insomnia. Studies were retrieved searching the Pubmed database animal studies, observational studies, clinical trials, and reviews published from 2009 to 2019 in English language. The MeSH keywords used were: ("Sleep Disorders" OR "Insomnia" OR "Circadian Rhythm" OR "Sleep Medicine Specialty" OR "Dyssomnias") AND "Phytotherapy". Titles and abstracts of studies matching our search strategy were screened and the full text of potentially eligible ones was evaluated. The bibliography of selected studies was also searched to retrieve additional articles. Most of the screened studies were clinical trials; some reviews were produced on selected compounds, while only few researches were on animal models. We included in the review the phytoterapics with at least two studies on humans available: valerian (*Valeriana officinalis*), chamomile (*Matricaria chamomilla/Matricaria Recutitia*), hops (*Humulus Lupulus*), jujube (*Ziziphus jujuba*), lemon balm (*Melissa officinalis*), and passionflower (*Passiflora incarnata*).

## 15.3 Valerian (*Valeriana officinalis*)

The medical properties of *Valeriana officinalis* are known since the fourth century BC, when Hippocrates suggested its use in digestive disorders, nausea and menstrual pain. Its properties in the treatment for sleep disorders were described for the first time by Galen in the II century AD, but it was primarily used for this indication in the sixteenth century [9], and only in 2016 this compound has been approved for use and marketing in Europe by the European Medicines Agency (EMA) [10]. In the USA, valerian is marketed as a food supplement and therefore is not regulated as a drug by the Food and Drug Administration.

The exact mechanism of action of valerian is unknown. It contains several substances (alkaloids, terpenes, organic acids, valepotriates, flavonoids) with potential physiological effect. In particular, one alkaloid (actinidine), and two terpenes (valerenic acid and velerenal aldehyde) may modulate GABA-ergic transmission. Actinidine has an allosteric interaction with the GABA receptor through an agonistic action on the benzodiazepine receptors. Valerenic acid interacts with the $GABA_A$ receptor, and its effects are absent in rats with point-mutation of the b3 subunit of the $GABA_A$ receptor, that thus seems to be the target of this substance [11]. This terpene also is a partial agonist of the 5HT receptor [12].

The pharmacological effects of valerian show high inter-individual variability, with considerable inter- and intra-subject variability. Furthermore, peak concentration, area under the curve of concentration, and clearance are inversely related to body weight [13].

In drosophila meganogaster, the addition of valerian extract to the sucrose medium of the fly

bottles induced a decrease of activity during both daytime and nighttime. In the same study, a mixture of valerian and hop (Cascade variety) upregulated the expression of mRNA for GABA receptors and, less strongly, serotonin receptors [14]. In a rodent model of pentobarbital-induced sleep, valerenic acid administration was associated with a reduction of latency sleep time and an increase in total sleep time [15]. A study on aqueous extract of Valeriana wallichii root showed similar results in freely-moving rats [16]. This study also showed a decrease in the monoamine (norepinephrine, serotonine, dopamine and hydroxy-indolacetic acid), levels in the frontal cortex.

With respect to safety, Xu et al. showed that doses up 1200 mg/kg/day did not cause harmful effects in mice and found a DL50 of 2000 mg/kg/day in mice [17]. Thus, recent evidence supports a positive effect of valerian on sleep in different animal models, with a favorable safety profile.

In the last decade, several reviews have been published reporting the effects of valerian in humans [7, 9, 18–22]; the most recent includes 6 randomized controlled trials published between 2009 and 2019. The effect of valerian varied according to the characteristics of the study participants: for example, in a sample of 100 postmenopausal women valerian was effective in improving both subjective and objective (Pittsburgh Sleep Quality Index) indices of sleep quality [23]. Instead, no positive effects were observed in patients with cancer [24] or restless leg syndrome [25].

Valerian has also been studied in association with other herbal remedies. In a randomized clinical trial, 91 patients with primary insomnia were randomized to a standardized mixture of valerian, passionflower, and hop or to zolpidem. Quality in sleep improved in both groups, without between-group differences, and also adverse events were similar in the two groups [26].

All the studies reported showed a good safety profile of valerian, confirming the findings of several other studies published in a time period outside the scope of this review. Furthermore, recently, Thomas et al. showed that a single daily dose of 1600 mg of valerian does not impact driving ability [27]. However, the EMA did not recommend the use of valerian in children <12 years of age and during pregnancy due to lack of clinical data, and warned about the potential to impact driving [10] (Table 15.1).

In conclusion, the studies published on the efficacy of valerian on sleep disorders in the last decade suffer from the same limitation of the rest of the literature on this topic: they are in general small, of medium/low quality, and the results are conflicting. Although part of the discrepancies may be due to differences in the population studied and in the dosage used, the evidence is still too weak to support the use of this plant in treating sleep disorders, and the American Academy of Sleep Medicine does not recommend it for sleep onset or sleep maintenance disturbances [28]. However, given its safety, a trial of valerian may be attempted in people at high risk for adverse drug reaction, such as elderly people at risk for falling.

## 15.4 Chamomile (*Matricaria chamomilla/Matricaria recutitia*)

Chamomile infusion is commonly used throughout the world. Several flavonoids and other phenolic compounds have been identified in various parts of the chamomile flower head, including apigenin, quercetin, patuletin, and luteolin, with relative concentrations varying within the different flower parts [29–31].

In mice, intra-peritoneal infusion of 360 mg/kg of chamomile induced a 57% reduction of motility without affecting motor coordination [32]. A purified extract containing apigenin (3 mg/kg i.p.) caused an increase of time spent in the open arms of an elevated plus-maze (a sign of reduction in anxiety) [33]. No effects of 10 mg/kg of apigenin were found on training and testing session performance of inhibitory or active avoidance and habituation to an open field, indicating no effect on memory [34]. The site of effect of apigenin is not clear. In a study on ovarictomized rats, inhaled vapours of chamomile oil reduced a stress-induced increase of ACTH and this effect was reversed with addition of a benzodiazepin receptor blocker (flumazenil) [35]. However,

**Table 15.1** Mechanism of action and available evidences on the effectiveness of herbal remedies on treating insomnia

| Compound | Mechanism of action | Studies on animals | Studies on human |
|---|---|---|---|
| Valerian | Possible modulation of GABAergic transmission by interaction with $GABA_A$ receptor | Effective in different animal models | RCTs available, but all with small sample size and of poor quality; results contrasting<br>Safe |
| Chamomile | Possible modulation of benzodiazepine receptor | Effective | Small sample size and poor quality; improves sleep quality |
| Hops | Not clear<br>Possible reduction of melatonin degradation by CYP1A2 | Tested together with valerian in Drosophila Melanogaster with effects on locomotor activity | Few studies<br>Small sample size and poor quality<br>Inconclusive results |
| Jujube | Possible modulation of expression of $GABA_A$ gene | Increases the expression of GABA receptor subunits a1, a 5, b2 in mice | Few studies<br>Small sample size and poor quality<br>Seems to improve insomnia |
| Lemon balm | Not known | Not available | Only 2 studies<br>Small sample size and poor quality<br>Seems to improve insomnia |
| Passionflower | Inhibits binding of GABA to $GABA_A$ and $GABA_B$ and inhibits GABA uptake | Not available | Only 2 clinical trials, one of which in combination with other herbal remedies<br>Positive results |

apigenin may interact with benzodiazepines receptor differently from benzodiazepines, as suggested by the above-reported study showing no effect on memory and in which the authors also found that, in contrast with diazepam, apigenin had no analgesic effects [34].

In a study on 60 nursing residents randomized to receive 200 mg of chamomile in capsules or a matching placebo, the active treatment group showed better scores at the PSQI compared to the placebo group at the end of a 4-weeks treatment. The difference was still observable at 6 weeks (2 weeks after the end of the treatment) [36]. Comparable results were reported in a similar population by a different research group [37].

The effect of chamomile was also studied in women in the post-partum period. Chang eta al. sequentially allocated 80 women after 6-weeks from delivery into an intervention group which drank a cup of chamomile (teabags with 2 g of dried flower infused in 300 ml of water for 10–15 min) for 14 days or a control group. The 14-item post-partum sleep quality score was used to grade the subjective quality of sleep. Women in the intervention group showed an improvement of physical symptoms associated with sleep disturbances at 2 weeks, with no differences in the other subscales of the PSQS. No differences were observed between the two groups after 4 weeks [38].

In a sample of community-dwelling persons aged 18–65 years with primary insomnia for at least 6 weeks, the effects of 540 mg of chamomile extract were tested in a randomized, placebo-controlled clinical trial. The authors reported only a modest effect on sleep latency and awakenings [39].

These studies, along with other two (one on patients receiving hemodialysis and one in postmanopausal women) were included in a meta-analysis by Hieu et coll., that found an overall beneficial effect of chamomile on sleep quality, but also acknowledged the poor quality of the studies [40] (Table 15.1).

## 15.5 Hops (*Humulus lupulus*)

Hops has been used in traditional western medicine for the treatment of sleep disturbances due its reported calming and sleep-promoting properties. It has been suggested that it exerts its action by decreasing melatonin degradation by CYP1A2 [41].

As reported elsewhere in this paper, a mixture of valerian and hop of the Cascade variety upregulated the expression of mRNA for GABA receptors and, less strongly, serotonin receptors in drosophila melanogaster, with a reduction of locomotor activity [14]; furthermore a mixture of hops, valerian, and passionflower did not show differences with zolpidem in improving quality of sleep in humans [26].

In patients with primary insomnia, hops in conjunction with polyunsaturated fatty acids was compared to olive oil to evaluate its effects on perceived sleep quality, sleep efficiency measured using actigraphy, and melatonin excretion. The authors found a marked improvement of all outcomes over the study period, but without any difference between the two groups [41].

A larger study on 171 volunteers with mild insomnia investigated the effects of a mixture of hops, casein extract hydrolysate, and *Zizyphus jujube* vs. placebo showed no differences between groups with respect to the primary endpoint (changes in the PSQI). Similarly, no differences were found for secondary endpoints: daytime functioning, physical fatigue, mood and anxiety, cognitive performance, and stress reactivity [14] (Table 15.1).

## 15.6 Jujube (*Ziziphus jujuba*)

*Ziziphus jujuba* has been used in Chinese medicine as a sedative, relaxing, and hypnotic agent. Jujube contains several substances that may contribute to these effects, including saponins, alkaloids, and flavonoids. The major active components seem to be the saponin jujuboside A (JuA) [42–44]. In mice, JuA inhibits the locomotory activities [45], and at high doses also inhibits the penicillin-induced hyperactivity of CA1 area in the hyppocampus [42]. It has been hypothesized that JuA exerts its action via the GABAergic system [46]. In vitro, JuA can induce influence the expression of $GABA_A$ □1, □5, and □2 subunit genes; thus, JuA seems to have a mechanism of action different from that of benzodiazepines [47].

A randomized clinical trial comparing a blend of herbs used in Chinese traditional medicine (Suan Zao Ren Tang – SZR – and Zhi Zi Chi Tang – ZZC), that is made by about 20% of jujube, with 0.5 mg of lorazepam enrolled 120 patients with primary insomnia. After 4 weeks of treatment, people taking SZR/ZZC showed a greater reduction in the PSQI score compared to the lorazepam group, the difference was mainly due to an effect on the sleep efficiency subscale [48].

A systematic review also including clinical trials published in Chinese literature underscored the poor methodological quality and the small sample size of the included studies and concluded that there is insufficient evidence to support efficacy of jujube [49] (Table 15.1).

## 15.7 Lemon Balm (*Melissa officinalis*)

*Melissa officinalis* has been used over the centuries for treating digestive disorders, infections, and anxiety, and recently it has been shown to have antioxidant properties [50]. Its leaf contains flavonoids (quercitrin, rhamnocitrin, luteolin), polyphenolic compounds (rosmarinic acid, caffeic acid, and protocatechuic acid), monoterpenoid aldehyde, monoterpene glycosides, triterpenes (ursolic and oleanolic acids), sesquiterpenes, tannins, and essential oils (citral), that may contribute to its effects on treating insomnia; however, the exact substances responsible for clinical effects are not known, and no pharmacokinetic nor pharmacodynamic data are available.

Our search strategy did not identify any study on the effect of lemon balm in animals. In humans, this plant (3 g/d) was able to improve sleep quality evaluated using the depression, anxiety and stress scale (DASS-21) test and

Pittsburgh sleep quality index in patients with stable coronary artery disease [51]. In a small pilot study, a group of people with mild-to-moderate anxiety disorders treated with melissa reduced clinician-rated sleep disturbances by 42% [52].

No other studies are available, and therefore no conclusion is possible on the effectiveness of this plant on sleep disturbances (Table 15.1).

## 15.8 Passionflower (*Passiflora incarnata*)

*Passiflora incarnata* is credited to have sedative properties and to act as anxiolytic; it is used to treat insomnia and also has spasmolytic effects. *Passiflora* acts by modulating the GABA transmission system, but at variance with valerian, it inhibits the binding of GABA to both $GABA_A$ and $GABA_B$ receptors, and by inhibiting the uptake of GABA [53].

No studies in animal models have been identified in the last decade and only two clinical trial were identified. In the first study, 41 young adults with sleep disturbances were administer passionflower infusion or placebo, with an improvement of sleep quality in the passionflower group [54]. The second study, above mentioned in the "Valerian" section, tested the association of passionflower, valerian and hop in comparison with zolpidem, and showed similar effects in the two treatment arms [26] (Table 15.1).

## 15.9 Conclusions

Despite the large number of studies published in the latest years, the available evidence on the effectiveness of plant-derived products for the treatment of insomnia remains inconclusive, primarily because of the poor quality and of the small sample size of most of the studies available on this topic. However, globally, these compounds seem to have a very low risk of poor side effects and to be well-tolerated in humans.

Large randomized clinical trials are needed to improve the quality of the available evidences.

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# 16 The Effects of Nutraceuticals and Herbal Medicine on *Candida albicans* in Oral Candidiasis: A Comprehensive Review

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**Abstract**

*Candida albicans* is part of the healthy flora in the oral cavity. It can also cause opportunistic infection, which can be deleterious. The most typical type of chronic oral candidiasis is denture stomatitis, and *C. albicans* is identified as the most crucial organism in this situation. Due to the development of the resistant form of candida, using conventional drugs can sometimes be ineffective. Herbs and naturally imitative bioactive compounds could become a new source for antimycotic therapy. Several review studies suggest that herbal medicine and natural bioactive compounds have antibacterial, antiviral and antifungal effects. Thus, it is hypothesized that these natural products might have beneficial effects on pathogenic oral fungal flora such as *C. albicans*. Although the effects of herbs have been investigated as antifungal agents in several studies, to the best of our knowledge, the effects of these natural products on *C. albicans* have not yet been reviewed. Thus, the aim of this study was to review the anti-candida activity (especially *C. albicans* in oral candidiasis) of herbal medicines and natural bioactive compounds. It is concluded that, in

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general, medicinal plants and nutraceuticals such as garlic, green tea, propolis, curcumin, licorice root, cinnamon, resveratrol, ginger, and berberine are useful in the treatment of *C. albicans* in oral candidiasis and could be considered as a safe, accessible, and inexpensive management option in an attempt to prevent and treat oral diseases. However, most of the evidence is based on the *in vitro* and animal studies, so more clinical trials are needed.

**Keywords**

*Candida albicans* · Oral candidiasis · Nutraceuticals · Herbal medicine

## 16.1 Introduction

*Candida albicans*, is the most predominant species found in oral candidiasis, belongs to healthy flora in the oral cavity of human [1–3]. It can convert into opportunistic pathogens due to predisposing factors such as poor oral hygiene, denture instability, systemic factors like immunologic and endocrine disease, medication such as steroids, broad-spectrum antibiotics, immunosuppressors, and various nutritional deficiencies [4]. Some pathogenic mechanism in *C. albicans* transformation phases includes epithelial adherence, production of hydrolytic enzymes, biofilm formation, phenotypic changes, and morphogenesis [5, 6]. Some of the clinical signs of oral candidiasis are thrush and creamy white crude like patches [2, 7].

The typical type of chronic oral candidiasis is denture stomatitis, and *C. albicans* is identified as the most crucial organism in this situation [8]. Some factors, such as poor oral health, instability, loosening of denture, irregularity, and pore of the inner surface of denture, which is considered as a reservoir of microorganism, are attribute to the predisposing factors [8, 9]. Furthermore, in severely compromised HIV patients, oropharyngeal candidiasis is prevalent, and *C. albicans* is the most common pathogen that is isolated from clinical specimens [10]. Difficulty in chewing and swallowing can occur if untreated and can lead to weight loss [7].

Conventional synthetic drugs such as imidazole related compounds (e.g. miconazole, fluconazole) or polyenic derivatives (e.g. nystatin) are used for the treatment of candidiasis [4]. However, drawbacks of these medications include the development of resistant strains or side effects such as bitter taste, allergic reaction, and adrenal insufficiency have necessitated a further search for alternative agents [5, 8].

In this context, herbs and naturally derived bioactive compounds become a new source of antimycotic therapy. Herbal medicine has antioxidants, anti-inflammatory, and antimicrobial properties due to their phytochemical constituents such as flavonoid and alkaloid [2, 11]. Several reviews have indicated that herbal medicine and natural bioactive compounds have antibacterial, antiviral and antifungal effects [12–16]. Thus, it is hypothesized that these natural products might have a beneficial effect on oral fungal flora such as *C. albicans*. Although the effects of herbs have been investigated as antifungal agents in several studies, to the best of our knowledge, the effects these natural products on *C. albicans* have not yet been reviewed. Thus, the aim of this study was to review the anti-candida activities of (especially against *C. albicans*) of herbal medicines and natural bioactive compounds. The methods and findings of reviewed studies are summarized in Tables 16.1, 16.2, 16.3 and 16.4.

### 16.1.1 Garlic

Garlic (Allium sativum) is one of the herbal medicines with antimicrobial properties, which improves the functioning of the immune system [8]. Garlic contains large amounts of organosulfur compounds, such as allicin and flavonoids, which may prevent oxidative damage and reduce blood pressure and hypercholesterolemia, which might lead to the reduction and prevention of cardiovascular diseases (CVDs) and certain types of cancers [58, 59]. In addition to several health benefits of garlic on chronic diseases, it is

**Table 16.1** The effects of nutraceuticals and herbal medicine on *Candida albicans* based on *in vitro* studies

| *Author, year* | *Type of article* | *Agent* | *concentration* | *Way of candida collection* | *Treatment duration* | *Intervention assigned to the control group* | *Type of application* | *Main outcome* |
|---|---|---|---|---|---|---|---|---|
| Thamburan et al. 2006 [10] | In vitro | Tulbaghia alliacea Tulbaghia violacea Allium sativum | 0.06% 0.15% 0.30% | The culture was obtained from the university | 24 h | Fluconazole drug | Aqueous Methanol Chloroform | Extracts of T.violacea were most inferior in preventing the growth of the fungus; they were inactive at the lowest concentration of 0.06% and only shown small zones of inhibition at the higher concentrations. In contrast, all extracts of T.alliacea at all concentrations exhibited antifungal activity |
| Motsei et al. 2003 [7] | In vitro | Tulbaghia violacea Allium sativum Other species | 100 mg/ml | From 5-month-old baby and patient in Christ The King hospital | 7 days | Amphotericin B | Aqueous | They had the best activity against candida- Albicans |
| Mendoza-Juache et al. 2017 [5] | In vitro | Allium sativum | 7.8–1000μg/ml | 56 patients who wear the denture | 48 h | Fluconazole | Essential oil | The essential oil of A. sativum is more effective than fluconazole |
| Behbehani et al. 2019 [17] | In vitro | EGCG | 0.98–62,5μg/ml | Collected from dental patient | 48 h | Fluconazole and ketoconazole | Solution | The synergistic effect of EGCG was supported in this study.EGCG alone can inhibit the growth of candida in planktonic and biofilm mode. |

(continued)

**Table 16.1** (continued)

| *Author, year* | *Type of article* | *Agent* | *concentration* | *Way of candida collection* | *Treatment duration* | *Intervention assigned to the control group* | *Type of application* | *Main outcome* |
|---|---|---|---|---|---|---|---|---|
| Antunes et al. 2015 [18] | In vitro | Green tea extract | 10 ml | Acrylic resin | 24 h | Distilled water | Aqueous | Green tea was significantly effective in the heat-cured group but not in microwave one. |
| Ota et al. 2001 [19] | In vitro/ in vivo | Brazilian propolis | 1–10 mg | Dental school patient | 48, 72, 96, 120 h | Ethanol | Solution | *C. albicans* was most sensitive strain to propolis, especially at 8 mg/ml |
| Freires et al. 2016 [20] | In vitro | Brazilian propolis (EEP3, EEP13) | 0.2–500μg/ml | Bank of microorganism | 24 h | Nystatin | Liquid | Strong antifungal activity was observed, and EEP 13 has a lower MIC than EEP3 |
| Martins et al. 2002 [21] | In vitro | Brazilian propolis (EEP 20%) | 20μl | 12 HIV-seropositive and seronegative patient | 48 h | Nystatin (100 IU)<br>Distilled water (20μl)<br>Fluconazole (25 mg)<br>Clotrimazole (50 mg)<br>Econazol(25 mg) | Solution | In seropositive patient: EEP and nystatin was effective against candida and candida was resistant to other drugs<br>In seronegative patient: Candida was vulnerable to all antifungal drugs and EEP |
| Gomaa et al. 2013 [22] | In vitro | Egyptian propolis (5%) | 25–125 ng/μl | Patient with acute pseudomembranous disease | 24 and 48 h | Culture control (no additional drug) | Solution | EEP was successful in preventing candida growth at 75 ng/μl |
| Fonseca Santos et al. 2019 [23] | In vitro | Curcumin | 5 mg/g | School of pharmaceutical sciences | 12 h | Curcumin in oleic acid<br>Fluconazole (512μg/ml)<br>Amphotericin B (16μg/ml) | Solution | The formulation was effective in improvement of candidiasis |
| | Ex-vivo | | 7.8–1000μg/ml | | 14 h | | | Curcumin was more potent than other groups |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mahattanadul et al. 2018 [24] | In vitro | Chitosan-curcumin | 0.15 | Bioprosthetic biofilm colonization model | 10 min + 24 h incubation time | CHX group 0.2 w/v 0.9% normal saline | Mouthwash | Curcumin in addition to chitosan act as a safe agent to the management of candidiasis |
| Daliri et al. 2019 [25] | In vitro | Curcumin | 10.2% | Did not mentioned | 30, 60, 120 s PDT time and 24 h incubation time | Positive control: Culture without PS Negative control: Culture without c-albicans Nystatin | Aqueous extract | CUR 10.2% in 30 s has the highest antifungal activity |
| Seleem et al. 2016 [26] | In vitro/ in vivo | Lichochalcone A (licorice root) | 2.8–280μM | Commercial strain ATCC 90028 | 24 h | Fluconazole (32–320μM) Nystatin (100 mm) Ethanol 1% | Solution | Antifungal activity of Lichochalcone A was comparable to antifungal drugs. |
| Oliveira et al. 2013 [27] | In vitro | G.glabra L (licorice root) | 0.19–11 mg/ml | Obtained from the oral cavity of pulmonary tuberculosis patient | 24 h | Untreated group | Solution | G.glabra L shows the highest cytotoxicity effect especially against candida albicnas strain |
| Roque et al. 2018 [11] | In vitro | G.glabra L (licorice root) | 10 mg/ml | Commercial strain ATCC 10231 | 24 h | Nystatin Glabridin DMSO | Toothpaste Oral gel Oral film | Ethanol extracts showed the highest antifungal activity and oral film with PLGA exhibit the most interaction with mucin |
| Irani et al. 2010 [28] | In vitro | G.glabra L (licorice root) | 4 and 8 mg/disk | Commercial strain ATCC 10231 | 24 h | Chloramphenicol (30μg) Solvent | Ethanol Aqueous | Leave extracts and ethanolic form had significant antifungal activity |
| Sharma et al. 2016 [29] | In vitro | G.glabra L (licorice root) | 50 g in 67% ethanol | Commercial strain ATCC 66027 | 24, 48, 72 h | Fluconazol (10 mcg) Itraconazol (10 mcg) Clotrimazol (10 mcg) | Solution | G.glabra L had greatest antifungal activity compared to fluconazole and itraconazole and insignificant activity in comparison with clotrimazole |

(continued)

**Table 16.1** (continued)

| *Author, year* | *Type of article* | *Agent* | *concentration* | *Way of candida collection* | *Treatment duration* | *Intervention assigned to the control group* | *Type of application* | *Main outcome* |
|---|---|---|---|---|---|---|---|---|
| Oliveira et al. 2014 [9] | In vitro/ in vivo | C.Z (cinnamon) | 2 ppm | Commercial strain ATCC 76485<br>30 HC acrylic resin | 1: 24 h<br>2: 15 days | 2: Artificial saliva Nystatin (100,000 UI/ ML) | 1:ESSENTIAL OIL<br>2:Mouthwash | Cinnamon inhibits the growth of *C. albicans*<br>The hardness of acrylic resins in cinnamon mouthwash influenced less than nystatin group |
| Taguchi et al. 2010 [30] | Invitro/ invivo | Cassia (cinnamon cassia) | 100% (0.2 mg/ml) | Commercial strain TIMM1768 | 18 h | DMSO 10% | Solution | Strongest effect was related to cinnamon |
| Latti et al. 2019 [31] | Invitro | Cinnamon | 100% (10 mg/ml) | Commercial strain ATCC2091 | 24 h | DMSO | Crude extract solution | Cinnamon had maximum antifungal activity in comparison with other drugs |
| Veilleux et al. 2019 [32] | Invitro | Cinnamon<br>Cinnulin pf | 1.25–0.195%<br>62.5–1000μg/ml | Commercial strain ATCC28366<br>And cases of systemic candidiasis | 24 h | Nystatin | Essential oil | Cinnamon oil in combination with cinnulin of can act as an antifungal agent and prevent adhesion of candida to the oral mucosa |
| Almeida et al. 2016 [33] | In vitro | Cinnamon<br>Citronella | 1:7.8–1000μg/ml<br>2:1 mg/ml | 1:Commercial strain ATCC90028 and<br>2:Human saliva | 1:24–48 h<br>2:Immersion for 3 min and incubation for 24 h | 1:Nystatin(0.5–64μg/ml) and fluconazole<br>2:PBS | Essential oil | Both essential oils exhibited antifungal and antibiofilm activity |
| Warnke et al. 2009 [34] | In vitro | Cinnamon | Did not mentioned | Commercial strain ATCC10231 | 18 h | Olive oil<br>Industrial paraffin oil<br>Ethanol 70%<br>H2O2 3%<br>CHX 0.15<br>Povidone-iodine | Essential oil | The largest effective zone belongs to cinnamon oil. |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lam et al. 2016 [35] | In vitro | Berberine Berberrubine | 100μg/ml | Obtain from the American type of culture collection | 48 h | Terbnafine DMSO | Solution | BBR in combination with terbinafine will produce larger inhibition zone in plates of *C. albicans*. But BBR or berberrubine alone did not inhibit the growth of *C. albicans* |
| Wei et al. 2011 [36] | In vitro | Berberine | 1.95–250 mg/l | Obtained from the college of dentistry | 24 h | Fluconazole (0.5–16 mg/l) Miconazole (0.125–4 mg/l) | Solution | A combination of BBR and miconazole or fluconazole showed strong antifungal activity. However, BBR showed antifungal activity alone. |
| Quan et al. 2006 [37] | In vitro | Berberine | 1–32μg/ml | 40 clinical isolated of fluconazole-resistant strains ATCC90028 | 24 h | ATCC90028 as the control group | Solution | BBR or fluconazole do not show significant Antifungal activity, but the combination of them revealed strong antifungal properties |
| Zoric et al. 2017 [38] | In vitro | Berberine | 5.10.25.50, 100μg/ml | ATCC90028 | 15 and 60 min | PBS | Solution | BBR showed strong antifungal activity against *C. albicans* |
| Silva et al. 2016 [39] | In vitro | Berberine | 0.125–64μg/ml | Blood sample at central public health | 24 h | Other strains of candida used as controls | Solution | BBR showed great antifungal activities. |
| Aghazadeh et al. 2016 [40] | In vitro | Zingiber officinale(ginger) | 0.625–80 mg/ml | Clinical isolate from the Iranian research organization | 24 h | No treatment in control Ethanol 70% | Mouthwash | 0.625–5 mg/ml can be used successfully in the oral cavity for antibiofilm activity against *C. albicans*. |

(continued)

**Table 16.1** (continued)

| *Author, year* | *Type of article* | *Agent* | *concentration* | *Way of candida collection* | *Treatment duration* | *Intervention assigned to the control group* | *Type of application* | *Main outcome* |
|---|---|---|---|---|---|---|---|---|
| Pozzatti et al. 2008 [41] | In vitro | Ginger rhizome | 50–3200μg/ml | ATCC0231<br>HIV positive wit oropharyngeal candidiasis<br>Immunocompromised with disseminated candidiasis<br>ATCC90028 | 48 h | No additional treatment | Solution | Ginger showed antifungal activity but it was not as potent as oregano, thyme, and cinnamon |
| Lee et al. 2018 [42] | In vitro | Ginger (gingerol, shogaol) | 0–1000μg/ml | DAY185 | 24 h | DMSO 0.1% | Solution | Ginger showed that antifungal activity in compound contains a smaller carbon side chain |
| Okamoto-Shibayama et al. 2010 [43] | In vitro | Resveratrol | 20–200μg/ml | SC5314 | 16 h | No additional treatment | Solution | Resveratrol is effective against the yeast form of *C. albicans* |
| Jung et al. 2007 [44] | In vitro | Resveratrol | 10–40μg/ml | Center for academic societies in Japan | 48 h | Amphotericin B | Solution | Resveratrol had antifungal activity at a final concentration of 10–20μg/ml |
| Weber et al. 2011 [45] | In vitro | Resveratrol (R5010) | 0.2–2-20μg/ml | ATCC90028<br>ATCC76615<br>SC5314 | 24 h | Fluconazole (0–128μg/ml) | Solution | Resveratrol did not inhibit candida's growth |
| Juin et al. 2019 [46] | In vitro | Resveratrol (EB487) | 0.81–20.32 mM | Atcc28367<br>Ca14-p | 24 h | Untreated group | Solution | Strong ability of EB487 was demonstrated to inhibit biofilm growth and preformed biofilm |

**Table 16.2** The effects of nutraceuticals and herbal medicine on *Candida albicans* based on clinical trials

| *Author, year* | *Type of article* | *Agent* | *Dose* | *Treatment duration* | *Number of participants* | *Mean age* | *Intervention assigned to the control group* | *Type of application* | *Main outcome* |
|---|---|---|---|---|---|---|---|---|---|
| Bakhshi, et al. 2012 [8] | Randomized double-blind clinical trial | Garlic | 40 mg/ml | 4 weeks | 40 | 73,52 ± 9.81 | Nystatin mouthwash | Aqueous solution | Improving the erythematous lesion |
| Sabitha et al. 2005 [47] | Randomized clinical trial | Garlic | Quantity sufficient to cover entire lesion | 2 weeks | 56 | More than 18 | Clotrimazole solution 1% | Paste | As effective as clotrimazole |
| Ghorbani et al. 2018 [48] | Clinical trial | Camellia sinensis | 0.5% | 1 and 7 and 14 days | 22 | 65 ± 11.3 | Nystatin suspension 1000 u/ml | Mouthwash | Length and width of lesion decreased in green tea groups as well as nystatin |
| Pina et al. 2017 [6] | Multicentric randomized trial | Brazilian propolis | 2% | 2 weeks | 40 | Over 60 years | Miconazole 20 mg/g | Gel | EEP-AP seems to be no inferior to miconazole and can be recommended as an alternative |
| Capistrano et al. 2013 [4] | Randomized clinical trial | Brazilian propolis | 2%<br>24% | 2 weeks | 45 | 60.76 ± 11.34 | Miconazole gel 2% | Gel<br>Mouthwash | Both forms of propolis have antifungal activity similar to miconazole |
| Santos et al. 2008 [49] | Clinical pilot study | Brazilian propolis | 10% | 1 week | 30 | 51 | Miconazole | Gel | Complete remission of the lesion in both groups was observed |
| Santos et al. 2005 [50] | Clinical trial | Brazilian propolis | 20% | 1 week | 18 | 42 | Nystatin 100.000 UI/ML | Solution (bottle) | Propolis group treated lesion similar to control group |
| Ota et al. 2001 [19] | Invitro/invivo | Brazilian propolis | 6% | 2 weeks | 12 | – | Hydroalcoholic without propolis | Mouthwash | Propolis group showed a significant reduction in number of candida |
| Mustafa et al. 2019 [51] | Single center RCT | Curcumin | 0.1% | 2 weeks | 30 | 58 | CHX mouthwash Chitosan 0.5% | Mouthwash | Efficacy of CHI-CUR mouthwash was better than other groups |
| Oliveira et al. 2014 [9] | Invitro/invivo | C.Z (cinnamon) | 625μg/ml | 15 days | 15 | 40–60 years | Compared to invitro part of the study | Mouthwash | None of patients showed signs and symptoms anymore |

**Table 16.3** The effects of nutraceuticals and herbal medicine on *Candida albicans* based on animal studies

| *Author, year* | *Animal type* | *Agent* | *Dose* | *Treatment duration* | *Number of animals* | *Intervention assigned to the control group* | *Type of assessment* | *Main outcome* |
|---|---|---|---|---|---|---|---|---|
| Rahayu et al. 2018 [1] | Wistar rats | Camellia sinensis | Green tea 1.25% EGCG 1% EGC 1% | 4 and 7 days | 35 | No additional treatment | Collection of blood serums | Green tea has an immunomodulatory and positive effect against candida more than two other type |
| Dovigo et al. 2013 [52] | Murine model | Curcumin | 7.4, 14.7 and 29.5 mg/ml | 20 min preactivation time 7 min LED | 45 | Positive: DMSO 10% Negative: Animals without immunosuppression | Samples swabbed from the tongue of mice | All curcumin and LED light were able to create a significant reduction in colonies count |
| Sakima et al. 2018 [53] | Murine model | Curcumin | 260μM | 5 days | 235 | No additional treatment | Samples swabbed from the tongue of mice | Cationic CUR-NP ± PDT, NYS1, NYS4, and free CUR+ PDT shows good antifungal activity |
| Karaman et al. 2011 [54] | BALB/c mice | Curcumin | 200 mg/kg | 5 days | 35 | Negative control: Without candida Positive control: Saline solution | Serum sample were assessed | Curcumin in combination with dexamethasone shows significant reduction in candida colonies |
| Seleem et al. 2016 [26] | BALB/c mice | Licorice | 7.5 mM | 5 days | 15 | Nystatin(100 m M) ETHANOL 1% | After euthanizing the mice tongues were sectioned | Lichochalcone A and nystatin show significant antifungal activity |
| Taguchi et al. 2010 [30] | In vitro/ in vivo | Cassia (cinnamon) | 5% 25% 100% (200 mg/ml) | 5 times after infection (3, 21, 27, 45, 41 h) | 101 | No additional control group | Samples swabbed from tongue of mice | 100% concentration exhibited antifungal activity and eradicated the symptom |

**Table 16.4** Summary of review studies regarding the effects of nutraceuticals and herbal medicine on *Candida albicans*

| Author, year | Type | Number of included studies | agent | Main outcome |
|---|---|---|---|---|
| Casaroto et al. 2010 [55] | Review | 3 in vitro<br>2 in vivo | Propolis | Propolis can be suggested for the treatment of candidiasis |
| Santezi et al. 2018 [56] | Review | Total: 28<br>Candida-albicans: 7 | Curcumin | PDT promote a reduction of c-albicans |
| Sidhu et al. 2018 [57] | Review | 3 animal<br>3 invitro | Licorice root | Licorice can be mentioned as a useful therapeutic agent and as an alternative drug for oral candidiasis. |

revealed that active components of garlic, allicin, and allicetaine (the most sulfur-containing compounds) have a therapeutic and antimicrobial properties [7]. Allicin and garlic extract causes an increase in the synthesis of cytokines, macrophage activity, lymphocytes, and other cells of the immune system [8]. It is effective against gram-positive and gram-negative bacteria, as well as *C. albicans* [7]. Chemical reaction with thiol groups of various enzymes is the significant antimicrobial effect of allicin [8]. Besides, the absorption of allicin and allicetaine through the digestive system and their further entrance in serum and blood could prevent the growth of micro-organisms [8]. Two native South African garlic species are Tulbaghia alliacea and Tulbaghia violacea, which are traditionally used as remedies for a variety of infections and diseases [10]. Regarding the antibacterial properties of garlic, several previous studies have assessed its impact on oral health, particularly *C. albicans*, the main findings of them are reviewed here.

In a randomized, double-blind clinical study, 40 aged people, who wear dentures, were divided into two groups. They were assigned to use garlic aqueous solution at a concentration of 40 mg/ml three times a day for 4 weeks and compared with a control group, in which nystatin mouthwash (medication used to fight fungal infections of the mouth) was used instead of the garlic. Results revealed that the application of nystatin and garlic produced a significant effect on the length and width of erythema. However, garlic aqueous had fewer side effects in comparison with nystatin, so the authors concluded that garlic might be considered as an appropriate replacement for nystatin in handling denture stomatitis [8].

In another randomized clinical trial, 74 patients aged above 18 years were divided into two groups. One group was assigned to use garlic paste (quantity sufficient to cover the entire lesion) with one drop of 2% lignocaine jelly for four times a day for 2 weeks. Another group was asked to use 1% clotrimazole solution. Eighteen patients (7- clotrimazole, 11 garlic paste) did not turn up for clinical evaluation. Results of 56 patients showed that in all, 61.5% of the garlic group showed complete suppression of the lesion compared with 50% of the clotrimazole group. The two treatments did not show statistically significant differences in the response rate [47].

In an *in vitro* study, solvent extractions of different concentrations (0.06%, 0.15%, 0.30%) of Tulbaghia alliacea and Tulbaghia violacea and Allium sativum were prepared, using a solvent of water, methanol, and chloroform. All solutions were tested for antifungal activity against candida-Albicans using agar plate disk. Results showed that extracts of Tulbaghia violacea were weakest in inhibiting the growth of candida. Tulbaghia alliacea and Allium sativum aqueous extracts, at 0.15% and 0.30% concentrations, T. alliacea exhibited a more significant zone of inhibition than A.sativum. In methanol extracts at all concentrations, T.alliacea had a broader area of inhibition. In chloroform extracts, in 0.30% concentration, the most significant zone belonged to the T.alliacea [10]. In an in vitro study, which studied traditional plants for managing candidiasis, they prepared plant material in an aqueous

extract with 100 mg/ml concentration. Then added extracts to sabouraud dextrose agar, which contain *C. albicans*. The aqueous extracts were divided into three parts stored at 4, 23, 33 °C for 7 days to assay antifungal activity. Plants extracts of Allium sativum and Tulbaghia violacea had the best activity against *C. albicans*. Allium sativum and Tulbaghia violacea remained active for 3 and 2 days, respectively, when stored at 4 °C. The Allium sativum extracts were the most stable in solution, although this activity decreased with longer storage [7] In an *in vitro* study, a total of 56 patients aged more than 40 years with acrylic dentures, who wore them at least 6 months, were included. The internal surface of denture was brushed with a sterile swab. Then swabs were suspended and prepared on CHROMagar candida at 36 °C for 2 days and subsequently another additional 7 days in 30 ± 1 °C to ensure reducing false-negative results. The essential oil of A.sativum with a final concentration of 7.8–1000μg/ml was used in this study. The control group was fluconazole with a level of 0.5–128μg/ml. The results of this study shown that the essential oil of A.sativum is more effective than fluconazole in the prevention of the growth of both planktonic and biofilm of *C. albicans* extracts from dentures [5].

### 16.1.2 Green Tea

Green tea is one of the most popular beverages, which has a high amount of flavonoids with antioxidant properties. Catechins which constitute about 20% of green tea flavonoids are the major constitution of green tea. In addition to several health benefits of green tea and catechins including anti-hypertensive [60], hypolipidemic [61, 62], anti-hyperglycemic [63, 64], anti-arteriosclerotic [65], anti-cancer [66], antioxidant [67], and anti-thrombotic properties [68, 69], green tea (Camellia Sinensis) contains some polyphenolic ingredients which show antifungal activities against Candida species [70, 71]. The potential antimicrobial properties of green tea are getting more prominent in recent decades [48].

Due to anti-inflammatory, antioxidant, anti-mutation, and anti-diabetic properties of green tea, it has an essential role in the improvement of erythema and mucosal inflammation [48]. Green tea contains catechin, which is consist of epicatechin (EC) epigallocatechin (EGC), EC gallate, and EGC gallate (EGCG). EGEC and EGC can influence the proliferation of lymphocyte T and cytokines production; thus, it can be assumed that green tea and its extracts (such as flavonoid) possess immunomodulatory effects against oral candidiasis. Flavonoids can induce synthesis of IL8, IL17, which are chemoattractants resources and can recruit neutrophil for phagocytosis to impede *C. albicans* colony formation [1]. Due to the antifungal activity of green tea, several studies have investigated the potential clinical applications of green tea on the oral cavity [72].

In a randomized clinical study, 22 patients with denture stomatitis were divided into two groups, namely nystatin and green tea. Candida infections of dentures were proved by culturing on CHROMagae candida medium; then patients received nystatin suspension 1000μ/ml and 15 ml green tea mouthwash (0.5%) four times a day. Then lesion size was measured in 1st, 7th, and 14th days. The results showed that the mean of candida colonies showed significant differences in green tea and nystatin groups before and after treatment. The average length and width of the lesion in these two groups decreased with the duration of treatment, and there were no statistically significant differences between nystatin and green tea [48]. In an animal study, which was conducted on 3 months old Wistar rats, monoclonal antibodies (IL8, IL17, HBD-2) were analyzed. Immunocompromised rats (induced by dexamethasone 0.8 mg/kg and tetracycline 12 mg/kg intraperitoneally for 7 days) were divided into seven groups: a control group and the group treated with green tea concentration of 1.25%, EGCG 1%, and EGC 1% for 4 and 7 days separately. Results showed that the expression of IL8, IL17, and HBD2 significantly increased in 7 days relative to 4 days. The expression of antibodies was considerably higher in green tea extracts group compared with EGC and EGCG. Also, there were insignificant differences

between the EGC and EGCG groups [1]. In an *in vitro* study, oral candida samples were collected from dental patients, and after preparation on CHROMagar medium, different concentrations of EGCG and different combination of EGCG and fluconazole or ketoconazole were prepared and added to the well after which incubated for 48 h. The effect of EGCG with or without antifungal drugs was measured in this study to assess the synergistic effect of herbal and chemical drugs. The result was in the following order: EGCG alone showed a significant inhibitory effect on the growth of the colony. It was indicated that the mature biofilms of candida were resistant to fluconazole and ketoconazole. When EGCGA was combined with these drugs, the synergistic effect was observed in the inhibitory effect against Candida species [17]. In another *in vitro* study, the effect of aqueous green tea was compared with free alcoholic mouthwash (Listerine) was analyzed. In this study, 60 specimens of heat-cured (HC) and microwaved cured (MV) acrylic resin were tested. The samples were treated with sabouraud broth and incubated for 24 h in a vertical position due to biofilm formation. After this time, they were immersed in the mouthwash or green tea extract for 15 min. CFU was measured, and results revealed that in HC group, green tea reduced colony formation significantly compared with the control group but in MW group, green tea decreased non-significantly the colony count. In both groups, mouthwash has a significant inhibitory effect on colony formation [18].

## 16.2 Propolis

Propolis is produced by honey bees from substances extracted from parts of some plants, buds and sap [73]. With regard to a wide range of biological constituents (more than 230 constituents), propolis as a natural resin has several biological activities, including antioxidant, anti-inflammatory, antibacterial, antiviral, fungicidal, hepatoprotective, free radical scavenging, immunomodulatory and anti-diabetic activity [74, 75]. For a long time, propolis was used to improve the health status of numerous diseases, such as mucocutaneous infections of fungal, bacterial and viral etiology and gastrointestinal disorders [76–78]. Along with the antifungal activity of propolis, there is a hypothesis that it may cause a change in the phenotype of the fungus without reducing it quantitatively [4, 6]. Because of this feature of propolis, several investigators have studied the antifungal action of propolis against *C. albicans* [21].

In a multicentric randomized trial, two groups of volunteers, with 20 patients per group, received treatment for denture stomatitis. The control group was treated with an oral gel containing 20 mg/g miconazole and propolis group used a gel with EPP-AP 2%. The treatment follows three times a day for 14 days. The results showed that a significant remission of the lesion during 2 weeks of treatment, and both groups achieved a 70% clinical cure rate. The CFU/ml count was reduced in the miconazole group, but the propolis group, besides its clinical improvement, did not show a significant reduction in the number of colonies [6].

In a randomized clinical trial, 45 patients, who were divided into three groups, were examined because of their denture stomatitis. One group received topical use of miconazole gel 2%, another one, propolis gel 2.5%, and the last one, propolis 24% in the form of mouthwash. The treatment lasts for 2 weeks, and four daily applications were used in these groups. The results presented that there were no statistical differences in efficacy among groups, and clinical remission of stomatitis, wholly or partially, was observed at the end of the treatment [4].

In a clinical pilot study, 30 patients were assigned to use a swab to dry the infected area beneath their complete denture and then apply drugs topically four times a day for 7 days. One group (n = 15) asked to use miconazole gel, and another group was allocated propolis gel. At the end of the week, no significant differences were observed among the two groups. All patients showed complete remission of palatal edema and erythema [49]. In another clinical trial, 18 patients (6 patients in the control group) accept the treatment. In the control group, they were

asked to use the solution of nystatin 100,000 UI/ML four times a day for 1 week. In the other group, they were asked to apply EEP 20% in the same way as used in control one. The results exhibited that all patients treated with EEP and antifungal drugs showed remission of the candidiasis lesions [50].

In an *in vitro/in vivo* study, antifungal activity of propolis was measured. First in the in vitro part, candida species were collected from dental school patients and cultured in M20 agar for 24 h. Then, 1–10 mg/ml of propolis was added to the agar plate and incubated for 48, 72, 96, and 120 h at 37 °C. Results showed that *C. albicans* was the most sensitive strain to propolis at 8 mg/ml concentration. In *in vivo* part, 12 patients, who wear a full denture and show stomatitis, were recruited to the study, five patients received hydroalcoholic solution 6% without propolis twice daily for 2 weeks. The rest of them received the hydroalcoholic solution of propolis (6%) in the form of mouthwash in the same way. Results revealed that all the patients in the propolis group show a significant reduction in the amount of candida in their saliva. However, the control group did not show any differences before and after treatment [19].

In an *in vitro* study, EEP3 and EEP13 were examined. Candida species were obtained from the bank of microorganisms. 0.2–500μg/ml concentration of EEP was added to the medium and incubated at 35 °C for 24 h. The control group was nystatin. In this study, the observation was in the following order: both extracts have vigorous antifungal activity, and EEP13 was stronger than EEP3 [20]. In another *in vitro* study, the effect of EEP on HIV-seropositive and HIV-seronegative with oral candida was evaluated. *C. albicans* were obtained from 12 HIV-positive and 12 normal patients. After culturing candida, EEP 20% applied to the agar surface and incubated at 37 °C for 48 h. The results were compared with the Econazole (25 mg), Clotrimazole (50 mg) and Fluconazole (25 mg). Also, 100 IU nystatin and 20μl sterile distilled water were used as the positive and negative control group, respectively. The results were in the following order: in seronegative patients with denture stomatitis, *C. albicans* showed significant vulnerability to all antifungal and EEP groups. In the seropositive group, clotrimazole and fluconazole did not inhibit the growth of candida. Econazole was ineffective against fungus and EEP, and nystatin, with no significant differences between them, produced a bigger inhibition zone against candida. Candida was significantly susceptible to EEP and nystatin [21].

In another *in vitro* study, Egyptian propolis was assigned to assessed adhesion and germ tube formation of oral candida. Candida species were taken from a patient with acute pseudomembranous and cultured in YPD agar plate. EEP 5% with concentration 25–125 ng/μl added to agar plate and colonies were counted after 24 and 48 h at 37 °C. Results showed that a gradual decrease in CFU occurred at 75 ng/μl, and propolis was successful in preventing germ tube formation [22]. In a review article that analyses phytomedicines for candida-associated denture stomatitis, pointed out that propolis may activate macrophage. Also, it is indicated that gel formulation could be used as an alternative topical choice for the improvement of denture stomatitis [55].

## 16.3 Curcumin

Curcumin is the bioactive pigment found in turmeric, which is known for its safety and multitude of pharmacological effects [79–88]. Photodynamic therapy (PDT) originates in the interaction between two items: a nontoxic photosensitizer (PS) and visible light. Interaction between these two factors releases reactive oxygen species (ROS) in front of oxygen. ROS are toxic and can destroy microorganisms [25]. Curcumin has excellent potential as a PS due to its ability to be activated by blue light [56]. Also, curcumin possesses antitumor, anti-inflammatory, antioxidant, and antimicrobial features [16, 25]. Regardless of these features, curcumin is lipophilic, so the clinical application of curcumin can be problematic. Numerous research performed to find out a more effective drug delivery system [53] and its potency as a PS, in addition to efficacy against oral candidiasis [51]. In a single-

center randomized clinical trial, 30 patients over 20 years old, who wears removable acrylic dentures and possess denture stomatitis, were divided into three groups. (1) Chitosan-curcuminoid (CHI- curcumin) mouthwash 0.1%, (2) chlorhexidine (CHX) mouthwash, and (3) vehicle formulation comprising chitosan 0.5% and PEG 400 (CHI). They were assigned to use them for 30 s three times a day for 14 days. Results showed that all groups had a high site activity in comparison with before treatment. CHI-curcumin was significantly stronger than other groups, which showed an 80% complete response [51]. In an animal study, which considered photodynamic inactivation of *C. albicans* in a murine model, 45 mice were divided into different groups. The groups are in the following order: one negative control group that contains five mice without any immunosuppression and one positive control group that treated only with sterile saline solution at 10% DMSO. Other groups received 7.4 (20μM), 14.7 (40μM), 29.5 (80μM), mg/l of curcumin with and without LED (455 nm, 89.2 MW/cm2). The last group received an LED light in the absence of curcumin. First of all, in treated groups, curcumin was topically applied to the dorsum of the tongue in a dark room for 20 min and was not swallowed. Then LED was performed for 7 min. The results exhibited that PDT was effective against candida, and all concentrations of curcumin produce a significant reduction in colonies. However, there were trivial differences between 40 and 80μM [52]. In another animal study, PDT by curcumin encapsulated in nanoparticles (curcumin-NPS) was conducted on the murine model, which were immunosuppressed to induce oral candidiasis. Two hundred thirty-five mice were selected in this study. Arrangement of groups was in the following order: the control group that received no treatment, free curcumin with and without PDT, anionic CUR-NP with and without PDT, cationic curcumin-NP with and without PDT. Nystatin (four times a day (NYS4) and once a day (Nys1). The maximum concentration of curcumin was 260μM, and treatment was given daily for 5 days. Results revealed that free curcumin without light and anionic CUR-NP (with and without light) did not show a significant reduction in colonies, whereas other groups decreased colony counts. Free curcumin with PDT was similar to cationic curcumin. There were no differences between NYS1 and NYS4. There were no differences between cationic CUR-NP with or without PDT [53].

In another animal study that analyzes the effect of curcumin on oropharyngeal candidiasis, 35 of BALB/c mice were divided into five groups. Group I received 1 mg/kg dexamethasone, group II has gotten 200 mg/kg curcumin dissolved in 2% carboxymethyl cellulose orally, group III received dexamethasone plus curcumin and group IV and V has gotten saline. Induction of candidiasis and asthma was not performed on group V and the treatment lasted for 5 days. Outcomes showed that groups II and III showed a significant reduction in candidiasis without differences between them. Also, group I indicated a significant lessening, but it was lower than other groups [54].

In the *in vitro/ex vivo* study, the antifungal potential of curcumin was assessed; in the ex-vivo part, retention of curcumin in the mucosa was measured. Experiments were carried out with curcumin, and formulation diluted to 30% with artificial saliva. Results compared to the control group (curcumin dissolved in oleic acid). Outcomes showed that retention values after 12 h in curcumin and formulation 30% were five times and three times higher than control, respectively suggesting that this system can be used for drug delivery. In the invitro part, curcumin with concentration from 7.8 to 1000μg/ml was added to the well and incubated at 37 °C for 48 h. Control groups were amphotericin B (16μg/ml) and fluconazole (512μg/ml). Results revealed that curcumin was more effective than control groups against candida colonies [23].

In the *in vitro/in vivo* study, the efficacy of chitosan-curcumin was evaluated. The *in vivo* study was carried on hamsters and evaluated ulcer healing, which is not the purpose of this review. Nevertheless, in the *in vitro* study, for determination against fungus in biofilm, 0.1% curcumin 0.5% chitosan mouthwash was used and compared to 0.2% CHX, blank formulation,

formulation with 0.5% chitosan only and 0.1% curcumin only. Disks contain candida were soaked in these solutions for 10 min and then incubated for 24 h at 37 °C. Results revealed that when curcumin combined with chitosan, its anti-candida efficacy was comparable to CHX mouthwash [24]. In another *in vitro* study, to assess PDT, an aqueous extract of curcumin 10.2% and methylene blue at 0.1% and 0.2% concentrations were used. Each solution measured with and without PDT and compared to the positive and negative control group, which was a culture without using PS and culture without *C. albicans*, respectively. As well, one group consist of 0.1 ml nystatin was compared. The highest antifungal activity was observed in 10.2% curcumin with 460 nm diode laser (25 MW) for 30 s. furthermore, curcumin was significantly more efficient than nystatin [25]. Recently, a review article determined the effectiveness of PDT on curcumin against several microorganisms and concluded that permanent DNA damage in candida occurred due to PDT. However, further investigations are needed in this aspect [56].

## 16.4 Licorice Root

Licorice root is composed of flavonoids and triterpenoids [89]. Some bioactive natural compound in licorice root is glycyrrhiza species, glabridin, liquiritin (a glycosidic form of liquiritigenin) and lichochalcone A [11, 29, 89]. These extracts show some antiinflammatory, antiulcer, antimicrobial, and antifungal activities [11]. The effectiveness of licorice root in oral diseases such as dental caries, candidiasis, periodontal disease has been great interest recently [57]. Several studies investigated the effect of licorice root on preventing and treating oral disease [57].

In a study that evaluates the antifungal activity of lichochalcone A against candidiasis, *in vitro* and *in vivo* studies were conducted. In the *in vitro* assay, commercial *C. albicans* strains and some resistant strain to fluconazole were selected. The final concentration of lichochalcone A was 2.8–280μM and used as a test group. Fluconazole (32–320μM) and nystatin (100 mM) used as positive control and 1% v/v ethanol as vehicle control. The drugs were applied to plates of candida and incubated for 24 h at 37 °C. Results demonstrated that in ordinary candida, lichochalcone A shows a comparable antifungal activity in similar potency of antifungal drugs, and in resistant strain, lower concentration of lichochalcone A was required to inhibit the growth of strain [26].

In the *in vivo* part of this study, 15 Balb/c mice were used. They were infected with oral candidiasis. The topical use of lichochalcone A (7.5 mM) for 30 s was applied twice daily for 5 days. Nystatin (100 mM) and ethanol 1% used as positive control and vehicle control, respectively. Mice were euthanized, and the tongue of them was sectioned and evaluated. There was a significant reduction of strains in the lichochalcone A and nystatin group. The tongue of these samples showed less severity of hyphal invasion compare to the vehicle control (39).

In an *in vitro* study, the effect of different herbal drugs such as glycyrrhiza glabra L (G.glabra L) was assessed against candida and bacterial strains. Strains were cultured, and extracts of G.glabra L (0.19–100 mg/ml) were applied on plates and incubated for 24 h at 37 °C and was compared to untreated control culture. It showed that the highest cytotoxicity was related to G.glabra L in contrast to other herbal extracts, and candida species were more sensitive to the extracts than bacterial one [27].

In a further *in vitro* study, bioadhesive nanoformulation of G.glabra L was measured. Different solvents, nanoparticles, and mucoadhesives dosage form were used that are named in the following order: solutions such as ethanol/acetone/dimethyl sulfoxide (DMSO)/water and hydroalcoholic. Nanoparticles (NPS) such as alginate, polylactic acid (PLA), polylactic-co-glycolic acid (PLGA), and NPS that were embedded into toothpaste, oral gel, and oral film. The final concentration of extracts was 10 mg/ml. They were applied on plates of c-albicans and incubated for 24 h at 37 °C. The results were compared with negative control (DMSO) and two positive ones (nystatin and free glabridin). The outcomes from this study showed that the most active extracts were ethanolic one (18%

w/w). NPS was produced successfully, and the antifungal activity of NPS was similar to free extracts of glabridin in MIC. Also, the oral film with PLGA NPs showed the highest interaction with the mucin. Besides, toothpaste with alginate NPs and oral gel with PLA NPs show high interaction with mucin [11].

In another *in vitro* study, different types of G.glabra extracts were examined including leaf ethanolic (5.4%), leaf aqueous (4%), root ethanolic (10%) and root aqueous (9.6%). They were added on c-albicans agar plates in 4 and 8 mg/disk and incubated for 24 h at 37 °C. The outcomes were compared to chloramphenicol (30μg) and solvent disk as the positive and negative control, respectively. Results showed that leaf extracts exhibited better activity than root extracts. As well, ethanolic extracts showed the most antifungal activity [28]. Moreover, an *in vitro* study, which examined antifungal activity of different medicinal plants, showed that extract of G.glabra L had a stronger inhibitory effect against other extracts. In comparison to antifungal agents, G.glabra L had better antifungal activity than fluconazole (10 mcg) and itraconazole (10 mcg) but lesser efficacy than clotrimazole (10 mcg) that was not significant statistically. In their method, they applied extracts (50 g powder dissolved in 67% ethanol) of different plants on plates of c-albicans and incubated for 24–48-72 h. G.glabra L showed maximum efficacy and was the most effective agent among all plants in this study [29]. In a review of studies such as those by Messier et al. (2011) [90], Utsunomiya et al. (2000) [91], Lee et al. (2009) [89] and Fatima et al. (2009) [92], and concluded that licorice is a therapeutic agent and can be used as a substitute agent for oral candidiasis [57].

## 16.5 Cinnamon

It has been shown that cinnamon possesses antiseptic, antimicrobial, analgesic properties [9]. This feature, especially antifungal activity, may be related to cinnamaldehyde. It inhibits amino acid decarboxylase activity. Cinnamaldehyde makes 50.5% of cinnamon bark and can involve in the biologic process due to its electronegative feature and reacts with the nitrogen-containing compound. This process inhibits the growth of microorganisms [31]. According to these characteristics, several studies have been conducted to displayed numerous beneficial of cinnamon against fungus like candida-albicans. In a study comprising of *in vitro* and *in vivo* assay, essential oil of Cinnamomum zeylanicum (C.Z) against oral candidosis was evaluated. In phase I study (*in vitro*), essential oil at a concentration of 2 ppm used as a solvent and compared to nystatin (control). Oil added to plates of candida and incubated for 24 h at 30 °C. In part II (*in vitro*), thirty HC acrylic resins were divided into three groups of artificial saliva (negative control) mouthwash, C.Z and nystatin. The acrylic resin was immersed for 1 min, three times a day for 15 days. Finally in phase III (clinical) 15 patients who showed signs and symptoms of denture stomatitis, assigned to use mouthwash (C.Z) 3 times a day for about 60 s in 15 days. Additionally, they received a container containing 500 ml of mouthwash to clean dental prostheses [9]. Results of these three phases were in the following order: all the test strains showed sensitivity to essential oil, and c-albicans showed the most sensitive behavior. For acrylic resin, the surface hardness of prosthesis was analyzed, and the mouthwash group causes a lower degree of changes in hardness when compared with the nystatin group. Finally, the clinical phase of the study revealed that using mouthwash at a concentration of 635μg/ml can heal the signs and symptoms [9]. In another study, the effect of Cassia (Cinnamomum cassia) on the murine model and culture of c-albicans was evaluated, In the *in vitro* condition, the effect of different herbs was measured, and cinnamon, DMSO (as control) were compared, too. They were added to the well and incubated for 18 h at 37 °C. Results showed that the inhibitory activity of cassia preparation was relatively stronger than other herbs. In the animal part of the study, 31 mice were examined for the cassia effect and 70 mice for other herbs. Cassia prepared in different concentrations 5% 25% and 100% (200 mg/ml). Control mice received no treatment. This preparation was administered by

force-feeding. Results exhibited that 100% concentration showed a great clinical score in the tongue, whereas 5 and 25% did not [30]. Furthermore, in an *in vitro* study. 100% (10 mg/ml) concentration of different extracts such as cinnamon was added to the standard strain of c-albicans and allowed to stand for 10 min, then incubated for 24 h at 37 °C. the control group was DMSO. Results showed that cinnamon extract showed the highest antifungal activity in comparison with other herbs such as cumin, dried black pepper, dried India bay leaves [31]. In an additional study that examined on antifungal activity of cinnamon bark and adherence to epithelial cells, they used cinnamon oil (1.25–0.195%), cinnulin pf (62,5–1000μg/ml) as test group and nystatin was used as a reference. Summary of the results is in the following order: cinnamon oil showed inhibition of *C. albicans* growth, but cinnulin pf did not have any effect on fungus even at maximum concentration. However, both of them significantly reduced biofilm formation. Also, either of them (cinnulin more than cinnamon oil) reinforcing the epithelial could prevent the invasion of the oral mucosa by oral pathogens. Cinnulin pf meaningfully weakened the adherence of *C. albicans* while no such effect was observed with cinnamon oil. In conclusion, to obtain the best way against the adherence of candida and inhibition of its growth is to combine two cinnamon fraction [32]. In another *in vitro* study, at first, 7.8–1000μg/ml concentration of cinnamon citronella essential oil was compared to nystatin and fluconazole (concentration range of 0.5–65μg/ml). Agents were added to the *C. albicans* culture and incubated for 24–48 h at 37 °C. Secondly, 27 HC acrylic resins were contaminated with human saliva that was collected from two healthy people, and different considerations were performed to allow adherence of candida to the surface of acrylic resins. Three groups of acrylic resin immersed in PBS (control), cinnamon essential oil, and citronella essential oil for 3 min. The results of two parts of the study exhibited that both of the essential oil had an antifungal and antibiofilm activity that can be due to the lipophilic nature of essential oil that disrupts the cell membrane of microorganisms [33].

Moreover, in the *in vitro* study, the activity of Indian medical plants such as Cinnamomum verum bark (C.V) against fluconazole-resistant *C. albicans* was assessed. 250, 500 and 1000μg/ml concentration of plants were prepared and added to the well and incubated for 24 h at 37 °C. The results showed that all extracts, specially C.V, exhibited antimycotic activity and can be used as an alternative drug against *C. albicans* [2]. Finally, another *in vitro* study that investigated the effect of different essential oil against microorganisms discovered the antibacterial and antifungal activity of all essential oil include cinnamon oil. Similarly, it showed that the largest active zone was attributed to cinnamon oil. In this study, their control group was olive oil, industrial paraffin oil, ethanol (70%) H2O2 (3%) CHX (0.1%), and povidone-iodine. Also, their incubation time was 18 h at 37 °C [34].

## 16.6 Resveratrol

Resveratrol is a major bioactive component in plant extracts that have been used for treating various human diseases as traditional medicine [44]. Some pharmacological effects of resveratrol, such as antiviral, anti-inflammatory, lifespan extension, have been demonstrated in various studies. But little evidence about antifungal activity exists [43]. In an *in vitro* study, 20–200μg/ml stock solution of resveratrol was added to the well of *C. albicans* and incubated for 16 h at 30 °C. To assess the morphological transition of *C. albicans*, they recognized that resveratrol was effective in both types of fungus (hyphal growth and yeast-form growth) and can inhibit *C. albicans* growth. Also, resveratrol impaired morphological transition in various situations. For instance, serum induction, nutrient starvation, and neutral ph. They mentioned that the inhibition of yeast form occurred at 100 or 200μg/ml concentration [43]. Moreover, another *in vitro* study has been indicated that the compound of resveratrol can disrupt the serum-induced fila-

mentous form of *C. albicans*. They stated that potential antifungal activity was at 10–20μg/ml concentration. Nevertheless, it was a little less potent than amphotericin B that used as a control group. They proposed that the antifungal activity of resveratrol is due to the induction of some intracellular physiological changes, Trehalose accumulation happens, and cell-cycle will be arrested [44]. On the contrary, an *in vitro* study that used 0.2, 2, and 20μg/ml solution of resveratrol and compared with fluconazole (0–0.128μg/ml) stated that no distinct reaction observed against *C. albicans* even at 20μg/ml. They incubated the plates with resveratrol and candida for 24 h at 37 °C.in their study, different experimental condition and type of candida strains were mentioned as a variable effect of resveratrol antifungal properties [45]. On the other hand, the latest study in 2019, which investigates the antibiofilm activity of a semi-synthetic molecule obtained from resveratrol, showed antifungal activity of this phytochemical. In this essay, 0.81–20.31 mM concentration of EB487 (resveratrol) was applied to the well and incubated at 37 for 24 h. They concluded that above 8.13 mM would inhibit biofilm growth regardless of the studied strains and 20.32 mM as the highest tested concentration inhibits 80% biofilm growth [46].

## 16.7 Ginger

Ginger is used to treating movement disorders, nausea, and vomiting during pregnancy in traditional medicine [40]. It consists of polyphenol compounds in its root and extract, which have considerable antioxidant activity. Several studies have shown antibiofilm activity against pathogenic bacteria [41] and antifungal properties against *C. albicans* isolated from the patient with genital candidiasis. However, there are only a few studies evaluating the effect of ginger on oral candidiasis [40]. In an *in vitro* study, the mouthwash form of ginger was applied to the well of candida and incubated for 24 h. different concentration of ginger (0.625–80 mg/ml) was compared to Muller-Hinton agar without ginger extracts and ethanol 70% as control groups. They concluded that biofilm reduction started at a concentration of 0.625 mg/ml, and at 40 mg/ml, no sign of biofilm formation was observed. Indeed, they mentioned that 0.625–5 mg/ml could be used successfully against candida colonization in the oral cavity [40]. In another *in vitro* study, the effect of essential oil of different plants such as cinnamon, ginger rhizome, rosemary, thyme, sage, and basil against *C. albicans* was investigated. *C. albicans* was selected from HIV positive patient that infected by oropharyngeal candidiasis or immunocompromised patient with disseminated fungal infection. Also, commercial strains used in this study as control strains. Candida species divided into two groups namely, fluconazole-susceptible and fluconazole-resistant [41]. The essential oil was prepared with 50–3200μg/ml concentrations applied to the well and incubated for 48 h at 35 °C. Results showed that ginger essential oil exhibited significant antifungal activity against both groups of candida, but its potency was the lowest in comparison with cinnamon or oregano and thyme. They stated that the fluconazole-resistant group was more susceptible to this essential oil [41]. Moreover, a further *in vitro* study evaluated different components of ginger; three gingerols (6- gingerol, 8- gingerol, 10, gingerol) and three shagaols (6- shagaols, 8-shagaols, 10-shagaols) against fluconazole-resistant *C. albicans*. They used DMSO 0.1% as the control group, and the concentration of extracts was 0–500μg/ml. Their outcome was in the following order: 6-gingerol, 8-gingerol, and 6-shogaol demonstrated antibiofilm activity at levels 10, 50, 100μg/ml, while 10-gingerol, 8-shogaol, and 10-shogaol showed no effect even at 100μg/ml. In reality, they concluded that the antibiofilm activity of the compound was attributed to the number of carbon side chains, as carbon side chain numbers become weighty, antibiofilm event appeared to decrease [42].

## 16.8 Berberine

Berberine is identified as a defense compound in plants and protects them against microorganisms [36]. Some pharmacological effects of berberine are recognized, such as antiarrhythmic, anti-inflammatory and anticancer properties [93]. Several studies assessed the pharmacology and clinical efficacy of berberine and have suggested its antimicrobial, antifungal, and antivirus effects [36]. In an *in vitro* study, the effects of berberine alone and in combination with fluconazole and miconazole were investigated. They used 1.95–250 mg/l for berberine, 0.125–4 mg/l for miconazole, and 0.5–16 mg/l for fluconazole and added to the plate of candida species and incubated for 24 h at 37 °C. Results revealed that berberine showed antifungal activity against candida species, but non-albicans strains were more vulnerable than *C. albicans*. Also, they stated that berberine plus miconazole or fluconazole showed a strong synergic effect against both forms of *C. albicans* (planktonic and biofilm) [36]. In another *in vitro* study, the potential effect of berberine alone and in combination with fluconazole were analyzed against fluconazole-resistant *C. albicans*. 0.125–64μg/ml of fluconazole and 1–32μg/ml of berberine were used, and outcomes demonstrated that berberine and fluconazole alone had a weak antifungal activity. In contrast, a combination of these substances produced a large and significant inhibition zone in *C. albicans* plates [37]. In another *in vitro* study, the sensitization of *C. albicans* to terbinafine by BBR and berberrubine were analyzed. They applied 100μg/ml of materials in the plates and incubated for 48 h at 35 °C. their result's revealed that berberine and berberrubine (it is analog) alone showed small or no antifungal activity, but the combination of 100μg berberine and 6μg terbinafine showed significant antifungal activity, even when compared with terbinafine alone. They stated that berberine, in conjunction with terbinafine, does not show any inhibition zone on the well [35]. In contrast, an *in vitro* study showed that BBR might enter *C. albicans* cell and act in both extracellular and intracellular sites. BBR treatment will decrease ergosterol, so it leads to the loss of membrane permeability and cause the cell death of *C. albicans* . They assessed 5, 10, 25, 50 and 100μg/ml of berberine and added to the well and compared them with PBS. They incubated at 37 °C for 15 and 60 min. They concluded that berberine accumulation at a dose of 50μg/ml is time-dependent and suggested that berberine may serve as an alternative treatment for candidiasis [38]. Furthermore, a further *in vitro* study demonstrated that after 24 h of treatment with berberine, mitochondrial dysfunction was observed in fluconazole-resistant *C. albicans* strains. They stated that BBR prompts the apoptotic mechanism in fungus, which are resistant to fluconazole. In their study, they examined 0.125–64μg/ml of concentration of berberine against *C. albicans*, and incubation time lasts for 24 h at 35 °C. They showed that berberine reduced the number of *C. albicans* in all level due to induction of instability in the cell membrane of fluconazole-resistant strains [39].

## 16.9 Conclusion and Future Perspective

This review has comprehensively assessed the effects of nutraceuticals and other diet ingredients on *C. albicans* in oral candidiasis based on pre-clinical and clinical trials. The results have shown that almost all of the nutraceuticals and specific diet ingredients discussed above such as garlic, green tea, propolis, curcumin, licorice root, cinnamon, resveratrol, ginger, and berberine are useful in the treatment of *C. albicans* in oral candidiasis (Fig. 16.1). These nutraceuticals are not expensive without any major side effects compared to other pharmacological agents. However, most of the evidence was based on the *in vitro* situation and animal studies, so it is strongly recommended to conduct more clinical trials to show the effectiveness of these phytochemicals on humans as well as the optimum dose and duration of treatment.

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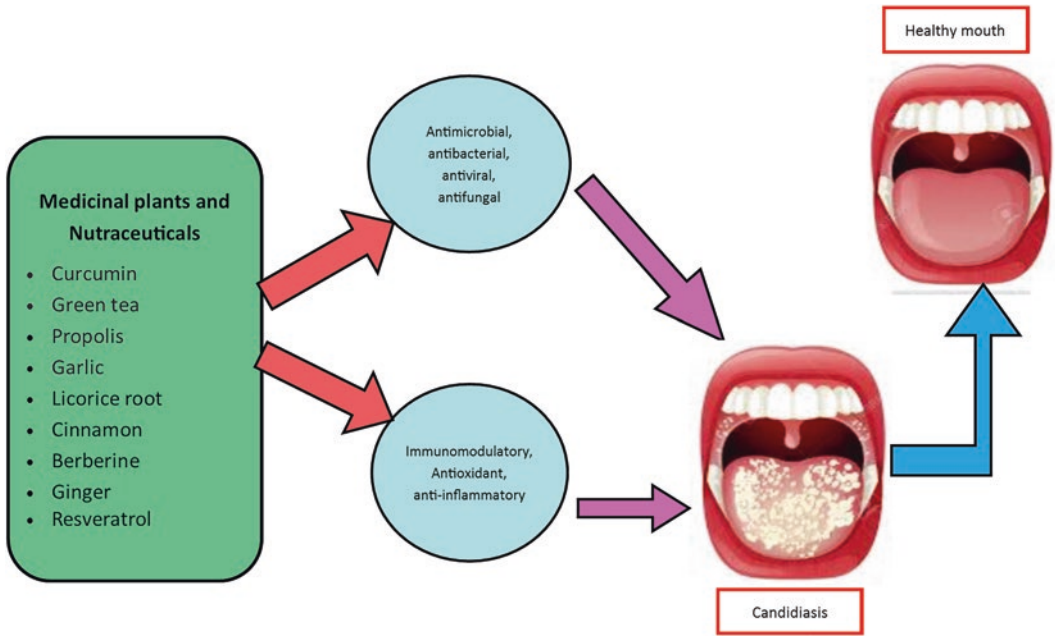


**Fig 16.1** Schematic summary of the major mechanisms underlying the beneficial effects of nutraceuticals and herbal bioactive compounds on oral candidiasis

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# 17

# Pharmacological Properties of the Plant-Derived Natural products Cannabinoids and Implications for Cardiovascular Health

Luca Liberale, Fabrizio Montecucco, and Federico Carbone

**Abstract**

The global march towards legalization of marijuana consumption is pursued in reason of the supposed harmless properties of this plant. Actually, a wide range of cannabinoids is endogenously produced and interacts with different classes of receptors ubiquitously distributed in the human body. Such endocannabinoid system (ECS) modulates several functions in health and disease. However, studies on synthetic ligands with selective agonist/antagonist activity on specific cannabinoid receptors, have clarified how complex the cannabinoid system is. The whole biological activity of cannabis sativa remains difficult to establish, due to the fact that it is a complex mixture of phytocannabinoids with different or even opposing effects. Δ9-tetrahydrocannabinol is the most represented phytocannabinoid in the marijuana plant and then the most studied compound. It has been widely associated with adverse CV effects in marijuana smokers. Conversely, less is known about the role of other phytocannabinoids. Here, we summarized the current knowledge about the effects of phytocannabinoids in CV disease, mainly focusing on atherosclerosis and myocardial infarction. We critically discussed clinical and experimental evidence linking phytocannabinoids to CV disease, attempting at explaining some controversies and suggesting the direction for future studies.

**Keywords**

Phytocannabinoid · Δ9-tetrahydrocannabinol · Cannabidiol · Atherosclerosis · Myocardial infarction · Cannabinoid receptor

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## 17.1 Introduction

Legalization of marijuana consumption for medical and/or recreational use is a global trend that many countries are pursuing in reason of the supposed harmless properties of this plant. However, some cannabis plant breeding exhibits nowadays up to 20% more Δ9-tetrahydrocannabinol (THC) than past decades, without accounting for the large amounts of heavy metals, pesticides and toxins accumulated from the soil. Furthermore, the THC content of synthetic cannabinoids now reaches 200 times that of the cannabis. Altogether, those factors not only justify the alert of health authorities [1, 2] but also represent a flywheel to deepen the pathophysiology of endocannabinoid system. Growing data are linking marijuana consumption with severe cardiovascular (CV) events but conflicting data exist. Such discrepancy may be due to biases in study design, as many variables influence clinical outcome: comorbidities, interactions between phyto- and endocannabinoids and concomitant abuses (e.g. tobacco, alcohol, other drugs). However, maybe more relevant is the discrepancy with experimental findings that are increasingly reporting the beneficial effects of targeting endocannabinoid system on CV health. Developing and testing of synthetic ligand with selective agonist/antagonist activity on specific cannabinoid receptors has contributed to uncover the complex biology of endocannabinoid system and paved the way for the study. Here we will focus on the role of phytocannabinoid in CV disease with particular regard to atherosclerosis and myocardial infarction.

## 17.2 Endocannabinoids and Atherosclerosis

The atherosclerotic plaque develops as a result of a multi-step process with deep alterations of the anti-thrombotic and anti-adhesive vascular endothelial layer to leading to chronic inflammation facilitating the deposition of altered lipids and inflammatory cells within the sub-endothelial space [3, 4]. Interestingly, atherosclerosis associates with an over-activation of the endocannabinoid system as a result of the altered balance between endocannabinoid synthesizing and degrading enzymes. Several experimental lines suggest that the endocannabinoid signalling plays important roles during the development of an atherosclerotic plaque including regulation of macrophage lipid uptake and recruitment of circulating leukocytes within the vessel wall. As a result, cannabinoids have been proposed as therapeutic targets to modulate vascular inflammation and plaque stability. Oxidized LDLs (OxLDL) are highly atherogenic particles formed in the sub-endothelial space and herein engulfed by monocytes through different scavenger receptors such as CD36 and LOX-1 [5]. Importantly, one of the non-psychoactive cannabis compound called cannabidiol have been reported to inhibit the activity of 15-lipoxygenase [6], the enzyme regulating active lipid oxidation thus potentially reducing plaque burden. This process drives the formation of foam cells releasing high amount of pro-inflammatory cytokines thus fuelling the atherosclerotic process [4]. Oppositely, the reverse cholesterol transport by ABCA1 and ABCG1 allows for the transfer of lipid to the anti-atherogenic HDLs and reduce the amount of lipid droplets within foam cells [7]. OxLDL have been shown to directly modulate endocannabinoid signalling in vitro by increasing 2-AG and AEA levels as well as CB1 and CB2 receptors (CB1 and CB2, respectively) expression in cell lines of mouse monocyte as well as in rat peritoneal macrophages. As a result, monocytes reduce the expression of expression of ABCA1 in RAW264.7 cells and increase that of scavenger receptor CD36 thus leading to increased intracellular cholesterol levels [8]. Interestingly, this effect was reversed by pre-treatment with the CB1 inhibitor AM251 suggesting this receptor as a possible target for anti-atherosclerotic therapies [8]. When overloaded by cholesterol, macrophages undergo necrosis and release DAMPs thus further entraining the vascular pro-inflammatory milieu and facilitating the formation of the necrotic core [4]. Here again, CB2 seem to play a beneficial role in facilitating macrophage apoptosis as opposed to necrosis in response to oxLDL via modulation of

the pro-survival Akt pathway [9]. Cholesterol accumulation impairs apoptotic cell clearance and facilitates inflammasome activation, of interest endocannabinoids have been shown to directly activate the NLRP3-IL1β pathway through CB1 in a mouse model of diabetes mellitus [10]. Although interesting, the extent to which those mechanisms might affect macrophage pathophysiology during atherosclerosis remains to be determined. The experimental role of endocannabinoid signalling in atherosclerosis has been investigated at different stage of the disease: from early endothelial dysfunction to the latest plaque rupture and thrombus formation. Interestingly, interference with the CB1 inverse agonist rimonabant seems to ameliorate endothelial dysfunction in isolated aortic rings of apolipoprotein E knockout ($ApoE^{-/-}$) mice [11]. Accordingly, when treated with AEA or others CB-1 agonists, primary human endothelial cells treatment showed reduced viability and increased ROS production [12]. Those deleterious features were successfully prevented by pre-treatment with CB1 antagonists, underlying an important role for this receptor in the development of vascular endothelial dysfunction [12]. This being said, the effect of CB1 antagonism on experimental atherosclerosis showed important differences between different animal strain with rimonabant successfully reducing plaque growth in the LDL receptor knockout ($Ldlr^{-/-}$) animals but not in $ApoE^{-/-}$ models [11, 13]. On the opposite, the anti-atherogenic role of CB2 have been shown by pharmacological and genetic (i.e. Cnr2−/− mice) models both in $ApoE^{-/-}$ and $Ldlr^{-/-}$ strains [14, 15], although some controversies still exist [16]. In this context, synthetic or plant-derived cannabinoids have shown important anti-inflammatory features by blunting the levels of pro-inflammatory cytokines thus reducing dysfunctional endothelial cell activation and macrophage infiltration [14, 17–19]. Specifically, THC has shown to blunt human T-cells proliferation and to inhibit the release of IFN-gamma thus reducing the formation of pro-inflammatory T-helper 1 lymphocytes [20]. Accordingly, CB2 is expressed at high levels by immune cells where it is supposed to play an anti-inflammatory role [14, 21]. Of interest, the anti-inflammatory and cytostatic properties of CB2 have been confirmed also in preliminary in vitro experiments employing human endothelial and vascular smooth muscle cells thus highlighting the potential translational value of those findings [22, 23]. Together with their receptors, some studies supported the role of endocannabinoids or plant-derived cannabinoids in atherosclerosis. Steffens and colleagues showed a decreased atherosclerotic progression in in murine models of atherosclerosis after oral administration of low-dose THC [17]. Also, they reported the presence of anti-inflammatory CB2 receptor in both human and mouse atherosclerotic plaques. Lymphocyte isolated from THC-treated resulted less active, while macrophage treated with THC in vitro showed blunted chemotaxis, an important process for atherosclerotic plaque growth [17]. Of importance, all these effects were completely reversed by a specific CB2 receptor antagonist underlying once more the important anti-inflammatory function of this receptor [17]. On the opposite, increased AEA signalling by genetic or pharmacological inhibition of its metabolizing enzyme FAAH associated with worsened atherosclerotic plaque development via enhanced CXCL1-mediated neutrophil recruitment as well as impaired vascular repair [24–26]. Less clear is the role of another molecule of the endocannabinoid superfamily, 2-AG. Initial reports using mice with genetic deficiency of its metabolizing enzyme (i.e. MAGL) on a $ApoE^{-/-}$ background suggested an anti-atherogenic role for this molecule through CB2-mediated reduction of lipid and inflammatory plaque content and enhanced collagen deposition and fibrous cap thickness [27]. Further experiments revealed a facilitating effect on the development of early plaques in $ApoE^{-/-}$ animals fed with high-fat diet and treated with the MAGL inhibitor JZL184 [28]. Lastly, another study supported an athero-protective effect of genetic or pharmacological MAGL blockade on atherosclerosis onset via increased CB2 and blunted CB1 signalling [29]. Although apparently controversial, these results might find an explanation in the different efficiency of MAGL inhibition which depends on the selected dose of the inhibitor, the

way and frequency of administration [30, 31]. Recently, the orphan-receptor GPR55 has been suggested to mediate endocannabinoid signalling by binding the endocannabinoid-like compound palmitoylethanolamide (PEA) as well as the endocannabinoids AEA and 2-AG [32, 33]. Interestingly, GPR55 is highly expressed on the surface of human leukocytes, specifically by monocytes and natural killer cells [34]. Here, in vitro data suggested GPR55 to play a role in oxLDL accumulation [34]. Recently, our group tested the potential role of this receptor in the setting of atherosclerosis by treating ApoE$^{-/-}$ mice under both normal chow and high-fat diet with the GPR55 antagonist CID16020046 [35]. Although, treatment with CID16020046 did not affect the plaque size, GPR55 blockade associated with features of plaque destabilization (i.e. increased intraplaque MMP9 and neutrophil content and reduced collagen deposition) only in animals fed with normal chow. Furthermore, we reported treatment with CID16020046 to induce degranulation of human neutrophil in vitro [35]. Hence, GPR55 might negatively regulates neutrophil chemotaxis and activation thus potentially playing a plaque stabilizing role in the setting of atherosclerosis [35]. Again, some of the anti-atherosclerotic effects of plant-derived cannabinoids might be mediated by this receptor as THC have been reported as its active ligand [36]. Lastly, another research group suggested GPR55 as the possible mediator for PEA anti-inflammatory and anti-atherogenic effects in ApoE$^{-/-}$ animals. In this work, treatment with PEA associated with reduced plaque formation and increased plaque stability via down-regulation pro-inflammatory macrophage activation. Mechanistically, PEA increased the expression of the phagocytosis receptor MerTK and enhanced macrophage efferocytosis in vitro, an effect blunted in macrophages obtained from GPR55 knockout animals [37]. In conclusion, cannabinoids might exert opposite pathophysiological roles in atherogenesis due to their actions on the different receptors. Among the endocannabinoid-related signalling pathways, CB1 seems to hold pathophysiological role in atherosclerosis as outlined by both experimental and clinical studies. Interfering with CB1 overactivation in selected patients might effectively reduce atherosclerotic burden directly by reducing vascular inflammation and indirectly through the improvements of metabolic risk factors.

## 17.3 Cannabinoids and Myocardial Injury

A growing body of evidence associates recreational use of marijuana with a wide range of myocardial injuries ranging from coronary thrombosis and subsequent myocardial infarction (MI), cardiomyopathies, heart failure and arrhythmias, up to sudden death [38]. Marijuana smoking increases the risk of MI up to about five-fold in the first hour after exposition [39]. During long-term follow-up, the risk drops down to 2.5-fold in the less than once for week smokers, whereas it has exacerbated in more frequently smokers [40]. Noteworthy, very long-term follow-up (up to 18 years) in MI survivors did not demonstrate any significant effect of marijuana use on mortality [41]. Nevertheless, a recent French epidemiological study on young consumers reported an increased number of severe CV complications that raised further concerns about the use of marijuana. Dose of marijuana certainly has a major role alongside with frequency of use, route of administration and duration of use. As reported in animal models, detrimental $CB_1$ receptor (CB1) activation occurs at high dose of THC with the most common effects represented by increase of heart rate and blood pressure lowering. Therefore, the dramatic increase of THC content in cannabis (from 2–3% up to 20%) may largely explain the epidemiological rise of CV complication related to marijuana use [1]. Conversely, very low dose of THC does not elicit substantial CB1 activation but rather acts as CB2 agonist with anti-inflammatory and anti-oxidant effects [17, 42]. Even, a single ultralow dose of THC exerts cardio-protective effect in experimental model of MI [43]. Chronic administration of low-dose THC could also induce a CB1 down-regulation, but data in this field are still controversial and limited to the central nervous system

[44, 45]. Although THC represents the prevalent compound of marijuana many other cannabinoids are contained within the plant. Among them, cannabidiol (CBD) is highly expressed in different varieties of marijuana and strongly differs from THC. CBD has low activity on CB1 and CB2 and preferentially acts as a CB2 inverse antagonist, property that may explain its anti-inflammatory activity [46]. This effect largely explains the cardio-protective role of CBD: a reduced inflammatory cell infiltration characterizes mouse model of MI, whereas no effect of CBD was observed in isolated heart model [47]. CBD activity on inflammatory cells involves bot canonical (CB1 and CB2) and non-canonical receptors, ultimately suppressing immune cell recruitment and activation [48, 49]. With the same mechanisms, beneficial effects of CBD were later demonstrated in animal models of doxorubicin-induced and diabetic cardiomyopathy and myocarditis as well. In addition, direct effects on cardiomyocytes were reported, including the restoring of mitochondrial function with consequent suppression of oxidative stress, NF-κB and cell death program [50–53]. Less important are other cannabinoids and terpenoids contained in the marijuana plant [54] excepted the tetrahydrocannabivarin, a CB2 agonist with a dose-dependent effect on CB1 (antagonist at low and agonist at high dose). Its beneficial effects are still limited to preclinical evidence on metabolic disorders [55].

## 17.4 Future Perspectives and Conclusion

The involvement of endocannabinoid system in CV health has been clearly widely demonstrated and strong is the claims for developing even more selective compounds. Conversely, clinical indications for the use of phytocannabinoid are still limited to end of life therapies (e.g. chemotherapy-induced nausea, AIDS- and cancer-related cachexia). Even the application for the treatment of pain is still discussed. Promising results are coming from the use of cannabidiol, even in CV disease. Conversely, the role of the other non-THC phytocannabinoid still remains unexplored in the context of CV health. Eventually, limiting or even eliminating THC from cannabis extract would have sense in order to clarify the potential therapeutic role of the other phytocannabinoids. In that case, for any potential future clinical application physicians will have to carefully monitor the development of adverse psychiatric effects.

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# 18 Beneficial Effects of Plant-Derived Natural Products on Non-alcoholic Fatty Liver Disease

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**Abstract**

Non-alcoholic fatty liver disease is becoming in one of the most prevalent liver diseases that leads to liver transplantation. This health problem is a multisystem disease with a complex pathogenesis that involves liver, adipose tissue, gut, and muscle. Although several pharmacological agents have been investigated to prevent or treat non-alcoholic fatty liver disease, currently there is no effective treatment for the management of this chronic liver disease. Nonetheless, the use of natural products has emerged as a alternative therapeutic for the treatment of hepatic diseases, including non-alcoholic fatty liver disease, due to its anti-inflammatory, antioxidant, anti-diabetic, insulin-sensitizing, antiobesity, hypolipidemic, and hepatoprotective properties. In the present review, we have discussed the evidence from experimental and clinical studies regarding the potential beneficial effects of plant-derived natural products (quercetin, resveratrol, berberine, pomegranate, curcumin, cinnamon, green tea, coffee, garlic, ginger, ginseng, and gingko biloba) for the treatment or prevention of non-alcoholic fatty liver disease.

**Keywords**

Natural products · Dietary supplements · Non-alcoholic fatty liver disease · NAFLD

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## 18.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as one of the main causes of liver disease, affecting 17–46% of adults in Western countries [1]. NAFLD is commonly associated with metabolic comorbidities such as obesity, type 2 diabetes, dyslipidemia, and metabolic syndrome [2]. This hepatic disease is characterized by >5% of liver fat which comprise a spectrum of different disorders that may progress from non-alcoholic fatty liver (NAFL), non-alcoholic ste-



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atohepatitis (NASH), fibrosis, cirrhosis, and finally hepatocellular carcinoma [1, 3]. The most difficult outcome of NAFLD, at the end-stage, is the need for liver transplantation [4]. Although liver biopsy remains the gold standard for NAFLD diagnosis, transient elastography by ultrasound is highly recommended to identify high-risk metabolic patients, prognosis, progression, and treatment response [1, 5, 6].

It is well-recognized that ethnic and genetic variants contribute to NAFLD etiology; however, its pathophysiology involves a complex interaction of hormonal and nutritional factors [7]. Novel evidence supports that NAFLD is a multisystem disease caused by "multiple-parallel hits" involving the liver, adipose tissue, gut, and muscle [8, 9]. In this regard, insulin resistance (IR) is the metabolic abnormality that plays a central role in the pathogenesis of NAFLD. In adipose tissue, IR decreases lipolysis resulting in increased lipid accumulation by the elevation of free fatty acids in the liver. Lipotoxicity eventually induces cell death through hepatic inflammation, mitochondrial disturbances, and endoplasmic reticulum stress. Furthermore, the realease of cytokines and chemokines triggers the inflammatory response and, consequently, the secretion of extracellular matrix proteins such as collagen, causing liver fibrosis [10].

In addition to the currently available treatment for NAFLD, such as weight loss diets, exercise, insulin sensitizer drugs, lipid-lowering drugs, and antioxidants [1], compounds derived from natural products have been used to ameliorate the symptoms of this liver disease. According to previous data, 65% of patients use natural supplementary products in addition to the prescribed drugs for the treatment of liver diseases [11, 12]. Thus, the use of alternative therapies, including dietary supplements, has increasing due to a potential hepatoprotective effect (Table 18.1). Herein, we aimed to review and summarize the current evidence on the positive effects of the most used natural products for NAFLD as well as their underlying mechanisms.

**Table 18.1** Protective effects of plant-derived natural products on non-alcoholic fatty liver disease

| Plant-derived natural products | Hepatoprotective effects | References |
|---|---|---|
| Quercetin | Reduction in oxidative stress, lipid levels, hepatic lipid accumulation, insulin resistance, and inflammation | [16–19, 23–25] |
| Resveratrol | Improvement of insulin sensitivity, lipid metabolism, inflammation, oxidative stress, and liver function. | [26–29, 31–35] |
| Berberine | Amelioration of hepatic fat content, oxidative stress, liver function, lipid levels, and insulin resistance. | [46–53] |
| Pomegranate | Improvement of oxidative stress, lipid metabolism, hepatic lipid accumulation, liver damage, and liver function. | [60, 66–69] |
| Curcumin | Reduction in lipid accumulation, oxidative stress, inflammation, liver damage, hepatic fibrosis, serum lipids, transaminases, and uric acid levels. | [75–85] |
| Cinnamon | Amelioration of hepatic lipid accumulation, lipid profile, liver function, insulin resistance, and inflammation. | [88–90] |
| Green tea | Decrease in body weight, hepatic fat accumulation, serum lipids, insulin resistance, transaminases, and inflammatory markers. | [94–97] |
| Coffee | Attenuation of liver damage, oxidative stress, inflammation, and liver steatosis. | [102–105] |
| Garlic | Inhibition of hepatic lipogenesis, lipolysis, lipid accumulation, apoptosis, inflammation, oxidative stress, and autophagy. | [111–117] |
| Ginger | Mitigation of lipid biosynthesis, oxidative stress, lipogenesis, insulin resistance, lipid production, and inflammation. | [121, 123, 124] |
| Ginseng | Reduction in body weight, lipid levels, liver steatosis, inflammation, oxidative stress, and liver damage. | [130–135, 137–139] |
| Gingko biloba | Improvement of lipid metabolism, liver inflammation, lipid accumulation, insulin resistance, liver function, and oxidative stress. | [145, 147–152] |

## 18.2 Natural Occurring Compounds for NAFLD

### 18.2.1 Quercetin

Quercetin (3, 3′, 4′ 5, 7-pentahydroxyflavone) is a major dietary flavonoid found in fruits and vegetables that exhibits antioxidant and anti-inflammatory properties [13, 14]. This natural compound is widely used as supplement for several liver diseases, and evidence derived from experimental studies supports that quercetin may improve some disorders involved in the pathogenesis of NAFLD (i.e., inflammation, oxidative stress, and cell death). Besides, hepatoprotective effects of quercetin have been examined against ethanol, metals, pesticides, drugs, chemicals, toxins, and viral hepatitis [15].

An *in vitro* study showed that quercetin reduces intracellular triglyceride levels and reactive oxygen species production in oleic acid-induced hepatic steatosis [16]. In this line, Li et al. described that quercetin decreases hepatic lipid accumulation and triglyceride levels in a NAFLD cell model [17]. Furthermore, quercetin treatment improves insulin resistance by tyrosine phosphorylation and down-regulates the expression of the sterol regulatory element-binding protein-1c (SREBP-1c) and fatty acid synthase (FAS) [17]. In agreement with the previous findings, Vidyashankar et al. observed a decreased triacylglycerol content, insulin resistance, and inflammatory cytokine release, while cellular antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) activities were increased by quercetin in oleic acid-induced hepatic steatosis in HepG2 cells [18]. Other experimental study revealed that quercetin decreases intracellular lipid accumulation by down-regulation of lipogenesis and up-regulation of lipolysis in Huh7.5 cells [19].

Studies in murine models have described mechanisms that alleviate the pathological characteristics of NAFLD such as the reduction of oxidative stress, decrease in lipid levels, improvement of insulin resistance and gut dysbiosis through a variety of signaling pathways including autophagy. Autophagy is a possible alternative mechanism for clearing ubiquitinated proteins under stress conditions, which may be implicated in the hepatoprotective effect of quercetin [20]. A previous study demonstrated that mice treated with quercetin significantly ameliorated liver damage, reduced hepatic cholesterol and oxidized low-density lipoprotein level by autophagy lysosomal pathway [20]. Zhu et al. suggested that quercetin improves NAFLD by inducing VLDL assembly and lipophagy via IRE1a/XBP1s pathway [21], an underlying mechanism that relieves endoplasmic reticulum stress.

The prebiotic activity of quercetin through the regulation of the intestinal microbiota composition has been recently proposed. An experimental study reported that quercetin supplementation enhances gut microbiota imbalance and related gut-liver axis activation by inhibiting TLR-4-NF-κB signaling pathway and endoplasmic reticulum stress in mice with induced NAFLD [22]. Additionally, quercetin improves the inflammatory process, oxidative stress, and lipid metabolism in mice with NAFLD [23]. Yang et al. [23] suggested that the hepatoprotective effect of quercetin may be due to the activation of farnesoid X receptor 1/Takeda G-protein-coupled 5 (FXR1/TGR5) signaling pathway. Another experimental study demonstrated that quercetin improves glucose and lipid metabolism through the upregulation of the expression and activity of sirtuin type 1 (SIRT1) and activation of Akt signaling pathway in diabetic rats [24].

Unfortunately, few clinical trials have been conducted to assess the effects of quercetin on NAFLD. In this context, Askari et al. observed that quercetin supplementation significantly decreased oxidative stress and inflammatory markers such as CRP and IL-6 in healthy subjects [25]. Thus, future human studies are mandatory to evaluate the efficacy of quercetin on NAFLD.

### 18.2.2 Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring polyphenol commonly found in grapes, berries, peanuts, and derivatives such as red wine [26]. To date, the therapeutic potential

of this natural agent has been widely explored in cancer, neurological disorders, cardiovascular diseases, diabetes, and NAFLD [27, 28]. Evidence from *in vitro* and *in vivo* studies have shown a beneficial effect of resveratrol on glucose and lipid metabolism, inflammation, and oxidative stress [26, 28]. Moreover, some molecular mechanisms of resveratrol have been described: (1) activation of AMPK that inhibits the novo lipogenesis by decreasing SREBP-1; (2) activation of cAMP signaling that increases insulin sensitivity through the activation of AMPK and SIRT1; (3) inhibition of NF-κB activity and attenuation of JAK2/STAT pathway related to pro-inflammatory cytokine secretion; and (4) induction of SIRT1/FOXO1/MnSOD/ROS pathway and upregulation of UCP2 that decrease reactive oxygen species production [27, 29].

It has been reported that resveratrol treatment increases mitochondrial biogenesis and the expression of mitochondrial respiratory complex subunits in a cell model of steatosis [30]. Recently, it was suggested that resveratrol improves lipid metabolism, redox homeostasis, and oxidative stress by the PKA/AMPK/PPARα signaling pathway resulting in protection against NAFLD [31]. In agreement with *in vitro* studies, Ding et al. observed that resveratrol supplementation prevents hepatic steatosis by up-regulation of the SIR-T1-autophagy signaling axis and decreasing endoplasmic reticulum stress [32].

Evidence from human studies have shown inconsistent results probably due to the small sample sizes and different treatment duration. In this regard, a double-blind, randomized, placebo-controlled trial showed a significant decrease in liver enzymes, glucose levels, insulin resistance, and lipid parameters after resveratrol administration in patients with NAFLD [33]. Another clinical trial revealed that resveratrol supplementation decreases alanine aminotransferase and hepatic steatosis in patients with NAFLD [34]. In this line, Heeboll et al. observed a significant reduction in liver function tests and liver fat accumulation following resveratrol treatment in NAFLD patients [35]. A randomized controlled clinical trial conducted in NAFLD patients found a modest weight loss but without favorable metabolic changes after resveratrol supplementation [36]. Furthermore, some clinical trials have reported no positive effects of resveratrol on body composition and characteristics of NAFLD [37–39]. Also, two recent meta-analysis suggested that current evidence is insufficient to support the efficacy of resveratrol in the management of NAFLD [40, 41]. Nonetheless, it is important to note that only four randomized clinical trials were included in these meta-analyses; therefore, further large-scale, well-designed, and population-based are required to clarify the potential beneficial effects of resveratrol in NAFLD.

### 18.2.3 Berberine

Berberine is an isoquinoline alkaloid of the protoberberine type that is often extracted from plants such as *Coptis chiensis*, *Rhizoma coptidis*, *Berbis aristata*, and *Hydrastis canadensis* [42]. This phytochemical has been widely used in traditional Eastern medicine to treat diarrhea and other gastrointestinal disturbances [43].

Both experimental and clinical studies have suggested positive effects of berberine on NAFLD. The body of evidence indicates that this herbal agent may improve clinical characteristics of NAFLD including hepatic steatosis, obesity, and hyperlipidemia [44, 45]. However, the molecular mechanisms involved in the hepatoprotective effects of berberine have not been elucidated.

In animal models, it has been demonstrated that berberine reduces hepatic fat content by increasing hepatic triglyceride export through a decreased methylation of the microsomal triglyceride transfer protein (MTTP) gene promoter [46]. Other study, in NAFLD rat model, revealed that berberine ameliorates liver function and histopathological changes accompanied by a significant reduction in liver index, AST, ALT, and triglyceride levels [47]. A recent research showed that berberine improves NAFLD by inhibiting hepatic lipid accumulation and hepatic oxidative stress via activation of the sirtuin 3 (SIRT3)/adenosine 5′-monophosphate (AMP)-activated pro-

tein kinase (AMPK)/acetyl-CoA carboxylase (ACC) and nuclear factor erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE) [48–50].

Additionally, the tentative mechanisms of berberine in NAFLD may be *de novo* lipogenesis suppression, increased fatty acid β-oxidation, and elevated extra-hepatic transport of triglycerides in the form of VLDL in the liver [51].

Data from human studies have suggested that the benefits of berberine are related to the improvement of hepatic insulin resistance and lipid-lowering effects in liver cells [52]. Also, berberine treatment plus lifestyle intervention exhibited a significant reduction of hepatic fat content, body weight, insulin resistance, and serum lipid concentrations in patients with NAFLD [53]. Besides, a meta-analysis of randomized clinical trials confirmed a significant decrease of total cholesterol, LDL, ALT, and HbA1c concentrations after berberine treatment in NAFLD patients [54].

### 18.2.4 Pomegranate

Pomegranate or *Punica granatum* is a fruit native from the region of Iran to the Himalayas in northern India, which has been extensively used for its medicinal properties [55, 56]. *In vitro* and *in vivo* investigations have shown that pomegranate has anti-diabetic, anti-inflammatory, antioxidant, and anti-obesity effects [57–59].

Different extracts of pomegranate (seed, flower, and peel) have been tested for their possible positive effects on hepatic lipid metabolism [60–62]. Additionally, this fruit contains several bioactive molecules including polyphenolic compounds, anthocyanins, hydrolyzable tannins, tannic acid, gallic acid, and ellagic acid, punicalin, punicalagin, pedunculagin, and flavanols [63–65].

It has been observed that pomegranate flower, at least in part, ameliorates diabetes and obesity-associated fatty liver by activating hepatic expression of genes responsible for fatty acid oxidation (peroxisome proliferator-activated receptor-α (PPAR-α) and acyl-CoA oxidase) [60]. Another study found that pomegranate extracts induce hepatic fatty acid oxidation by upregulating CPT-1 and PPAR-α along with the regulation of cholesterol homeostasis by increasing hepatic expression of ApoA1 and LDL receptor in induced NAFLD [66]. Further, polyphenols from pomegranate flower reduce hepatic lipid accumulation through the regulation of lipid metabolism by SirT3, AMPK, ACC2, and CPT-1 expression in NAFLD mice [67]. Moreover, pomegranate confers protection against induced fatty liver by reducing histological abnormalities, dilatation and congestion of central veins, blood sinusoids and portal veins [63].

Evidence from clinical trials evaluating the effects of pomegranate or its extracts in the treatment of NAFLD is scarce. In this regard, a previous study revealed that pomegranate seed oil combined with fucoxanthin reduce body and liver fat content and improve liver function tests in obese NAFLD women [68]. Furthermore, a clinical trial in patients with NAFLD showed a significant decrease in liver enzymes (AST, ALT) and body mass index as well as an increase in total antioxidant capacity after pomegranate treatment [69]. Hence, further clinical trials are required in order to confirm the protective role of pomegranate against NAFLD.

### 18.2.5 Curcumin

Curcumin is a polyphenolic compound that is commonly isolated from rhizome of *Curcuma longa*, also known as Turmeric [70]. In India, for many years, this natural agent has been widely used for the treatment of different diseases due to its antioxidant, anti-inflammatory, anti-carcinogenic, and hepatoprotective properties [71–74].

The potential role of curcumin on liver function and hepatotoxicity has been described in several studies. In this context, curcumin reduced lipid accumulation and oxidative stress damage through suppression of superoxide dismutase (SOD)-2 expression and activation of peroxi-

some proliferator-activated receptors (PPARs) in an *in vitro* model of liver steatosis [75]. Evidence from NAFLD/NASH animal models has consistently shown that curcumin exhibits a potent inhibition of oxidative stress and inflammation. Thus, it has been reported that administration of curcumin protects against the development and progression of fibrosis by inhibiting hepatic oxidative stress in mice with NASH [76]. Also, curcumin therapy prevented liver injury by decreasing pro-inflammatory and pro-oxidant responses and intrahepatic $CD4^+$ cells accumulation in mouse [77]. Besides, results from a NASH/hepatocellular carcinoma mouse model revealed that curcumin reduces the progression of NASH and liver damage via inhibiting HMGB1-NF-κB translocation [78].

A recent randomized clinical trial found a significant decrease of hepatic fibrosis, NF-κB activity, circulating liver enzymes, and TNF-α levels, although these changes were not superior to lifestyle modification in patients with NAFLD [79]. A previous study observed that curcumin supplementation significantly decreases serum lipids and uric acid concentrations in subjects with NAFLD [80]. A randomized controlled trial indicated that curcumin administration improves liver fat and reduces serum transaminase levels in NAFLD patients [81]. Results of another randomized clinical trial demonstrated that curcumin supplementation attenuates hepatic fibrosis in patients with NAFLD [82].

A recent systematic review of clinical trials suggested a significant decrease in ALT and AST levels as well as a mitigation in NAFLD severity [83]. In this line, a meta-analysis of randomized controlled trials revealed that curcumin significantly reduces LDL-C, triglycerides, fasting plasma glucose, insulin resistance, weight, and AST levels in NAFLD patients [84]. Another recent meta-analysis found a significant decrease on serum concentrations of ALT and AST following curcumin therapy in patients with NAFLD [85]. Nonetheless, it is important to note that only 4 studies were included in both meta-analysis; therefore, future clinical trials are warranted to confirm these results.

### 18.2.6 Cinnamon

Cinnamon is one of the most important spices used in different cultures around the world. This food additive is obtained from different tree species of the genus *Cinnamomum*. The cinnamon tree is an herbaceous perennial plant native to Vietnam, Indonesia and other Southeast Asian countries. For many years, cinnamon has been used in traditional medicine for respiratory, digestive, and gynecological disorders [86].

Previous studies, *in vitro* and *in vivo,* reported that cinnamon has anti-inflammatory, antimicrobial, antidiabetic, and antioxidant activities [87]. Additionally, it has been indicated that cinnamon exhibits a positive effect on nonalcoholic fatty liver disease (NAFLD). In this regard, Lopes et al. [88] found that treatment with cinnamon aqueous extract lowers hepatic triacylglycerol content by suppressing the expression of diacylglycerol O-acyltransferase 2 (DGAT2) and sterol regulatory element-binding transcription factor 1 (SREBF1) in rat liver. In consistency, a recent study observed that treatment with cinnamon powder reduces hepatic lipid accumulation through the downregulation of SREBF1 and sterol regulatory element-binding transcription factor 2, resulting in the improvement of liver steatosis in a zebrafish model [89].

According to clinical trials, Askari et al. [90] demonstrated therapeutic benefits of cinnamon on lipid profile, liver enzymes, insulin resistance, and C-reactive protein in NAFLD patients. Since evidence from human studies is still insufficient, further long-term and larger clinical trials are required to corroborate these findings.

### 18.2.7 Green Tea

Green tea is derived from the leaves and buds of the plant *Camellia sinensis*, and it is a widely consumed beverage worldwide [91]. This beverage contains a great number of bioactive compounds mainly flavonoids. Catechins (flavan-3-ols), a type of flavonoids, constitute 30–42% of green tea [92]. Several health benefits

of catechins have been reported including anti-diarrheal, anti-inflammatory, anti-oxidative, anti-bacterial, anti-diabetic, anti-aging, anti-cancer, anti-parkinsonism, anti-stroke, and anti-atherosclerotic properties [93].

Moreover, the hepatoprotective effect of green tea has been examined in experimental and clinical trials. With this regard, Bae et al. [94] observed that treatment with green tea extract inhibits hepatic fat accumulation, decreases triglyceride and glucose levels, and improves insulin resistance by activating the sirtuin 1 and AMP activating protein kinase pathway, leading to the prevention of the development and progression of NAFLD in mice. In consistency, Huang et al. [95] found that green tea polyphenol treatment reduces body weight, mesenteric fat mass, fasting glucose, insulin resistance, serum cholesterol, and severity of fatty liver by decreasing bile acid and lipid absorption in mice.

Results from clinical trials have also shown a positive effect of green tea therapy against NAFLD. A previous double-blind, placebo-controlled, randomized clinical trial revealed a significant reduction in ALT and AST levels after green tea extract supplementation in patients with NAFLD [96]. Besides, it has been suggested that catechin contained in green tea induces degradation of fatty acids through the activation of hepatocellular mitochondrial β-oxidation, resulting in a potential protective mechanism for NAFLD [96]. Another randomized placebo-controlled trial conducted in NAFLD patients reported a significant improvement in body weight, body mass index, insulin resistance, lipid profile, aminotransferases, and inflammatory markers, as well as a regression of fatty liver following green tea extract therapy [97].

### 18.2.8 Coffee

Coffee is one of the most popular and common drinks in the world, which has been part of human consumption since the fifteenth century [98]. Coffee is prepared from the roasted seeds of the coffee plant and it contains nitrogenous compounds such as caffeine, trigonelline, chlorogenic and carboxylic acids, and lipids [99]. Previous studies have described that coffee intake is associated with reduced incidence of some chronic diseases including cardiovascular disease, diabetes, and neurodegenerative disorders [100, 101]. Also, experimental and epidemiological studies have observed that coffee exerts beneficial effects on NAFLD through its antioxidant, anti-inflammatory, and antifibrotic activities [102–104]. In this regard, Amer et al. [103], reported that caffeine consumption attenuates histological changes and liver function by decreasing oxidative stress and inflammatory markers in induced hepatotoxicity. A recent experimental study demonstrated that coffee treatment prevents NAFLD by reducing liver macrovesicular steatosis, serum cholesterol, and alanine aminotransferase through regulation of liver fat oxidation, intestinal cholesterol efflux, energy metabolism and gut permeability [104]. Furthermore, it has been suggested that coffee administration may protect against NASH by decreasing hepatic fat accumulation, systemic and liver oxidative stress, liver inflammation, and reducing the hepatic concentrations of TNF-α and interferon-γ and increasing interleukin-4 and interleukin-10 [105].

A previous meta-analysis of observational studies revealed that coffee consumption is inversely associated with the risk of NAFLD [106]. In this line, another meta-analysis found a significant reduced risk of NAFLD among coffee drinkers and decreased risk of liver fibrosis in patients with NAFLD drinking coffee [107]. However, evidence from clinical trials is lacking; hence, future studies are mandatory in order to confirm the potential beneficial impact of coffee on NAFLD.

### 18.2.9 Garlic

Garlic (*Allium sativum*), an herb frequently used in traditional medicine in Asia, Egypt, and the Mediterranean regions [108], contains several extracts with biological activity such as S-allyl cysteine, S-allymercaptocysteine, diallyl disulfide, cinnamoyloctopamines, and garlicnin D [28, 109, 110]. These garlic extracts have antioxi-

dant, antifibrotic, anti-inflammatory, and anti-apoptosis properties, which strongly suggest positive effects of garlic in the management of NAFLD [28, 111].

Experimental studies in animal models have consistently demonstrated therapeutic benefits of garlic on NAFLD. With this regard, S-allyl cysteine prevents NAFLD through the reduction of hepatic lipogenesis and lipid accumulation by activation of AMPKα pathway and downregulation of SREBP-1 in human HepG2 cells [112]. Additionally, it has been observed that administration of garlic-derived S-allylmercaptocysteine protects against NAFLD through inhibition of apoptosis and improving autophagy [113]. Also, garlic essential oil was effective in preventing NAFLD by reducing lipid accumulation, inflammation, and oxidative damage through the improvement of lipid metabolism and oxidative stress [111]. It has been reported that administration of garlic-derived S-allymercaptocysteine attenuates NAFLD-induced liver injury, fat accumulation, collagen formation and free fatty acids through the decrease of lipogenesis and lipolysis, expression levels of pro-fibrogenic factors, liver oxidative stress, and inflammation [114]. Further, treatment with garlic-derived antioxidant S-allylmercaptocysteine protects the liver from chronic injury by decreasing the number of apoptotic cells through the reduced activity of LKB1/AMPK and PI3K/Akt pathways, and by reducing autophagic markers and mTOR activity in a NAFLD rat model [115]. Besides, it has been revealed that aged black garlic exerts hepatoprotective effects on fatty liver by inhibiting the elevation of AST and ALT [116], reducing triglyceride accumulation in the liver and improving insulin resistance and the intestinal microbiota in ddY-H mice [117].

Nonetheless, epidemiological and clinical studies evaluating the potential benefits of garlic for the treatment of NAFLD in humans are scarce. A recent population-based study found that raw garlic intake is inversely associated with NAFLD [118]. Additionally, a double-blind, randomized, placebo-controlled trial indicated that treatment with fermented garlic extracts improves serum levels of gamma-glutamyl transpeptidase and alanine aminotransferase in adults with elevated serum levels of gamma-glutamyl transpeptidase [119]. Another randomized placebo-controlled trial reported that garlic powder administration significantly reduces body weight and fat mass in patients with NAFLD [120]. Thus, the aforementioned findings suggest that garlic and its extracts might be used as hepatoprotective agents against NAFLD.

### 18.2.10 Ginger

Ginger (*Zingiber officinale*), a spice extensively used in different traditional medicine systems, exhibits hypolipidemic, antioxidant, and anti-inflammatory activities [121–123]. However, experimental studies in animal models evaluating the efficacy of ginger on NAFLD are scarce. Lai et al. [121] observed that ginger essential oil mitigates lipid biosynthesis and oxidative stress through downregulation of SREBP-1c, acetyl-CoA carboxylase, fatty acid synthase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and cytochrome P450 2E1 in induced NAFLD. Additionally, 6-gingerol (the main active component of ginger) improves lipid metabolism by increasing β-oxidation and decreasing lipogenesis through activation of PPAR-α and CPT1α and inhibition of DGAT-2. Also, this ginger extract attenuates oxidative stress and ameliorates mitochondrial function by upregulating mitochondrial enzymes such as NOX, SDH, and SIRT3 in hepatic steatosis. Besides, 6-gingerol enhances insulin sensitivity through the regulation of Akt signaling pathway [123]. Thus, these biological effects suggest that ginger could be an effective dietary supplement to ameliorate NAFLD.

Regarding human studies, a randomized, double-blind, placebo-controlled clinical trial showed a significant reduction in alanine aminotransferase, γ-glutamyl transferase, inflammatory cytokines, insulin resistance, and hepatic steatosis after ginger supplementation in patients with NAFLD [124]. Nevertheless, evidence from clinical trials is still insufficient and further research is needed to corroborate the benefits of ginger for the treatment of NAFLD.

### 18.2.11 Ginseng

Panax ginseng, one of the ginseng species, is an oriental herb native of China, Korea, and Russia that has been widely used for more than 2000 years. This natural agent contains mainly triterpenic saponins, which are responsible of its medicinal effects [125, 126]. Several studies have reported that ginseng exerts beneficial effects on liver diseases such as fatty liver, liver fibrosis, liver carcinoma, and alcohol-induced liver damage [127, 128].

Mollah et al. [129] observed that Panax ginseng increases the expression of lipoprotein lipase and peroxisome proliferators-activated receptors-gamma in adipose tissue, as well as the regulation of glucose transporter 4 (GLUT4) and of the insulin receptor in skeletal muscle and liver. Furthermore, Miranda-Henriques et al. [130] found that ginseng treatment reduces weight gain, glucose and lipid levels, and prevents non-alcoholic steatohepatitis by decreasing steatosis and inflammation in animal models.

Ginsenoside Rg1 is one of the active components of ginseng with positive effects in the treatment of liver diseases. Xu et al. [131] indicated that Rg1 prevents the development of NAFLD through the reduction of lipid deposition, oxidative stress, and inflammatory response. Additionally, previous studies demonstrated that Rg1 improves liver damage in acute liver failure and alcoholic liver disease by anti-inflammatory and anti-apoptosis actions [132–135].

Korean red ginseng (KRG), an extract of Panax ginseng, and its associated ginsenosides have shown several biological effects [136]. In this regard, KRG reduced proinflammatory cytokines and increased adiponectin levels in overweight patients with NAFLD [137]. Besides, this natural agent regulates the expression of FABP4 and reduces NASH related inflammation [138]. A previous experimental study revealed that the administration of KRG improves the lipid profile and natural killer cell activity and inhibits the development of steatohepatitis in rats [139].

Ginseng seeds is another product of ginseng that has a positive impact in NAFLD due to its high content of unsaturated fatty acids, phytosterols, and ginsenosides [140–143]. Kim et al. [143] reported that, *in vivo* and *in vitro*, ginseng seed oil suppresses lipogenesis and induces degradation of fatty acids leading to hepatoprotective effect on NAFLD.

Although ginseng might be an alternative therapeutic option for the treatment of NAFLD, clinical studies are lacking and further research is needed in this field.

### 18.2.12 Gingko biloba

Gingko biloba (GB), from the family Ginkgoaceae, is one of the oldest medicinal plants native of China, Korea and Japan. The leaves and seeds of this herb have been used for years to treat bronchial asthma, stroke, hypertension, hypercholesterolemia, and cerebrovascular diseases [144]. Although more than 200 components have been identified in the leaf of G. biloba, its pharmacological properties are mainly attributed to flavonoids, terpenes, trilactones and proanthocyanidins [145]. Currently, Ginkgo biloba extract (GBE) is widely used as a dietary supplement or phytomedicine in Western countries [146]. Wang et al. [147] observed that GBE reduces triglyceride and fatty acid concentrations, and increases the expression and activity of the enzyme carnitine palmitoyltransferase 1a in rat liver. Additionally, Xie et al. [148] found that GBE regulates HMG-CoA reductase activity and inhibits the cholesterol influx in HepG2 cells.

Ginkgolide A (GA), a derived from GB leaf, induces CYP3A protein expression and enzyme activity, and decreases liver inflammation through inhibition of NF-κB activity by NF-κB-specific suppressor IκBα in human hepatocytes [149, 150]. Jeong et al. [151] showed that GA suppress cellular lipogenesis and lipid accumulation by causing mitochondrial oxidative stress in steatotic HepG2 cells; while this extract also improves NAFLD through the induction of lipoapoptosis and inhibition of cellular inflammation in high-fat diet mice.

Another of the bioactive components of GB with pharmacological properties at the liver level are the polysaccharides. In this context, Yan et al.

[145] revealed that the polysaccharides exert a protective effect on NAFLD by decreasing insulin resistance, improving liver function, ameliorating antioxidant defense system, and reducing lipid peroxidation.

Moreover, it has been suggested that GB treatment may prevent the development of NAFLD by mitigating fatty degeneration and inflammatory process in the liver through the reduction of TNF-α expression, hepatic enzymes, and lipid levels [152]. Further, Yang et al. [153] observed that GBE decreases circulating lipids, ameliorates liver function and fatty degeneration in hepatocytes, and reduces inflammation by increasing lipase activity, decreasing liver free fatty acids, increasing the degradation of triglycerides and reducing its synthesis, resulting in the prevention of NASH in animal model.

Given the biological and pharmacological effects of GB, this medicinal herb could be an effective therapy for prevention of NAFLD; however, clinical trials are required in order to confirm the results obtained from the experimental studies.

## 18.3 Conclusion

It has been projected that NAFLD will be the leading cause of liver related morbidity and mortality as well as the main indication of liver transplantation worldwide [154]. Hence, more effective therapies are required to prevent and treat NAFLD. Thus, given the beneficial molecular effects of herbal agents on lipid metabolism, oxidative stress, insulin resistance, inflammation, and liver damage, plant-derived natural products emerge as an alternative therapeutic option for the management of NAFLD (Fig. 18.1). However, although evidence from experimental studies support the potential benefits of natural agents against NAFLD, results from clinical trials is still insufficient and long-term clinical studies are mandatory in this field.

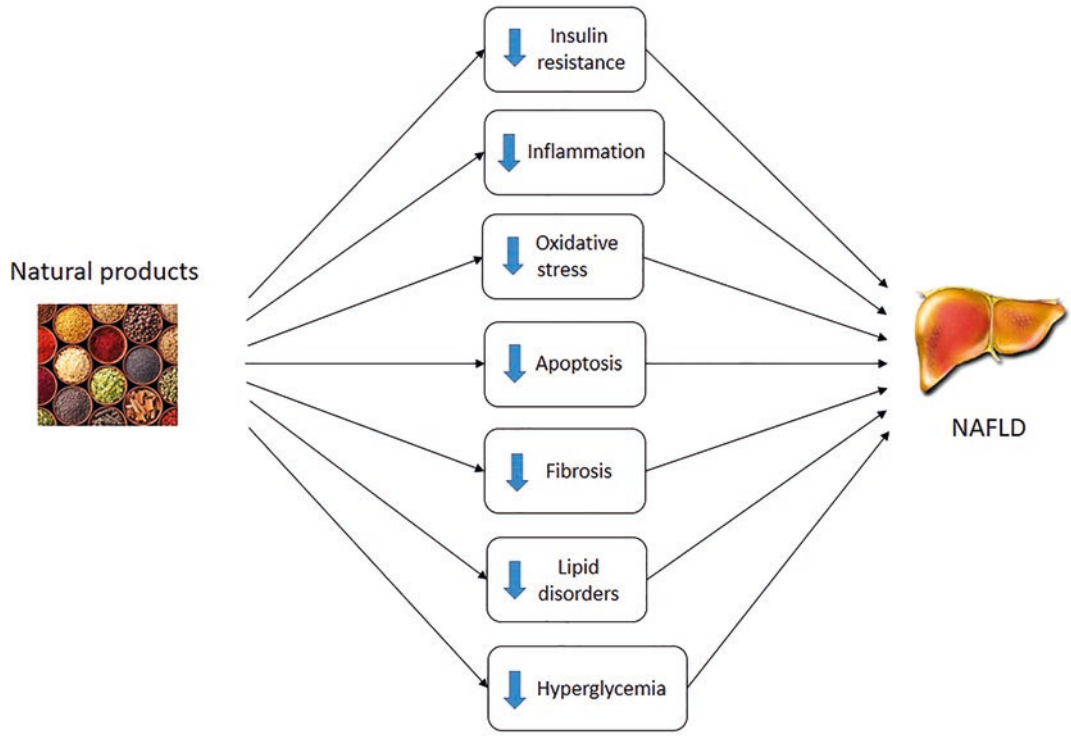


**Fig. 18.1** Natural products in non-alcoholic fatty liver disease

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# 19 Crocin Improves Oxidative Stress in Testicular Tissues of Streptozotocin-Induced Diabetic Rats

Habib Yaribeygi, Stephen L. Atkin, George E. Barreto, and Amirhossein Sahebkar

**Abstract**

Crocin has been shown to have potent antioxidant properties, but its potential antioxidative effects on testicular tissue during uncontrolled diabetes is unknown. Wistar rats were randomly divided into four separate groups; normal, normal-treated, diabetic and diabetic treated (n = 6 per group). Diabetes was induced by a single intravenous injection of streptozotocin (45 mg/kg). Two treated groups of animals (diabetic and non-diabetic) received Crocin daily for 56 days (40 mg/kg/intraperitoneally). At the end of the 56th day, animals were sacrificed and blood and testicular tissue obtained. The level of nitrate, malondialdehyde, glutathione, and the activities of superoxide dismutase and catalase enzymes were determined. Crocin therapy moderated the increased oxidative stress in testicular tissue induced by diabetes with a significant reduction in nitrate and malondialdehyde, whilst reducing superoxide dismutase and catalase enzyme activities in diabetes ($p < 0.001$), though glutathione was unaffected. Treatment by Crocin in normal rats also modestly improved parameters of oxidative stress ($p < 0.05$). Crocin has a protective effect on diabetes induced oxidative stress in testicular tissue in an animal model, though it is unclear if this is a direct antioxidant effect.

**Keywords**

Crocin · Oxidative stress · Testis · Malondialdehyde

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## 19.1 Introduction

Oxidative stress plays an important pathophysiological role in inflammatory disease processes [1]. Oxidative stress and resultant damage is caused by increased free radical production that is over and above the body's normal intrinsic antioxidant defenses (ADS) [2]. Under homeostatic conditions, free radical generation is neutralized by the components of the ADS, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH) [3]. However, in pathological conditions, such as chronic hyperglycemia, there is enhanced free radical production that overcomes the ADS, leading to cellular damage [4].

Oxidative stress causes cellular damage via several mechanisms, such as lipid peroxidation, DNA damage, structural protein injury and induction of apoptotic pathways [2, 5]. These injuries ultimately result in cellular death and tissue dysfunction. Thus, enhancing the intrinsic ADS is an attractive strategy to prevent oxidative damage [6]. Oxidative damage is a prominent contributory cause in cardiovascular disease, renal failure, Alzheimer disease as well as testicular disorders [1, 7–9].

Oxidative damages is directly associated with various testicular disorders, such as varicocele, cryptorchidism, cancer and testicular torsion [10]. It is thought that male infertility is also related to increased excessive free radical production in the testis [11, 12]. Physiologically, free radicals are vital for normal healthy sperm activity, but excessive levels have a deleterious effect on spermatozoa, affecting their fertilizing capacity [12, 13]. Free radical overload in testicular tissues has been shown to peroxidize lipids, with toxic byproduct formation including malondialdehyde (MDA), and results in single or double-stranded DNA breaks [14, 15]. It has been suggested that oxidative stress induces apoptotic pathways, leading to cellular death and infertility [16, 17]. Mammalian spermatozoa are very sensitive to free radical attack due to the high concentration of polyunsaturated fatty acids in their membrane [18], and antioxidative agents such as vitamin E and ascorbic acid have been proposed as effective treatments for male infertility [18]. The origin of free radical production in testicular tissue is mainly via NADPH oxidase enzyme activity and mitochondrial dysfunction [19].

Crocin is a water soluble beta carotene which is mainly found in Saffron (*Crocus Sativus* L.) and Gardenia plants [4]. This phytochemical has been shown to have potent antioxidant activity as well as other beneficial effects [4, 20–22] that can explain several putative medicinal properties of saffron [23–27]. The antioxidant effect of Crocin has been shown in previous studies in renal tissue [4], hepatic cells [4], myocardium [28], retina [29], and neuronal cells [30]. More recently, studies have demonstrated that it may have an antioxidant effect in testicular tissue [31, 32], and, hypothetically, could protect against modulators of oxidative stress, such as diabetes.

In this study, we have evaluated the antioxidant potential of Crocin in testes in an animal model of diabetes-induced oxidative stress.

## 19.2 Materials and Methods

### 19.2.1 Experimental Animals

Male Wistar rats (220–240 g) purchased from the Pasteur Institute (Tehran, Iran) were kept in standard medium size polyester cages (two rats/cage). The animals were kept in standard conditions of temperature (22 ± 2 °C) and humidity (%55 ± 5) in a 12-h light/dark cycle and had free access to water and standard rodent food. They were randomly divided into four separate groups (n = 6) as normal (N); normal treated (NC); diabetic (D) and diabetic treated (DC).

### 19.2.2 Ethical Considerations

All protocols for the animal studies were approved by the local ethics committee and followed the NIH Guidelines for care and use of experimental animals.

### 19.2.3 Diabetes Induction

Streptozotocin (STZ) (40 mg/kg) (Sigma Aldrich, St. Louis, MO, USA) and then dissolved in cold saline. Diabetes was induced by an intravenous injection of STZ (45 mg/kg) into the tail vein. After 72 h, blood samples were obtained from the tail for blood glucose measurement using a standard glucometer (Bionime, Switzerland). Rats with a blood glucose above than 400 mg/dl were considered as diabetic and were randomly divided into two diabetic groups (treated and non-treated).

### 19.2.4 Treatment

Crocin was purchased from Sigma Aldrich (USA) and dissolved in distilled water that was made up daily. Two treatment groups of rats (control and diabetic) were treated daily with Crocin for 56 days (40 mg/kg/day/intraperitoneally).

### 19.2.5 Sampling

At the end of 8 weeks, rats were sacrificed their testicular tissue collected to assess for malondialdehyde (MDA), nitrate (Nitrate), catalase (CAT) and superoxide dismutase (SOD) enzymes and glutathione (GLT) content. Blood samples were collected directly from the heart, then the serum was separated immediately by centrifuge (3000 rpm for 15 min).

### 19.2.6 Blood Glucose Analyzing

Blood glucose concentration was evaluated using available commercial kits (Pars-Azmoon, Iran).

### 19.2.7 Tissue Preparation

Testicular tissue (500 mg) was weighed, after which homogenization medium (phosphate buffer (0.1 mol, pH = 7.4)) was added. Following homogenization of the tissue on ice using an electric homogenizer, the samples were centrifuged (20 min at 4 °C and 4000 rpm) and the supernatants were removed as the cytosolic extract of the testicular tissue. Supernatants were stored in −80 °C for subsequent biochemical assessment.

### 19.2.8 SOD Enzyme Activity

The activity of SOD enzyme, as a main element of ADS in testicular cells, was determined using the Winterbourn method [33]. This method was developed based on the ability of the SOD enzyme to inhibit the reduction of nitro-blue tetrazolium by superoxide. Briefly, 0.067 Moles of potassium phosphate buffer (pH 7.8) was added to 0.1 Mole EDTA containing 0.3 mM sodium cyanide, 1.5 mM nitro-blue tetrazolium and 0.1 ml of the stored testicular supernatant. Next, 0.12 mM of riboflavin was added to the samples to activate the reaction and incubated for about 10 min, the sample optic absorbance recorded at 560 nm in 5 min on a spectrophotometer. The amount of enzyme required to produce 50% inhibition was taken as 1 unit (U). The final results were expressed as U/mL.

### 19.2.9 CAT Enzyme Activity

The activity of CAT enzyme, as another major antioxidative agent in testes, was assessed by the Aebi method [34]. Briefly, a mixture containing of 0.85 ml of potassium phosphate buffer 50 mM, pH 7.0 and 0.1 ml of testicular homogenate were incubated for 10 min at room temperature. The reaction was activated by adding 0.05 ml of $H_2O_2$ (30 mM prepared in potassium phosphate buffer 50 mM, pH 7.0). The decrease of optic absorbance was recorded for 3 min at 240 nm. The CAT enzyme activity was expressed as 1 μmole $H_2O_2$ decayed U/mL.

### 19.2.10 Assessment of GLT Content

GLT content was determined using the Tietz method [35]. The protein content of the testicular sample was precipitated by adding 5% sulfosalicylic acid, then removed by centrifuge (2500 g/10 min). GLT content in the resulting supernatant was assessed by the following steps: 100 µl of protein-free supernatant was added to 800 µl of 0.3 mM $Na_2HPO_4$ and 100 µl of 0.04% 5–5′-dithiobis [2- nitrobenzoic acid] in 0.1% sodium citrate. The 5–5′-dithiobis [2-nitrobenzoic acid] absorbance was recorded at 412 nm for 5 min. A standard curve for GLT was produced and the sensitivity of measurement determined to be between 1 and 100 µM. The concentration of GLT was expressed as nmol/mL.

### 19.2.11 Nitrate Content Assay

The Nitrate level of testes cytosolic extract, as a major index of nitrous free radicals, was assessed by colorimetric reaction using the Griess method [36]. Briefly, 0.1 ml of cytosolic extract was deproteinized by adding 0.2 ml of zinc sulfate solution, then centrifuged for 20 min at 4000 rpm and 4 °C to allow supernatant separation. Then 0.05 ml of sulfanilamide (0.01%) and 0.05 ml N-[1-naphthyl] ethylenediamine dihydrochloride (NED, 0.01%) were incubated together at 37 °C for 30 min in a dark room. The absorbance of this mixed solution was determined at a wavelength of 540 nm. Nitrite concentration was assessed using a standard curve generated from the absorbance of each sodium nitrate solution. The level of nitrate content was expressed as µg/mg protein.

### 19.2.12 Lipid Peroxidation Assay (MDA Content)

The level of MDA, as the end product of lipid peroxidation, was assessed using the Satoh method [37]. Briefly, 0.5 ml of tissue homogenate was added to 1.5 ml of 10% trichloroacetic acid, mixed and incubated at room temperature for 10 min. Afterwards, 1.5 ml of supernatant and 2 ml of thiobarbituric acid (0.67%) were added and placed in a boiling water bath in sealed tubes for 30 min. Then, samples were allowed to cool to room temperature, about 20 min. Then, 1.25 ml N-butanol was added and the mixture centrifuged for 5 min at 2000 g. The resulting supernatant was removed and its optical absorbance detected at 532 nm on a spectrophotometer. MDA content was determined by using 1,1,3,3-tetraethoxypropane. MDA concentration was expressed as nmol/ml.

### 19.2.13 Statistical Analyses

Data was analyzed by one-way analysis of variance (ANOVA) and Tukey post-hoc tests. $P < 0.05$ was considered to be significant. The results are expressed as Mean ± SD.

## 19.3 Results

Serum glucose concentrations in rats at the completion of the study (56 days) are shown in Table 19.1. As anticipated, the STZ injection significantly increased blood glucose, up to 402 mg/dl. Crocin caused a reduction in glucose levels in both normal and diabetic rats.

The changes of GLT levels in the experimental groups are presented in Fig. 19.1. The mean value of GLT content in the normal group was 0.178±0.01 and Crocin increased GLT content to 0.318 ± 0.03 in normal-treated rats (P = 0.0001).

**Table 19.1** Serum glucose concentrations in rats at the completion of the study (56 days)

| Groups | Serum glucose ± SD |
|---|---|
| Normal (N) | 95 ± 12 |
| Normal Treated by Crocin (NC) | 82 ± 13 |
| Diabetic (D) | 402 ± 25 |
| Diabetic Treated by Crocin (DC) | 368 ± 22 |

Sreptozotocin injection significantly increased blood glucose, up to 402 mg/dl. Crocin caused a reduction in glucose levels in both normal and diabetic rats

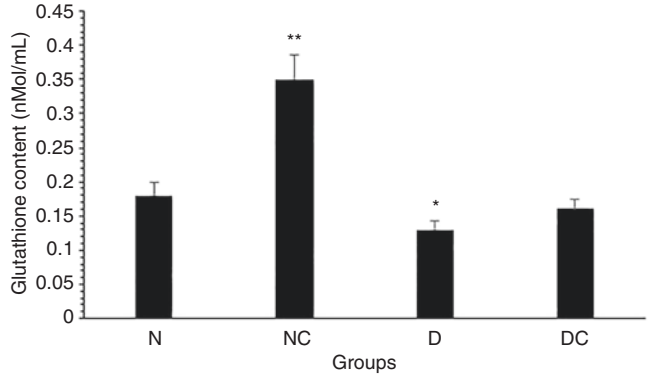


**Fig. 19.1** Changes of GLT levels (nMol/mL) in experimental groups. The mean value of GLT content in the normal group was 0.178 ± 0.01. Crocin increased GLT content to 0.318 ± 0.03 in normal-treated rats (P = 0.0001). Diabetes reduced GLT content to 0.13 ± 0.01, and Crocin did not alter this in diabetic rats

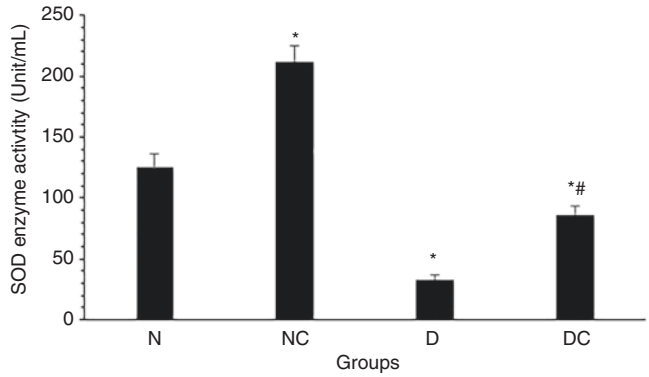


**Fig. 19.2** SOD enzyme activity (Unit/mL) in all groups. In normal rats, the mean value of SOD activity was 127 ± 11.5. Crocin markedly increased this to 215 ± 12.4 (P = 0.0001). Diabetes reduced SOD enzyme activity to 32 ± 3.6 (P = 0.000). Crocin significantly increased SOD enzyme activity to 82.6 ± 7.2 in diabetic animals (P = 0.0001), but this was still reduced relative to the control group (P = 0.0001)

Diabetes reduced GLT content to 0.13 ± 0.01, and Crocin did not alter this in diabetic rats.

SOD enzyme activity in all groups is shown in Fig. 19.2. In normal rats, the mean value of SOD activity was 127 ± 11.5 and was markedly increased by Crocin to 215 ± 12.4 (P = 0.0001). Diabetes reduced SOD enzyme activity to 32 ± 3.6 (P = 0.0001), and Crocin significantly increased SOD enzyme activity to 82.6 ± 7.2 in diabetic animals (P = 0.0001), but this was still reduced relative to the control group (P = 0.0001).

CAT enzyme activity for all groups is shown in Fig. 19.3. In the normal group, the CAT enzyme activity was 0.46 ± 0.01 that was increased by Crocin to 0.073 ± 0.01 in normal-treated rats (P = 0.005). Diabetes reduced CAT enzyme activity to 0.027 ± 0.002 (P = 0.07) that was partially reversed by Crocin that significantly increased this value to 0.047 ± 0.002 (P = 0.05).

MDA content in testicular tissues in all groups is shown in Fig. 19.4. The mean value of MDA content in normal rats was 2.42 ± 0.39 that was lowered by Crocin to 0.41 ± 0.02 in non-diabetic animals (P = 0.01). Diabetes markedly increased MDA content to 13.6 ± 1.6 (P = 0.0001) that was decreased Crocin treatment in diabetic rats to 4.49 ± 0.68 (P = 0.0001).

Nitrate content in all animals is shown in Fig. 19.5. In normal rats, the mean Nitrate content was 2.33 ± 0.036. Crocin did not change Nitrate content in normal treated rats. Diabetes significantly enhanced Nitrate content to

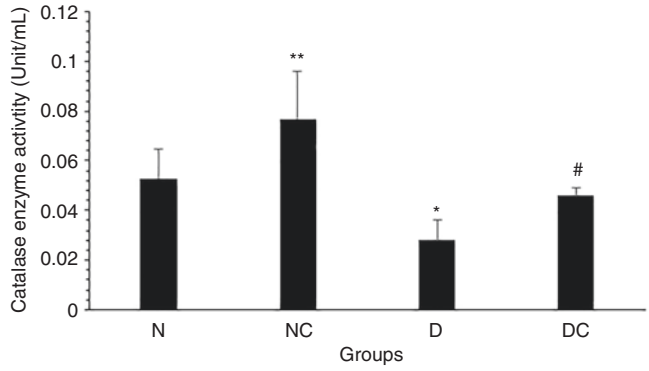


**Fig. 19.3** CAT enzyme activity (Unit/mL) in all animals. In the normal group, the CAT enzyme activity was 0.46 ± 0.01. Crocin increased it to 0.073 ± 0.01 in normal-treated rats (P = 0.005). Diabetes reduced CAT enzyme activity to 0.027 ± 0.002 (P = 0.07). Also, Crocin significantly increased this value to 0.047 ± 0.002 (P = 0.05)

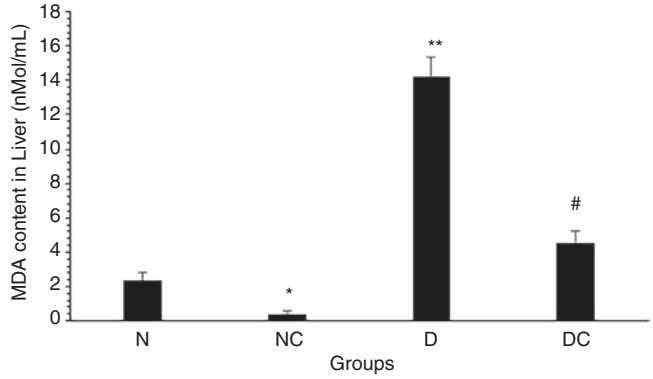


**Fig. 19.4** MDA content in testicular tissues (nMol/mL) in all experimental rats. The mean value of MDA content in normal rats was 2.42 ± 0.39. Crocin lowered it to 0.41 ± 0.02 in non-diabetic animals (P = 0.01). Diabetes markedly increased MDA content to 13.6 ± 1.6 (P = 0.0001). Treatment by Crocin in diabetic rats decreased MDA content to 4.49 ± 0.68 (P = 0.0001)

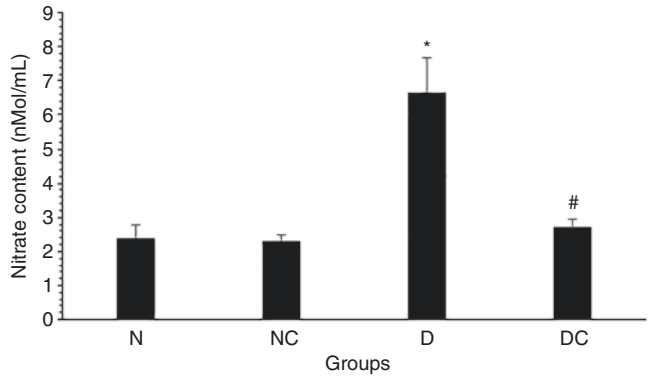


**Fig. 19.5** Nitrate content (nMol/mL) in all animals. In normal rats, the mean Nitrate content was 2.33 ± 0.036. Crocin did not change Nitrate content in normal treated rats. Diabetes significantly enhanced Nitrate content to 6.4 ± 1.0 (P = 0.001), and Crocin significantly decreased it in the diabetic animals to 2.94 ± 0.24 (P = 0.001)

6.4 ± 1.0 (P = 0.001), and Crocin significantly decreased it in the diabetic animals to 2.94 ± 0.24 (P = 0.001).

## 19.4 Discussion

In this diabetes model the endogenous ADS was reduced whilst the measures of enhanced free radical generation in testicular tissue, nitrate and MDA, were increased. These measures were improved by Crocin with a reduction in SOD and CAT reflecting the endogenous ADS system in testicular tissue and reduced oxidative damage by lowering MDA production and nitrate levels. Oxidative stress is considered to be a major factor in testicular dysfunction [10]. Previous studies have shown that oxidative damage due to free radical overload in testicular tissues can cause various testicular complications as well as infertility [11, 12].

The potent antioxidant ability of Crocin has been reported in the literature [4, 38]. In our current study, Crocin reduced nitrous free radical levels in testicular tissues and increased SOD and CAT enzyme potency (the main elements of cellular ADS). While Crocin did not have the same effect on glutathione content, it did reduce oxidative damage in testicular tissues by lowering MDA production. We are unaware of any other study that has evaluated the effects of Crocin in diabetes-induced oxidative stress in testis. However, beneficial antioxidative effects of Crocin have been shown in other tissues [4, 38, 39] and Crocin may improve oxidative damage by scavenging free radical species or via up-regulation of antioxidative elements [40, 41].

In this study STZ-induced hyperglycemia enhanced nitrate levels (a marker of nitrous free radicals) and so increased free radical generation. This finding was in accord with previous findings [42], demonstrating that diabetes increases free radical generation in different tissues [42]. Our results are in accord with others who reported that diabetes increases free radical production and induces oxidative damage in testis [43]. Our data suggest that diabetes can induce oxidative stress by either free radical overload or via inhibition of the ADS system. Earlier, we have reported that uncontrolled diabetes can reduce ADS potency of liver tissues by decreasing SOD and CAT enzymes activities [4]. Salceda et al. in 1998 and Kakkar et al. in 1999 showed that diabetes disrupts the normal physiologic redox state in brain, liver and pancreas tissues [44, 45]. Moreover, Shrilatha et al. in 2007 and Asadi et al. in 2017 reported the same observations in testis [46, 47]. Thus, our findings are in accord with previous resports and suggest that free radical overload (nitrate level) or inhibition of ADS (SOD and CAT enzymes activities) may underlie the diabetes-induced disruption of the redox state.

In conclusion it was shown that Crocin can exert a potent antioxidant effects on testicular tissue by lowering free radical generation and potentiating ADS parameters, thereby improving diabetes-induced oxidative stress. This suggests that Crocin therapy may have utility for readjusting the redox state in testis, and may be a possible therapeutic agent in male infertility.

**Conflict of Interests** All of the authors declare that there is no conflict of interest.

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# 20 Beneficial Medicinal Plants for Memory and Cognitive Functions Based on Traditional Persian Medicine

Bahareh Sadat Yousefsani, George E. Barreto, and Amirhossein Sahebkar

**Abstract**

Alzheimer's disease (AD) is one of the most important causes of dementia, especially in the elderlies. Due to the failures of recent clinical trials in finding effective medications, it appears the use of complementary therapies such as Traditional Persian Medicine (TPM) and the rich sources of effective herbs as well as their constituents for improving memory function could be beneficial. The aim of this study was to evaluate the recommended natural remedies in the TPM and examine their pharmacological properties. For this purpose, the data were collected by searching the recommended prescriptions of the seminal TPM textbooks. Then, the names of the most freuqently mentioned plants were extracted from the natural remedies and evaluated for their pharmacological properties. The sources included recently published articles cited in the major scientific databases. A total of 262 plants were identified in 96 evaluated prescriptions; 20 plants were identified with the most frequency of report (i.e. more than 10 times). Their neuroprotective effects, antioxidant features, and anti-AD properties were discussed. Based on our results, TPM has introduced many effective treatments for AD. Hence, more clinical studies are warranted to verify their efficacy and safety.

**Keywords**

Alzheimer's disease · Natural products · Medicinal plants · Traditional Persian Medicine (TPM)

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## 20.1 Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disorder, has shown considerable statistical growth in recent years. Estimations have demonstrated that the number of people that succumb to AD will triple by 2050 [1]. The rapid increase in the prevalence of AD has signaled the importance of more research designs into the treatment of AD due to the limited therapeutic options and lack of sufficient effects. Ethnopharmacological experiments have unveiled that traditional medicine is a source of herbal remedies that can help find new therapies [2, 3].

Numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as AD. Based on the pamphlets and textbooks of the Traditional Persian Medicine (TPM), it seems that AD signs and symptoms are consistent with "Nesyan" disease [4]. Therefore, investigating the herbal remedies introduced in the treatment of AD can improve memory and cognitive disorders and improve symptoms of AD as well as brain atrophy [5, 6].

Ethno-pharmacological research has revealed potential new medications from natural sources for cognitive disorders. There are various medications available in medicine, especially in Western medicine, which have been totally isolated from natural sources including the plants. For example, galantamine and anticholinesterase (anti-ChE) alkaloids extracted from a plant (*Galanthus caucasicus*) have been approved for the treatment of AD and is now in clinical use. Furthermore, boswellic acids, elicited from *boswellia carterii*, which has been used in TPM, is effective in AD by showing acetylcholinesterase inhibitory effects [7]. Various other plant species have also revealed an interdependent relationship between the pharmacological activities and the treatment of cognitive disorders, indicating the potential of TPM for therapeutic uses in mental disorders such as AD. This study probed some of the natural remedies that have been used in TPM for their reputed effects on cognitive-enhancement or memory improvement. As documented in previous studies, plants have antioxidant and neuroprotective activities [8, 9]. Moreover, they enhance the cholinergic function in the central nervous system (CNS). All of these pharmacological activities which may be pertained to the treatment of cognitive disorders are discussed in this review.

## 20.2 Materials and Methods

In this study, natural remedies that have been used in TPM for the treatment of diseases were used as the research data. For this purpose, 6 important medicinal and pharmaceutical books were thoroughly analyzed. In terms of temporal distribution, efforts have been made to select books from different historical periods, that is from the fifth to the thirteenth century Hijri (AH). Therefore, the books like *Ghanoon fel-teb (the canon of medicine)*, *Gharabadin Shafa'i, Gharabadin Kabir, Exir-e-Azam, Daqaeq al-Alaj* and *Gharabadin Azam* were selected [10–15].

The data were garnered through the keywords of "Nesyan", "Faramoshy", "Zekr", and "Hafezeh". Each of these keywords was searched separately in each of the mentioned books and the results were stored in a file. The repeated remedies in a book were deleted; if a remedy was repeated in several books; however, they would not be deleted and would be included for the data collection because of the importance of that remedies. Eventually, 4 remedies of *the canon fel-teb*, 16 formulas of *Gharabadin Shafa'i*, 26 remedies of *Gharabadin kabir*, 24 remedies of the *Exir-e Azam*, 6 remedies of the *Daqaeq al-Alaj* and 20 remedies of the *Gharabadin Azam* were entered into the data analysis. To identify the plants most commonly used in the remedies, the plants of each remedies were listed in a table containing the plants' names and their numbers in each book. Given that some of the plants were only mentioned in one or two books or were not frequent enough, it was decided to continue analyzing the plants with a frequent repetition of 10 times or more.

The temperaments of the plants were extracted from the TPM reference books. The scientific names and pharmacological properties of these plants were also searched in the scientific data-

bases (e.g. Google Scholar, PubMed, Medline, and Scopus). The results are displayed in Table.

## 20.3 Results

262 plants were mentioned in the TPM books, of which20 plants were the most frequent ones, that is repeated more than 10 times. Most of these plants have protective pharmacological effects on the central nervous system (CNS) as confirmed in the clinical studies and some require further specific clinical studies on AD. Table 20.1 exhibits theses effective plants and their pharmacological properties. (Table 20.1).

## 20.4 Discussion

Based upon the therapeutic effects of the existing medications for AD and the therapeutic pitfalls of many new medications in the late stages of clinical trials as well as the growing-apace prevalence of this disease, the application of other alternative therapies seems to be essential. Studies have demonstrated the efficacy of some of the herbs for the AD treatment; in other words, it has been adequately established that traditional medicine can be indeed helpful in this regard [70].

Some state-of-the-art studies give support to the usefulness of some of these natural remedies. Specifically, the pharmacological properties of most of these plants have revealed antioxidant compounds which, due to the role of oxidative stress in the development of Alzheimer's disease, can be effective in the treatment of memory impairment. In addition, the anti-AD effects of many of the TPM recommended remedies have been endorsed in prior studies.

Studies have reported that herbs have beneficial effects on cognitive function. For example, Piper (*Piper nigrum L.*) is at the top of the list in this table and contains Piperine –a major alkaloid of black pepper— which has anti-inflammatory, anticonvulsant and antidepressant effects [71]. It also has neuroprotective effects in *in vivo* and *in vitro* models [35]. Antioxidant activity of Aqueous and ethanolic extracts of this plant ameliorates Parkinsonian and AD [17, 18]. Therefore, black pepper is considered to be a potential functional food for enhancing the brain function [16, 72].

Another plant, Cinnamomum extract contains proanthocyanidin and cinnamaldehyde, which can inhibit amyloid fibril formation according to the *in vitro* studies. It has the free radical cleansing capacity; moreover, the antioxidant activities of the methanolic extract of the Cinnamomum verum leaf has been well documented in other studies [19, 20].

Another plant is Ginger (*Zingiber officinalis Rosc*). Ginger extract can increase the epinephrine, norepinephrine, dopamine, and serotonin contents in the brain. That said, it seems the ginger extract plays a neuroprotective role. The antioxidant activity of the ginger extract has been confirmed in some studies; thus, it is a natural preservative substance, applicable in pharmaceutical industries [23, 24].

Studies have demonstrated that Black- or chebulic myrobalan (*Terminalia chebula Retz*) compounds such as hydrolysable tannins, phenolic compounds, and flavonoids have antioxidant activity in different and specific pathways [25, 27]. Furthermore, these components have an acetylcholinesterase inhibitory activity that nowadays is applied for the management of AD [26].

Frankincense olibanum (*Boswellia carterii Bird*) is recognized for its aromatic resin. This resin has antioxidant activity, elevates the acetylcholine level and reduces the acetylcholinesterase activity in the brain. The mentioned features have been histopathologically confirmed to improve the neurodegenerative characteristics of ADs in rats [29, 30, 73]. Incensole acetate, derivated from serrata, has anti-inflammatory effect. All in all, it inhibited the hippocampal neurodegeneration and boosted cognitive capability as identified through the object recognition test [28].

Finally, the stigma of saffron (*Crocus sativus*) is dried for use as a spice and medicinal plant in many cultures. In TPM, aside from its utilization for mental diseases such as anxiety, depression, insomnia and tension, saffron has been used as a cardioprotective medication [74, 75]. Studies

**Table 20.1** Pharmacological properties of herbal plants used to treat Alzheimer's disease in Persian medicine

| Common name | Scientific name | Persian name | Family name | Part used | Pharmacological effect | | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | | neuroprotective | Anti-Alzheimer | Anti-oxidant | |
| Piper | *Piper nigrum* | Filfil | Piperaceae | Fruit | * | * | * | [16–18] |
| Cinnamon | *Cinnamomum verum* | Darchin | Lauraceae | Stem bark | * | * | * | [19–21] |
| Ginger | *zingiber officinale Rosc* | Zanjabil | Zingiberaceae | Rhizome | * | * | * | [22–24] |
| Black- or chebulic myrobalan | *Terminalia chebula Retz* | Halileh | Combretaceae | Fruit | * | * | * | [25–27] |
| Frankincense, olibanum | *Boswellia carterii* | Kondor | Burseraceae | Oleogum resin | * | * | * | [28–30] |
| Saffron | *Crocus sativus* | Zafaran | Iridaceae | Stigma | * | * | * | [31–33] |
| Long pepper | *Piper longum* | Darfilfl | Piperaceae | Fruit | * | * | * | [34–36] |
| Sedge galingale | *Cyperus rotundus.* | So'd | Cyperaceae | Tuber | | * | * | [37, 38] |
| Clove | *Dianthus barbatus* | Mikhak | Caryophyllaceae | Fruit | * | * | | [39, 40] |
| Indian valerian | *Nardostachys Jatamansi* | Sonbol al-Tib | Caprifoliaceae | Root | * | * | * | [41–43] |
| Mastic | *Pistacia lentiscus* | Mastaki | Anacardiaceae | Oleo gum-resin | * | * | * | [44–46] |
| Rose | *Rosa damascene* | Gol-e Sorkh | Rosaceae | Flower, rosewater, rose oil | | | * | [47] |
| Sweet flag | *Acorus calamus* | Vaj | Araceae | Rhizome | * | * | * | [48–50] |
| Amla or amalaki | *Phyllanthus emblica L.* | Amele | Phyllanthaceae | Fruit | * | * | * | [51–53] |
| Asarum or wild ginger | *Asarum europaeum* | Osaron | Aristolochiaceae | Rhizome | * | * | * | [54, 55] |
| Lavender | *Lavandula angustifolia mill.* | Ostokhoddus | Lamiaceae | Aerial part | * | * | * | [56–58] |
| Lemon balm | *Melissa Officinalis* | Badranjbuyeh | Lamiaceae | Aerial part | | | | [59–61] |
| Nutmeg or mace | *Myristica fragrans* | Joz boa | Myristicaceae | Seed | * | * | * | [62–64] |
| Green or true cardamom | *Elettaria cardamomum* | Hel | Zingiberaceae | Fruit | * | * | * | [65–67] |
| Lemon grass | *Cymbopogon schoenanthus* | Ezkher maki | Poaceae | Leaves | * | * | * | [68, 69] |

have reported that saffron is effective for the treatment of AD in cognitive clinical dementia [76].

## 20.5 Conclusions

Although in the current paper 20 herbs with the highest frequency of usefulness have been introduced and evaluated, a thorough introduction and analysis of all 262 plants adopted in the PM sources along with their pharmacological properties and clinical effects will definitely be valuable.

It should also be borne in mind that these plants can have potential side effects that ought to be taken into account. Although the safety of the traditional remedies has been tested and confirmed to some extent in successive generations for several hundred years, in order to prove their comprehensive safety, especially the combination of plants, it is necessary to carry out wider and more cogent studies with scientific methodology and evidence-based medicine (77). In a nutshell, future clinical studies with a particular focus on their potential effects in the prevention of AD are warranted.

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# 21

# The Use of Medicinal Plants for the Treatment of Alopecia in the Canon of Avicenna: An Evidence-Based Review

Zahra Boghrati, Sara Sabouri-Rad, Seyed Ahmad Emami, Mohsen Tahaghoghi Saber, Amirhossein Sahebkar, and Zahra Tayarani-Najaran

**Abstract**

Although mostly seen in the scalp, alopecia can occur in any hair- bearing site of the body. In spite of various modern treatments, total cost, efficacy, safety and drug dependency have caused a global willing towards natural remedies. The aim of this chapter is to focus on medicinal plants mentioned in Canon of Avicenna, one of the most primary medicinal books, for the treatment of alopecia. Databases like PubMed, Scopus and Google Scholar were searched for plants mentioned in Canon for managing alopecia to find studies on their clinical efficacy or mechanisms, which may have attributed to the treatment of alopecia. 25 plants belonging to 16 families have been mentioned in Canon. Most of them have a history of use in ethno-medicine and some are used in hair growth products nowadays. Investigating literatures has shown that anti- inflammatory and immunomodulatory properties are the proposed mechanisms for the treatment of some types of alopecia. Islamic traditional

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medicine can give new insights for development of multiple natural treatment, which their use in human have been tested for thousands of years. By confirming their efficacy and safety, traditional herbal remedies are appropriate alternatives for chemicals mainly used for alopecia.

**Keywords**

Alopecia · Avicenna · Canon · Medicinal plants

## 21.1 Introduction

Scalp hair has always had a huge impact on overall appearance and aesthetic perception of human beings as if its loss might result in psychological suffering, low self-esteem and impaired quality of life. Thanks to laboratory and clinical research, not only numerous internal and external factors have been identified contributing to hair loss but also various medicinal and surgical approaches are now developed to assist patients to cope with the problem. However, those seeking for a quick and easy replacement may even end in wearing artificial hair or wigs, which are no perfect substitute for real hair due to unnatural look and lack of acceptability by most people [15, 74, 86].

Whether the diagnosed culprits are hormonal abnormalities, genetic backgrounds, poor nutrition, severe stressful periods, medicinal side effects and chemical exposures, appropriate approaches ranging from popular prescription drugs like mionxidil commencing on many types of hair loss to complex formulated novel medicines like oral JAK/STAT pathway inhibitors (e.g. tofacitinib and ruxolitinib) prescribed for recalcitrant alopecia areata as well as procedures including low- frequency laser therapy, micro needling and hair transplantation primarily done for androgenetic alopecia will be adopted [14, 19, 26, 36, 39, 48, 53, 73, 76, 81, 82, 87, 95, 102–104].

Patients' major concerns of each therapeutic method in terms of total cost, efficacy, safety and drug dependency would lead them to take natural products and herbal remedies which introduced whether by ancient botanists or physicians or are the products of modern contemporary technology. Current positive attitudes towards medical herbs for hair loss incited our team to review types of hair loss and the medicinal herbs mentioned in Canon, a book by the great Iranian philosopher and physician, Avicenna who described at least two types of hair loss entitling dā-ol ḥayye (alopecia furfuracea) and dā-ol tha'lab (alopecia) as well as two mechanisms including either excessive dryness or moisture of the regional hair and skin [27]. The authors believe these terms might relatively refer to telogen effluvium, hair loss due to seborrheic dermatitis or ichthyosis and alopecia areata universalis type in modern dermatology respectively.

It is noteworthy to utter that medical terminology for hair loss is alopecia which is obtained from the Greek word *alopekia*, meaning fox mange, a highly contagious skin disease in canidae caused by the *Sarcoptes scabiei* mite [52].

Nowadays different types of alopecia, obviously with several separate pathogenic mechanisms, are diagnosed including androgenetic alopecia, telogen effluvium, traction alopecia, alopecia areata, trichotillomania, pressure-induced alopecia, temporal triangular alopecia, lipedematous alopecia, TNF-α inhibitor induced psoriasiform alopecia, congenital alopecia with papules, central centrifugal cicatricial alopecia, lich planopilaris, frontal fibrosing alopecia, discoid lupus erythematosus Acne keloidalis nuchae, dissecting cellulitis and other types of hair loss due to hair shaft abnormalities (Sperling, 2018). In the present review, after a brief description of the most common types of alopecia, we only focused on alopecias in which inflammation and autoimmune mechanisms are the principal interfering factors in order to provide some scientific support on the use of medicinal herbs in view of modern medicine. The efficacious plants were elicited from Canon and their scientific names

were identified by the use of reliable resources [28, 91].

## 21.2 Alopecia in View of Avicenna

Avicenna has given a description and some common treatments for hair loss that can be noteworthy nowadays:

It should be noted that the causative agent of dā-ol tha'lab (alopecia) (in Arabic dā' = disease and tha'lab = fox) is a poorly substituted sputum in the skin and hair follicles that damages the hair roots and prevents proper nutrition. The origin of the name dā-ol tha'lab implies a similar disease affecting fox family. There is also another type of hair loss called as dā-ol hayye (al-hayye = snake). The difference between them lies in the fact that the latter illness not only causes hair loss, but also results in cutaneous scaling. The underlying cause of these two diseases is bile sputum. The phlegm, black bile sputum and bad blood sputum may also contribute. To detect the pathogenic sputum, one should first shave the affected hair area and then intensely massage the skin, depending on the created color, the disease agent can be determined (yellow indicates the presence of bile and the colors black, white and red indicate the involvement of black bile, phlegm and blood, respectively) [27].

## 21.3 Common Treatments of Alopecia Mentioned in Canon by Avicenna

Undoubtedly, the best treatment is to remove the inductive sputum from the body. For this purpose, appropriate drugs or phlebitis are used. After that, the abnormal sputum is discharged from the head by using suitable mouthpiece (inhaling drugs), incense and gargles. In the last step, the inappropriate sputum settled in the skin should be drained and in order to prevent its substitution, some medical interventions are necessary. Since dry skin can exacerbate hair loss, temperate oils should accompany hot and strong medicines such as tāfsiā/*Ruta sylvestris* (one of the main drugs used to treat this condition), in order to moderate the drug strength. It is also necessary to reduce the number of strong drugs and to remove them quickly after their application, the vice versa is true for the poorly prescription medications. It is also crucial for these medicines to be subtle to penetrate through the skin and have some kind of astringency (not be too high to prevent their own penetration into pores) to prevent the penetration of improper sputum to the head. The treatment should be gradual; first, the weakest drugs and the least amount of them should be applied. If not efficient, the potency and quantity can be increased. Considering this prevents injury or inflammation.

Blushing of the skin by massage is an indication of the proper effect of the drug. Massage can be done by a rough texture and if it was not effective, the affected area should be cut with a razor and then be rubbed by garlic. If severe purgation of an unpleasant and inappropriate sputum is required, leech therapy, cupping or blistering agents can be used. Wearing a felt hat day and night may accelerate the treatment process by causing sweating which reduces the improper sputum [27].

## 21.4 A Brief Review on the Most Common Types of Alopecia and their Treatments in Modern Medicine

### 21.4.1 Alopecia Areata

Alopecia areata is an inflammatory and organ specific autoimmune disease of the hair follicles in which $CD8^+$ T cells producing Interferon gamma (IFNγ) are the initial operating agents. Subsequently, gamma-chain cytokines such as IL2, IL7, IL15 and IL21 signal via JAK/STAT pathway which is at least one significant underlying mechanism in this alopecia [97]. The disease mostly presents with symptomless, reversible hair loss patches on any hair bearing areas of the skin [43]. This type can also promote to either involving the entire scalp (alopecia totalis) or the entire body and scalp (alopecia universalis) [5].

#### 21.4.1.1 Treatment

The first-line therapies for AA are topical corticosteroids (clobetasol preparations), intralesional corticosteroids (triamcinolone acetonide) [63]. Topical irritants such as anthralin cream 0.5–1% topical minoxidil (2% or 5%), topical or oral photo chemotherapy (PUVA), excimer laser and contact immunotherapy (like 2, 3-diphenylcyclopropenone) [53]. The therapeutic effects of immunosuppressive drugs such as systemic corticosteroids, methotrexate and cyclosporine have shown efficacy, yet, with high rates of relapses and major toxicities [32, 71]. More recently, as mentioned above JAK/STAT inhibitors have had promising effects. A few in vivo studies on simvastatin/ezetimibe combination for AA treatment have also shown positive results. The adverse effects and relapse rates were minor or not observed. According to these studies, this combination can serve as a new promising treatment for AA [11, 44]

### 21.4.2 Androgenetic Alopecia

Androgenetic alopecia (AGA) is the most common form of alopecia, affecting both men and women to some degrees during their lives. Genetics and androgen disorders are considered as the main causes of AGA [66]. The frequency and severity of this type increase with age [92].

#### 21.4.2.1 Treatment

The first therapy for AGA is topical minoxidil 5% which is safe and efficient [59, 99]. Besides, finasteride in men [34] and spironolactone in women [1, 10] are sometimes prescribed. Topical minoxidil and oral finasteride have been approved by the US Food and Drug Administration (FDA) for male pattern hair loss [92]. Minoxidil is the first FDA approved treatment for AGA and finasteride is the approved drug only for men [82]. For females, topical minoxidil (2% solution) is an approved treatment. In addition, oral contraceptives and spironolactone may be beneficial in hyper- androgenemia. Hair transplantation and scalp reduction can also be considered as alternative approaches [92].

### 21.4.3 Telogen Effluvium

In healthy normal status, only 10% of scalp hair follicles are in telogen phase of hair cycling, however, pathologic events including malnutrition, severe chronic illnesses, endocrinopathies, prolonged psychological stress, certain types of drug consumption and even physiologic conditions such as postpartum period could alter the biologic clock of hair follicles leading to shedding of more than 50–200 hairs daily. Diameter reduction of hairs are observed on both entire scalp and on pubis as well as axilla [67].

#### 21.4.3.1 Treatment

Hair regrowth without treatment as well as excellent prognosis after removing the possible culprit might be observed. Obviously, the baldness is not something to be expected in patients [75].

## 21.5 Scientific Evaluation of Medicinal Plants Mentioned for the Management of Alopecia in Canon

The medicinal herbs mentioned for the management of alopecia in Canon and some scientific evidences substantiating their efficacy are described individually in this section. Besides, the traditional application of some plant are also reported. All the collected results are shown in Tables 21.1, 21.2, 21.3, and 21.4.

### 21.5.1 Indigofera tinctoria

The leaves of *I. tinctoria* are used in herbal shampoos as hair growth promoters [61]. The leaf decoction of the plant has been used in Indian ethno-medicine as a treatment for skin diseases [20]. An herbal gel formulation containing *I. tinctoria* extract showed a reduction in the initiation and total time of hair growth in treated group by 30%. Besides, the texture and quality of the grown hair was significantly enhanced [42]. The plant is also used in a formulation of poly-herbal

**Table 21.1** Medicinal plants mentioned in Canon for treatment of alopecia

| No. | Scientific names | Synonym(s) | Arabic name | Common English name | Family |
|---|---|---|---|---|---|
| 1 | *Adiantum capillus-veneris* L. | | barshiāoshān | maidenhair fern | Pteridaceae |
| 2 | *Aegilops neglecta* Req. ex Bertol. | *Triticum ovatum* Raspail | dausar | three-awn goat grass | Poaceae |
| 3 | *Agrimonia eupatoria* L. | *Eupatorium dioscoridis* Bubani | ghāfith | agrimony | Rosaceae |
| 4 | *Allium cepa* L. | | basal | onion | Amaryllidaceae |
| 5 | *Allium sativum* L. | | thūm | garlic | Amaryllidaceae |
| 6 | *Apium graveolens* L. | | karafs | celery | Apiaceae (Umbelliferae) |
| 7 | *Artemisia abrotanum* L. | | qaisūm | southern wood | Asteraceae (Compositae) |
| 8 | *Artemisia absinthium* L. | | afsantin | absinthe | Asteraceae (Compositae) |
| 9 | *Artemisia maritima* L. | | shih | worm wood | Asteraceae (Compositae) |
| 10 | *Asphodelus tenuifolius* Cav. | | serish | asphodel | Xanthorrhoeaceae |
| 11 | *Beta vulgaris* L. | | silq | beet | Amaranthaceae |
| 12 | *Brassica nigra* K.Koch | *Sinapis nigra* L. | khardal | black mustard | Brassicaceae (Cruciferae) |
| 13 | *Cynara scolymus* L. | | hurshuf | globe artichoke | Asteraceae (Compositae) |
| 14 | *Delphinium staphisagria* L. | | miwizaj | mountain raisin | Ranunculaceae |
| 15 | *Drimia maritima* Stearn | *Scilla maritima* L.; *Squilla maritima* Steinh. *Urginea maritima* Baker | unṣul | squill | Asparagaceae |
| 16 | *Ferula assa-foetida* L. | *Ferula foetida* St.-Lag.. | haltith | asafoetida | Apiaceae (Umbelliferae) |
| 17 | *Indigofera tinctoria* L. | | nil | true indigo | Leguminosae |
| 18 | *Narcissus tazetta* L. | | nargis | narcissus | Amaryllidaceae |
| 19 | *Nymphaea lotus* L. | | nilufar | water lily | Nymphaeaceae |
| 20 | *Phragmites australis* (Cav.) Trin. ex Steud. | *Arundo donax* Forssk.; *Phragmites communis* Trin. | qaṣab | giant cane | Poaceae (Gramineae) |
| 21 | *Ranunculus asiaticus* L. | *Cyprianthe asiatica* Freyn | kabikaj | Persian buttercup | Ranunculaceae |
| 22 | *Raphanus raphanistrum* subsp. *sativus* Domin | *Raphanus sativus* L. | fujl | radish | Brassicaceae (Cruciferae) |
| 23 | *Ruta graveolens* L. | *Ruta hortensis* Mill. | sodhāb | common rue | Rutaceae |
| 24 | *Semecarpus anacardium* L.f. | | balādhur | marking nut | Anacardiaceae |
| 25 | *Thapsia garganica* L. | *Thapsia decussata* Lag. | tāfsiā | deadly carrot | Apiaceae (Umbelliferae) |

hair oil, according to Ayurvedic Formulary of India, prepared for the treatment of hair fall and premature hair greying [8].

Furthermore, the ethanol extract of the leaves has shown anti-inflammatory activity when administered orally [80] and the aqueous extract has exhibited immunomodulatory effects on humoral and cellular immune response [93].

**Table 21.2** Recent *In vivo* studies on medicinal plants mentioned in Canon for treatment of alopecia

| Plant | Part/extract | Active constituent | Method | Animal | Effect | Ref. |
|---|---|---|---|---|---|---|
| *Semecarpus anacardium* | nut milk extract | _ | aflatoxin B -induced hepatocellular carcinoma | male Wistar rats | immunomodulatory | [65] |
| | stem bark/ ethyl acetate extract | _ | carrageenan induced rat paw edema | male Sprague-Dawley rats | moderate anti- inflammatory | [84] |
| | seeds/ethyl acetate and methanol extracts | tetrahydroamento flavone (THA) | carrageenan induced rat paw edema | male Sprague–Dawley rats | anti- inflammatory | [83] |
| | nut milk extract | _ | carrageenan induced rat paw edema/cotton pellet granuloma | male Wistar rats | anti- inflammatory | [69] |
| | | _ | high fat diet, streptozotocin-induced type 2 diabetic rat | male Sprague–Dawley rats | anti- inflammatory | [37] |
| | | _ | xylene-induced ear edema, formalin induced inflammation/ myeloperoxidase (mpo) activity | male albino Wistar rats | immunomodulatory and anti-inflammatory | [70] |
| | nuts/ chloroform extract | _ | carrageenan induced rat paw edema | male albino Haffkine strain rats | anti- inflammatory | [79] |
| *Apium graveolens* | leaves/hydro alcoholic extract | _ | croton-oil-induced ear edema | male CD-1 mice | anti-inflammatory | [51] |
| *Beta vulgaris* | leaves/ aqueous extract | _ | carrageenan induced rat paw edema/cotton pellet granuloma | albino wistar rats | anti-inflammatory | [30] |
| | roots/ ethanol extract | _ | gentamicin-induced nephrotoxicity | albino Wistar rats | anti-inflammatory | [16] |
| | betalain-rich dye | _ | carrageenan induced rat paw edema/ myeloperoxidase kinetic-colorimetric assay/ carrageenan-induced peritonitis | male Swiss mice | anti-inflammatory/ anti- oxidant | [50] |
| | roots/ methanol extract | _ | humoral antibody response/delayed type hypersensitivity response | Swiss albino mice | immunomodulatory | [96] |

(continued)

**Table 21.2** (continued)

| Plant | Part/extract | Active constituent | Method | Animal | Effect | Ref. |
|---|---|---|---|---|---|---|
| *Indigofera tinctoria* | leaves/ aqueous extract | _ | carrageenan induced rat paw edema | albino Wistar rats | anti-inflammatory | [80] |
| | leaves/ ethanol extract | _ | noise stress induction test | albino Wistar rats | immunomodulatory | [93] |
| | leaves/ coconut oil extract/ Siddha formulation | _ | | albino Wistar rats | hair growth enhancing | [42] |
| *Apium graveolens* | stalks | apiuman | endotoxin shock model | male A/ HeJ mice | anti-inflammatory | [60] |
| | aerial parts/ ethanol extract | _ | carrageenan induced rat paw edema | inbred albino Wistar rats | anti-inflammatory | [3] |
| | seeds/ aqueous and hexane fractions | _ | xylene-induced ear edema/formalin test | male NMRI mice | anti-inflammatory | [68] |
| | seeds/ ethanol extract | _ | xylene-induced ear edema/cotton pellet granuloma | male Swiss mice | anti-inflammatory | [6] |
| *Ferula assa-foetida* | oleo gum resin | _ | carageenan induced rat paw edema | male albino mice | anti-inflammatory | [7] |
| *Narcissus tazetta* | bulbs | nartazin | mitogenic activity of murine spleen and bone marrow cells | BALB/c mice | immunomodulatory | [13] |
| *Artemisia absinthium* | leaves/ aqueous extract | _ | polycystic ovary syndrome rat model | female Wistar rats | anti-inflammatory | [77] |
| | aerial parts/ methanol extract | _ | venom and carrageenan induced rat paw edema | albino Wistar rats | anti-inflammatory | [55] |
| | aerial parts/ aqueous extract | _ | carbon tetrachloride (CCL4)-induced chemical liver injury/bacillus Calmette–Guerin and lipopolysaccharide (BCG/LPS)-induced immunological liver injury in mice | male Kunming mice/male NIH mice | immunomodulatory and anti-inflammatory | [4] |
| | leaves/ aqueous extract, essential oil | _ | carrageenan induced paw edema | albino mice | anti-inflammatory | [21] |
| | stem/ methanol extract | _ | carrageenan induced paw edema | albino mice | anti-inflammatory | [2] |

(continued)

**Table 21.2** (continued)

| Plant | Part/extract | Active constituent | Method | Animal | Effect | Ref. |
|---|---|---|---|---|---|---|
| *Adiantum capillus-veneris* | whole plant/ethanol extract | _ | carrageenan induced rat paw edema | albino Wistar rats | anti-inflammatory | [22] |
| | fronds/ethanol extract | capillirone 4α-hydroxyfilican-3-one | carrageenan induced rat paw edema | albino Wistar rats | anti-inflammatory | [23] |
| | fronds/ethanol extract | _ | LPS-induced inflammatory cytokines production | CD-1 mice | anti-inflammatory | [105] |
| *Agrimonia eupatoria* | aerial parts/infusion and ethyl acetate fraction | _ | carrageenan induced rat paw edema | male Wistar rats | anti-inflammatory | [78] |
| *Ruta graveolens* | aerial parts/methanol extract | skimmianine (quinoline alkaloid) | carrageenan induced rat paw edema | male Wistar rats | anti-inflammatory | [72] |
| | leaves/methanol extract | _ | carrageenan induced rat paw edema | albino rats | anti-inflammatory | [47] |
| *Allium cepa* | bulbs/aqueous extract | _ | evaluation of vascular inflammation in FFR | fructose-fed male Wistar rats | anti-inflammatory | [100] |
| | bulbs/methanol extract | _ | evaluation of pro-inflammatory cytokines | atypical prostatic hyperplasia induced Wistar rats | immunomodulatory and anti-inflammatory | [17] |
| *Allium sativum* | bulbs/aqueous extract | _ | evaluation of vascular inflammation in FFR | fructose-fed male Wistar rats | anti-inflammatory | [100] |
| | bulbs/aqueous extract | _ | determination of tumor necrosis factor-alpha gene and protein expression- Griess method | diabetic male Wistar rats | anti-inflammatory | [56] |
| | aged garlic extract | _ | western blotting | male ApoE-KO mice | anti-inflammatory | [54] |

**Table 21.3** Recent *in vitro* studies on medicinal plants mentioned in Canon for treatment of alopecia

| Plant | Part/extract | Active constituent | Assay | Effect | Ref. |
|---|---|---|---|---|---|
| *Allium cepa* | Bulbs | Quercetin | induced osteoclast genesis in RAW 264.7 | anti- inflammatory: ↓NF-κB activation, ↓ IL-1α and IL-6, ↑ IL-3 and IL-4 | [58] |
| | bulbs/essential oil | _ | inhibition of bovine serum albumin denaturation (Anti-denaturation assay) | anti- inflammatory | [18] |
| | bulbs/aqueous extract | onion lectin | RAW264.7 and rat PECs (peritoneal exudates cells) | immunomodulatory: inducing Th1 immune response, ↑IFN-γ and IL-2, ↑NO | [64] |
| | sprouts/aqueous and dichloromethane extract | _ | inhibition of 15-lipoxygenase (LISA Kit) | anti- inflammatory | [94] |
| *Allium sativum* | bulbs/essential oil | _ | inhibition of bovine serum albumin denaturation (Anti-denaturation assay) | anti- inflammatory | [18] |
| | bulbs/aqueous extract | _ | lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages | anti- inflammatory: ↓NO production, ↓iNOS level and activity | [98] |
| *Semecarpus anacardium* | seeds/ethanol extract | _ | peripheral blood and synovial fluid mononuclear cells of healthy individuals and rheumatoid arthritis patients | anti- inflammatory and immunomodulatory | [90] |
| | seeds/, ethyl acetate and methanol extracts | etrahydroamentoflavone (THA) | *in vitro* COX-1 catalyzed prostaglandin biosynthesis assay | inhibition of COX-1 and COX-2 | [83] |
| | stem bark/ethyl acetate extract | butein and 7,3′,4′-trihydroxyflavone | *In vitro* COX-1 and COX-2 catalyzed prostaglandin biosynthesis assay | inhibition of COX-1 and COX-2 | [84] |

(continued)

**Table 21.3** (continued)

| Plant | Part/extract | Active constituent | Assay | Effect | Ref. |
|---|---|---|---|---|---|
| *Apium graveolens* | leaves/ethanol/water 1:1 | apiin | | inhibition of NO release and iNOS expression | [51] |
| | stem and leaves/aqueous extracts | flavonoids (quercetin, rutin) and coumarins (bergapten, isopimpinellin, xanthotoxin) | lymphocyte transformation, ELISA assay and flow cytometry on human peripheral blood mononuclear cells (PBMC) | immunomodulatory | [12] |
| *Artemisia absinthium* | whole plant/methanol extract | p7f (a tetramethoxy-hydroxyflavone) | LPS-stimulated RAW 264.7 macrophages | anti- inflammatory: inhibition of NF-κB activity and the expression or production of pro-inflammatory mediators such as COX-2/PGE(23) and iNOS/NO. | [46] |
| | aerial parts/essential oil | _ | soybean 5-lipoxygenase (5-LOX) enzyme inhibition | anti-inflammatory: 5- lipoxygenase Inhibition | [88] |
| | flowers | cardamonin | THP-1 monocytes, RAW264.7 macrophages | anti-inflammatory: ↓TNFα and NO synthesis | [25] |
| *Raphanus raphanistrum* | seeds/80% aqueous methanolic extract | 4-methylthio-butanyl derivatives | LPS-stimulated murine microglia BV2 cells | anti- inflammatory: ↓ NO levels | [38] |
| | seeds/aqueous extract | sinapic acid | LPS-stimulated RAW264.7 macrophages | anti- inflammatory: ↓ inflammatory mediators and inhibition of NF-κB activity | [40] |
| *Phragmites australis* | leaves/aqueous extract | _ | LPS-stimulated RAW264.7 macrophage | anti- inflammatory: ↓ NO, TNF-α and IL-β | [106] |
| *Adiantum capillus-veneris* | fronds/ethanol extract | _ | TNF-α- induced inflammation in HepG2 and HEK293 cell line | anti- inflammatory: inhibition of NF-κB activity | [105] |
| *Agrimonia eupatoria* | aerial parts/infusion and ethyl acetate fraction | _ | LPS-stimulated RAW264.7 macrophages | anti- inflammatory: ↓ NO | [78] |
| *Delphinium staphisagria* | seeds/traditional preparation | _ | matrigel capillary assay in Human Umbilical Vein Endothelial Cells (HUVECs) | angiogenenic | [41] |

**Table 21.4** Clinical studies on medicinal plants mentioned in Canon for treatment of alopecia

| Plant | Treatment group | Control group | Study design | Number of patients | Treatment duration | Result | Ref. |
|---|---|---|---|---|---|---|---|
| *Agrimonia eupatoria* | aerial parts tea | no preparation | uncontrolled pilot clinical trial | 19 | 30 days | ↓serum IL-6 | [24] |
| *Allium sativum* | aged garlic extract | placebo capsules | parallel, double-blind, placebo-controlled, randomized clinical trial | 51 | 6 weeks | ↓ IL-6 and TNF-α | [101] |
| | 5% garlic gel in combination with betamethasone cream | placebo gel in combination with betamethasone cream | randomized, double-blind, controlled clinical trial | 40 | 3 months | hair re-growth | [24] |
| *Allium cepa* | crude onion juice | tap water | single blind, placebo-controlled clinical study | 62 | 2 months | hair re-growth | [98] |

### 21.5.2 Beta vulgaris

The betalain-rich dye and different extracts of *B. vulgaris* provoked prominent anti-inflammatory effects in different animal models by reducing pro-inflammatory cytokines [16, 30, 50]. The *in vivo* immunomodulatory activity of the methanol extract was also comparable to that of levamisole [96].

Canon: The decoction or extract of beet leaves have been considered useful for hair loss. Avicenna has suggested to wash the affected organs with sodium nitrate before using a plaster of the herb [28].

### 21.5.3 Allium cepa

The application of crude onion was tested on patchy AA in comparison with tap water. The results showed hair re-growth in 86.9% patients [89]. It has been suggested that the presence of some minerals such as zinc and iron helps in maintaining the hair health [35]. The onion juice has also demonstrated anti-dandruff properties [33].

The essential oil, bulb extract and quercetin which constitutes 2.5% of the onion extract, showed positive effects on inhibiting the inflammation [18, 58]. The onion lectin had immunomodulatory properties by inducing Th1 immune response *in vitro* [64]. Moreover, the onion sprouts volatile and water extracts provoked prominent anti-inflammatory effect by inhibiting hydro peroxide production [94]. In rats, the aqueous and methanol extracts of bulbs reduced inflammation by various mechanisms [17, 100].

### 21.5.4 Allium sativum

The adjuvant therapy of topical garlic gel and betamethasone valerate cream has resulted in a significant response in the AA treated patients after 3 months [24]. The aged garlic extract could decrease inflammation cytokines in rats and in adults with obesity [54, 101].

The bulbs essential oil and aqueous extract have been determined to possess anti-inflammatory properties by various mechanisms in animals and cell cultures [18, 56, 100].

Canon: The mixture of ash of garlic dispensed in honey may help in the treatment of alopecia [28].

### 21.5.5 Semecarpus anacardium

The plant has been used traditionally in India as a hair growth promoter [85]. Tetrahydroamentoflavone, the major bioactive compound from the seeds, as well as different

extracts of *S. anacardium*, exhibited cyclooxygenase inhibitory and dose-dependent anti-inflammatory activity [83]. Moreover, butein and 7,3′,4′-trihydroxyflavone, two bioactive molecules isolated from the stem bark, exhibited selective COX-1 and moderate COX-2 inhibitory effects [84].

The crude ethanol extract of the seeds inhibited the production of pro-inflammatory cytokines (IL-1β and IL-12p40) in healthy and rheumatoid arthritis patients and suppressed the nitric oxide production in mouse macrophage cell line [90].

The traditionally prepared nut milk extract of *S. anacardium* as well as its nut chloroform extract, have revealed anti-inflammatory and immunomodulatory activities both in both animal and human studies [37, 65, 69, 70, 79].

### 21.5.6 Drimia maritima

A mixture of several plants including *D. maritima* and *Adiantum capillus-veneris* has been patented (patent number: US20070110705A1) to be used against excessive sebum and hair fall and also for promoting hair growth [49]. To be concerned, in 2007, Polat et al. claimed a case report of contact dermatitis with topical administration of *Urginea maritima* (Synonym for *D. maritima*) [62].

Canon: Due to caustic characteristic, *D. maritima* has been suggested to be painted or massaged in a mixture of olive oil, pine resin or honey, when used topically [28].

### 21.5.7 Apium graveolens

The aerial parts of *A. graveolens* have revealed a higher inhibitory activity on inflammation than acetylsalicylic acid [3]. The leaves extract has exhibited a significant and dose-dependent anti-inflammatory activity which was confirmed by the inhibition of NO release and iNOS expression due to the presence of polyphenol constituent, apiin [51]. The ethanol extract was also able to suppress chronic inflammation [6]. Apiuman, a pectic polysaccharide from the stalks of *A. graveolens*, has exhibited anti-inflammatory effects by reducing proinflammatory and increasing anti-inflammatory cytokines, as well as attenuating neutrophil migration to the affected area [60].

In addition, immunomodulatory activities of celery together with some of its known flavonoids and coumarins were reported by Cherng et al. [12] [12].

### 21.5.8 Adiantum capillus-veneris

Among the crude ethanol extract fractions of the plant, the ethyl acetate fraction possessed the best inflammation inhibition, comparable to that of Indomethacin [22]. Furthermore, two triterpenoids, capillirone and 4α-hydroxyfilican-3-one, yielded in this plant, also showed similar efficiency [23]. The ethanol extract of the fronds also showed anti-inflammation effects both *in vitro* and *in vivo* by inhibition the NF-κB pathway and inflammatory cytokines production, respectively [105]. Additionally, topical administration of 1% *A. capillus-veneris* on testosterone-induced alopecia in male albino mice prevented the manifestation of hair loss. Thus, it could be suggested as a potential preparation for AGA [57].

Canon: Application of ashes of *A. capillus-veneris* in vinegar and olive oil benefits alopecia while in the mixture with the oil of myrtle and wine, lengthens the hair and prevents hair fall [28].

### 21.5.9 Pilosella officinarum (syn: *Hieracium pilosella*)

Little evidence was found in *Pilosella officinarum*. However, an old study has shown the anti-inflammatory activity of *H. pilosella* in rats [9].

Canon: The combination of the herb with vinegar is useful for alopecia [28].

### 21.5.10 Artemisia absinthium

The anti-inflammatory properties for the aqueous extract and the essential oil of the aerial parts of *A. absinthium* have been reported [21, 88]. The immunomodulatory activity of the aqueous extract was comparable to that of silymarin in

hepatic injury [4]. Furthermore, *A. absinthium* could decrease inflammatory cytokines and testosterone in rats with polycystic ovary syndrome [77]. Methanol extract exhibited a significant anti-inflammatory activity at the dose of 1000 mg/kg in carrageenan induced paw edema in albino mice [2] also inhibited the venom-induced inflammation in rats, preferably at dose of 37.5μg/kg [55]. Cardamonin and P7F (a flavonoid) isolated from *A. absinthium* inhibited NO and TNFα production [25] also NF-κB, COX-2 and iNOS/NO activity [45].

### 21.5.11 Ferula assa-foetida

The oleogumresin has been found to have anti-inflammatory effect by inhibition of 15- lipoxygenase. This activity can be due to the presence of terpenoids [7].

Canon: A liquid poultice comprises *F. assa-foetida*, vinegar and pepper, can be prepared in the case of alopecia [28].

### 21.5.12 Narcissus tazetta

A glutamine rich peptide, called nartazine isolated from the bulbs of *N. tazetta*, was reported to have immunomodulatory effects through mitogenic response in murine splenocytes, anti-proliferative activity in leukemia cells and proliferative activity in bone marrow cells [13].

Canon: The roots are suggested for alopecia that may be contributed to the mentioned effects [28].

### 21.5.13 Artemisia abrotanum

The immunomodulatory activity of *A. abrotanum* was reported in humoral and cellular immunity [31].

Canon: The application of the burnt plant in castor or radish oils has been mentioned for alopecia. The herb decoction in warming and deobstruent oils has been reported to increases the growth of beard [28].

### 21.5.14 Brassica nigra

Despite the lack of studies on anti-inflammatory and immunomodulatory activity of *B. nigra*, a recent research on sinigrin, mainly found in *B. nigra* seeds, has proved its ability to suppress inflammation by inhibition of COX-2 and PGE2 expression and IL-1β and IL-18 production [46].

### 21.5.15 Raphanus raphanistrum (syn: *Raphanus sativus*)

Among the 4-methylthio-butanyl derivatives isolated from the seeds of the plant, sinapoyl desulfoglucoraphenin decreased NO production with $IC_{50}$ of 45.36μM *in vitro* [38]. Moreover, sinapic acid has been identified as the main constituent responsible for the anti- inflammation activity of the seeds [40].

Canon: When mixed with the flour of darnel (*Lolium temulentum*), *R. raphanistrum* improves the growth of hair [28].

### 21.5.16 Phragmites australis

The aqueous extract of leaves has shown *in vitro* anti-inflammatory effects [106].

Canon: The barks and roots are suggested for alopecia which also act as detergent [28].

### 21.5.17 Agrimonia eupatoria

In a clinical trial on daily tea consumption of *A. eupatoria* for 1 month, the outcomes showed a significant reduction in serum levels of IL-6 [29]. Moreover, both the infusion and the ethyl acetate fraction exhibited anti-inflammatory activity *in vitro* and *in vivo* [78].

Canon: When used in the treatment of initial stages of alopecia, *A. eupatoria* might come in useful [28].

### 21.5.18 Delphinium Staphisagria

In a study, the seed extract of *D. staphisagria* significantly induced angiogenesis in HUVECs, which was considered as a mechanism for its hair growth effect [41].

Canon: *Delphinium staphisagria* should be painted locally for chronic alopecia [28].

### 21.5.19 Ruta graveolens

The anti-inflammatory effects of methanol extract and skimmianine, isolated from the aerial parts of *R. graveolens*, have been proved [47, 72].

## 21.6 Concluding Remarks

Traditional and folklore medicine have always been great sources for discovery of new medications. Time-honored medicinal ITM books such as Canon are valuable treasures and can act as exemplify for modern pharmaceutical world. In Canon a wide variety of medicinal plants with various pharmacological and therapeutic effects are described. In this review the treatments regarding alopecia, the condition of losing hair, are elicited from Canon and their possible mechanisms are evaluated. The remedies are natural based formulations, which are mostly medicinal plants and herbal mixtures. Among the 25 plants mentioned in Canon, some possible scientific evidences were found for 18. Different mechanisms of action could be considered for these medicinal plants including anti-inflammatory, immunomodulatory and angiogenic activities. Similar molecular pathways might also be the therapeutic cause for un-investigated plants. Since most of the inspected plants have shown anti-inflammatory activity, this effect can be considered as the major mechanism for prevention or treatment of alopecia.

All in all, investigating the enriched ITM books can lead to discovery of practical natural medications for treatment of various disorders such as alopecia. However, work on the underling mechanisms of anti-hair loss effect of all the surveyed plants, the synergism between components, the possible main active constituents and the efficacy and safety of these treatments may further elucidate the use of the mentioned remedies in Canon.

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# 22

# Ethnobotanical Uses, Phytochemistry and Pharmacology of Different *Rheum* Species (Polygonaceae): A Review

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**Abstract**

Today, there is an increased tendency to use herbal remedies. Rhubarb refers to several species of the genus *Rheum* L. in the Polygonaceae family. This species-rich genus is mainly distributed in Asian countries. Several medicinal effects have been attributed to the *Rheum* spp. in the traditional and modern medicine such as healing lungs, liver, kidney, womb and bladder diseases, cancer, diabetes, insect bites, relapsing fevers, diarrhea and constipation. Various *in vitro*, *in vivo* and clinical studies have investigated the therapeutic effect of extracts, fractions and pure compounds isolated from different species of this genus. Considering the positive findings, several pharmaceutical formulations contain-

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ing rhubarb extract like capsules, drops, mouthwashes and different topical formulations are now present in the market. However, there are other traditional therapeutic effects of rhubarb that have not been studied yet and it is of great importance to perform confirmatory experiments or clinical investigations. The current review summarizes general information regarding botany, phytochemistry, ethnobotany and pharmacological aspects of *Rheum* spp. It is hoped that the present review could motivate subsequent research on the other medicinal properties of these plants that have been neglected until today.

**Keywords**

Rhubarb · *Rheum* · Polygonaceae · Traditional medicine · Ethnobotany

## 22.1 Introduction

*Rheum* spp.(Polygonaceae) as one of the oldest and frequently used herbal medicines is mainly distributed in the Asian countries such as China, India, Nepal, Korea, Bhutan, Pakistan, Turkey, Iran, Russia and Tibet [1–3]. In the Islamic traditional medicine (ITM), various medicinal effects have been attributed to the *Rheum* genus such as healing liver, kidney, womb and bladder diseases, hiccups, diarrhea, constipation, insect bites and relapsing fevers [4, 5]. Several phytochemical studies have demonstrated that the main structures present in different species of this genus are anthraquinones, anthrones and different phenolic compounds such as stilbenes, flavonoids and tannins. These compounds have demonstrated a wide range of pharmacological activities including purgative [6], anti-cancer [7], anti-diabetes [8], anti-oxidative [9], hepatoprotective [10] and nephroprotective [11] in *in vitro*, *in vivo* and clinical studies. In the current chapter we present a general report on botany, phytochemistry, ethnobotany and pharmacological activities of *Rheum* genus.

## 22.2 Botany

### 22.2.1 Botanical Description

*Rheum* L. (Polygonaceae) is a species-rich genus, comprises a total of 44 accepted species (The Plant List, 2013). *Rheum* species, commonly called rhubarb, includes perennial, stout herbs with procumbent to erect basal leaves and heights range from the procumbent (*R. palaestinum*) to 2 m tall (*R. palmatum* and *R. webbianum*). The mountainous and desert regions of the Qinghai–Tibetan Plateau area (the highest and largest plateau in the world) and adjacent areas of central Asia are putatively centers of both origin and diversification of *Rheum*, owing to its extremely diversified morphology and high endemism at both species and section level [12]. It is suggested that the rich geological and ecological diversity of these regions, coupled with the habitat isolation due to oscillating climatic conditions during and after the uplifts of the plateau, might have caused the fast radiation and speciation of *Rheum* [13]. Furthermore, it seems that polyploidy have played an important role in driving diversification and speciation of this genus. More than 50% of taxonomically circumscribed species in *Rheum* taxa were involved in the polyploidy during their diversification histories [14]. The taxonomy of this genus remains complex, due to the convergent evolution and random fixation of unique morphological characters, which might explain the substantial inconsistencies among gross morphology, pollen exine pattern and trnL-F phylogeny within it [13]. The main habitats preferred by most *Rheum* taxa are cold and dry alpine meadow, steppe desert and dry slopes [15].

*Rheum* species are perennial plants, possess roots long, stout. Stem erect, hollow, sulcate, glabrous or strigose. Leaves basal and cauline, simple, sinuatedentate or palmate, the basal ones sparse, dense, or in a rosette, larger than the alternate cauline leaves, the latter sometimes lacking, ocrea usually large, membranous, margin entire, inflorescence simple or branched, usually paniculate, or spike-like or spherical, pedicel articulate, flowers bisexual or polygamo-monoecious, perianth persistent, tepals 6, stamens mostly 9

(6 + 3), rarely 7 or 8, styles 3, short, horizontal, stigmas inflated, recurved, achenes trigonous, winged [16].

According to the website 'TPL' (http://www.theplantlist.org.), there are 121 scientific plant names of species rank for the genus *Rheum*, of these 44 are accepted species names. Table 22.1 summarizes all synonyms of *Rheum* taxa.

### 22.2.2 Authentication and Detection of Adulteration

Because of some morphological similarities of the plants and their misidentification by the vendors and consumers, the crude medicinal plants are often substituted or adulterated in commerce which may lead to poor clinical efficacy and adverse effects. In Iran, a number of *Rheum* taxa are traded in traditional medicine markets and shops such as *R. palmatum, R. ribes* and *R. turkestanicum*. Taxonomic assessment revealed that some of them should be considered as adulterated and substituted samples. For instance, *R. ribes* are admixed with *R. turkestanicum* and are sold in the market which degrades its quality and efficacy [17, 18]. In various parts of India, *R. emodi* are adulterated with *R. webbianum* and *R. Spiciforme* [19]. In Chinese markets, many adulterants include *R. franzenbachii*, *R. undulatum*, *R. rhaponticum*, *Rumex crispus*, and *R. dentatus* are commonly admixed with official Da-huang (the dried rhizomes and roots of *R. palmatum*, *R. tanguticum*, and *R. officinale*), because of similar morphological traits. The results of several previous studies have shown significant differences in the chemical composition of rhubarbs, and it is recommended that clinical practice should be performed for each species individually [20]. Moreover, due to the increasing demand both domestically and internationally and the short supply of official rhubarb, some *Rheum* taxa, like *R. hotaoense* has also been used as commercial substitutes in certain regions [21]. Therefore, it is essential to provide an authentic tool for realizing the distinction between different *Rheum* species and their adulterants. **Authentication** of these crude medicinal plants

**Table 22.1** Scientific names and synonym(s) of reported *Rheum* species worldwide [according to The Plant List (2013)]

| No | *Rheum* species (Accepted names) | Synonyms |
|---|---|---|
| 1 | *Rheum acuminatum* Hook. f. & Thomson | *Rheum orientalixizangense* Y.K. Yang, J.K. Wu & Gasang. |
| 2 | *Rheum alexandrae* Batalin | – |
| 3 | *Rheum altaicum* Losinsk. | *Rheum rhaponticum* Herder |
| 4 | *Rheum australe* D. Don | *Rheum emodi* Wall. ex Meisn. |
| 5 | *Rheum compactum* L. | *Rheum nutans* Pall. |
| | | *Rheum orientale* Losinsk. |
| 6 | *Rheum delavayi* Franch. | *Rheum strictum* Franch. |
| 7 | *Rheum forrestii* Diels | – |
| 8 | *Rheum glabricaule* Sam. | – |
| 9 | *Rheum globulosum* Gage | – |
| 10 | *Rheum hotaoense* C.Y. Cheng & T.C. Kao | – |
| 11 | *Rheum* × *hybridum* Murray | – |
| 12 | *Rheum inopinatum* Prain | – |
| 13 | *Rheum kialense* Franch. | *Rheum micranthum* Sam. |
| 14 | *Rheum laciniatum* Prain | |
| 15 | *Rheum lhasaense* A.J. Li & P.G. Xiao | |
| 16 | *Rheum likiangense* Sam. | *Rheum ovatum* C.Y. Cheng & T.C. Kao |
| 17 | *Rheum lucidum* Losinsk. | *Rheum korshinskyi* Titov ex Losinsk. |
| 18 | *Rheum macrocarpum* Losinsk. | *Rheum ferganense* Titov |
| | | *Rheum lobatum* Litv. ex Losinsk. |
| | | *Rheum nuratavicum* Titov |
| | | *Rheum plicatum* Losinsk. |
| | | *Rheum vvedenskyi* Sumner |
| | | *Rheum zergericum* Titov |
| 19 | *Rheum maculatum* C.Y. Cheng & T.C. Kao | – |

(continued)

**Table 22.1** (continued)

| No | *Rheum* species (Accepted names) | Synonyms |
|---|---|---|
| 20 | *Rheum moorcroftianum* Royle | – |
| 21 | *Rheum nanum* Siev. ex Pall**.** | *Rheum cruentum* Siev. ex Pall. |
| | | *Rheum leucorrhizum* Pall. |
| 22 | *Rheum nobile* Hook. f. & Thomson | – |
| 23 | *Rheum officinale* Baill. | – |
| 24 | *Rheum palmatum* L. | *Rheum potaninii* Losinsk. |
| | | *Rheum qinlingense* Y.K.Yang, D.K.Zhang & J.K.Wu |
| 25 | *Rheum przewalskyi* Losinsk. | – |
| 26 | *Rheum pumilum* Maxim. | – |
| 27 | *Rheum racemiferum* Maxim. | – |
| 28 | *Rheum reticulatum* Losinsk. | – |
| 29 | *Rheum rhabarbarum* L. | *Rheum franzenbachii* Münter |
| | | *Rheum franzenbachii* var. *mongolicum* Münter |
| | | *Rheum undulalum* L. |
| | | *Rheum undulatum* var. *longifolium* C.Y. Cheng & T.C. Kao |
| 30 | *Rheum rhaponticum* **L.** | |
| 31 | *Rheum rhizostachyum* Schrenk | *Rheum aplostachyum* Kar. & Kir. |
| 32 | *Rheum rhomboideum* Losinsk. | – |
| 33 | *Rheum ribes* L. | – |
| 34 | *Rheum spiciforme* Royle | *Rheum scaberrimum* Lingelsh. |
| 35 | *Rheum subacaule* Sam. | – |
| 36 | *Rheum sublanceolatum* C.Y. Cheng & T.C. Kao | – |
| 37 | *Rheum tanguticum* Maxim. ex Balf. | *Rheum palmatum* subsp. *dissectum* Stapf |
| | | *Rheum palmatum* f. *rubiflora* Stapf |
| | | *Rheum tanguticum* var. *viridiflorum* Y.K. Yang & D.K. Zhang |
| 38 | *Rheum tataricum* L.f. | *Rheum caspicum* Pall. |
| | | *Rheum songaricum* Schrenk |
| 39 | *Rheum tibeticum* Maxim. ex Hook. f. | – |
| 40 | *Rheum turkestanicum* Janisch. | *Rheum megalophyllum* Sumner |
| | | *Rheum renifolium* Sumner |
| | | *Rheum rupestre* Litv. ex Losinsk. |
| | | *Rheum turanicum* Litv. |
| 41 | *Rheum uninerve* Maxim. | – |
| 42 | *Rheum webbianum* Royle | – |
| 43 | *Rheum wittrockii* C.E. Lundstr. | – |
| 44 | *Rheum yunnanense* Sam. | – |

is very necessary since without correct identification, the efficacy and safety of products cannot be guaranteed.

### 22.2.3 Threat Categorization and Conservation Prioritization

Several ***Rheum*** species, particularly from Kashmir Himalaya are under tremendous risk and have been considered as "threatened" by several agencies such as International Union for Conservation of Nature (IUCN), United Nations Environment Programme (UNEP) and World Wide Fund for Nature (WWF) [22]. Multiple factors such as habitat loss and extensive collection from the wild have caused a significant decline in the natural resources of them. In India, various

sub-endemic plant taxa belonging to the genus *Rheum*, were identified as threatened including *R. moorcroftianum* "critically endangered", *R. webbianum* "Endangered" and *R. australe* "vulnerable", due to a critical decrease in their population [23]. *R. wittrockii* is an endangered and rare species that grows in Kazakhstan. It is a very useful medicinal plant and is being used for cooking in a variety of dishes [24]. Some *Rheum* species, such as *R. alexandrae* and *R. nobile* are monocarpic perennial species, meaning that they only produce flowers once in a lifetime and are only reproduced through their seeds. These plants are "potentially endangered" and overexploitation of their wild resources should be forbidden [25]. Both of these rare noteworthy taxa-endemic to the high eastern Himalayas-are being used in traditional Tibetan medicine [26, 27]. Three other *Rheum* species including *R. tanguticum, R. officinale* and *R. palmatum* are endemic and "endangered" plant species that grow in China. In recent years, due to the overutilization and the loss of habitat of *R. tanguticum*, it has been named as "endangered" in the China higher plants endangered list [28, 29]. Moreover, *R. palmatum* and *R. officinale* wild resources have decreased due to a decrement in *R. tanguticum* natural resources, thus, both are considered as "threatened" taxa in China [30]. There is a strong need for conservation priorities and management strategies of such valuable *Rheum* gene pool through establishment of herbal gardens and medicinal plants nurseries for *ex situ* conservation, coupled with education and awareness programs for large-scale cultivation [23]. If overharvesting and habitat destruction of these valuable species continues, they may vanish from the area within a few years.

## 22.3 Phytochemical Constituents

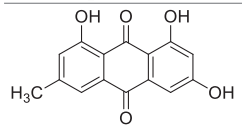
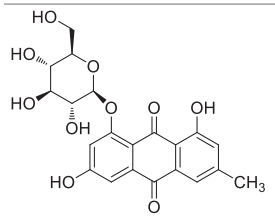
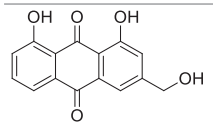
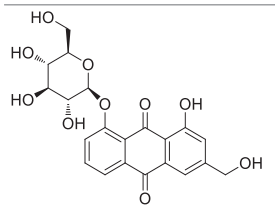
According to previous studies, the most important chemical structures from *Rheum* taxa are anthraquinones, anthrones and phenolic compounds (stilbenes, flavonoids, phenolic glycosides, phenolic acids, cinnamic acid derivatives and tannins). These compounds have been classified in Table 22.2.

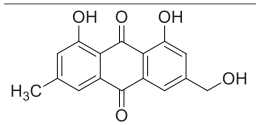
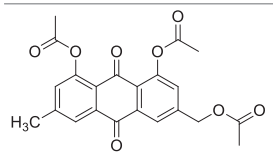
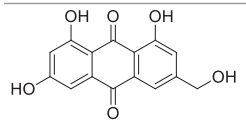
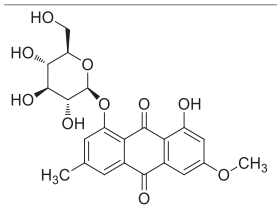
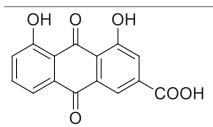
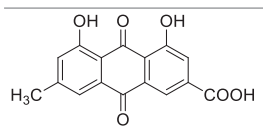
## 22.4 Ethnobotanical and Ethnomedicinal Knowledge

The traditional uses of *Rheum* species in **ethnomedicine** mainly originate from Asia and Europe. **25** species of *Rheum* have been reported to be beneficial among which *R. australe*, *R. palmatum*, *R. ribes* and *R. webbianum* have the highest number of citations in the world. Different parts of *Rheum* taxa including roots, petioles, fruits and seeds have been used as **ethnomedicine** for a long time. There are some reports on the traditional uses of *R. palmatum* and ***R. rhaponticum*** in European countries, particularly Bulgaria and Spain. In these countries, the roots are the most used part for treating fever, heart problems, stomachache and jaundice [60, 61]. Stems of *R. rhabarbarum* commonly known as "Rhabarber" have been recommended as depurative in Germany [62]. In Kazakhstan, *R. wittrockii* was used by Kazakhs against gastro-enteric and skin **ailments**. Moreover, *R. altaicum* was advised as an anti-inflammatory and for treating skin problems. Stalks of *R. compactum* and *R. wittrockii* were eaten by local people [63].

China represents most of the distribution range of the *Rheum* taxa in the world [16]. Many species of this genus are used in **traditional** Chinese **medicine** and many reports are found highlighting their traditional and ethnomedicinal applications. Among them, *R. officinale, R. palmatum* and *R. tanguticum* are the official rhubarbs. In Chinese markets, dried roots and rhizomes of *R. tanguticum* and *R. palmatum* are called "north rhubarb", while that of *R. officinale* are called "south rhubarb" [28, 29]. These taxa are known for their purgative, anti-bacterial, astringent, anti-carcinogenic, and stomachic properties [64]. Further, the roots and rhizomes of *R. palmatum*-commonly known as Zhang Ye Da Huang or Chinese rhubarb-have been recommended to treat abdominal distension, constipation and stomach pain [65]. Also, t**he roots of** *R. officinale,* known as Da Huang, have been recognized as a wound healing agent and purgative [66, 67]. Leaf petioles of *R. acuminatum, R. austral, R. globulosum, R. inopinatum, R. lhasaense,*

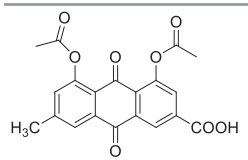
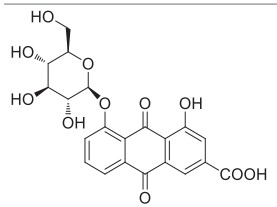
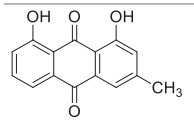
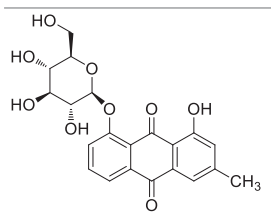
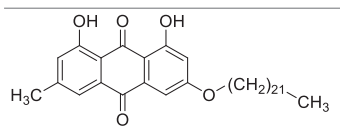
**Table 22.2** The most important chemical structures isolated from different parts of *Rheum* spp.

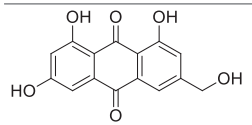
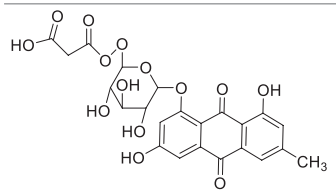
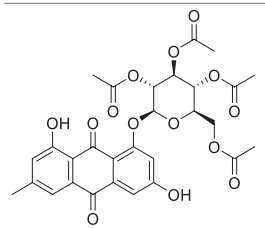
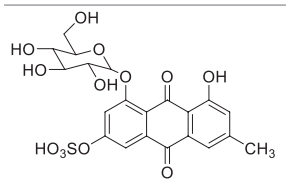
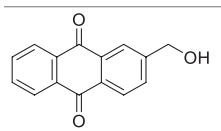
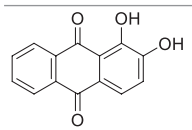
| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
| **Anthraquinone derivatives** | | | | |
|  | Emodin | *R. nobile* | Rhizomes | [31] |
| | | *R. emodi* | Roots and rhizomes | [32–34] |
| | | *R. acuminatum* | Roots | [32] |
| | | *R. palmatum* | Rhubarb powder | [32, 35] |
| | | *R. spiciforme* | Different plant parts | [36] |
| | | *R. webbianum* | Different plant parts | [36] |
| | | *R. tanguticum* | – | [32] |
|  | Emodin-8-O-$\beta$-D- glucopyranoside (emodin 8-glucoside) | *R. nobile* | Rhizomes | [31] |
| | | *R. emodi* | Rhizomes, roots and rootstalks | [20, 33, 37–39] |
| | | *R. palmatum* | Roots | [20] |
| | | *R. tanguticum* | Roots | [20] |
| | | *R. officinale* | Roots | [20] |
| | | *R. franzenbachii* | Roots | [20] |
| | | *R. hotaoense* | Roots | [20] |
| | | *R. emodi* | Rhizomes | [33, 34] |
| | | *R. franzenbachii* | Roots and rhizomes | [20, 40] |
|  | Aloe-emodin | *R. emodi* | Rhizomes | [41] |
| | | *R. spiciforme* | Different plant parts | [36] |
| | | *R. webbianum* | Different plant parts | [36] |
| | | *R. rhabarbarum* | Stalks | [42] |
| | | *R.palmatum* | – | [32, 43] |
| | | *R. acuminatum* | Roots | [32] |
| | | *R. tanguticum* | – | [32] |
|  | Aloe-emodin glucoside | – | Rhizomes | [40] |

| | | | | |
|---|---|---|---|---|
|  | 6-Methyl-aloe-emodin | *R. emodi* | Rhizomes | [39] |
|  | 6-Methyl-aloe-emodin-triacetate | *R. emodi* | Rhizomes | [39] |
|  | Physcion | *R. emodi* | Roots and rhizomes | [32, 33, 41] |
| | | *R. acuminatum* | – | [32] |
| | | *R. officinale* | Rhizomes, rhubarb samples | [32, 44, 45] |
| | | *R. tanguticum* | – | [32] |
| | | *R. palmatum* | – | [32, 43] |
|  | Physcion-8-O-$\beta$-D-glucopyranoside | *R. nobile* | – | [31] |
| | | *R. emodi* | Roots and rootstalks | [38] |
|  | Rhein | *R.emodi* | Roots and rhizomes | [32, 41] |
| | | *R.officinale* | Rhizomes, rhubarb samples | [32, 44, 45] |
| | | *R. tanguticum* | – | [32] |
| | | *R. palmatum* | – | [32, 43] |
| | | *R. spiciforme* | Different plant parts | [36] |
| | | *R. webbianum* | Different plant parts | [36] |
|  | 6-Methyl-rhein | *R. emodi* | Rhizomes | [39] |

(continued)

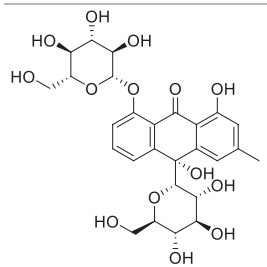
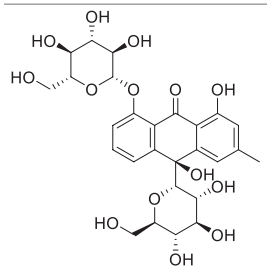
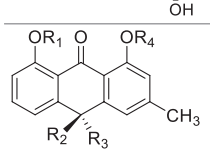
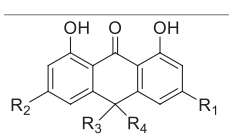
**Table 22.2** (continued)

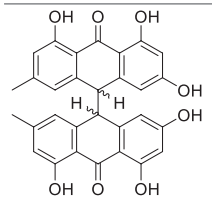
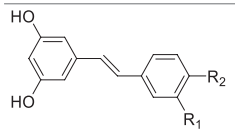
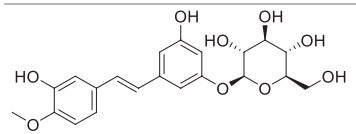
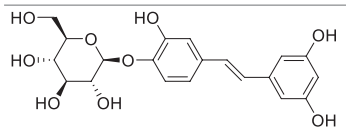
| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
|  | 6-Methyl-rhein-diacetate | *R. emodi* | Rhizomes | [39] |
|  | Rhein 8-O-glucoside | *R. officinale* | Roots | [20] |
|  | Chrysophanol | *R. nobile* | Rhizomes | [31] |
| | | *R. emodi* | Roots and rhizomes | [32–34] |
| | | *R. acuminatum* | Roots | [32] |
| | | *R. officinale* | Rhizomes, rhubarb samples | [32, 44, 45] |
| | | *R. palmatum* | – | [32, 43] |
| | | *R. tanguticum* | Rhizomes | [32, 44] |
| | | – | Rhizomes, rhubarb samples | [40, 46] |
| | Chrysophanic acid (chrysophanol) | – | Rhubarb samples | [46] |
|  | Chrysophanol glucoside | *R. emodi* | Roots and rhizomes | [20, 33, 34] |
| | | *R. officinale* | Roots | [20] |
| | | *R. palmatum* | Roots | [20] |
| | | *R. tanguticum* | Roots | [20] |
| | | *R. franzenbachii* | Roots | [20] |
| | | *R. hotaoense* | Roots | [20] |
| | Chrysophanol-8-O-$\beta$-D-glucopyranoside | *R. nobile* | Rhizomes | [31] |
|  | Revandchinone-3 | *R. emodi* | Rhizomes | [47] |

| | | | | |
|---|---|---|---|---|
|  | Citreorosein | – | – | [48] |
|  | Emodin 8-O-(6′-O-malonyl)-glucoside | *R. emodi* | Roots | [20] |
| | | *R. officinale* | Roots | [20] |
| | | *R. palmatum* | Roots | [20] |
| | | *R. tanguticum* | Roots | [20] |
| | | *R. franzenbachii* | Roots | [20] |
| | | *R. hotaoense* | Roots | [20] |
|  | Emodin 8-O-(2′, 3′, 4′, 6′-tetraacetyl)-glucoside | *R. emodi* | Roots | [49] |
|  | Emodin 8-O-$\beta$-D-glucopyranosyl-6-O-sulfate | *R. emodi* | Roots | [49] |
|  | 2-Hydroxymethyl anthraquinone | *R. rhabarbarum* | Stalks | [42] |
|  | Alizarin | *R. emodi* | Rhizomes | [50] |

(continued)

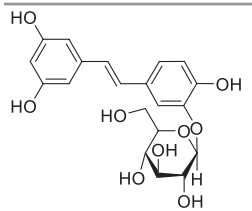
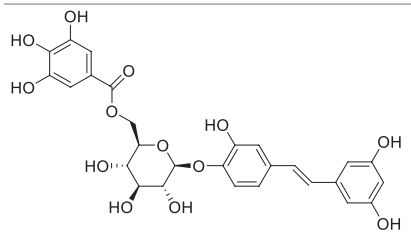
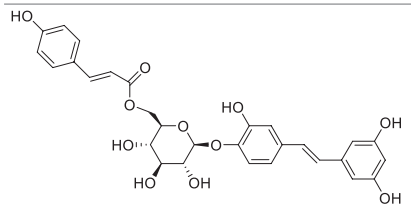
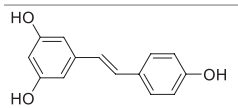
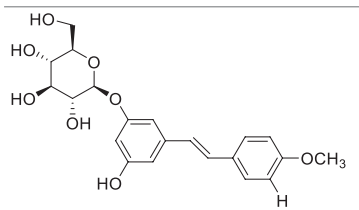
**Table 22.2** (continued)

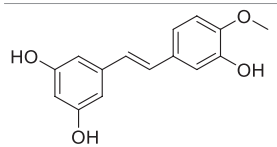
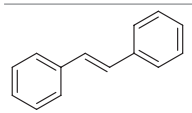
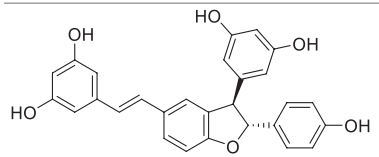
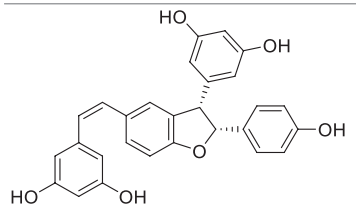
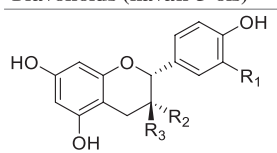
| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
| **Anthrone derivatives** | | | | |
|  | 10-Hydroxycascaroside C (anthrone C-glucosides) | *R. emodi* | Roots | [51] |
|  | 10-Hydroxycascaroside D | *R. emodi* | Roots | [51] |
|  | 10R-Chrysaloin1-O-$\beta$-D-glucopyranoside: $R_1$ = H, $R_2$ = H, $R_3$ = Glu, $R_4$ = Glu | *R. emodi* | Roots | [51] |
| | Cascaroside C: $R_1$ = Glu, $R_2$ = Glu, $R_3$ = H, $R_4$ = H | *R. emodi* | Roots | [51] |
| | Cascaroside D: $R_1$ = Glu, $R_2$ = H, $R_3$ = Glu, $R_4$ = H | *R. emodi* | Roots | [51] |
| | Cassialoin: $R_1$ = H, $R_2$ = OH, $R_3$ = Glu, $R_4$ = H | *R. emodi* | Roots | [51] |
|  | Revandchinone-1: $R_1$ = $CH_3$, $R_2$ = $OCH_3$, $R_3$ = H, $R_4$ = O-CO-$(CH_2)_7$-CH=CH-$(CH_2)_7$-$CH_3$ | *R. emodi* | Rhizomes | [47] |
| | Revandchinone-2: $R_1$ = $CH_3$, $R_2$ = H, $R_3$ = H, $R_4$ = O-CO-$(CH_2)_{26}$-$CH_3$ | *R. emodi* | Rhizomes | [47] |
| | Revandchinone-4: $R_1$ = $CH_2OH$, $R_2$ = OH, $R_3$ = OH, $R_4$ = O-$(CH_2)_{17}$-$CH_3$ | *R. emodi* | Rhizomes | [47] |

| | | | | |
|---|---|---|---|---|
|  | *trans*-Emodindianthrone: 10–10′ *trans*<br>*cis*-Emodindianthrone: 10–10′ *cis* | *R. nobile* | Rhizomes | [31] |
| **Stilbenes** | | | | |
|  | Piceatannol: $R_1$ = OH, $R_2$ = OH | *R. emodi* | Roots | [32, 37, 38] |
| | | *R. acuminatum* | Roots | [32] |
| | Piceatannol-4′-O-*β*-D-(6″-O-acetyl)-glucoside: $R_1$ = OH, $R_2$ = O-(6′-O-acetyl)-Glu | *R. nobile* | Rhizomes | [31] |
| | Piceatannol-4′-O-*β*-D-glucoside: $R_1$ = OH, $R_2$ = O-Glu | *R. nobile* | Rhizomes | [31] |
| | Resveratrol-4′-O-*β*-D-glucoside (resveratroloside): $R_1$ = H, $R_2$ = O-Glu | *R. nobile* | Rhizomes | [31] |
| | | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
| | Desoxyrhaponticin: $R_1$ = H, $R_2$ = $OCH_3$ | *R. emodi* | Rhizomes | [53] |
| | | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
|  | Rhaponticin | *R. rhabarbarum* | Stalks | [42] |
| | | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
|  | Piceatannol-4′-O-*β*-D-glucopyranoside | *R. emodi* | Roots, rhizomes, rootstalks | [37, 38] |

(continued)

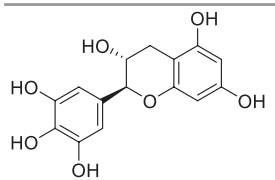
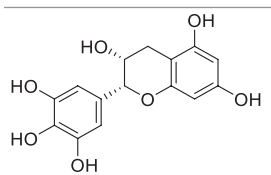
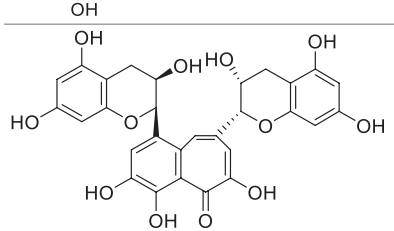
**Table 22.2** (continued)

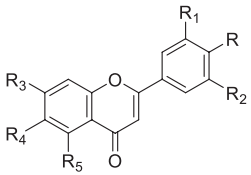
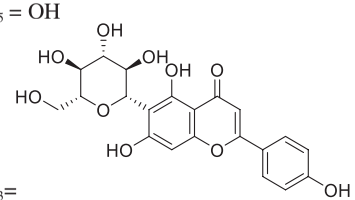
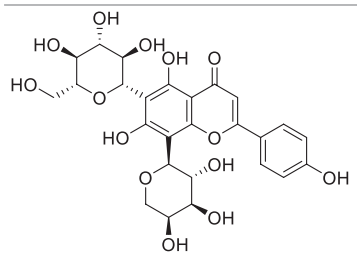
| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
|  | Piceatannol-3′-O-*β*-D-glucopyranoside | *R. emodi* | Roots and rootstalks | [38] |
| | | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
|  | Piceatannol-4′-O-*β*-D-(6″-O-galloyl)-glucopyranoside | *R. emodi* | Roots and rhizomes | [37] |
|  | Piceatannol-4′-O-*β*-D-(6″-O-*p*-coumaroyl)-glucopyranoside | *R. emodi* | Roots and rootstalks | [38] |
|  | Resveratrol | *R. australe* | Roots | [32] |
| | | *R. undulatum* | Rhizomes | [54] |
| | | *R. acuminatum* | Roots | [32] |
|  | Desoxyrhapontigenin | *R. emodi* | Rhizomes | [53] |
| | | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |

| | | | | |
|---|---|---|---|---|
|  | Rhapontigenin | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
|  | *trans*-Stilbene | *R. undulatum* | Rhizomes | [54] |
|  | Maximol A | *R. maximowiczii* | Roots | [55] |
|  | Maximol B | *R. maximowiczii* | Roots | [55] |
| **Flavonoids (flavan-3-ols)** | | | | |
|  | (+)-Catechin: $R_1$ = OH, $R_2$ = H, $R_3$ = OH | *R. nobile* | Rhizomes | [31] |
| | | *R. emodi* | Roots and rhizomes | [37] |
| | | *R. rhabarbarum* | Leaves, petioles, rhizomes | [52] |
| | | *R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
| | (−)-Epicatechin: $R_1$ = OH, $R_2$ = OH, $R_3$ = H | *R. nobile* | Rhizomes | [31] |
| | | *R. emodi* | Rhizomes | [53] |
| | (−)-Epicatechin-3-O-gallate: $R_1$ = OH, $R_2$ = O-Glu, $R_3$ = H | *R. nobile* | Rhizomes | [31] |
| | (−)-Epiafzelechin: $R_1$ = H, $R_2$ = OH, $R_3$ = H | *R. nobile* | Rhizomes | [31] |

(continued)

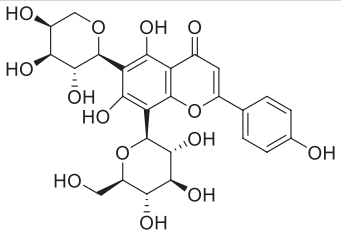
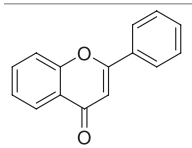
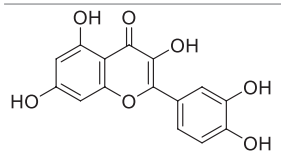
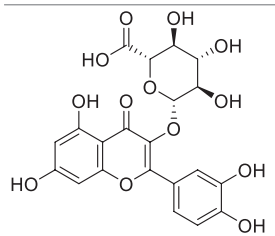
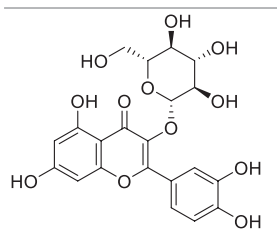
**Table 22.2** (continued)

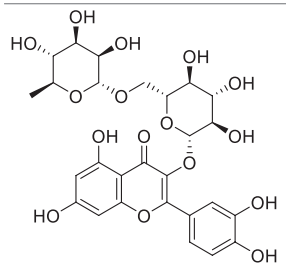
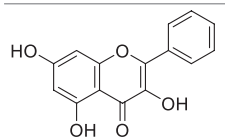
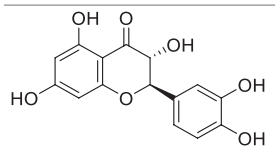
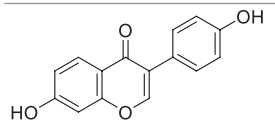
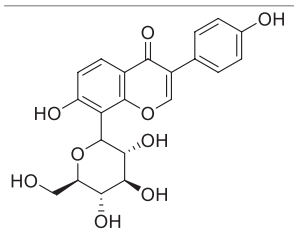
| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
|  | (−)-Gallocatechin | *R. emodi* | Rhizomes | [50] |
|  | (-)-Epigallocatechin | *R. emodi* | Rhizomes | [50] |
|  | Theaflavin | *R. emodi* | Rhizomes | [50] |

| **Flavonoids (flavones)** | | | | |
|---|---|---|---|---|
|  | 7,4′-dihydroxyflavone: R = OH, $R_1$ = H, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H | *R. emodi* | Rhizomes | [50] |
| | Chrysin: R = OH, $R_1$ = $OCH_3$, $R_2$ = $OCH_3$, $R_3$ = OH, $R_4$ = H, $R_5$ = OH | *R. emodi* | Rhizomes | [50] |
| | Genkwanin: R = OH, $R_1$ = H, $R_2$ = H, $R_3$ = $OCH_3$, $R_4$ = H, $R_5$ = OH | *R. emodi* | Rhizomes | [50] |
| | Luteolin: R = OH, $R_1$ = H, $R_2$ = OH, $R_3$ = OH, $R_4$ = H, $R_5$ = OH | *R. emodi* | Rhizomes | [50] |
| | Luteolin-3′, 7-di-O-glucoside: R = OH, $R_1$ = H, $R_2$ = O-D-Glu, $R_3$ = O-D-Glu, $R_4$ = H, $R_5$ = OH | *R. emodi* | Rhizomes | [50] |
| | Diosmetin-7-O-rhamnoside (diosmin): R = $OCH_3$, $R_1$ = H, $R_2$ = OH, $R_4$ = H, $R_5$ = OH  $R_3$= | *R. emodi* | Rhizomes | [50] |
| | Sinensetin: R = $OCH_3$, $R_1$ = H, $R_2$ = $OCH_3$, $R_3$ = $OCH_3$, $R_4$ = $OCH_3$, $R_5$ = $OCH_3$ | *R. emodi* | Rhizomes | [50] |
|  | Isovitexin | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |

(continued)

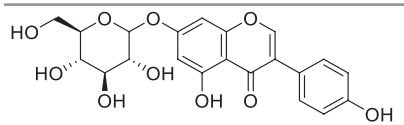
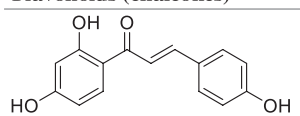
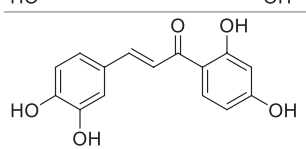
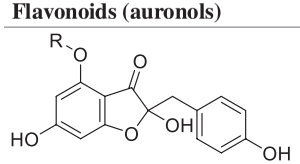
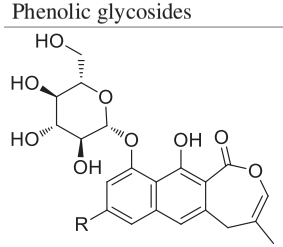
**Table 22.2** (continued)

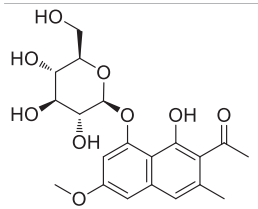
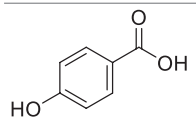
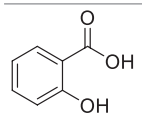
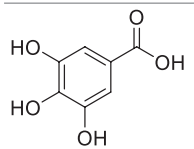
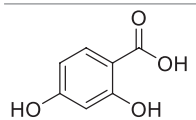
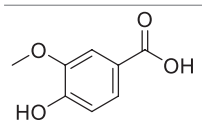
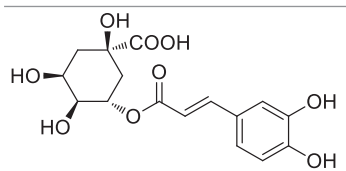
| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
|  | Schaftoside: 6-C-$\beta$-D-glucosyl-8-C-$\beta$-D-arabinosylapigenin | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
|  | Isoschaftoside: 6- C-$\beta$-D-arabinosyl-8-C-$\beta$-D-glycosylapigenin | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
| **Flavonoids (flavonols)** | | | | |
|  | Flavonol | *R. emodi* | Rhizomes | [50, 56] |
|  | Quercetin | *R. emodi* | Rhizomes | [56] |
| | | *R. tataricum* | Leaves and seeds | [57] |
|  | Miquelianin: (quercetin-3-O-glucuronide) | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |

| | | | | |
|---|---|---|---|---|
|  | Isoquercitrin | *R. rhabarbarum* | – | [42] |
|  | Rutin | *R. rhabarbarum R. rhaponticum* | Leaves, petioles, rhizomes | [52] |
|  | Galangin | *R. emodi* | Rhizomes | [50] |
| **Flavonoids (flavanonols)** | | | | |
| | (+)-Taxifolin | *R. emodi* | Rhizomes | [56] |
| **Flavonoids (isoflavones)** | | | | |
|  | Daidzein | *R. emodi* | Rhizomes | [56] |
|  | Puerarin: daidzein-8-O-glucoside | *R. emodi* | Rhizomes | [50, 56] |

(continued)

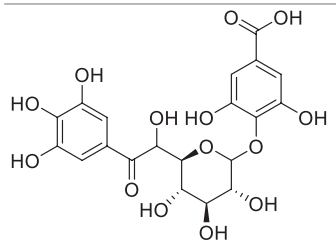
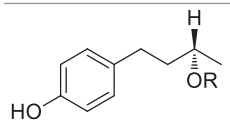
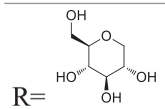
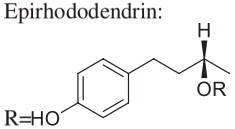
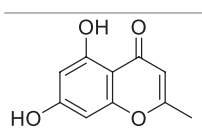
**Table 22.2** (continued)

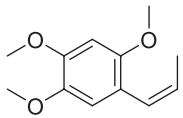
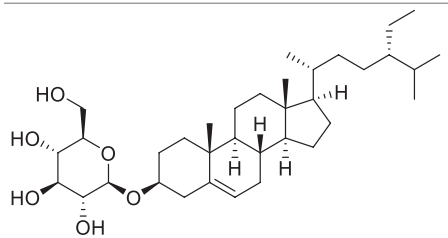
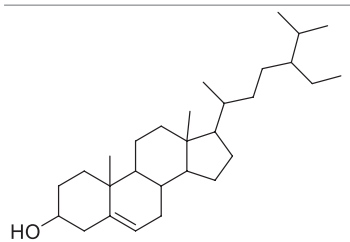
| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
|  | Genistin (genistein-7-O-glucoside) | *R. emodi* | Rhizomes | [50] |
| **Flavonoids (chalcones)** | | | | |
|  | Isoliquiritigenin | *R. emodi* | Rhizomes | [50] |
|  | Butein | *R. emodi* | Rhizomes | [50] |
| **Flavonoids (auronols)** | | | | |
|  | Carpusin (marsupsin): R = $CH_3$ | *R. emodi* | Roots | [49] |
| | Maesopsin: R = H | *R. emodi* | Roots | [49] |
| Phenolic glycosides | | | | |
|  | 8-Methoxyl-rheumone A: R = $OCH_3$ | *R. nobile* | Rhizomes | [31] |
| | Rheumone A: R = H | *R. nobile* | Rhizomes | [31] |

| | | | | |
|---|---|---|---|---|
|  | Torachrysone-8-O-$\beta$-D-glucopyranoside | *R. nobile* | Rhizomes | [31] |
| | | *R. emodi* | Roots and rhizomes | [49, 53] |
| **Phenolic acids** | | | | |
|  | *p*-Hydroxybenzoic acid | *R. emodi* | Rhizomes | [50] |
|  | *o*-Hydroxybenzoic acid | *R. emodi* | Rhizomes | [50] |
|  | Gallic acid | *R. emodi* | Rhizomes | [50, 56] |
|  | $\beta$-Resorcylic acid | *R. emodi* | Rhizomes | [56] |
|  | Vanillic acid | *R. emodi* | Rhizomes | [50] |
| **Cinnamic acid derivatives** | | | | |
|  | Chlorogenic acid (caffeoylquinic acid) | *R. emodi* | Rhizomes | [50] |

(continued)

**Table 22.2** (continued)

| Structure | Name of compound | Species | Part used | References |
|---|---|---|---|---|
| **Tannins** | | | | |
|  | Digalloyl glucose (gallic acid 4-O-(6-galloylglucoside)) | – | Commercial rhubarb | [58] |
| **Miscellaneous** | | | | |
|  | (+)- Rhododendrol:<br>R = H | *R. maximowiczii* | Roots and barks | [55, 59] |
| R=  | Epirhododendrin:<br>  | *R. maximowiczii* | Roots and barks | [55, 59] |
| | (S)-4-(4-Hydroxyphenyl)-2-butanol 2-O-sulfate: R = $SO_3H$ | *R. maximowiczii* | Roots | [55] |
| | (S)-4-(4-Hydroxyphenyl)-2-butanol 2-O-(6-O-galloyl)-D-glucopyranoside:<br>R = 6-galloyl-$\beta$-D-Glcp | *R. maximowiczii* | Roots | [55] |
| | (S)-4-(4-Hydroxyphenyl)-2-butanol 2-O-(6-O-acetyl)-D-glucopyranoside:<br>R = 6-O-acetyl-$\beta$-D-Glcp | *R. maximowiczii* | Roots | [55] |
| | (S)-4-(4-Hydroxyphenyl)-2-butanol 2-O-[6-O-(3,5-dimethoxy-4-O-L-rhamnopyranosyl galloyl)-D-glucopyranoside]:<br>R = $\alpha$-L-rhamnopyranosyl | *R. maximowiczii* | Roots | [55] |
|  | Noreugenin | *R. emodi* | Rhizomes | [53] |

| | | | | |
|---|---|---|---|---|
| **Phenyl propanoids** | | | | |
|  | *β*-Asarone | *R. emodi* | Rhizomes | [47] |
| **Phytosterols** | | | | |
|  | Daucosterol | *R. emodi* | Roots, rhizomes and rootstalks | [37, 38] |
|  | *β*-Sitosterol | *R. emodi* | Roots, rhizomes and rootstalks | [37, 38] |

*R. palmatum, R. pumilum, R. rhomboideum* and *R. tanguticum* are used as condiment in China [68].

In Nepal, the crushed and boiled roots of *R. acuminatum* and *R. australe* are used for indigestion, menstruation problems and blood purification. Besides, the roots paste is applied externally on fractured and broken bones. The petioles of *R. acuminatum* have been used to treat diarrhea, constipation, cold, cough and headache and also to make pickles [69].

In Iranian traditional medicine, people use the roots of *R. turkestanicum* to treat diabetes, hypertension and cancer [70]. The roots of *R. palmatum* are traditionally used for liver diseases, constipation, and backache and as cardiac tonic, appetizer and anti-lithiatic. Moreover, different parts of *R. ribes* (Persian name: Rivas) are used for various **ailments** of human and animals.

Fresh young stems of *R. tibeticum* -known as Sheepod in Pakistan- are being used as vegetable and mild laxative agent [71]. In the Pakistan folk medicine, the powdered rhizomes of *R. australe* are used topically for treating wounds and orally for curing constipation [72]. Furthermore, different parts of *R. spiciforme* have been recognized for curing digestive disorders and as blood purifier and tonic for livestock [73].

In India, *R. australe* has been used to treat abdominal pain and constipation, loss of appetite, asthma, bronchitis, fever, cuts, dysentery, eye disorders, sprains, swellings, ulcers and wounds. *R. moorcroftianum* has been recommended to treat colds and internal injuries. *Rheum webbianum* has been advised as an astringent agent and purgative and is used for curing wounds, abdominal disorders and boils [23]. The most frequent traditional uses of *Rheum* taxa in different countries is to treat gastrointestinal **diseases**, skin problems, abdominal complaints, kidney **ailments,** jaundice, diabetes, bronchitis, worms and boils. Apart from their medicinal applications, several species of *Rheum* are used as natural dyes. The roots of *R. acuminatum, R. austral, R. moorcroftianum* and *R. webbianum* are good sources to obtain yellow color which is used in cosmetics, textile industry or as a food colorant [68, 69, 74].

## 22.5 Nature of *Rheum* spp. Described in ITM

In Islamic Traditional Medicine (ITM) four genus of *Rheum* have been described including *R. palmatum* (Râvand Sini), *R. rhaponticum* (Râvand Shâmi), *R. turkestanicum* (Râvand Torki) and *R. ribes* (Ribâs). The temperament (Mizaj) of *R. palmatum, R. rhaponticum* and *R. turkestanicum* are mentioned as warm and dry in second degree, but *R. ribes* is described as cold and dry in second degree (Table 22.3). In different traditional references, Rivand is mentioned as resolving (moḥallel), cutting (moqaṭṭeʿ), deobstruent (mofaṭṭeḥ), purifying (monaqi), attenuating (molaṭṭef), and also astringent (qabeḍ). Ribas (*R. ribes*) is attenuating, astringent and gastrointestinal tonic [75].

## 22.6 Medicinal Uses of *Rheum* spp. in ITM

The different properties of *Rheum* spp. are categorized according to the organs of the body on which they exert their effects.

### 22.6.1 Respiratory System

One of the most important therapeutic applications of *R. palmatum* and *R. rhaponticum* in ITM is respiratory problems. These rhubarb species are considered as expectorant, corrosive moisture desiccant and anti-cold cough. They also improve asthma, hemoptysis and orthopnea [4, 5, 75]. In contrast, *R. ribes* is considered as a harmful plant for respiratory system in ITM [5].

**Table 22.3** *Rheum* spp. used in ITM

| *Rheum* taxa | Part used | Temperament |
|---|---|---|
| *R. palmatum* | Roots | Warm/Dry |
| *R. rhaponticum* | Roots | Warm/Dry |
| *R. turkestanicum* | Roots | Warm/Dry |
| *R. ribes* | Stems and leaves | Cold/Dry |

### 22.6.2 Central and Peripheral Nervous System

*Rheum palmatum* and *R. rhaponticum* are known as good remedies for brain purgation, headache and memory [4]. It has been reported that *R. ribes* is deleterious for central and peripheral nervous system [5].

### 22.6.3 Liver, Kidney and Spleen

Avicenna, in his book (Canon), together with several other scientists such as Ibn Al-Baytâr have recommended rhubarb preparations as anti-weakness and analgesic of liver and kidney [4, 5]. It is liver deobstruent and is able to break kidney and bladder stones. It also removes rigidity and edema from spleen and is useful in treating dropsy [5, 75].

### 22.6.4 Gastrointestinal System

According to the studied texts, rhubarb is very useful for internal organs, especially stomach and liver. It is believed that all of the species can attenuate stomach problems [4, 5, 75]. A dense extract of *R. ribes* is used to reduce thirst and to cure nausea and hemorrhoids. The astringent property of *R. ribes* seeds can be effective in curing diarrhea. The other species of *Rheum* can be beneficial in treating flatulence, hiccups, diarrhea, constipation and intestinal ulcers [4, 76].

### 22.6.5 Skin

In ITM, rhubarbs have a good potential for eliminating skin freckles and lentigo as well as healing dermatitis and psoriasis [4, 75]. *Rheum ribes* is used for erysipelas, rosacea acne and herpes in a preparation with barely (*Hordeum vulgare* Linn) flour [75].

### 22.6.6 Reproductive Organs

*Rheum* taxa. Except *R. ribes*, reduce libido and remove the womb pain [75].

### 22.6.7 Joints and Muscles

*Rheum* spp. except *R. ribes* are used as anti-sciatic and analgesic. They also improve muscle weakness and nerve rupture [4, 75].

### 22.6.8 Heart

According to ITM, *R. ribes* can empower the principle organs of the body meaning heart, brain and liver. It can also improve tachycardia [4].

### 22.6.9 Mental Properties

*Rheum ribes* is anti-depressant and exhilarant. Moreover, it attenuates melancholia and misanthropy [76].

### 22.6.10 Bacterial Infections

Rhubarbs are mentioned as anti-recurrent fevers in ITM [5]. Also, *R. ribes* is considered as an anti-smallpox and anti-measles herb [4].

### 22.6.11 Other Properties

Topical use of *R. ribes* extract is useful for improving eyesight [5, 76].

## 22.7 Pharmacological Aspects

At present, various pharmacological activities of *Rheum* species including anti-cancer, anti-viral, anti-fungal, anti-bacterial, anti-oxidative, anti-dermatitis, hypoglycemic, hypolipidemic and kidney and liver protective have been shown in different *in vitro*, *ex vivo* and *in vivo* studies (Tables 22.4, 22.5, 22.6). The effect of different preparations of rhubarb in treating atherosclerosis, acute bleeding of the upper gastrointestinal tract, constipation, dysenteric diarrhea and depression has been demonstrated in some clinical trials as well (Table 22.7).

**Table 22.4** *In vitro* studies of *Rheum* spp.

| Pharmacological effect | Species | Part used | Constituents/Preparations | Tested pathogen/cell | Results | References |
|---|---|---|---|---|---|---|
| Anti-cancer | *R. officinale* | – | Aqueous extract | A549 (lung)<br>MCF-7 (breast) | Decreasing cell number, DNA fragmentation and single DNA strand breakage | [77] |
| | | | | | $IC_{50}$[1] for A549: 620 ± 12.7μg/mL<br>$IC_{50}$ for MCF-7: 515 ± 10.1μg/mL | |
| | *R. emodi* | Rhizomes | MeOH and aqueous extracts | MDA-MB-435S (breast)<br>Hep3B (liver) | Demonstrating considerable cytotoxicity in both cell lines | [56] |
| | | | | | MeOH extract:<br>$IC_{50}$ for MDA-MB-435S: 8.50 ± 3.70μg/mL<br>$IC_{50}$ for Hep3B: 38.43 ± 6.00μg/mL<br>Aqueous extract:<br>$IC_{50}$ for MDA-MB-435S: 33.00 ± 8.31μg/mL<br>$IC_{50}$ for Hep3B: 85.58 ± 3.60μg/mL | |
| | *R. turkestanicum* | Roots | *n*-hexane, EtOAc and aqueous extracts | HeLa (cervix)<br>MCF-7<br>Human blood lymphocytes (non-malignant control) | Decreasing cell viability in malignant cells but not in non-malignant cells by *n*-hexane and EtOAc extracts | [70] |
| | *R. palmatum* | – | Crude extract | LS1034 cells (human colon adenocarcinoma) | Inducing apoptosis and DNA damage<br>Decreasing the percentage of viable cells in a dose-dependent manner | [78] |
| | *R. emodi* | Leaves and rhizomes | MeOH and EtOAc extracts<br>Pure compounds (emodin, chrysophanol and their glycosides) | MIAPaCa-2 (pancreas)<br>HCT-116 (colon)<br>MCF-7<br>T47D (breast) | Reducing the viability of MIAPaCa-2 and HCT-116 cells by rhizomes MeOH extract and leaves EtOAc extract | [79] |
| Anti-metastatic | *R. palmatum* | Bark | Hydro-alcoholic extract | MDA-MB-231 | Inhibiting migration, mobility and invasion at non-toxic concentrations | [80] |
| Anti-metastatic and anti-cancer | *R. palmatum* | – | Crude extract | U-2 OS human osteosarcoma cells | Inhibiting migration and invasion of the cells<br>Decreasing the percentage of viable cells in a dose-dependent manner | [81] |
| | *R. palmatum* | – | Hydro-alcoholic extract | SCC-9 (squamous carcinoma of the tongue)<br>SAS (oral) | Inhibiting motility, invasion and migration at non-toxic concentrations<br>Decreasing the viability at concentrations>20μg/mL | [82] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Anti-HBV | *R. palmatum* | Roots | Aqueous extract | HepG2 2.2.15 (liver) | Reducing the level of extracellular HBV[2] virion DNA at 64–128μg/mL<br>Preventing the secretion of HBsAg[3] dose dependently | [83] |
| | *R. palmatum* | Rhizomes | EtOH extract | HepAD38 (liver) | Preventing the production of HBV-DNA and HBsAg dose-dependently | [84] |
| | *R. palmatum* | Rhizomes | EtOH extract | HepG2 2.2.15 (liver) | Preventing the production of HBV-DNA and HBsAg expression dose-dependently | [85] |
| Anti-$CVB_3$ | *R. palmatum* | Roots and rhizomes | EtOH extract | $CVB_3$[4] propagated in HEp-2 (human laryngeal carcinoma) | Inhibiting the activity of $CVB_3$ ($IC_{50}$: 4μg/mL) | [86] |
| Anti- JEV | *R. palmatum* | Crude extract powder | MeOH and aqueous extracts<br>Pure compounds (chrysophanol and aloe-emodin) | BHK-21 (baby hamster kidney) | Reducing JEV[5] plaque<br>Exhibiting virucidal activity<br>Inhibiting residual infectivity compared to controls | [87] |
| Anti-HIV | *R. palmatum* | Roots | Aqueous MeOH extract | Jurkat (human T-lymphoblastoid cells)<br>HEK293T (human embryonic kidney cells) | Preventing the HIV-1[6] RNase H activity ($IC_{50}$: 0.9μg/mL) | [88] |
| | *R. officinale* | | | | Preventing the HIV-1 RNase H activity ($IC_{50}$: 0.25μg/mL) | |
| Anti-H1N1 | *R. tanguticum* | Roots and rhizomes | EtOH extract | Influenza virus A (H1N1) (propagated in MDCK cells) | Inhibiting viral entry<br>Preventing viral attachment and penetration into the host cells<br>Blocking haemagglutinin-mediated fusion | [89] |
| Anti-fungal | *R. emodi* | Rhizomes | MeOH extract | *Candida albicans*<br>*Cryptococcus neoformans*<br>*Sporotrichum schenckii*<br>*Trichophyton mentagrophytes*<br>*Aspergillus fumigatus* | MIC[7]:<br>*C. albicans*: 250μg/mL<br>*C. neoformans*: -<br>*S. schenckii*: -<br>*T. mentagrophytes*: 250μg/mL<br>*A. fumigatus*: 250μg/mL | [41] |
| | *R. emodi* | Rhizomes | MeOH extract | *Aspergillus niger*<br>*C. albicans* | MIC:<br>*A. niger*: 50 mg/mL<br>*C. albicans*: 16.66 mg/mL | [90] |
| Anti-biofilm formation | *R. undulatum* | Roots | Extract | *C. albicans* | Blocking the adhesion of *C. albicans* biofilms to polystyrene surfaces<br>Damaging the cell membrane integrity | [91] |

(continued)

**Table 22.4** (continued)

| Pharmacological effect | Species | Part used | Constituents/Preparations | Tested pathogen/cell | Results | References |
|---|---|---|---|---|---|---|
| Anti-cariogenic | *R. undulatum* | Roots | Dichloromethane extract | *Streptococcus mutans* and *S. sobrinus* | Inhibiting the caries-inducing factors<br>Preventing *in vitro* dental plaque formation | [92] |
| | *R. undulatum* | Roots | Dichloromethane extract | *S. mutans* | Reducing the initial rate of glycolytic acid production of *S. mutans* biofilms | [93] |
| Anti-bacterial | *R. palmatum*<br>*R. undulatum*<br>*R. rhaponticum* | Roots | EtOH extract | *Staphylococcus aureus* and *S. epidermidis*<br>*Escherichia coli*<br>*Klebsiella pneumoniae*<br>*Proteus mirabilis* | All the extracts were more active against *Staphylococcus* spp. in comparison to gram negative strains<br>*R. undulatum* extract had the strongest inhibitory effect on *Staphylococcus* spp | [94] |
| | *R. ribes* | Roots, stalks and leaves | MeOH extract | 1. *S. aureus*<br>2. *E. coli*<br>3. *K. pneumonia*<br>4. *Pseudomonas aeruginosa*<br>5. *Shigella flexneri* | All extracts had good anti-bacterial activity against test organisms<br>All extracts were found to be more active against *S. flexneri* and *K. pneumonia* | [95] |
| | *R. ribes* | Roots and rhizomes | EtOH and aqueous extracts | *E. coli*<br>*S. aureus*<br>*P. aeruginosa*<br>*P. mirabilis* | Both extracts showed significant zones of inhibition against all the tested microorganisms<br>The EtOH extract showed a higher zone of inhibition range in comparison with the aqueous extract | [96] |
| | *R. ribes* | Flowers | *n*-hexane extract<br>Essential oil (EO) | *S. aureus* and *S. epidermidis*<br>*S. pneumoniae*<br>*E. coli*<br>*K. pneumonia*<br>*Neisseria gonorrhoeae*<br>*P. aeruginosa*<br>*Salmonella typhimurium* | Moderate anti-bacterial activity of extract and EO on *S. pneumonia*, *S. epidermidis*, *K. pneumonia* and *S. typhimurium*<br>*S. epidermidis* was the most sensitive organisms affected by the hexane extract and EO | [97] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Anti-oxidant | *R. ribes* | Roots and stems | Chloroform and aqueous MeOH extracts | – | All four extracts exhibited stronger activity than known standards (BHT[8] and α-tocopherol)<br>Higher anti-oxidant activity of root extract than stem extract was observed | [98] |
| | *R. ribes* | Roots and stems of flowers (peels and flesh) | Ether, EtOH and aqueous extracts | – | All extracts showed radical and superoxide scavenging activity<br>A significant difference between the control and extracts in linoleic acid peroxidation was observed | [99] |
| | *R. emodi* | Rhizomes | MeOH and aqueous extracts | – | MeOH extract was a stronger anti-oxidant in comparison with aqueous extract<br>Aqueous extract showed efficiency in DNA protection | [56] |
| | *R. emodi* | Roots | MeOH, aqueous MeOH, acetone, aqueous acetone and aqueous extracts | – | Aqueous MeOH extract was the most active extract against radicals | [100] |
| | *R. emodi* | Roots | MeOH, chloroform and EtOAc extracts | – | All extracts showed radical scavenging activity<br>MeOH extract was the most active radical scavenger | [101] |
| | *R. officinale* | Roots and tubers | EtOH extract | – | Demonstrating strong radical scavenging activity | [102] |
| Pancreatic insulin secretion | *R. ribes* | Roots and rhizomes | Aqueous extract | Pancreatic β-cells (MIN6) | Stimulating insulin secretion | [103] |
| α-glucosidase inhibitory effect | *R. emodi* | Rhizomes | MeOH extract | α-glucosidase from yeast and rat intestinal acetone powder | Inhibiting yeast and mammalian α-glucosidase activity | [53] |
| | *R. rhabarbarum* | Peels | MeOH extract | α-glucosidase from *Saccharomyces cerevisiae* | Inhibiting α-glucosidase activity with an $IC_{50}$ value of 0.013 ± 0.002 mg/mL | [104] |
| | *R. palmatum* | Roots | MeOH extract | α-glucosidase from *Saccharomyces cerevisiae* | Inhibiting α-glucosidase activity with an $IC_{50}$ value of 0.014 ± 0.0001 mg/mL | |
| | *R. palmatum* | Roots | EtOAc extract | α-glucosidase from *Saccharomyces cerevisiae* | Inhibiting α-glucosidase activity with an $IC_{50}$ value of 0.016 ± 0.0002 mg/mL | |
| Hepatic stellate cells migration in liver fibrosis | *R. palmatum* | Roots and rhizomes | EtOH extract | HSC-T6 (hepatic stellate cells of rat) | Attenuating TGF-β1[9]-mediated migration of HSCs by possible interference in Smad2/3[10] phosphorylation, the MAPK[11] pathway, and MMP-2[12] activity | [105] |

(continued)

**Table 22.4** (continued)

| Pharmacological effect | Species | Part used | Constituents/Preparations | Tested pathogen/cell | Results | References |
|---|---|---|---|---|---|---|
| Intestinal epithelial apoptosis | *R. tanguticum* | Roots | *R. tanguticum* Polysaccharides | HIEC cells (normal human intestinal epithelium) | Elevating cell survival<br>Decreasing MDA[13] and LDH[14] activity<br>Reducing cell apoptosis | [106] |
| Radiation-induced intestinal mucosal injury | *R. tanguticum* | Roots | *R. tanguticum* Polysaccharides | IEC-6 cells (rat intestinal crypt epithelial cells) | Inhibiting cell death<br>Reducing the formation of intracellular ROS[15]<br>Inhibiting apoptosis partially | [107] |
| Bone protection | *R. rhaponticum* | Roots | ERr 731® extract | U2OS-ER$\alpha$[16] (osteosarcoma)<br>U2OS-ER$\beta$[17] (osteosarcoma) | Activating ER$\alpha$ significantly<br>Stimulating the ER$\beta$-dependent reporter gene activity | [108] |
| Tyrosinase and melanin biosynthesis inhibitory | *R. officinale* | Rhizomes | Aqueous MeOH, acetone, EtOAc and aqueous extracts<br>Different fractions of EtOAc extract<br>Pure compounds (2 glycosylated hydroxystilbenes) | Mushroom tyrosinase/B-16 mouse melanoma cells (for pure compounds) | Inhibiting tyrosinase activity by all the mentioned extracts, fractions and pure compounds<br>Inhibiting melanin biosynthesis by the glycosylated hydroxystilbenes | [109] |

Abbreviations: [1]Half maximal inhibitory concentration, [2]Hepatitis B virus, [3]The surface antigen of the hepatitis B virus, [4]Coxsackievirus B3, [5]Japanese encephalitis virus, [6]Human immunodeficiency virus, [7]Minimum inhibitory concentration, [8]Butylated hydroxytoluene, [9]Transforming growth factor beta 1, [10]A family of structurally similar proteins that are the main signal transducers for receptors of the TGF-β, [11]Mitogen-activated protein kinase, [12]Matrix metalloproteinase 2, [13]Malondialdehyde, [14]Lactate dehydrogenase, [15]Reactive oxygen species, [16]Estrogen receptor α, [17]Estrogen receptor β

**Table 22.5** *Ex vivo* studies of *Rheum* spp.

| Pharmacological effect | Species | Part used | Constituents/Preparations | Tested animal/tissue or cell | Results | References |
|---|---|---|---|---|---|---|
| Laxative | *R. palmatum* | Roots | EtOH extract | Sprague–Dawley rats/ileum | Affecting the $Na^+$-$K^+$-$2Cl^-$ cotransporter more directly than $Na^+$-$K^+$ ATPase on the serosal side of the intestinal epithelial cells | [110] |
| Hypotensive | *R. undulatum* | Rhizomes | Aqueous extract | Sprague–Dawley rats/thoracic aortae | Dilating vascular smooth muscles (vasorelaxation) | [111] |
| | *R. undulatum* | Rhizomes | MeOH extract | Sprague–Dawley rats/thoracic aortae | Dilating vascular smooth muscles (vasorelaxation) | [112] |

**Table 22.6** *In vivo* studies of *Rheum* spp.

| Disease | Species | Part used | Constituents/Preparations | Animal | Study design | Results | References |
|---|---|---|---|---|---|---|---|
| $CVB_3$ infection | *R. palmatum* | Roots and rhizomes | EtOH extract in normal saline | BALB/c mice | i.p.[1] administration of the extract at 0.3 g/kg/d starting from 24 hours post-virus exposure | Alleviating clinical signs<br>Increasing survival rate<br>Decreasing viral titer | [86] |
| Hypoglycemic effect in healthy mice | *R. ribes* | Roots | Hydro-alcoholic extract, chloroform and water fractions of hydro-alcoholic extract in normal saline (containing Tween 80) | NMRI mice | Single dose oral administration of extracts at 50 mg/kg to healthy mice | Reducing blood glucose in healthy mice by water fraction | [113] |
| Glucose and starch tolerance test | *R. ribes* | Roots and rhizomes | Aqueous extract | Sprague-Dawley rats | Oral administration of extract at 125, 250 and 500 mg/kg | Improving glucose homeostasis through retarding carbohydrate digestion | [114] |
| Diabetes | *R. franzenbachii* | Roots and rhizomes | EtOH extract in NaCMC[2] solution | Wistar rats | Oral administration of the extract at 125, 250 and 500 mg/kg/d for 14 days | Reducing plasma glucose level and MDA<br>Increasing catalase activity | [115] |
| | *R. ribes* | Rhizomes | Aqueous extract fractions in normal saline | Swiss-Webster mice | i.p. administration of fractions at 12.5, 25 and 50 mg/kg | Decreasing blood glucose<br>Improving peripheral nerve function | [116] |
| | *R. turkestanicum* | Roots | Hydro-alcoholic extract in water | Wistar rats | Oral administration of extract at 100, 200 and 300 mg/kg/d for 4 weeks | Decreasing blood glucose, HbA1c[3], TG[4], total Chol[5], and LDL[6]<br>Suppressing body weight loss<br>Reducing ALT[7], AST[8] and LDH activity | [117] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Dyslipidemia in diabetes | *R. palmatum* | Rhizomes | Aqueous extract in saline | Wistar rats | Oral administration of extract at 150 and 300 mg/kg, and 300 mg/kg (plus 0.2 mg/kg atropine) | Decreasing postprandial hypertriglyceridemia at 150 and 300 mg/kg by promoting intestinal transit in a dose-dependent manner | [118] |
| | *R. turkestanicum* | Rhizomes | Water decoction | Wistar rats | Oral administration at 200, 400 and 600 mg/kg/d for 3 weeks. | Decreasing TG levels in comparison to untreated diabetic rats<br>Reducing cholesterol levels at the doses of 400 and 600 mg/kg as compared to the control group | [119] |
| Dyslipidemia in high-fat diet | *R. rhabarbarum* | Rhizomes | EtOH extract in 0.8% CMC solution | C57BL/6 mice | Oral administration of extract at 100 mg/kg for 8 weeks | Blocking body weight gain<br>Reducing feed efficiency, liver weight and total and LDL-cholesterol levels | [120] |
| Dyslipidemia | *R. emodi* | Rhizomes | EtOH extract and different fractions (hexane, chloroform, butanol-soluble and butanol-insoluble) of EtOH extract in 0.2% w/w aqueous gum acacia solution | Charles foster rats | Oral administration of the extract and fractions at 200 mg/kg | Decreasing total cholesterol, phospholipid, TG, VLDL[9] and LDL besides an increase in HDL[10] by EtOH extract and butanol-soluble fraction | [121] |
| Nephrotoxicity | *R. emodi* | Rhizomes | Water-soluble and insoluble fractions of MeOH extract in 1% CMC solution | Wistar albino rats | Oral administration of extracts at 350 mg/kg/d | Water-soluble fraction: protecting all the proximal tubule segments<br>Water-insoluble fraction: protecting S2 segment of proximal tubule beside enhancing gentamicin nephrotoxicity | [122] |
| | *R. palmatum* | Roots and rhizomes | EtOH extract, total anthraquinones, total tannins and remaining compounds fractions in 0.5% CMC solution | Sprague-Dawley rats | Treatment with different fractions at different doses for 7 days. | Only total tannin fraction protected the kidney function in $K_2Cr_2O_7$-injured rats | [123] |

(continued)

**Table 22.6** (continued)

| Disease | Species | Part used | Constituents/Preparations | Animal | Study design | Results | References |
|---|---|---|---|---|---|---|---|
| Chronic renal failure | *R. officinale* | Roots | Petroleum ether (PE), EtOAc, and butanol (BU) fractions | Sprague-Dawley rats | Oral administration of PE, EtOAc and BU fractions at 800, 200 and 600 mg/kg, respectively for 6 weeks | All of the fractions resulted in:<br>Lowering creatinine and $BUN^{11}$ levels<br>Enhancing creatinine clearance<br>Improving renal tubulointerstitial injury | [124] |
| Diabetic nephropathy | *R. officinale* | Roots | Water decoction | Wistar rats | Oral administration of water extract at 125 mg/kg/d for 80 days | Ameliorating high blood and urinary glucose levels<br>Improving hyperlipidemia and creatinine excretion | [125] |
| $HgCl_2$ renal toxicity | *R. turkestanicum* | Roots | Hydro-alcoholic extract in saline | Wistar rats | i.p. administration of extract at 100 and 200 mg/kg/d for 3 days | Improving necrosis and atrophy of the kidney<br>Decreasing serum urea, creatinine and renal MDA | [126] |
| $HgCl_2$ hepatotoxicity | *R. turkestanicum* | Roots | Hydro-alcoholic extract in saline | Wistar rats | i.p. administration of extract at 100 and 200 mg/kg/d for 3 days | Improving liver function by reducing serum ALT and AST<br>Decreasing MDA and inflammatory infiltration in the liver | [126] |
| Chronic liver injury | *R. palmatum* | Roots and rhizomes | Hydro-alcoholic extract in normal saline | Sprague-Dawley rats | Intragastric administration of rhubarb extract at 2, 5.40, 14.69 and 40 g/kg/d for 12 weeks | Hepatoprotection for injured rats at 2 and 5.4 g/kg/d<br>Hepatic injury for normal rats at all the tested doses and injured rats at 14.69 and 40 g/kg/d | [127] |
| Acute liver failure | *R. palmatum* | Roots and rhizomes | – | ICR mice | i.p. administration of *R. palmatum* at 1.5 g/kg/d | Reducing ALT, AST and inflammatory factors<br>Regulating the expression of apoptosis-related proteins | [128] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Nonalcoholic fatty liver disease | *R. palmatum* | Roots and rhizomes | Aqueous extract in normal saline | Sprague-Dawley rats | Oral administration of extract at 690 and 1300 mg/kg/d for 6 weeks | Reducing liver weight, blood glucose, ALT enzyme and liver steatosis | [129] |
| Hepatotoxicity | *R. emodi* | Roots and rhizomes | Flavonoid-containing fractions in normal saline | Wistar rats | Oral administration of different fractions every 6 hours | Decreasing ALT, AST, ALP[12] and bilirubin | [130] |
| Hepatocellular carcinoma | *R. palmtum* | Roots | MeOH extract | Wistar rats | Oral administration of extract at 100 mg/kg/d for 12 weeks | Reducing the elevated ALT and AST<br>Increasing total proteins, albumin and globulin<br>Reducing the tumor markers (AFP[13] and GGT[14]) levels | [131] |
| TNBS-induced colitis | *R. Tanguticum* | – | *R. tanguticum* polysaccharides | Sprague-Dawley rats | Oral administration of polysaccharides at 200 mg/kg/d for 5, 7, 10 and 14 days | Reducing diarrhea, mortality, colon mass and ulcer area | [132] |
| | *R. tanguticum* | – | *R. tanguticum* polysaccharides | Sprague-Dawley rats | Oral administration of extract alone or in combination with 5-ASA at 200 mg/kg/d for 5 days | Both groups resulted in:<br>Attenuating histological signs<br>Decreasing NF-κBp65[15] and TNF-α[16] expressions<br>Inhibiting the overexpression of COX-2[17] | [133] |
| Gastric ulcer | *R. ribes* | Leaves | Aqueous and MeOH extracts in 0.5% acacia gum solution | Wistar rats | Oral administration of the extracts at 200 mg/kg/d for 5 days | MeOH extract increased the level of muco proteins and reduced ulcer scores | [134] |

(continued)

**Table 22.6** (continued)

| Disease | Species | Part used | Constituents/Preparations | Animal | Study design | Results | References |
|---|---|---|---|---|---|---|---|
| Uterotrophy model | *R. rhaponticum* | Roots | ERr 731® extract in Caster oil | Wistar rats | s.c.[18] administration of estradiol at 0.5µg/kg/d alone or in combination with ERr 731® at 0.1, 1, 10 and 100 mg/kg/d for 3 days<br>s.c. administration of estradiol at 4µg/kg/d and ERr 731® at 0.1, 1, 10 and 100 mg/kg/d, for 3 days | Reducing the estradiol-induced uterine growth stimulation when combined with estradiol | [135] |
| Endometrial safety | *R. rhaponticum* | Roots | ERr 731® extract | Wistar rats | Oral administration of the extract at 1 mg/kg or 1 g/kg for 90 days | No stimulatory activity on proliferation in the uterus<br>No effect on the bone mineral density | [136] |
| Experimental atopic dermatitis | *R. Tanguticum* | Rhizomes | Hydro-alcoholic extract | NC/Nga mice | Oral administration of the extract at 30–300 mg/kg/d for 5 weeks | Ameliorating skin leisons<br>Inhibiting dermatitis | [137] |
| Pigmentation | *R. officinale* | Roots and rhizomes | Raspberry ketone (RK) in Vaseline | C57BL/6 J mice | Topical application of 0.2 or 2% RK twice daily for 3 weeks | Increasing the degree of skin whitening within 1 week of treatment | [138] |
| Irradiation-induced immune damage | *R. tanguticum* | – | A polysaccharide component in saline | Kunming mice | Oral administration of the component at 200, 400 and 800 mg/kg/d for 14 days before irradiation | Promoting the innate immune function by increasing spleen and thymus index, phagocytic function of macrophages, and rate of carbon clearance<br>Improving humoral and cellular immune function by increasing serum hemolysin and NK cells[19] activity, respectively | [139] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Yeast-induced pyrexia | *R. palmatum* | Roots | MeOH extract in normal saline | Sprague-Dawley rats | Oral administration of extract at 3.5 g/kg 1 h before and 3 hrs after pyrexia induction | Decreasing rectal temperature from 4–12 hrs after yeast induction<br>Inhibiting $PGE_2$[20] production | [140] |
| Alzheimer's disease | *R. ribes* | Roots and rhizomes | Hydro-alcoholic extract | Wistar rats | i.p. administration of extract at 250 and 500 mg/kg/d for 20 days | Improving memory deficits induced by bilateral nucleus basalis of Meynert lesions | [141] |

Abbreviations: [1]Intraperitoneal, [2]Sodium carboxymethyl cellulose, [3]Hemoglobin A1c, [4]Triglyceride, [5]Cholesterol, [6]Low-density lipoprotein, [7]Alanine aminotransferase, [8]Aspartate aminotransferase, [9]Very low-density lipoprotein, [10]High-density lipoprotein, [11]Blood urea nitrogen, [12]Alkaline phosphatase, [13]α-fetoprotein, [14]Gamma-glutamyl transferase, [15]Nuclear factor κB, [16]Tumor necrosis factor α, [17]Cyclooxygenase 2, [18]Subcutaneous, [19]Natural killer cell, [20]Prostaglandin $E_2$

**Table 22.7** Clinical studies of *Rheum* spp.

| Disease | Species | Part used | Preparations | Study design | Participants | Dose | Results | References |
|---|---|---|---|---|---|---|---|---|
| Atherosclerosis | *R. officinale* | Roots | Capsules (from aqueous extract) | Randomized, double-Blind, placebo-controlled, clinical trial | 103 patients aged between 45–65 years, | Trial group: routine medications plus rhubarb capsules at 50 mg/kg<br>Control group: Routine medications (metoprolol and aspirin) plus placebo capsules containing starch | Improving endothelial function (which might be mainly due to its lipid-lowering effect) | [142] |
| Acute bleeding of the upper gastrointestinal tract | – | – | Raw rhubarb powder<br>Raw rhubarb tablet<br>Roasted rhubarb powder | – | 400 patients aged between 13–81 years | 3 g 2–4 times daily until occult blood ceased to occur in the stool | Ceasing the bleeding rapidly<br>Decreasing the loss of blood and need for anti-coagulant drugs<br>Disappearing the absorption fever<br>Increasing appetite<br>Correcting anemia | [143] |
| Constipation after operation for lumbar vertebral fracture | *R. officinale* | – | Gauze smeared by powder | – | 74 patients aged between 28–66, failed to pass gas in 6 h after the internal fixation for lumbar vertebral fracture, coupled with abdominal distention and discomfort | Observation group: Ordinary treatment plus daily topical application of rhubarb at acupoint Shenque for 6 h for 3 days<br>Control group: Ordinary treatment | Lowering the time to conduct the first flatulence and defecation | [144] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dysenteric diarrhea in children | *R. ribes* | Dried fruits | Syrup (from aqueous extract) | Randomized, double-blind, placebo controlled and parallel-group clinical trial | 150 children aged between 12–72 months with suspected *Shigella* dysentery | 2.5 mL for children less than 15 kg, or 5 mL for children more than 15 kg, every 6 hrs. For 5 days+standard antibiotic treatment | Alleviating the severity of fever and diarrhea Reducing the duration of dysentery, fever and abdominal pain | [145] |
| Primary dysmenorrhea | *R. emodi* | Roots and rhizomes | Capsules (from roots and rhizomes powder) | Randomized, single-center, single-blind, standard controlled Trial | 45 unmarried participants between ages of 15–25 years, having regular menstrual cycles with moderate to severe dysmenorrhea | Experimental group: 3 capsules of rhubarb twice a day starting from 2 days before menstruation and continuing until first 3 days of menstruation for 3 consecutive cycles Control group: 1 capsules of mefenamic acid 3 times a day after meal with the same protocol | Decreasing the menstrual pain by both treatments after three-cycle intervention | [146] |
| Major depressive disorder | *R. ribes* | Stalks | Capsules (from hydro-alcoholic extract) | Randomized, double blind, parallel-group trial | 33 patients aged between 18–60 years, having mild to moderate major depressive disorder | Oral administration of 400 mg capsules 3 times daily for 6 weeks | Reducing depressive symptoms in week 4 and 6 | [147] |

## 22.8 Conclusion

Traditional medicine around the world plays an important role in exploring new drugs. Utilizing from accurate instructions of famous scientists, ITM texts are valuable sources for detecting new drugs. In this review, the applications and lucrative properties of *Rheum* spp. were investigated in ITM books, and adapted with the results reported in pharmacological studies.

Rhubarb is said to possess a wide range of therapeutic applications in the traditional and folklore medicine such as healing gastrointestinal, liver, kidney, womb and bladder diseases, diarrhea and constipation [4, 5], skin problems, diabetes, bronchitis and boils. The medicinal effects of rhubarb may owe to the several chemical compounds present in this plant specifically anthraquinones, anthrones and different phenolic compounds such as stilbenes, flavonoids and tannins. The effect of this herb in treating both constipation and diarrhea is due to anthraquinones and tannins, respectively. At low doses, rhubarb is said to act as anti-diarrheal, while at higher doses it is cathartic [148]. Many effects of rhubarb including anti-cancer, anti-microbial, anti-oxidant, anti-diabetic, anti-dyslipidemic, anti-pigmentation, nephroprotective, hepatoprotective and immunoprotective have been shown in *in vitro* and *in vivo* studies. In addition, some clinical trials have indicated the efficacy of this plant in treating atherosclerosis, gastrointestinal bleeding, diarrhea and dysmenorrhea which is in agreement with the traditional and folklore medical applications of rhubarb. The results of previous studies have shown significant differences in the chemical composition of *Rheum* genus, and it is recommended that clinical practice should be performed for each species individually [20]. Though many pharmacological activities of *Rheum* spp. have been investigated, further studies especially clinical trials are needed to fill the present gaps in our knowledge of different aspects of this plant. According to the potency of rhubarb in treating different diseases, the demand for this plant in international and domestic markets is growing leading to excessive exploration and a sharp drop in the wild resources of rhubarb as well as damage to the wildlife [149]. Several ***Rheum*** species are under immense risk and have been considered as "threatened" and it is of great importance to stop overharvesting and habitat destruction and establish rhubarb gardens in combination with education and awareness programs for large-scale cultivation and conservation of this genus [23].

Taken together, rhubarb is a valuable medicinal plant that has been useful in treating several diseases for centuries. Due to the different therapeutic effects mentioned in the ITM texts and folklore medicine as well as various pharmacological and clinical studies and notable phytochemicals isolated from *Rheum* spp., this genus is highly recommended to the herbal pharmaceutical industry for manufacturing several oral and topical formulations beside exploring new lead compounds and drugs for treating skin, gastrointestinal, metabolic and reproductive diseases.

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**Conflict of Interest** None.

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# Genus *Rosa*: A Review of Ethnobotany, Phytochemistry and Traditional Aspects According to Islamic Traditional Medicine (ITM)

23

Zahra Ayati, Mahin Ramezani, Mohammad Sadegh Amiri, Amirhossein Sahebkar, and Seyed Ahmad Emami

**Abstract**

*Rosa* spp. is an important genus in the Rosaceae family which is a source of medicinal natural products, particularly polyphenolic and terpenoid compounds and is used in several traditional medicines such as Islamic Traditional Medicine (ITM) to cure various diseases. Plants in this genus are known to possess anti-inflammatory, antidiabetic, anti-constipation, cardioprotective and neuroprotective activities. Furthermore, phytochemical investigations have reported *Rosa* species to contain a wide range of chemical compounds including quercetin, kaempferol, catechin, citronellol, limonene, lycopene, carvacrol, thymol, ascorbic acid (vitamin C), rosmarinic acid, etc. The current review is an attempt to cover the available findings on the ethnobotany and photochemistry of this genus as well as its medicinal aspects in ITM.

**Keywords**

*Rosa* · Rosaceae · Nasrin · *R. canina* · ITM · Traditional medicine · *R. damascena*

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## 23.1 Introduction

Genus *Rosa* is one of the most widespread and important members of Rosaceae family with approximately 200 species. It contains valuable active compounds, including polyphenols and



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terpenes and is economically important as a source of essential oil for perfumes.

*Rosa* has a long history of medicinal use in different folklore **and** traditional medicines as well as Islamic Traditional Medicine (ITM). It has been used for the treatment of **various disorders** including heart and brain spasms, respiratory and psychiatric problems [1]. In folk medicines it has been used for several purposes such as laxative, diuretic, anti-gout and anti-rheumatism [2].To extend our view towards future indication of *Rosa* spp. for medicinal purposes, this study was an attempt to review phytochemical, ethnobotany and ITM applications of *Rosa* spp.

## 23.2 Botany, Taxonomy and Conservation Status of *Rosa* Taxa

The Rose family (Rosaceae), in the order Rosales, is the 19th largest family of flowering plants [3]. It comprises about 95 genera and 2800 species which are cosmopolitan to sub-cosmopolitan, particularly in the Northern hemisphere. The family is extremely important economically as the source of many cultivated fruits, including *Fragaria* (strawberry), *Malus* (apples), *Prunus* (almond, apricot, cherry, peach, plum), *Pyrus* (pear) and *Rubus* (blackberry, raspberry), as well as essential oils (e.g., *Rosa*), and numerous ornamental taxa. The Rosaceae is traditionally classified into four subfamilies: the Rosoideae, Prunoideae, Maloideae and Spiraeoideae [4]. However, phylogenetic analysis by Potter, divides it into three subfamilies: Rosoideae, Dryadoideae and Spiraeoideae (incl. Amygdaloideae and Maloideae) [5]. The genus *Rosa* L. is one of the most important genera in the subfamily Rosoideae, which includes approximately 200 shrubby species distributed widely throughout the temperate and subtropical habitats of the northern hemisphere. Roses have worldwide economic importance as a source of essential oils for perfumes, medicinal properties, ornamental plants and cut flower industry [6, 7]. Mandenova, stated that the Anatolia region is considered as a major center of rose differentiation [8]. The taxonomy and phylogenetic relationships of this genus remain obscure due to problems such as inadequate anatomical and morphological characters to sufficiently discriminate between species and the human impact by rose breeding, biological phenomena in reproductive biology, and low levels of nuclear and chloroplast genome variation [6, 7]. The genus has been divided into four subgenera *Rosa*, *Hesperhodos* Cockerell, *Platyrhodon* (Hurst) Rehder and *Hulthemia* (Dumort.) Focke. Subgenus *Rosa* encompasses about 95% of all species and is subdivided into ten sections [6]. *Rosa* taxa are deciduous or evergreen shrubs, erect or climbing. The stems are mostly prickly or rarely unarmed. The leaves show an alternate form of growth. They are imparipinnate and they have stipule, or rarely simple without stipule. The flowers of these taxa are solitary or in a corymb, or panicle. Sepals 5. Petals 5. The stamens are numerous, inserted at disc. The carpels are numerous. The styles are free or connate in a long column, protruding through the orifice of the disc. The number of ovule is one. The achenes are numerous [9]. The cultivation of roses for many purposes has been widespread throughout the world. Apart from their ornamental and medicinal usage, several species of *Rosa* are used for miscellaneous applications. For example, *R. pulverulenta* is drought resistant and used for afforestation in Turkey. *Rosa spinosissima* is also planted along the European coast to stabilize sandy areas and prevent erosion [10, 11]. Some species of *Rosa* are resistant to plant diseases and cold-hardiness, like *R. rugosa* which is known as Mei Gui, in Japan. Because this wild rose is winter-hardy and is immune to black spot and most other diseases that affect European garden roses, it was introduced into Europe and North America [12]. Altitude alterations are considered as a barrier in the extension of some *Rosa* taxa. *R. sempervirens* is one of the *Rosa* species most susceptible to cold and higher altitudes represent a barrier to its extension. However, in some other taxa, like *R. pisiformis*, lower altitudes represent a barrier to its extension [10]. This taxon is endemic to Turkey with a very restricted range at high alti-

tudes (1600–2000). Due to its endemicity, its reduced range and the several threats it faces, such as low seed production, fuelwood and tourism, it is rated vulnerable [10]. There is an urgent need to take immediate steps for management strategies and conservation priorities for all *Rosa* taxa assigned to a threat category. If overutilization and habitat destruction of such valuable endemic taxa continues, we will lose them within the next few decades.

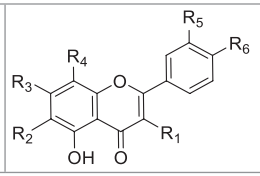
## 23.3 Phytochemical of *Rosa* Species

Several studies have been carried out to identify the composition of *Rosa* species. Many chemical compositions have been identified based on these studies. Nevertheless, two groups, including phenolic compounds and terpenes compounds, are the most important that have been extracted and identified. Flavonoids are a large family consisting of 5000 hydroxylated polyphenol compounds that perform key functions, including adsorption of insects and combating environmental stresses, in plants [13]. Based on chemical structures, flavonoids are divided into 12 major subgroups, five of which including anthocyanidins, flavonols, flavones, flavonoids, flavonoids and isoflavones are reviewed in this paper. Also, one of the large polyphenols is tannins, as shown in Table 23.1. Most people use essential oils for their therapeutic effects or for perfume. Essential oils are composed of hydrocarbon species and can be classified as terpenes, alcohol, esters, aldehydes, ketones and phenols, and so on (Table 23.2). The chemical compositions of the *Rosa* species were presented in Tables 23.1, 23.2, and 23.3.

## 23.4 Ethnobotanical and Ethnomedicinal Uses

*Rosa* L. is one of the major economically important genera of ornamental plants, and its parts are used in different traditional medicines and nutritional products. The reports concerning the traditional applications of *Rosa* species mainly originate from Europe, Asia and American continents. As illustrated in Table 23.4, thirty-four species of *Rosa* have been reported to show benefits data in ethnobotanical uses. Various parts of *Rosa* taxa including hips (fruits), flowers, leaves, barks, roots and branches have been used in ethnobotany since long time in different countries. Literature review shows that, *R. canina*, as the most famous species of the *Rosa* genus, has remarkable traditional medicinal properties for alleviating a wide spectrum of diseases. It is a well-known plant in the European and Asian Traditional Medicine. Different parts of *R. canina* have been commonly used for various diseases related to the urinary, digestive, nervous, respiratory and circulatory systems as well as to infectious ailments (Table 23.4). There are considerable reports on the traditional uses of different roses in European countries, particularly Turkey and Italy. In Turkey, roses have a broad habitation and many of them are important in folk medicine among which *R. canina, R. damascena, R. gallica, R. pulverulenta, R. dumalis, R. villosa, R. foetida, R. Phoenicia* and *R. heckeliana,* are the most popular medicinal plants [10, 11]. The fruits of *R. pulverulenta* are rich in ascorbic acid and minerals which are used to make jam, marmalade and fruit juice [47]. *R. Phoenicia* fruits commonly known as "Kusburnu, it burnu, Okuzgotu" have been recommended to curing cold, flu and diabetes [48]. *R. pisiformis*is native to Turkey (North East and East Anatolia) and indigenous people have traditionally used it as laxative. *R. heckeliana* fruits, known as Şilank, are eaten as antitussive and treatment of colds [49]. The hips of *R. villosa* have been advised as laxative, diuretics, treatment of cold, pain of menstruation, making marmalade and also used as a tea substitute [10]. In Italy, the petals of *R. sempervirens*, known as Reusa, are applied to treat headache and its fruits are used in ophthalmic [50]. In northern Japan, where *R. rugose* known as Mei Gui, grows naturally, the dried petals have been consumed as antidiarrheal and hemostatic agents [12]. In Korea, the fruit of *R. davurica* has been used to treat of gastroenteric disorder. Furthermore, the root of *R. multiflora* has been recognized to treat of neuralgia [51]. In tradi-

**Table 23.1** Major phenolic components of *Rosa* species of different plant parts

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| Phenolic compounds | | | | | |
| | |  | | | |
| 1 | Kaempferol: $R_1$ = OH, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. rugosa* | Aerial parts | [12] |
| | | | *R. agrestis* | Leaves | [14] |
| 2 | Kaempferol O-hexoside-deoxyhexoside | | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| 3 | Kaempferol 3-O-glucoside: $R_1$ = O-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. sempervirens* | Rose hip[a] | [16] |
| | | | *R. rugosa* | Leaves | [12] |
| | | | *R. agrestis* | Leaves | [14] |
| | | | *R. canina* | Leaves | [15] |
| | | | *R. chinensis* | Flower | [17] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. gallica* | Petal | [19] |
| 4 | Kaempferol 7-O-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-glucoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. canina* | Leaves | [15] |
| 5 | Kaempferol 3-O-α-L-arabinofuranoside: $R_1$ = O-α-L-arabinofuranoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. gallica* | Petal | [19] |
| 6 | Kaempferol 3-O-(2″-O-β-$_D$-glucosyl)-β-$_D$-xyloside: $R_1$ = O-(2″-O-β-$_D$-glucosyl)-β-$_D$-xyloside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. rugosa*,<br>*R. maikwai* | Petal | [20] |
| | | | *R. gallica* | Petal | [19] |
| 7 | Kaempferol 3-O-(2″-*O*-galloyl)-β-$_D$-glucoside: $R_1$ = O-(2″-O-galloyl)-β-$_D$-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. gallica* | Petal | [19] |
| 8 | Kaempferol 3-O-sophoroside: $R_1$ = O-sophoroside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. rugosa*,<br>*R. maikwai* | Petal | [20] |
| | | | *R. gallica* | Petal | [19] |

| | | | | |
|---|---|---|---|---|
| 9 | Kaempferol 3-O-rutinoside (nicotiflorin): $R_1$ = O-rutinoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| | | *R. chinensis* | Flower | [17] |
| 10 | Kaempferol 3-O-β-$_D$-galactoside: $R_1$ = O-β-$_D$-galactoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *R. gallica* | Petal | [19] |
| 11 | Kaempferol O-rhamnoside | *R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| | | *R. chinensis* | Flower | [17] |
| 12 | Kaempferol 3-O-α-$_D$-rhamnoside (afzelin): $R_1$ = O-α-$_D$-rhamnoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *R. gallica* | Petal | [19] |
| | | *R. damascena* | | [21] |
| 13 | Kaempferol 3-O- arabinoside: $R_1$ = O- arabinoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *R. chinensis* | Flower | [17] |
| 14 | Kaempferol-O-rutinoside | *R. rubiginosa* | Herbal tea | [22] |
| 15 | Kaempferol 4′-O-β-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = O-glucoside | *R. pimpinellifolia* | Leaf | [23] |
| 16 | Kaempferol 3-O-β-$_D$-glucopyranosyl(1→4)-β-$_D$-xylopyranoside: $R_1$ = β-$_D$-glucopyranosyl(1→4)-β-$_D$-xylopyranoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *R. damascena* | | [21] |
| 17 | Quercetin: $R_1$ = OH, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. rugosa* | Leaves | [12] |
| | | *R. agrestis* | Leaves | [14] |
| | | *R. woodsia* | Rose hip with seed | [24] |
| | | *R. pimpinellifolia* | Fruit<br>Flower | [25] |
| | | *R. heckeliana* | Root | [26] |
| | | *R. nutkana* | Fruit | [27] |
| | | *R. chinensis* | Flower | [17] |
| | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | *R. rubiginosa* | Herbal tea | [22] |

(continued)

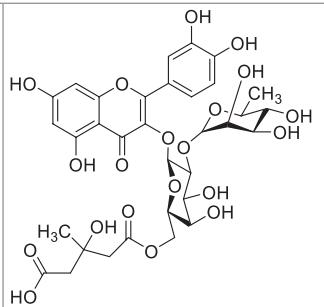
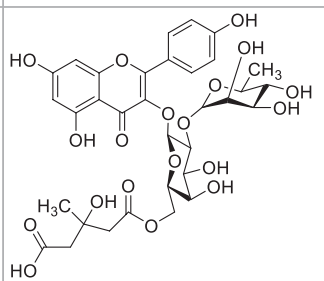
**Table 23.1** (continued)

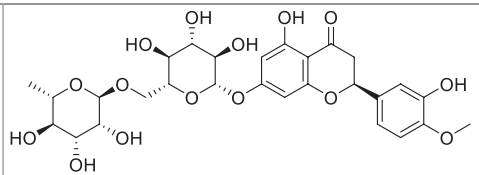
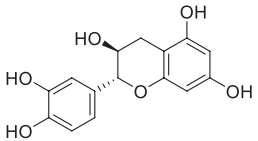
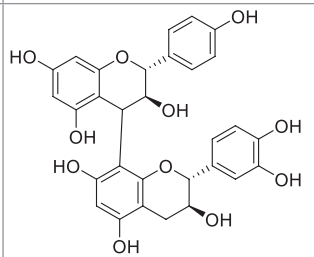
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 18 | Quercetin-3-O-glucoside (isoquercetin): $R_1$ = O-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *R. sempervirens* | Rose hip | [16] |
| | | | *R. rugosa* | Aerial parts | [12] |
| | | | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| | | | *R. chinensis* | Flower | [17] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. gallica* | Petal | [19] |
| | | | *R. damascena* | | [21] |
| | | | *R. pimpinellifolia* | Fruit<br>Flower | [25] |
| | | | *R. nutkana* | Fruit | [27] |
| 19 | Quercetin-3-O-galactoside (hyperoside): $R_1$ = O-galactoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *R. agrestis* | Leaves | [14] |
| | | | *R. sempervirens* | Rose hip | [16] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. gallica* | Petal | [19] |
| | | | *R. pimpinellifolia* | Leaf | [23] |
| 20 | Quercetin 3-O-rhamnoside (quercitrin): $R_1$ = O-rhamnoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *R. sempervirens* | Rose hip | [16] |
| | | | *R. chinensis* | Flower | [17] |
| | | | *R. agrestis* | Leaves | [14] |
| | | | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |

| | | | | |
|---|---|---|---|---|
| 21 | Quercetin 3-O-rutinoside (rutin): $R_1$ = O-rutinoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. woodsia* | Rose hip with seed | [24] |
| | | *R. rugosa* | Root | [12] |
| | | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| | | *R. pimpinellifolia* | Fruit<br>Flower | [25] |
| | | *R. canina* | Fruit | [28–30] |
| 22 | Quercetin 3-xyloside: $R_1$ = O-xyloside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. agrestis* | Leaves | [14] |
| 23 | Quercetin 3-O-arabinoside: $R_1$ = O-arabinoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. chinensis* | Flower | [17] |
| 24 | Quercetin 3-O-β-$_D$-xylopyranoside: $R_1$ = O-β-$_D$-xylopyranoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. gallica* | Petal | [19] |
| 25 | Quercetin 3-O-α-$_L$-arabinofuranoside: $R_1$ = O-α-L-arabinofuranoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. gallica* | Petal | [19] |
| 26 | Quercetin 3-O-(2″-O-β-$_D$-glucosyl)-β-$_D$-xyloside: $R_1$ = O-(2″-O-β-$_D$-glucosyl)-β-$_D$-xyloside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. rugosa*,<br>*R. maikwai* | Petal | [20] |
| | | *R. gallica* | Petal | [19] |
| 27 | Quercetin 3-O-(2″-O-galloyl)-β-$_D$-glucoside: $R_1$ = O-(2″-O-galloyl)-β-$_D$-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. gallica* | Petal | [19] |
| 28 | Quercetin 3-O-(2″-O-β-$_D$-glucosyl)-β-$_D$-galactoside: $R_1$ = O-(2″-O-β-$_D$-glucosyl)-β-$_D$-galactoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. gallica* | Petal | [19] |
| 29 | Quercetin 3-O-sophoroside: $R_1$ = O-sophoroside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. rugosa*,<br>*R. maikwai* | Petal | [20] |
| | | *R. gallica* | Petal | [19] |
| 30 | Quercetin-3-O-β-glucuronide: $R_1$ = O-glucuronide, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *R. pimpinellifolia* | Leaf | [23] |
| 31 | Quercetin-hexoside | *R. woodsia* | Rose hip with seed | [24] |
| | | *R. rubiginosa* | Herbal tea | [22] |
| 32 | Quercetin di-O-hexoside | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |

(continued)

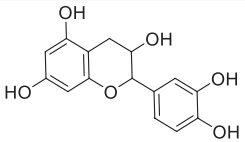
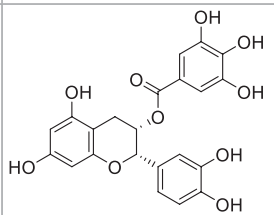
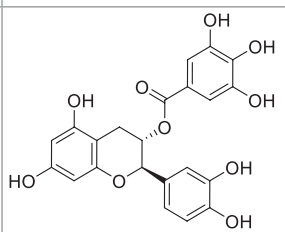
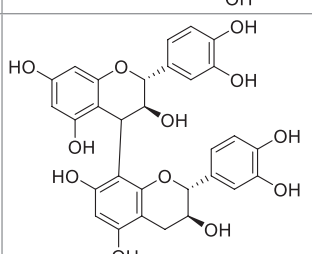
**Table 23.1** (continued)

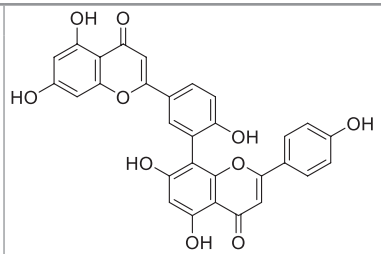
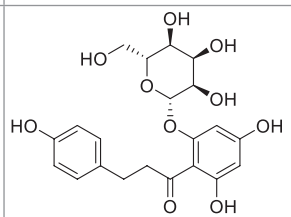
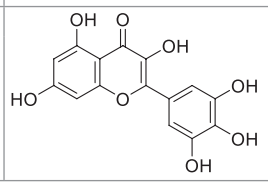
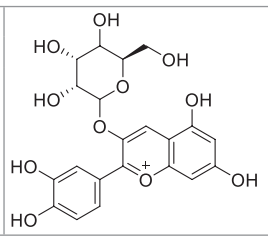
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 33 | Quercetin O-pentoside | | *R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| | | | *R. rubiginosa* | Herbal tea | [22] |
| 34 | Quercetin-O-deoxyhexoside | | *R. rubiginosa* | Herbal tea | [22] |
| 35 | Aromadendrin O-hexoside-deoxyhexoside | | *R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| 36 | Apigenin: $R_1$ = H, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. rugosa* | Leaves | [12] |
| 37 | Apigenin-7-glucoside: $R_1$ = H, $R_2$ = H, $R_3$ = O-glucoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | | *R. damascena* | Petal | [31] |
| 38 | Diosmetin (5,7,3′-trihydroxy-4′-methoxyflavone): $R_1$ = H, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OMe | | *R. agrestis* | Leaves | [14] |
| 39 | Quercetin 3-O-α-$_L$-rhamnopyranosyl-(1→2)-[6-O-(3hydroxy-3-methylglutaryl)-β-$_D$-galactopyranosides] |  | *R. pimpinellifolia* | Leaf | [23] |
| 40 | Kaempferol 3-O-α-$_L$-rhamnopyranosyl-(1→2)-[6-O-(3hydroxy-3-methylglutaryl)-β-$_D$-galactopyranosides] |  | *R. pimpinellifolia* | Leaf | [23] |

| | | | | | |
|---|---|---|---|---|---|
| 41 | Hesperidin |  | *R. rubiginosa* | Herbal tea | [22] |
| 42 | Catechin |  | *R. woodsia* | Rose hip with seed | [24] |
| | | | *R. rugosa* | Root | [12] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. rubiginosa* | Herbal tea | [22] |
| | | | *R. sempervirens* | Rose hip | [16] |
| | | | *R. laevigata* | | [32] |
| | | | *R. heckeliana* | Root | [26, 33] |
| | | | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| 43 | Afzelechin (4α→8)-catechin |  | *R. rugosa* | Root | [12] |

(continued)

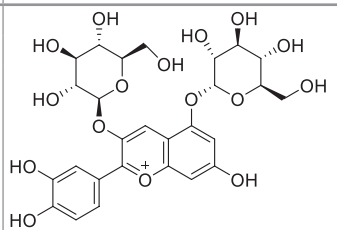
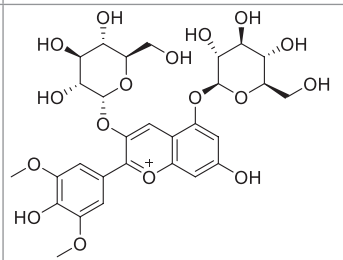
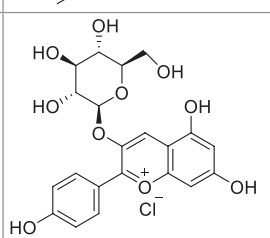
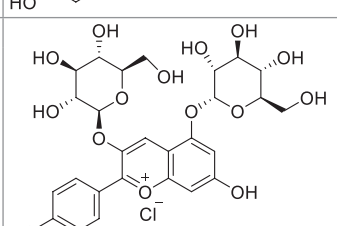
**Table 23.1** (continued)

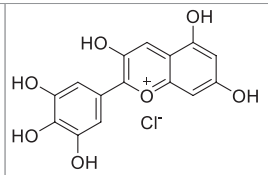
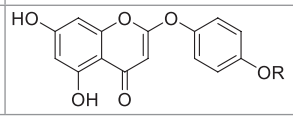
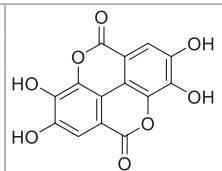
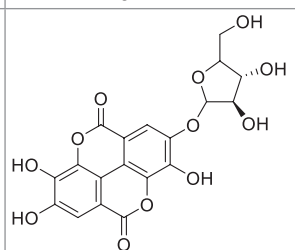
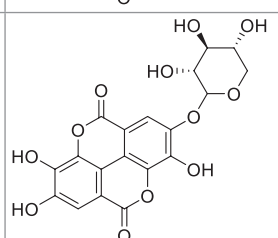
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 44 | Epicatechin |  | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. rubiginosa* | Herbal tea | [22] |
| | | | *R. laevigata* | | [32] |
| | | | *R. heckeliana* | Root | [26] |
| 45 | Epicatechin gallate |  | *R. canina*<br>*R. moschata*<br>*R. sempervirens* | Leaves | [15] |
| 46 | Catechin gallate |  | *R. nutkana* | Fruit | [27] |
| 47 | Procyanidin B-3 |  | *R. rugosa* | Root | [12] |

| | | | | | |
|---|---|---|---|---|---|
| 48 | Amentoflavone |  | *R. sempervirens* | Rose hip | [16] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| 49 | Phloridzin |  | *R. woodsia* | Rose hip with seed | [24] |
| 50 | Myricetin |  | *R. canina* | Fruit | [28] |
| | | | *R. woodsia* | Rose hip with seed | [24] |
| 51 | Isorhamnetin-O-rutinoside rabdosiin | | *R. rubiginosa* | Herbal tea | [22] |
| 52 | Cyanidin 3-O-glucoside |  | *R. damascena* | Petal | [31] |
| | | | *R. damascena* | | [21] |
| | | | *R. pimpinellifolia* | Fruit<br>Flower | [25] |

(continued)

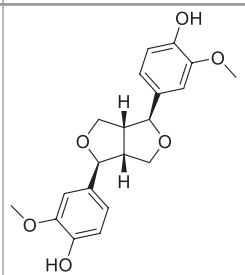
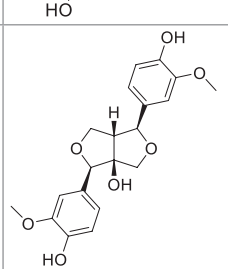
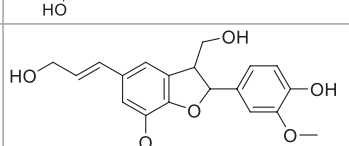
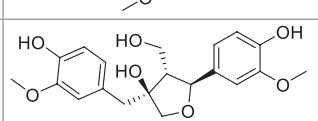
**Table 23.1** (continued)

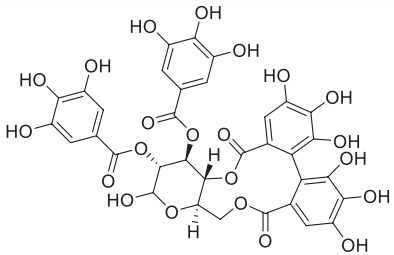
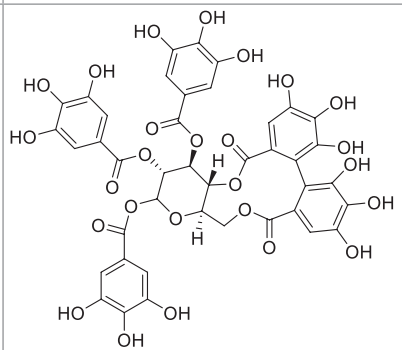
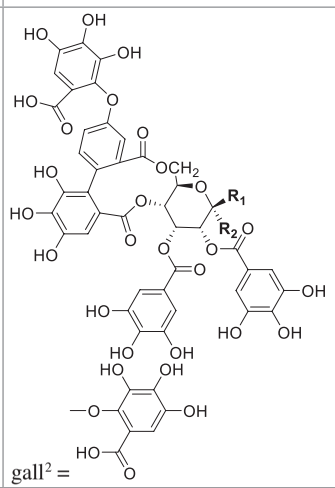
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 53 | Cyanidin-3,5-di-O-glucoside |  | *R. damascena* | Petal | [31] |
| | | | *R. chinensis* | Flower | [17] |
| | | | *R. pimpinellifolia* | Fruit Flower | [25] |
| 54 | Malvidin-3,5-diglucoside |  | *R. damascena* | Petal | [31] |
| 55 | Pelargonidin-3-O-glucoside chloride |  | *R. damascena* | Petal | [31] |
| 56 | Pelargonidin-3,5-di-O-glucoside chloride |  | *R. damascena* | Petal | [31] |
| | | | *R. chinensis* | Flower | [17] |

| | | | | | |
|---|---|---|---|---|---|
| 57 | Delphinidin chloride |  | *R. damascena* | Petal | [31] |
| | |  | | | |
| 58 | 6-demethoxy-4'-O-methylcapillarisin: R = $CH_3$ | | *R. rugosa* | Leaves | [12] |
| 59 | 6-demethoxycapillarisin: R = H | | *R. rugosa* | Leaves | [12] |
| 60 | Ellagic acid |  | *R. sempervirens* | Rose hip | [16] |
| | | | *R. Heckeliana* | Root | [26] |
| | | | *R. Heckeliana* | Root | [33] |
| 61 | 4′-O-arabinfuranosyl ellagic acid |  | *R. laevigata* | | [32] |
| 62 | 4′-O-xlyopyranosyl ellagic acid |  | *R. laevigata* | | [32] |

(continued)

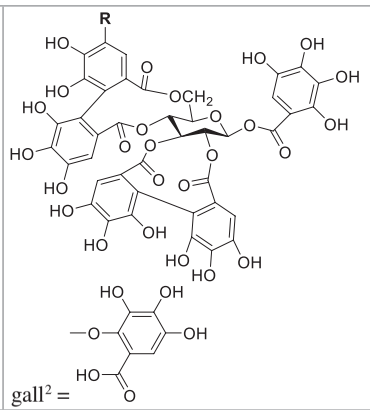
**Table 23.1** (continued)

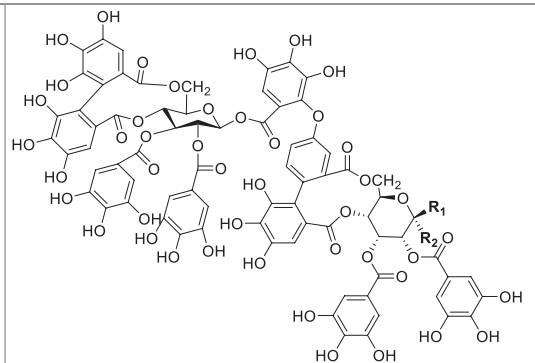
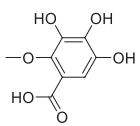
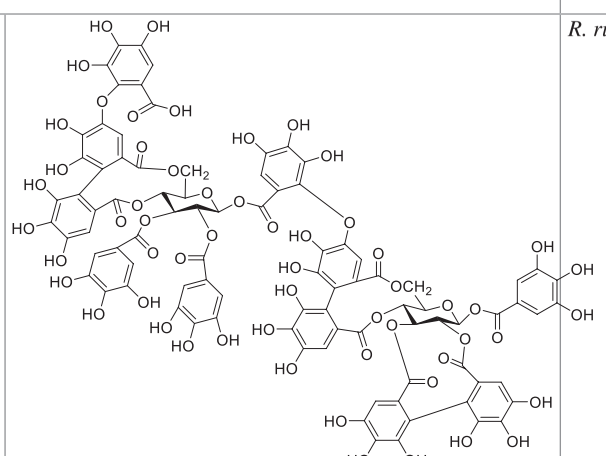
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 63 | (+)-pinoresinol |  | *R. multiflora* | Root | [34] |
| 64 | (+)-8-hydroxypinoresinol |  | *R. multiflora* | Root | [34] |
| 65 | (−)-dehydrodiconiferyl alcohol |  | *R. multiflora* | Root | [34] |
| 66 | (−)-olivil |  | *R. multiflora* | Root | [34] |

| | | | | | |
|---|---|---|---|---|---|
| 67 | Tellimagrandin I |  | *R. rugosa* | Flower petals | [12] |
| 68 | Tellimagrandin II |  | *R. rugosa* | Flower petals | [12] |
| | |  gall[2] = | | | |

(continued)

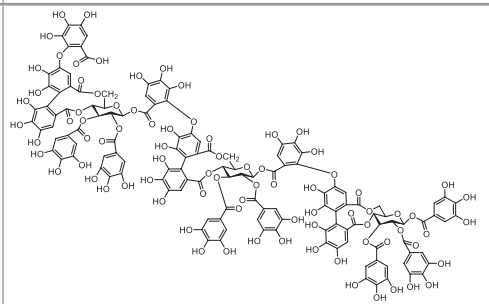
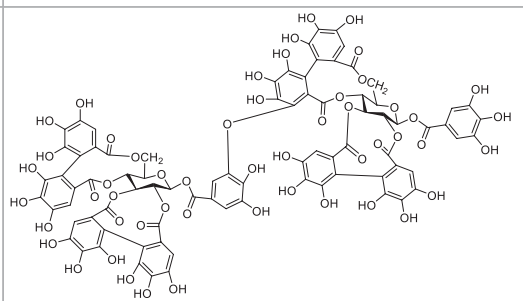
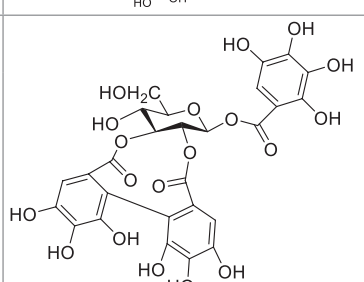
**Table 23.1** (continued)

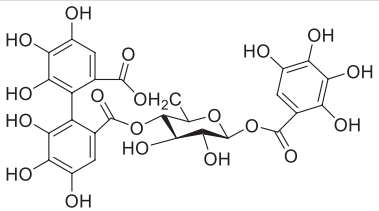
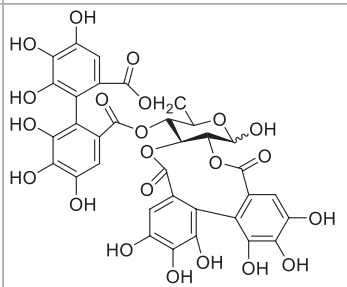
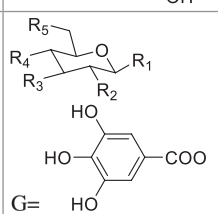
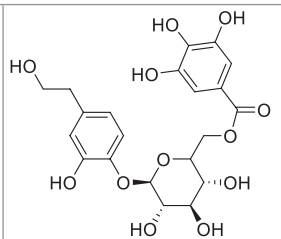
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 69 | Rugosin A: $R_1$ = O-gall, $R_2$ = H | | *R. rugosa* | Flower petals | [12] |
| 70 | Rugosin B: $R_1$ = H, $R_2$ = OH | | *R. rugosa* | Flower petals | [12] |
| | | | *R. chinensis* | Flower | [17] |
| | |  gall[2] = | | | |
| 71 | Rugosin C: R = O-gall | | *R. rugosa* | Flower petals | [12] |
| | | | *R. chinensis* | Flower | [17] |
| 72 | Casuarictin: R = OH | | *R. rugosa* | Flower petals | [12] |

| | | | | | |
|---|---|---|---|---|---|
| | |  gall[2] =  | | | |
| 73 | Rugosin D: $R_1$ = O-gall, $R_2$ = H | | *R. rugosa* | Flower petals | [12] |
| 74 | Rugosin E: $R_1$ = H, $R_2$ = OH | | *R. rugosa* | Flower petals | [12] |
| 75 | Rugosin F |  | *R. rugosa* | Flower petals | [12] |

(continued)

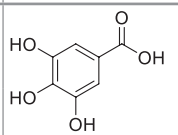
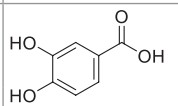
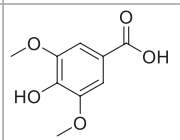
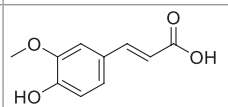
**Table 23.1** (continued)

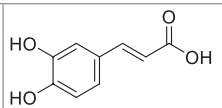
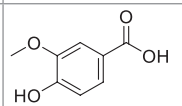
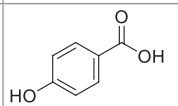
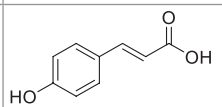
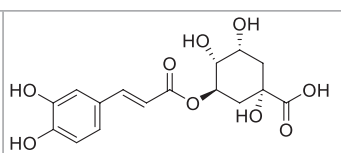
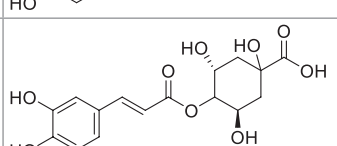
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 76 | Rugosin G |  | *R. rugosa* | Flower petals | [12] |
| 77 | Sanguiin |  | *R. chinensis* | Flower | [17] |
| 78 | Isostrictinin |  | *R. rugosa* | Flower petals | [12] |
| | | | *R. laevigata* | | [32] |

| | | | | | |
|---|---|---|---|---|---|
| 79 | Strictiniu |  | *R. rugosa* | Flower petals | [12] |
| 80 | Pedunculagin |  | *R. rugosa* | Flower petals | [12] |
| | |  | | | |
| 81 | 1,2,3,4,6-penta-O-galloyl-β-$_D$-glucoside: $R_1 = R_2 = R_3 = R_4 = R_5 = G$ | | *R. nutkana* | Fruit | [27] |
| 82 | 1,6-digalloyl-β-$_D$-glucoside: $R_1 = R_5 = G$, $R_2 = R_3 = R_4 = OH$ | | *R. nutkana* | Fruit | [27] |
| 83 | 3-hydroxyl-4-O-[(6″-O-galloyl)-glucopyranoside] Phenethyl alcohol |  | *R. laevigata* | | [32] |

(continued)

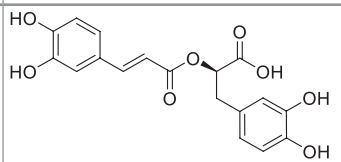
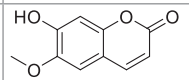
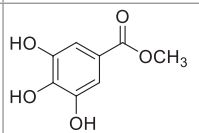
**Table 23.1** (continued)

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 84 | Gallic acid |  | *R. woodsii* | Rose hip with seed | [24] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. laevigata* | | [32] |
| | | | *R. heckeliana* | Root | [26] |
| | | | *R. sempervirens* | Rose hip | [16] |
| 85 | Protocatechuic acid |  | *R. woodsii* | Rose hip with seed | [24] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. sempervirens* | Rose hip | [16] |
| 86 | Syringic acid |  | *R. canina* | Fruit | [28] |
| 87 | Ferulic acid |  | *R. woodsia* | Rose hip with seed | [24] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. sempervirens* | Rose hip | [16] |

| | | | | | |
|---|---|---|---|---|---|
| 88 | Caffeic acid |  | *R. canina* | Fruit | [28] |
| | | | *R. woodsia* | Rose hip with seed | [24] |
| | | | *R. rubiginosa* | Herbal tea | [22] |
| | | | *R. heckeliana* | Root | [26, 33] |
| 89 | Vanillic acid |  | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| 90 | *p*-hydroxybenzoic acid |  | *R. sempervirens* | Rose hip | [16] |
| | | | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| 91 | *p*-coumaric acid |  | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. woodsia* | Rose hip with seed | [24] |
| | | | *R. heckeliana* | Root | [26] |
| 92 | *p*-coumaric acid-O-hexoside rutin | | *R. rubiginosa* | Herbal tea | [22] |
| 93 | 3-caffeoyl quinic acid (chlorogenic acid) |  | *R. woodsia* | Rose hip with seed | [24] |
| 94 | 4-O-caffeoylquinic acid |  | *R. rubiginosa* | Herbal tea | [22] |

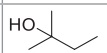
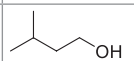
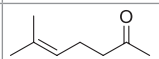
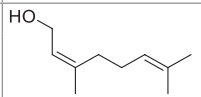
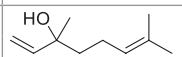
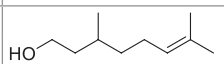
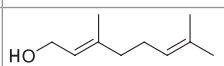
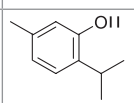
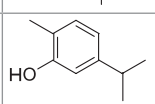
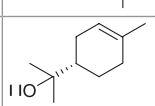
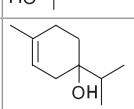
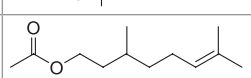
(continued)

**Table 23.1** (continued)

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 95 | Rosmarinic acid |  | *R. rubiginosa* | Herbal tea | [22] |
| 96 | Scopoletin |  | *R. heckeliana* | Root | [26] |
| 97 | Methyl gallate |  | *R. rugosa* | Leaves | [12] |
| 98 | Methyl gallate-hexoside | | *R. rubiginosa* | Herbal tea | [22] |
| 99 | Mono-O-galloyl-β-$_D$-glucopyranoside | | *R. chinensis* | Flower | [17] |
| 100 | di-O-galloyl-β-$_D$-glucopyranoside | | *R. chinensis* | Flower | [17] |
| 101 | Tri-O-galloyl-β-$_D$-glucopyranoside | | *R. chinensis* | Flower | [17] |
| 102 | Rosmarinic acid-O-hexoside | | *R. rubiginosa* | Herbal tea | [22] |
| 103 | Caffeoyl hexoside | | *R. woodsia* | Rose hip with seed | [24] |
| 104 | Phloretin-O-hexoside | | *R. rubiginosa* | Herbal tea | [22] |

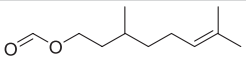
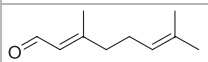
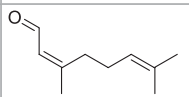
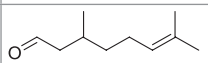
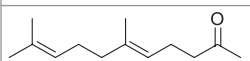
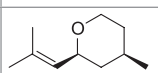
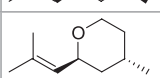
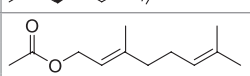
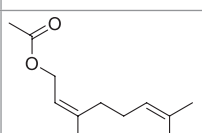
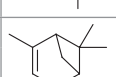
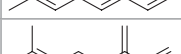
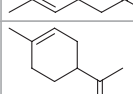
[a]Rose hip is the accessory fruit of the rose plant
[b]Galloyl group

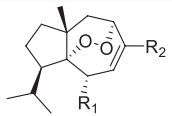
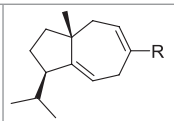
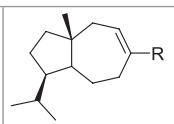
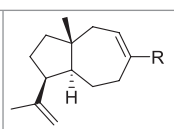
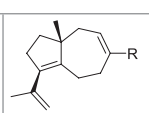
**Table 23.2** Components of obtained essential oils from different parts of *Rosa* species

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| | | **Hemi-, mono- and sesquiterpenoids** | | | |
| 1 | 2-methylbutan-2-ol |  | *R. rugosa* | Flower | [12] |
| 2 | 3-methylbutan-l-ol |  | *R. rugosa* | Flower | [12] |
| 3 | 6-methyl-hept-5-en-2-one |  | *R. rugosa* | Pollen | [12] |
| 4 | Nerol |  | *R. rugosa* | Leaves | [12] |
| | | | *R. centifolia* | Petal | [35] |
| 5 | Linalool |  | *R. rugosa* | Leaves | [12] |
| | | | *R. damascena* | Flower | [36] |
| 6 | Citronellol |  | *R. rugosa* | Floral parts | [12] |
| | | | *R. centifolia* | Petal | [35] |
| 7 | Geraniol |  | *R. rugosa* | Floral parts | [12] |
| | | | *R. centifolia* | Petal | [35] |
| | | | *R. hemisphaerica* | Aerial parts | [37] |
| 8 | Thymol |  | *R. foetida* | Flower | [38] |
| 9 | Carvacrol |  | *R. foetida* | Flower | [38] |
| 10 | α-terpineol |  | *R. rugosa* | Floral parts | [12] |
| 11 | Terpinen-4-ol |  | *R. damascena* | Flower | [36] |
| 12 | Citronellyl acetate |  | *R. rugosa* | Floral parts | [12] |
| | | | *R. damascena* | Flower | [36] |

(continued)

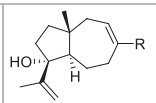
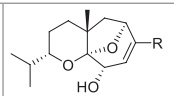
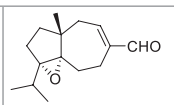
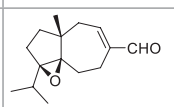
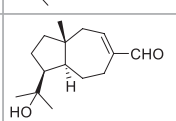
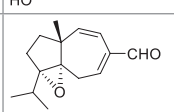
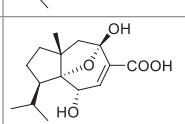
**Table 23.2** (continued)

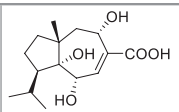
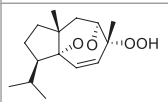
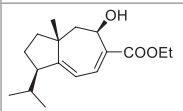
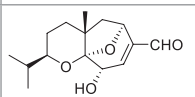
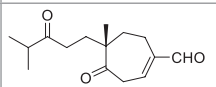
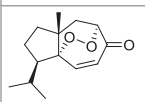
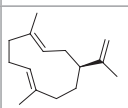
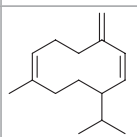
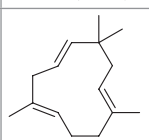
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 13 | Citronellyl formate |  | *R. rugosa* | Floral parts | [12] |
| 14 | Geranial |  | *R. rugosa* | Floral parts | [12] |
| 15 | Neral |  | *R. rugosa* | Floral parts | [12] |
| 16 | Citronellal |  | *R. rugosa* | Floral parts | [12] |
| 17 | Geranylacetone |  | *R. rugosa* | Floral parts | [12] |
| 18 | *cis*-rose oxide |  | *R. rugosa* | Floral parts | [12] |
| 19 | *trans*-rose oxide |  | *R. rugosa* | Floral parts | [12] |
| 20 | Geranyl acetate |  | *R. rugosa* | Floral parts | [12] |
| | | | *R. centifolia* | Petal | [35] |
| | | | *R. domescena* | Flower | [36] |
| 21 | Neryl acetate |  | *R. rugosa* | Floral parts | [12] |
| 22 | α-pinene |  | *R. rugosa* | Floral parts | [12] |
| | | | *R. damascena* | Flower | [36] |
| 23 | *E*-β-ocimene |  | *R. rugosa* | Floral parts | [12] |
| 24 | β-myrcene |  | *R. damascena* | Flower | [36] |
| 25 | Limonene |  | *R. rugosa* | Floral parts | [12] |

| | | | | |
|---|---|---|---|---|
| |  | | | |
| 26 | Rugosal A: $R_1$ = OH, $R_2$ = CHO | *R. rugosa* | Leaves | [12] |
| 27 | Rugosic acid A: $R_1$ = OH, $R_2$ = COOH | *R. rugosa* | Leaves | [12] |
| 28 | Rugosic acid A methyl ester: $R_1$ = OH, $R_2$ = COOMe | *R. rugosa* | Leaves | [12] |
| 29 | 1,5-epidioxy-2-hydfoxycarot-3-ene: $R_1$ = OH, $R_2$ = Me | *R. rugosa* | Leaves | [12] |
| 30 | 1,5-epidioxy-2--hydroperoxyearot-3-en-14-al: $R_1$ = OOH, $R_2$ = CHO | *R. rugosa* | Leaves | [12] |
| 31 | 1,5-epidioxy-2-hydroperoxyearot-3-en-14-oic acid: $R_1$ = OOH, $R_2$ = COOH | *R. rugosa* | Leaves | [12] |
| |  | | | |
| 32 | Carom-l,4-dienaldehyd: R = CHO | *R. rugosa* | Leaves | [12] |
| 33 | Carota-l,4-dienoic acid: R = COOH | *R. rugosa* | Leaves | [12] |
| 34 | Carota-l,4.Dien: R = Me | *R. rugosa* | Leaves | [12] |
| 35 | Carota-l,4-dienol: R = $CH_2OH$ | *R. rugosa* | Leaves | [12] |
| |  | | | |
| 36 | Daueenai: R = CHO | *R. rugosa* | Leaves | [12] |
| 37 | Daueene: R = Me | *R. rugosa* | Leaves | [12] |
| |  | | | |
| 38 | Isodaucenal: R = CHO | *R. rugosa* | Leaves | [12] |
| 39 | Isodaucenoic acid: R = COOH | *R. rugosa* | Leaves | [12] |
| 40 | Isodaucene: R = Me | *R. rugosa* | Leaves | [12] |
| 41 | Isodaucenol: R = $CH_2OH$ | *R. rugosa* | Leaves | [12] |
| |  | | | |

(continued)

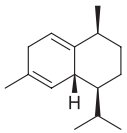
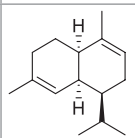
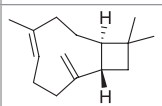
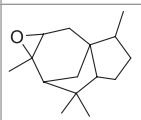
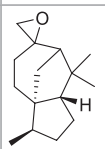
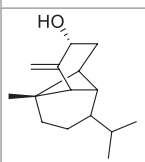
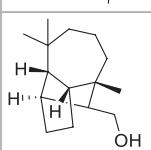
**Table 23.2** (continued)

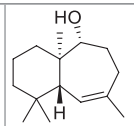
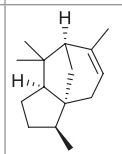
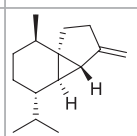
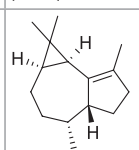
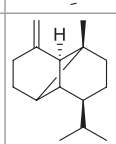
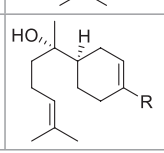
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 42 | 11,12-dehydrodaucenal: R = CHO | | *R. rugosa* | Leaves | [12] |
| 43 | 11,12--dehydrtxlaucenoic acid: R = COOH | | *R. rugosa* | Leaves | [12] |
| | |  | | | |
| 44 | 10-hydroxyisodaueenal: R = CHO | | *R. rugosa* | Leaves | [12] |
| 45 | 10-hydroxyisodaueene: R = Me | | *R. rugosa* | Leaves | [12] |
| | |  | | | |
| 46 | Rugosal D: R = CHO | | *R. rugosa* | Leaves | [12] |
| 47 | Rugosic acid D: R = COOH | | *R. rugosa* | Leaves | [12] |
| 48 | Epoxydaucenal A |  | *R. rugosa* | Leaves | [12] |
| 49 | Epoxydaucenal B |  | *R. rugosa* | Leaves | [12] |
| 50 | 11 -hydroxy-<br>12-hydroisodaucenal |  | *R. rugosa* | Leaves | [12] |
| 51 | Rosacarotanal |  | *R. rugosa* | Leaves | [12] |
| 52 | Rugosic acid B |  | *R. rugosa* | Leaves | [12] |

| | | | | | |
|---|---|---|---|---|---|
| 53 | Rugosic acid C |  | *R. rugosa* | Leaves | [12] |
| 54 | 1,5-epidioxy-4-hydroperoxycarot-2-ene |  | *R. rugosa* | Leaves | [12] |
| 55 | 5-hydroxyearota-l,3-dienoic acid Ethyl ester |  | *R. rugosa* | Leaves | [12] |
| 56 | Epirugosal D |  | *R. rugosa* | Leaves | [12] |
| 57 | Secocarotanal |  | *R. rugosa* | Leaves | [12] |
| 58 | 1,5-epidioxy-14-norearot-2-en-4--one |  | *R. rugosa* | Leaves | [12] |
| 59 | Germacrene A |  | *R. foetida* | Flower | [39] |
| 60 | Germacrene D |  | *R. damascena* | Flower | [36] |
| 61 | α-humulene |  | *R. damascena* | Flower | [36] |

(continued)

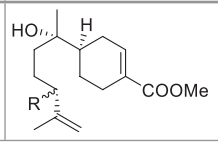
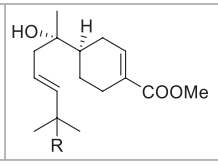
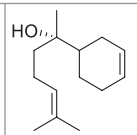
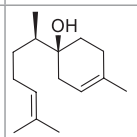
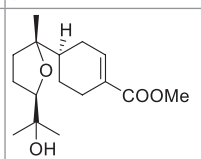
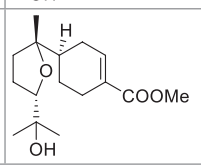
**Table 23.2** (continued)

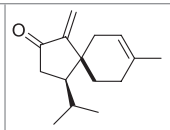
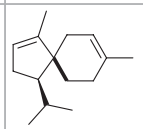
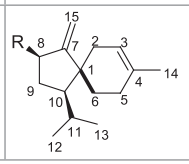
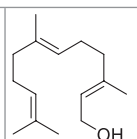
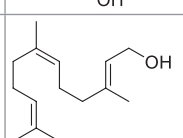
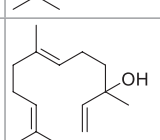
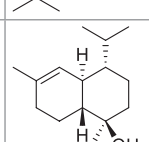
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 62 | *trans*-cadina-1,4-diene |  | *R. foetida* | Flower | [39] |
| 63 | α-cadinene |  | *R. foetida* | Flower | [39] |
| 64 | *trans*-caryophyllene |  | *R. damascena* | Flower | [36] |
| 65 | α-cedrene epoxide |  | *R. foetida* | Flower | [39] |
| 66 | β-cedrene epoxide |  | *R. foetida* | Flower | [39] |
| 67 | β-copaen-4α-ol |  | *R. foetida* | Flower | [39] |
| 68 | Longifolol |  | *R. foetida* | Flower | [39] |

| | | | | | |
|---|---|---|---|---|---|
| 69 | Allohimachalol |  | *R. foetida* | Flower | [39] |
| 70 | 2-epi-α-funebrene |  | *R. foetida* | Flower | [39] |
| 71 | β-cubebene |  | *R. foetida* | Flower | [39] |
| 72 | α-gurjunene |  | *R. foetida* | Flower | [39] |
| 73 | β-copaene |  | *R. foetida* | Flower | [39] |
| | |  | | | |
| 74 | (+)-4-*epi*-α-bisabolol: R = $CH_3$ | | *R. rugosa* | Leaves | [12] |
| 75 | Hamanasal A: R = CHO | | *R. rugosa* | Leaves | [12] |
| 76 | Hamanasie acid A: R = COOH | | *R. rugosa* | Leaves | [12] |
| 77 | Bisaborosaol A: R = COOMe | | *R. rugosa* | Leaves | [12] |

(continued)

**Table 23.2** (continued)

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| | |  | | | |
| 78 | Bisaborosaol C1/2: R = OOH | | *R. rugosa* | Leaves | [12] |
| 79 | Bisabomsaol E1/2: R = OH | | *R. rugosa* | Leaves | [12] |
| | |  | | | |
| 80 | Bisaborosaols D: R = OOH | | *R. rugosa* | Leaves | [12] |
| 81 | Bisabomsaol F: R = OH | | *R. rugosa* | Leaves | [12] |
| 82 | 7-nor-a-bisabolol |  | *R. rugosa* | Leaves | [12] |
| 83 | *epi*-β-bisabolol |  | *R. foetida* | Flower | [39] |
| 84 | Bisaborosaol B1 |  | *R. rugosa* | Leaves | [12] |
| 85 | Bisaborosaol B2 |  | *R. rugosa* | Leaves | [12] |

| | | | | | |
|---|---|---|---|---|---|
| 86 | Rosaeorenone |  | *R. rugosa* | Leaves | [12] |
| 87 | Acoradiene III |  | *R. rugosa* | Leaves | [12] |
| | |  | | | |
| 88 | Acora-3(4),7(15)miene: R = H | | *R. rugosa* | Leaves | [12] |
| 89 | Rosacorenol: R = OH | | *R. rugosa* | Leaves | [12] |
| 90 | *E,E* -famesol |  | *R. rugosa* | Flower | [12] |
| 91 | *E,Z*-famesol |  | *R. rugosa* | Flower | [12] |
| 92 | Nemlidol |  | *R. rugosa* | Flower | [12] |
| 93 | α-cadinol |  | *R. foetida* | Flower | [39] |

(continued)

**Table 23.2** (continued)

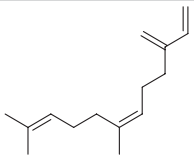
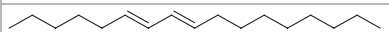
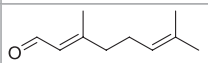
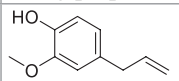
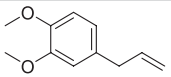
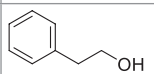
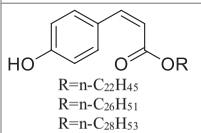
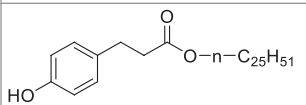
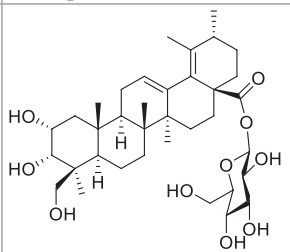
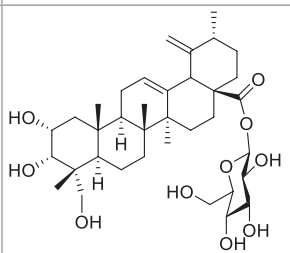
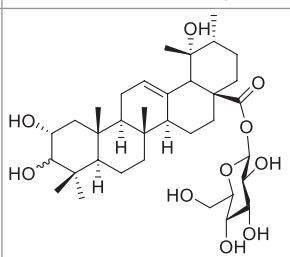
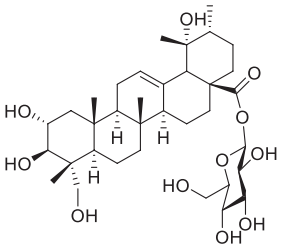
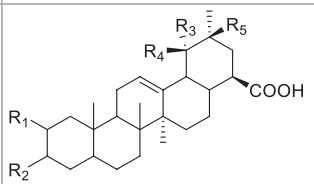
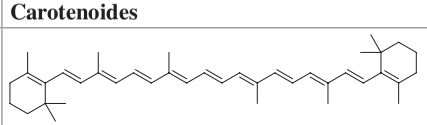
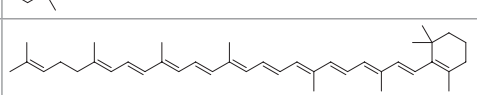
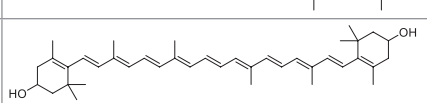
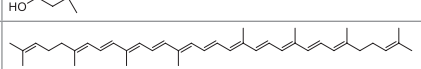
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 94 | (*Z*)-β-farnesene |  | *R. foetida* | Flower | [39] |
| 95 | 6(*E*),8(*E*)-heptadecadiene |  | *R. foetida* | Flower | [39] |
| 96 | *E*-citral |  | *R. damascena* | Flower | [36] |
| | | **Phenylpropanoids** | | | |
| 97 | Eugenol |  | *R. rugosa* | Floral fragrance | [12] |
| 98 | 4-methyleugenol |  | *R. rugosa* | Floral fragrance | [12] |
| 99 | β-phenylethyl alcohol |  | *R. rugosa* | Petals | [12] |
| 100 | 4′-hydroxy-Z-cinnamic acid alkyl esters |  R = n-$C_{22}H_{45}$ R = n-$C_{26}H_{51}$ R = n-$C_{28}H_{53}$ | *R. rugosa* | Leaves | [12] |
| 101 | 4′-hydroxy-2,3-dihydrocinnamic acid pentacosyl Ester |  | *R. rugosa* | Leaves | [12] |

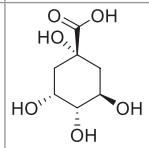
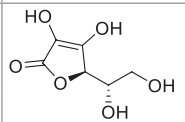
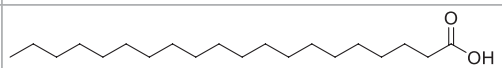
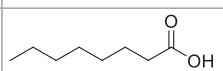
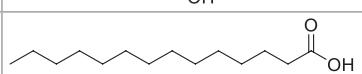
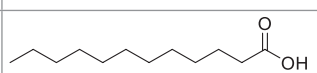
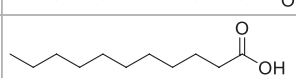
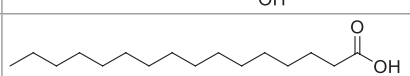
**Table 23.3** Other compounds extracted from different parts of *Rosa* species

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| | | **Triterpenoids** | | | |
| 1 | 2α,3α,24-trihydroxyurs-12,18-dien28-oic acid β-$_D$-glucopyranosyl ester |  | *R. laevigata* | Root | [40] |
| 2 | 2α,3α,23-trihydroxyurs-12,19(29)-dien-28-oic acid β-$_D$-glucopyranosyl ester |  | *R. laevigata* | Root | [40] |
| | |  | | | |
| 3 | 2α,3β,19α-trihydroxyurs-12-en-28-oic acid β-$_D$.glucopyranosyl ester: β | | *R. laevigata* | Root | [40] |
| 4 | 2α,3α,19α-trihydroxyurs-12-en-28-oic acid β-$_D$-glucopyranosyl ester: α | | *R. laevigata* | Root | [40] |

(continued)

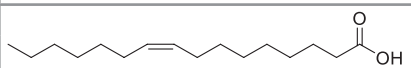
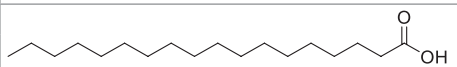
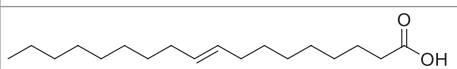
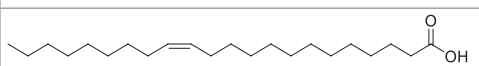
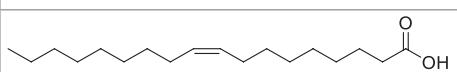
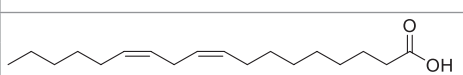
**Table 23.3** (continued)

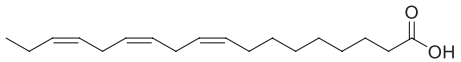
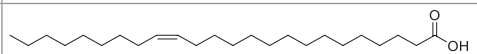
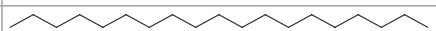
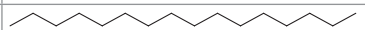
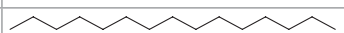
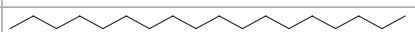
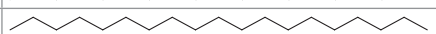
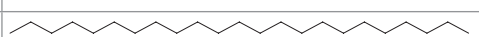
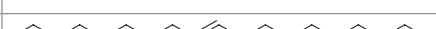
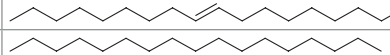
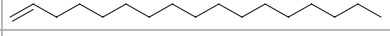
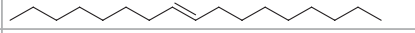
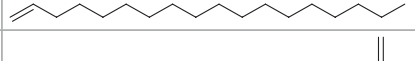
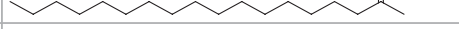
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 5 | 2α,3β,19α,23-tetrahydroxyurs-12-en-28--oic acid β-D-glucopyranosyl ester |  | *R. laevigata* | Root | [40] |
| | |  | | | |
| 6 | Tormentic acid: $R_1$=, $R_2$=, $R_3$ = OH, $R_4$ = $CH_3$, $R_5$ = H | | *R. nutkana* | Fruit | [27] |
| 7 | Euscaphic acid: $R_1$=, $R_2$=, $R_3$ = OH, $R_4$ = $CH_3$, $R_5$ = H | | *R. nutkana* | Fruit | [27] |
| 8 | Ursolic acid: $R_1$ = H, $R_2$=, $R_3$ = H, $R_4$ = $CH_3$, $R_5$ = H | | *R. nutkana* | Fruit | [27] |
| 9 | Maslinic acid: $R_1$=, $R_2$=, $R_3$ = H, $R_4$ = H, $R_5$ = $CH_3$ | | *R. nutkana* | Fruit | [27] |
| | | **Carotenoides** | | | |
| 10 | β-Carotene |  | *R. pimpinellifolia* | Fruit<br>Flower | [25] |
| 11 | γ-Carotene |  | *R. pimpinellifolia* | Fruit | [25] |
| 12 | Zeaxanthin |  | *R. pimpinellifolia* | Fruit<br>Flower | [25] |
| 13 | Lycopene |  | *R. pimpinellifolia* | Fruit | [25] |

| | | | | | |
|---|---|---|---|---|---|
| | | **Hydrocarbons and derivatives** | | | |
| 14 | Quinic acid |  | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| 15 | Ascorbic acid (vitamin C) |  | *R. canina*<br>*R. phoenicia* | Rose hip with seed | [18] |
| | | | *R. rubiginosa* | Herbal tea | [22] |
| | | | *R. nutkana* | Fruit | [27] |
| | | | *R. centifolia*<br>*R. agrestis*<br>*R. pulverulenta*<br>*R. montana* | Fruit | [41] |
| 16 | Arachidic acid |  | *R. pulverulenta*<br>*R. canina*<br>*R. heckeliana* | Seed | [42] |
| | | | *R. agrestis*<br>*R. canina*<br>*R. rubiginosa* | Rose hips | [43] |
| 17 | Caprylic acid |  | *R. montana* | Seed | [44] |
| 18 | Myristic acid |  | *R. montana* | Seed | [44] |
| 19 | *n*-dodecanoic acid (lauric acid) |  | *R. foetida* | Flower | [38] |
| 20 | Undecanoic acid |  | *R. montana* | Seed | [44] |
| 21 | Palmitic acid |  | *R. montana* | Seed | [44] |
| | | | *R. agrestis*<br>*R. canina*<br>*R. rubiginosa* | Rose hips | [43] |
| | | | *R. pulverulenta*<br>*R. canina*<br>*R. Heckeliana* | Seed | [42] |

(continued)

**Table 23.3** (continued)

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 22 | Palmitoleic acid |  | *R. montana* | Seed | [44] |
| | | | *R. pulverulenta*<br>*R. canina*<br>*R. Heckeliana* | Seed | [42] |
| 23 | Stearic acid |  | *R. montana* | Seed | [44] |
| | | | *R. agrestis*<br>*R. canina*<br>*R. rubiginosa* | Rose hips | [43] |
| | | | *R. pulverulenta*<br>*R. canina*<br>*R. Heckeliana* | Seed | [42] |
| 24 | Elaidic acid |  | *R. montana* | Seed | [44] |
| 25 | Erucic acid |  | *R. agrestis*<br>*R. canina*<br>*R. rubiginosa* | Rose hips | [43] |
| 26 | Oleic acid |  | *R. montana* | Seed | [44] |
| | | | *R. agrestis*<br>*R. canina*<br>*R. rubiginosa* | Rose hips | [43] |
| | | | *R. acicularis* | Seed | [45] |
| | | | *R. pulverulenta*<br>*R. canina*<br>*R. heckeliana* | Seed | [42] |
| 27 | Linoleic acid |  | *R. montana* | Seed | [44] |
| | | | *R. agrestis*<br>*R. canina*<br>*R. rubiginosa* | Rose hips | [43] |
| | | | *R. acicularis* | Seed | [45] |
| | | | *R. pulverulenta*<br>*R. canina*<br>*R. Heckeliana* | Seed | [42] |

| | | | | | |
|---|---|---|---|---|---|
| 28 | Linolenic acid |  | *R. pulverulenta*<br>*R. canina*<br>*R. heckeliana* | Seed | [42] |
| | | | *R. agrestis*<br>*R. canina*<br>*R. rubiginosa* | Rose hips | [43] |
| | | | *R. acicularis* | Seed | [45] |
| 29 | Nervonic acid |  | *R. montana* | Seed | [44] |
| 30 | *n*-heptadecane |  | *R. foetida* | Flower | [38] |
| | | | *R. foetida* | Flower | [39] |
| 31 | *n*-nonadecane |  | *R. foetida* | Flower | [38] |
| | | | *R. centifolia* | Petal | [35] |
| 32 | *n*-hexadecane |  | *R. foetida* | Flower | [39] |
| | | | *R. damascena* | Flower | [36] |
| 33 | *n*-pentadecane |  | *R. foetida* | Flower | [39] |
| 34 | *n*-octadecane |  | *R. foetida* | Flower | [39] |
| 35 | *n*-nonadecane |  | *R. foetida* | Flower | [39] |
| | | | *R. hemisphaerica* | Aerial part | [37] |
| 36 | *n*-heneicosane |  | *R. foetida* | Flower | [39] |
| | | | *R. centifolia* | Petal | [35] |
| | | | *R. hemisphaerica* | Aerial part | [37] |
| 37 | Tricosane |  | *R. centifolia* | Petal | [35] |
| | | | *R. hemisphaerica* | Aerial part | [37] |
| 38 | Pentacosane |  | *R. centifolia* | Petal | [35] |
| | | | *R. hemisphaerica* | Aerial part | [37] |
| 39 | 9-nonadecene |  | *R. hemisphaerica* | Aerial part | [37] |
| 40 | Heptadecane |  | *R. hemisphaerica* | Aerial part | [37] |
| 41 | 1-heptadecene |  | *R. foetida* | Flower | [38] |
| 42 | 8-heptadecene |  | *R. hemisphaerica* | Aerial part | [37] |
| 43 | 1-octadecene |  | *R. foetida* | Flower | [39] |
| 44 | 2-methyl-1-octadecene |  | *R. foetida* | Flower | [39] |
| 45 | (Z)-2-eicosene |  | *R. foetida* | Flower | [39] |

(continued)

**Table 23.3** (continued)

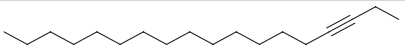
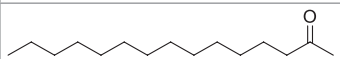
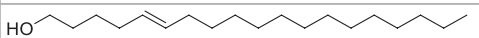
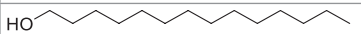
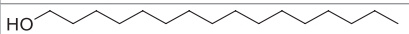
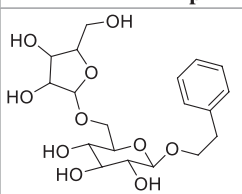
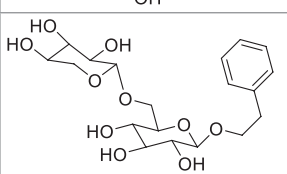
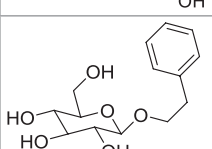
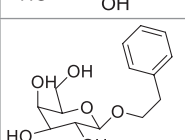
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 46 | 3-octadecyne |  | *R. hemisphaerica* | Aerial part | [37] |
| 47 | 2-pentadecanone |  | *R. foetida* | Flower | [39] |
| 48 | 5-nonadecen-1-ol |  | *R. hemisphaerica* | Aerial part | [37] |
| 49 | *n*-tetradecanol |  | *R. foetida* | Flower | [39] |
| 50 | 1-hexadecanol |  | *R. foetida* | Flower | [39] |
| | | **Miscellaneous compounds** | | | |
| 51 | 2-phenylethyl 6-*O*-α-$_L$-arabinofuranosyl- β-$_D$-glucopyranoside |  | *R. damascena* | Flower | [46] |
| 52 | 6-*O*-β-$_D$-xylopyraranosyl- β-$_D$--glucopyranoside |  | *R. damascena* | Flower | [46] |
| 53 | 2-phenylethyl β-$_D$-glucopyranoside |  | *R. damascena* | Flower | [46] |
| 54 | β-$_D$-galactopyranoside |  | *R. damascena* | Flower | [46] |

**Table 23.4** Some of the most important ethnobotanical uses of *Rosa* species in different countries

| No | *Rosa* species | Vernacular name | Country | Part used | Ethnobotanical uses | References |
|---|---|---|---|---|---|---|
| 1 | *R. acicularis* Lindl. | Khoht'an | Alaska | Hips, petals | Jelly and jam | [74] |
| 2 | *R. agrestis* Savi | – | Croatia | Fruit | Eaten by coastal households | [75] |
| 3 | *R. alba* L. | Chittagulab | Pakistan | Flower | Treatment of diabetes | [76] |
| 4 | *R. beggeriana* Schrenk ex Fisch. & C.A.Mey. | Korik | Iran | Flower | Treatment of cardiac disorders | [60] |
| | | Soori | Pakistan | Fruit, branches | Tonic, source of fuel wood (fruits), fencing (branches) | [72] |
| | | Nastaran | Iran | Fruit | Antihypertensive, diuretic, treatment of kidney stone | [59] |
| | | Chaphirosa | Bolivia | Flowers, root | Ophthalmia and sinusitis treatment (flowers), psychological depression (root) | [77] |
| | | Kuin | India | Fruits, leaves, flowers | Edible (fruits), fodder, fencing (leaves), sweet smell (flower) | [73] |
| | | Nastaran | Iran | Fruit | Treatment of blood pressure, kidney stones, diuretic | [56] |
| | | Shilan | Iraq | Flowers, fruits | Diuretic, blood cell disorders, sedation | [78] |
| | | Quaregl, uva raja | Italy | Leaves, fruits, fresh flowers | Conjunctivitis and redden eyes treatment (leaves)<br>Eaten fresh or in jam (fruits) | [79] |
| 5 | *R. canina* L. | Ward Barri | Lebanon | Fruit, root | Astringent, antidiarrheal and antiscorbutic (fruit juice)<br>Dyspnea and rheumatism treatment (decoction of roots and fruits) | [55] |
| | | Trendafiliiege¨r | Macedonia | Flowers, fruits | Respiratory problems (cough, bronchitis and cold) | [80] |
| | | ˇSipurak, divljaruˇza | Montenegro | Fruit | Colds, vitamin C deficiency, urinary tract disorders and kidney stones | [81] |
| | | Nar lbared | Morocco | Leaf, flower, fruit | Laxative, febrifuge, diuretic, vermifuge, anemia | [82] |
| | | Sipkinje, ˇSipak | Serbia | Fruit | Astringent, tonic herb, rich in vitamins A, B, C and K. used for colds and influenza (tea) | [83] |
| | | Kusburnu, it burnu, Okuzgotu | Turkey | Fruit | Cold, flu, cough<br>Diabetes, appetizer | [48] |
| | | Nametek | Uzbekistan | Fruit | Against common cold | [84] |
| 6 | *R. centifolia* L. | Rosa de Castilla | Mexico | Flower | Colic, spots, eyes, tea (oral) and used to wash the affected part (topical) | [68] |
| | | Rosa de castilla | Ecuador | Flower | Conjunctivitis, relaxant | [69] |
| 7 | *R. chinensis* Jacq. | – | China | Flower | Menstrual disorder, menstrual colic treatment, ornamental plant | [52] |
| | | Gulab | Pakistan | Flower | Treatment of abdominal pain and pneumonia | [63] |

(continued)

**Table 23.4** (continued)

| No | *Rosa* species | Vernacular name | Country | Part used | Ethnobotanical uses | References |
|---|---|---|---|---|---|---|
| 8 | *R.* × *damascena* Herrm. | Gulab | Afghanistan | Flower | Treatment of abdominal pain, anorexia, pneumonia, earache | [62] |
| | | Gul | Turkey | Flower | Pain relief, skin problems, gastrointestinal diseases | [11] |
| | | Gole Mohammadi | Iran | Flower | Anti-hemorrhoid, laxative, calmative | [59] |
| 9 | *R. davurica* Pall. | Saengyeolgwinamu | Korea | Fruit | Gastro enteric disorder | [51] |
| 10 | *R. dumalis* Bechst. | – | Turkey | Fruit | Laxative, diuretics, treatment of cold and pain of menstruation, making marmalade or jam | [10] |
| 11 | *R. foetida* Herrm. | Nastaran zard | Iran | Petals | Exertion of kidney and bladder stones | [85] |
| | | Gole zard | Iran | Flower | Ovary tonic, emmenagogue | [59] |
| | | – | Turkey | Fruit, flower | Laxative, ornamental shrubs | [10] |
| 12 | *R. gallica* L. | Ormang¨ul¨u | Turkey | Flower | Anti-diarrhea | [86] |
| | | Golsorh | Iran | Flower | Aphrodisiac, menopause hot flashes | [87] |
| | | Gul | Turkey | Fruit | Laxative, soothing, antiseptic, treatment of psoriasis | [10] |
| 13 | *R. gymnocarpa* Nutt. exTorr. & A.Gray | Baldhip rose | Canada | Hips, leaves and stalks | Tea | [88] |
| 14 | *R. heckeliana* Tratt. | Şilank | Turkey | Fruit | Antitussive, colds | [49] |
| 15 | *R. hemisphaerica* Herrm. | Okuzgotu | Turkey | Fruit | Eaten as fresh, foodstuff | [89] |
| 16 | *R.indica* L. | Ghulab | Pakistan | Floral part | Anti-constipation and abdominal problems | [64] |
| 17 | *R. laevigata* Michx. | Jinyingzi | China | Leaf, fruit | Promoting blood circulations, eliminating stasis to stop pain, dispelling wind and cold, removing dampness, clearing away heat and toxic, the fruit is edible materials | [54] |
| 18 | *R. macrophylla* Lindl. | Seghu | Nepal | Fruit | Eaten raw as fruit or a snack | [90] |
| 19 | *R. montana* Chaix ex Vill. | – | Turkey | Fruit | Marmalade, jam and fruit juice | [10] |
| 20 | *R. moschata* Herrm. | Gangligulab | Pakistan | Flowers, fruits | Anti-constipation | [65] |
| 21 | *R. multiflora* Thunb. | Jjilrekkot | Korea | Root | Neuralgia | [51] |
| 22 | *R. nutkana* C.Presl | Nootka rose | Canada | Hips, leaves, branches, roots, petals, inner bark | Tonic tea, diarrhea (fruits), poultice for bee stings (leaves), eyewash for sore eyes (roots) | [70] |
| 23 | *R. phoenicia* Boiss. | Kusburnu, it burnu, Okuzgotu | Turkey | Fruit | Cold, flu, diabetes | [48] |
| 24 | *R. pisiformis* (H.Christ) Sosn. | – | Turkey | Fruit, flower | Laxative, ornamental values | [10] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 25 | *R. pulverulenta* M.Bieb. | Ward Dabek | Lebanon | Fruit | Astringent, anti-diarrheal, diuretic and antiscorbutic (fruit juice) | [55] |
| 26 | *R. roxburghii* Tratt. | Cili | China | Fruit | Detoxifying effect, inducing saliva and slakes thirst, digestion | [54] |
| 27 | *R. rubiginosa* L. | Rosa musqueta | Argentina | Fruit | Edible, antitussive, dermatological benefits | [91] |
| 28 | *R. rugosa* Thunb. | Mei Gui | Japan | Petals | Antidiarrheal and haemostatic agents | [12] |
| 29 | *R. sempervirens* L. | Reusa | Italy | Petal, fruit | Headache (petals), ophthalmic disorders (fruits) | [50] |
| 30 | *R. sericea* Wall. ex Lindl. | Jangly Gulab | India | Root, flower, fruit | Uterine diseases, edible (fruits) | [67] |
| 31 | *R. spinosissima* L. | – | Turkey | Fruit, flower | Tea substitute | [10] |
| 32 | *R. villosa* L. | – | Turkey | Hips | Laxative, diuretics, treatment of cold and dysmenorrhea, marmalade, tea substitute | [10] |
| 33 | *R. webbiana* Wall. ex Royle | Siamarpho | Pakistan | Floral part and bark | Skin inflammation | [64] |
| | | Shuli | India | Fruit | Treatment of impotency and jaundice | [66] |
| | | Chyrir, Röhloy | Afghanistan | Hips, wood | Mixed with mother's milk and applied to children's ear-ache (ashes of hips), stomach trouble, fever, bloody cough and high blood pressure (decoction of hips), used as fire-wood | [61] |
| 34 | *R. woodsii* Lindl. | Champes, rosa de costilla | USA | Fruit, flower | Eaten raw or used to make jelly (fruits), babies colic treatment, alleviate intestinal cramps (tea of flower petals) | [71] |

tional Chinese medicine, the flowers of *R. chinensis* are used to curing of menstrual disorder and menstrual colic. It is originated and widely cultivated in China. It is considered to be an important ancestor of modern roses. It is not only a popular ornamental plant, but also is widely used in food and cosmetic industry [52, 53]. Furthermore, the fruit of *R. roxburghii* is edible and used for digestion, heat-clearing and detoxifying effect [54]. In Lebanon, the fruits of *R. canina* and *R. pulverulenta* have been recognized to treat various diseases. The fruit juice of them is orally used as astringent, anti-diarrhoeic, diuretic and antiscorbutic. Moreover, decoction of roots and fruits of *R. canina* is taken to treat dyspnoea and rheumatism [55]. In Iranian traditional medicine, the fruits of *R. canina* have been recommended as diuretic and curing of blood pressure and kidney stones [56]. *R. damascena*, known as GoleMohammadi, is one of the most important species of this genus in Iran. Apart from the use of it as ornamental plants in houses, gardens, and parks, it is principally cultivated for using in medicine, food and perfume industry [57, 58]. The flower of *R. damascena* has been advised as anti-hemorrhoid, laxative and calmative. The flowers of *R. foetida*, known as GoleZard, are consumed as ovary tonic and emmenagogue [59]. Furthermore, the flowers of *R. beggeriana* has been recognized to curing of cardiac disorders [60]. In Afghanistan, the ashes of hips of *R. webbiana* is mixed with mother's milk and applied to children's ear-ache. Decoction of hips is advised for stomach trouble, fever, bloody cough and high blood pressure [61]. Moreover, the flower of *R. damascena* is applied as treatment of abdominal pain, anorexia, pneumonia and earache [62]. Several species of this genus are used in the Pakistan folk medicine and many reports are found highlighting their ethnobotanical and traditional uses. For example, the flowers of *R. chinensis,* known as Gulab, are used for treatment of abdominal pain and pneumonia [63]. *R. indica* flowers are used as curing of constipation and abdominal problems [64]. The fruits and flowers of *R. moschata,* known as Gangligulab, are consumed for treatment of constipation [65]. *R. webbiana*, is commonly known as Shuli, in India and its fruit has been consumed to curing of jaundice and impotency [66]. Furthermore, *R. sericea* has been recognized to treat of uterine diseases [67]. In Argentina, the fruit of *R. rubiginosa* is edible and used as antitussive and dermatologic [67]. In Ecuador and Mexico, the flowers of *R. centifolia,* have been recommended as relaxant, conjunctivitis and treat of colic [68, 69]. *Rosa nutkana* is native to British Columbia, Canada. Indigenous people have traditionally used it as a source of food and medicine. The hips (fruits) are consumed for children suffering from diarrhea. The leaves are used as a poultice for bee stings and the roots used as eyewash for sore eyes. Local women have used the roots for the treatment of sore throats. The branches are applied to treat various women's complaints, diarrhea, and vomiting. A tonic tea is made from the fruits, leaves, petals, branches, and inner bark [70]. In America, the fruits of *R. woodsii* were eaten raw or used to make jelly. The flower petals of it are used in making a tea which is given to babies with colic. The tea is also used to alleviate "torzones de tripas" (intestinal cramps) [71]. The most frequent traditional applications of *Rosa* taxa in different countries seems to be treatment of kidney ailments, menstrual disorder, gastrointestinal diseases, nervous ailments, respiratory diseases, skin problems, abdominal complaints, diabetes and jaundice. *Rosa* taxa are reportedly used for a multitude of ethnobotanical purposes besides nutritional and medicine consumption. Various species of *Rosa* have ornamental values such as *R. canina, R. foetida, R. villosa, R. elymaitica* and *R. hemisphaerica* [10]. *Rosa beggeriana* and *R. canina* are also used to build natural fences [72, 73]. Furthermore, *R. beggeriana* and *R. webbiana* are used as fuel [61, 72]. Table 23.4 provides a summary of the ethnobotanical and traditional applications of *Rosa* taxa in various cultures of the world.

## 23.5 *Rosa* in Islamic Traditional Medicine (ITM)

### 23.5.1 Nature of *Rosa* spp. Described in ITM

Among different species of *Rosa*, 3 are represented in ITM, which are as follow: (a) *R. canina* L. (Nastaran), (b) *R. moschata* Herrm. (Nasrin), (c) *R.damascena* (Ward) [92]; In Canon of Avicenna, the temperament (Mizaj) of *R. canina* and *R. moschata* is described as hot and dry and for *R. domescena* is cold and dry [93]. In most of the studied books Nasrin and Nastaran have been mentioned as a temperate warmer, resolving (mohallel), attenuant (molattef), deobstruent (mofatteh), purifying (monaghi) and also bile and phlegm laxative [93–98].Ward is astringent and potentially is attenuate, deobstruent, resolving and tonic [99].

The seeds of Ward are more astringent and desiccant (Table 23.5).

### 23.5.2 Medicinal Uses of *Rosa* spp. in ITM

#### 23.5.2.1 Gastrointestinal System

One of the most important applications of Nasrin *(R. canina* and *R. moschata)* in ITM is due to gastrointestinal problems. It is mentioned as anti-gastritis, anti-nausea, anti-flatulence and anti-dyspepsia in the form of decoction, infusion, powder, jam and compote [95, 97, 98]. Avicenna, in his book (Canon), together with several other scientists such as Ibn Nafis Qarshi and Ibn Al-Baytâr recommended *R. canina* preparations as anti-nausea and vomiting. To the mentioned purpose fourteen grams of wild *R. canina* flower is recommended [93, 95, 96]. It is also mentioned to be effective for the treatment of hiccough [93, 95, 96, 100]. Another medicinal use of Nasrin in ITM is treating constipation [99]. Ward (*R. domescena*) could improve internal body parts and act as stomach moisture dehumidifier [99]. To this purpose, one of the famous formulation of *R. domescena* in ITM, "Gol angabin" (rose

**Table 23.5** Major ITM books and their authors that described medicinal properties and nature of *Rosa* spp.

| No | Book | Language | Author | Living period |
|---|---|---|---|---|
| 1 | Al-Shamel fi al-Tibbe | Arabic | Ibn Nafis Qarshi | 1210-1288 A.D. |
| 2 | Al-Qânun fi al-Tibbe | Arabic | Ibn Sina, HA. | 980–1037 A.D. |
| 3 | Zakhireh khârazmshâhi | Persian | Jorjâni, SI. | 1042–1136 A.D. |
| 4 | Al-Aghrâz al-Tibbe wa al-Mabâhethi al-Alâiiah | Persian | Jorjâni, SI. | 1042–1136 A.D. |
| 5 | Al-Hâwi fi al-Tibbe | Arabic | Razi, MZ. | 865–925 A.D. |
| 6 | Al-Mo'tamad fi al-adwiyah al-Mofradah | Arabic | Torkamâni YO. | 1222-1294 A.D. |
| 7 | Al-Jâmee le Mofradât al-Adwiah wa al-Aghziah | Arabic | Ibn Al-Baytâr, AA. | 1193–1248 A.D. |
| 8 | Tohfah al-Momenin | Persian | Husseini Tonekaboni, MM. | 17 $^{th}$ century |
| 9 | Al-Abniyah an Haqâyeq al-Adwiah | Persian | Herawi, AR. | 10 th century |
| 10 | Hadiqat al-Azhâr fi Mâhiyyat al-ushb wa al-uqqâr | Arabic | Ghasani, AM. | 1547–1611 A.D. |
| 11 | Al-Saydanah | Arabic | Biruni, MA. | 937–1048 A.D. |
| 12 | Makhzan al-Adwiah | Persian | Aqili Khorasani, MH. | 18 th century |
| 13 | Tadhkirat Oli al-Albâb wa al-Jâme le al-Ajb al-Ujâb | Arabic | Antaki, DO. | 1535–1599 A.D. |
| 14 | Qarabâdin Kabir | Persian | Aqili Khorasani, MH. | 18 th century |

honey) which is contain of *R. domescena* flower and honey and has the temperament of hot and dry, is recommended. Rose honey with *Carum carvi* improves food digestion [101]. Topical application of *R. domescena* with *Lens esculenta* and *Myrtus communis* could improve ulcers [102]. Avicenna believes that oral and topical application of *R. domescena oil* reduces the stomach inflammation also can help the digestion. In his view point oral application of rose water is a good remedy for gastric weakness. It reduces the pain of rectal diseases in topical usage when some scars happen [93]. *R. domescena* is also recommended to improve some oral diseases such as thrush aphtha and smallpox [102].

#### 23.5.2.2 Liver and Kidney

The beneficial properties of Nasrin oil for the treatment of liver obstructions have been described by one of the ITM scientists, Aqili Alavi Khorasani in his book (Qarabadine-kabir) [103]. Also, in some of the reviewed books, it increases bile release [97, 101].

Gol-angabin is recommended for fracturing the bladder and kidney stones and to remove urinating difficulty [101].

#### 23.5.2.3 Central and Peripheral Nervous System

Nasrin was believed to be useful for cleaning brain from waste humors. Ibne Nafis Qarashi in his famous book (Al-Shamel fi al-Tibbe) states that it has potentials for warming the head as well as resolving dense wind and ejects it by sneeze. It has been mentioned as a brain obstruction opener [96]. In some of the investigated books, both Nasrin and Ward are believed to be useful as brain and sense tonic, to this purpose they are smelled continuously [93, 96, 97, 99, 104]. *R. domescena* oil is mentioned to improve brain hot and cold swollen if added to vinegar and use it frequently in topical form [95]. Gol-angabin when used after taking food could block the vapor to brain [101]. *R. domescena* is tonic for nervous system and can prevent insomnia [99]. It is recommended for hot as well as wet headache [93, 102]. In ITM, Nasrin is warm and dry in second grade, and topical application of its oil can improve nervous diseases especially in elderly and wet people [102]. It is also reported to reduce cold nervous pains [93, 95, 96]. For this reason, one of the most cited applications of Nasrin in ITM is its use in cold headache. Topical administration of rose oil on frontal and temporal areas was recommended to alleviate headache; to this purpose Razes and Jorjani prescribed it in combination with *Aloe* and vinegar [105].

#### 23.5.2.4 Heart and Arterials

The beneficial properties of *Rosa* spp. to improving heart have been described by some ITM scientists such as Aqili Alavi Khorasani, in his book, *Makhzan al-Adwiah* (Drug Treasure). He believes that beside improving the heart, Nasrin is good for the treatment of cold tachycardia and [99] *R. domescena* (Ward) is highly recommended to improve hot tachycardia; to this purpose it is suggested to be drinked slowly [99]. Nasrin has been reported to open obstructions and could exit chest (including heart) flatus [96].

Ibn Nafis believes that topical application, drinking and smelling of rose water (from *R. domescena*) could improve heart and smelling of it has beneficial properties for hot tachycardia [96].

#### 23.5.2.5 Respiratory System

One of the important and most cited applications of *Rosa* in ITM is due to its use for the treatment of respiratory diseases. They prescribed Nasrin for cleansing the lungs from harmful humors and vapors [96, 97, 99, 101]. It is mentioned to characteristically improve phlegmy and melanotic pleurisy. The benefit of *R. canina* preparations can be used as anti-congestion and for the treatment of throat swollen and tonsillitis [96, 99].

Avicenna believes that topical application of boiled seeds of *R. domescena* strengthens the gums and also reduces earache. Besides, rose water improves hemoptysis [93].

#### 23.5.2.6 Psychiatric

According to ITM, Ward, Nasrin and Nastaran are refreshing and bracing. They also strengthen

spiritual power [99]. Nasrin is recommended to improve some psychiatric disorders such as obsessive compulsive disorder and mania [101].

#### 23.5.2.7 Skin and Hair

According to the studied texts *R. canina* and *R. moschata* could exert hair tonic properties in topical usage forms. Nasrin was used for blackening of the hair and removing dandruff. To this purpose topical administration of 2.5 to 5 gr of dry powder is recommended [99]. Besides this, it has the potential to remove the skin freckles. For the mentioned purpose, rose flowers were blended and administered topically [96]. *R. domescena* is recommended to remove itches of the skin. To this purpose it is added to cool water and vinegar [102]. Ibn Nafis believes that continuous use of *R. domescena* could whiten the hair [96].

#### 23.5.2.8 Joints and Muscles

In ITM, *R. canina* and *R. moschata* had been cited several times to be effective in recovering joint problems which are due to coldness. Ward (*R. domescena*) in the form of Gol-angabin is also recommended for joint's pain and gout [101].

#### 23.5.2.9 Reproductive (Genital) Organs

According to ITM, Nasrin and Nastaran have also been reported to be useful as emmenagogue [97, 104]. Avicenna recommended Ward to reduce womb's pain [93].

## 23.6 Conclusion

The genus *Rosa* has been used in folklore and ITM as a treatment for a wide range of disorders. In this review, ethnobotanical and photochemistry of *Rosa* spp. as well as its beneficial properties in ITM were investigated.

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# 24

# Ethnobotany, Phytochemistry, Traditional and Modern Uses of *Actaea racemosa* L. (Black cohosh): A Review

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**Abstract**

*Actaea racemosa* (AR) also known as *Cimicifuga racemosa*, is a perennial plant from Ranunculaceae family which was used as traditional remedies in treatment of various condition like rheumatoid muscular pain, headache, inflammation and dysmenorrhea. *Actaea racemosa* was basically native to Canada and the Eastern United State. This chapter proposed the ethnopharmacological uses of *Actaea racemosa*, and its phytochemical properties. Specifically, in this article we focused on use of *Actaea racemose* for menopausal and post-menopausal symptoms management. Electronic databases including PubMed and Scopus were searched for studies on *Actaea racemose* and its administration in management of menopausal symptoms. Chem Office software was also used in order to find chemical structures. The key words used as search terms were *Cimicifuga racemose*,

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*Actaea racemose*, Ranunculaceae, Black cohosh, Menopausal symptoms. We have included all relevant animal and human studies up to the date of publication. The analysis on *Actaea racemose* showed various indications for different plant's extracts. Approximately 131 chemical compounds have been isolated and identified from *Actaea racemosa*. According to recently studies, the most important chemicals known of the *Actaea racemosa* are phenolic compounds, chromones, triterpenoids, nitrogen-containing constituents. In addition, in vivo and in vitro studies reported wide range of pharmacological activities for Black cohosh like attenuating menopausal symptoms. Mechanism of action for some ethnomedicinal indications were made clear while some of its activities are not confirmed by pharmacological studies yet. Further investigations on its pharmacological properties are necessary to expand its clinical effective use. Also, additional large clinical trials are recommended for clarifying the effect of Black cohosh.

**Keywords**

*Cimicifuga racemosa* · *Actaea racemosa* · Ranunculaceae · Black cohosh · Menopausal symptoms

## 24.1 Introduction

*Actaea racemosa* (AR) is a perennial plant from buttercup (Ranunculaceae) family, known as Black cohosh, bugbane, black snakeroot, rattle weed and wanzenkraut, has been identified as popular herbal medicine to cure wide range of female concerns. Notably, the rhizome was considered as a remedy for kidney disorders, sore throat, malaria, malaise and menstrual cramps by Native Americans. Also it was used as antidote to rattlesnake bite and Chorea (St. Vitus's dance) [1]. Thereafter, around 1850 Black cohosh became one of the most popular remedies specially to treat rheumatoid muscular pain, headache, inflammation and dysmenorrhea [2]. A report recorded in 1855 describes 160 childbirths that were eased by using a tincture of fresh roots of the plant [3].

Recently the pharmaceutical component, fresh or dried rhizome, has been specified to have anti-osteoporosis, anti-diabetes and anti-polycystic syndrome effect and premenstrual and menopausal symptom(including hot flashes, mood disturbances, diaphoresis, palpitations, and vaginal dryness) in various *in vivo* and *in vitro* pharmacological studies [3].

Numerous studies have been conducted on menstruation and menopausal symptoms. Moreover, as the plant shows selective estrogenic effects, it is hoped to become one of the important remedies in post-menopausal symptoms and also an efficient cure for breast cancer.

Plants as medicines or food are used from several millennia ago and today various strategies are being used to find novel effective medications from natural sources. Exploring folk and traditional medicine systems is a rational strategy for this purpose. In the present study, we aimed to summarize the information about AR described in available traditional and modern resources. To prepare this review, electronic searches were carried out in several databases (including PubMed, Cochrane Library, SCOPUS, EMBASE, HerbMed) from inception to February 2018. Search terms included the common name(s), scientific name(s), and all listed synonyms. No restrictions were placed on quality of publications.

## 24.2 Botany, Ethnobotany and Authentication Profile

The plant described as tall-stemmed plant with 1 to 1.5 m height and flowers originating from a wide base of serrated green foliage [4]. The leafy plant has strong black rhizome which is cylindrical, knotted and thick, whereas roots are straight, strong and dark brown. White flowers consist of 3 to 8 petalsand casbergs surround flower buds [3].

Although *Cimicifuga*is native to Canada and the Eastern United States and was traditionally used by Native Americans, it has been raised in Europe [1].

In late 17 century Plukenet stated that Black cohosh or *Actaea racemosa (Cimicifuga racemosa*) belonged to Ranunculaceae family. Although Pursh change the classification as *Cimicifuga racemosa* and place it in *Macroty* [3]. Lately, based on morphological investigations and DNA sequence mapping, the genus *Cimicifuga* changes back to *Actaea*. *Actaea* genus consists of erect perennial plants that produce large, compound leaves with toothed edges from an underground rhizome.

Recently, based on morphological characters and DNA evidence, the genus *Cimicifuga* has been reclassified into the genus *Actaea* (Ranunculaceae) [5]. The genus *Actaea* comprises a total of 28 taxa distributed throughout the Northern Hemisphere, which is represented by eight endemic to North America and the other 20 found in Asia and Europe. Among them, *Actaea racemosa* L. (=*Cimicifuga racemosa* (L.) Nutt.) is a well-known ethnomedicinal plant, primarily for management of menopausal symptoms and is also one of the most popular botanical dietary supplements in America and Europe [6–8]. It described as perennial plant with 1 to 1.5 m height and flowers originating from a wide base of serrated green foliage [4]. The leafy plant has strong black rhizome which is cylindrical, knotted and thick, whereas roots are straight, strong and dark brown. White flowers consist of 3 to 8 petalsand casbergs surround flower buds [3]. It is naturally found in the deciduous forests of North America and spread mostly from the south of Ontario to central Georgia, north to Wisconsin, and west to Missouri and Arkansas [5]. Nowadays, AR, is renowned as "woman's herb" for its therapeutic values and is also considered as one of the 10 top-selling herbals in the United States. However, several concerns regarding its adulteration, substitution and misidentification have been voiced in herbal markets and trades which may have posed a consumer safety risk. Because of its similar appearance and growing locales, AR was adulterated with some other *Actaea* species as well as blue cohosh (*Caulophyllum thalictroides* (L.) Michx.), which are described as poisonous [7, 9, 10]. It is usually harvested (>90%) from native wild growth where its range overlaps with the closely related *Actaea cordifolia* DC., *A. pachypoda* Elliott (white cohosh), *A. podocarpa* DC. (yellow cohosh), and *A. rubra* (Aiton) Willd [6, 7, 11]. Therefore, correct identification and distinguishing of AR from other species is a critical initial step to ensure its quality, safety, and efficacy. It is also essential to adoption of fingerprinting techniques and find more appropriate marker compounds for botanical authentication. Literature ***review*** shows that the ethnobotanical applications of AR mainly originate from American continent. Traditionally, the dried roots and rhizomes of AR have been used by Native Americans and early colonists to treat a broad spectrum of disorders such as general malaise, malaria, rheumatism, insomnia, abnormalities in kidney function, sore throat, rattlesnake bites, gynecological problems, and assist with childbirth [12, 13]. In America, it is also well documented for its remarkable uses in Haudenosaunee traditional medicine, which is widely used as antirheumatic and also is applied in Orthopedic [14]. In North American Indian medicine, AR is believed to be efficaceous in the treatment of a variety of conditions including joint aches and myalgias, as well as gynecological problems [15]. In Mexico, AR is widely used in traditional medicine as a remedy for the menopausal symptoms [16]. In Brazil, it is considered very useful in the treatment of female sexual dysfunction [17]. In Guatemala, it is considered very useful in the treatment of menopausal and other female disorders [18]. Furthermore, in ethno veterinary medicine (EVM), its rhizome is also used for uterine infections in pets in British Columbia, Canada [19]. In the European Traditional Medicine, AR is commonly recommended as an effective treatment for menopausal ailments, and used as an alternative to hormone-replacement therapy [20]. In Spain, traditional healers have recommended the use of *A. racemosa* for the treatment of post-menopausal symptoms [21]. In Sweden, it is considered a safe alternative for women in whom oestrogen therapy is contraindi-

cated [22]. Moreover, in the United Kingdom, *A. racemosa* has been prescribed to treat of epilepsy [23].

## 24.3 Phytochemicals

The biological effects of the rhizome is due to the presence of active compounds including the triterpene glycosides (actein, 27-deoxy actein, cimicifugoside), phenyl propan derivatives like isoferulic acid and quinolizidine alkaloids including cytisine and methylcytisine [24].

According to recently studies, the most important chemicals known of the AR are phenolic compounds, chromones, triterpenoids, nitrogen-containing constituents (Table 24.1). Among these, derivatives of triterpenoid can be observed in abundance in this species. Triterpenes are a batch of chemical compounds which consists of three units of terpene, most of which are found in nature as cyclic triterpenes, consisting of 1–5 rings systems. Although the most abundant tricyclic and tetracyclic triterpenes in nature have five carbon rings, however in many plants, there is pentacyclic triterpenes, free or mixed with sugars in glycosides (saponins) [25, 26]. Phenylpropanoid esters are another important component in AR, these compounds are from the phenolic group. The combinations (cimiracemates A-D) of this category are listed in Table 24.1.

## 24.4 Standardized Extracts- Dosage

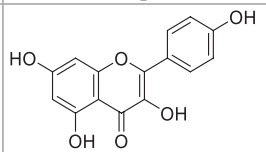
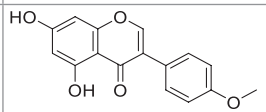
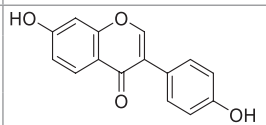
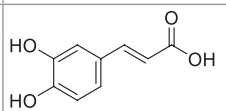
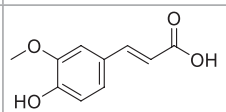
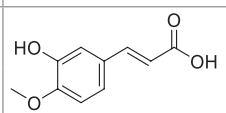
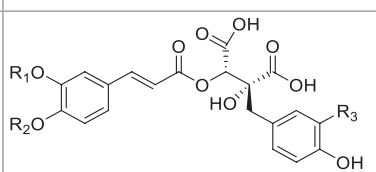
Many commercial standardized extracts of black cohosh root and rhizome are available [4]. Products were analyzed by looking at four bioactive triterpene constituents (R-actein, 23-epi-26-deoxyactein, S-actein, and 26-deoxyactein) standardized to 5.6% of the active triterpene glycosides.Remifemin®, a 40% isopropanolic extract by volume, is standardized to have 1 mg of triterpenes, measured as 27-deoxyacteine per 20 mg tablet [20]. Remifemin® formulation and production process have been evolved over the past 20 years, therefore the amount of extract that is used by several studies differ. A standardized liquid formulation of Remifemin® was used in some studies [40].

Phyto-Female Complex was prepared as combination product of standardized extract of black cohosh, dong quai, milk thistle, red clover, American ginseng, and chaste-tree berry (SupHerb, Netanya, Israel) Standardized extracts of black cohosh [*Cimicifuga racemosa*(L.) Nutt.] root extract0.1 mg (2.5 mg of triterpene glycoside, 2.5%), dong quai [*Angelica sinensis*(Oliv.) Diels]root extract 75 mg (7.5 mg of ligustilides, 1%), milk thistle (*Silybum marianum*Gaertn.) herb extract 75 mg (60 mg of silymarin, 80%), red clover (*Trifolium pratense* L.) flower extract 50 mg (4 mg of isoflavone, 8%), American ginseng (*Panax quinquefolius* L.) root extract 50 mg (12.5 mg of ginsenosides, 25%), and chaste-tree berry (*Vitex agnus-castus* L.) fruit extract 50 mg (2.5 mg of vitexin, 5%) were encapsulated as a single dose oral formulation [41]. Also, administrating of AR extract as a Pharmaceutical-grade product (Lot BC191) was reported in a study to have no significant outcome on anxiety associated with menopause [42]. 7.27 mg of triterpene glycosides standardized as a black cohosh product has been administered in some studies [43, 44]. However, some studied black cohosh products lacked information on the label as to where the product originated [45]. Other products included the method to determine bioavailability [46, 47].

Also BNO 1055 (Menofem/Klimadynon) a 58% ethanol extract by volume was prepared from AR corresponding to 21.5 mg root per tablet [47].

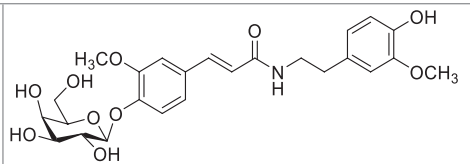
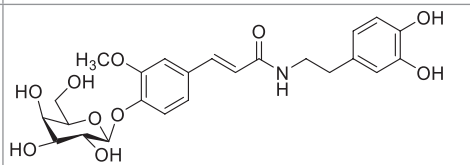
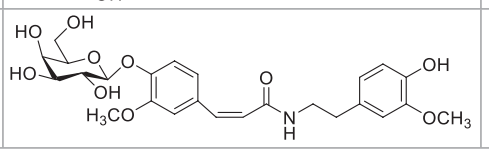
In a randomized, double-blind, clinical dose comparison trial on climacteric complains, it has been specified that there was no difference between 40 mg daily and 127 mg per day administration of a special isopropanolic extract of root of the herb [48]. Also some studies use 200 to 400 mg of dried rhizome or 0.4 to 2 ml of a 1:10 60% ethanol tincture [49]. Other studies suggest various preparations of AR extract. For instance a study in 2005 conducted by using either 40 mg isopropanolic aqueous AR extract daily compared with low-dose transdermal estradiol [50].

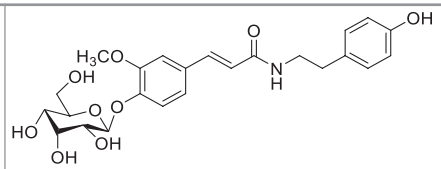
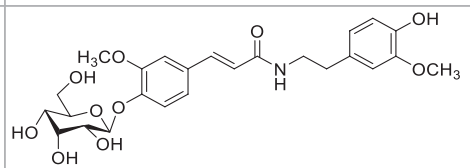
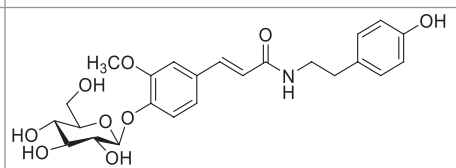
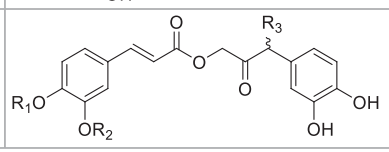
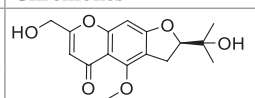
**Table 24.1** Chemical composition of AR

| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| | | **Phenolic compounds** | | |
| 1 | Kaempferol |  | Rhizome | [27] |
| 2 | Biochanin A |  | Rhizome | [27] |
| 3 | Formononetin |  | Rhizome | [28] |
| | | | Roots and rhizomes | [29] |
| | | | Roots | [30] |
| 4 | Caffeic acid |  | Rhizome | [31] |
| | | | Roots and rhizomes | [29, 32] |
| 5 | Ferulic acid |  | Roots and rhizomes | [29, 32] |
| 6 | Isoferulic acid |  | Rhizome | [31] |
| | | | Roots | [30] |
| | | | Roots and rhizomes | [29, 32] |
| | |  | | |

(continued)

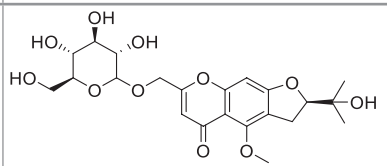
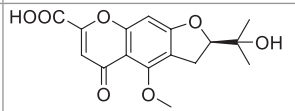
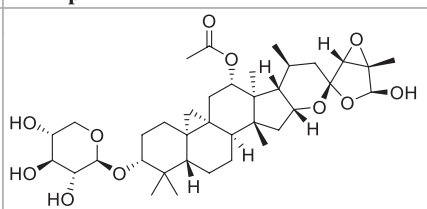
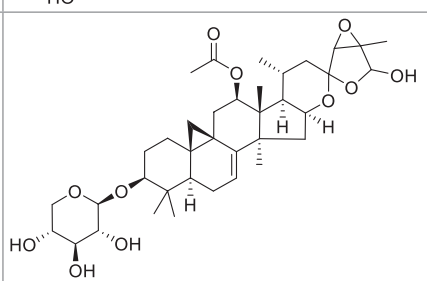
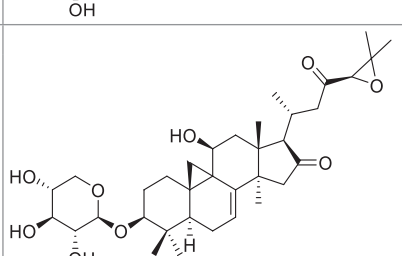
**Table 24.1** (continued)

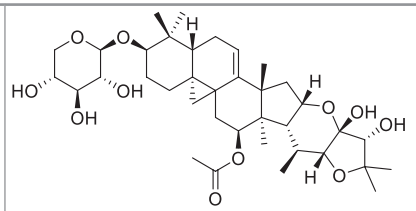
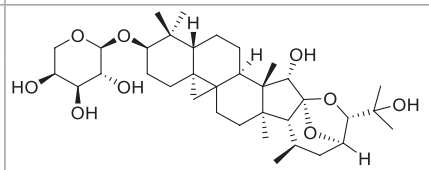
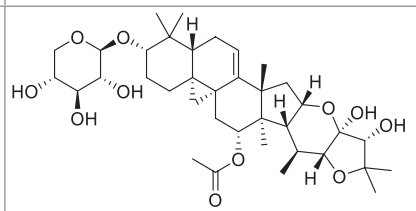
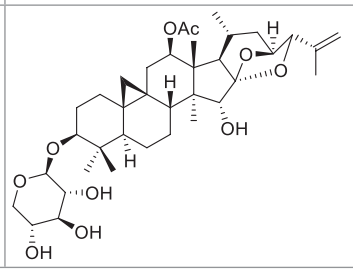
| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 7 | Fukinolic acid: $R_1$ = H, $R_2$ = H, $R_3$ = OH | | Roots and rhizomes | [29, 32] |
| | | | Rhizome | [33] |
| 8 | Cimicifugic acid A: $R_1$ = $CH_3$, $R_2$ = H, $R_3$ = OH | | Roots and rhizomes | [29, 32] |
| | | | Rhizome | [33] |
| 9 | Cimicifugic acid B: $R_1$ = H, $R_2$ = $CH_3$, $R_3$ = OH | | Roots and rhizomes | [29, 32] |
| | | | Rhizome | [33] |
| 10 | Cimicifugic acid D: $R_1$ = H, $R_2$ = H, $R_3$ = H | | Roots and rhizomes | [32] |
| 11 | Cimicifugic acid E: $R_1$ = $CH_3$, $R_2$ = H, $R_3$ = H | | Roots and rhizomes | [32] |
| | | | Rhizome | [33] |
| 12 | Cimicifugic acid F: $R_1$ = H, $R_2$ = $CH_3$, $R_3$ = H | | Roots and rhizomes | [32] |
| | | | Rhizome | [33] |
| 13 | Cimicifugamide |  | Rhizome/roots | [34] |
| 14 | Cimicifugamide A |  | Rhizome/roots | [34] |
| 15 | Isocimicifugamide |  | Rhizome/roots | [34] |

| | | | | |
|---|---|---|---|---|
| 16 | Trans-feruloyl-tyramine 4-O-β-D-alloside |  | Rhizome/roots | [34] |
| 17 | Transferuloyl-(3-O-methyl)<br>Dopamine 4-O-β-D-alloside |  | Rhizome/roots | [34] |
| 18 | Trans-feruloyl tyramine 4-O-β-glucopyranoside |  | Rhizome/roots | [34] |
| | |  | | |
| 19 | Cimiracemates A: $R_1$ = Me, $R_2$ = H, $R_3$ = H | | Rhizome | [35] |
| 20 | Cimiracemates B: $R_1$ = H, $R_2$ = Me, $R_3$ = H | | Rhizome | [35] |
| 21 | Cimiracemates C: $R_1$ = Me, $R_2$ = H, $R_3$ = OMe | | Rhizome | [35] |
| 22 | Cimiracemates D: $R_1$ = H, $R_2$ = Me, $R_3$ = OMe | | Rhizome | [35] |
| | | **Chromones** | | |
| 23 | Cimifugin |  | Roots and rhizomes | [29] |
| | | | Rhizome | [36] |

(continued)

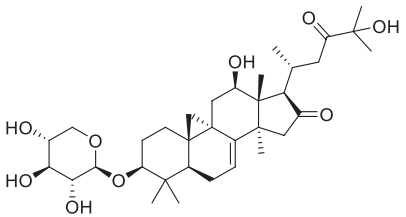
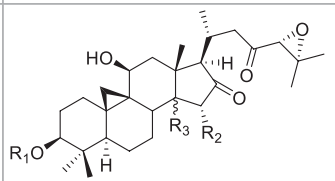
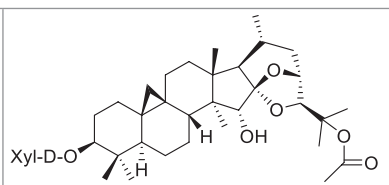
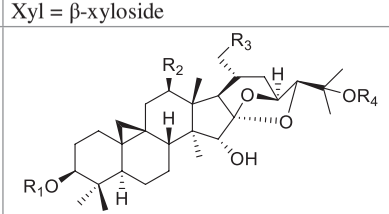
**Table 24.1** (continued)

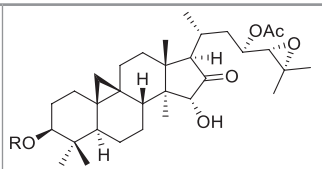
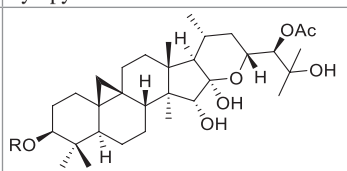
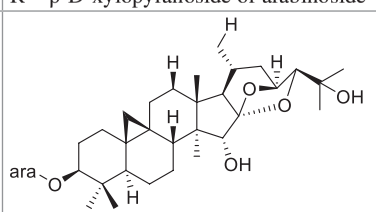
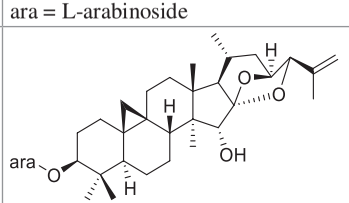
| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 24 | Prim-O-glucosylcimifugin |  | Rhizome | [36] |
| 25 | Divaricatacid |  | Rhizome | [36] |
| | | **Triterpenoids** | | |
| 26 | Actein |  | Rhizome | [28, 37] |
| | | | Roots and rhizomes | [29] |
| | | | Roots | [30] |
| 27 | Cimicifugoside |  | Rhizome | [27] |
| 28 | Cimicifugoside H-1 |  | Rhizome | [31] |
| | | | Roots and rhizomes | [29] |

| | | | | |
|---|---|---|---|---|
| 29 | Cimiracemoside A |  | Rhizome | [28, 31] |
| 30 | Cimiracemoside C |  | Roots and rhizomes | [29] |
| | | | Rhizome | [36] |
| 31 | Cimiracemoside F |  | Roots and rhizomes | [29] |
| 32 | Cimiracemoside K |  | Rhizome | [36] |

(continued)

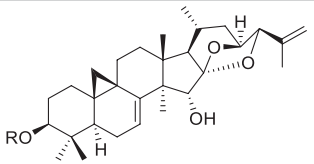
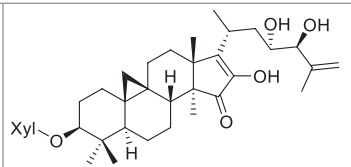
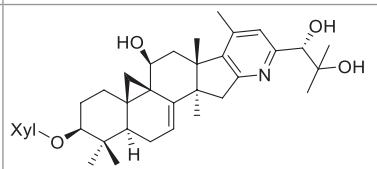
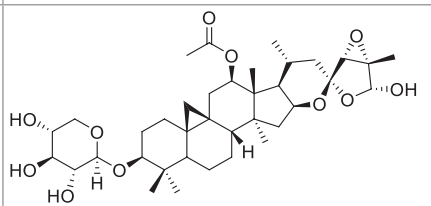
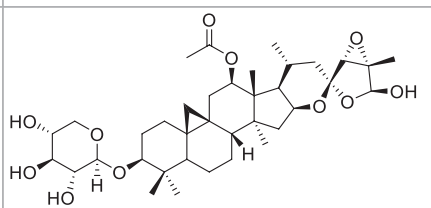
**Table 24.1** (continued)

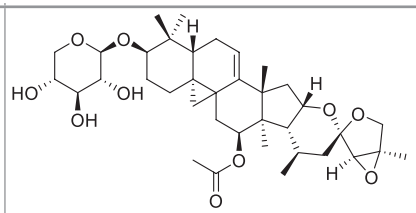
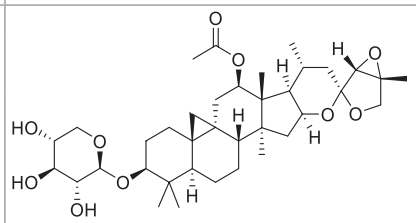
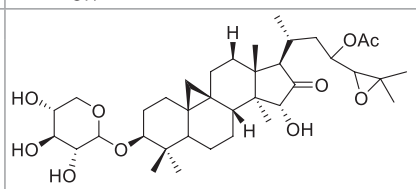
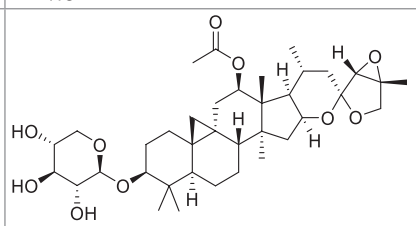
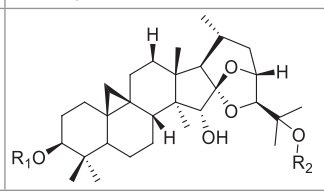
| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 33 | Cimicifugoside H-2 |  | Rhizome | [31] |
| | |  | | |
| 34 | Cimicifugoside H-5: $R_1$ = xyl, $R_2$ = OH, $R_3$ = α-$CH_3$ | | Rhizome | [36] |
| 35 | Shengmacichun dixyloside: $R_1$ = xyl-(1→3)-xyl, $R_2$ = H, $R_3$ = β-$CH_3$ | | Rhizome | [36] |
| 36 | 25-acetyl cimigenol 3-xyloside |  <br>Xyl= β-xyloside | Roots and rhizomes | [29] |
| | | Xyl = β-xyloside | | |
| 37 | 12′,21-dihydroxycimigenol-3-O-l-arabinoside |  | Rhizome | [36] |
| | | $R_1$= arabinoside, $R_2$= OH, $R_3$= OH, $R_4$= H | | |
| | | $R_1$ = arabinoside, $R_2$ = OH, $R_3$ = OH, $R_4$ = H | | |

| | | | | |
|---|---|---|---|---|
| 38 | 23-O-acetylshengmanol-3-O-β-D-glucopyranoside(1–3)-β-D-xylopyranoside or arabinoside |  <br>R = β-D-glucopyranoside(1–3)-β-D-xylopyranoside or arabinoside | Rhizome | [36] |
| 39 | 24-O-acetylhydroshengmanol-3-O-β-D-xylopyranoside or arabinoside |  <br>R= β-D-xylopyranoside or arabinoside<br>R = β-D-xylopyranoside or arabinoside | Rhizome | [36] |
| 40 | 12β,21-dihydroxycimigenol-3-O-L-arabinoside |  <br>ara= L-arabinoside<br>ara = L-arabinoside | Rhizome | [36] |
| 41 | (23R,24S)-25-anhydrocimigenol-3-O-α-L-arabinose |  <br>ara= L-arabinoside<br>ara = L-arabinoside | Rhizome | [36] |

(continued)

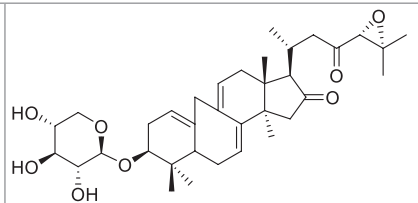
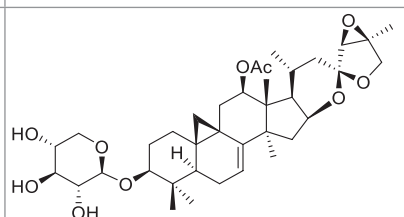
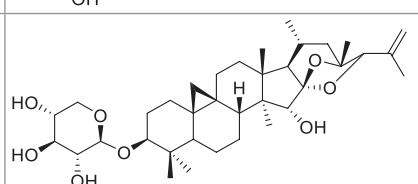
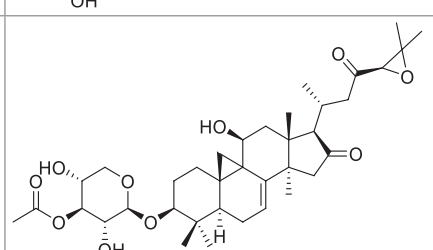
**Table 24.1** (continued)

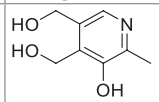
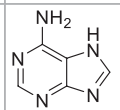
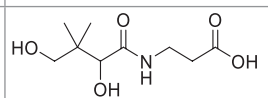
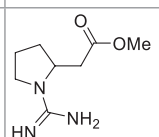
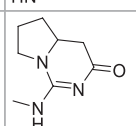
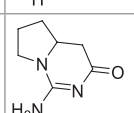
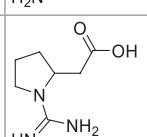
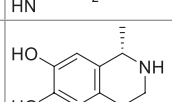
| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| | |  | | |
| 42 | (23R,24R)-7-en25anhydrocimigenol-3-O-β-D-xylopyranoside: R = β-D-xylopyranoside | | Rhizome | [36] |
| 43 | (23R,24R)-7-en-25-anhydro cimigenol-3-O-α-L-arabinoside: R = α-L-arabinoside | | Rhizome | [36] |
| 44 | Cimidahuside G |  | Rhizome | [36] |
| 45 | cimicifineA(syn. cimicifugadine) |  | Rhizome | [36] |
| 46 | (26R)-actein |  | Rhizome | [31] |
| 47 | (26S)-actein |  | Rhizome | [31] |

| | | | | |
|---|---|---|---|---|
| 48 | 26-deoxycimicifugoside |  | Rhizome | [31] |
| 49 | 23-epi-26-deoxyactein |  | Rhizome | [28, 31] |
| | | | Roots and rhizomes | [29] |
| | | | | [38] |
| 50 | 23-OAc-shengmanol-3-O-β-d-xyloside, |  | Rhizome | [31] |
| | | | | [38] |
| 51 | 26-deoxyactein |  | Rhizome | [31] |
| | |  | | |

(continued)

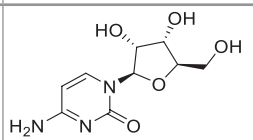
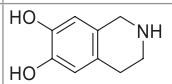
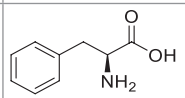
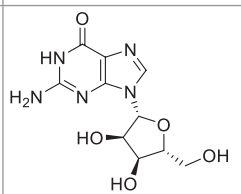
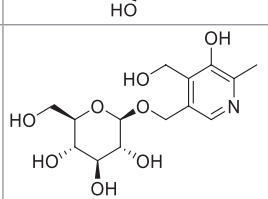
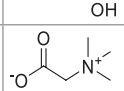
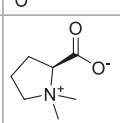
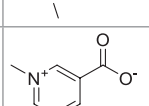
**Table 24.1** (continued)

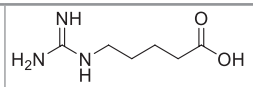
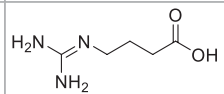
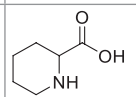
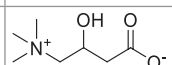
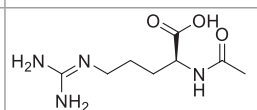
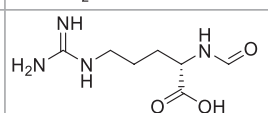
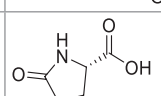
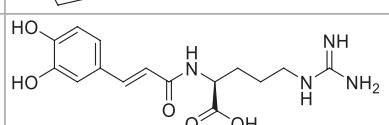
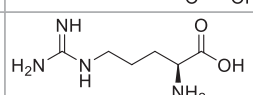
| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 52 | 25-OAc-cimigenol-3-O-α-l-arabinoside: $R_1$ = 3-O-α-l-arabinoside, $R_2$ = OAc | | Rhizome | [31] |
| 53 | 25-OAc-cimigenol-3-O-β-d-xyloside: $R_1$ = 3-O-β-d-xyloside, $R_2$ = OAc | | Rhizome | [31] |
| | | | Roots and rhizomes | [29] |
| | | | | [38] |
| 54 | cimigenol3-O-α-l-arabinoside: $R_1$ = 3-O-α-l-arabinoside, $R_2$ = H | | Rhizome | [31] |
| 55 | Cimigenol-3-O-β-d-xyloside: $R_1$ = 3-O-β-d-xyloside, $R_2$ = H | | Rhizome | [31] |
| 56 | Isocimipodocarpaside |  | | [38] |
| 57 | 23-epi-26-deoxycimicifugoside |  | | [38] |
| 58 | 25-anhydrocimigenol xyloside |  | | [38] |
| 59 | 3′-O-acetylcimicifugoside H-1 |  | | [38] |

| | | | | |
|---|---|---|---|---|
| | | Nitrogen-containing constituents | | |
| 60 | Pyridoxine |  | Roots/rhizome | [39] |
| 61 | Adenine |  | Roots/rhizome | [39] |
| 62 | Pantothenic acid |  | Roots/rhizome | [39] |
| 63 | Cimipronidine methyl ester |  | Roots/rhizome | [39] |
| 64 | N-methyl cyclocimipronidine |  | Roots/rhizome | [39] |
| 65 | Choline |  | Roots/rhizome | [39] |
| 66 | Cimipronidine |  | Roots/rhizome | [39] |
| 67 | Salsolinol |  | Roots/rhizome | [39] |

(continued)

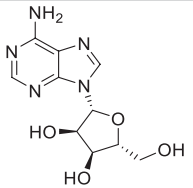
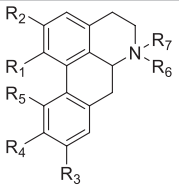
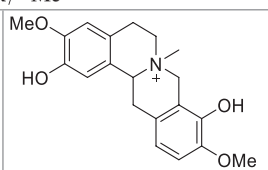
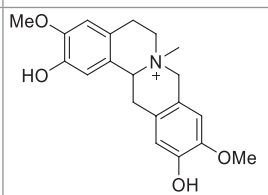
**Table 24.1** (continued)

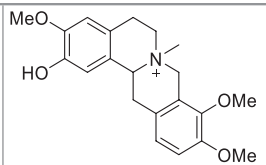
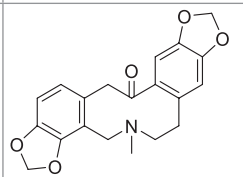
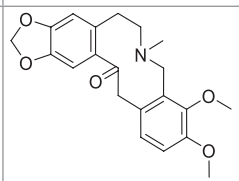
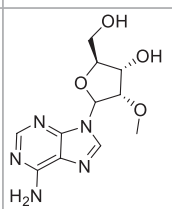
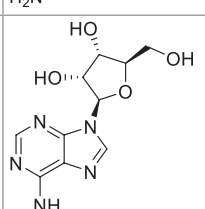
| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 68 | Cytidine |  | Roots/rhizome | [39] |
| 69 | Norsalsolinol |  | Roots/rhizome | [39] |
| 70 | Phenylalanine |  | Roots/rhizome | [39] |
| 71 | Guanosine |  | Roots/rhizome | [39] |
| 72 | 5′-O-(β-D-glucopyranosyl) Pyridoxine |  | Roots/rhizome | [39] |
| 73 | Glycine betaine |  | Roots/rhizome | [39] |
| 74 | Proline betaine |  | Roots/rhizome | [39] |
| 75 | Trigonelline |  | Roots/rhizome | [39] |

| | | | | |
|---|---|---|---|---|
| 76 | δ-Guanidinovaleric acid |  | Roots/rhizome | [39] |
| 77 | γ-Guanidino butyric acid |  | Roots/rhizome | [39] |
| 78 | Pipecolic acid |  | Roots/rhizome | [39] |
| 79 | L-carnitine |  | Roots/rhizome | [39] |
| 80 | α-N-acetyl arginine |  | Roots/rhizome | [39] |
| 81 | N-formyl arginine |  | Roots/rhizome | [39] |
| 82 | Pyroglutamic acid |  | Roots/rhizome | [39] |
| 83 | Caffeoyl arginine |  | Roots/rhizome | [39] |
| 84 | Arginine |  | Roots/rhizome | [39] |

(continued)

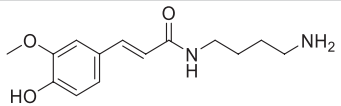
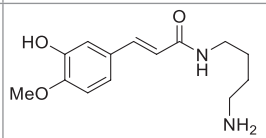
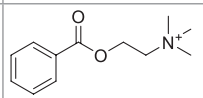
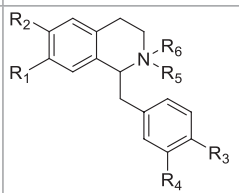
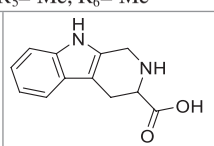
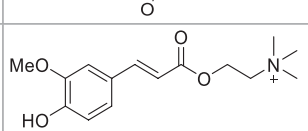
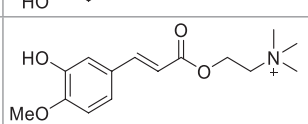
**Table 24.1** (continued)

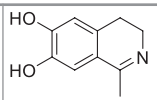
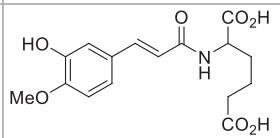
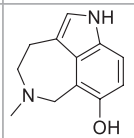
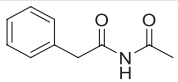
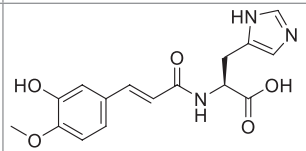
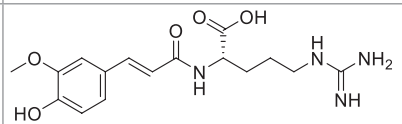
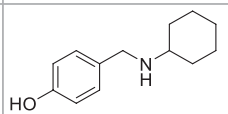
| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 85 | Adenosine |  | Roots/rhizome | [39] |
| | |  | | |
| 86 | Magnoflorine: $R_1$ = OH, $R_2$ = OMe, $R_3$ = H, $R_4$ = OMe, $R_5$ = OH, $R_6$ = Me, $R_7$= Me | | Roots/rhizome | |
| 87 | Laurifoline: $R_1$ = OH, $R_2$ = OMe, $R_3$ = OH, $R_4$ = OMe, $R_5$ = H, $R_6$ = Me, $R_7$= Me | | Roots/rhizome | |
| 88 | Laurolitsine: $R_1$ = OMe, $R_2$ = OH, $R_3$ = OH, $R_4$ = OMe, $R_5$ = H, $R_6$ = H, $R_7$ = H | | Roots/rhizome | |
| 89 | Menisperine: $R_1$ = OMe, $R_2$ = OMe, $R_3$ = H, $R_4$ = OMe, $R_5$ = OH, $R_6$= Me, $R_7$= Me | | Roots/rhizome | |
| 90 | Laurotetanine: $R_1$ = OMe, $R_2$ = OMe, $R_3$ = OH, $R_4$ = OMe, $R_5$ = H, $R_6$ = H, $R_7$ = H | | Roots/rhizome | |
| 91 | Xanthoplanine: $R_1$ = OMe, $R_2$ = OMe, $R_3$ = OH, $R_4$ = OMe, $R_5$ = H, $R_6$ = Me, $R_7$= Me | | Roots/rhizome | |
| 92 | Cyclanoline |  | Roots/rhizome | [39] |
| 93 | Phellodendrine |  | Roots/rhizome | [39] |

| | | | | |
|---|---|---|---|---|
| 94 | N-methyl tetrahydrocolumbamine or isomer |  | Roots/rhizome | [39] |
| 95 | Protopine |  | Roots/rhizome | [39] |
| 96 | Allocryptopine |  | Roots/rhizome | [39] |
| 97 | 2′-O-methyladenosine |  | Roots/rhizome | [39] |
| 98 | N-methyladenosine |  | Roots/rhizome | [39] |

(continued)

**Table 24.1** (continued)

| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 99 | Feruloyl putrescine |  | Roots/rhizome | [39] |
| 100 | Isoferuloyl putrescine |  | Roots/rhizome | [39] |
| 101 | Benzoyl choline |  | Roots/rhizome | [39] |
| | |  | | |
| 102 | Magnocurarine: $R_1$ = OH, $R_2$ = OMe, $R_3$ = OH, $R_4$ = H, $R_5$ = Me, $R_6$= Me | | Roots/rhizome | [39] |
| 103 | Norcoclaurine: $R_1$ = OH, $R_2$ = OH, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = H | | Roots/rhizome | [39] |
| 104 | Reticuline: $R_1$ = OH, $R_2$ = OMe, $R_3$ = OMe, $R_4$ = OH, $R_5$ = H, $R_6$= Me | | Roots/rhizome | [39] |
| 105 | Isomer of magnocuranine (oblongine): $R_1$ = OMe, $R_2$ = OH, $R_3$ = OH, $R_4$ = H, $R_5$= Me, $R_6$= Me | | Roots/rhizome | [39] |
| 106 | 1,2,3,4,-tetrahydro-β-carboline-3carboxylic acid |  | Roots/rhizome | [39] |
| 107 | Feruloyl choline |  | Roots/rhizome | [39] |
| 108 | Isoferuloyl choline |  | Roots/rhizome | [39] |

| | | | | |
|---|---|---|---|---|
| 109 | 1,2-dehydrosalsolinol |  | Roots/rhizome | [39] |
| 110 | N-isoferuloyl glutamic acid |  | Roots/rhizome | [39] |
| 111 | Cimitrypazepine |  | Roots/rhizome | [39] |
| 112 | *N*-phenylacetyl acetamide |  | Roots/rhizome | [39] |
| 113 | *N*-isoferuloyl histidine |  | Roots/rhizome | [39] |
| 114 | *N*-feruloyl arginine |  | Roots/rhizome | [39] |
| 115 | *N*-cyclohexyl-4-hydroxy benzylamine |  | Roots/rhizome | [39] |

(continued)

**Table 24.1** (continued)

| No. | Name of compounds | Structures | Plant parts | References |
|---|---|---|---|---|
| 116 | γ-Guanidino butanol | | Roots/rhizome | [39] |
| 117 | Histidine betaine | | Roots/rhizome | [39] |
| 118 | Choline hexoside | | Roots/rhizome | [39] |
| 119 | γ-Guanidino butyric acid methylester | | Roots/rhizome | [39] |
| 120 | *N*-isoferuloyl arginine | | Roots/rhizome | [39] |
| 121 | γ-Guanidino butyric acid ethylester | | Roots/rhizome | [39] |
| 122 | δ-Guanidinovaleric acid methyl ester | | Roots/rhizome | [39] |
| 123 | *N*-feruloyl dopamine-4′-O-hexoside | | Roots/rhizome | [39] |
| 124 | *N*-isoferuloyl dopamine-4′-O-hexoside | | Roots/rhizome | [39] |
| 125 | *N*(2)-methyl-6-hydroxy-3,4-dihydro-β-carboline | | Roots/rhizome | [39] |
| 126 | *N*-feruloyl phenylalanine-4′-O-hexoside | | Roots/rhizome | [39] |
| 127 | *N*(2)-methyl-6-hydroxy-1,2,3,4-tetrahydro-β-carboline | | Roots/rhizome | [39] |
| 128 | *N*-isoferuloyl arginine methylester | | Roots/rhizome | [39] |
| 129 | *N*-feruloyl tyramine-4‴-O-hexoside | | Roots/rhizome | [39] |
| 130 | *N*-isoferuloyl arginine ethylester | | Roots/rhizome | [39] |
| 131 | *N*-feruloyl-3‴-methoxytyramine-4‴-O-hexoside | | Roots/rhizome | [39] |

## 24.5 Preclinical Studies

### 24.5.1 Hormonal Effect

Black cohosh has been approved in many countries for premenstrual discomfort (weight gain, swelling, mood fluctuations, and breast tenderness), dysmenorrhea, and perimenopausal symptoms, and it is also recommending alone or combined with a nonprescription remedy (such as dietary isoflavones and vitamin E) for the relief of mild vasomotor symptoms related to menopause. Commercial products containing AR and *Hypericum perforatum* L. are available for the treatment of menopausal depression, as well as premenstrual depression. Moreover, in Chinese Medicine, the herb was used to treat the above-mentioned disorders, as well as to treat measles in pre-cutaneous rash stage.

The mechanism of action of black cohosh remains unclear. Actually, increase in lutein hormone(LH) which leads to estrogen level elevation, can reduce the severity of menopausal disorders. The compounds in the rhizome of this plant attached to estrogen receptors, leading to suppressed selective LH secretion without any effect on follicle stimulating hormone(FSH). The result of this process is a kind of estrogenic effect that reduces the symptoms of menopause, such as hot flashes, sweating and mental disorders in women [51, 52]. Nevertheless some controversial researches suggests a lack of estrogen-like activity in this plant and proposed other mechanisms for its activity in premenstrual and menopausal disorders [53, 54].They said that the plant didn't have any effect on the concentration of FSH, LH, sex-linked hormones bound to globulin (SHBG), prolactin and estradiol. Otherwise a study by Wuttke et al. suggests decrease in LH level followed by direct effect of AR extract on hypothalamus and reduction of gonadotropin-releasing-hormone level. The study also rejects the hypothesis of suppression of pituitary by the extract due to no significant change in LH level in ovariectomized rats. It was concluded that administration of 62.5 mg AR extract in ovariectomized rats lessen serum LH level beside control and estrogen-treated groups. Therefore it can be inferred that AR extract causes hypothalamo-pituitary axis to suppress pituitary LH secretion [55].

In a study, they evaluated the influence of a standardized isopropanolic extract of black cohosh on an animal model of endometrial cancer. Ectopic growth of the primary tumor as well as the incidence and localization of metastases were examined, partly in the setting of a combination treatment with tamoxifen. In contrast to the endometrial estrogen agonist tamoxifen, black cohosh did not further growth or metastasizing potential of the primary tumor, proposing that black cohosh demonstrated no estrogen-agonistic activity in mammary cells [56].

In vitro studies specified that AR exerted antagonist effect on estrogen receptor-positive breast cancer cells and may act as a selective estrogen receptor modulator (SERM) [57, 58].

The extract of this plant does not stimulate the growth of cellular estrogen-dependent breast cancer cells (MCF-7). Moreover, significant activation of neither human estrogenic (hER) alpha nor beta receptor has not been reported. Additionally, the results suggest that the extract has antagonistic estrogen effects on the receptor, which has positive response to estrogen in breast cancer cells, and its high concentrations suppress proliferation of breast adenocarcinoma cells (MCF-7). Besides, the use of AR extract reduces the proliferation of estrogen-dependent cells, which can be reversed by increasing estradiol concentrations [57].

Although cancer-free women as well as breast cancer patients and survivors use black cohosh to mitigate vasomotor symptoms, there is limited data about its potential effect on breast cancer development or progression. In a study on MMTV-*neu* mouse model, using an adjusted dose for the mice in correlation with the recommended dose in women (40 mg/d), no differences were observed in the incidence or onset of mammary tumors in black cohosh-treated versus control group. The lack of effect on mammary tumor development suggests that black cohosh may have no effect on breast cancer risk if given to women before tumor formation. In contrast, black cohosh significantly increased the inci-

dence of lung metastases in tumor-bearing animals compared with mice fed the isoflavone-free control diet [59].

A case report mentioned how to use homeopathic products of AR for induction and strengthening of labor but there is a need for further studies [60]. In an animal study simulating menopause model, it was shown that the isopropanol AR extract with the brand name of Rimifemin can have additional effects beside estradiol valerate in preventing menopausal osteoporosis [61]. So, based on available information it seems that the black cohosh could be helpful in management of premenstrual and postmenopausal symptoms. However, the exact mechanism of its action should be determined in future studies.

### 24.5.2 Analgesic Effects

It has been shown that methanol extract derivate from the rhizome of the black cohosh has a kind of analgesic effect in rats [62]. Another study was conducted to investigate the effect of black cohosh extract on opiate receptors. The study revealed that 100% methanol, 75% ethanol and 40% 2-propanol extracts of black cohosh possess compounds which act as mixed competitive ligand and partial agonist in the human mu opiate receptor [63, 64].

Significant increases in μ-opioid receptor binding potential was recorded during a clinical trial in brain regions involved in emotional and cognitive function ranging from 10% to 61% across regions [63]. Also, a study was performed on effect of AR extract on gamma-aminobutyric acid (GABA) receptors. The results suggest that positive allosteric effect on $GABA_A$ may evolve in treatment of climacteric symptoms by AR extract [65]. The vasodilatory effects of the AR extract may be involved in this effect [60].

### 24.5.3 Antidepressants Effects

The result of a study indicates that the extract derivate from the plant has anti-depressant effects in female mice. In this study, tail suspension test (TST) was used as a screening test to evaluate the anti-depressant effect of this plant. Furthermore, administration of AR extract was comparable to that of imipramine (antidepressant drug), resulted in dramatically reduction of immobilization of these animals.

### 24.5.4 Anti-inflammatory Effects

Results of a study provide scientific evidence that methanol extract derivate from the rhizome of black cohosh exerted anti-inflammatory effect on rats [62]. The outcome of these investigations proved that administration of ferulic acid, isoferulioc acid or derived extract of the rhizome of *Cimicifuga* species to virus infected mice, cause reduction in interleukin-8 level and exuded neutrophils in broncoalveolar lavage process, in comparison with the placebo group [66].

### 24.5.5 Anti-diabetes Effect

*Actaea racemosa* extract Ze 450 has been reported to have anti diabetes effects. This compound activated AMP-activated protein kinase to the same extent as metformin. Its oral or intraperitonal administration decreased significantly average daily and cumulative weight gain, daily food and water intake, while metformin had no effect. In summary, the results show that Ze 450 reduced significantly body weight, plasma glucose, improved glucose metabolism and insulin sensitivity in diabetic ob/ob mice. However, longer studies in other animal models or patients with disturbed glucose tolerance or diabetes may be useful for further investigation [67].

### 24.5.6 Anti-osteoporosis Effect

Actein's of AR has been shown to have protective effects on bone-forming cells against the oxidative damage caused by antimycin A toxin, which can be important in the treatment of osteoporosis [68].In a study on effect of AR extract on osteoporosis, decrease in development of osteoporosis

was reported due to reduction in load of bone marrow fat and pro-inflammatory cytokine secretion by AR BNO 1055 extract. In this study rats were ovx and fed with food containing either AR BNO 1055 or its triterpene-saponin or polar constituents or with E2 for 4 weeks. Ovx rats lost significant amounts of trabecular BMD, surface and nodes while the number of free trabecular ends and fat load in the marrow increased. This was completely prevented by E2 and partially by AR BNO 1055 and the triterpene-saponin but not by the polar fraction [69].

### 24.5.7 Cytotoxic and Anti-proliferative Effect

The antioxidant and anti-cancer effects of the AR extract and derived compounds have been observed against various classes of cancer cells that are hormone-dependent, such as breast and prostate [70]. Also anti-proliferative effects and increasing expression of pro-apoptotic gene were observed in an investigation on rhizome extract of AR in estrogen receptor positive human breast cancer cell line MCF-7 [71]. Besides, based on clinical experience with animal, it was shown that AR extract has an anti-proliferative effect on the LNCaP cell line. Upon histological evaluation, the amount of tumor tissue in the control animals was significantly larger than in the AR-treated animals. The AR treatment did not affect serum testosterone levels significantly regardless of tumors development. It is concluded that compounds in AR inhibit tumor development, proliferation and dignity following subcutaneous inoculation of LNCaP cells. So, the AR extract may prove to be efficient in preventing and treatment of prostate cancer [72].

Hepatotoxicity was reported occasionally with AR. for liver toxicity evaluation ethanolextract of AR was administered orally to rats, and liver sections were analyzed by electron microscopy. Microvesicular steatosis was found in rats treated with >500 μg/kg body weight *Actaea* extract. In vitro, cytotoxicity was apparent at concentrations ≥75 μg/mL, and mitochondrial β-oxidation was impaired at concentrations ≥10 μg/mL. Apoptotic cell death was the main mechanism of this effect [73].

## 24.6 Clinical Trials

### 24.6.1 Clinical Trials on Menopause Symptom

LH and FSH are critical hormones which are represented to play critical role in women health matters. Fluctuation in level of these hormones leads to major mood and physical disorders through women cycles. As in vitro and animal studies suggest hormonal effect of black cohosh extract, many clinical studies are conducted to evaluate that effect.

Various studies have been done on the use of this plant in the treatment of menopausal symptoms and its complications [74–76]. Reported studies were designed by administrating dried rhizome extract, standardized extract called Remifemin® and isopropanolic or aqueous-isopropyl alcohol extract of AR in duration of 2 months to 3.6 year.

Improvement in menopausal symptoms were identified by measuring several scales, including hot flashes, nights sweats, number of awakenings at night, and scores for the Menopause Rating Scale (MRS), Kupperman Index [37], Hamilton Depression and Anxiety Rating Scales, Symptom Rating Test, Greene Climacteric Scale (GCS), Vaginal Maturation Index, Clinical Global Impression scale, Self-Assessment Depression Scale, and Back Depression Inventory (BDI). However, most of the mentioned studies have evaluated menopausal symptoms by using Kupperman Index [37].

Several controlled trials with small sample size showed beneficial effects of black cohosh on menopausal symptoms while higher-quality trials suggest no significant improvement in mentioned symptoms. Due to conflicting results achieved on several studies no proven effect of black cohosh on menopausal symptoms were announced. However a study of large patients showed efficacy and safety of the product from black cohosh [76].

A review article published in Lancet on 2005 about treatment of menopausal symptoms has revealed that, according to the results of randomized-controlled trials, the plant cannot be used as a useful medication for the treatment of menopausal hot flashes. However, there are some exceptions. For example, a study suggested that administration of high doses of this plant to women with breast cancer who treated with tamoxifen made a significant reduction in the frequency of hot flashes [77].

Another systematic review on randomized, placebo-controlled clinical trials conducted to evaluate the effects of this plant and other non-hormonal treatments to improve symptoms associated with menopause. The results suggested that although numerous clinical trials have been performed on this plant, the qualities of these trials are very different and their results are varied. The time interval of most randomized placebo controlled trials was relatively short (12 weeks or less) to assess the effects of the plant in relieving menopausal symptoms and the sample size was also small [78].

In an article published in 2006 in Journal of Alternative and Complementary Therapies, it was noted that several studies conducted during 2005 and 2006 provided new information on the plant. Two of these studies have shown that the plant has positive effects on menopausal symptoms. The results of one of the studies has been mixed up, and in another, it has been stated that the plant does not affect menopausal symptoms [79].Also an article was published in the New England Journal of Medicine on 2006 which mainly concluded negatively about the effect of this plant on the frequency and severity of menopausal hot flashes. However, in the trials mentioned in this article, the sample size or duration of treatment is not mentioned [80].

The trial of the Herbal Alternatives for Menopause (HALT) Study was relatively a large and long-term research. A1-year randomized, double-blind, placebo-controlled trial on 351 women measured the effects of AR on Rate and intensity of vasomotor symptoms (1 = mild to 3 = severe), by using Wiklund Vasomotor Symptom Subscale. The results indicated that the plant alone or as a component of the mixture has little power in treating or relieving hot flashes or night sweats in menopausal women and it does not reduce the frequency or severity of these cases. The study lacks complete information about methodology and data gathering [81].

In 1982 a multi-center study on 629 patients suffering from menopausal symptoms (with an average age of 51 years) showed that in 80% of these patients, the symptoms of the disease were either better or completely eliminated by consuming the plant extract. The plant was tolerated very well, so that only 7% of the patients during study had mild gastrointestinal problems [82].

It was proposed that in comparison with conjugated estrogen (CE), AR extract was equally effective in menopausal symptoms contro lbased on a 12 weeks multi-center randomized placebo-controlled double-blinded trial. Post-menopausal volunteers between 40–60-year-old who at least 6 months passed from their last menstrual period and at least 3 episodes of hot flashes per day were observed at 0, 4, 8 and 12 weeks. Treatment was conducted in 3 groups of AR BNO 1055 extract (40 mg per day of herbal drug; n = 20), placebo (n = 20) and CE (0.6 mg per day; n = 22). Menopausal symptoms were assessed by the menopause rating scale (MRS) and a diary. AR BNO 1055 had no effect on endometrial thickness, which was significantly increased by CE but vaginal superficial cells were increased by both of them. It is proposed that AR BNO 1055 contains substances with SERM activity, i.e. with acceptable effects in the brain/hypothalamus, bone and vagina, but without exerting uterotrophic effects [83].

To determine the effects of commercial preparation of AR (Remifemin®) on secretion of FSH, LH, a placebo-controlled investigation was performed on 110 postmenopausal women. Two months after daily administration of 8 mg of this extract, FSH concentration was similar to placebo subjects, while LH secretion was significantly decrease in patients treated with the extract. This suggests the presence of estrogenic effects in this plant products [51].

Additionally, to assess the effects of this plant, two-arm randomization, placebo-controlled,

double blind trial was conducted on 85 women with a history of breast cancer. Patients were randomly assigned to black cohosh (one tablet of AR extract twice daily with meals) or placebo, stratified on tamoxifen use for 60 days. Before starting to take the pills and at 30 and 60 days, they completed a 4-day hot flash diary. At the final visit, they completed another menopausal symptom questionnaire. FSH and LH level were measured the first and the last visits in some of patients. Patients on both groups indicated declines in number and also severity of hot flashes. No significant differences between groups were found regarding improvements in menopausal symptoms, Hot flashes and FSH or LH level [84].

A randomized-controlled double-blinded parallel group study was conducted on 123 premenopausal and postmenopausal women, with kupperman index ≥20, who received two different doses (39 mg and 127.3 mg) of Remifemin® for 12 weeks. No difference is reported in the reduction of menopausal symptoms between two groups. In addition, in neither of these two doses, the estrogen-like effects on the uterus were observed. At the end of 12th week of this study, the percentage of patients with appropriate treatment response (with a Kupperman menopausal index of less than 15) was 70% and 72% respectively for the group receiving the standard dose of the drug and the group consuming large amount of plant, respectively. This study did not have a control group [85].

In a randomized, double-blind, cross-over trial in two 4-week period that targets to estimate efficacy of *CR* (1 capsule containing 20 mg per day of AR) in hot flush on 132 postmenopausal women, similar results were obtained and neither significant decrease in the frequency nor in severity of hot flashes among the patients administered this medicinal product was reported. The study did not succeed in providing evidence of effectiveness for reducing hot flashes in comparison to placebo [86].

In a randomized, double-blind, placebo-controlled trial with parallel groups for evaluation of AR efficacy in the treatment of menopausal induced anxiety disorder, patients were divided in two groups receiving either the extract of the aforementioned plant as 32 mg capsules containing 4 active titerpenes standardized to 5.6% of the active triterpene glycosides (n = 15) or placebo (n = 13) for 12 weeks. Using different criteria including Hamilton Anxiety Rating (HAM-A) scale, Beck Anxiety Inventory [61], Green Climacteric Scale (GCS), and Psychological Well Being (PGWB) index, there was not a significant difference between two groups [42].

In a randomized, double-blind, placebo-controlled clinical trial in Thailand 54 women over 40 years old with moderate to severe menopausal symptoms based on Kupperman index, received 40 mg of AR extract (n = 27) daily or placebo (n = 27) for 12 weeks. Results were evaluated by measuring KI scores, frequency of hot flashes, Menopause-Specific Quality of Life (MENQOL) score, participants global satisfaction and safety outcomes. Finally, there was no significant difference between the extract and the placebo in reducing symptoms [87].

In an open-label trial the effect of AR extract (Remifemin®) was evaluated by administration 40 drops of the extract twice daily to 50 patients with menopausal disorders for 3 months. The women included in study were refusing to use hormonal drugs or they were prohibited from administering these drugs. The efficacy of AR was measured by using the Kuppermann index of the Profile of Mood States (POMS) and the Clinical Global Impressions (CGI) scale. Based on these scales' scores, the extract had led to a significant improvement in menopausal discomfort [88].

In a randomized controlled, comparative clinical trial 60 hysterectomized patients less than 40 years old who had at least one intact ovary and still complained of climacteric symptoms, were treated with estriol (1 mg), conjugated estrogens (1.25 mg), estrogen-gestagen sequential therapy or an extract from AR known as Remifemin® (8 mg) after randomized distribution into 4 equal groups. Therapy was controlled after 4, 8, 12 and 24 weeks with a modified Kupperman-Index that also included trophic disorders of the genitals, and also by serum-FSH and -LH measurement. In all groups, the modified Kupperman-Index became significantly lower, but the parallel

decrease of gonadotropins could not be confirmed statistically. There were no significant differences between groups concerning therapy success [52].

Additionally, the results of a double-blinded randomized clinical study with 2 doses of isopopanolic extract of AR (40 and 127 mg) on 60 patients for 6 month indicated significant decrease in menopausal symptoms with both doses. At the end of study 90% of participants respond to therapy (kupperman index more than 15) and LH, FSH, Prolactin, Estradiol, vaginal cytological parameters do not have significant changes. So it could be suggested that the extract had non-hormonal effect [48].

Another study performed on 136 breast cancer survivors who experienced hot flashes, aged between 35–52 years old receiving tamoxifen. A control group receiving 20 mg/day of tamoxifen ($n$ = 46) and a group receiving same dose of tamoxifen with AR BNO 1055 (Menofem®/Klimadynon®), corresponding to 20 mg of herbal drug (n = 90). After 12 months of treatment in 46.7% of patients treated with the plant extract, the hot flashes were eliminated, nevertheless no one in the control group observed such an outcome. Moreover, 24.4% of patients treated with this plant and 73.9% of patients receiving placebo still had severe complications of menopause. In 28.9% of patients treated with this plant, mild disease related complications were seen, which was estimated to be 26.1% in the control group [89].

A randomized, double-blind, multi-center, placebo-controlled trial, carried out on 122 postmenopausal women to evaluate the efficacy and safety of the AR extract (6.5 mg dried rhizome extract) by measuring Kupperman Index and Menopause Rating Scale. This 12-weeks study demonstrated the superior effects of the plant extract in comparison with the placebo on patients whose Kupperman index score was 20 or higher. Reduction of the scores was 47% and 21% in black cohosh and placebo groups, respectively. But there was no significant difference in weekly weighted scores of hot flashes and the Menopause Rating Scale scores between two groups [90].

In a post-marketing follow-up study, 2016 women aged 40 to 56 years with menopausal symptoms score of 20 (based on the Kupperman index) received the isopropanol extract of the plant. At the beginning and the end of the fourth, eighth and 12th weeks of the study, changes in individual symptoms of menopause including hot flashes, sweating, insomnia and anxiety were significantly decreased in those consuming the extract, with an average of 17.64% based on the Kupperman index [91].

Also, a prospective study on 120 healthy women with menopausal symptoms with a follow-up period of 6 months was conducted on hot flashes. Compared with fluoxetine, black cohosh (Remixin) showed more potency for alleviation of hot flashes and night sweats. On the other hand, improvement in Beck's Depression Scale was much greater with fluoxetine than the AR extract [92].

In a double-blind, randomized, placebo-controlled clinical trial on 84 women at the beginning of the menopause, subjects received either 6.5 mg of AR or placebo as a tablet once a day for 8 weeks. Measurement of menopausal symptoms was conducted by the Greene climacteric scale (GCS) at the beginning of the study and at the end of the 4th and 8th weeks. Results showed that according to GCS subscale scores, patients in the intervention group significantly improved in vasomotor, psychological, physical and sexual symptoms. These changes were significantly higher at the end of the eighth week [93].

In a double-blind, randomized, placebo-controlled clinical trial in China, 244 patients with menopausal symptoms aged 40–60 years, randomly received 40 mg daily AR isopropanol extract (N = 122), or 2.5 mg/day tibolone orally (N = 122) for 3 months. Then, patients with at least one uterine fibroid, 34 patients in the medicine group and 28 patients in the tibolone group, were examined for alteration of fibroid size by transvaginal ultrasonography. At the end of the study, the results showed significant reduction in size of the uterus myoma in AR extract consumers compared with tibolone, so they concluded that the product can be effective in treating the

symptoms of menopause and uterine fibroids [94].

Some other studies were conducted on products containing two or more compounds with probable effect on vasomotor symptoms. A 12-month randomized, placebo-controlled clinical trial on 351 postmenopausal women who reported at least 2 hot flashes per day were performed. Participants in this trial were randomly assigned to one of the following three drug regimens:

(A) Daily intake of 160 mgofAR extract
(B) Receiving an herbal drug consisting of 200 mg of the abovementioned plant and 9 other components
(C) Receiving a multi-component herbal medicine along with a diet containing soya

Other women participating in the trial were randomly assigned to receive hormones (estrogen with or without progesterone) or placebo. The medical regimen containing AR or other medicinal herbs did not provide significant clinical relief for menopausal hot flashes. At the end of the third, 6th and 12th months of trial, hot flushes were observed in subjects who administered one of these three regimens as well as placebo recipients. But in patients undergoing estrogen administration, vasomotor symptoms were significantly reduced [95].

In a 16-week randomized, double-blind clinical trial on 301 women with menopausal complications including emotional disturbances, positive effects of AR mixture with St john's wort (*Hypericum perforatum*) in improvement of menopausal problems was presented. Each pill included standardized extract of AR (equivalent to one milligram of triaferenic glycosides), and standardized extract of *Hypericum perforatum* (equivalent to 0.25 mg of hypercytinum). Participants randomly assigned to the treatment group received 2 tablets twice a day during the first 8 weeks, and then one tablet twice daily. This study, demonstrated the superior effects of the mixture in comparison with placebo for menopausal symptom relief (50% vs. 19.6%, respectively). Moreover, the severity of depression evaluated by using Hamilton Depression Rating Scale total score whichreduced 41.8% and 12.7%, respectively in medicine and placebo groups [96].

A randomized, double-blind, placebo-controlled trial examined a Phyto-Female Complex's effect on 50 healthy pre and postmenopausal women, aged 44–65 years two times a day for 3 months. A structured questionnaire on the frequency and intensity of menopausal symptoms was completed weekly from one week before treatment and throughout the 3-month treatment period for patients, followed by biochemical tests, breast examination, and transvaginal ultrasonography. Volunteers receiving Phyto-Female Complex reported a significantly superior mean reduction in menopausal symptoms than the placebo group. The study concluded Phyto-Female Complex as a safe and effective remedy to eliminate hot flashes and sleep disturbances in pre- and postmenopausal women after at least for 3 months use [41]. A randomized, four-arm, double-blind clinical trial on vasomotor symptoms in 89 patients compare the effect of standardized black cohosh (ethanol extract of black cohosh below-ground parts (128 mg/d standardized to 7.27 mg triterpene glycosides)), red clover (ethanol extract of the aerial parts of red clover (398 mg/d standardized to 120 mg isoflavones), placebo and 0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate (CEE/MPA;). Reductions in vasomotor symptoms after 12-month intervention were as follows: black cohosh (34%), red clover (57%), placebo (63%), and CEE/MPA (94%), thereafter only treatment with CEE/MPA was significantly effective on symptoms. Both consumed extracts were considered to be safe during the study [44].

In a randomized clinical trial, the efficacy of the 40 mg per day aqueous-isopropyl alcohol extract of this plant on menopausal problems was evaluated in comparison with the low dose (25 μg) of estradiol administered via transdermal administration for 3 months on 64 postmenopausal women. As a result, it was found that the plant is a valid alternative to the use of transdermal estradiol for the treatment of menopausal

complications, especially for women who do not prefer or were not allowed to use conventional medicines [50].

Considering all performed clinical trials, the products containing black cohosh could be effective options in reduction of post-menopausal symptoms including hot flashes and psychological disturbances. However, the exact mechanism of its action is not clear.

Various clinical trials on efficacy of Black cohosh in controlling menopausal symptoms are organized in Table 24.2.

### 24.6.2 Clinical Trials on Cancer

Although menopausal symptoms shown to be treated by hormonal medications, it should be noted that some hormonal medications used to treat various diseases lead to an increased risk of breast cancer occurrence. So several studies conducted on effects of black cohosh on cancers. Besides, investigation of AR extract supplementation benefit in patients with breast cancer under chemotherapy which can lead to menopausal symptoms, is an important issue.

Regarding the risks of using hormonal treatments for menopausal symptoms, beside the efficacy and tolerability of AR extract (AR BNO 1055- daily dose of 40 mg herbal drug), the incidence of hormone-dependent tissue cancers, was studied in a prospective, open-label, multinational, multicenter one-year follow-up study of 400 postmenopausal women with estrogen deficiency symptoms. The uterus tissues health was studied by testing the samples obtained from the uterine biopsy. To evaluate the safety of the drug for endometrial tissue, the product was administered for 52 weeks and endometrial biopsy did not show any hyperplasia or endometrial dysfunction in these patients. After one year no uterus infiltration was specified in the subjects, endometrial thickness was not increased in ultrasound evaluation, despite significant reduction of the hot flashes. Overall drug tolerability was also documented well [97].

A retrospective observational pharmacoepidemiologic cohort study indicated no significant difference in efficacy of isopropanol extract and the plant itself for reduction of breast cancer recurrence possibility. Totally 18,861 patients included in the study while 1102 of them had received an isopropanolic AR extract therapy (Remifemin® or Remifemin® plus) and were observed for a mean period of 3.6 years. The study showed that treatment with this plant can play a role in the long-term survival of the patients and risk of breast cancer recurrence had not improved in group consuming isopropanolic extract in comparison with group receiving placebo [98].

In an open randomized clinical trial, in order to evaluate the safety of the herb for treatment of menopausal symptoms in healthy women, the effect of its isopropanol extract (40 mg daily) on breast density was determined by mammograms and evaluation of breast epithelial cells' infiltration. During a 6-months treatment period, it was found that the extract neither had side effects on breast tissue, nor had any side effects on the health of the uterus and general health of the individual [99].

In a prospective clinical study, 50 patients with breast cancer who received tamoxifen were given 1 to 4 tablets containing 2.5 mg AR isopropanol extract daily for 6 months. Participants' complaints were rated using the menopause rating scale (MRS II) at 1, 3, and 6 months after the onset of the intervention. Results showed a significant reduction in total MRS II score. Furthermore, symptoms such as hot flashes, sweating, sleep disturbances and anxiety enhanced, but no significant modification reported in musculoskeletal and urogenital complications. None of the reported side effects were belonged to complementary therapy and 90% patients informed tolerance to the drug well and very well. Therefore, researchers announced that using this supplement beside tamoxifen in breast cancer patients with psychovegetative symptom is reasonable [100].

By reviewing available randomized controlled and uncontrolled trials, and some observational studies, the current evidences do not support the association or dependence of the use of the plant and increased risk of breast cancer. There is also

**Table 24.2** Clinical studies of AR.

| No. | Type of study | Population under observation | Adminstration Dose | Duration of adminstration | Goal | Result | Conclusion | Ref. |
|---|---|---|---|---|---|---|---|---|
| 1 | Open-label trial | 50 patients with menopausal disorders | 40 drops of extract twice daily | 3 months | Improvement in menopausal discomfort | Eficacy of AR measured by Kupperman index of the profile of mood states (POMS) and the clinical global impressions (CGI) scale | Extract had led to a significant improvement in menopausal discomfort | [88] |
| 2 | Double-blind, randomized, placebo controlled clinical trial | 80 patients | Isopropanolic AR extract | 12-week | Investigate iAR for efficacy as measured by the KMI in 80 patients | Significant superiority of iAR to placebo for alleviating climacteric complaints | iAR extract demonstrated anti Climateric activities | [123] |
| 3 | Randomized controlled, comparative clinical trial | 60 hysterectomized patients less than 40 years old who had at least one intact ovary | Estriol (1 mg), conjugated estrogens (1.25 mg), estrogen-gestagen and 8 mg Remifemin® | 24 weeks | Compare Remifemin ® with hormonal therapy in reducing menopausal symptoms | The modified Kupperman-index became significantly lower in 4 groups | No significant differences between 4 groups concerning therapy success. | [52] |
| 4 | Placebo controlled | 110 postmenopausal women | 8 mg of remifemin® | 2 months | Effects of commercial preparation of AR (Remifemin®) on secretion of FSH, LH, | FSH concentration was similar to placebo subjects, while LH secretion was significantly decrease in patients treated with the extract. | Presence of estrogenic effects in the plant products | [51] |
| 5 | Double-blind and randomized clinical study | 60 patients | 40 mg and 127 mg daily use of isopropanolic extract | 6 months | Improvement in menopausal discomfort | 90% of participants respond to therapy (kupperman index more than 15) | Significant decrease in menopausal symptoms in 2 doses by non-hormonal effect | [48] |
| 6 | Two-arm randomization, placebo-controlled, double blind trial | 85 women with a history of breast cancer | Black cohosh extract one tablet twice daily with meals | 60 days | Improvement in menopausal discomfort | Decrease in number and also severity of fluttering in both groups | Significant difference between groups in improvements in menopausal symptoms, hot flashes and FSH or LH level | [84] |

(continued)

**Table 24.2** (continued)

| No. | Type of study | Population under observation | Adminstration Dose | Duration of adminstration | Goal | Result | Conclusion | Ref. |
|---|---|---|---|---|---|---|---|---|
| 7 | Randomized-controlled double-blinded parallel group study | 123 premenopausal and postmenopausal women, with kupperman index ≥20 | (39 mg and 127.3 mg daily) of Remifemin® | 12 weeks | Evaluate efficacy and safety of AR rhizoma (40 mg/day) and higher dose and its associated physiological effects | Treatment response (Kupperman menopausal index of less than 15) was 70% and 72% respectively for the group receiving the standard dose and large amount | No difference is in reduction of menopausal symptoms and estrogen-like effect on uterus between groups. | [85] |
| 8 | Multi-center randomized placebo-controlled double-blinded trial | 62 post-menopausal women (40–60 y) with at least 6 month of their last bleeding period and at least 3 hot flashes per day | AR BNO 1055 extract (40 mg per day of herbal drug, placebo and CE (0.6 mg per day) | 12 weeks | Compare, AR BNO 1055 with standard hormone therapy in improving climacteric complaints and effects in vagina and bone metabolism | Measuring:<br>Menopause rating scale<br>CrossLaps<br>Alkaline phosphatase | Use of *AR* and CE increases the activity of osteoblasts | [83] |
| 9 | Two-armed, randomized and open-label study | 136 breast cancer survivors aged 35–52 year receiving tamoxifen | 20 mg per day tamoxifen with or without AR BNO 1055 (20 mg of herbal drug) | 12 months | Reducing hot flashes | In 46.7% of patients treated with the plant hot flashes were eliminated | Reduction in the severity and frequency of hot flashes | [89] |
| 10 | Randomized, multicenter, double-blind clinical trial | 304 patients | 40 mg per day of isoprpanolic extract | 12 weeks | Compared the efficacy and tolerability of the isopropanolic black cohosh extract in the treatment of climacteric complaints compared with placebo | Decrease in menopausal rating scale units similar to recent hormone replacement therapies. The most effectiveness was on hot flushes. | The isopropanolic extract of black cohosh root stock as effective substance for relieving climacteric symptoms, especially in early climacteric women | [110] |

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| 11 | Multicenter, randomized, placebo-controlled, double-blind study | 124 peri-menopausal women | Supplementary one tablet daily containing100mg AR extract (providing 8 mg deoxyacetein) or placebo | 12 weeks | Effects of a novel dietary supplement containing soy isoflavones and AR on climacteric symptoms | Improvement of modified Kupperman index and Greene climacteric scale were not significant between groups | No significant clinical improvement were reported | [124] |
| 12 | Randomized, double-blind, multi-center, placebo-controlled parallel group trial | 122 postmenopausal women | (6.5 mg dried rhizome extract) | 12 weeks | Efficacy and safety of the AR extract | Measuring Kupperman index and menopause rating scale | No significant difference in weekly weighted scores of hot flashes and the menopause rating scale between two groups but significant reduction in Kupperman index | [90] |
| 13 | Post-marketing follow-up study | 2016 women aged 40 to 56 years with menopausal symptom | 2 tablets daily of Remifemin ® (the isopropanol extract) | 12 weeks | Improvement of menopausal problems | Average decrease in kupperman index was 17.64 points, as hot flashes, sweating, insomnia and anxiety scores decrease too. | Severity of symptoms significantly decreased by consuming extract | [91] |
| 14 | Randomized clinical trial | 64 postmenopausal women | 40 mg per day aqueous-isopropyl alcohol extract and 25 µg transdermal estradiol | 3 months | Treatment of menopausal complications | Same efficacy of two drugs on reduction of vasomotor, depression symptoms. | AR extract as a valid alternative to the use of transdermal estradiol for the treatment of menopausal complications | [50] |
| 15 | Prospective, open-label, multinational, multicenter study | 400 postmenopausal women with estrogen deficiency symptoms | AR BNO 1055- daily dose of 40 mg herbal drug | 52 weeks | Measuring risk of hormonal effects beside efficacy and tolerability | Measuring incidence of hormone-dependent tissue cancers | The extract had no effect of hyperplasia or endometrial dysfunction. Or endometrial thickness Occurrence of hot flashes was significantly reduced. | [97] |

(continued)

**Table 24.2** (continued)

| No. | Type of study | Population under observation | Adminstration Dose | Duration of adminstration | Goal | Result | Conclusion | Ref. |
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| 16 | 1-year randomized, double-blind, placebo-controlled trial | 351 women 52% in menopausal transition and 48% postmenopausal (45 to 55 years) | Black cohosh, 160 mg daily; multibotanical with black cohosh, 200 mg daily, and 9 other ingredients; multibotanical plus dietary soy counseling; conjugated equine estrogen, 0.625 mg daily, with or without medroxyprogesterone acetate, 2.5 mg daily; placebo | 12 months | Effectiveness and safety of three naturopathic measures as compared to hormone therapy and placebo | Measuring rate and intensity of vasomotor symptoms Wiklund vasomotor symptom subscale | In contrast to hormone therapy, herbal medication had no significant difference to placebo | [81] |
| 17 | Randomized, placebo-controlled clinical trial | 351 postmenopausal women who reported at least 2 hot flashes per day | 160 mg daily of AR extract- herbal drug consisting of 200 mg of the mentioned plant and 9 other components-multi-component herbal medicine-hormone(E ± P) | 12-month | Relief for menopausal hot flashes | In third, sixth and 12th months of trial, hot flushes were observed in subjects who administered one of these three regimens as well as placebo recipients | No significant clinical relief for menopausal hot flashes | [95] |
| 18 | Randomized, double-blind, corss-over trial | 132 postmenopausal women | 1 capsule, AR 20 mg BID | Two 4-week periods | Relief for menopausal hot flashes | The product had no effectiveness for reducing hot flashes in comparison to placebo | No significant decrease in the frequency and severity of hot flashes | [86] |
| 19 | Randomized, double-blind clinical trial | 301 women with menopausal complications | 2 tablets twice a day (equivalent to 2 milligram triaferenic glycosides) in combination with 0. 5 mg of hypercytinum | 16 weeks | Improvement of menopausal problems | Hamilton's depression scale, decrease in severity of depression was 41.8% and 12.7%, in intervention and control group respectively. | Superior effects of the mixture in comparison with placebo for symptom relief and depression in postmenopausal women | [96] |

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| 20 | Double-blind study | Sixty-two postmenopausal women | AR (daily dose corresponds to 40 mg of herbal drug), conjugated estroged (CE: 0.6 mg/day), or placebo | 12 weeks | Effects of the preparation AR BNO 1055 on markers of bone metabolism, hormones, sex hormone-binding globulin (SHBG), lipometabolism, vaginal maturity, and routine laboratory parameters | AR and CE both had beneficial effect on bone density while strong estrogenic vaginal mocousa effect was shown with CE. | AR is suggested for increasing bone remodeling and weak estrogen-like effects in the vaginal mucosa. | [118] |
| 21 | Randomized, double-blind, controlled study in 5 centers | 244 menopausal patients aged 40–60 years and with a Kupperman Menopause Index (KMI) > or = 15 | 40 mg iAR daily or tibolone 2.5 mg daily orally | 12 months | Efficacy-safety balance of the isopropanolic extract of AR (iAR, Remifemin) in comparison with tibolone | Statistical significant non-inferiority of iAR to tibolone in treatment but less adverse effect (vaginal bleeding -breast and abdominal pain -leukorrhea) | Influence of 2 remedies on climatrict symptoms were the same while Remifemin was shown to have superiority in safety profile | [115] |
| 22 | Prospective, controlled open-label observational study | 6141 women at 1287 outpatient gynecologists | Remifemin® (monotherapy) 1 tablet twice a day or Remifemin® plus John's wort (combination therapy) 1–2 tablets twice daily based on physician consideration | 12 months | Effectiveness and safety of black cohosh alone or in fixed combination with St. John's wort | Improvement by both therapies was maintained at 6 and 12 months was measured by MRS sub- score PSYCHE | Decrease in climacteric mood symptoms with combination of black cohosh and St. John's wort was superior to black cohosh alone. | [125] |
| 23 | Prospective study | 120 healthy women with menopausal symptoms | Remixin® 40 mg per day or 20 md daily of fluoxetine | 6 months | Improvements in symptoms | Fluoxetine is more effective in improvements shown on Beck's depression scale | Black cohosh (Remixin) shows more potency to lower hot flashes and night sweats than fluoxetine | [92] |

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**Table 24.2** (continued)

| No. | Type of study | Population under observation | Adminstration Dose | Duration of adminstration | Goal | Result | Conclusion | Ref. |
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| 24 | Retrospective observational pharmaco-epidemiologic cohort study | 18,861 patients join in the study while 1102 of them had received an isopropanolic AR extract therapy | Isopropaolic extract(Remifemin® or Remifemin ® plus) | 3.6 years mean overall observation | Risk of breast cancer recurrence | 14% recurrence of breast cancer was observed after 2 and 6.5 years in control group and AR consuming group consequently. | Treatment with extract causes the long-term survival of the disease and risk of breast cancer. Recurrence do not increase in comparison with placebo | [98] |
| 25 | Non-prospective, open randomized clinical trial | 65 women | 40 mg black cohoshisopropanol extract daily | 6 months | Evaluate the safety of the herb for treatment of menopausal symptoms | Breast density was determined by mammograms and infiltration of breast epithelial cells. | Isopropanolic extract does not cause adverse effects on breast tissue. Endometrial or general safety concerns was not reported | [99] |
| 26 | Randomized, double-blind, placebo-controlled trial | 50 pre and postmenopausal 44–65 y women | Phyto-female complex two times a day | 3 months | Frequency and intensity of menopausal symptoms | Examining biochemical tests, breast check, and transvaginal ultrasonography | Phyto-female complex is a safe and effective remedy to lower of hot flushes and sleep disturbances. | [41] |
| 27 | Mechanistic open labelled study | 6 post-menopausal women | 40 mg per day of Remifemin | 12 weeks | Black cohosh effect on the central endogenous opioid system | Remifemin effects was measured by saline/naloxone [123] challenge and positron-emission tomography [124] imagingwith carfentanil | Examination of central opioid function demonestrated a neuropharmacologic action of black cohosh treatment in postmenopausal women | [63] |
| 28 | Randomized, double-blind, placebo-controlled trial | 28 post- and pre-menopausal women | 32 mg capsules containing 4 active titerpenes standardized to 5.6% of the active triterpene glycosides | 12 weeks | Menopausal induced anxiety disorder | Examining – Hamilton Anxiety Rating (HAM-A) scores – Beck Anxiety Inventory [61] -green climacteric scale (GCS) -psychological well being (PGWB) index | No significant difference between two groups | [42] |

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| 29 | Randomized, four-arm, double-blind clinical trial | 89 women | Ethanol extract of black cohosh (128 mg/d standardized to 7.27 mg triterpene glycosides), red clover, CEE/MPA | 12 months | Reductions in vasomotor symptoms | Reduction in vasomotor symptoms was black cohosh (34%), red clover (57%), placebo (63%), and CEE/MPA (94%) | Only treatment with CEE/MPA significantly effects on symptoms | [44] |
| 30 | Prospective clinical study | 50 patients with breast cancer | Tamoxifen with daily 1–4 tablets (containing 2.5 mg AR isopropanol extract) | 6 months | Improvement psychovegetative symptom in breast cancer | Significant reduction in total menopause rating scale (MRS II)<br>No significant modification reported in musculoskeletal and urogenital complications | Using the supplement beside tamoxifen in patients with psychovegetative symptom in breast cancer is reasonable | [100] |
| 31 | Prospective, double-blind, placebo-controlled study | 209 post-menopausal women | Estradiol 2 mg/ norethisterone acetate 1 mg (E2/NETA), tibolone 2.5 mg or placebo or Remifemin (20 mg) twice daily | 6 months | Effects of continuous combined hormone therapy, tibolone, black cohosh, and placebo on digitized mammographic breast density | Mammograms by digitized quantification of breast density revealed no increase in tissue density by placebo and black cohosh | Unlike other treatments, black cohosh had no effect on breast tissue density | [126] |
| 32 | Meta-analysis of randomized, double-blind, and controlled clinical trials | 1020 Perimenopausal and postmenopausal women | 40–128 mg of iAR daily | 3–6 months | Evaluated the efficacy and safety of the isopropanolic black cohosh extract (iAR) | No difference in hepatic enzymes were observed during 5 clinical trials | Meta-analysis of five randomized, double-blind, and controlled clinical trials showed no evidence that iAR has any adverse effect on liver function. | [127] |

(continued)

**Table 24.2** (continued)

| No. | Type of study | Population under observation | Adminstration Dose | Duration of adminstration | Goal | Result | Conclusion | Ref. |
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| 33 | Multicenter, randomized, double-blind, placebo-controlled study | 101 menopausal women | Oral BRN-01 tablets (with AR extract and 4 other extracts) treatment (2 to 4 tablets per day) or placebo | 12 weeks | Evaluate the efficacy of the non-hormonal treatment BRN-01 in reducing hot flashes | The combination therapy of several extracts suggest lowered incidence of hot flashes | The tablet would be useful in patients suffering from symptoms | [128] |
| 34 | Double-blind, randomized, placebo-control clinical trial | 84 women at the beginning of the menopause | 6.5 mg of AR or placebo in a pill once a day | 8 weeks | Improvement in menopausal discomfort | Measurement of menopausal symptoms was conducted by the Greene climacteric scale (GCS) | Significant improved in vasomotor, psychological, physical and sexual symptoms by the extract | [93] |
| 35 | Double-blind, randomized, placebo-controlled clinical trial | 244 patients with menopausal symptoms aged 40–60 years | 40 mg daily AR isopropanol extract or 2.5 mg/day tibolone orally | 3 months | Improvement in menopausal discomfort and treatment of uterine fibroids | Significant reduction in size of the uterus myoma in consumption of AR extract compared with tibolone | Be effective in treating the symptoms of menopause and uterine fibroids | [94] |
| 36 | Randomized study | 116 women who had endometriosis | Remifemin 20 mg twice a day or Tibolone 2.5 mg per day oral after GnRH-a injection | 12 weeks | Investigate clinical efficacy and safety of Remifemin on peri-menopausal symptoms in endometriosis patients with a post-operative GnRH-a therapy | Remifemin with fewer adverse effects than tibolone has the same effect on treating hot flashes and sweating score | Efficacy on clinical symptoms and safety was higher with Remifemin in comparison to tibolone in patients receiving GNRH- a therapy | [129] |

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| 37 | Randomized, double-blind and placebo-controlled | 42 postmenopausal womenwith sleep disturbance | 20 mg of crude drug (2.5 mg of extract) daily or placebo | 6 months | Effect of black cohosh on both objective and subjective sleep in early postmenopausal women with sleep complaints | In comparison with placebo significant polysomnographic changes was reported with black cohosh | Improved sleep was the result on black cohosh treatment with a wide safety index | [130] |
| 38 | Randomized, double-blind, placebo-controlled clinical trial | 54 women over 40 years old with moderate to severe menopausal symptoms based on Kupperman index [37], | 40 mg of AR extract daily and placebo | 12 weeks | Improvement in menopausal discomfort | Measuring:<br>KI<br>frequency of hot flashes<br>Menopause-specific quality of life (MENQOL) score<br>participants global satisfaction and safety outcomes | No significant difference between the extract and the placebo in reducing symptoms | [87] |

no enough evidence to suggest the benefit of this supplement in reducing stroke risk in patients with breast cancer. Finally, due to promising and, incidentally, controversial observations, more experiments are necessary in this field [101].

### 24.6.3 Clinical Trials on Osteoporosis

Due to hormonal effect of black cohosh it was hypothesized that the extract may also have beneficial effects on bone density and mechanism of bone formation. Results of a study that was performed on Sixty-two evaluable postmenopausal women using the AR BNO 1055 extract (containing 40 mg herbal drug), conjugated estrogen (CE) (0.6 mg) or placebo for 12 weeks indicates the positive effects of the plant on bone metabolism. Menopause rating scale were used for menopausal symptom measurements and diary levels of Cross Laps (marker of bone degradation) by ELECSYS system and alkaline phosphatase as metabolic index indicative of bone formation were also determined. It was shown that treatment with AR BNO 1055 extract for 12 weeks increased the amount of bone alkaline phosphatase but the level of this enzyme did not change in the patients who received conjugated estrogen or placebo. However, consumption of the plant and conjugated estrogen also increased serum levels of triglycerides. Therefore, it seems that the use of this plant and CE increases the activity of osteoblasts. On the other hand, no hazardous side effects have been reported from the consumption of the plant [76].

### 24.6.4 Clinical Trials on Polycystic Ovary Syndrome

In a randomized controlled trial was performed on 147 women aged less than 35 years with unexplained infertility and recurrent clomiphene resistance for ovulation induction. Anovulatory participants which was diagnosed by serum oestradiol <200 ng/ml and absence of a dominant ovarian follicle on day 9 of the menstrual cycle, were excluded (n = 28). Complete data sets available for 119 women. All women received Clomiphene citrate (clomiphene) 150 mg on menstrual cycle days 3–7. A randomized group also took AR 20 mg/day between days 1–12. AR described as 'phytoestrogens' was provided in the commercial preparation Klimadynon®, manufactured by Norica in Germany. A trigger injection (human chorionic gonadotropin, 10,000 IU) and timed intercourse was recommended when a dominant follicle >17 mm was observed. Pregnancy rate was 20.3% and 43.3% in clomiphene alone and the clomiphene plus AR group respectively ($P < 0.01$). Clinical pregnancy rate in the combination group was 36.7% versus 13.6% in the clomiphene alone group ($P < 0.01$). Endometrial thickness in combination group was 8.9 (±1.4) versus 7.5 (±1.3) ($p < 0.001$). Days to ovulation in clomiphene alone group was 13.0 ± 1.1 and in the clomiphene plus AR group 14.2 ± 1.3 (n.s.). Luteal progesterone peak (ng/ml) in combination group was 13.3 (±3.1) versus 9.3 (±2.0) in clomiphene alone group ($p < 0.01$). All other hormone measures were not significantly different [102]. In another prospective randomized controlled trial in Egypt, 100 women with PCOS were allocated into one of two groups: one group (n = 50) received clomiphene citrate 100 mg daily for 5 days, and the other group (n = 50) received AR 20 mg daily for 10 days. Both groups received medication starting from the second day of the cycle for three consecutive cycles. The groups were similar in terms of age, clinical presentation and hormonal levels before treatment. Following treatment, significant favourable changes in LH level and FSH/LH ratio ($p = 0.007$ and 0.06, respectively) were seen in the Klimadynon group. In this group the progesterone level was higher from the first treatment cycle, indicating better ovulation ($p = 0.0001$), and endometrial thickness was greater ($p = 0.0004$). The pregnancy rate was higher in the Klimadynon group but the difference between the groups was not significant ($p = 0.1$) [103]. Based on these studies, AR extract as a phyto-oestrogen can be used as an alternative to clomiphene citrate for ovulation induction in women with polycystic ovarian syndrome.

## 24.7 Contraindications

This plant should not be used during pregnancy as it may cause fetal abortion. The use of this plant during lactation is prohibited.

## 24.8 Side Effects and Warnings

Although no serious and risky complications have been reported following the correct administration of this plant, the onset of some side effects caused by the intake of the plant, including inflammation of the gastric and intestinal mucosa, nausea and vomiting is probable. There is one case of muscle damage following the use of this plant [104]. A case of severe hyponatremia and altered mental status associated with the use of black cohosh during prolonged labor was reported. A 39-year-old primigravida at 38 weeks of gestational age presented to the emergency department after she became disoriented and lethargic while laboring at home with a midwife. She had consumed several doses of black cohosh to induce labor. On presentation, she was nonverbal and unable to follow commands. Her serum sodium was 114 mmol/L (range, 132–145 mmol/L), serum osmolality was 253 mOsm/kg (range, 275–300 mOsm/kg), urine osmolality was 190 mOsm/kg (range, 300–900 mOsm/kg), and urine sodium was <10 mmol/L. The patient soon became uncooperative and combative and a cesarean section was performed. Her mental status returned to baseline and she was subsequently discharged home without further complication [105]. Further investigation is needed to evaluate the safety and efficacy of black cohosh during pregnancy and labor. In another case report about a 55-year-old woman who used an herbal mixture containing AR and *Hypericum perforatum* for treatment of menopausal hot flashes, after 17 months she suffered from abdominal pain and discomfort during urination. The imaging showed a large myoma in her uterus, which forced her to undergo surgery [106]. In another case report a 46-year-old woman who was hospitalized for orbicular dyskinesia due to facial, oral and lingual movement disorder, it was found that two doses of AR 20 mg and *Panax ginseng* C.A.Mey. 50 mg has been used to treat menopausal complications. The mechanism of this complication seems to be due to the effect of AR on dopaminergic, serotonergic and gabaergic systems [107]. A case of subacute liver failure was reported in a 60-year-old woman who used this herbal to treat menopausal symptoms, leading to liver loss and need for liver transplantation. This case should always be considered in prescribing the herb [108]. However, in a review article, it has been mentioned that, despite the existing case reports, this plant should not be introduced as a potential cause of liver toxicity [109]. As mentioned in a review article, the side effects of this plant, which include gastroesophageal complications and rash, are rare, mild, and reversible (Borrelli and Ernst 2008). In an animal study, administration of this plant increased incidence of pulmonary tumors [56]. One case of severe muscular damage and a severe increase in blood levels of creatine phosphokinase and lactate dehydrogenase associated with consuming this herb has been reported after being used for the treatment of menopausal symptoms, which was disappeared by stopping its consumption [104].

## 24.9 Toxicity and Safety Studies

Black cohosh safety was evaluated in lots of previous studies and it was acceptably well tolerated in patients. Based on a review article collecting data from 35 clinical investigations and one meta-analysis on 5 published randomized, controlled studies on AR safety, 13,492 people used registered (n = 11,961, 88.6%) and un-registered (n = 1531, 11.4%) preparations of AR. All users demonstrated good tolerability to the extracts and no difference in severity and frequency of side effects were specified for the AR extract compared to the placebo [44, 81, 83, 90, 96, 98, 110]. Furthermore, no drug interaction was recorded for AR [98]. Specifically, 2 studies mentioned no relevant interaction between extract and cytochrome P- isoenzymes or P-glycoprotein [111, 112]. In clinical studies also safety of the extract for specific tissues was evaluated. Estrogen-

sensitive tissues like breast and uterus were exposed to the extract and no significant clinical alteration in hormonal parameters were mentioned [44, 50, 83, 85, 101, 113, 114]. Additionally, several studies on endometrium revealed the same results [44, 50, 83, 97, 99, 115]. Even more, a study suggested reduction of breast cancer risk in patients receiving AR [116]. Another study which was conducted on 9900 patients showed no change in risk of breast cancer using ethanol extract of AR and also a decrement in the risk of breast cancer in patients using isopropanol extract [110, 117].

Another concern about AR extract was its negative effect on liver function. Liver function was evaluated before and after consuming the extract in 11 studies. There was no clinical adverse event on using AR extracts [45, 50, 90, 109, 110, 115, 118, 119]. However, there are several case reports on hepatotoxicity induced by AR food supplements. The most severe illness occurred in a 47-year-old woman who was taking black cohosh for symptoms related to the menopause [120]. These evidences suggested that food supplements of the plant are not controlled as the medical extracts do [121].

In the United States, the Food and Drug Administration (FDA) lists it as an "herb of undefined safety" [1]. But there are no restrictions on the use of black cohosh in Australia. They mentioned that it contains a mixture of alkaloids, tannins and terpenoids and has not previously been reported to have hepatotoxic effects.

## 24.10 Drug Interactions

*High risk:

Concomitant use of this plant with azathioprine or cyclosporine may decrease the effect of these immunosuppressive drugs and result in rapid removal of the transplanted organ. It is recommended that patients consuming azathioprine to maintain transplantation should refrain from using the supplements, teas and herbal mixtures containing the plant. If it is determined that the herbal product consumed by this group of patients contains mentioned plant, the product should immediately discontinue and control the patient in terms of signs and symptoms of transplant rejection.

The plant could have effect on vagous nerve, which can lead to hypotension and exacerbates the effects of drugs used during general anesthetic induction, anesthetic drugs, antihypertensive and sedative medications. So, 2 weeks prior to any surgery, the use of herbal medicines should be discontinued [122].

Using this herb with antihypertensive drugs may increase the effect of these drugs and their concomitant use is prohibited.

-The concomitant use of AR extract with tamoxifen may increase its effects. On the other hand, the results of in vitro studies indicate that in the absence of estrogen, the extracts prepared from this plant significantly inhibit human breast adenocarcinoma (MCF-7) cells. Consequently, the plant increases the effect of tamoxifen if used simultaneously [57].

Besides, if it is used in conjunction with iron products, the tannin in the plant may combine with iron and lead to the formation of insoluble complexes. Patients using iron supplements should take these two medicines at least 2 hours apart.

## 24.11 Conclusions

The plant AR has been used by native Americans as a remedy for a wide range of disorders. In this review, ethnobotanical and phytochemistry of AR were discussed as well as pharmacological properties. On the basis of the results of our study, some researches showed positive effect of different commercial and noncommercial products based on black cohosh extract, as well as different effects on osteoporosis, cell proliferation, hormone secretion, diabetes, analgesic and depression in preclinical studies. However, further pharmacological and clinical studies are needed to find unexplored aspects and to reveal these reported effects of Black cohosh and other members of Ranunculacea family.

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# 25

# Ethnobotany, Phytochemistry and Pharmacological Features of *Centella asiatica*: A Comprehensive Review

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**Abstract**

*Centella asiatica* (CA) or Gotu cola is an herbal plant from the Apiaceae family with a long history of usage in different traditional medicines. It has long been used for the treatment of various ailments such as central nervous system (CNS), skin and gastrointestinal disorders especially in the Southeast Asia. This chapter focused on the phytochemical constituent and pharmacological activities of CA based on preclinical and clinical studies. Additionally, botanical description and distribution, traditional uses, interactions, and safety issues are reviewed. Electronic data-

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bases of Google Scholar, Scopus, PubMed, and Web of Science were searched to obtain relevant studies on the pharmacological activities of CA. Approximately, 124 chemical compounds including triterpenoids, polyphenolic compounds, and essential oils have been isolated and identified from CA. Ethnomedicinal applications of CA mostly include treatment of gastrointestinal diseases, wounds, nervous system disorders, circulatory diseases, skin problems, respiratory *ailments*, diabetes and sleep disorders in various ethnobotanical practices. Pharmacological studies revealed a wide range of beneficial effects of CA on CNS, cardiovascular, lung, liver, kidney, gastrointestinal, skin, and endocrine system. Among them, neuroprotective activity, wound healing and treatment of venous insufficiency, as well as antidiabetic activity seem to be more frequently reported. At the moment, considering various health benefits of CA, it is marketed as an oral supplement as well as a topical ingredient in some cosmetic products. Additional preclinical studies and particularly randomized controlled trials are needed to clarify the therapeutic roles of CA.

**Keywords**

*Centella asiatica* · Herbal plant · Ethnobotany · Phytochemistry · Pharmacology · Clinical studies

## 25.1 Introduction

Medicinal plants perhaps are the main source for new chemical entities or may be used in their intact form as a medicine. They have proven their therapeutic potentials along the time and nowadays investigates for active compounds, by *in vivo*, *in vitro*, and clinical studies for confirming their usages as a medicine or an adjuvant or a supplement for standard treatments of diseases [1].

*Centella asiatica* (L.) Urb. is anayurvedic (an Indian system of medicine) and Chinese traditional medicine plant belonging to Apiaceae family (previously known as Umbelliferae) (Fig. 25.1) [2]. It also named *Hydroctyle asiatica* and Indian pennywort, GotuKola (Europe and America), Pegaga (Malaysia), Mandukaparni (India), Kaki Kuda or Pegagan (Indonesia), Gong Gen or Tung chain (China) are its other common names in different countries [3, 4]. *Centella asiatica* (CA) has been listed in the Indian Herbal Pharmacopoeia, the Pharmacopoeia of the People's Republic of China, the European Pharmacopoeia, and the German Homeopathic Pharmacopoeia as a drug [5].

**Fig. 25.1** An illustration of *Centella asiatica*. Note: This figure was published in the internet

It traditionally is applied for different conditions such as some infectious and inflammatory diseases, seizure, tumor, and also psychosis [6, 7]. CA also improved some neurological and psychological conditions such as general anxiety disorder, dementia and cognitive disorders [8, 9]. It is established that extract of CA is an antioxidant and an anti-inflammatory agent and also it has showed anti-hyperglycemia and anti-hyperlipidemia effects in various studies [10–13]. It promoted wound healing and improved filtration and some other functions of venous and general circulation in diabetic and hypertensive patients or patients who have venous insufficiency or other disorders such as anal fissure [14–17]. It was helpful for treatment of some dermal disorders like erythema, edema, crust, excoriation and lichenification [8, 18]. CA seems to

be safe for human use [15, 19, 20]. So, CA logically has enough potential for investigation as a medicinal herb for different applications.

In present chapter, we attempt to incorporate a comprehensive, detailed, and up to date findings about its ethnobotanical uses, phytochemistry, preclinical and clinical studies, interactions, and safety issues, focusing on the pharmacological and pharmacognostic aspects.

## 25.2 Botanical Description and Distribution

The genus *Centella* L. is a member of the Apiaceae family (formerly Umbelliferae) with important medicinal species, containing a total of 59 accepted species worldwide [21]. Among them, the most popular and commercially important is *Centella asiatica* (L.) Urb., which naturally found in the tropical and subtropical regions, of the Old and New World [22, 23]. *Centella asiatica* is a stoloniferous perennial plant, with a height which can reach up to 15 cm. The stem is creeping and glabrous. The leaves are orbicular or reniform, 1–3 from each node of stems, sheathing leaf base, crenate margins, glabrous on both sides. The flowers are fascicled umbels, each umbel consisting of 3–4 white to purple flowers. The fruit is schizocarp with oblong and globular shape of 5 cm long. The seeds have pendulous embryo [22]. It is commonly found in the damp and marshy areas of South-Southeast Asia, Australia, Madagascar, Southern and Central Africa, some Pacific Islands and several regions of South Eastern United States and South Central America [24].

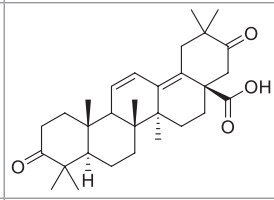
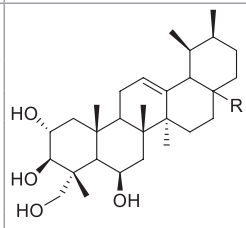
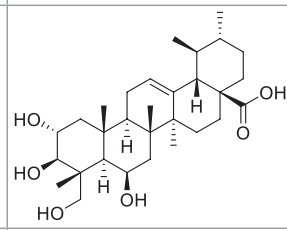
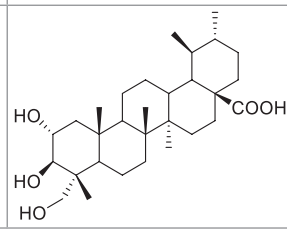
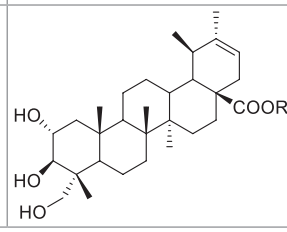
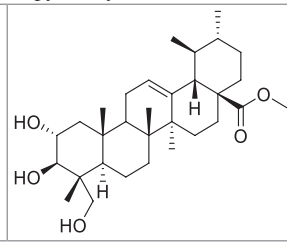
## 25.3 Phytochemistry Study

Phytochemical studies on CA indicate the presence of several categories of chemical compounds such as triterpenoids, polyphenolic compounds, and essential oils. These compounds are shown in Table 25.1.

## 25.4 Ethnobotanical and Ethnomedicinal Uses

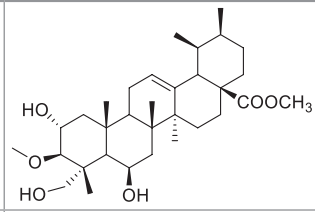
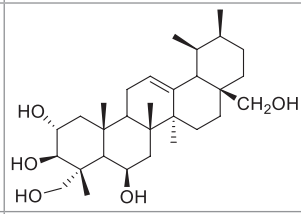
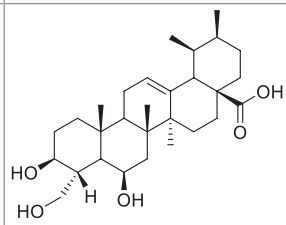
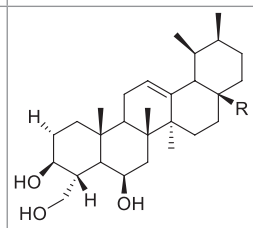
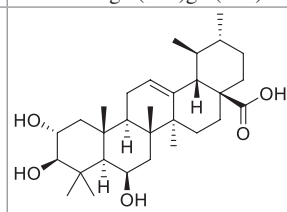
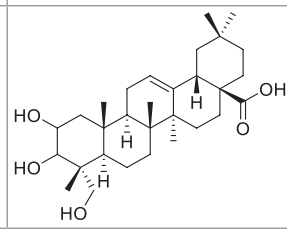
*Centella asiatica* (L.) Urb. has a long history of uses both as edibles and as ethnomedicinal plant in different ethnobotanical practices around the world. *Literature review demonstrates that*, CA, as the most famous species of the *Centella* genus, has noticeable traditional applications which mainly originate from Asia, Africa and Europe continents. In Asian countries, particularly India, Nepal, Bangladesh and China, there are remarkable reports on the traditional applications of CA. In India, it is applied as a traditional drug to treat asthma [25]. In the Indian Traditional Medicine, its leaves commonly known as Vallarai, which are widely employed as for children as a memory enhancer [26]. Furthermore, the whole plant is used for the treatment of stomach worm, and its leaf is also taken as an effective treatment for leucorrhoea, epilepsy and mental disorder [27]. In Pakistan, its leaves commonly known as Barhami, are considered very useful in the treatment of skin diseases, syphilitic, rheumatism, dysentery and fevers [28]. In China, CA is considered very useful in the treatment of hepatitis [29]. In the *Traditional* Chinese *Medicine*, it is also consumed as heat-clearing, detoxification, sunstroke and gallstones cleaning [30]. In Nepal, the paste of whole plant is applied to relieve muscular swelling and joint pains. It is also used to cure skin diseases such as eczema and pimples. Moreover, a decoction of it is given to cure fever, indigestion, uric acid and dysentery. It is also recommended for children to enhance memory power [31]. In addition, the leaves and roots of CA are extensively used in folk medicine to treat wound, gastritis and anorexia by local communities of the Kali Gandaki Watershed Area, Nepal [32]. In Bhutan, traditional medicine practitioners recommended it as appetizer [33]. In Bangladesh, the decoction of its leaves are used for curing of hypertension. Leaf paste is applied for healing of wounds, burns, and skin lesion [34]. In Philippines, its leaves are recommended to treat of urination difficulty, sore eyes and burns [35].

**Table 25.1** Chemical composition of *Centella asiatica*

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| | | **Triterpenoids** | | |
| **1** | Papyriogenin A |  | Leaves | [106] |
| **2** | Madecassoside (brahminoside) |  R = COO-glc(1–6)glc(1–4)rha | Leaves | [106, 107] |
| | | | | [108] |
| | | | | [109] |
| | | | | [53] |
| | | | | [110] |
| | | | Leaves | [111] |
| | | | Whole plants | [112] |
| **3** | Madecassic acid |  | Leaves | [106, 111] |
| **4** | 2α,3ß,20,23-tetrahydroxy-urs-28-oic acid |  | | [108] |
| | |  | | |
| **5** | 2α,3ß,23-trihydroxy-urs-20-en-28-oic acid: R = H | | | [108] |
| **6** | 2α,3ß,23-trihydroxy-urs-20-en-28-oic acid O-α-L-rhamnopyranosyl- (1–4)-O-ß-D-glucopyranosyl-(1–6)-O-ß-Dglucopyranosyl ester: R = rha(1–4)-glc(1–6)-glc- | | | [108] |
| **7** | Methyl asiatate |  | | [108] |

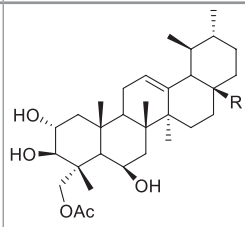
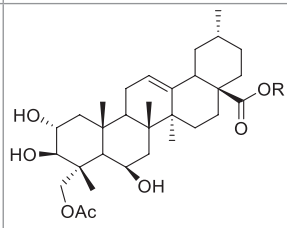
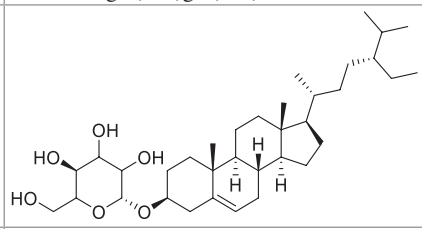
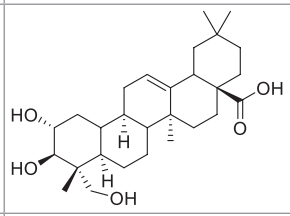
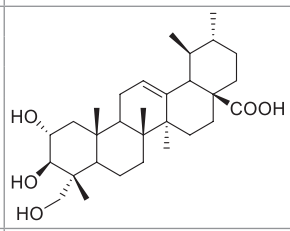
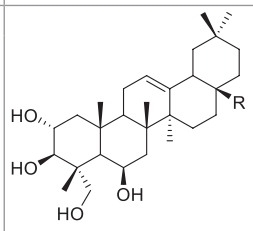
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **8** | Methyl brahmate |  | | [108] |
| **9** | Brahmol |  | | [108] |
| **10** | Isothankunic acid (3α,5α,6ß,24-tetrahydroxy-urs-12-en-28-oic acid) |  | | [108] |
| **11** | Isothankuniside |  R = COO-glc(1–6)glc(1–4)rha | | [108] |
| **12** | Madasiatic acid |  | | [108] |
| **13** | 2,3,23-trihydroxy-olean-12-en-28-oic acid |  | | [108] |

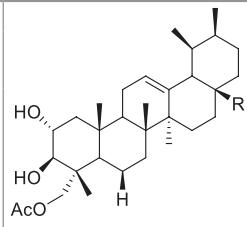
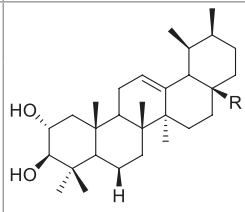
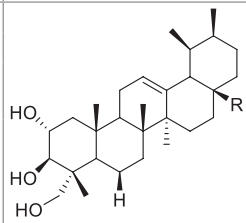
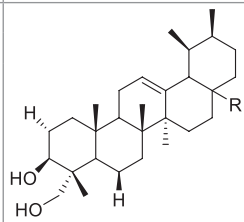
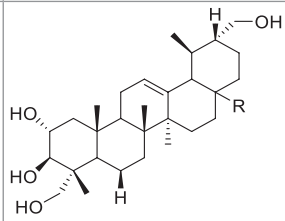
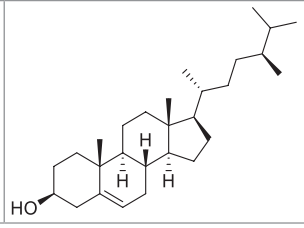
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **14** | 23-O-acetylmadecassoside |  R = COO-glc(1–6)glc(1–4)rha | Leaves | [111] |
| **15** | 23-O-acetylasiaticoside B |  R = COO-glc(1–6)glc(1–4)rha | Leaves | [111] |
| **16** | Sitosterol 3-O-β-glucoside |  | Leaves | [111] |
| **17** | Arjunolic acid |  | | [2] |
| **18** | Asiatic acid (2α,3ß,23-trihydroxy-urs-12-en-28-oic acid) |  | | [53]<br>[108]<br>[24]<br>[110] |
| **19** | Asiaticoside B |  R = COO-glc(1–6)glc(1–4)rha | | [108]<br>[53] |

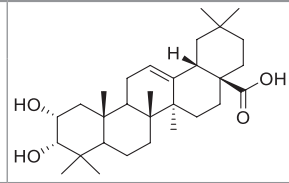
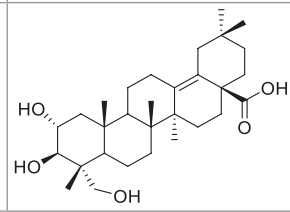
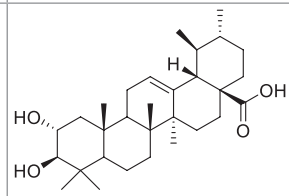
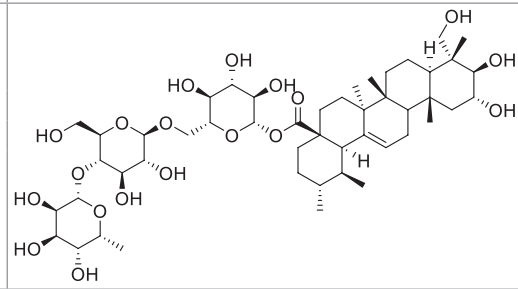
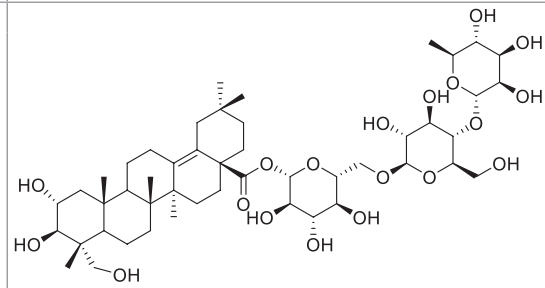
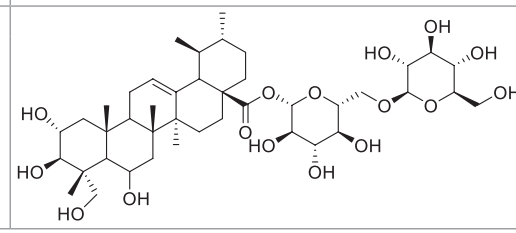
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **20** | Asiaticoside C |  R = COO-glc(1–6)glc(1–4)rha | | [108] |
| | | | Whole plants | [112] |
| | | | Leaves | [111] |
| **21** | Asiaticoside D |  R = COO-glc(1–6)glc(1–4)rha | | [2] |
| | | | | [108] |
| | | | Whole plants | [112] |
| **22** | Asiaticoside E |  R = COO-glc(1–6)glc | | [2] |
| | | | | [108] |
| | | | Whole plants | [112] |
| | | | Fresh mature plants | [113] |
| **23** | Asiaticoside F |  R = COO-glc(1–6)glc(1–4)rha | | [108] |
| | | | | [2] |
| | | | Whole plants | [112] |
| | | | Leaves | [111] |
| **24** | Asiaticoside G |  R = COO-rha(1–4)glc(1–6)glc | | [2] |
| **25** | Campesterol |  | | [24] |

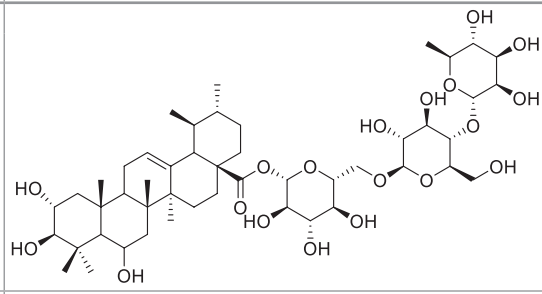
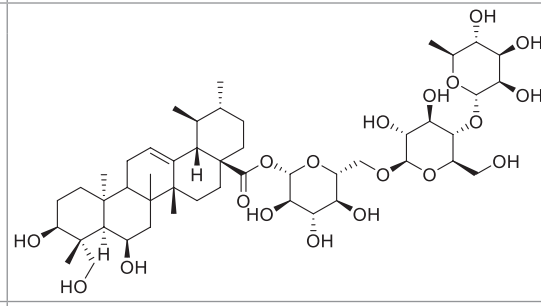
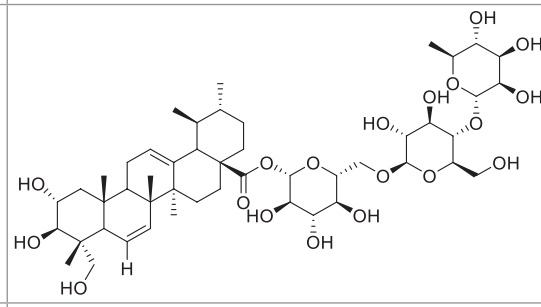
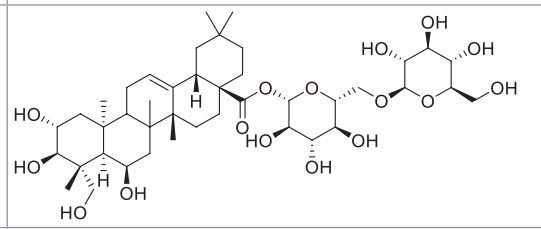
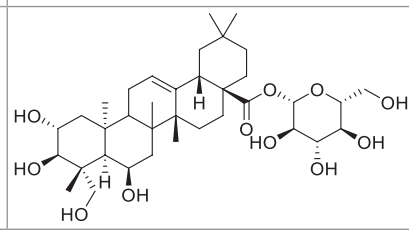
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **26** | 3-epimaslinic acid |  | Aerial parts | [114] |
| | | | | [24] |
| **27** | Centellasapogenol A |  | | [2] |
| | | | | [108] |
| **28** | Corosolic acid |  | Aerial parts | [114] |
| **29** | Asiaticoside |  | Leaves | [106, 107] |
| | | | | [115] |
| | | | | [109] |
| | | | | [53] |
| | | | | [110] |
| | | | Whole plants | [112] |
| | | | Leaves | [111] |
| **30** | Centellasaponin A |  | | [108] |
| | | | | [2] |
| **31** | Centellasaponin B |  | | [2] |
| | | | | [108] |

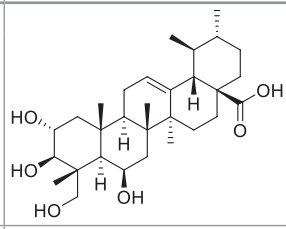
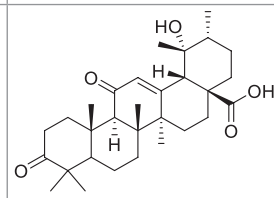
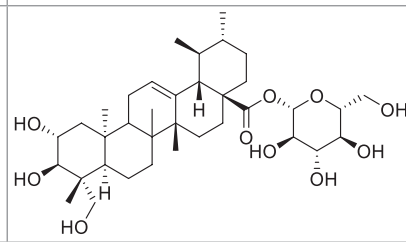
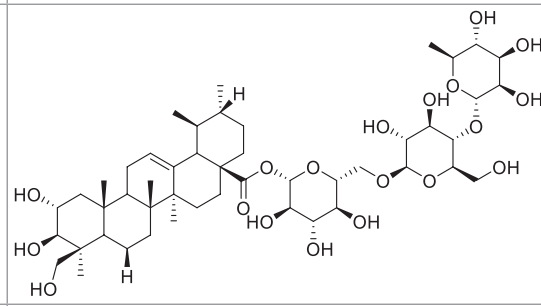
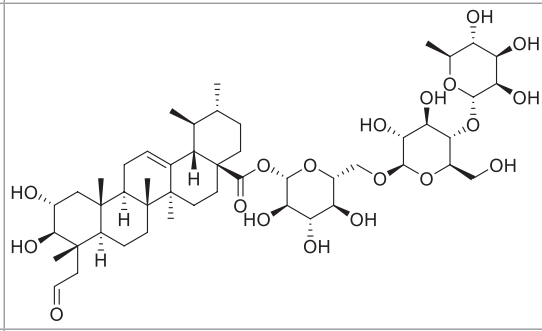
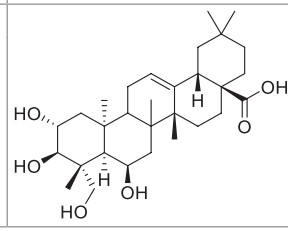
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **32** | Centellasaponin C |  | | [2]<br>[108] |
| **33** | Centellasaponin D |  | | [108]<br>[2] |
| **34** | Centelloside E |  | | [2] |
| **35** | Centelloside D |  | | [2] |
| **36** | Chebuloside II |  | | [2] |

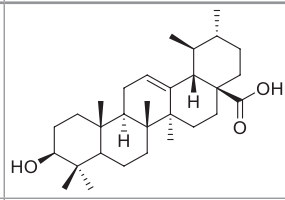
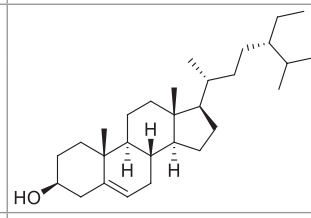
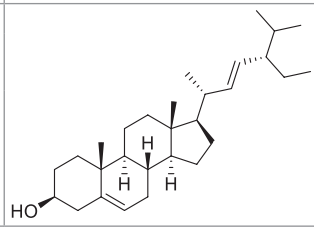
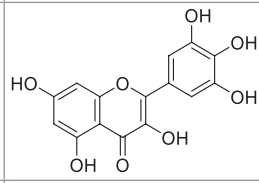
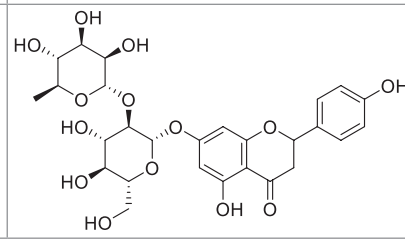
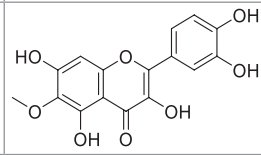
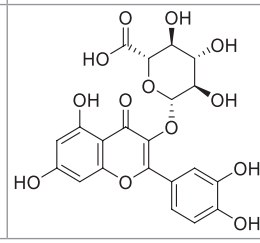
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **37** | Madecassic acid (brahmic acid) |  | | [53] [108] [110] |
| **38** | Pomolic acid |  | Aerial parts | [114] |
| **39** | Quadranoside IV |  | | [2] |
| **40** | Scheffuroside F |  | | [2] |
| **41** | Scheffuroside B |  | | [108] |
| **42** | Terminolic acid |  | | [53] [108] |

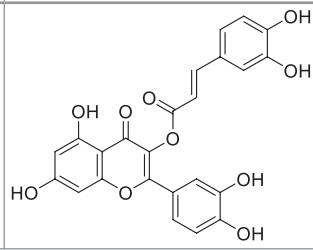
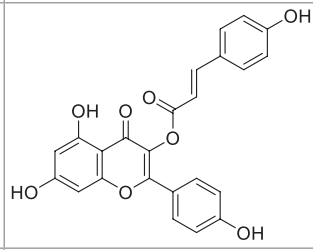
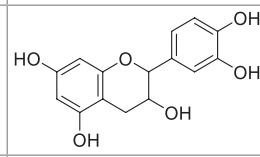
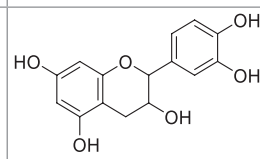
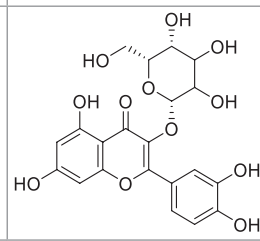
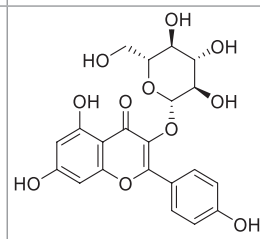
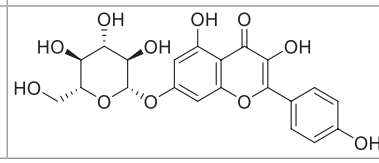
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **43** | Ursolic acid |  | Aerial parts | [114] |
| **44** | Sitosterol |  | Aerial parts | [114] |
| | | | Aerial parts | [116] |
| **45** | Stigmasterol |  | | [110] |
| | | | Aerial parts | [116] |
| | | | | [115] |
| | | **Polyphenolic compounds** | | |
| **46** | Myricetin |  | | [110] |
| **47** | Naringin |  | Leaves | [117] |
| **48** | Patuletin |  | | [110] |
| **49** | Querectin-3-O-β-D-glucuronide |  | Leaves | [111] |

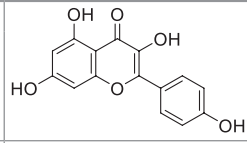
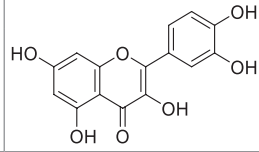
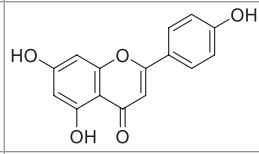
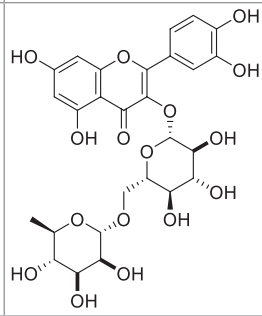
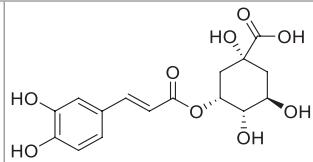
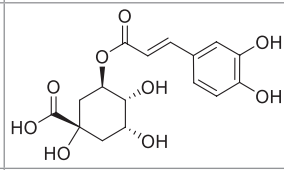
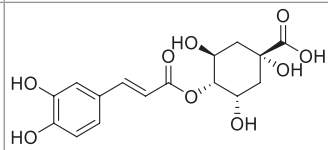
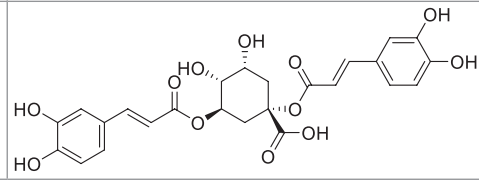
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **50** | Castillicetin |  | | [110] |
| **51** | Castilliferol |  | | [110] |
| **52** | Catechin |  | | [24] |
| **53** | Epicatechin |  | | [24] |
| **54** | 3-glucosylquercetin |  | | [110] |
| **55** | 3-glucosylkaemferol |  | | [110] |
| **56** | 7-glucosylkaemferol |  | | [110] |

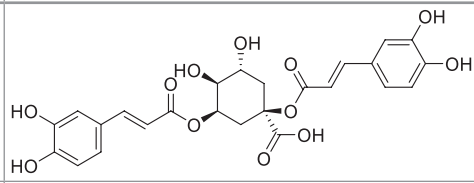
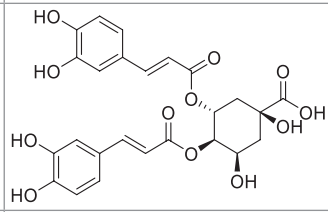
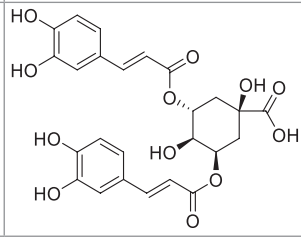
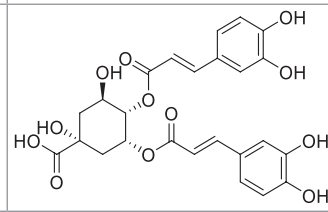
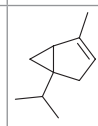
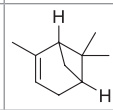
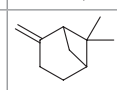
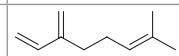
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **57** | Kaempferol |  | | [110] |
| | | | | [24] |
| **58** | Quercetin |  | Leaves | [117] |
| | | | | [110] |
| **59** | Apigenin |  | | [110] |
| **60** | Rutin |  | Leaves | [117] |
| | | | | [110] |
| **61** | Neochlorogenic acid (5-O-dicaffeoylquinic Acid) |  | Leaves | [23] |
| **62** | Chlorogenic acid (3-O-caffeoylquinic acid) |  | Leaves | [23] |
| **63** | Cryptochlorogenic acid, (4-O-caffeoylquinic acid) |  | Leaves | [23] |
| **64** | 1,3-dicaffeoylquinic acid |  | Leaves | [23] |

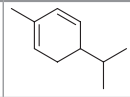
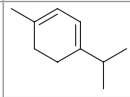
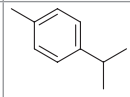
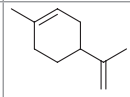
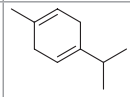
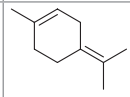
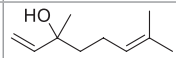
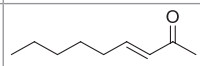
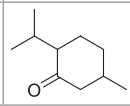
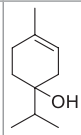
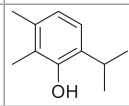
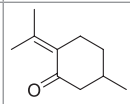
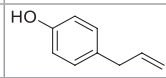
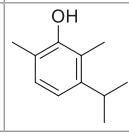
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **65** | 1,5-dicaffeoylquinic acid |  | Leaves | [23] |
| **66** | 3,4-dicaffeoylquinic acid (isochlorogenic acid B) |  | Leaves | [23] |
| **67** | 3,5-dicaffeoylquinic acid (isochlorogenic acid A) |  | Leaves | [23] |
| **68** | 4,5-dicaffeoylquinic acid (isochlorogenic acid C) |  | Leaves | [23] |
| | | **Essential oils** | | |
| **69** | α-Thjuene |  | Fresh mature plants | [113] |
| **70** | α-Pinene |  | Fresh mature plants | [113] |
| **71** | Camphene |  | Fresh mature plants | [113] |
| **72** | β-Pinene |  | Fresh mature plants | [113] |
| **73** | Myrcene |  | Aerial parts | [114] |
| | | | | [53] |
| | | | Fresh mature plants | [113] |

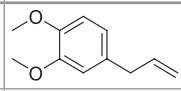
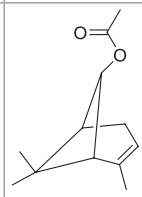
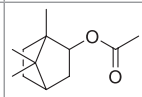
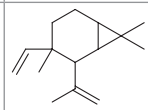
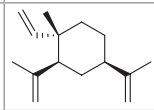
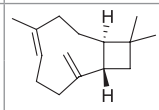
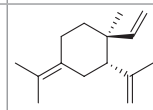
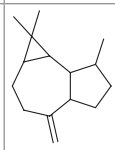
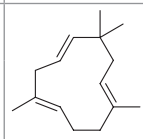
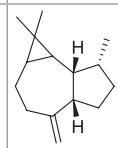
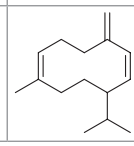
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **74** | α-Phellandrene |  | Fresh mature plants | [113] |
| **75** | α-Terpinene |  | Fresh mature plants | [113] |
| **76** | p-cymene |  | Fresh mature plants | [113] |
| **77** | Limonene |  | Fresh mature plants | [113] |
| **78** | γ-Terpinene |  | Fresh mature plants | [113] |
| **79** | Terpinolene |  | Fresh mature plants | [113] |
| **80** | Linalool |   | Fresh mature plants | [113] |
| **81** | 3-nonen-2-one |   | Fresh mature plants | [113] |
| **82** | Menthone |   | Fresh mature plants | [113] |
| **83** | Terpinen-4-ol |   | Fresh mature plants | [113] |
| **84** | Methyl thymol |   | Fresh mature plants | [113] |
| **85** | Pulegone |   | Fresh mature plants | [113] |
| **86** | Chavicol |   | | [53] |
| **87** | Methyl carvacrol |   | Fresh mature plants | [113] |

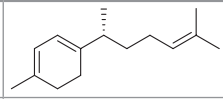
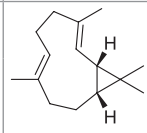
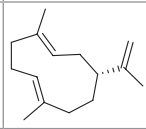
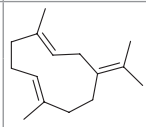
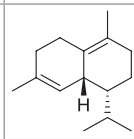
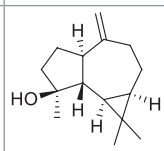
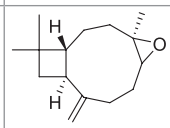
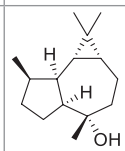
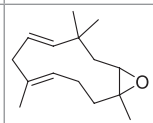
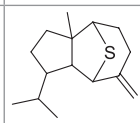
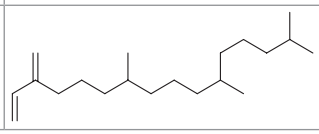
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **88** | Methyleugenol |  | | [53] |
| **89** | Chrysanthenyl acetate |  | Fresh mature plants | [113] |
| **90** | Bornyl acetate |  | Fresh mature plants | [113] |
| **91** | Bicycloelemene |  | Fresh mature plants | [113] |
| **92** | β-Elemene |  | Fresh mature plants | [113] |
| **93** | β-Caryophyllene |  | Aerial parts | [114] |
| | | | Fresh mature plants | [113] |
| **94** | γ-Elemene |  | Fresh mature plants | [113] |
| **95** | Aromadendrene |  | Fresh mature plants | [113] |
| **96** | α-Humulene |  | Aerial parts | [114] |
| | | | Fresh mature plants | [113] |
| **97** | Allo-aromadendrene |  | Fresh mature plants | [113] |
| **98** | Germacrene D |  | Fresh mature plants | [113] |

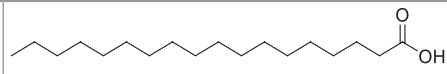
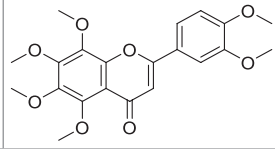
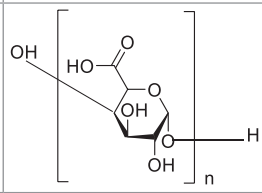
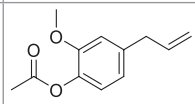
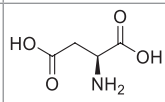
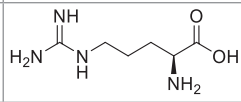
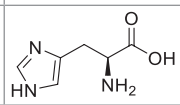
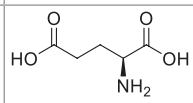
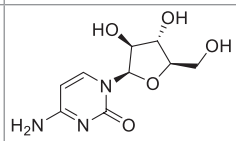
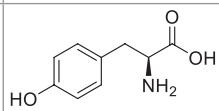
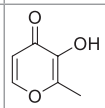
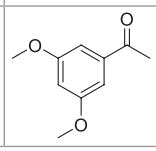
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **99** | γ-Curcumene |  | Fresh mature plants | [113] |
| **100** | Bicyclogermacrene |  | Aerial parts | [114] |
| | | | Fresh mature plants | [113] |
| **101** | Germacrene A |  | Fresh mature plants | [113] |
| **102** | Germacrene B |  | Aerial parts | [114] |
| | | | Fresh mature plants | [113] |
| **103** | δ-Cadinene |  | Fresh mature plants | [113] |
| **104** | Spauthulenol |  | Fresh mature plants | [113] |
| **105** | Caryophyllene oxide |  | Fresh mature plants | [113] |
| **106** | Viridiflorol |  | Fresh mature plants | [113] |
| **107** | Humulene epoxide |  | Fresh mature plants | [113] |
| **108** | Mintsulfide |  | Fresh mature plants | [113] |
| **109** | Neophytadiene |  | Fresh mature plants | [113] |

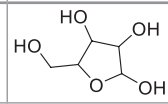
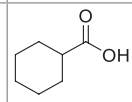
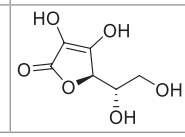
(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **110** | n-octadecanoic acid |  | Whole aerial parts of | [118] |
| | | **Other compounds** | | |
| **111** | Nobiletin |  | Leaves | [106] |
| **112** | Pectic acid |  | | [115] |
| **113** | Eugenol acetate |  | | [53] |
| **114** | Aspartic acid |  | Aerial parts | [116] |
| **115** | Arginine |  | Aerial parts | [116] |
| **116** | Histidine |  | Aerial parts | [116] |
| **117** | Glutamic acid |  | Aerial parts | [116] |
| **118** | Arabinoside |  | | [108] |
| **119** | Tyrosine |  | Aerial parts | [116] |
| **120** | Methyl pyromeconic acid (maltol) |  | Leaves | [106] |
| **121** | 3′,5′-dimethoxyacetophenone |  | Leaves | [106] |

(continued)

**Table 25.1** (continued)

| No. | Name of compounds | Structures | Plant parts | Ref. erences |
|---|---|---|---|---|
| **122** | Beta-D-ribofuranoside |  | Leaves | [106] |
| **123** | Cyclohexanecarboxylic acid |  | Leaves | [106] |
| **124** | Ascorbic acid |  | Aerial parts | [116] |
| | | | | [115] |

It is also well-documented for its remarkable uses in the African Traditional Medicine. In Tanzania, the decoction of whole plant is believed to be efficacious in the treatment of malaria [36]. In Cameroon, its leaves are applied to treat pharyngitis and dysmenorrhoea convulsion [37]. Furthermore, the decoction of whole plant, is also taken as an effective treatment for vomiting and appendicitis [38]. In Uganda, the decoction of its leaves are used traditionally in the treatment of Ulcers [39]. In Guinean traditional medicine, the decoction of whole plant is prescribed for diabetes [40]. In Nigeria, its root decoction is taken as an effective treatment for haemorrhoids [41]. In South Africa, the root of CA is used by Bapedi traditional healers to treat diabetes mellitus [42]. Its root and leaves commonly known as Inyongwane, are also reported as a traditional drug to treat stomach disorders, dysentery and diarrhoea [43].

CA is also a well-known ethnomedicinal plant in the European Traditional Medicine. In Greece, its leaves commonly known as Sentella, which are widely applied as blood circulation stimulant and as a remedy for hypertension, phlebitis, uric acid, cellulites, and menstruation disorders [44]. In Russia, the leaves and barks of CA are employed as a traditional drug to treat depression [45]. In Turkey, its aerial parts are considered very useful in the treatment of neurological disorders [46].

Furthermore, in different geographical areas of American continent, remarkable reports of its traditional uses, are found. In Brazil, the leaves and barks of CA are extensively used in folk medicine to treat hypertension and as dermal lesions [47]. It is also taken as an effective treatment for weight loss among Mexican-American women [48]. In other geographical regions like Madagascar, its leaves popularly known as Viliantsahona, are applied externally to treat pimples [49]. In the folk phytotherapy of the Yaegl Aboriginal community in northern New South Wales, Australia, the leaves of it, also are prescribed for the treatment of arthritis [50].

The most frequent ethnomedicinal applications of CA appears to be treatment of gastrointestinal diseases, wounds, nervous system disorders, circulatory diseases, skin problems, respiratory *ailments*, diabetes and also as sedative in various ethnobotanical practices throughout the world. In addition to its medicinal usages, it is commonly eaten as fresh vegetable in Malaysia, China, Sri Lanka, India and Indonesia [51]. In Thailand, its aerial parts are eaten as wild food plant [52].

## 25.5 Pharmacological Aspects

So far, many *in vivo* and *in vitro* pharmacological studies have been investigated different biological activities of CA, mostly about its traditional uses. These studies, mainly focused on titrated extract of CA(TECA), total triterpenoid fraction of CA (TTFCA), total triterpenic fraction (TTF)

or each triterpene derivative, alone such as Asiatic acid, Asiaticoside, Madecassic acid, and Madecassoside [53].

It is revealed that CA acts on central nervous system (CNS) as neuroprotective agent, memory enhancer, tranquilizer, anxiolytic, sedative, antidepressant, anticonvulsant, and nerve regenerator in Alzheimer and Parkinson disease [54].The neuroprotective effects of CA has been revealed to be due to different mechanisms, such as reduction of oxidative stress parameters and inhibition of acetylcholine esterase activity [55], decrease of amyloid-β plaques and protection of cornu ammonis pyramidal neurons in the hippocampus [56, 57], modulation of neurotransmitter activity in the synaptic gap [58], and etc.

It also has lots of beneficial effects to prevent and reduce the complications of metabolic syndrome, considering its positive effects on the lipid profile [13], as the aqueous leaf extract of CA reduced the level of total cholesterol, triglyceride (TG), low-density lipoprotein (LDL) and elevated the level of high-density lipoprotein (HDL) in a high cholesterol-fed rat model. In an *in vitro* model of tumor necrosis factor alpha (TNF-α)-induced atherosclerosis in human aortic endothelial cells, Asiatic acid significantly reduced endothelial hyper-permeability and secretions of cell adhesion molecules [59]. Asiatic acid in a rat model of renovascular hypertension, ameliorated hemodynamic alterations, renin-angiotensin system (RAS) activation, inflammation and oxidative stress comparable with captopril (an angiotensin-converting enzyme (ACE) inhibitor) [60]. In a rat model of spontaneous type 2 diabetes, administration of Asiatic acid decreased insulin resistance and blood glucose and also protected islet cells from fibrosis [61].

Protective effects of CA against fibrosis, inflammation and hypertrophy in heart, kidney, liver and lung have been proven in some studies. Progression of myocardial remodeling and left ventricular hypertrophy attenuated with oral administration of Asiatic acid in a mouse model of cardiac hypertrophy [62]. Alcoholic extract of CA in a rat model of isoniazid induced-hepatorenal damage, significantly improved the histology of the liver and kidney, reduced the hematological and oxidative parameters and also lowered liver and kidney function markers to near-normal levels [63]. Also oral administration of Madecassoside in a mouse model of pulmonary fibrosis mediated by bleomycin, ameliorated the pathological changes and reduced the collagen deposition in the lungs [64].

It dramatically heals and prevents gastric ulcers in models of gastric lesions. Aqueous leaf extract of CA in a rat model of indomethacin-induced gastric ulceration, significantly protected and also accelerated the ulcer healing process [65]. Leaf extract of CA demonstrated anti-*Helicobacter pylori* activity both *in vivo* and *in vitro* [66] and also the work of Guo et al. (2015) showed that Asiatic acid had beneficial influences in amelioration of ulcerative colitis through anti-inflammatory effects [67].

CA is effective in acceleration of small, hypertrophic, diabetic or burn wound healing and also in treatment of scleroderma, psoriasis, cellulite, photoaging skin and striae gravidarum [68]. Dermatologic effects of the CA has been suggested to result from elevation of intracellular fibronectin content and collagen synthesis, increasing the fibroblast proliferation and epithelization, promotion of the tensile strength and also inhibition of inflammatory response in keloids and hypertrophic scars [69].

Furthermore, there are two studies carried out about the beneficial effects of Madecassoside and Asiaticoside in prevention of osteoporosis. Results showed that they suppressed receptor activator of nuclear factor-κB ligand (RANKL)-induced bone resorption and osteoclast differentiation in a dose-dependent manner [70, 71].

Taken together, it seems that CA performs beneficial effects in the most parts of body (Fig. 25.2), and plays a positive role in the human health. We attempt to provide a summary of CA pharmacological activities and mechanisms of action, categorized according to the body organs or systems which are presented in Tables 25.2 and 25.3.

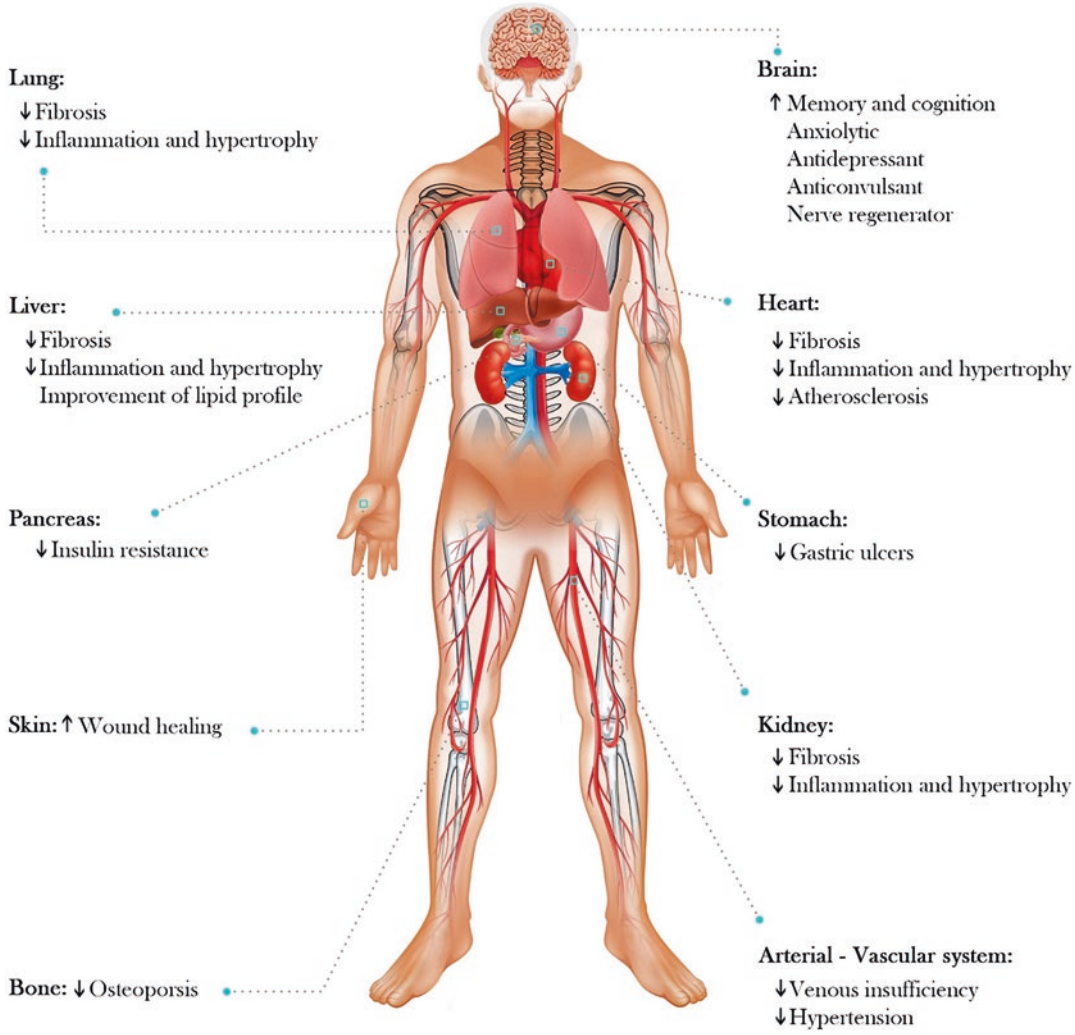



**Fig. 25.2** Activity sites of *Centella asiatica* in human body. Note: This figure provided with the authors

## 25.6 Clinical Studies

Despite many *in vivo* and *in vitro* studies have been carried out about different pharmacological and traditional uses of CA, but there are only a limited number of clinical studies about some effects of CA. Most of these clinical studies include CNS, cardiovascular, and dermatological effects, which are categorized in details in Table 25.4.

The results of a systematic review and meta-analysis about the effects of CA on cognitive function and mood related outcomes extracted from eleven randomized controlled trials, demonstrated that CA improves mood and decreases anger. However, it's revealed that CA doesn't have significant effect on cognitive function in comparison with placebo, at all [72].

Daily oral administration of selected triterpenes of CA (120 to 240 mg) for 52 weeks in 43 type 2 diabetic patients significantly reduced total symptom scores of neuropathy and paresthesia [73]. Topical administration of Madecassoside 0.1% with vitamin C 5% cream for 6 months twice daily in 20 healthy postmeno-

**Table 25.2** *In vivo* studies of *Centella asiatica*

| Activity/Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | References |
|---|---|---|---|---|---|
| **Central nervous system** | | | | | |
| Anti-depressant | Extract of CA | Mouse and rat model | Anti-depressant activity | Modulation of $D_2$ receptor and cholinomimetic activity | [119] |
| Anti-anxiety | Aqueous extract of CA (25 mg/kg) | Mouse model,i.p. | ↓spontaneous motor activity and delayed pentylenetetrazole-induced convulsions | – | [120] |
| Learning and memory | Fresh leaf aqueous extract of CA | Rat model / two compartment passive avoidance task | Improvement of learning and memory | ↓norepinephrine, dopamine, serotonin and their metabolites | [121] |
| Cognitive Impairment | Aqueous extract CA (200 mg/kg) | Rat model, oral, QD, 14 days | ↑learning and memory | ↓lipid peroxidation, ↑endogenous antioxidant enzymes in brain | [122] |
| Anticonvulsant | Crude drug, methanolic and solubulised extract of CA (500, 1000 mg/kg) | Rat model, oral, 1, 3, 6, 24 hours | Anticonvulsantactivity comparable with phenytoin (30 mg/kg) | – | [123] |
| Cognitive impairment | Aqueous extract of CA (200, 300 mg/kg) | Rat model, oral, QD, 21 days | ↑cognition | ↓malondialdehyde, ↑glutathione and catalase | [124] |
| Cognitive impairment and epilepsy | Aqueous extract of CA (300 mg/kg) | Rat model, oral | ↓seizure score, ↑learning and memory | – | [125] |
| Anti-depressant | TTFCA | Mouse model / forced swimming test | ↓immobility time, | Amelioration of imbalance of amino acid levels | [126] |
| Anticonvulsant | Hydroalcoholic leaf extract of CA (100 and 200 mg/kg) | Mouse model, oral | Anticonvulsant, antioxidant, and central nervous system depressant actions | – | [127] |
| Nerve regeneration | Ethanolic extract of CA (300–330 mg/kg) | Rat model, oral, QD, 18 days | ↑functional recovery, ↑axonal regeneration | – | [128] |
| Cognition | Aqueous extract of CA (200 mg/kg) | Mouse model, oral, QD, 15 days | ↑learning and memory, | ↑acetylcholine esterase activity, ↑dendritic arborization of hippocampal CA3 neurons | [129] |
| Anticonvulsant | Ethyl acetate fraction of CA | Mouse model, oral | Synergism with antiepileptic drugs (phenytoin, valproate, and gabapentin) | – | [130] |
| Anti-depressant | TTFCA | Rat model | ↓corticosterone level in serum and ↑serotonin, norepinephrine, dopamine and their metabolites in brain | Ameliorating the function of HPA axis, ↑monoamine neurotransmitters | [131] |

| | | | | | |
|---|---|---|---|---|---|
| Anxiolytic | Methanolic and ethyl acetate extracts of CA and Asiaticoside | Rat model | Anxiolytic activity | – | [132] |
| Neuronal dendritic growth | Fresh leaf juice of CA (4 and 6 mL/kg) | Rat model, oral, QD, 4 and 6 weeks | ↑dendritic length and dendritic branching in amygdaloid neurons | – | [133] |
| Neuronal dendritic growth | Fresh leaf extract of CA (v6 mL/kg) | Rat model, oral, QD, 6 weeks | ↑dendritic length and dendritic branching in hippocampal CA3 neurons | – | [134] |
| Cognitive impairment | Aqueous extract of CA (150 and 300 mg/kg) | Rat model, oral, QD, 25 days | ↓memory impairment | ↓oxidative damage, ↑acetylcholinesterase activity | [135] |
| Alzheimer's disease | Extract of CA (2.5, 5 g/kg) | Mouse model, oral, QD, 2 and 8 months | ↓amyloid β plaques in hippocampus | ↓amyloid β neurotoxicity and antioxidant properties | [136] |
| Cerebral ischemia | Asiatic acid (30, 75, 165 mg/kg) | Mouse model, oral, 1 and 7 days | ↓infarct volume and improvement of neurological outcome | ↓blood–brain barrier permeability, ↓mitochondrial injury | [137] |
| Parkinson's disease | Aqueous extract of CA (300 mg/kg) | Rat model, oral, QD, 21 days | Neuroprotective effect | ↓mitochondrial damage and oxidative stress | [138] |
| Epilepsy | n-hexane, chloroform, ethyl acetate and n-butanol extract of CA (200 mg/kg) | Rat model, oral, QD, 1 week | Anticonvulsant and neuroprotective activity | ↑$Na^+/K^+$, $Mg^{2+}$ and $Ca^{2+}$ -ATPase activities | [139] |
| Parkinson's disease | Asiaticoside (15, 30 and 45 mg/kg) | Rat model, oral, QD, 14 days | Neuroprotective effects | Antioxidant activity, maintaining the metabolic balance of dopamine, and ↑ ratio of Bcl-2/Bax | [140] |
| Alzheimer's disease | Aqueous extract of CA (200 mg/kg) | Mouse model, oral, 2 weeks | Improvement of behavioral deficits | Attenuation of amyloid-β associated behavioral abnormalities | [141] |
| Alzheimer's disease | Aqueous extract of CA (100 mg/kg) | Rat model, oral, 6 weeks | ↑time spent in goal quarter, ↓memory deficits | Antioxidant action | (121) |
| Anxiolytic and anti-depressant | Asiatic acid (30 mg/kg) | Rat model, i.p. | ↑open arm time, ↓time mobile, ↑mean movement time, ↓fecal pellet output | Modulation of benzodiazepine site on the $GABA_A$ receptor | [142] |
| Migraine | Asiaticoside (10 and 30 mg/kg, 100 μg/rat) | Rat model, oral and nasal, acute and 7-days subacute | Significant anti-nociception activity, reversal of the nitroglycerine-induced hyperalgesia, ↓number of vocalization | Modulation of 5-HT1A and 5-HT1B | [58] |
| Cognitive impairment | Aqueous extract of CA (2 mg/mL) in drinking water | Mouse model, 5 weeks | ↑cognition | ↑expression of mitochondrial and antioxidant response genes in the brain and liver | [143] |
| Anxiolytic | ECa233 | Mouse model | Anxiolytic activity | Modulation of neuronal activity on intercalated neurons of amygdala | [144] |

(continued)

**Table 25.2** (continued)

| Activity/Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | References |
|---|---|---|---|---|---|
| Neuroprotection | Asiatic acid (30 mg/kg) | Rat model, oral, QD, 20 and 40 days | Inhibition of p21 (cell cycle arrest) and MDA (lipid peroxidation product) in the hippocampus that produced by 5-fluorouracil | ↑Notch1, SOX2, nestin, DCX, and Nrf2 expression in the hippocampus | [145] |
| Cognitive impairment | Aqueous extract of CA (2 mg/mL) in drinking water | Mouse model, 2 weeks | Improvement of memory and executive function | ↑synaptic density, ↓mitochondrial dysfunction, ↓oxidative stress | [146] |
| Learning And memory | Ethanolic extract of CA (30 mg/kg) | Rat model, oral, QD, 14 days | Improvement of spatial learning and memory, not significant effect on locomotor activity | ↑AMPA receptor GluA1 subunitin the CA1 and CA2 sub regions of the hippocampus | [147] |
| Parkinson's disease | ECa 233 (10, 30, 100 mg/kg) | Rat model, oral, QD, 20 days | Protection of locomotor deficits, ↓dopaminergic neuronal death in the substantianigra | ↑mitochondrial complex I activity, ↑antioxidants activity | [148] |
| Alzheimer's disease | Aqueous extract of CA (200, 400, 800 mg/kg) | Rat model, oral, QD, 70 days | Alleviation of cognitive impairments | ↓pathological changes in the hippocampus CA1 pyramidal cells | [149] |
| Alzheimer's disease | Aqueous extract of CA(2 mg/mL) in drinking water | Mouse model, 14 days | ↑learning and memory | ↓amyloid-β plaques in the hippocampus, ↓mitochondrial dysfunction, ↓oxidative stress | [56] |
| Learning and memory | Hydroalcoholic extract of CA (100, 300, 600 mg/kg) | Rat model, oral, QD, 14 days | ↑learning and memory | ↑expression of AMPA receptors GluA1 and GluA2 subunits, differential expression of NMDA receptors GluN2 A and GluN2B subunits in the hippocampal subfields and entorhinal cortex | [57] |
| Learning and memory | ECa 233 (10, 30 mg/kg) | Rat model, oral, BID, 30 days | ↑learning and memory | ↑synaptic plasticity and plasticity-related proteins in hippocampus | [150] |
| Neuroprotection | Ethanolic leaf extract of CA (150,300, 600 mg/kg) | Rat model, oral, QD, 28 days | Neuroprotection in the hippocampus | ↓TNF-$\alpha$ and ↑brain-derived neurotropic factor in the hippocampus | [151] |
| Alzheimer's disease | Hydroalcoholic extract of CA (200, 400, 800 mg/kg) | Rat model, QD, 10 weeks | ↓cognitive deficits, ↓morphological aberrations in the CA3 region of hippocampus | ↓GSK-3β, ↑protein phosphatase2 | [149] |
| Amnesia | Ethanolic extract of CA (250, 500 mg/kg) | Mouse model, oral, 14 days | Neuroprotective effects | ↑antioxidant activity, inhibition of acetylcholine esterase | [152] |

| | | | | | |
|---|---|---|---|---|---|
| **Gastrointestinal** | | | | | |
| Gastric ulcer | CA | Rat model | Significantly inhibition of gastric ulceration mediated by cold restraint stress | GABAergic activity of CA | [153] |
| Gastric ulcer | Alcoholic root extract of CA (100 mg/kg) | Rat model, oral, QD, 16 days | ↓number and severity of the ulcers | Anti-stress activity of CA | [154] |
| Gastric ulcer | Aqueous leaf extract of CA (250, 500 mg/kg) | Rat model, oral, QD | Inhibition of gastric ulceration, 42.6% and 100%, respectively | – | [155] |
| Gastric ulcer | Aqueous extract of CA (50, 250, 500 mg/kg) | Rat model, oral, QD | Prevention of gastric lesions | Anti-inflammatory effect through reduction of mucosal MPO | [156] |
| Gastric ulcer | Fresh juice of CA (200, 600 mg/kg) | Rat model, oral, BID, 5 days | Significantly inhibition of gastric ulceration | Augmentation of defensive mucosal factors (↑gastric mucin secretion and mucosal cell glycoproteins) | [157] |
| Gastric ulcer | Aqueous extract of CA (100, 250 mg/kg) or Asiaticoside (5, 10 mg/kg) | Rat model, oral, QD, 7 days | Accelerating the ulcer healing process | Anti-inflammatory effect through reduction of iNOS synthesis | [158] |
| Gastric ulcer | Aqueous leaf extract of CA (10, 20 mg/kg) | Rat model, oral, QD | Prevention of gastric ulceration mediated by indomethacin | – | [159] |
| Gastric ulcer | Alcoholic leaf extract of CA (100, 200, 400 mg/kg) | Rat model, oral, QD | Significantly protection of the gastric mucosa | – | [160] |
| Gastric ulcer | CA, *Hericiumerinaceus* and *Amomumvillosum* | – | Significantly inhibition of gastric ulcer | – | [161] |
| Ulcerative colitis | Asiatic acid (3, 10, 30 mg/kg) | Mouse model, oral, QD, 10 days | Significantly ameliorates ulcerative colitis | ↓inflammatory cytokines (IFN-γ, TNF-α, IL-1β, and IL-6), ↓caspase-1 activation in peritoneal macrophages | [67] |
| Anti-*Helicobacter pylori* | Leaf extract of CA (50 mg/kg) | Mouse model, oral, 3 weeks | ↓*H. pylori* colonization in mice gastric mucosa | – | [66] |
| Gastric ulcer | Aqueous leaf extract of CA (50, 250 mg/kg) | Rat model, oral, QD | Significantly gastroprotective | ↓malondialdehyde, TNF, COX-2 and iNOS | [65] |
| **Cardiovascular** | | | | | |
| Cardiomyopathy | Aqueous extract of CA (200 mg/kg) | Rat model, oral | Restored the enzyme activities to near normal levels | – | [162] |
| Myocardial Infarction | Alcoholic extract of CA (100–1000 mg/kg) | Rat model, oral | ↓necrosis of the myocardium, ↓lipid peroxide levels in serum and heart tissue | Free radical scavenging activity or enhancing the endogenous antioxidants level | [163] |

(continued)

**Table 25.2** (continued)

| Activity/Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | References |
|---|---|---|---|---|---|
| Myocardial failure | Aqueous extract of CA (200 mg/kg) | Rat model, oral | Cardioprotective effect against myocardial failure | Inhibition of oxidative stress and mitochondrial dysfunction | [164] |
| Hyperlipidemia | CA (1000, 2000 mg/kg) | Rat model, oral, QD | ↓TG and total cholesterol | – | [10] |
| Cardiac hypertrophy | Asiatic acid (100 mg/kg) | Mouse model, oral, 2 weeks | ↓(heart weight to body weight, interventricular septal end-diastolic dimension, left ventricular end diastolic posterior wall dimension and left ventricular end-diastolic diameter) | Inhibition of TGF- β1 | [165] |
| Cardiac hypertrophy | Asiatic acid)100 mg/kg) | Mouse model, oral | Attenuation of the pressure overload progression of left ventricular hypertrophy and heart failure | Blocking the activation of mitochondrial and death receptor-dependent apoptotic signaling pathways, blocking of TGF-β1/Smad and IL-6 signaling activation | [62] |
| Myocardial infarction | Asiatic acid)5, 25, 50 mg/kg) | Rat model, oral, QD, 4 weeks | Preservation of cardiac function and inhibition of left ventricular remodeling | Blocking the phosphorylation of p38 MAPK and ERK1/2 in the infarct border zone of the ischemic myocardium | [166] |
| Hyperlipidemia | Aqueous extract of CA (0.25, 0.5, 1 g/kg) | Rat model, oral, QD, 4 weeks | ↓(TG, total cholesterol, LDL), ↑HDL | – | [13] |
| Hypertension | Asiatic acid (30 mg/kg) | Rat model, oral, QD, 4 weeks | Amelioration of hemodynamic alterations, RAS activation, inflammation and oxidative stress | Direct effect on RAS activation, inflammation and oxidative stress and/or ACE inhibitory effect on Angiotension II-$AT_1$ receptor-NADPH oxidase-NF-κB pathway | [60] |
| Myocardial ischemia/reperfusion | Asiatic acid (2.5, 5, 10 mg/kg) | Rat model of ischemia/reperfusion, oral, single dose pretreatment | ↑cardiac function indexes, ↓size of myocardial infarction, ↓LDH and ↓creatine kinase activities, ↓cardiomyocyte apoptosis | Activation of Akt/GSK-3β signaling pathway in the myocardium to inhibit glycogen breakdown through PPARɣ activation and GLUT4 translocation | [167] |
| **Liver** | | | | | |
| Liver fibrosis | Total glucosides of CA | Rat model, 6 weeks | Improvement of histology, ↓AST, ALT and hyaluronic acid to near-normal levels | – | [168] |
| Liver damage | Aqueous extract of CA (100 mg/kg) | Mouse model | Preserves hepatocytes from abnormality, ↓binucleated cells | – | [169] |

| | | | | | |
|---|---|---|---|---|---|
| Acute liver injury | Asiaticoside (5, 10, 20 mg/kg) | Mouse model, QD, 3 days | Decrease of the magnitude of hepatocytes necrosis and leukocyte infiltration, ↓TNF-α, ALT, AST, caspase-3 activity and MAPK | Inhibition of TNF-α and MAPKs | [170] |
| Liver fibrosis | Asiatic acid (0.5, 2, 8 mg/kg) | Rat model, oral, QD, 6 weeks | Anti-fibrotic activity | Blocking of TGF-β/Smad signaling pathway | [171] |
| Liver injury | Aqueous extract of CA (200 mg/kg) | Rat model, oral, QD, 7 days | Significantly restored marker enzyme levels, total protein and albumin levels | Antioxidant action | [172] |
| Liver and immune organ damage | Triterpenesaponins of CA (250 mg/kg) | Rat model, oral, QD, 30 days | Improvement of histology of the liver and immune organs | Restoring the cytokine production and antioxidant system | [107] |
| Liver and kidney damage | Ethanolic extract of CA (100 mg/kg) | Rat model, oral, QD, 30 days | Improvement of histology, ↓(hematological parameters, oxidative status, liver and kidney function markers to near-normal levels) | – | [63] |
| Liver injury | Aqueous leaf extract of CA (100, 200 mg/kg) | Rat model, oral, QD, 5 days | Hepatoprotective | ↑antioxidant enzymes, ↓inflammatory mediators | [173] |
| Cardiac and hepato-renal injury | Asiatic acid (5, 10, 20 mg/kg) | Rat model, oral, QD, 7 days | Improvement of histology, ↓(oxidative stress, serum creatinine and serum blood urea nitrogen) | ↑Nrf2 protein expression | [174] |
| Liver fibrosis | Asiatic acid (5, 10 mg/kg) | Rat model, 6 weeks | Attenuates liver injury and fibrosis | Regulation of Bcl-2/Bax and PI3K/AKT/mTOR signaling pathway | [175] |
| Acute liver failure | Madecassoside (20, 40 mg/kg) | Mouse model, oral, QD, 10 days | Suppressing the production of inflammatory cytokines and recovering antioxidant enzyme activity, ↓iNOS and COX-2 | Inhibition of p38/NF-κB and activation of Nrf2/HO-1 signaling pathways | [176] |
| **Kidney** | | | | | |
| Chronic renal failure | CA | Rat model, enema, QD, 30 days | Improvement of electrolyte levels, hematocrit, RBC counts and hemoglobin content | – | [177] |
| Diabetic nephropathy | Asiatic acid | Rat model | Protective effects | Upregulation of nephrin in the podocyte, inhibition of JNK signaling pathway | [178] |
| Tubulointerstitial fibrosis | Asiatic acid (4, 16 mg/kg) | Mouse model, oral, QD, 6 days | ↓tubular injury, fibroblast activation and extracellular matrix accumulation | ↓α-smooth muscle actin by inhibition of Smad-dependent TGF-β1 signaling pathway | [179] |
| Nephropathy | Asiatic acid (8, 16, 32 mg/kg) | Rat model, oral, QD, 4 weeks | Improvement of histology, ↓proteinuria, ↓total cholesterol, ↑serum albumin, | ↑mRNA and protein levels of synaptopodin, nephrin and podocin, ↓mRNA and protein levels of desmin | [180] |

(continued)

**Table 25.2** (continued)

| Activity/Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | References |
|---|---|---|---|---|---|
| Renal fibrosis | Asiatic acid (5 mg/kg) and naringenin | Mouse model, i.p., QD, 7 days | Anti-fibrotic effect on unilateral ureteral obstruction nephropathy | Rebalancing of TGF-β/Smad signaling pathway | [181] |
| Diabetic nephropathy | CA (5.6, 11.2, 16.8 mg/kg) | Rat model, oral, QD, 16 weeks | Prevention and cure of diabetic nephropathy | Regulating of TGF-β1/Smad signaling pathway | [182] |
| Diabetic nephropathy | Asiatic acid (10, 20, 40 mg/kg) | Rat model, oral, QD, 8 weeks | Protection of podocytes | Antioxidant effect, protection of podocytes, ↓activation of JNK signaling pathway | [183] |
| Acute kidney injury | Asiatic acid (50 mg/kg or 100 mg/kg) | Mouse model, i.p., single dose pretreatment | ↓(serum creatinine, blood urea nitrogen and histological changes) | Anti-apoptosis and anti-inflammation mechanisms with upregulation of the apoptosis inhibitor survivin and suppression of IL-1β, TNF-α, MCP-1, caspase-1, and upregulation of Smad7 | [184] |
| **Lung** | | | | | |
| Pulmonary fibrosis | Madecassoside (40 mg/kg) | Mouse model, oral, QD, 21 days | Amelioration of Bleomycin-induced pulmonary fibrosis | Increases the activity of PPAR-γ, which subsequently increases hepatocyte growth factor expression in colonic epithelial cells | [64] |
| Acute lung injury | Asiatic acid (25, 50, 100 mg/kg) | Mouse model | Inhibition of LPS-induced acute lung injury | Inhibition of the inflammatory cytokines production via blocking of the TLR4/NF-κB signaling pathway | [185] |
| Pulmonary inflammation | Asiatic acid (15, 30 mg/kg) | Mouse model, oral, QD, 11 days | Effectively inhibits pulmonary inflammatory response | Suppression of inflammatory mediators | [186] |
| Scleroderma | Asiatic acid (2, 8 mg/kg) | Mouse model, oral, QD, 2 weeks | Prevents the development of interstitial lung disease mediated by hypochlorous | Inhibition of Smad2/3 activation | [187] |
| **Endocrine** | | | | | |
| Diabetes | Asiatic acid (25 mg/kg) | Rat model, oral, QD, 2 weeks | ↓blood glucose, ↑serum insulin | Preserves β-cells in the pancreatic islet by inducing AKT kinase activation expression and Bcl-xL expression | [170] |
| Diabetes | Ethanolic extract of CA (200 mg/kg) | Rat model, oral, QD, 15 days | Significantly anti-diabetes activity but not significantly influence on the level of serum insulin | – | [188] |
| Diabetes | Asiatic acid (5, 10, 20 mg/kg) | Rat model, oral, QD, 45 days | ↓ blood glucose, ↑ insulin secretion from β-cells and modulated hepatic enzymes including AST and ALT and ALP responsible for glucose metabolism to near-normal levels | – | [189] |

| | | | | | |
|---|---|---|---|---|---|
| Diabetes | Asiatic acid (20 mg/kg) | Rat model, oral, QD | ↓lipid peroxidation and hyperglycemia, ↑antioxidant status | – | [190] |
| Diabetes | Ethanolic extract of CA(250, 500, 1000 mg/kg) | Rat model, oral, BID, 4 weeks | ↓(hyperglycemia, serum LDL and cholesterol), ↑HDL | Inhibition of intestinal disaccharidase enzymes and α-amylase, glucose fiber binding | [191] |
| Hypoglycemic activity | CA (1000, 2000 mg/kg) | Rat model, oral | ↓plasma glucose | – | [10] |
| Diabetes | Madecassic acid (0.05% and 0.1% diets) | Mouse model, oral, QD, 6 weeks | ↓(plasma glucose, TG, cholesterol, oxidative and inflammatory stress), ↑plasma insulin | — | [192] |
| Spontaneous type 2 diabetes | Asiatic acid (25 mg/kg) | Rat model, oral, QD, 4 weeks | Improvement of insulin resistance, ↓glucose level and islet fibrosis | Inhibition of fibronectin (a key protein related to islet fibrosis) | [61] |
| Diabetes | CA (300 mg/kg) | Rat model, oral, QD, 4 weeks | Reversed the glucose and lipid levels, tricarboxylic acid cycle and amino acid metabolic disorders, ↑production of insulin | – | [193] |
| Type 2 diabetes | Methanolic leaf extract CA (500, 1000 mg/kg) | Rat model, oral, QD, 14 days | ↑activity of muscle glycolytic enzymes, ↑glycogenesis in the skeletal muscle, ↓histological damage of skeletal muscle fibers | ↑skeletal muscle glycogen with target muscle glucose and glycogen metabolism | [194] |
| **Skin** | | | | | |
| Wound healing | Cothyline®; Asiaticoside | Guinea pig model, topical, TDS, 1 week | Accelerated the healing process | – | [195] |
| Wound healing | Aqueous extract of CA (1%) | Rat model, topical ointment, cream and gel, TDS, 24 days | ↑cellular proliferation, collagen synthesis and tensile strength | – | [196] |
| Wound healing | Asiaticoside (0.2, 0.4%) | Rat model, topical solution, BID, 7 days | ↑(hydroxyproline content, tensile strength, collagen content and epithelization) | ↑levels of enzymatic and non-enzymatic antioxidant | [197, 198] |
| Wound healing | Asiaticoside (0.2%) | Guinea pig model, topical solution, BID, 7 days | ↑(hydroxyproline content, tensile strength, collagen content and epithelization) | – | [197] |
| Wound healing | Asiaticoside (1 mg/kg) | Guinea pig model, oral, 7 days | ↑(hydroxyproline content, tensile strength, collagen content and epithelization) | – | [197] |
| Wound healing | TECA (asiatic acid, madecassic acid and asiaticoside) | Rat model, SC, 28 days | ↑(dry weight, DNA, protein, hydroxyproline, collagen, peptidichydropr oline,glycosaminoglycan synthesis) | – | [199] |

(continued)

**Table 25.2** (continued)

| Activity/Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | References |
|---|---|---|---|---|---|
| Wound healing | Ethanolic leaf extract of CA | Rat model, oral, 10 days | ↑(epithelization, contraction, tensile strength) | – | [200] |
| Burn wound healing | Madecassoside (6, 12, 24 mg/kg) | Mouse model, oral, 20 days | Significant wound-healing activity | Antioxidant activity, collagen synthesis and angiogenesis | [201] |
| Burn wound healing | Asiaticoside (100 mg) | Mouse model, topical ointment, 19 days | Burn wound healing most strongly at very low concentrations | ↑angiogenesis | [202] |
| Wound healing | Hexane, methanolic, ethyl acetate and aqueous extracts of CA | Rat model, topical, 14 days | ↑wound healing in both incision and burn wounds | – | [203] |
| Wound healing | Methanolic extract of CA | Rat model, electrospun gelatin Nanofibers containing CA | Presented the highest recovery rate | Promoting fibroblast proliferation and collagen synthesis and exhibits antibacterial activity | [204] |
| Burn wound healing | Asiaticoside or madecassoside (500 μL) | Rat model, topical, QD, 14 days | ↑wound healing | – | [205] |
| **Others** | | | | | |
| Osteoporosis | Madecassoside (10 mg/kg) | Mouse model of ovariectomy,i.p., QOD, 6 weeks | Protection and prevention of estrogen deficiency-inducedbone loss | Inhibition of osteoclast activity | [70] |
| Glaucoma | Asiatic acid ($2 \times 10^{-5}$ and $2 \times 10^{-4}$ μmol) | Rat model, intravitreally injection | ↑retinal ganglion cell survival and function, prevention of retinal ganglion cell apoptosis | Upregulating the expression of the antiapoptotic protein Bcl-2 and downregulating the expression of the pro-apoptotic proteins Bax and caspase-3 | [206] |

**Table 25.3** *In vitro* studies of *Centella asiatica*

| Activity / Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | Ref. erences |
|---|---|---|---|---|---|
| **Central nervous system** | | | | | |
| Neuronal damage | Ethanolic extract of CA (100 μg/mL) | Human SH-SY5Y cell lines | ↑neurite outgrowth | – | [128] |
| Alzheimer's disease | Hydroalcoholic extract of CA (100–150 μg/mL) | Spectrophotometric method of Ellman | Inhibition of acetylcholinesterase with IC50 values of 19.87 μg/mL in comparison with Physostigmine (IC50 value of 0.076 μg/mL) | – | [207] |
| Anxiolytic | Aqueous and ethanolic extracts of CA (1 mg/mL) | Rat brain homogenate assay, spectrophotomethric method | Stimulation of glutamic acid decarboxylase activity by over 40% | – | [208] |
| Alzheimer's disease | Leaf extract of CA (1, 5, 10, 100, 200 μg/mL) | Neuroblastoma cell line expressing amyloid-β and rat embryonic cortical primary cell culture | ↑phosphorylation of cAMP response element binding protein (CREB) | ERK/RSK signaling pathway | [209] |
| Neuropsychiatric disease | Aqueous extract of CA (125–500 mg/mL) | Rat cerebellum, radio-enzymatic assay | Inhibition of $Ca^{2+}$-independent $PLA_2$ and cytosolic $PLA_2$ | – | [210] |
| Alzheimer's disease | Aqueous leaf extract of CA (100 μg) | Thioflavin-T assay and transmission electron microscopy | Not inhibition of amyloid-β aggregation, not disintegration of preformed fibrils | – | [211] |
| Alzheimer's disease | Aqueous extract of CA (200 μg/mL) | SH-SY5Y and MC65 human neuroblastoma cells | Protection of SH-SY5Y and MC65 cells from toxicity induced by exogenously added and endogenously generated amyloid-β, respectively, prevention of intracellular amyloid-β aggregate formation in MC65 cells | – | [141] |
| Neuroprotection | Caffeoylquinic acid andaqueous extract of CA (50 and 100 ug/mL) | MC65, SH-SY5Y and neuroblastoma cells | ↓intracellular ROS and $Ca^{2+}$ levels, ↑expression of antioxidant response genes | Attenuation of amyloid-β-induced oxidative stress and mitochondrial dysfunction | [212] |
| Parkinson's disease | Asiatic acid (0.01, 0.1, 5, 10, and 100 nM) | SH-SY5Y cells | ↓ ROS, mitochondrial dysfunction and apoptosis | Antioxidant, mitoprotective and anti-apoptotic properties | [55] |
| **Gastrointestinal** | | | | | |
| Ulcerative colitis | Asiatic acid | Human monocytic THP-1 cells | Inhibition of NLRP3 inflammasome (multi-protein complexes which activates caspase-1 and maturates pro-inflammatory cytokine IL-1β), ROS and mitochondria dysfunction | – | [67] |

(continued)

**Table 25.3** (continued)

| Activity / Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | Ref. erences |
|---|---|---|---|---|---|
| Anti-*Helicobacter pylori* | Leaf extract of CA (2 mg/mL) | Agar dilution method | Inhibition of *H. pylori* | – | [66] |
| **Cardiovascular** | | | | | |
| Arteries and vascular disease | Alcoholic extract of CA | Normal human colon fibroblasts cells | ↑fibroblast cell attachment and tissue plasminogen activator | – | [213] |
| Hyperlipidemia | Alcoholic extract of CA (20 µL) | Pancreatic lipase solution (6 µL) | Anti-lipase activity with $IC_{50}$ of 759.14 µg/mL | – | [10] |
| Cardiac hypertrophy | Asiatic acid (2.5–30 µM) | Neonatal rat ventricular myocytes | Significantly attenuation of hypertrophic response in cardiomyocytes | ↓ANP mRNA expression, ↓TGF-β1-stimulated increase in the levels of p38 and ERK1/2 phosphorylation, ↓NF-κB binding activity | [165] |
| Atherosclerosis | Asiatic acid (10–40 µM) | Human aortic endothelial cells | ↓endothelial hyper-permeability and secretions of cell adhesion molecules protein expression that triggered by TNF-α (10 ng/ml) | Inhibition of NFκB-activation | [59] |
| **Liver** | | | | | |
| Liver fibrosis | Asiatic acid (20, 30 µM) | Hepatic stellate cell line | Significantly inhibition of TGF-β1-inudced collagen I and α-smooth muscle actin mRNA expression | Blocking of TGF-β/ Smad signaling pathway | [171] |
| **Kidney** | | | | | |
| Anti-fibrotic | Methanolic extract of CA (1250 µg/mL) | Normal renal mammalian fibroblasts | Induced apoptosis | – | [214] |
| Renal fibrosis | Asiatic acid (20, 30 µM) | Renal tubular epithelial cells | Anti-fibrotic effect | Inhibition of Smad3 phosphorylation | [181] |
| **Endocrine** | | | | | |
| Hypoglycemic activity | CA (50 µg/mL) | Differentiating 3 T3-L1 adipocytes | ↑lipogenesis as did Troglitazone (an anti-diabetic drug) | Reverses lipid metabolism disorders | [215] |
| Hypoglycemic activity | Alcoholic extract of CA (25, 50 µL) | Colorimetric method | Inhibition of α-glucosidase with $IC_{50}$ of 42.27 µg/mL and α-amylase with $IC_{50}$ of 536.51 µg/mL | – | [10] |
| Vascular complications of diabetes | CA (10, 25 µg/mL) | Human umbilical vein endothelial cells | ↓expression and exposure of vascular adhesion molecules, ↑vascular reactivity | Inhibition of nitro-oxidative stress and down-regulation of MAPK and NF-κB activation | [216] |

| | | | | | |
|---|---|---|---|---|---|
| **Skin** | | | | | |
| Wound healing | TTFCA (25 μg/mL) | Human skin fibroblast | ↑collagen and fibronectin synthesis | – | [217] |
| Wound healing | TECA (Asiatic acid, Madecassic acid and Asiaticoside) | Human foreskin fibroblast monolayer cultures | ↑proline level and collagen synthesis | – | [199] |
| Wound healing | Asiatic acid, Madecassic acid, Asiaticoside (4.5, 4.5, 6 μg/mL respectively) | Human skin fibroblast | ↑type I collagen synthesis | – | [218] |
| Wound healing | Asiaticoside, Madecassoside | Human fibroblast culture | ↑type I and III collagen synthesis | – | [219] |
| Wound healing | Asiaticoside (40 μg/disk) | Chick chorioallantoic membrane model | ↑angiogenesis | – | [197] |
| Wound healing | TECA (Asiatic acid, Madecassic acid, Asiaticoside, Madecassoside) | Human fibroblasts, DNA microarrays analysis | Stimulation of wound healing | Changes of genes expression responsible for angiogenesis and wound healing | [220] |
| Wound healing | Asiaticoside (30 μg/mL) | Human dermal fibroblasts, DNA microarray analysis | ↑fibroblast proliferation and extracellular matrix synthesis | Changes of genes expression involved in cell proliferation, cell cycle and extracellular matrix | [221, 222] |
| Wound healing | Asiaticoside (10 μM) | Human dermal fibroblasts | ↑type I collagen synthesis | Activation of TβR1 kinase-independent Smad pathway | [223] |
| Burn wound healing | Madecassoside (10, 30, 100 μmol/L) | Rat aortic ring assay | ↑endothelial cell growth | – | [201] |
| Wound healing | Asiaticoside | THP-1 and human keratinocyte cell line | Influence on the level of cytokines, ↑angiogenesis, stimulation of VEGF production, MCP-1, IL-1 | – | [202] |
| Wound healing | Ethanolic leaf extract of CA (50 mg/mL) | Human fibroblast cells | ↑collagen synthesis | – | [224] |
| Hypertrophic scar | Asiaticoside (100, 250, 500 mg/L) | Keloid-derived fibroblasts, RT-PCR and Western blot and MTT | Normalization of healing process | Suppresses collagen expression and TGF-b/Smad signaling through inducing Smad7 and inhibiting TGF-βRI and TGF-βRII in keloid fibroblasts | [225] |
| Wound healing | Asiaticoside (62.5, 125, 250, 500, 1000 μM) | Human skin fibroblasts | ↑migration and proliferation of the fibroblasts, ↑extracellular matrix synthesis | – | [226] |
| Wound healing | Aqueous extract of CA (1000 ppm) | Rabbit corneal epithelial cells | ↑cell migration, changes of proliferation and cell cycle | – | [227] |

(continued)

**Table 25.3** (continued)

| Activity / Disease | Active constituent | Study design | Result(s) | Proposed mechanism(s) | Ref. erences |
|---|---|---|---|---|---|
| Burn wound healing | Asiaticoside or Madecassoside (100 ng/mL) | The human acute monocytic leukemia cell line (THP-1) | ↑monocyte chemoattractant protein-1 production, but not significant effect on vascular endothelial growth factor production | – | [205] |
| Anti-psoriatic | Aqueous extract of CA (210, 238 mg/mL) or Asiaticoside (8.4 μM) or Madecasosside (8.6 μM) | SVK-14 keratinocytes | Inhibition of growth of SVK-14 keratinocytes | – | [228] |
| UVB protection | TECA (1, 2, 5 μg/mL) | Normal human HaCaT keratinocytes | ↓UVB toxicity | mRNA alteration caused inhibition of apoptosis and cell proliferation | [227] |
| Skin whitening | TECA (10, 25, 50, 100, 200 μg/mL), Asiatic acid (50 μM), Madecassic acid (200 μM), or Asiaticoside (200 μM) | B16F10 mouse melanoma cells | ↓hyperpigmentation | ↓melanin content in melanocytes by inhibition of tyrosinase mRNA expression | [229] |
| **Others** | | | | | |
| Periodontal tissue healing | Asiaticoside (25, 50, 100 mg/mL) | Human periodontal ligament cells | ↑type I collagen synthesis and osteogenic differentiation | ↑mRNA and proteins of fibronectin and type I collagen, ↓metalloproteinase-I mRNA expression | [230] |
| Osteoporosis | Madecassoside (5, 10 μmol/L), 5 days | Bone marrow monocytes | Suppression of RANKL-induced osteoclast differentiation dose-dependently | Inhibition of osteoclastogenesis via inhibition of NFATc1, c-Fos and blocking of $Ca^{2+}$ oscillation, MAPK and NF-κB signaling pathways | [70] |
| Osteoporosis | Asiaticoside (5, 10, 20 μmol/L) | Bone marrow macrophages | Suppression of RANKL-induced bone resorption and osteoclast formation dose-dependently | Inhibition of Ctsk, Atp6v0d2, Nfatc1, Acp5, Dc-stamp and blocking of $Ca^{2+}$ oscillation, NF-κB and NFATc1 signaling pathways | [71] |

**Table 25.4** Clinical studies of *Centella asiatica*

| Activity /Disease | Active constituent | Study design | Participants | Result(s) | Ref. erences |
|---|---|---|---|---|---|
| **Central nervous system** | | | | | |
| Cognition | Extract of CA (250, 500, 750 mg) | Randomized, placebo-controlled, double-blind study, QD, 2 months | 28 healthy elderly participants | ↑cognition and mood | [231] |
| Mild cognitive impairment | CA (50 mg/kg) | Cross-design clinical study, 2 months | 41 healthy middle age and elderly adults | Attenuate age-related decline in memory function | [232] |
| Mild cognitive impairment | Aqueous extract of CA (500 mg) | Oral, BID, 6 months | 60 elderly subjects of age group 65 years and above | Improvement of mild cognitive impairment | [233] |
| Generalized anxiety disorder | Hydroalchoholic extract CA (500 mg) | Hamilton's brief psychiatric rating scale, oral, BID, 60 days | 33 participants | ↓anxiety related disorders, stress and depression | [9] |
| **Gastrointestinal** | | | | | |
| Gastric ulcer | Fresh juice of CA (60 mg/kg) | Oral, QD | 15 patients with peptic or duodenal ulcer | Improvement in subjective symptoms (93%) and ulcers healing (73%) | [234] |
| **Cardiovascular** | | | | | |
| Vascular disease | TTFCA (60 mg/day) | Comparative clinico-instrumental study, 30 days | 80 patients with venous insufficiency of the lower limbs | Improvement of venous reflux | [235] |
| Vascular disease | Extract of CA | Randomized controlled trials, TDS, 4 weeks | 17 patients with chronic venous insufficiency | Improvement of clinical observations of venous insufficiency and venous tone | [236] |
| Vascular disease | TTFCA (60, 120 mg/day) | Randomized, double blind, placebo controlled, 8 weeks | 94 patients with venous insufficiency of the lower limbs | ↓pain, heaviness, edema and improve in venous distensibility | [237] |
| Vascular disease | TTFCA (60 mg/day) | Oral, QD, 3 months | 20 patients with varicose vein | ↑vascular integrity | [238] |
| Venous hypertension | TTFCA (60 mg) | Oral, TDS, 2 weeks | 44 patients with venous hypertension | ↑microcirculation and capillary permeability | [239] |
| Post phlebitic syndrome | TTFCA | Oral, QD, 3 weeks | Patients with postphlebitic syndrome | Significantly returned circulating endothelial cells to the normal level | [240] |
| Vascular disease | TFCA (30, 60 mg) | Randomized controlled trials, BID, 60 days | 87 patients with chronic venous hypertensive microangiopathy | Effective | [15] |

(continued)

**Table 25.4** (continued)

| Activity /Disease | Active constituent | Study design | Participants | Result(s) | Ref. erences |
|---|---|---|---|---|---|
| Vascular disease | CA, tocopherol, rutin and melilotus | Clinical practice, 30 days | 30 patients with chronic venous insufficiency | Significantly improvement of the clinical symptom | [241] |
| Vascular disease | TTFCA (60 mg) | Randomized controlled trials, BID, 8 weeks | 40 patients with severe venous hypertension, ankle swelling, and lipodermatosclerosis | ↑microcirculation, ↓leg volume | [242] |
| Venous hypertension | TTFCA (30, 60 mg) | Prospective, placebo-controlled, randomized, TDS, 4 weeks | 62 patients with venous hypertension | ↓capillaryfiltrationrate, anklecircumferenceandankleedema | [243] |
| Vascular disease | TTFCA (60, 120 mg/day) | A single-blind, controlled, randomized placebo-study, 8 weeks | 99 patients with venous hypertensive microangiopathy | Significantly improve of venous hypertensive microangiopathy | [242] |
| Atherosclerosis | TTFCA (100 mg) and Pycnogenol | Observational pilot substudy, oral, QD, 30 months | 824 patients with femoral or carotid stenosing plaques | ↓plaques progression, events (hospital admission, specialized care), the need for risk factor management and oxidative stress | [77] |
| **Liver** | | | | | |
| Chronic hepatic disorder | Titrated extract of CA | – | 12 patients | Therapeutic effects in 5 patients | [244] |
| **Endocrine** | | | | | |
| Diabetic microangiopathy | TTFCA (60 mg) | Clinical prospective randomized trial, oral, BID, 6 months | 50 diabetic microangiopathy patients | ↑microcirculation, ↓ capillary permeability | [245] |
| Diabetic microangiopathy | TTFCA (60 mg) | Clinical prospective randomized trial, oral, BID, 12 months | 50 diabetic microangiopathy patients | ↓capillary filtration and edema | [14] |
| Diabetic wound | Capsule of Asiaticoside (100 mg), | Prospective randomized control study, oral, TDS, 21 days | 200 diabetic wound patients | Effective in the wound healing promotion and suppresses the scars | [17] |
| Diabetic cystoid macular edema | CA (30 mg) with flavonoids and Melilotus | Prospective, interventional, controlled study, oral, QD, 14 months | 40 diabetic cystoid macular edema patients without macular thickening | Preserving retinal sensitivity | [246] |

| | | | | | |
|---|---|---|---|---|---|
| Diabetic foot ulcer | Cream of CA and *Plectranthusamboinicus* | Single-center, randomized, topical, BID, 2 weeks | 24 diabetic patients with foot ulcer | Slightly improvement but not significant | [247] |
| Diabetic cystoid macular edema | CA (15 mg) with flavonoids and Melilotus | Prospective, interventional, controlled study, oral, QD, 36 months | 70 diabetic cystoid macular edema patients without macular thickening | Preserving retinal sensitivity | [19] |
| Diabetic neuropathy | Selected triterpenes of CA (120 escalated to 240 mg) | Randomized, double-blind, placebo-controlled, pilot clinical study, oral, 52 weeks | 43 type 2 diabetic patients | ↓total symptom score and paresthesia | [73] |
| **Skin** | | | | | |
| Wound healing | Asiaticoside | Topical, TDS | 20 patients with dirty wounds and chronic or recurrent atony refractory | Accelerated the healing process | [195] |
| Wound healing | Madecassoside (0.1%) with vitamin C (5%) | Randomized double-blind study, topical, BID, 6 months | 20 healthy postmenopausal female volunteers with actinically aged facial, neck and forearm skin | Significantly improved the clinical score of deep and superficial wrinkles, suppleness, firmness, roughness and skin hydration | [74] |
| Striaegravidarum | Cream of CA triterpenes, hydroxyprolisilane-C, rosehip oil and vitamin E | Randomized, double-blind, placebo-controlled trial, topical, BID, 30 days | 183 pregnant women | ↓severity of the striae during pregnancy, prevents the appearance of new striae and halts progression | [248] |
| Scleroderma | Madecassol; tablet (10 mg), ointment, powder | TDS for tablet and BID for ointment, 6 months | 54 patients with systemic and focal scleroderma | ↓indurative lesions, hyperpigmentation, vascular trophic disorders | [75] |
| Healing of skin graft | Cream of CA (7%) | Prospective randomized, controlled, double-blind trial, topical, BID, 12 weeks | 30 adult patients with a split-thickness skin graft, 2 weeks after completion of epithelialization | Significant improvement of Vancouver scar scale | [249] |
| Cutaneous stretchmarks | Centellicum®; CA (250 mg) | Oral, TDS, 6 weeks | 78 women with stretchmarks, 6 months postpartum | ↓visible stretchmarks, ↑skin thickness, ↑collagen components, improve of the grey scale median, skin perfusion, temperature and elasticity | [250] |

pausal female volunteers with actinically aged facial, neck and forearm skin, significantly improved the clinical score of deep and superficial wrinkles, suppleness, firmness, roughness and skin hydration [74]. Also topical and oral formulations of Madecassol in 54 patients with systemic and focal scleroderma significantly reduced indurative lesions, hyperpigmentation and vascular trophic disorders after 6 months of administration [75]. The efficacy of CA for the treatment of chronic venous insufficiency was investigated in a systematic review according to eight randomized controlled trials. Qualitative data of three study showed that CA significantly reduced pain, leg heaviness and oedema. Also quantitative data of five other studies, indicated significant improvement of microcirculatory parameters including venoarteriolar response, severity of ankle swelling and transcutaneous partial pressure of $O_2$ and $CO_2$ [76]. Furthermore, daily oral administration of 1000 mg TTFCA with Pycnogenol (the extract of French maritime pine bark) for 30 months in 824 patients with femoral or carotid stenosing plaques, significantly lowered plaques progression and events regarding to atherosclerosis [77].

## 25.7 Herb-Drug Interactions

According to *in vitro* studies, ethanolic extract of CA competitively inhibited cytochrome P450 (CYP) 1A2 and CYP2C9 and noncompetitively inhibited CYP3A4. Inhibition of CYP1A2 and CYP3A4 may be due to flavonoids of the plant. Methanolic extract of CA has noncompetitive inhibitory effect on CYP2D6 [78, 79]. An *in vitro* study showed that standardized extract of CA, ECa233, only inhibited CYP3A4, CYP2D6 and CYP2B6. Similar standardized extract was found inhibitor of CYP2B1 and CYP2B2 and decreased sulfotransferase activity of liver in rats [80–82]. These effects may explain reduction of clearance and increase of the area under the curve (AUC) of amitriptyline by CA in rats. So, it seems that CA may increase concentration of medicines metabolized by these enzymes [83]. Some data suggested ethyl acetate extract of the plant had additive antiepileptic effect in combination with gabapentin, valproate, phenytoin, and reduced effective dose of these medicines but, that was determined some constituents of CA may decline protective effect of phenytoin, phenobarbital and carbamazepine against seizure [84, 85]. Nevertheless, there is no herb-drug interaction documented in human study. Due to CNS depression activity of CA, it is advised to avoid concomitant use of this plant with CNS depressant medications. Data suggested that CA theoretically may increase effect of hypoglycemic agent and hepatotoxicity of medicines determined hepatotoxic [86–88]. Taken together, more data is needed to confirm herb-drug interactions of CA.

## 25.8 Pregnancy and Lactation

CA perhaps was found to be safe in pregnant female rats and was not teratogenic for male rats [89, 90]. Human studies suggested topical application of CA is possibly safe but, due to limitation of teratology data and some abortions reported from chronic oral use of the plant in rats, pregnant and nursing mothers are advised to avoid to ingestion of formulations congaing CA [91–93].

## 25.9 Toxicology

In human clinical studies, no serious adverse effect was reported from CA in oral or topical use. In clinical studies, the usual dose for oral administration was two capsule containing 500 mg hydro-ethanolic extract of CA once a day for up to 60 days or three capsule containing 50 mg of extracted Asiaticoside for up to 21 days. In another study volunteers took one capsule containing standardized extract of CA in dose up to 500 mg for 7 days and show no adverse effect [9, 17, 83, 91, 94–96]. Asiaticoside isolated from CA and oral administration of 1 g/kg showed no toxicity in previous human studies [97–99]. However, it may cause allergy in some patients, especially in topically application. So, it is important to care about probable contact dermatitis.

There was a report from hepatotoxicity effect following ingestion of CA resulting in jaundice in human. This adverse reaction was resolved after discontinuation of the plant [87].

In animal studies, CA made no toxicity up to 1000 mg orally in a single dose or daily for up to 90 days. In these studies, CA increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), *blood urea nitrogen* (BUN) and creatinine significantly after a month, but all parameters still were within normal range and no death occurred. According to this, perhaps the medium lethal dose (LD50) and no-observed-adverse-effect-level (NOAEL) for its standardized extract is greater than 2000 mg/kg or even 4000 mg/kg and 1000 mg/kg respectively in rats. The standardized extract causes no acute toxicity sign in dose up to 10 g/kg in mice. These results were reported from single dose, acute and subchronic evaluations of CA toxicity [20, 100–103]. Some data from animal studies suggested CA may have antifertility effect in male rat and also may cause abortion in female rat [92, 104, 105].

## 25.10 Conclusions

In summary, CA is a herbal medicine which is found almost all over the world and has been used since prehistoric and immemorial times in many traditional systems of medicine as a curative agent for a wide range of ailments. In recent years, CA significantly drawn the attention of researchers because of its health-promoting potentials. According to the results of our study, CA demonstrated to have CNS, cardiac, pulmonary, liver and kidney protective, antiulcer, wound healing, and antidiabetic effects. On the basis of the experimental evidence, some chemical isolates and herbal preparations of CA have been launched in the market as oral supplements or topical ingredients in cosmetic products.

Although a large number of studies have investigated the biological activities and underlying mechanisms of CA over the past decades, documented data and findings of these studies are still limited. Similarly, there is limited information about interactions, adverse effects and toxicity of CA. Hence, it seems that further scientific studies and organized clinical trials are required to validate and justify the safety and efficacy of CA.

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# 26

# Ethnomedicinal Uses, Phytochemistry and Pharmacology of Different *Cichorium* Species (Asteraceae): A Review

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**Abstract**

*Cichorium* species have been used widely in traditional medicine universally. It is reported as a treatment for various respiratory and gastrointestinal disorders, as well as diabetes and rheumatism. A range of constituents including phenolic and poly phenolic compounds, fatty and organic acids and essential oils comprise the chemical composition of *Cichorium* species. Furthermore, modern investigations on these species has shown different pharmacological activities such as antioxidant, antiproliferative, anti-inflammation, antibacterial, anti-hyperglycemic, antidiabetic and hepatoprotective effects which are associated with divers molecular pathways and mechanisms. In this chapter, we have summarized comprehensive information regarding traditional and ethnomedicinal uses, phytochemical analysis and pharmacological aspects of *Cichorium* species.

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**Keywords**

*Cichorium* · Asteraceae · Traditional medicine · Ethnobotany · Pharmacology

## 26.1 Introduction

*Cichorium* L. from the family Asteraceae (Compositae) is globally used for numerous medicinal benefits; particularly as anti-diabetic, digestion improvement and hepatoprotective agent. This herb is mainly distributed in Europe and Asia. The species *C. endivia*, *C. intybus*, *C. pumilum* and *C. spinosum* are among the most famous ones used in traditional and folklore medicines and culinary purposes. In Islamic traditional medicine, (ITM) *Cichorium* is renowned for its effect as a liver protective agent and has been prescribed by many great physicians of that era.

The small genus of *Cichorium* has become noteworthy because of its two commercially species: *C. endivia* (endive) and *C. intybus* (chicory), which are cultivated through the world [1].

Many chemical constituents have been isolated from this genus, among them phenolic and polyphenolic compounds are the major group. Besides, different parts of the plants have shown various pharmacological effects by varied mechanism *in vivo* and *in vitro*.

This article summerizes comprehensive information regarding traditional and ethnomedicinal uses, phytochemical analysis and pharmacological aspects of *Cichorium* species.

## 26.2 Botanical Profile and Taxonomy of *Cichorium* Taxa

*Cichorium* L. belongs to the family Asteraceae, which is the largest family of flowering plants with approximately 23,000 species in 1535 genera [2]. The genus *Cichorium* encompasses about ten species with major distribution areas in Europe and Asia. Although, *Cichorium* is a small genus, but it has gained importance because of its two commercially species: *C. endivia* L. (endive) and *C. intybus* L. (chicory), which are cultivated globally [1]. The genus *Cichorium* L. contains annual or perennial herbs. The stems are usually solitary, branched. The leaves are runcinate-pinnatifid or dentate. The capitula are numerous, terminal and axillary. Involucre cylindrical, consisting of 2 rows of bracts, the outer shorter. Receptacle more or less flat, without scales. The ligules are usually blue. The fruit is achene and obovoid in shape, more or less angled, truncate at apex; pappus of 1–2 rows of short, obtuse scales [3]. According to The Plant List (The Plant List, 2013), there are 92 scientific plant names of species rank for the genus *Cichorium*, of these, 10 are accepted species names. Table 26.1 summarizes all synonyms of *Cichorium* species based on the website 'TPL' (http://www.theplantlist.org.).

## 26.3 Phytochemical Study

Various analytical studies on *Cichorium* species show that essential oil constituents and sesquiterpene lactones derivatives are the major compounds of this species. Besides, some significant phenolic and polyphenolic compounds have also been exhibited. Table 26.2 shows the chemical structures of isolated compounds from different parts of *Cichorium* species.

## 26.4 Ethnobotanical and Ethnomedicinal Uses

Ethnobotanical literature survey indicates that *C. endivia* L., *C. intybus* L., *C. pumilum* Jacq. and *C. spinosum* L. are the famous species of the *Cichorium* genus, which have extraordinary traditional medicinal properties and folk applications in Europe, Asia and American continents.

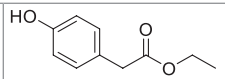
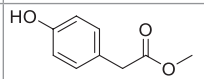
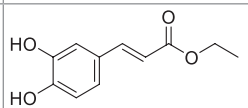
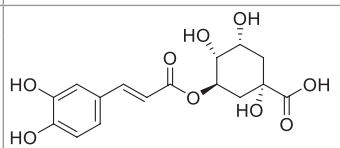
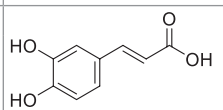
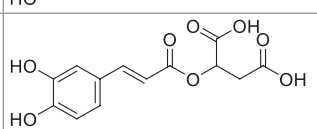
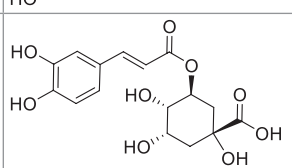
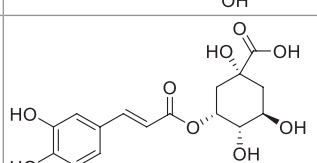
Various parts of *Cichorium* species including root, leaf, fruit, and aerial part have been traditionally used for a wide spectrum of folk uses. Some of their exemplary uses are given below while the others are summarized in Table 26.1.

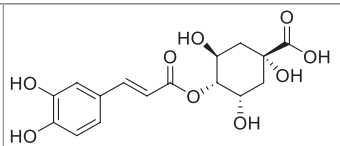
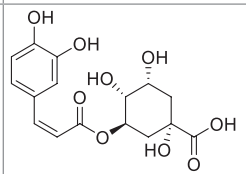
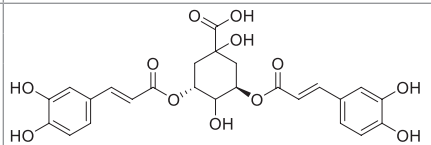
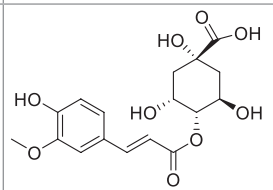
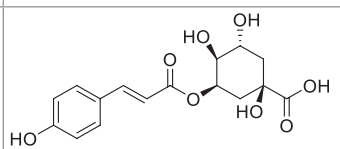
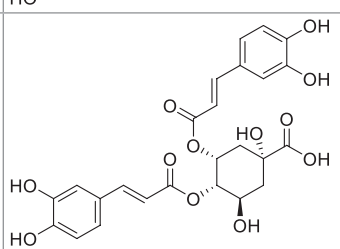
**Table 26.1** Scientific names and synonyms of reported *Cichorium* species [according to The Plant List (2013)]

| NO | *Cichorium* species (accepted names) | Synonym(s) |
|---|---|---|
| 1 | *C. alatum* Hochst. & Steud. | |
| 2 | *C. bottae* Deflers | |
| 3 | *C. callosum* Pomel | |
| 4 | *C. calvum* Sch.Bip. ex Asch. | *C. calvum* Sch.Bip. |
| 5 | *C. dubium* E.H.L.Krause | |
| 6 | *C. endivia* L. | *C. casnia* Wall. |
| | | *C. casnia* C.B.Clarke |
| 7 | *C. hybridum* Halácsy | |
| 8 | *C. intybus* L. | *C. balearicum* Porta |
| | | *C. byzantinum* Clementi |
| | | *C. cicorea* Dumort. |
| | | *C. commune* Pall. |
| | | *C. divaricatum* Heldr. ex Nyman |
| | | *C. glabratum* C.Presl |
| | | *C. glaucum* Hoffmanns. & Link |
| | | *C. hirsutum* Gren. |
| | | *C. illyricum* borb. |
| | | *C. officinale* Gueldenst. ex Ledeb. |
| 9 | *C. pumilum* Jacq. | *C. ambiguum* Schult. |
| | | *C. dichotomum* Link |
| | | *C. divaricatum* Schousb. |
| | | *C. glandulosum* Boiss. & A.Huet |
| | | *C. minimum* Port. |
| | | *C. nanum* Port. ex Nyman |
| | | *C. noeanum* Boiss. |
| | | *C. polystachyum* Pomel |
| 10 | *C. spinosum* L. | |

Traditionally, *C. endivia* L. was cultivated for culinary; either cooked or eaten raw in salads. However, its popularity is attributed to its healthy properties, which are mainly due to its high levels of antioxidant compounds. It is the only taxon of this genus which is not known from the wild flora [31]. *Cichorium intybus* L. (common name: chicory), as a wild weed or cultivated is a medicinally important plant in different regions around the world. It is a hardy plant and can endure extreme temperatures during both vegetative and reproductive growth stages [1]. This plant is reputed as hepatoprotective and due to its prevalent distribution, various parts of the plant have been consumed in folk medicines globally [32]. Historically, it was used by the ancient Egyptians as a medicinal species, coffee substitute, and vegetable crop and was occasionally consumed for animal forage [32]. In Indian traditional therapy, chicory is widely consumed as a traditional remedy for diabetes mellitus. It has a long history of use and is particularly of great value for its tonic effects upon the liver and digestive tract. The roots and the leaves are appetizer, cholagogue, depurative, digestive, diuretic, hypoglycemic, laxative and tonic. A decoction of the roots has been utilized in the healing of jaundice, liver enlargement, gout and rheumatism [33]. Moreover, its seeds are one of the main ingredients of Jigrine, a commercial product of India consumed as a traditional remedy for the different illnesses of the liver [34]. In Serbia, the infusion of chicory flowers have traditionally been used for the treatment of diarrhea [35]. The people native to the areas of Monte Vesole and Ascea, Cilento National Park (Campania, Southern Italy) use the leaf and root of *C. intybus* L. (Cicoria) against kidney disorders. Furthermore, the decoction of the roots is utilized against hypertension and leaves are added to soups, eaten as salad or fried [36]. In the Traditional Chinese Medicine (TCM), *C. intybus* L. and *C. pumilum* Jacq. are considered to be folk medicines used for the treatment of liver diseases [13]. In the Eastern Mediterranean regions, *C. pumilum* leaves are consumed to treat diabetes, bacterial infections, poisoning and rheumatism. In Jordan, its leaves are widely used in salads, after being blanched, as the unblanched leaves are bitter [37]. As illustrated in Table 26.1, the most commonly ethnomedicinal uses of *Cichorium* taxa seems to be treatment of gastrointestinal ailments, urinary system disorders, skin problems, abdominal complaints, respiratory problems, menstrual disorders, diabetes, kidney stones, and also act as anti-allergic in different traditional medicine systems around the world (Table 26.3).

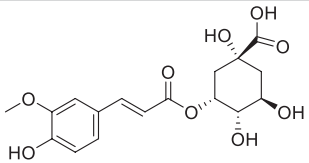
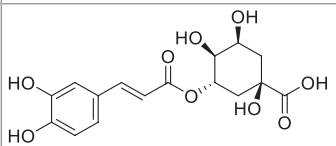
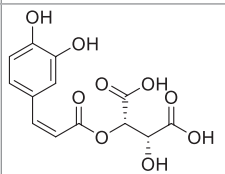
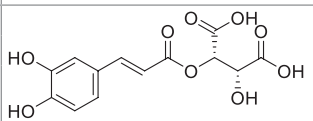
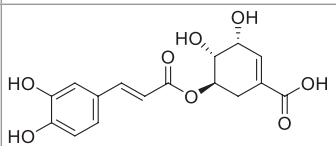
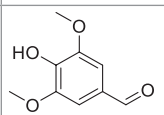
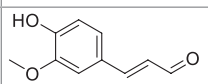
**Table 26.2** Chemical composition from different parts of *Cichorium* species

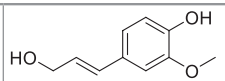
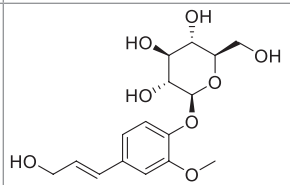
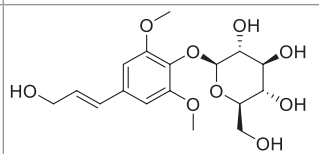
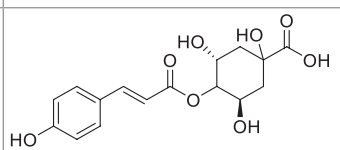
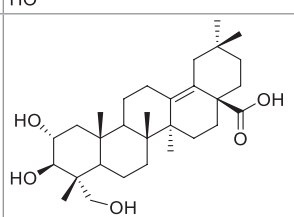
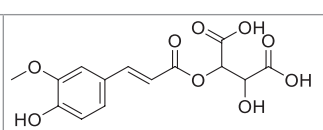
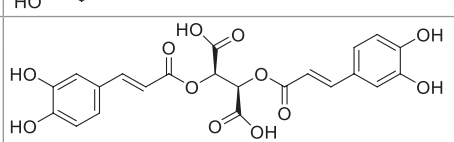
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| **Phenolic and polyphenolic compounds** | | | | | |
| 1 | Ethyl *p*-hydroxyphenylacetates |  | *C. endivia* | Roots | [4] |
| 2 | Methyl *p*-hydroxyphenylacetates |  | *C. endivia* | Roots | [4] |
| 3 | Ethyl *trans*-caffeate |  | *C. endivia* | Roots | [4] |
| 4 | Chlorogenic acid |  | *C. intybus* | | [5] |
| | | | *C. spinosum* | Edible parts | [6] |
| | | | *C. intybus* | Leaves | [7] |
| | | | *C. intybus* | Roots | [8] |
| 5 | Caffeic acid |  | *C. intybus* | Leaves | [9] |
| | | | *C. spinosum* | Edible parts | [6] |
| 6 | Caffeoylmalic acid |  | *C. spinosum* | Edible parts | [6] |
| 7 | 3-caffeoylquinic acid |  | *C. intybus* | Leaves | [9] |
| 8 | 5-caffeoylquinic acid |  | *C. intybus* | Leaves | [9] |

| | | | | | |
|---|---|---|---|---|---|
| 9 | 4-caffeoylquinic acid |  | *C. intybus* | Leaves | [9] |
| 10 | *cis*-5-caffeoylquinic acid |  | *C. intybus* | Leaves | [9] |
| 11 | 3,5-dicaffeoylquinic acid |  | *C. intybus* | Roots | [8] |
| | | | *C. intybus* | Leaves | [9] |
| | | | *C. spinosum* | Leaves | [10] |
| 12 | 4-O-feruloylquinic acid |  | *C. intybus* | Leaves | [9] |
| 13 | 3-O-*p*-coumaroyl quinic acid |  | *C. intybus* | Flowers | [11] |
| 14 | 4,5-dicaffeoylquinic acid |  | *C. intybus* | Roots | [8] |

(continued)

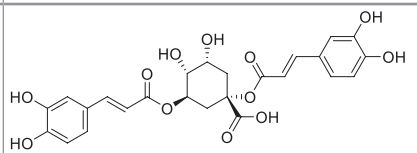
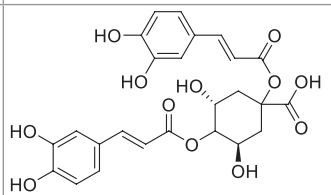
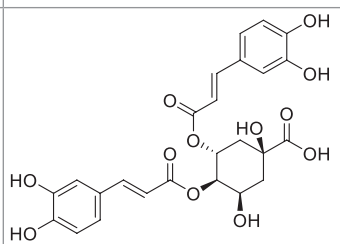
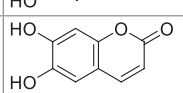
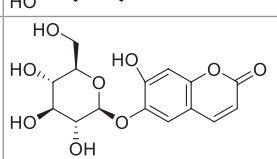
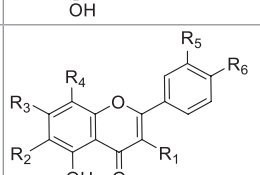
**Table 26.2** (continued)

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 15 | 5-O-feruloylquinic acid |  | *C. intybus* | Leaves | [9] |
| 16 | 5-O-caffeoylquinic acid |  | *C. spinosum* | Leaves | [10] |
| 17 | *cis*-caftaric acid |  | *C. intybus* | Leaves | [9] |
| 18 | *trans*-caftaric acid |  | *C. intybus* | Leaves | [9] |
| | | | *C. spinosum* | Leaves | [10] |
| | | | *C. spinosum* | Edible parts | [6] |
| 19 | 5-caffeoylshikimic acid |  | *C. intybus* | Leaves | [9] |
| 20 | Syringaldehyde |  | *C. pumilum* | Roots | [12] |
| 21 | Coniferyl aldehyde |  | *C. pumilum* | Roots | [12] |

| | | | | | |
|---|---|---|---|---|---|
| 22 | Coniferyl alkohol |  | *C. pumilum* | Roots | [12] |
| 23 | Coniferin |  | *C. pumilum* | Roots | [12] |
| 24 | Syringin |  | *C. pumilum* | Roots | [12] |
| 25 | 5-*p*-coumaroylquinic acid |  | *C. intybus* | Leaves | [9] |
| 26 | Coutaric acid |  | *C. spinosum* | Leaves | [10] |
| | Coutaric acid hexoside | | *C. spinosum* | Leaves | [10] |
| 27 | Fertaric acid |  | *C. spinosum* | Leaves | [10] |
| 28 | Dicaffeoyltartaric acid (chicoric acid) |  | *C. intybus* | Leaves | [9] |
| | | | *C. intybus* | Leaves | [7] |
| | | | *C. spinosum* | Edible parts<br>Leaves | [6, 10] |

(continued)

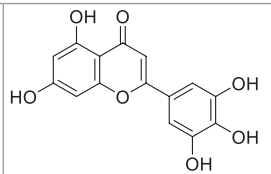
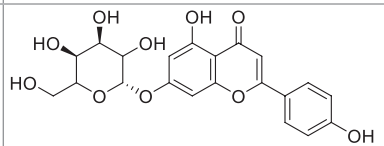
**Table 26.2** (continued)

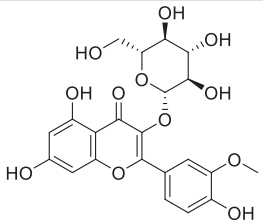
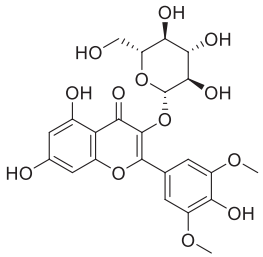
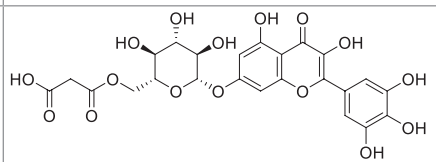
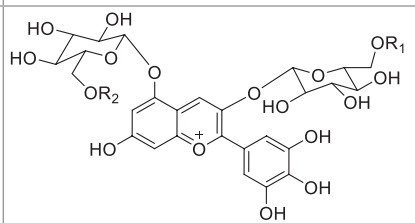
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 29 | 1,3-dicaffeoylquinic acid |  | *C. intybus* | Leaves | [9] |
| 30 | 1,4-dicaffeoylquinic acid |  | *C. intybus* | Leaves | [9] |
| 31 | 3,4-dicaffeoylquinic acid |  | *C. intybus* | Leaves | [9] |
| 32 | Esculetin |  | *C. intybus* | Roots | [1] |
| 33 | Esculin |  | *C. intybus* | Roots | [1] |
| | |  | | | |

| | | | | |
|---|---|---|---|---|
| 34 | Kaempferol: $R_1$ = OH, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. endivia* | | [13] |
| 35 | Kaempferol-3-O-glucosyl-7-O-(6′′-O-malonyl)-glucoside: $R_1$ = O-glucosyl, $R_2$ = H, $R_3$ = O-(6′′-O-malonyl)-glucoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 36 | Kaempferol-7-O-glucosyl-3-O-(6′′-malonyl)-glucoside: $R_1$ = O-(6′′-malonyl)-glucoside, $R_2$ = H, $R_3$ = O-glucosyl, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 37 | Kaempferol-3-O-β-$_D$-glucuronide: $R_1$ = O-β-$_D$-glucuronide, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. spinosum* | Edible parts | [6] |
| 38 | Kaempferol-3-O-sophoroside: $R_1$ = O-sophoroside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 39 | Kaempferol-7-O-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-glucoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 40 | Kaempferol-7-O-rutinoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-rutinoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 41 | Kaempferol-7-O-(6′′-O-malonyl)-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-(6′′-O-malonyl)-glucoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 42 | Kaempferol-7-O-glucuronide: $R_1$ = OH, $R_2$ = H, $R_3$ = O-glucuronide, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 43 | Kaempferide-3-O-(6′′-O-malonyl)-glucoside: $R_1$ = O-(6′′-O-malonyl)-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 44 | Kaempferol-3-O-glucuronide: $R_1$ = O-glucuronide, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| | | *C. spinosum* | Leaves | [10] |
| 45 | Kaempferol-3-O-glucuronide-7-O-glucoside: $R_1$ = O-glucuronide, $R_2$ = H, $R_3$ = O-glucoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 46 | Kaempferol-3-O-(6′′-O-malonyl)-glucoside: $R_1$ = O-(6′′-O-malonyl)-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 47 | Kaempferol-3-O-rutinoside: $R_1$ = O-rutinoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. spinosum* | Leaves | [10] |
| 48 | Kaempferol-3-O-glucoside: $R_1$ = O-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| | | *C. endivia* | | [13] |
| 49 | Kaempferol-7-O-neohesperidoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-neohesperidoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 50 | Kaempferol-7-O-(6′′-O-acetyl)-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-(6′′-O-acetyl)-glucoside, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 51 | Kaempferol-3-O-(6′′-O-acetyl)-glucoside: $R_1$ = O-(6′′-O-acetyl)-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = H, $R_6$ = OH | *C. spinosum* | Leaves | [10] |
| 52 | Quercetin-7-O-galactoside: Quercetin: $R_1$ = OH, $R_2$ = H, $R_3$ = O-galactoside, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *C. intybus* | Leaves | [9] |
| 53 | Quercetin-3-O-(6′′-O-malonyl)-glucoside: $R_1$ = O-(6′′-O-malonyl)-glucoside, $R_2$ = H, $R_3$ = O-galactoside, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | *C. intybus* | Leaves | [9] |

(continued)

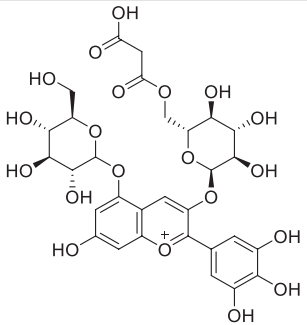
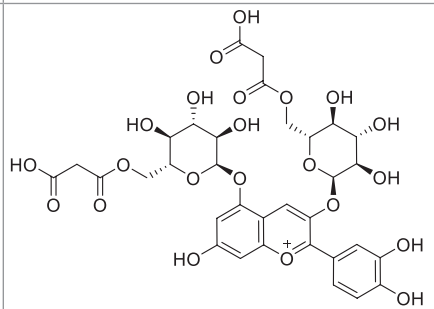
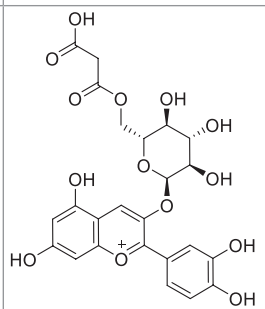
**Table 26.2** (continued)

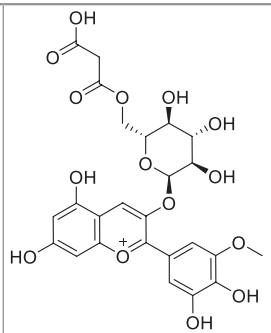
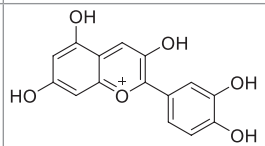
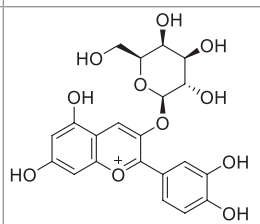
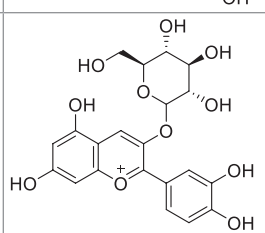
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 54 | Quercetin-7-O-*p*-coumaroylglucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-*p*-coumaroylglucoside, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 55 | Quercetin-7-O-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-glucoside, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 56 | Quercetin-7-O-glucuronide: $R_1$ = OH, $R_2$ = H, $R_3$ = O-glucuronide, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 57 | Quercetin-7-O-(6′′-O-acetyl)-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-(6′′-O-acetyl)-glucoside, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| | | | *C. spinosum* | Leaves | [10] |
| 58 | Quercetin-3-O-β-$_D$-glucuronide: $R_1$ = O-β-$_D$-glucuronide, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *C. spinosum* | Edible parts | [6] |
| | | | *C. spinosum* | Leaves | [10] |
| 59 | Quercetin 3-O-*β*-$_D$-glucoside (isoquercitrin): $R_1$ = O-*β*-$_D$-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *C. intybus* | Flowers | [11] |
| 60 | Quercetin-3-O-glucuronide-7-O-(6′′-O-malonyl)-glucoside: $R_1$ = O-glucuronide, $R_2$ = H, $R_3$ = O-(6′′-O-malonyl)-glucoside, $R_4$ = H, $R_5$ = OH, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 61 | Isorhamnetin-7-O-(6′′-O-acetyl)-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-(6′′-O-acetyl)-glucoside, $R_4$ = H, $R_5$ = $OCH_3$, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 62 | Isorhamnetin-7-O-neohesperidoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-neohesperidoside, $R_4$ = H, $R_5$ = $OCH_3$, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 63 | Isorhamnetin-7-O-glucoside: $R_1$ = OH, $R_2$ = H, $R_3$ = O-glucoside, $R_4$ = H, $R_5$ = $OCH_3$, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 64 | Isorhamnetin-7-O-glucuronide: $R_1$ = OH, $R_2$ = H, $R_3$ = O-glucuronide, $R_4$ = H, $R_5$ = $OCH_3$, $R_6$ = OH | | *C. intybus* | Leaves | [9] |
| 65 | Isorhamnetin-3-O-glucuronide: $R_1$ = O-glucuronide, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = $OCH_3$, $R_6$ = OH | | *C. spinosum* | Leaves | [10] |
| 66 | Isorhamnetin-3-O-(6′′-O-acetyl)-glucoside: $R_1$ = O-(6′′-O-acetyl)-glucoside, $R_2$ = H, $R_3$ = OH, $R_4$ = H, $R_5$ = $OCH_3$, $R_6$ = OH | | *C. spinosum* | Leaves | [10] |
| 67 | Hieracin II |  | *C. endivia* | Roots | [4] |
| 68 | Apigenin-7-O-glucoside |  | *C. intybus* | Leaves | [9] |
| 69 | Apigenin-O-glucuronide | | *C. spinosum* | Leaves | [10] |

| | | | | | |
|---|---|---|---|---|---|
| 70 | Chrysoeriol-3-O-glucoside |  | *C. intybus* | Leaves | [9] |
| 71 | Tricin-3-O-glucoside |  | *C. intybus* | Leaves | [9] |
| 72 | Myricetin-7-O-(6′‘-O-malonyl)-glucoside |  | *C. intybus* | Leaves | [9] |
| | |  | | | |
| 73 | Delphinidin 3,5-di-O-(6-O-malonyl-β-$_D$-glucoside): $R_1$ = manolyn, $R_2$ = manolyn | | *C. intybus* | Flowers | [11] |
| 74 | Delphinidin 3-O-(6-O-malonyl-*β*-$_D$-glucoside)-5-O-*β*-$_D$-glucoside: $R_1$ = manolyn, $R_2$ = H | | *C. intybus* | Flowers | [11] |
| 75 | Delphinidin 3-O-*β*-$_D$-glucoside-5-O-(6-O-malonyl-*β*-$_D$-glucoside): $R_1$ = H, $R_2$ = manolyn | | *C. intybus* | Flowers | [11] |
| 76 | Delphinidin 3,5-di-O-*β*-$_D$-glucoside: $R_1$ = H, $R_2$ = H | | *C. intybus* | Flowers | [11] |

(continued)

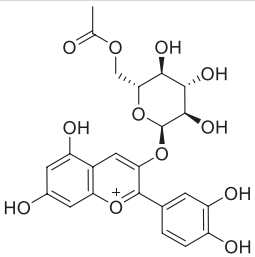
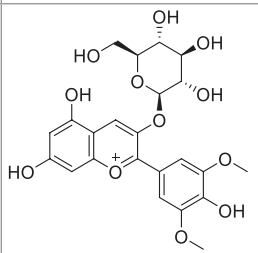
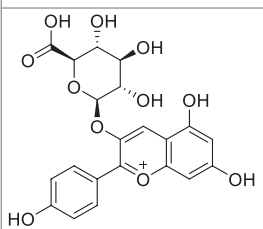
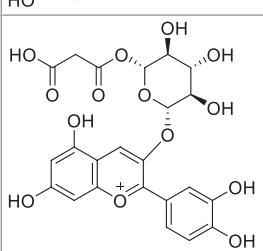
**Table 26.2** (continued)

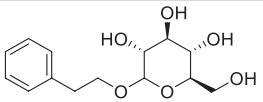
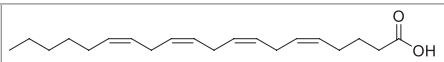
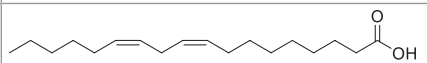
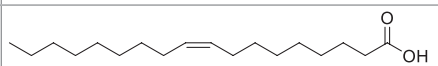
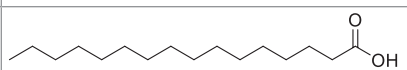
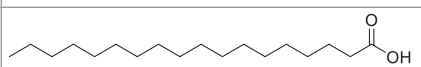
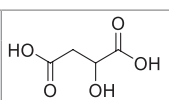
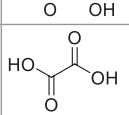
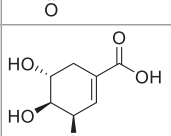
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 77 | Delphinidin-3-O-(6′′-O-malonyl)-glucoside-5-O-glucoside: |  | *C. intybus* | Leaves | [9] |
| 78 | Cyanidin-3,5-di-O-(6′′-O-malonyl)-glucoside |  | *C. intybus* | Leaves | [9] |
| 79 | Cyanidin-3-O-(6′′-O-malonyl)-glucoside |  | *C. intybus* | Leaves | [9] |

| | | | | | |
|---|---|---|---|---|---|
| 80 | Petunidin-3-O-(6′′-O-malonyl)-glucoside |  | *C. intybus* | Leaves | [9] |
| 81 | Cyanidin |  | *C. intybus* | Leaves | [9] |
| 82 | Cyanidin-3-O-galactoside |  | *C. intybus* | Leaves | [9] |
| 83 | Cyanidin-3-O-glucoside |  | *C. intybus* | Leaves | [9] |

(continued)

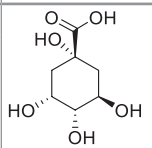
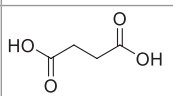
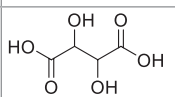
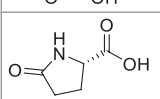
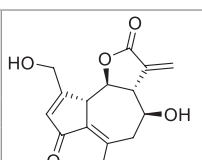
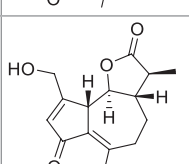
**Table 26.2** (continued)

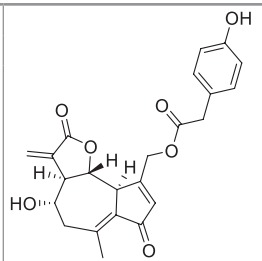
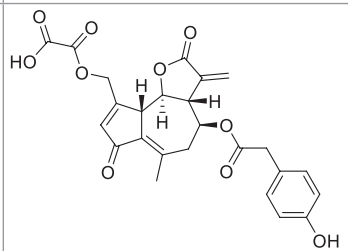
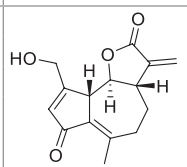
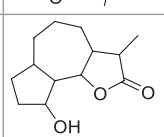
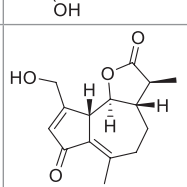
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 84 | Cyanidin-3-O-(6″-O-acetyl)-glucoside |  | *C. intybus* | Leaves | [9] |
| 85 | Malvidin-3-O-glucoside |  | *C. intybus* | Leaves | [9] |
| 86 | Pelargonidin-3-O-monoglucuronide |  | *C. intybus* | Leaves | [9] |
| 87 | Cyanidin<br>3-O-*β*-(6-O-malonyl)-$_{D}$- glucopyranoside |  | *C. intybus* | Flowers | [11] |

| | | | | | |
|---|---|---|---|---|---|
| 88 | 2-phenylethyl-β-$_{D}$-glucopyranoside |  | *C. unshiu*<br>*C. endivia* | Leaves | [14]<br>[13] |
| **Fatty acids** | | | | | |
| 89 | Arachidonic acid |  | *C. intybus* | Dried Plant Powder | [15] |
| 90 | Linoleic acid |  | *C. intybus* | Dried Plant Powder | [15] |
| 91 | Oleic acid |  | *C. intybus* | Dried Plant Powder | [15] |
| 92 | Palmitic acid |  | *C. intybus* | Dried Plant Powder | [15] |
| 93 | Stearic acid |  | *C. intybus* | Dried Plant Powder | [15] |
| **Organic acids** | | | | | |
| 94 | Malic acid |  | *C. intybus*<br>*C. spinosum* | Leaves<br>Edible parts | [9]<br>[6] |
| 95 | Oxalic acid |  | *C. intybus* | | [16] |
| 96 | Shikimic acid |  | *C. intybus* | | [16] |

(continued)

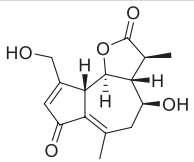
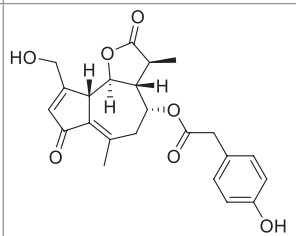
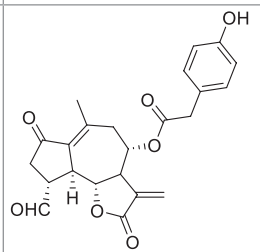
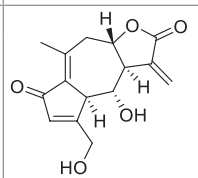
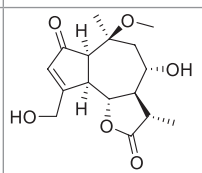
**Table 26.2** (continued)

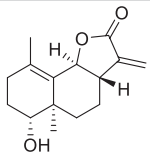
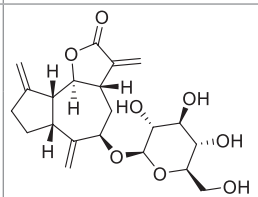
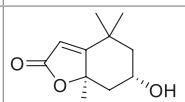
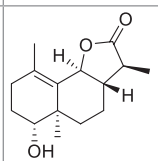
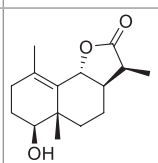
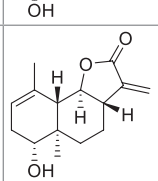
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 97 | Quinic acid |  | *C. intybus* | | [16] |
| | | | *C. spinosum* | Edible parts | [6] |
| 98 | Succinic acid |  | *C. intybus* | | [16] |
| 99 | Tartaric acid |  | *C. spinosum* | Edible parts | [6] |
| 100 | Pyroglutamic acid |  | *C. spinosum* | Edible parts | [6] |
| **Essential oils** | | | | | |
| 101 | Lactucin |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. intybus* | Roots | [18, 19] |
| | | | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| 102 | Jacquinelin |  | *C. pumilum* | Roots | [12] |

| | | | | | |
|---|---|---|---|---|---|
| 103 | Lactucopicrin |  | *C. intybus* | Roots | [18, 19] |
| | | | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| 104 | Lactucopicrin-15-oxalate |  | *C. intybus* | Dried Plant Powder | [15] |
| 105 | 8-deoxylactucin |  | *C. intybus* | Roots | [19] |
| | | | *C. intybus* | | [20] |
| | | | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| 106 | Chicoralexin |  | *C. intybus* | Dried Plant Powder | [15] |
| 107 | Jacquilenin |  | *C. intybus* | Roots Leaves | [17] |

(continued)

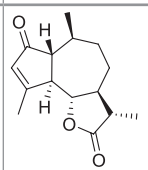
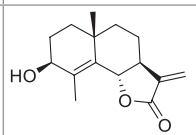
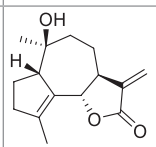
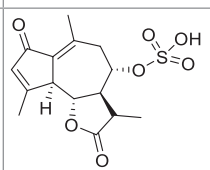
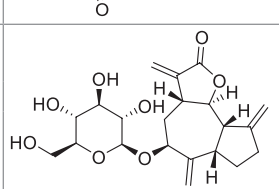
**Table 26.2** (continued)

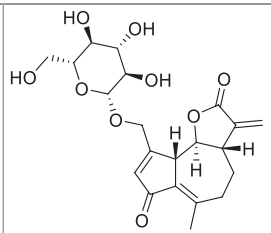
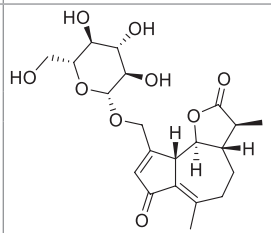
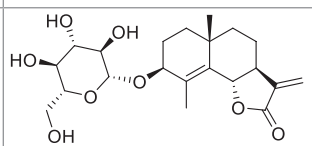
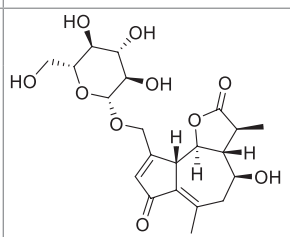
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 108 | 11$\beta$,13-dihydrolactucin |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| 109 | 11,13-dihydrolactucopicrin |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| 110 | 3,4$\beta$-dihydro-15-dehydrolactucopicrin |  | *C. intybus* | Roots<br>Leaves | [17] |
| 111 | Intybulide |  | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| 112 | 10$\beta$-methoxy-1$\alpha$, 11$\beta$,13-tetrahydrolactucin |  | *C. endivia* | Roots | [4] |

| | | | | | |
|---|---|---|---|---|---|
| 113 | Magnolialide |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| 114 | Ixerisoside D |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. intybus* | Roots | [21] |
| | | | *C. endivia* | Roots | [4] |
| 115 | Loliolide |  | *C. intybus* | Roots<br>Leaves | [17] |
| 116 | Artesin |  | *C. pumilum* | Roots | [12] |
| 117 | 11-epiartesin |  | *C. pumilum* | Roots | [12] |
| 118 | Santamarine |  | *C. pumilum* | Roots | [12] |

(continued)

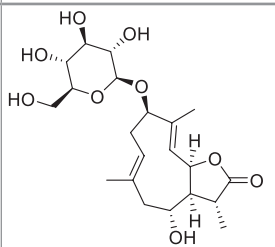
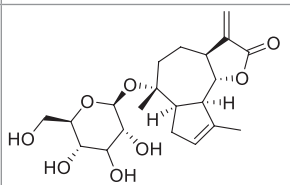
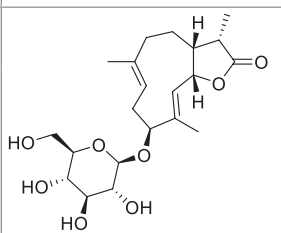
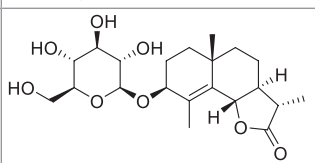
**Table 26.2** (continued)

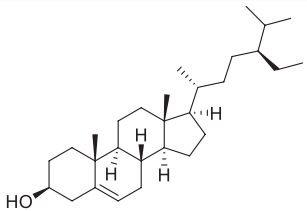
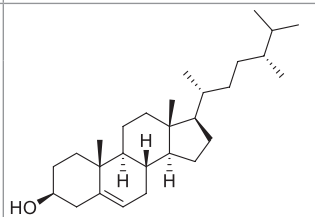
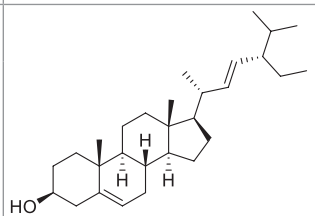
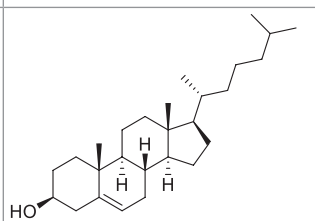
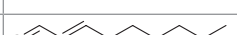
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 119 | Cichoralexin |  | *C. intybus* | Leaves | [22] |
| 120 | Cichoriolide A |  | *C. intybus*<br>*C. endivia* | | [23] |
| 121 | Cichopumilide |  | *C. pumilum* | Roots | [17, 24] |
| 122 | 8-deacetylmatricarin-8-O-sulphate |  | *C. spinosum* | Edible parts | [6] |
| 123 | Ixerisoside D |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. intybus* | Roots | [21] |
| | | | *C. endivia* | Roots | [4] |

| | | | | | |
|---|---|---|---|---|---|
| 124 | Crepidiaside A |  | *C. intybus* | Roots | [8, 21] |
| 125 | Crepidiaside B |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. endivia* | Roots | [4] |
| | | | *C. pumilum* | Roots | [12] |
| | | | *C. intybus* | Roots | [21] |
| 126 | Cichorioside |  | *C. intybus*<br>*C. endivia* | Roots | [23] |
| 127 | Cichorioside B |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. intybus*<br>*C. endivia* | Roots | [23] |

(continued)

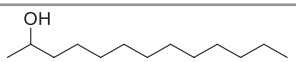
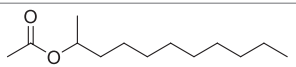
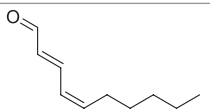
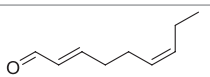
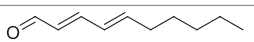
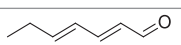
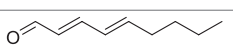
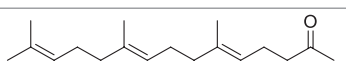
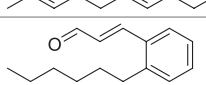
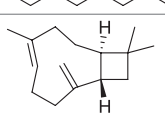
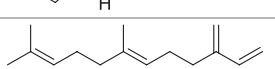
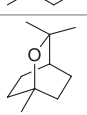
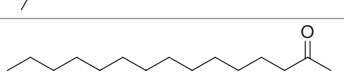
**Table 26.2** (continued)

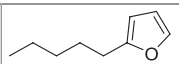
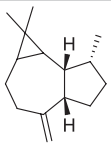
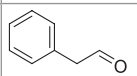
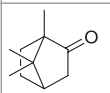
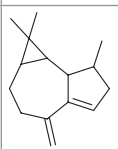
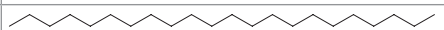
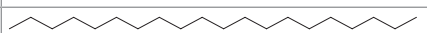
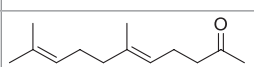
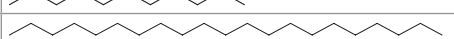
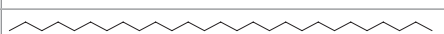
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 128 | Cichorioside C |  | *C. intybus*<br>*C. endivia* | Roots | [23] |
| 129 | Macrocliniside G |  | *C. endivia* | Roots | [4] |
| 130 | Sonchuside A |  | *C. intybus* | Roots<br>Leaves | [17] |
| | | | *C. endivia* | Roots | [4] |
| 131 | Sonchuside C |  | *C. intybus* | Roots<br>Leaves | [17] |

| | | | | | |
|---|---|---|---|---|---|
| 132 | *β*-sitosterol |  | *C. intybus* | Leaves<br>Floral stalks | [25] |
| | | | *C. intybus* | Roots | [26] |
| | | | *C. intybus* | Dried<br>Plant<br>Powder | [15] |
| 133 | Campesterol |  | *C. intybus* | Leaves<br>Floral stalk | [25] |
| | | | *C. intybus* | Dried<br>Plant<br>Powder | [15] |
| 134 | Stigmasterol |  | *C. intybus* | Leaves<br>Floral stalks | [25] |
| | | | *C. intybus* | Dried<br>Plant<br>Powder | [15] |
| 135 | Cholesterol |  | *C. intybus* | Dried<br>Plant<br>Powder | [15] |
| 136 | *n*-heneicosane |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 137 | (2*E*)-nonen-1-al |  | *C. intybus* | Aerial parts<br>Roots | [27] |

(continued)

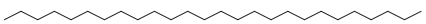
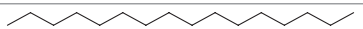
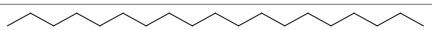
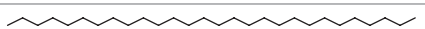
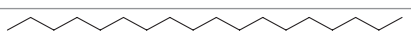
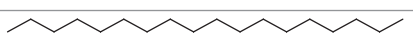
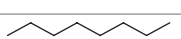
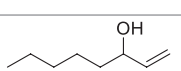
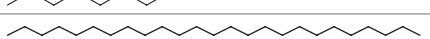
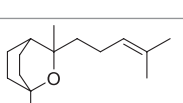
**Table 26.2** (continued)

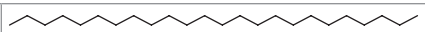
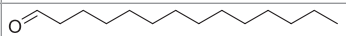
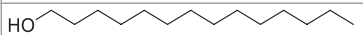
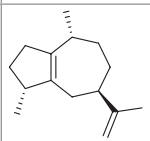
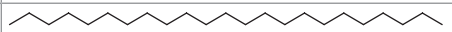
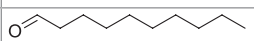
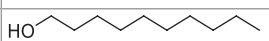
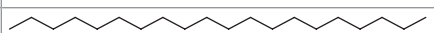
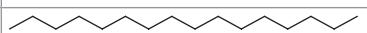
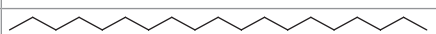
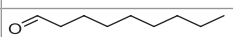
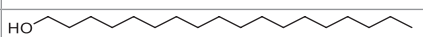
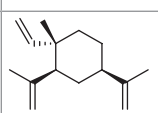
| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 138 | (2*E*)-tridecanol |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 139 | (2E)-undecanol acetate |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 140 | (2*E*, 4*Z*)-decadienal |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 141 | (2*E*, 6*Z*)-nonadienal |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 142 | (2*E*,4*E*)-decadienal |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 143 | (2*E*,4*E*)-heptadienal |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 144 | (2*E*,4*E*)-nonadienal |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 145 | (5*E*,9*E*)-farnesyl acetone |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 146 | (*E*)-2-hexylcinnamaldehyde |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 147 | (*E*)-caryophyllene |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 148 | (*E*)-*β*-farnesene |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 149 | 1,8-cineole |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 150 | 2-pentadecanone |  | *C. intybus* | Aerial parts<br>Roots | [27] |

| | | | | | |
|---|---|---|---|---|---|
| 151 | 2-pentyl furan |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 152 | Allo-aromadendrene |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 153 | Benzene acetaldehyde |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 154 | Camphor |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 155 | Dehydro-aromadendrene |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 156 | Docosane |  | *C. intybus* | Dried<br>Plant<br>Powder | [15] |
| 157 | Eicosane |  | *C. intybus* | Dried<br>Plant<br>Powder | [15] |
| 158 | Geranyl acetone |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 159 | Heneicosane |  | *C. intybus* | Dried<br>Plant<br>Powder | [15] |
| 160 | Heptacosane |  | *C. intybus* | Dried<br>Plant<br>Powder | [15] |

(continued)

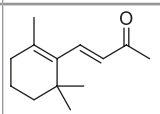
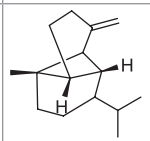
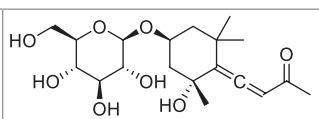
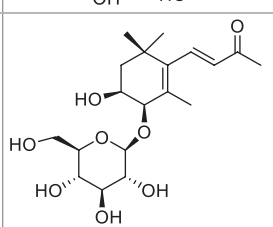
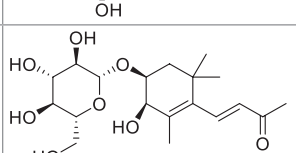
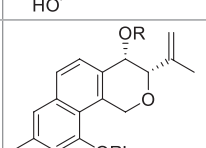
**Table 26.2** (continued)

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 161 | Heptadecane |  | *C. intybus* | Dried Plant Powder | [15] |
| 162 | Hexacosane |  | *C. intybus* | Dried Plant Powder | [15] |
| 163 | Hexadecane |  | *C. intybus* | Dried Plant Powder | [15] |
| 164 | Nonodecane |  | *C. intybus* | Dried Plant Powder | [15] |
| 165 | Octacosane |  | *C. intybus* | Dried Plant Powder | [15] |
| 166 | Octadecane |  | *C. intybus* | Aerial parts Roots | [27] |
| 167 | Octadecane |  | *C. intybus* | Dried Plant Powder | [15] |
| 168 | Octane |  | *C. intybus* | Aerial parts Roots | [27] |
| 169 | Octen-3-ol |   | *C. intybus* | Aerial parts Roots | [27] |
| 170 | Pentacosane |  | *C. intybus* | Dried Plant Powder | [15] |
| 171 | Pentadecane |  | *C. intybus* | Aerial parts Roots | [27] |
| 172 | Sesquicineole |   | *C. intybus* | Aerial parts Roots | [27] |

| | | | | | |
|---|---|---|---|---|---|
| 173 | Tetracosane |  | *C. intybus* | Dried Plant Powder | [15] |
| 174 | Tetradecanal |  | *C. intybus* | Aerial parts Roots | [27] |
| 175 | Tetradecanol |  | *C. intybus* | Aerial parts Roots | [27] |
| 176 | *trans*-*β*-guaiene |  | *C. intybus* | Aerial parts Roots | [27] |
| 177 | Tricosane |  | *C. intybus* | Dried Plant Powder | [15] |
| 178 | *n*-decanal |  | *C. intybus* | Aerial parts Roots | [27] |
| 179 | *n*-decanol |  | *C. intybus* | Aerial parts Roots | [27] |
| 180 | *n*-eicosane |  | *C. intybus* | Aerial parts Roots | [27] |
| 181 | *n*-hexadecane |  | *C. intybus* | Aerial parts Roots | [27] |
| 182 | *n*-nonadecane |  | *C. intybus* | Aerial parts Roots | [27] |
| 183 | *n*-nonanal |  | *C. intybus* | Aerial parts Roots | [27] |
| 184 | *n*-octadecanol |  | *C. intybus* | Aerial parts Roots | [27] |
| 185 | *n*-tridecane |  | *C. intybus* | Aerial parts Roots | [27] |
| 186 | *β*-elemene |  | *C. intybus* | Aerial parts Roots | [27] |

(continued)

**Table 26.2** (continued)

| No. | Name of compounds | Structures | Species | Plant parts | References |
|---|---|---|---|---|---|
| 187 | *β*-ionone |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| 188 | *β*-ylangene |  | *C. intybus* | Aerial parts<br>Roots | [27] |
| **Other compounds** | | | | | |
| 189 | Staphylionoside D |  | *C. calvum* | Aerial parts | [28] |
| 190 | Saussureoside B |  | *C. calvum* | Aerial parts | [28] |
| 191 | Komaroveside A |  | *C. calvum* | Aerial parts | [28] |
| | |  | | | |

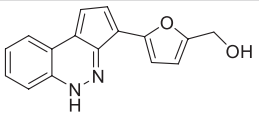
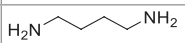
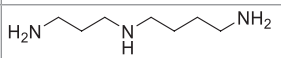
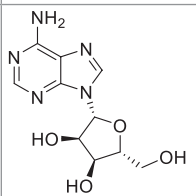
| | | | | | |
|---|---|---|---|---|---|
| 192 | Cichorins A: R = H, R′ = $CH_3$ | | *C. intybus* | Roots | [29] |
| 193 | Cichorins B: R = R′ = $CH_3$ | | *C. intybus* | Roots | [30] |
| 194 | Cichorins C: R = R′ = H | | *C. intybus* | Roots | [30] |
| 195 | 2-furanmethanol-(5′→11)-1,3-cyclopentadiene[5,4-c]-1H-cinnoline |  | *C. endivia* | | [13] |
| 196 | Putrescine |  | *C. intybus* | Leaves<br>Floral stalks | [25] |
| 197 | Spermidine |  | *C. intybus* | Leaves<br>Floral stalks | [25] |
| 198 | Adenosine |  | *C. endivia* | | [13] |

**Table 26.3** Some of the most important ethnobotanical uses of *Cichorium* species in different countries

| NO. | *Cichorium* species | Country | Vernacular name | Part used | Ethnobotanical uses | References |
|---|---|---|---|---|---|---|
| 1 | *C. endivia* | Italy | Radicciùn sarvègo, Radiccia | Leaves | Depurative of intestine and blood | [38] |
| | | Spain | Escarola | Leaves | Treatment of hoarseness | [39] |
| | | Italy | Shkarol | Leaves | Eaten raw in salads | [40] |
| | | India | Kaasani, Bustaani | Roots | Demulcent, febrifuge, diuretic, used in dyspepsia, as a tonic for liver and digestive system | [41] |
| 2 | *C. intybus* | Afghanistan | Kasni | Roots | Cholagogue, febrifuge, painkiller, treatment of kidney pain, sunstroke, typhoid, jaundice, severe headache and prevention of malaria | [42] |
| | | America | Chicory | Leaves | Treatment of sweats, menopausal symptoms, hot flashes, nervousness | [43] |
| | | Bulgaria | Sinja zlatchka | Roots, aerial parts | Cholagogue, stimulant for gastric secretion, hypoglycemic | [44] |
| | | Greece | Kihorio, Agrio radiki | Aerial parts | Spasmolytic, ↓cholesterol, antiseptic, treatment of liver disorders | [45] |
| | | India | Chicory | Whole plant | Diabetes, appetizer, cholagogue, depurative, digestive, diuretic, hypoglycemic, laxative, tonic, treatment of jaundice, liver enlargement, gout and rheumatism. | [33] |
| | | Iran | Kasni | Aerial parts | Depurative, antipyretic, treatment of boils, icterus and allergic dermatitis | [46] |
| | | Iran | Kasni | Whole plant | Febrifuge, blood cleanser and cooling, treatment of jaundice | [47] |
| | | Iran | Kasni | Aerial parts | Appetizer, depurative, febrifuge, anti-allergic, treatment of jaundice and palpitation | [48] |
| | | Iraq | Çeqçeqe | Roots, leaves | Treatment of constipation, blood cholesterol, anemia, colon problems, urinary system problems, prostate problems, skin sensitivity | [49] |
| | | Italy | Girasole | Leaves | Diuretic, treatment of hypertension | [50] |
| | | Italy | Cicoria, Scarra | Aerial parts | Diuretic, laxative, purgative | [51] |
| | | Italy | A cicoria catalogna | Leaves | Laxative | [52] |

(continued)

**Table 26.3** (continued)

| NO. | *Cichorium* species | Country | Vernacular name | Part used | Ethnobotanical uses | References |
|---|---|---|---|---|---|---|
| | | Jordan | Chicory | Roots | Strengthening stomach, diuretic | [53] |
| | | Morocco | El-eokif | Whole plant | Treatment of kidney problems, diabetes | [54] |
| | | Pakistan | Kasini | Whole plant | Tonic, diuretic, treatment of fevers and vomiting | [55] |
| | | Pakistan | Kasni | Whole plant | Treatment of asthma | [56] |
| | | Palestine | Hindiba | Aerial parts | Treatment of coughing and diarrhea | [57] |
| | | Spain | Achicoria, Salmero'n | Basal leaves | Used in stewed, salads, drinks (coffee substitute), eaten raw as a snack | [58] |
| | | Turkey | Hindiba, I˙ndibahar, I˙ndiba | Leaves | Treatment of kidney stones and abdominal pains | [59] |
| | | Turkey | Acı günek, Çiftlik otu | Leaves | Painkiller, treatment of stomach diseases | [60] |
| 3 | *C. pumilum* (= *C. glandulosum*) | Lebanon | Hendbe Barrie | Leaves | Treatment of rheumatism | [61] |
| | | China | – | Whole plant | Treatment of liver diseases | [13] |
| | | Palestine | – | Leaves | Treatment of diabetes, bacterial infections, poisoning and rheumatism | [62] |
| 4 | *C. spinosum* | Greece | Stamnagathi | Leaves | Consumed as leafy vegetables | [63] |

## 26.5 Nature of *Cichorium* spp. in ITM

Among different species of *Cichorium*, *C. intybus* is represented in ITM and is called "Hendbā Bostāni" or "Garden Kāsni". Avicenna has described Kasni as follow: *The garden Cichorium is of two varieties, one with big and long leaves which is called "Hendbā Shāmi", and one with smaller leaves and flowers and bitter taste which is called "Hendbā Bāqi*". The temperament of Kāsni is described as cold in the end of the first degree; the dried Kāsni is dry in the first degree and the fresh one is wet in the first degree. The garden Kāsni is more cold and wet [64, 65]. In all of the studied literature, *C. intybus* is introduced as a strong deobstruent plant with detergent (jālli) properties. It is also mentioned as an astringent (qābeḍ) and bitter agent [66]. Most of the medicinal activities of the plant can be attributed to these general effects. Several scientists believed that unwashed Kāsni is much more powerful than the washed one, since washing may diminish its deobstruent properties [65, 67, 68].

## 26.6 Medicinal Properties of *Cichorium* spp. in ITM

In ITM, a considerable attention has been paid to *Cichorium* especially toward its beneficial properties due to liver disorders. Frequently, *Cichorium* is described as a liver protective agent. It is suggested that *Cichorium* can treat obstructions of the liver and jaundice, and is a liver cleansing agent [66]. It is also used for dropsy

and biliary fevers [67]. For this purpose, the juice of unwashed *C. intybus* leaves are prescribed. Some scientists have recommended the mixture of *C. intybus* and *Foeniculum vulgare* juice as the best remedy for the treatment of obstructive jaundice [65, 68].

Regarding gastrointestinal problems, *Cichorium* has cleansing properties and can act as a gastric tonic. Wild *Cichorium* has been considered to be more effective in treatment of stomach disorders. Topical application of *Cichorium* on stomach has anti-inflammatory effects and can improve edema [66, 67, 70]. *Cichorium* is also prescribed for other warm swollen and edemas. Avicenna believed that the topical application of *C. intybus* juice together with vinegar and zinc oxide (Esfi d̲ āj) is very effective in cooling the hot parts of body (which have some sorts of inflammation) [64, 67, 70]. Moreover, *Cichorium* is administered for the treatment of gout, eye inflammation and cold fevers [64, 66, 71].

In ITM, *Cichorium* is also believed to be beneficial for cardiovascular system and can treat tachycardia, especially when used with barley flour in topical preparation [64, 70]. Rāzi, Ibn al-Baytār and some other ITM physicians have recommended *C. intybus* root for the treatment of scorpion bite and a good remedy for hemoptysis [64–67, 69, 70].

## 26.7 Pharmacological Aspects

So far, various pharmacological activities have been reported from *Cichorium* spp., including antioxidant, antibacterial, antiproliferative, antidiabetic, anticonvulsant, anti-hyperlipidemia, hepatoprotective, anti-cancer, anti-osteoporotic and gastroprotective. Some *in vivo* and *in vitro* studies regarding these activities are summarized in Tables 26.4 and 26.5.

## 26.8 Conclusion

*Cichorium* has a long history of use in traditional and folklore medicine. It has been used for the treatment of several disorders such as diabetes, rheumatism, respiratory and gastrointestinal disorders. The most remarkable use of *Cichorium* might be its application for the treatment of jaundice and liver disorders which is also highly recommended by Iranian traditional physicians. Pharmacological studies on *Cichorium* species not only substantiate the use of *Cichorium* in traditional medicine, but also presents some new functions for this species; for example: antioxidant, anti-cancer, anti-microbial and lipid modulatory activities.

The main chemical composition of *Cichorium* is reported to be sesquiterpene lactones derivatives and essential oil compounds. Although there is no specific study on the activity of these constituents, they seem to have a distinctive role in the pharmacological effects of *Cichorium*.

**Acknowledgements** This work was supported by Research Affairs of Mashhad University of Medical Sciences, Mashhad, Iran.

**Conflict of Interest** None.

**Table 26.4** *In vitro* studies of *Cichorium* spp.

| Species | Part used | Type of extraction/ phytochemical | Activity | Tested pathogen/cell/assay | Result(s) | References |
|---|---|---|---|---|---|---|
| *C. endivia* | Whole plant | Ethanol extract (95%) | Antioxidant | *tert*-butyl hydroperoxide-induced HepG2 cells oxidative stress injury | ↓intracellular ROS[1] production | [72] |
| *C. endivia* | – | Ethanol extract (95%) | Antioxidant | $H_2O_2$-induced $HepG_2$ cells oxidative stress injury | ↓ROS level in a concentration-dependent manner | [73] |
| *C. endivia* | – | Methanol extract | Cytotoxic | Human lymphoma cell line U-937 GTB | Potent activity at 10μg/ml | [74] |
| *C. glandulosum* | Seeds | Total flavonoids | Antioxidant | DPPH[2], ABTS[3], scavenging activity of hydroxyl radicals and superoxide anion assay, FRAP[4] assay | DPPH: $IC_{50}$ = 7.33 ± 0.093μg/ml, ABTS: $IC_{50}$ = 9.24 ± 0.100μg/ml, hydroxyl radicals: $IC_{50}$ = 154.33 ± 11.38μg/ml, superoxide anion: $IC_{50}$ = 256.7 ± 4.86μg/ml | [75] |
| | | | α-Glucosidase inhibitory | Inhibition assay for α- glucosidase activity | ↓α-glucosidase and α-amylase activities at 8–64 mg/ml range | |
| | | | α-Amylase inhibitory | Inhibition assay for α-amylase activity | | |
| *C. glandulosum* | Whole plant | Ethanol extract (70%) | Lipid modulatory | Free fatty acid- induced HepG2/ acetaminophen-induced L02 | ↓serum and cellular lipid, protecting the cells from inflammation and injury | [76] |
| *C. spinosum* | Leaves | Water decoction | Antioxidant | Normal human neonatal foreskin fibroblast strain | ↓intracellular ROS levels | [6] |
| *C. intybus* | Leaves | Hydro alcoholic extract | Antioxidant | DPPH | $IC_{50}$: 67.2 ± 2.6μg/ml | [77] |
| *C. intybus* | Seeds | Sodium phosphate citrate buffer and sodium acetate buffer/ proteins and peptides | Antibacterial | bacterial strains *Staphylococcus aureus* (ATCC 25923), Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* | Zone of inhibition: 10 mm (*P. aeruginosa)*, 11 mm (*E. coli*) and 10 mm (*P. vulgaris)* | [78] |

(continued)

**Table 26.4** (continued)

| Species | Part used | Type of extraction/ phytochemical | Activity | Tested pathogen/cell/assay | Result(s) | References |
|---|---|---|---|---|---|---|
| *C. intybus* | Roots | Ethanol extract (70%) | Mitogenic | Human peripheral blood lymphocytes and thymocytes | Inhibitory effect on lymphocyte proliferation (SI < 0.36) and stimulatory effect on the mixed lymphocyte reaction (SI range 1.24–1.70) | [79] |
| *C. intybus* | Seeds | Methanol extract/ various compounds | α-Glucosidase inhibitory | Inhibition assay for α- glucosidase activity | Glucosidase inhibition: Cichoridiol ($IC_{50}$ = 51.9μM) Acetylated cichoridiol ($IC_{50}$ = 10± 0.62μM) Vanillic acid ($IC_{50}$ = 69 ± 0.008μM) | [80] |
| *C. intybus* | Roots | Chicoric acid extract | Anti-hyperglycemic effect | Rat insulinoma-derived INS-1 β-cells/ L6 myocyte cells | Stimulating insulin release at 50μg/ mL, ↑glucose uptake | [81] |
| *C. intybus* | Leaves/ roots | Ethanol extract (70%) | Antiproliferative | Amelanotic melanoma C32 | Significant activity of leaves extract: 30.8% of inhibition at 100μg/ml | [82] |
| *C. intybus* | Leaves | Methanol extract (50%) | Antiproliferative | MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) | Antiproliferative activity against: MCF7 (78.07 ± 0.59%) A431 (73.38 ± 2.59%) | [83] |
| *C. intybus* | Whole leaves / only the red parts of the leaves | Methanol extract | Antioxidant/ antiproliferative | Human colon carcinoma (Caco-2) cells | Higher activity of the red parts of the leaves | [84] |
| *C. intybus* | Leaves | Hydro alcoholic extract | Antioxidant | DPPH | $IC_{50}$: 67.2 +/− 2.6μg/ml | [77] |
| *C. intybus* | Whole plant | Methanol extract (50%) | Antioxidant | DPPH, hydrogen peroxide-scavenging and iron-ion chelating tests | Exhibited antioxidative activities in all tests | [85] |
| *C. intybus* | Roots | Chicoric acid extract | Antidiabetic/ antioxidant | L6 rat myocyte cells/ DPPH | ↑glucose uptake, ↑oxidative stress ($H_2O_2$) survival test, antioxidant activity: $IC_{50}$ = 61.4 ± 1.75μg/ mL | [86] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. intybus* | Leaves | Sesquiterpene lactone-enriched extracts, lactucin, 8-deoxylactucin, and lactucopicrin | Anthelmintic | *Haemonchus contortus* egg | 8-deoxylactucin showed the highest activity | [87] |
| *C. intybus* | Whole plant | Methanol extract | Anti-mycobacterial/cytotoxic | *Mycobacterium bovis* BCG/ human colon carcinoma (DLD-1) cells | Activity against *M. bovis*: $IC_{50}$ > 500μg/ mL Cytotoxicity against DLD-1 cells: $IC_{50}$ > 100μg/ mL | [88] |
| *C. intybus* | Leaves, flowers | Ethanol extract | Antiproliferative | MCF7 | % survival ± standard deviation = 105.15 ± 5.74 | [89] |
| *C. intybus* | Roots/aerial Parts | Petroleum ether, chloroform, ethyl acetate and methanol extracts | Larvicidal | Larvae of *Anopheles stephensi*, *Aedes aegypti, Culex quinquefasciatus* | Highest larvicidal activity: Root methanol extract | [90] |
| *C. intybus* | Roots | Lactucin, Lactucopicrin | Antimalarial | HB3 clone of strain *Honduras-1* of *Plasmodium falciparum* | Antimalarial activity | [18] |
| *C. intybus* | Leaves | Water decoction | Antioxidant | Normal human neonatal foreskin fibroblast strain | ↓intracellular ROS levels | [6] |
| *C. intybus* | Leaves | Ethanol extract (70%) | Inhibition of fat digestion | Lipase activity determination | ↓pancreatic lipase activity | [91] |
| *C. intybus* | Leaves | Ethanol extract (70%) | Antioxidant | DPPH, bovine brain peroxidation assay, β-carotene bleaching test | DPPH: $IC_{50}$ = 26μg/ml, β-Carotene test: $IC_{50}$ = 50μg/ml, Peroxidation activity: $IC_{50}$ = 127μg/ml | [92] |
| | Roots | | | | DPPH: $IC_{50}$ = 76μg/ml, β-Carotene test: $IC_{50}$ = 23μg/ml, Peroxidation activity: $IC_{50}$ = 74μg/ml | |
| *C. intybus* | _ | Ethanol extract (96%) | Antimicrobial | Agar diffusion method | Antibacterial effect against *Pseudomonas aeruginosa*: MIC = 16μg/ml | [93] |
| *C. intybus* | Areal parts | Methanol extract | Anticancer | K562, Jurkat and Raji cell lines | $IC_{50}$ for Jurkat cell line = 138μg/ mL | [94] |
| *C. intybus* | Areal parts | Ethanol extracts (70% and 99%) | Antimicrobial | *Bacillus* sp. | Moderate activity | [95] |

[1]reactive oxygen species, [2]2, 2-diphenyl-1-picrylhydrazyl, [3]2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), [4]ferric-reducing antioxidant power

**Table 26.5** *In vivo* studies of *Cichorium* spp.

| Species | Part used | Extraction/constituents | Activity | Study design | Result(s) | References |
|---|---|---|---|---|---|---|
| *C. endivia* | Whole plant | Ethanol extract (95%) | Anti-hepatotoxic | ICR mice | Inhibition of the pathological changes of liver injury | [72] |
| *C. glandulosum* | Whole plant | Ethanol extract (70%) | Lipid modulatory | Male Sprague-Dawley rats | ↓serum and cellular lipid | [76] |
| *C. glandulosum* | Seeds | Water (WE), petroleum ether (PE), chloroform (CE), ethyl acetate (EE), and n-butanol (BE) extracts | Ultraviolet B protection | Ultraviolet B-induced liver damage in female Sprague–Dawley rat | Protection activity: EE (100 and 1000 mg/ml), BE (100 and 1000 mg/ ml), and WE (1000 mg/ml) | [96] |
| *C. glandulosum* | Aerial parts | Sesquiterpene-rich fraction (SRF) | Anti-hepatotoxic | $CCl_4$[1] induced hepatotoxicity in mice | ↓impact of $CCl_4$ toxicity<br>↓ AST[2], ALT[3], TBIL[4] | [97] |
| | | | | LPS[5]-induced Liver injury in mice | Restoration of enzymatic levels, ↓AST, ALT, TBIL | |
| *C. glandulosum* | Seeds | Total flavonoids | Antioxidant | $CCl_4$ induced hepatotoxicity In rats | ↓ MDA[6], restoration of superoxide dismutase and glutathione levels,↑catalase and glutathione peroxidase levels | [75] |
| *C.glandulosum* | Roots | Ethanol extract (95%) | Anti-hepatotoxic | $CCl_4$ and galactosamine-induced hepatotoxicity in mice | ↓impact of $CCl_4$ toxicity (10 mL/kg, i.p.), ↓serum aspartate aminotransferase, alanine aminotransferase and ALP[7] | [98] |
| *C.glandulosum* | Seeds | Total flavonoids (TFs), ethanol extract (70%) | Anti-hepatotoxic | $CCl_4$ induced liver damage in rats | ↓levels of AST, ALT, ALP, TBIL, and TBARs[8] substances, ↓pancreatic lipase: $IC_{50}$ = 1.318 ± 0.164 mg/ml. | [99] |
| *C.glandulosum* | Seeds | Ethanol extract (70%) | Anti-hepatotoxic | Cyclophosphamide-induced hepatotoxicity in mice | Amelioration of ALT, AST, GSH[9], T-SOD[10] and MDA | [100] |
| *C. intybus* | Leaves | Ethanol extract (80%) | Anti-hyperglycemic | Nicotinamide-streptozotocin–induced diabetic mice | Antidiabetic effect at dose of 5.369–0.183 μIU/mL | [101] |
| *C. intybus* | Seeds | Methanol extract | Anti-hepatotoxic | $CCl_4$ induced hepatotoxicity in albino rats | Potent anti-hepatotoxic activity comparable to silymarin | [34] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. intybus* | Seeds | Cichotyboside | Anti-hepatotoxic | $CCl_4$ induced hepatotoxicity in Wistar rats | ↑TBIL, ↓ALP, ↓SGOT[11], ↓SGPT[12] | [102] |
| *C. intybus* | Aerial parts | Ethanol extract (70%) | Anticonvulsant | Electrically and chemically induced seizure in albino rats | Protection against PTZ[13] -induced and maximal electroshock seizures | [103] |
| *C. intybus* | Roots | Methanol extract | Anti-hepatotoxic | $CCl_4$ induced hepatotoxicity in rats | Biochemical and cellular restoration of hepatotoxic alteration | [104] |
| *C. intybus* | Roots | Chicoric acid extract | Anti-hyperglycemic | Intraperitoneal glucose tolerance test on male Wistar rats | ↓hyperglycemia and ↑glucose tolerance at 15 and 30 mg/ kg | [81] |
| *C. intybus* | Roots | Chicoric acid extract | Antidiabetic | Streptozotocin-induced diabetic Wistar rats | ↓hyperglycemia ↓glycosuria | [86] |
| *C. intybus* | Seeds | Aqueous extract | Antidiabetic | Streptozotocin-induced diabetic Wistar rats | ↓body-weight loss ↓FBS[14],TG[15]and HbA1c[16] ↑NO[17] | [105] |
| *C. intybus* | Leaves | Aqueous extract | Anti-obesity/ anti-hyperlipidemia | Female Sprague-Dawley rats | ↑free T3 and T4, ↓ALT and AST, ↓body weight gain | [106] |
| *C. intybus* | Leaves | Powder | Neuroprotective/ antidiabetic | Alloxan induced diabetes in Wistar rats | ↓blood glucose level, ↓MDA, ↓GSH, ↑brain catalase activity, ↓lipid peroxidation, | [107] |
| *C. intybus* | Leaves | Ethanol extract (80%) | Anti-lipid peroxidase | $CCl_4$ induced hepatotoxicity in male Sprague–Dawley rats | ↓MDA | [108] |
| *C. intybus* | Leaves | Aqueous extract | Offspring sex ratio | Male and female Wistar rats | Male to female ratio 10.23%, ↑$Na^+$ and $K^+$ levels | [109] |
| *C. intybus* | Leaves | Powder | Anti-hepatotoxic | Hypercholesterolemia induced male albino SD rats | ↓ALT, AST and ALP, ↓LDL[18] and VLDL[19], ↑HDL[20], ↓total cholesterol, ↓TG | [110] |
| *C. intybus* | Roots | Inulin | Anti-colon cancer | (AOM[21] induced ACF[22] in rats | ↓AOM-induced ACF | [111] |
| *C. intybus* | Seeds | Aqueous extract | Antidiabetic | Streptozotocin-induced diabetic Wistar rats | ↓serum glucose, ↓TG | [112] |
| *C. intybus* | Leaves | Ethanol extract (70%) | Anti-inflammation | Croton oil-induced ear edema in male CD-1 mice | Edema reduction: 36% | [92] |
| | Roots | | | | Edema reduction: 22% | |

(continued)

**Table 26.5** (continued)

| Species | Part used | Extraction/constituents | Activity | Study design | Result(s) | References |
|---|---|---|---|---|---|---|
| *C. intybus* | Roots | Ethanol extract (80%) | Tumor inhibitory | EAC[23] in Swiss mice | Inhibition of EAC tumor at 300–700 mg/kg/day | [113] |
| *C. intybus* | Leaves | Aqueous extract | Anti-osteoporotic | Glucocorticoid-induced osteoporosis in female albino rats | Osteoporosis prevention | [114] |
| *C. intybus* | Seeds | Ethanol extract (75%) | Metabolic syndrome disorders inhibition | Diet-induced metabolic disturbances in male Wistar rats | Improve glycaemia, ↓serum atherogenicity, ↑blood antioxidant status | [115] |
| *C. intybus* | Whole plant | Powder | Anti-hyperlipidemia | Triton WR-1339-induced hyperlipidemia in male albino rats | Improve lipid profile | [116] |
| *C. intybus* | Seeds | Ethanol extract (95%), fractions of hexane, chloroform, and *n*-butanol | Postcoital contraceptive | Female Sprague-Dawley rats | ↓number of implantations | [117] |
| *C. intybus* | Seeds | Methanol extract | Antioxidant/ anti-hepatotoxic | $CCl_4$ induced liver toxicity in albino Wistar rats | ↑glutothione peroxidase,↑superoxide dismutase, ↓glutathione, ↑ SGOT, ↑ SGPT, ↑ ALKP, ↑direct bilirubin | [118] |
| *C. intybus* | Roots | – | Gastroprotective | H. Shay's model of experimental ulcer in rats | ↓gastric secretion, ↑defense barrier function of the gastric mucosa | [119] |
| *C. intybus* | Whole plant | Ethanol extract | Anti-hepatotoxic | $CCl_4$ induced liver fibrosis in rats | ↓ALT, ↓AST, ↓MDA, ↑antioxidant enzymes | [120] |
| *C. intybus* | Esculletin | – | Antidepressant/ anti- inflammation | LPS-induced neuroinflammatory processes and depressive-like behavior in mice | ↓immobility time in TST[24] and FST[25], ↓ pro-inflammatory cytokines, inhibition of nuclear factor-κB (NF-κB) pathway in hippocampus,upregulations of brain derived neurotrophic factor and phosphorylated tyrosine kinase B protein expression in hippocampus. | [121] |
| *C. intybus* | Roots /root callus | Aqueous extract | Anti-hepatotoxic | $CCl_4$ induced hepatotoxicity in Wistar rats | More hepatoprotection for root callus extract than natural root extract | [122] |

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|---|---|---|---|---|---|---|
| *C.intybus* | Leaves and roots | Lactucin, lactucopicrin,11β,13-dihydrolactucin | Analgesic/ sedative | Hot plate test in albino Swiss mice<br>Tail-flick test in albino Swiss mice<br>Locomotor activity test | Analgesic effects at doses of 15 and 30 mg/kg in the hot plate test.<br>The most potent analgesic: Lactucopicrin<br>Sedative properties in the spontaneous locomotor activity test: Lactucin and lactucopicrin, | [123] |
| *C. intybus* | Fresh flowering aerial parts | Ethanol extract | Acute oral toxicity | Both sexes of adult albino mice | ↓ acute oral toxicity symptoms (LD50) > 5000 mg/kg) | [124] |
| | | | Male fertility | Male Wistar rats | ↓weight of testes, epididymis, seminal vesicle and ventral prostate, ↓motility, count and viability of sperms, ↓serum levels of testosterone, FSH[26]and LH[27] with hyperprolactinemia, ↓testicular and epididymal function | |
| *C. intybus* | Whole plant | Ethanol extract | Anti-hepatotoxic | $CCl_4$ induced liver damage in rat | ↓raised serum liver enzyme levels, less fatty changes and marked regenerative activity in the livers possesses significant antihepatotoxic | [125] |
| *C. intybus* | Aerial parts, leaves, roots | Methanol extract, *n*-hexane, dichloromethane, ethyl acetate and n-butanol fractions | Wound healing | Wound models in rats | Potent wound healing activity of methanol extract and dichloromethane fraction | [126] |
| *C. intybus* | Roots | Ethanol extract | Anti-hepatotoxic | $CCl_4$ induced hepatotoxicity in rats | Significant hepatoprotective activity in comparison to silymarin. ↓ALT, AST and bilirubin ↑SOD and GSH, LPO[28] | [127] |
| *C. intybus* | – | Ethanol extract | Antioxidant | Streptozotocin-induced diabetic rats | ↓blood glucose, TG, total cholesterol, LDL-cholesterol, ↑ HDL-cholesterol. ↑body weight, ↑reduced GSH, SOD, glutathione-S-transferase and CAT[29], ↓MDA | [128] |

(continued)

**Table 26.5** (continued)

| Species | Part used | Extraction/constituents | Activity | Study design | Result(s) | References |
|---|---|---|---|---|---|---|
| *C. intybus* | Fruits | – | Anti-hepatotoxic | 4-*tert*-octylphenol induced liver injury and oxidative stress in male rats | ↓ liver TBARS and bilirubin, AST, ALT, ALP and GGTP[30] activities, ↑GSH, ↑SOD, ↑CAT | [129] |
| *C. intybus* | – | Inulin | Whole-body bone mineral density increase | WBBMC[31], WBBA[32] and WBBMD[33] in rat | ↑WBBMC ($P < 0.05$) and WBBMD ($P < 0.001$) without any changes on WBBA, ↑calcium absorption, ↑ mineral parameters in whole-body bones (dose –dependently) | [130] |
| *C. intybus* | Seeds | Aqueous extract | Anti-inflammation | Induced early stage and late stage diabetes in rats | Preventing and delaying diabetes onset, ↓mRNA, protein expression levels of IKKβ[34], and P65 genes, ↓ DNA-binding capacity of NF-κB[35] | [131] |
| *C. intybus* | – | Crude extract, solvent soluble fractions | Anti-microbial | *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus epidermidis,* methicillin-resistant *Staphylococcus aureus, Bacillus subtilis* | Potential activities against *P. aeruginosa*, *B. subtilis*, *S. aureus* and *S. epidermidis* | [132] |
| | | | Antifungal | *Aspergillus flavus, Fusarium solani, Aspergillus fumigatus, Aspergillus Niger* | Aqueous and ethyl acetate fractions: Promising activity against *F. solani* and *A. niger* chloroform fraction: Highly active against *F. solani* | |
| *C. intybus* | Seeds | Aqueous, chloroform, ethanol, and hexane extracts | Antibacterial | *P. aeruginosa, S. aureus, E. coli*, *Acinetobacter. baumannii* | Highest antibacterial activity: Aqueous and ethanol extracts | [133] |
| *C. intybus* | – | Ethanol extract | Anti-hepatotoxic | Thioacethamide-induced hepatotoxicity in rats | ↓activity of aminotransferases and ALP, ↓bilirubin | [134] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *C. intybus* | Roots, aerial parts | Ethanol extract | Anti-pancreatitis | Cerulein-induced acute pancreatitis in mice | ↓amylase and lipase, ↓inflammatory features like edema and leukocyte infiltration in pancreatitis tissue | [135] |
| *C. pumilum* | Flowers/aerial parts | Ethanol extract (50%) | Anti-cancer | Drug-induced benign breast tumor in female Sprague-Dawley rats | ↓lobular hyperplasia, ↓fibro adenoma, ↓number of estrogen receptor -positive cells | [136] |

Abbreviations
[1]carbon tetrachloride, [2]aspartate transaminase, [3]alanine transaminase, [4]total bilirubin, [5]lipopolysaccharide, [6]malondialdehyde, [7]alkaline phosphatase, [8]thiobarbituric acid reactive, [9]glutathione, [10]total superoxide dismutase, [11]serum glutamic-oxaloacetic transaminase, [12]serum glutamic-pyruvic transaminase, [13]pentylenetetrazole, [14]fasting blood sugar, [15]triglycerides, [16]hemoglobin A1c, [17]nitric oxide, [18]low-density lipoprotein, [19]very-low-density lipoprotein, [20]high-density lipoprotein, [21]azoxymethane, [22]aberrant crypt foci, [23] Ehrlich ascites carcinoma, [24] tail suspension test, [25] forced swimming test, [26] follicle stimulating hormone, [27] luteinizing hormone, [28] lipid peroxidase, [29] catalase, [30] gamma-glutamyl transpeptidase, [31] whole-body bone mineral content, [32] whole-body bone area, [33] whole-body bone mineral density, [34] inhibitor of nuclear factor kappa-B kinase subunit beta, [35] nuclear factor kappa-light-chain-enhancer of activated B cells

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# 27

# Medicinal Species of the Genus *Berberis*: A Review of Their Traditional and Ethnomedicinal Uses, Phytochemistry and Pharmacology

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**Abstract**

Discovering new drugs for treating different health problems is one of the basic needs of human societies. There are various strategies to find new lead compounds. One of the most important strategies in this regard is using the knowledge in traditional and folk medicines as a valuable guide. Islamic traditional medicine (ITM) is a well reputed school of medicine with a long history. In the textbooks of this medical system, the properties and applications of many medicinal plants have been described. As a part of an ongoing project on plants used in ITM, in this study we investigated botany, traditional uses, phytochemistry, and pharmacology of *Berberis* spp. The great genus *Berberis* (Berberidaceae) consists of 594 species worldwide which have been used in different traditional medicines for a wide range of diseases. In ITM reference books such as Al-Hâwi fi'aṭ-Ṭibbe (Comprehensive Book of Medicine), Kâmel al-Sinâh aṭ-Ṭibbiyah (Complete Book of the Medical

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Art), Al-Qânun fi' aṭ-Ṭibbe (Canon of Medicine), Ḏakhireh Khârazmshâhi (Treasure of Khârazmshâh), and Makhzan al-Adwyah (Drug Treasure), different parts of *B. vulgaris* and *B. integrimma*, known as Zereshk, have been mainly prescribed for skin, liver, stomach, kidney, and eye problems. There are several pharmacological studies reporting beneficial effects of various *Berberis* plants confirming traditional uses. Most of the activities reported for *Berberis* is attributed to the presence of an important class of alkaloids namely isoquinolines. Nevertheless, clinical studies are necessary to confirm the therapeutic effects of different species of this genus.

**Keywords**

Traditional medicine · *Berberis* spp. · Berberidaceae · Drug discovery · Berberine · Barberry

## 27.1 Introduction

Natural products have played important roles to comply essential needs of human beings including health problems since ancient times. These needs led to discovering the properties of various natural resources particularly plants. Gradually, the findings increased and different traditional medical systems were founded. There have been several traditional schools of medicine in the world, of which traditional Chinese medicine, Ayurveda, Kampo, and Islamic traditional medicine (ITM) are some of the most well-known ones.

As individual plants and preparations have been used for many decades in different traditional system of medicine, these schools could be an invaluable guide to discover new drugs. In ITM textbooks written by famous scientists like ar-Rhâzi (Rhazes) and Ibn- Sinâ (Avicenna), the qualities of many individual herbs such as their nature, natural habitat, energy pattern, effects, indications, duration of action, relationships to the body organs, toxicity and contraindications, dosage, types of preparations, and antidotes have been described.

*Berberis* spp. belonging to Berberidaceae family consists of 594 species worldwide. *Berberis* spp. have been used since ancient times to treat various diseases in traditional and folk medicines. However, among these species, *B. vulgaris* stands out the rest as a remedy and food additive. Almost all parts of these plants including fruit, root, bark and leaf have medicinal and nutritional uses [1]. *Berberis vulgaris* and *B. integrimma* are called Zereshk in ITM. In Iran, Zereshk, in addition to its therapeutic properties, is a food additive and used as juice [2].

The principal objective of this chapter was to investigate botany, traditional and ethno medicinal uses, phytochemistry and pharmacological activities of *Berberis* spp.

## 27.2 Botanical Profile and Taxonomy

*Berberis* L. (Berberidaceae family), is the largest woody plant genus of the basal eudicots in the flowering plants [3]. Plants belonging to this genus are distributed all over the world mainly in India, Pakistan, Japan, China, Central and West Asia, South-East Asia, Europe, East Africa, South America and North America [4]. The taxonomy of Berberis is still somewhat uncertain due to variable characters in its species. The occurrence of hybridization and some degree of introgression in transitional zones has produced intermediate forms that cause complexity in Berberis taxonomy [5]. Some taxonomists have reported different numbers of species in this genus. However, based on the website 'TPL' (http://www.theplantlist.org.), there are 1373 scientific plant names of species rank for the genus *Berberis*, of these 594 are accepted species/infraspecific taxa names.

*Berberis* taxa are shrubs, often evergreen and spiny, gregarious or sporadic, usually with yellow woods [6]. The stem or branches are red-brown to pale or whitish. The leaves are on long shoots usually modified into 1–3(−7)-partite spines. The inflorescence is short, on lateral branches, racemiform, umbellate, fascicled or panicled. The flowers of these plants are yellow to orange, 3-merous pedicellate. The perianth

segments are usually in three whorls subequal to unequal; outer two whorls forming the sepals; inner whorl forming the petals, each beset with two basal glands. Stamens (4-) 6, usually shorter than petals. The ovary is 1-carpellate, oblong to ellipsoid, 1-locular, (1-) 2–6(−15, very rarely more) - ovuled on basal placenta; ovules anatropous; stigma is usually broad or peltate, sessile or subsessile, sometimes on distinct style. Berry is ellipsoid, subglobose, ovoid, obovoid to oblong, usually red or bluish-black. The seeds are mostly oblong-ellipsoid, copiously albuminous with straight embryo [7].

## 27.3 Phytochemistry

Several investigations have been carried out to identify phytochemical constituents and bioactive components from different species of *Berberis* genus. Studies show that alkaloids such as bisbenzylisoquinolines, benzylisoquinolines, isoquinolines and dimeric isoquinolines are one of the main phytochemical class found in these plants. However, many other compounds belonging to various classes of secondary metabolites such as phenolic and organic acids, flavonoids, phthalates, terpenes including mono-, sesqui-, di-, and tri-terpenes, phenyl propanoids, steroids, and fatty acids are reported from *Berberis* spp. (Table 27.1).

## 27.4 Ethnobotanical and Ethnomedicinal Uses

Several *Berberis* species have been used in folk medicine since time immemorial to remedy a broad spectrum of human and animal diseases. The different parts of *Berberis* taxa including its fruit, leaf, bark and root have been used as traditional medicine for a long time in various countries. Among all species of this genus, 26 have been reported to be mainly used according to ethnobotanical and traditional data (Table 27.2).

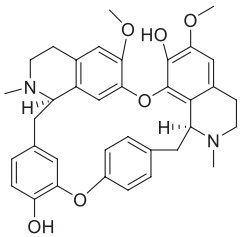
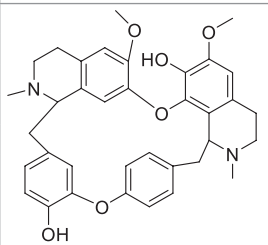
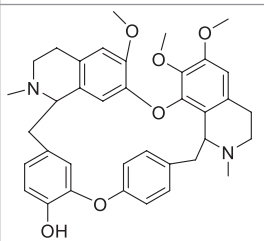
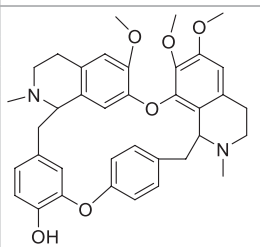
A literature survey shows that *B. vulgaris*, as the most popular species of *Berberis* genus, has remarkable traditional medicinal properties to treat a wide range of illnesses. It is a well-known plant in Asian and European systems of traditional medicine. For instance, in Bulgaria, *B. vulgaris* has been used to treat a variety of diseases such as cholecystitis, indigestion, diarrhea, and dysentery [47]. In Hungary, *B. vulgaris* has been used as a snack and a substitute for vinegar [48]. In Afghanistan, the fruits of this plant have been prescribed for the treatment of liver diseases, indigestion, and traumatic pain [49]. In Iran, *B. vulgaris*, known as "Zereshk" have been widely cultivated in the South-Khorasan province of Iran [50]. In this country, *B. vulgaris* has a long reputation and its fruits have been commonly used as a food additive or as juice [2]. Besides, it has been used as depurative, antipyretic, anthelmintic, anti-gout and anti-dysentery agents [51]. Although, *B. vulgaris* is the most common and popular species of *Berberis* genus, there are several other species with beneficial properties (Table 27.2).

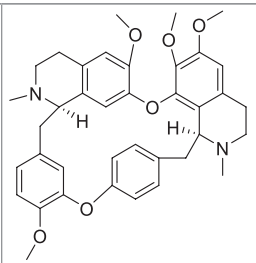
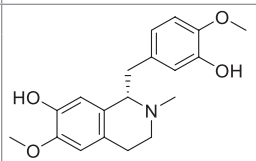
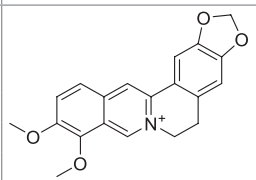
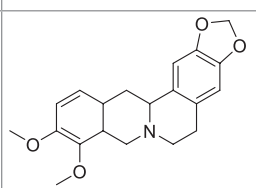
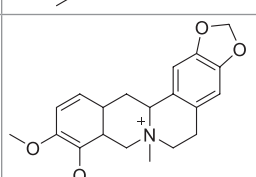
## 27.5 Medicinal Uses of Zereshk in Islamic Traditional Medicine

Aqili Khorasani has described Zereshk as: "It is a thorn tree growing mostly in mountains and hillsides. There are two kinds of Zereshk, one has fruits with seeds inside and the other is seedless. Seedless Zereshk is the best type and widely used as medicine and food" [76]. Avicenna has also mentioned that there are two kinds of Zereshk. He said: "one kind of Zereshk that grows in plains is round and red but the other one that grows in mountainous regions is dark and rectangular" [77]. In all studied sources (Table 27.3), the temperament (mizaj) of Zereshk is described as cold and dry. In most of the textbooks, Zereshk has been used as a laxative of bile, astringent (qabez), restraint (rade), incisor and anti-dote agent [76–78].

The hepato-protective and anti-inflammatory properties of *Berberis* spp. is mentioned in almost all traditional textbooks. In Makhzan al-Adwyah, the oral administration of barberry with aqueous

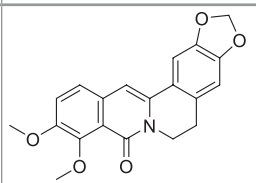
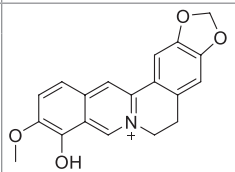
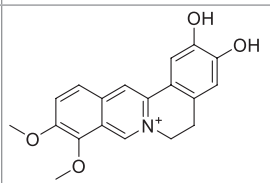
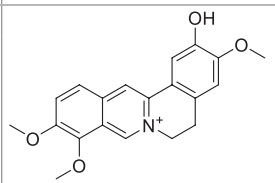
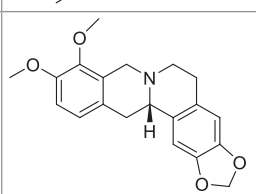
**Table 27.1** Chemical structures of some phytochemicals reported from *Berberis* species

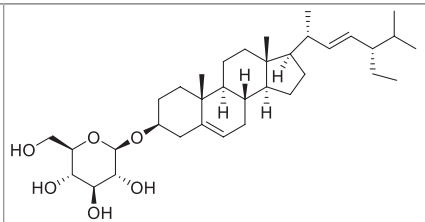
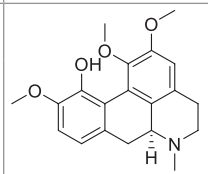
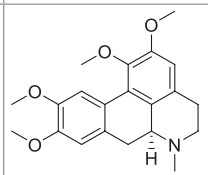
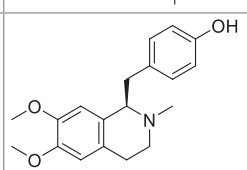
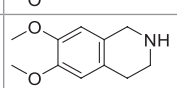
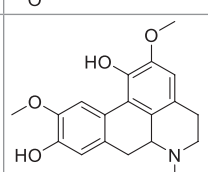
| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| Alkaloids | | | | | |
| 1 | Aromoline |  | *B. vulgaris* | Root | [8–12] |
| | | | *B. aristata* | Bark | [11] |
| 2 | Berbamine |  | *B. vulgaris* | Root, bark | [13] |
| | | | *B. tinctoria* | Bark | [14] |
| | | | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. aristata* | Bark | [11] |
| | | | *B. asiatica* | Leaf, root | [16] |
| | | | *B. jaeschkeana* | – | [17] |
| | | | *B. lycium* | Root | [16, 18] |
| 3 | Baluchitine |  | *B. lycium* | Root | [19] |
| 4 | Oxyacanthine |  | *B. vulgaris* | Stem, bark | [20] |
| | | | *B. aristata* | Bark | [11] |
| | | | *B. asiatica* | Leaf, Root | [16] |
| | | | *B. baluchistanica* | Root | [20] |
| | | | *B. integerrima* | Leaf | [21] |

| | | | | | |
|---|---|---|---|---|---|
| 5 | Obaberine |  | *B. baluchistanica* | Root | [20] |
| 6 | Reticuline |  | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. integerrima* | Leaf | [21] |
| 7 | Berberine |  | *B. vulgaris* | Bark, root, fruit | [10, 13, 22, 23] |
| | | | *B. tinctoria* | Bark | [14] |
| | | | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. aristata* | Bark | [11, 24] |
| | | | *B. asiatica* | Leaf, root | [16] |
| | | | *B. lycium* | Root | [16, 18] |
| | | | *B. umbellata* | Root | [25] |
| | | | *B. jaeschkeana* | – | [17] |
| 8 | Tetrahydroberberine |  | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| 9 | *N*-methyl tetrahydroberberine |  | *B. petiolaris* | Fruit, leaf, root, stem | [15] |

(continued)

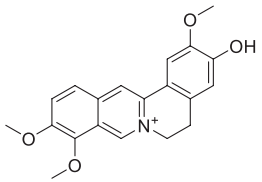
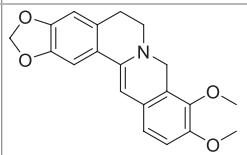
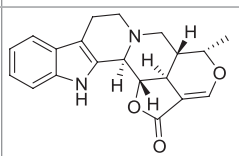
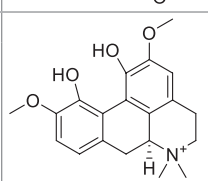
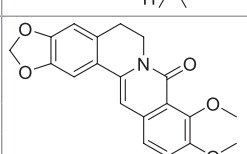
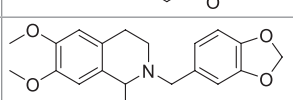
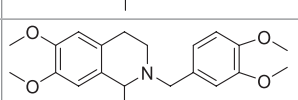
**Table 27.1** (continued)

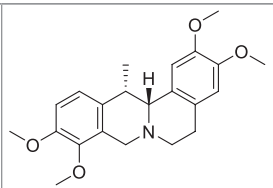
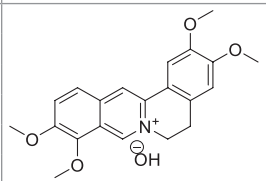
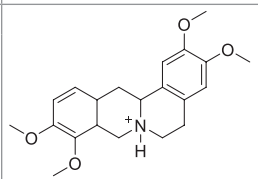
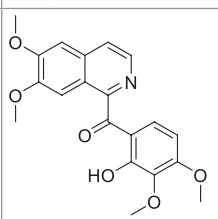
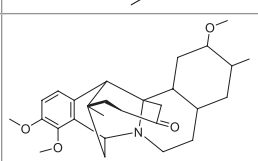
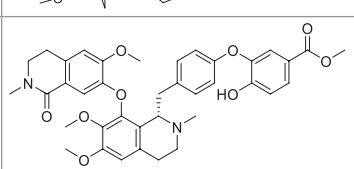
| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| 10 | Berlambine |  | *B. vulgaris* | Root, stem bark | [10, 26] |
| 11 | Berberrubine (thalifendine) |  | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. vulgaris* | Stem, bark | [26] |
| 12 | Demethyleneberberine |  | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| 13 | Columbamine |  | *B. vulgaris* | Root, bark | [10, 27] |
| | | | *B. aristata* | Root | [24, 28] |
| 14 | Canadine |  | *B. tinctoria* | Bark | [14] |

| | | | | | |
|---|---|---|---|---|---|
| 15 | Hydroxycanthine |  | *B. vulgaris* | Root, stem, bark | [10, 29] |
| 16 | Isocorydine |  | *B. vulgaris* | Root, stem, bark | [10, 27, 30] |
| | | | *B. integerrima* | Leaf | [21] |
| 17 | Glaucine |  | *B. integerrima* | Leaf | [21] |
| 18 | Armepavine |  | *B. integerrima* | Leaf | [21] |
| 19 | Heliamine |  | *B. integerrima* | Leaf | [21] |
| 20 | Isoboldine |  | *B. integerrima* | Leaf | [21] |

(continued)

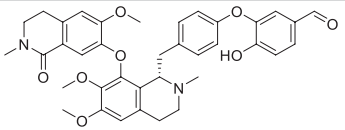
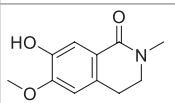
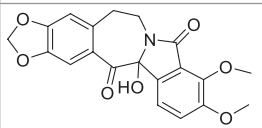
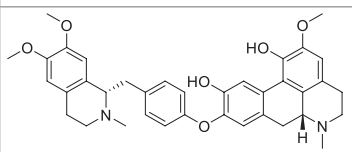
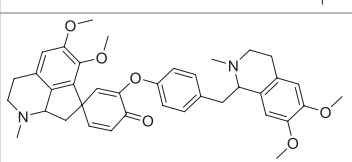
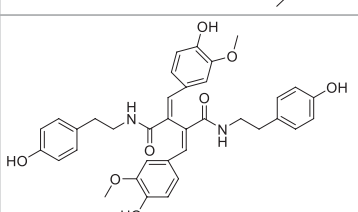
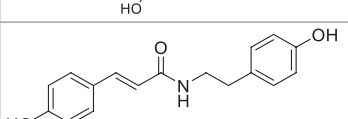
**Table 27.1** (continued)

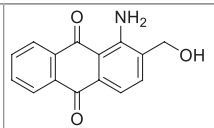
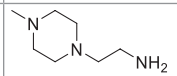
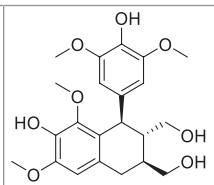
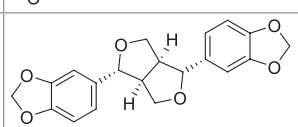
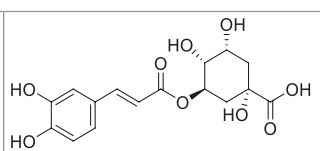
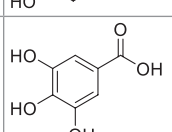
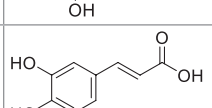
| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| 21 | Jatrorrhizine |  | *B. vulgaris* | Root, bark | [13] |
| | | | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. aristata* | Bark | [11, 24] |
| | | | *B. asiatica* | Leaf, root | [16] |
| | | | *B. umbellata* | Root | [25] |
| 22 | Lambertine |  | *B. vulgaris* | Root, bark | [27, 31] |
| 23 | Magniflorine |  | *B. vulgaris* | Root, bark | [27, 32] |
| 24 | Magnoflorine |  | *B. vulgaris* | Root | [33–35] |
| | | | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| 25 | Oxyberberine (8-oxoberberine) |  | *B. vulgaris* | Root, bark | [10, 13, 27] |
| | | | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. aristata* | Bark | [11, 24] |
| | | | *B. asiatica* | Leaf, root | [16] |
| 26 | Intebrinine |  | *B. integerrima* | Leaf | [21] |
| 27 | Intebrimine |  | *B. integerrima* | Leaf | [21] |

| | | | | | |
|---|---|---|---|---|---|
| 28 | Corydaline |  | *B. baluchistanica* | Root | [20] |
| 29 | Palmatine |  | *B. vulgaris* | Root, bark | [13] |
| | | | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. aristata* | Bark | [11, 24] |
| | | | *B. asiatica* | Leaf, root | [16] |
| | | | *B. lycium* | Root | [16, 18] |
| | | | *B. jaeschkeana* | – | [17] |
| 30 | Tetrahydropalmatine |  | *B. petiolaris* | Fruit, leaf, root, stem | [15] |
| | | | *B. asiatica* | Bark | [11, 24] |
| 31 | Taxilamine |  | *B. aristata* | Root | [12] |
| 32 | Karachine |  | *B. aristata* | Root | [12] |
| 33 | (−)-Tejedine |  | *B. vulgaris* | Root, stem, bark | [10, 36] |

(continued)

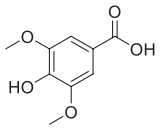
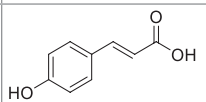
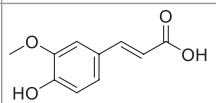
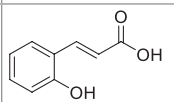
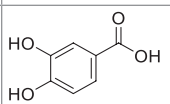
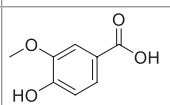
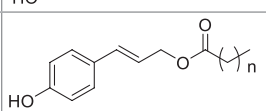
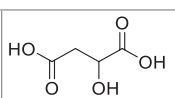
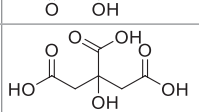
**Table 27.1** (continued)

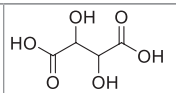
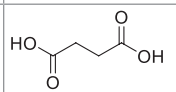
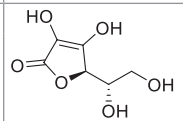
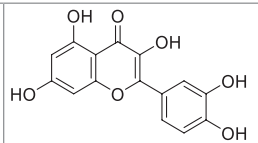
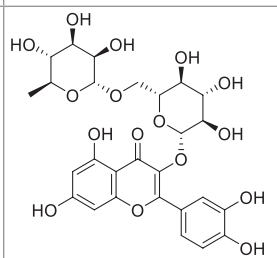
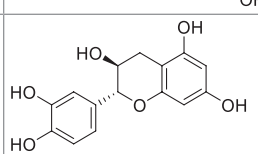
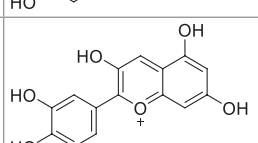
| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| 34 | Baluchistanamine |  | *B. lycium* | Root | [19] |
| | | | *B. baluchistanica* | Root | [20] |
| | | | *B. vulgaris* | Root, bark | [13] |
| 35 | Thalifoline |  | *B. vulgaris* | Root, bark | [13] |
| 36 | Chilenine |  | *B. vulgaris* | Root, bark | [13] |
| 37 | Pakistanine |  | *B. baluchistanic*a | Root | [37] |
| 38 | Pakistanamine |  | *B. baluchistanic*a | Root | [37] |
| 39 | Cannabisin G |  | *B. vulgaris* | Root, bark | [27] |
| 40 | *N*-(*p*-*trans*-Coumaroyl)tyramine |  | *B. vulgaris* | Root, bark | [27] |

| | | | | | |
|---|---|---|---|---|---|
| 41 | 1-Amino-2-(hydroxymethyl) anthraquinone |  | *B. vulgaris* | Wood, bark | [38, 39] |
| 42 | 1-Piperazineethanamine, 4-methyl- |  | *B. tinctoria* | Bark | [14] |
| 43 | Pyridine |  | *B. tinctoria* | Bark | [14] |
| Lignans | | | | | |
| 44 | (±)–Lyoniresinol |  | *B. vulgaris* | Root, bark | [27] |
| 45 | Sesamin |  | *B. orthobotrys* | Leaf | [40] |
| Phenolic acid derivatives | | | | | |
| 46 | Chlorogenic acid |  | *B. vulgaris* | Fruit, aerial parts | [22, 41] |
| 47 | Gallic acid |  | *B. vulgaris* | Fruit | [41] |
| 48 | Caffeic acid |  | *B. vulgaris* | Fruit | [41] |

(continued)

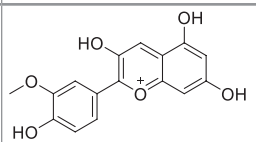
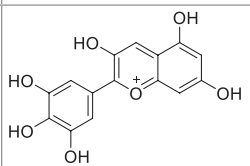
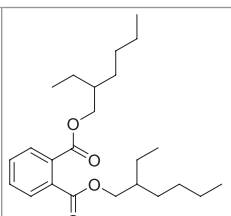
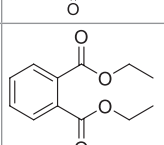
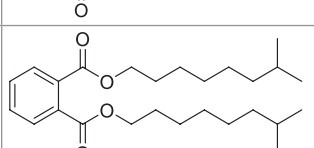
**Table 27.1** (continued)

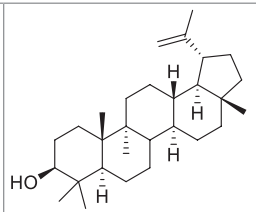
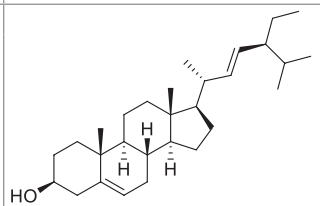
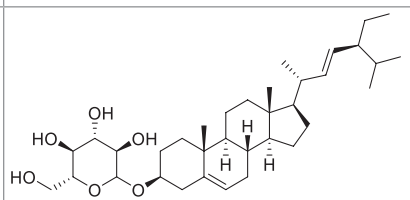
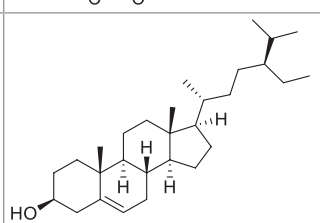
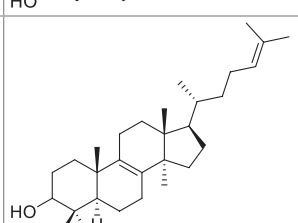
| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| 49 | Syringic acid |  | *B. vulgaris* | Fruit | [41] |
| 50 | *ρ*-Coumaric acid |  | *B. vulgaris* | Fruit | [41] |
| 51 | Ferulic acid |  | *B. vulgaris* | Fruit | [41] |
| 52 | *o*-Coumaric acid |  | *B. vulgaris* | Fruit | [41] |
| 53 | Protocatechuic acid |  | *B. vulgaris* | Fruit | [41] |
| 54 | Vanillic acid |  | *B. vulgaris* | Fruit | [41] |
| 55 | (2Z)-3-(4-Hydroxyphenyl)-2-propen-1-yl esters |  | *B. vulgaris* | Fruit | [32] |
| Organic acids | | | | | |
| 56 | Malic acid |  | *B. vulgaris* | Fruit | [41] |
| 57 | Citric acid |  | *B. vulgaris* | Fruit | [41] |

| | | | | | |
|---|---|---|---|---|---|
| 58 | Tartaric acid |  | *B. vulgaris* | Fruit | [41] |
| 59 | Succinic acid |  | *B. vulgaris* | Fruit | [41] |
| 60 | Ascorbic acid |  | *B. vulgaris*<br>*B. integerrima* | Fruit | [42] |
| Flavonoids (free and glycosylated) | | | | | |
| 61 | Quercetin |  | *B. vulgaris* | Aerial parts, root, stem, bark | [10, 22, 41, 43] |
| 62 | Rutin |  | *B. vulgaris* | Aerial parts, root, stem, bark | [10, 22, 26, 41] |
| 63 | Catechin |  | *B. vulgaris* | Fruit | [41] |
| 64 | Cyanidin |  | *B. vulgaris* | Fruit | [42] |

(continued)

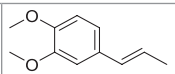
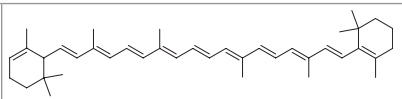
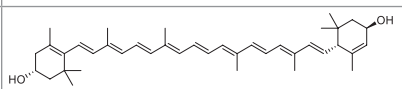
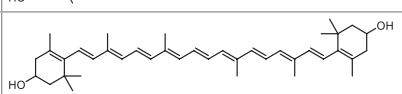
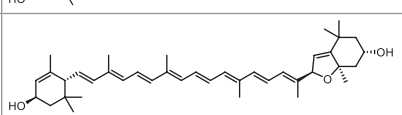
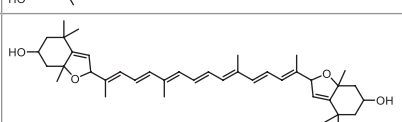
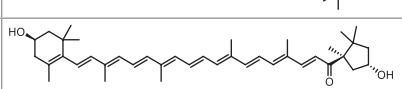
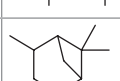
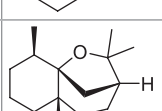
**Table 27.1** (continued)

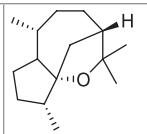
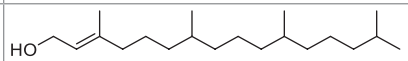
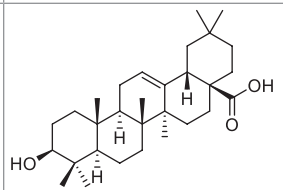
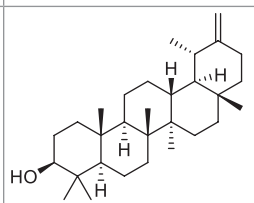
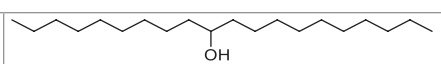
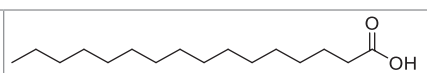
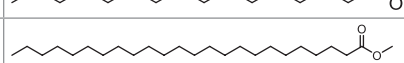
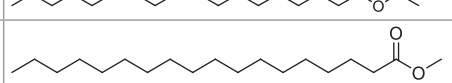
| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| 65 | Peonidin |  | *B. vulgaris* | Fruit | [42] |
| 66 | Delphinidin |  | *B. vulgaris* | Fruit | [42] |
| Phthalates | | | | | |
| 67 | Di-(2-ethylhexyl)phthalate |  | *B. vulgaris* | Wood bark | [38] |
| 68 | Diethyl phthalate |  | *B. tinctoria* | Bark | [14] |
| 69 | 1,2-Benzenedicarboxylic acid, diisononyl ester |  | *B. vulgaris* | Wood bark | [38] |

| | | | | | |
|---|---|---|---|---|---|
| Steroids | | | | | |
| 70 | Lupeol |  | *B. vulgaris* | Fruit | [23] |
| 71 | Stigmasterol |  | *B. vulgaris* | Fruit | [23] |
| | | | *B. tinctoria* | Bark | [14] |
| 72 | Stigmasterol glucoside |  | *B. vulgaris* | Fruit | [23] |
| 73 | *β*-Sitosterol |  | *B. tinctoria* | Bark | [14] |
| | | | *B. vulgaris* | Fruit | [44] |
| | | | *B. orthobotrys* | Leaf | [40] |
| 74 | Lanosterol |  | *B. tinctoria* | Bark | [14] |

(continued)

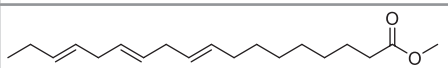
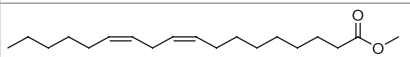
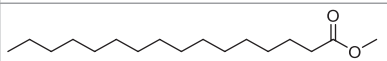
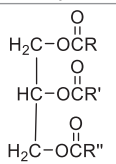
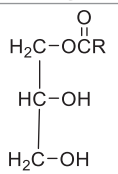
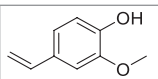
**Table 27.1** (continued)

| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| Phenyl propanoids | | | | | |
| 75 | Benzene,1,2-dimethoxy-4-(1-propenyl)- |  | *B. tinctoria* | Bark | [14] |
| Terpenes | | | | | |
| 76 | $\alpha$-Carotene |  | *B. vulgaris* | Fruit | [42] |
| 77 | Lutein |  | *B. vulgaris* | Fruit | [42] |
| 78 | Zeaxanthin |  | *B. vulgaris* | Fruit | [42] |
| 79 | Flavoxanthin |  | *B. vulgaris* | Fruit | [42] |
| 80 | Auroxanthin |  | *B. vulgaris* | Fruit | [42] |
| 81 | Capsanthin |  | *B. vulgaris* | Fruit | [42] |
| 82 | Dihydropinene |  | *B. tinctoria* | Bark | [14] |
| 83 | Deoxybaimuxinol |  | *B. vulgaris* | Wood bark | [38, 39] |

| | | | | | |
|---|---|---|---|---|---|
| 84 | Epi-ligulyl oxide |  | *B. vulgaris* | Wood bark | [38, 39] |
| 85 | Phytol |  | *B. tinctoria* | Bark | [14] |
| 86 | Oleanolic acid |  | *B. aristata* | | [11] |
| 87 | Taraxasterol |  | *B. tinctoria* | Bark | [14] |
| Waxes | | | | | |
| 88 | 10-Eicosanol |  | *B. orthobotrys* | Leaf | [40] |
| Fatty acid derivatives | | | | | |
| 89 | *n*-Hexadecanoic acid |  | *B. tinctoria* | Bark | [14] |
| 90 | Tetracosanoic acid, methyl ester |  | *B. vulgaris* | Wood bark | [38, 39] |
| 91 | Linoleic acid ethyl ester |  | *B. tinctoria* | Bark | [14] |
| 92 | Octadecanoic acid, methyl ester |  | *B. tinctoria* | Bark | [14] |

(continued)

**Table 27.1** (continued)

| No. | Compounds | Structure | Species | Plant part | References |
|---|---|---|---|---|---|
| 93 | 9,12,15 – Octadecatrienoic acid, methyl ester, (Z,Z,Z) |  | *B. tinctoria* | Bark | [14] |
| 94 | 9,12- Octadecadienoic acid (Z,Z) -, methyl ester |  | *B. tinctoria* | Bark | [14] |
| 95 | Hexadecanoic acid, methyl ester |  | *B. tinctoria* | Bark | [14] |
| 96 | Fatty acid esters | RCOOR′<br>R, R′ = long chain fatty acids | *B. vulgaris* | Fruit | [44] |
| 97 | Fatty alcohols | $RCH_2OH$<br>R = long chain fatty acids | *B. vulgaris* | Fruit | [44] |
| 98 | Triacyl glycerols |  <br>R, R′, R″= long chain fatty acids<br>R, R′, R″ = long chain fatty acids | *B. vulgaris* | Fruit | [44] |
| 99 | Monoacyl glycerols |  <br>R= long chain fatty acids<br>R = long chain fatty acids | *B. vulgaris* | Fruit | [44] |
| Miscellanous | | | | | |
| 100 | 2-Methoxy 4-vinylguaiacol |  | *B. vulgaris* | Wood bark | [38] |

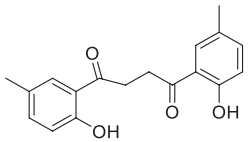
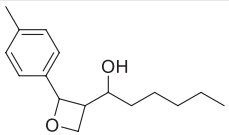
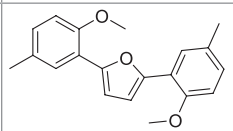
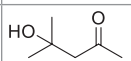
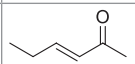
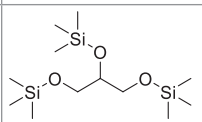
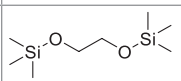
| | | | | | |
|---|---|---|---|---|---|
| 101 | 1,4-Bis-(2′-hydroxy-5′-methylphenyl)-butan-l: cdione |  | *B. coriaria* | Root | [45] |
| 102 | 2-(4-Methylphenyl)-3-(1-hydroxyhexyl) oxetane |  | *B. vulgaris* | Wood bark | [38, 39] |
| 103 | 2,5-Bis-(2′-methoxy-5′-methylphenyl)-furan |  | *B. umbellata* | Root | [46] |
| 104 | 2-Pentanone,4-hydroxy-4-methyl- |  | *B. tinctoria* | Bark | [14] |
| 105 | 3-Hexen-2-one |  | *B. tinctoria* | Bark | [14] |
| 106 | Glycerol, tris (trimethylsilyl) ether |  | *B. tinctoria* | Bark | [14] |
| 107 | 1,2-Bis(trimethylsiloxy)ethane |  | *B. vulgaris* | Stem bark | [39] |

**Table 27.2** Some of the most important ethnobotanical uses of *Berberis* species in different countries

| Species | Vernacular name | Country | Part used | Ethnobotanical uses | References |
|---|---|---|---|---|---|
| *B. aristata* | – | India | Root, stem, fruit | Skin and eye problems, jaundice, food additive | [52] |
| *B. asiatica* | – | Nepal | Bark, root | Fever, cough, conjunctivitis, blood purification | [53] |
| *B. atrocarpa* | Jiu zi ca ma | Tibet | Leaf, fruits | Food additive | [54] |
| *B. baluchistanica* | Zarch | Pakistan | Whole plant | Stomachache, digestion problems, jaundice | [55] |
| | Zarch, karwaskai | Pakistan | Root, leaf, fruit | Snake bite, gynecological problems | [56] |
| *B. brandisiana* | Shugloo | Pakistan | Leaf, fruits, bark | Dysentery and sore throat, food additive, tonic, wound healing, and arthritis. | [56] |
| *B. calliobotrys* | – | Pakistan | Root bark | Internal wounds | [57] |
| *B. crataegina* | Kızılcık | Turkey | Fruit | Food additive | [58] |
| *B. holstii* | – | Malawi | Leaf, stem bark | Cough, malaria, stomachache, sexually transmitted infections, and pneumonia | [59] |
| *B. integerrima* | Zereshk kuhi | Iran | Fruit | Hypoglycemic, anti-hypertensive, anti-pyretic, and anti-gout agents | [51] |
| | Zelk | Iran | Fruit, root | Analgesic | [51] |
| | Zereshk kuhi | Iran | Fruit | Jaundice, blood and liver cleanser, hypoglycemia, hypertension, fever | [60] |
| *B. jaeschkeana* | Kingora | India | Root | Astringent, diuretic, blood purifier, eye disorders, menorrhea, jaundice and skin diseases | [61] |
| | Kaymali | India | Root, fruit | Eye problems, fever, stomach disorders, skin diseases, blood cleanser, jaundice, dysmenorrhea | [62] |
| | – | India | Root, stem | Eye problems | [52] |
| *B. kongboensis* | Jiu zi ca ma | China | Leaf, fruit | Food additive | [63] |
| *B. kumaonensis* | Jhuri | India | Root | Astringent, diuretic, blood purifier, eye disorders, dysmenorrhea, jaundice and skin diseases | [61] |
| *B. libanotica* | – | Lebanon | Root | Rheumatism and neuralgic diseases | [64] |
| | Sumbal | Pakistan | Leaf, seed | Diarrhea and dysentery | [57] |
| *B. lycium* | – | India | Root, stem, fruit | Constipation | [52] |
| | Zyarlargay | Pakistan | Root, fruit, bark | Carminative, febrifuge, eye problems, chronic diarrhea, toothache, jaundice, diabetes and tonic | [52] |
| *B. microphylla* | – | Argentina | Whole plant | _ | [65] |
| *B. orthobotrys* | Ishkeen, churkee | Pakistan | Leaf, fruit, root | Inflammation, diabetes, injuries, bone fracture, wounds, ophthalmic diseases, dysentery | [66] |

(continued)

**Table 27.2** (continued)

| Species | Vernacular name | Country | Part used | Ethnobotanical uses | References |
|---|---|---|---|---|---|
| *B. pachyacantha* | Karpa | India | Root, fruit | Stomach problems, fever | [62] |
| *B. petiolaris* | – | India | Flower | Food additive | [67] |
| *B. pruinosa* | Sai mang | China | Branches, root, fruit | Diarrhea | [54] |
| | Pakkad | India | Root, fruit | Intestinal disorders, eye problems, oxytocic agent | [62] |
| *B. pseudumbellata* | – | India | Root | Eye irritation, pain, cold, cough, fever | [68] |
| | Skiorbu | Pakistan | Flower, fruit, seed | Jaundice | [69] |
| *B. temolaica* | Si sen | China | Root | Dye | [54] |
| *B. tinctoria* | Oosikala | India | Leaf bunch | Snake bite, indigestion | [70] |
| *B. ulicina* | Sinskingnama | India | Fruit | Ring worm | [71] |
| *B. umbellata* | – | India | Root, stem | Skin problems, fever | [52] |
| *B. vulgaris* | – | Bulgaria | Bark, roots, fruit | Cholecystitis, indigestion, diarrhoea, dysentery | [72] |
| | Zereshk | Iran | Fruit | Antipyretic, anthelmintic, anti-gout, anti-dysentery agent | [51] |
| | Zerk | Afghanistan | Fruit | Hepatic problems, indigestion, traumatic pain | [49] |
| | – | Switzerland | Fruit | Food additive | [73] |
| | Alrera, alrro | Spain | Young leaf | Appetizer, antipyretic agent | [74] |
| | Fajisóska, nyúlsom, sóska, sóskaborbolya | Hunguary | Fruit, shoot | Snack, substitute for vinegar | [48] |
| *B. wallichiana* | – | India | Root | Natural dye | [75] |

extract of saffron is prescribed as a good remedy for dropsy and hardness of liver. The fruits of this plant has cleansing properties due to the sour taste and temperament. Thus, they are able to open liver obstructions. It has also been used as a liver tonic [76, 79–81].

The beneficial effects of Zereshk is mentioned frequently in traditional textbooks for the treatment of gastrointestinal-related diseases. Zereshk, due to its astringent property, can strengthen digestive system and is helpful for the treatment of cold temperament-induced gastrointestinal disorders and prevent accumulation of harmful humors in related organs. In addition, astringent property of Zereshk makes it a useful agent for the treatment of constipation caused by warm and humid temperament [78]. Indeed, it has been used as a strong laxative to expel viscous phlegm and bile humors from stomach [80].

Another important gastrointestinal-related property of Zereshk is its anti-diarrhea activity. For instance, due to its astringent nature, cold and dry temperament, it is a good remedy for the treatment of biliary diarrhea. In addition, a mixture of Zereshk with warm temperament medicines is a useful treatment for diarrhea caused by phlegm, cold and poor liver function [1, 78, 79, 82, 83].

In fact, Zereshk has been reported to be used for a wide range of gastrointestinal problems mainly because of its astringent property and sour taste. In several ITM books, oral administration of aqueous extract of Zereshk has been prescribed for the treatment of nausea and vomiting [76, 79, 81, 84]. The oral administration of Zereshk to heal stomach inflammation has been prescribed by famous physicians [76, 77, 80, 82, 85]. In various sources of traditional medicine, the fruit of Zereshk is mentioned as an appetizer. For example, oral administration of a syrup made from Zereshk, apple and lemon juice has been prescribed for the treatment of appetite deficiency

**Table 27.3** The main textbooks of Islamic traditional medicine used in this study

| Author | Book | Language | Published in |
|---|---|---|---|
| Mohammad Ibn Zakariyâ Râzi (Rhazes) (865-925 A.D) | Al-Hâwi fi aṭ-Ṭibbe (Comprehensive Book of Medicine) | Arabic | Beirut, 2002 |
| Ali Ibn Abbas Ahwâzi Arjâni (930-994 A.D) | Kâmel al-Sinâh aṭ-Ṭibbiyah | Arabic | Qom, 2009 |
| Hossein Ibn Ali Ibn Sinâ (Avicenna) (980-1037 A.D) | Al-Qânun fi aṭ-Ṭibbe (Canon of Medicine) | Arabic | New Delhi, 1987 |
| Sayyed Esmâil Jorjâni (1042-1136 A.D) | Ḏakhireh Khârazmshâhi (Treasure of Kharazmshah) | Persian | Tehran, 1976 |
| Sayyed Esmail Jorjâni (1042-1136 A.D) | Al-Aghrâḍ aṭ-Ṭibbiah wa al-Mabâhethi al-Alâiiah (Medical Goals and Alaii's Discussions) | Persian | Tehran, 2006 |
| Ḍiâ al-Din Ibn Beytâr (1193-1248 A.D) | Al-Jâmee le Mofradâ t al-Adwiah wa al-Aghḏiah (Comprehensive Book in Simple Drugs and Foods) | Arabic | Beirut, 2001 |
| Ibn Nafis Qarshi (1210-1288 A.D) | Ash −Shamel fi aṭ-Ṭibbe | Arabic | Tehran, 2009 |
| Malek Mozaffar Ghasâni Torkmani (1222–1294 A.D) | al-Mo'tamad fi al-Adwiyah al-Mofradah | Arabic | Beirut, 2000 |
| Ali Ibn Hussein Anṣâri Shirâzi (1329-1403 A.D) | Ekhtiyârât Badiʿi | Persian | Tehran, 1993 |
| Abolqasem Ibn Muhammad Ghasâni (1547–1611 A.D) | Ḥadiqat al-Azhâr fi Mâḥiyyat al-Uʿshb wa al-U̲ʿqqâr | Arabic | Beirut, 1985 |
| Dawoud Anṭâki (1599 A.D) | Taḏkirat Oli al-Albâb (Memorandum Book) | Arabic | Beirut, 2000 |
| Hakim Mohammad Momen Tonekâboni (16th century A.D.) | Toḥfat al- Moʿmenin (Rarity of the Faithful) | Persian | Tehran, 1959 |
| Mohammad Hussein Aqili Khorâsâni (18th century A.D.) | Makhzan al-Adwyah (Drug Treasure) | Persian | Tehran, 2014 |

[76, 79, 80]. Ibn Beytâr believed that the fruit of Zereshk can heal intestinal ulcers. In addition, it is mentioned that continuous consumption of Zereshk can treat bleeding of hemorrhoids. Other scientists also used the fruit of this plant alone or in combination with other appropriate medications to treat intestinal ulcers and prevent scratching of this organ [1, 77, 83].

In ITM references, it is mentioned that consumption of Zereshk can produce flatus in some people. In these cases, it should be prescribed with *Syzygium aromaticum* (L.) Merr. & L.M.Perry. In addition, Zereshk is better to be used with sugar and sweets to prevent constipation [79, 80]. It is worth mentioning that in Ekhtiyârât Badiʿi, Zereshk has been prescribed with Arabic gum to reduce intestine complications [86].

In most ITM texts, Zereshk poultice has been prescribed for the treatment of a wide range of skin problems including hot inflammation, solidity and hot swelling [1, 76, 78, 80, 86]. For example, Ibn Nafis prescribed Zereshk poultice to prevent swelling expansion [1]. Topical administration of a mixture from Zereshk and borax has been reported to be an effective treatment for scrofula [86].

In some ITM books, Zereshk poultice has been prescribed for the treatment of a wide range of skin problems including hot inflammation, solidity and hot swelling [1, 76, 78, 80, 86]. For example, Ibn Nafis prescribed Zereshk poultice to prevent swelling expansion [1]. Topical administration of a mixture from Zereshk and borax has been reported to be an effective treatment for scrofula [86].

Ibn Nafis has stated in his book (Al Shâmel fi aṭ-Ṭibbe) that Zereshk is an eye strengthening agent and a good remedy for eye problems. For this purpose, it has been usually prescribed as kohl [78]. It has been also used as a treatment for eye pain, as a mixture with egg or milk [86] and conjunctivitis (plant root) [85]. Although preparations from different parts of *B. vulgaris* are

reported in traditional textbooks as a tonic and disinfectant agent for eyes frequently.

In ITM, there are various reasons for headaches. It is mentioned that one of these reasons is the movement of harmful humors from stomach to the head. The fruit of this plant due to their astringent properties can strengthen the stomach and decrease the mobility of these humors upward [78].

From the perspective of Ibn Nafis Qarshi, Zereshk could suppress urine due to its astringent property [78]. In one of the ITM books, it is mentioned: "putting a lint impregnated with honey and Zereshk in ear continuously for several days could heal ear wound and eliminate infected earwax [86].

Aphthous stomatitis is a common mouth disease that causes painful necrotic lesions in the oral mucosa, tongue and gum. Administration of barberry rootlets are one of the highly recommended treatment for this disease in ITM textbooks [86]. In some ITM texts, Zereshk has been prescribed for the treatment of snake bites, deadly poisons, palpitation, nausea and anorexia. For this purpose, a concentrated syrup prepared from barberry, apple and lemon juice with sugar was prescribed. In case of making great antidote, known as "Great Theriac", citron juice and pearl should be added to the prepared syrup [76, 79, 80]. Due to cold and dry temperament, Zereshk is a useful treatment for fevers with hot and biliary origin [78, 80].

## 27.6 Pharmacological Aspects of *Berberis* spp.

Several pharmacological studies have been carried out on different *Berberis* spp. Although some of these studies confirm the properties reported in traditional medicine, most of the traditional applications need to be investigated. *In vitro*, *in vivo*, and clinical trials support the anti-inflammatory, hepato-protective, and anti-colitis activities mentioned in ITM textbooks for these plants. *B. vulgaris* (Zereshk) is the most reported species both in traditional books and scientific databases. However, there are many reports on other species belonging to this genus. For example, properties like analgesic, hypoglycemic, hypotensive and antimicrobial activities are reported for *B. aristata*, *B. calliobotrys* and *B. asiatica*. Table 27.4 shows the activities for various *Berberis* spp. in modern medicine. Antioxidant, anti-microbial, hypolipidemic, and hypoglycemic activities are the most reported properties for *Berberis* plants in modern medicine.

The hepato-protective activities of *Berberis* spp. is well investigated in modern studies. It is shown that *B. vulgaris* bark extract/β-cyclodextrin complex has increased protection of hepatic cells via suppression of apoptosis and lipogenesis pathways [87]. In addition, *B. vulgaris* extract may have hepato-protective effects through the enhancement of antioxidant status, chelating abilities and free radicals quenching activities. It could significantly prevent toxic compound accumulation, increase alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), total cholesterol (TC), and total bilirubin (TB), inhibit lipid peroxidation and protein carbonyls (PCO) formation. Additionally, it could normalize the antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) [88, 89].

*Beberis aristata* has shown promising hepato-protective activities and the number of reports on its hepato-protective effect is even more than *B. vulgaris*. Different parts of this plant including stems and roots have decreased the toxic effects in various model of hepatotoxicity [90, 91]. There are also some other species from this genus such as *B. integerrima* Bge [92]., *B. asiatica* [93], *B. coriaceae* [94] and *B. lyceum* [95] with a few reports on their protective effect on liver [94]. Besides, berberine, as one of the active component in this genus, is reported to have inhibitory effects on hepatic stellate cell activation via targeted fibrogenic TGF-β1 pathway and extracellular MMP-2 deposition [96].

*Berberis* spp. and particularly berberin, one of the main alkaloids in *Berberis* spp., are reported to have anti-diarrheal effects which is probably through inhibition of intestinal fluid accumulation, inhibition of gastrointestinal motility [97],

**Table 27.4** Pharmacological studies of *Berberis* spp.

| Species | System | Activity | Study design | References |
|---|---|---|---|---|
| *B. vulgaris* | Integumentary system | Leishmaniasis | Mice, 10% root bark alcohol extract | [120] |
| | | Anti-apoptotic and anti-inflammatory effects on keratinocytes | *In vitro*, berberine | [106] |
| | Digestive system | | | [121] |
| | | Increase intestinal mucosa | Broiler chickens, aqueous extract, fruit | |
| | | Anti-colitic | Rats, ethanol extract, fruit, 10 mg/kg | [122] |
| | | Hepato-protective | Mice, aqueous extract | [88] |
| | Nervous system | | | |
| | | Reduce the acquisition and reinstatement of morphine-induced conditioned place preference | Mice, aqueous extract, 200 mg/kg | [123] |
| | | Epilepsy | Rats, aqueous extract, fruit | [2] |
| | | Anti-convulsant | Mice, berberine | [124] |
| | | Anti-convulsant | Rats, berberine (50, 100 and 200 mg/kg, ip) | [125] |
| | | Parkinson's disease | Mice, berberine, 50 mg/kg, oral | [126] |
| *B. aristata* | Digestive system | Hepato-protective | Rats, root, extract | [90] |
| | | Hepato-protective | Rats, ethanol extract, 400 mg/kg/day orally | [91] |
| | | Up-regulating the hepatic low density lipoprotein receptor expression | Human hepatoma cells, berberine | [127] |
| | Visual system | | | [128] |
| | | Eye protection | Rabbits, eye drop, stem wood | |
| | | Anti-microbial agent for eyes | Methanol extract, stem, *in vitro* | [129] |
| | Integumentary system | Anti-acne | Stem (gel, in combination with other plants) | [130] |
| *B. asiatica* | Digestive system | Hepato-protective | Rats, methanol extract, aerial parts | [93] |
| *B. calliobotrys* | Nervous system | Analgesic | Mice, aqueous methanol extract, stem, (250 mg/kg and 500 mg/kg) | [131] |
| | | Anti-convulsant | Mice, 500 and 1000 mg/kg orally, ethyl acetate and *n*-butanol fractions | [132] |
| | Cardiovascular system | Anti-thrombotic | Rabbits, butanol fraction (100 mg/kg), | [133] |
| | Skeletal system | Anti-arthritic | Rats, 70% methanol extract and *n*-butanol and aqueous fractions, 200 mg/kg, stem | [134] |
| *B. crataegina* | Nervous system | Analgesic | Mice and rats, roots and barks, aqueous extract | [135] |
| *B. holstii* | Cardiovascular system | Anti-plasmodial | *In vitro*, $IC_{50}$ = 0.17μg/ml, the crude alkaloid extract, roots and leaf | [136] |

(continued)

**Table 27.4** (continued)

| Species | System | Activity | Study design | References |
|---|---|---|---|---|
| *B. integerrima* | Digestive system | Hepato-protective | Rats, root, aqueous extract, (500 mg/kg bw) intra gastric | [92] |
| | Reproductive system | Protective effects on testicular injury | Rats, methanol extract, root, 500 mg/kg bw | [137] |
| | Nervous system | Anti-convulsant | Mice, methanol extract, hydromethanol, and chloroform fraction, root | [138] |
| *B. libanotica* | Cardiovascular system | Inhibits the viability of adult t cell leukemia | *In vitro*, ethanol fraction, stem | [139] |
| *B. lyceum* | | Anti-coccidial | Broiler chickens, root bark, berberine | [140] |
| *B. microphylla* | Cardiovascular system | Hypoglycemic | *In vitro*, ethanol, root | [141] |
| *B. orthobotrys* | Skeletal system | Anti-arthritic | *In vivo*, aqueous-methanol extract, root | [142] |
| | Cardiovascular system | Anti-hyperlipidemic | Rats, aqueous methanol extract, | [143] |
| | | Anti-hypertensive | rats, aqueous-methanol (70:30) extract, 100 mg/kg | [144] |
| *B. tinctoria* | Cardiovascular system | Antioxidant and antihemolytic | *In vitro*, fruit | [145] |
| | | Antibacterial | *In vitro*, root, extract | [146] |
| | | Antiviral | | |

ameliorating impaired gastrointestinal function, and anti-inhibition of smooth muscle contraction as well as anti-inflammatory and anti-microbial activities [97]. A novel mechanism for anti-diarrheal activity of berberine includes reinforcing the tight junctions, reducing epithelial permeability in the gut, and increasing transepithelial electrical resistance [98, 99].

The anti-inflammatory and protective effects of *Berberis* preparations in different skin problems are reported in several studies. The most reported species in this regard is *B. aristata*. For example, preparations from *B. aristata* have shown anti-psoriatic [100, 101] and anti-hyperpigmentation activities [102]. In addition, hydro-alcoholic extract of *B. aristata* root, as an ingredient of an herbal mixture, has been found out to be effective against skin damages induced by ultraviolet-rays *in vivo* [103]. Studies have shown that due to inhibitory effect on basal and UV-induced matrix metalloproteinases 1 and 9 expressions, berberine exerts its anti-skin aging and protective activities [104]. Not only *B. aristata*, but also preparations from *B. vulgaris* has shown skin protective effects.

Administration of aqueous extract and fruit juice of *B. vulgaris* orally in patients with acne vulgais could significantly reduce the number of lesions as well as mean Michaelson's acne severity score [105].

The anti-inflammatory and protective effects of berberine have been evaluated in different skin problem models. In a recent study, post-treatment of berberine in sulfur mustard exposed keratinocytes have decreased apoptosis significantly [106]. In addition, it has shown potential activities for the treatment of skin swelling, cellulite and skin slimming which may be attributed to its inhibitory effects on subcutaneous pre-adipocytes differentiation and facilitating lipolysis in adipocytes [107]. It is also reported that berberine can disperse the pigment of the skin in Bufo melanostictus melanophores by stimulation of beta-2 adrenergic receptors [108].

Berberine is reported to be a useful agent for inhibiting the rewarding effects of drugs of abuse such as morphine probably through decreasing hippocampal brain-derived neurotrophic factor mRNA expression and blocking the increase in hypothalamic CRF expression and TH expres-

sion in the locus coeruleus, cocaine via modulating the central dopaminergic system, and nicotine as well as ethanol [109].

Studies show that berberine could ameliorate toxicity of chemical toxins in brain, heart, kidney, liver and lung in part through antioxidant, anti-inflammatory, anti-apoptotic, modulation of mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) signaling pathways [110]. We could not find evidences in modern medicine studying the direct effects of this plant for eye problems. However, there are some studies reporting different preparations from *B. vulgaris* and berberine as analgesic [111] and anti-microbial [112–114] agents which may be the mechanisms of action for the beneficial effects of *B. vulgaris* as an eye remedy. There are just a few studies showing the eye protective effects of *B. aristata* (Table 27.4).

Pharmacological studies show that *B. vulgaris* and berberine have many beneficial effects on various CNS-related problems including, autoimmune encephalomyelitis and multiple sclerosis, convulsion, depression, Parkinson and Huntington diseases and properties such as analgesic and neuro-protective effects [109].

*Berberis vulgaris* and berberine have shown anti-urolithic activities *in vivo* [115, 116]. Aqueous root bark extract of *B. vulgaris* exhibited its activity through inhibition of calcium oxalate crystal deposition in renal tubules and protection of the kidneys against polyuria and impaired renal function. Other studies imply the activity of this plant against urolithiasis via antioxidative pathways. For instance, a homeopathic preparation of *B. vulgaris* could alleviate the renal calculi-associated oxidative damage by upregulating the antioxidant status [116].

Both *B. vulgaris* and *B. aristata* have been reported to protect kidneys in different renal toxicity models [117, 118]. Antioxidative and metal chelating properties are probably the most important mechanisms of action. Moreover, there is a study investigating the nephron-protective activity of *B. baluchistanica* root extract. This methanol extract could exert strong nephro-protective effects at doses 100, 200 and 300 mg/kg body weight by restoring various biomarkers such as creatinine, urea, serum uric acid levels in plasma and urine output creatinine clearance, urinary protein and γ-glutamyl transferase level in urine indicative of an antioxidant pathway [119].

## 27.7 Conclusion

Instructions, practices, skills and knowledge in different systems of traditional medicine are a helpful guide in drug discovery. Since the preparations used in traditional and ethno medicine have been prescribed over generations, their properties, indications, probable toxicities and adverse effects are well identified and the long route to find a potential drug candidate would be shortened. Plants belonging to *Berberis* spp. have a long reputation for medicinal as well as nutritional uses. In Islamic Traditional Medicine (ITM), *B. vulgaris* and *B. integrimma* (known as Zereshk) have been used for the treatment of a wide range of diseases. However, other species of this genus are also reported to have been prescribed to heal many health problems in various traditional systems of medicine. Comparing the knowledge from traditional medicine and findings from pharmacological studies shows that these plants are useful for the treatment of gastrointestinal, hepato-, urinary and skin problems as well as a strong anti-dote. However, clinical studies are required to evaluate the efficacy of these plants.

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# 28

# Protective Effects of Intravenous Magnesium Sulfate in Stroke Patients Receiving Amiodarone: A Randomized Controlled Trial

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**Abstract**

Anti-arrhythmic agents, like amiodarone, interfere at different stages of the ischemic stroke. However, amiodarone was accompanied with immunological pulmonary complications and adverse neurological effects. We hypothesize that magnesium sulfate in combination with amiodarone holds promise for stroke treatment. Thirty-six patients with confirmed diagnosis of ischemic stroke and atrial fibrillation who received bolus amiodarone were randomly assigned to magnesium sulfate every 24 h or similar volume of normal saline (as placebo)

The trial was registered in the Iranian Registration of Clinical Trials (IRCT; ID: IRCT201701011165N16). URL: http://en.irct.ir/trial/315

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for 5 days. Various severity test scores were used to evaluate the symptoms. Routing biochemistry were also measured at days 1 and 5. Treatment with MgSO4 results in a significant reduction in serum levels of NGAL, Hb, T.Bill, IL-6, IL-8, SNSE, S100B, EGF, PAF, CRP and IgG. Also, MgSO4 treatment significantly improved the RASS, Candida, SOFA, NIHSS and APACHE scores. Moreover, reduction of IL-6, IL-8, SNSE, EGF and APACHE score and increase in RASS score were significantly higher in MgSO4 group compared with placebo. Intravenous administration of MgSO4 in amiodarone-treated stroke patients improved the inflammatory, immunological and neurological indicators and reduced disability in ICU-admitted AIS patients, suggesting that this treatment scheme may prevent amiodarone-induced complications in these patients.

**Keywords**

Ischemic stroke · Amiodarone · Magnesium sulfate · Ischemia · Severity · Neuroprotection

## 28.1 Introduction

Ischemic stroke, in which a blood vessel leading to or within the brain is obstructed by a clot and followed by the impaired blood flow, induces neuronal cell death [1], and is the main cause of adult inability and the second leading cause of mortality worldwide [2–5]. Tissue plasminogen activator (tPA), a thrombolytic which targets the thrombus within the blood vessel, is the approved treatment of acute ischemic stroke (AIS) [1, 3, 4]. However, intravenous thrombolysis is not always helpful because most patients with AIS lose the narrow therapeutic time window. Also, recanalization does not always happen, while even if it is achieved, due to the re-obstruction, neurological decay can occur [3]. In this regard, it was reported the recurrent stroke risk is elevated gradually up to 8–12% (within 7 days), 12–15% (within 30 days) and 17–19% (within 90 days) [6].

Development of neuroprotective agents in order to interfere at different stages of the ischemic cascade, to increase the window for recanalization therapy and to save ischemic neurons in the brain from irreversible injury, was as much considerable as thrombolytic treatments [3, 7]. As much as ion channels play a crucial role in the development of ischemic brain injury, amiodarone as a multiple ion channel blocker has neuroprotective effects on the ischemic brain [8]. In addition, due to 50–60% maintenance of sinus rhythm, amiodarone is better than other agents, including dronedarone in treatment of atrial fibrillation (AF) [9], which is associated with significantly high risk of a cerebrovascular accident and elevated morbidity and mortality. Such that, amiodarone treatment was proposed for the prevention of AF after coronary artery bypass grafting [10, 11], while immunological pulmonary complications and adverse neurological effects was reported in amiodarone treated patients [12]. Moreover, it was reported that amiodarone treatment in non-valvular AF patients was accompanied with 1.81-fold increased risk of stroke [13].

Magnesium (Mg), an N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, as another neuroprotective agent, acts through several mechanisms such as blood flow elevation to ischemic brain areas, cellular energy metabolism improvement and glutamate dependent necrosis inhibition in the hippocampal neurons [4, 14]. Deficiency of Mg in acute and chronic cerebral ischemia leads to hypoxia and further death in

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cells. Therefore, there is a need of using this neuroprotective and neurotrophic drug in the treatment and prevention of cerebrovascular disease [15]. IMAGES study reported that Mg treatment for acute stroke was potentially safe, cheap, effective [16] and showed no significant adverse side effects [17]. In addition, magnesium sulfate (MgSO4) has shown to be effective for both rate and rhythm control in critically ill patients with new-onset AF and might reduce the need for antiarrhythmic drugs such as amiodarone [18]. It was illustrated that combined postoperative use of MgSO4 with amiodarone was proficient in decreasing the incidence of postoperative AF and MgSO4-mediated reduction was significantly higher than amiodarone alone therapy [19]. Since the combination therapy holds promise for stroke treatment [20], we aimed to investigate the supportive effects of magnesium on the prevention of amiodarone-induced immunological and inflammatory pulmonary complications in patients with stroke.

### 28.1.1 Materials and Methods

The trial was registered in the Iranian Registration of Clinical Trials (IRCT; ID: IRCT201701011165N16). The trial protocol was approved by the Tehran University of Medical Sciences Ethics Committee (ID: IR.TUMS.VCR.REC.1396.2257).

This randomized double-blind placebo-controlled clinical trial was performed on patients with ischemic stroke admitted to the intensive care unit in the Baqiyatallah Hospital and Sina Hospital (Tehran, Iran). The study was approved by the Ethics Committee of the Baqiyatallah University of Medical Sciences and a written informed consent was obtained from patients prior to inclusion in the study. Thirty-six patients were enrolled in this study. The inclusion criteria were: (1) confirmed stroke by CT Scan, (2) diagnosis of AF, and (3) receiving bolus amiodarone (300 mg bolus followed by 50 mg/hour for 5 consecutive days). Patients with serum creatinine levels greater than 2 mg/dl and those with hypermagnesemia were excluded from the study.

Patients were randomly assigned (using permuted blocks) to either magnesium sulfate or matched placebo (normal saline). In group one, subjects received 15 cc (7.5 g) of magnesium sulfate every 24 h. Similar volume of normal saline was administered to the subjects in the group two.

Post stroke neurologic deficit were evaluated by NIHSS (NIH Stroke Score) during the study. Level of consciousness was monitored by GCS (Glasgow Coma Scale) and RASS (Richmond Agitation Sedation Scale). SOFA (Sequential organ failure assessment) score was used to track multiple organs status such as cardiovascular systems, respiratory systems, nervous systems, liver, coagulation and kidney. APACHE II (Acute Physiology and Chronic Health Evaluation II) scale was used to measure severity of diseased condition based on the acute physiological parameters. Candida score as a bedside scoring system for candida infection in the patients admitted to the ICU was evaluated. Mortality rate during the hospitalization period and up to 3 months' post discharge, hospitalization period, heart rate, blood pressure, respiratory rate, arterial blood gas (ABG) and adverse drug reactions were also recorded daily for each patient during the study.

Laboratory data such as complete blood count with differentials (CBC-diff), pH, lactate, blood urea nitrogen (BUN) and serum levels of creatinine (Scr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), neuron-specific enolase (SNSE), S100B, glucose, total bilirubin, sodium, potassium, magnesium and calcium were measured before and after the intervention (days 1 and 5) in both of the study groups. Immunological parameters such as IgM, IgG, interleukin 6 (IL-6), interleukin 8 (IL-8), platelet-activating factor (PAF), plasminogen activator inhibitor-1 (PAI-1), neutrophil gelatinase-associated lipocalin (NGAL) protein, epidermal growth factor (EGF) protein, transforming growth factor β (TGFβ) proteins were measured at day 1 and day 5 using commercial ELISA kits.

## 28.2 Statistical Analysis

The obtained data were analyzed using the Statistical Package Social Sciences (SPSS) software for Microsoft Windows (SPSS 17.0, SPSS Inc., and Chicago, IL, USA). Within-group (before vs. after) comparisons were made using paired samples t-test and Wilcoxon signed-rank test in case of normal and non-normal distribution of data, respectively. Between-group changes were performed using independent samples t-test and Mann-Whitney U test in case of normal and non-normal distribution data, respectively. $P < 0.05$ were considered as statistically significant.

## 28.3 Results

### 28.3.1 Baseline Characteristics

Thirty-six subjects (18 in each group) completed the study with no drop-out and death during the study. There were no significant differences between MgSO4 versus placebo groups in the case of sex, mean ± SD age (65.78 ± 12.13 *vs.* 66.5 ± 12.58) and the percentage of hypertensive (56% *vs.* 33.3%), hyperlipidemic (16.7% *vs.* 11.1%) and diabetic (27.8% *vs.* 22.2%) patients ($P > 0.05$). Baseline values of weight ($P = 0.020$), BMI ($P = 0.015$), IL-6 ($P = 0.043$), IL-8 ($P < 0.001$), SNSE ($P < 0.001$), PAF ($P < 0.001$), PAI ($P < 0.001$), TGF ($P = 0.003$), IgG ($P < 0.001$), PLT ($P < 0.001$), AST ($P = 0.008$) and BUN ($P < 0.001$) showed statistically significant differences between groups. Baseline characteristics of the study population are summarized in Table 28.1.

### 28.3.2 Effect of MgSO4 on Biochemical Parameters

Using of MgSO4 significantly reduced the serum concentrations of NGAL ($P = 0.034$), Hb ($P = 0.031$), T.Bill ($P = 0.021$); however, the reduction in levels of FBS, WBC, ALT, BUN, Scr, and K and increase in the levels of PLT, AST, NA and lactate were not statistically significant in MgSO4 group ($P > 0.05$). In placebo group, except of PLT concentration ($P = 0.000$) which reduced, the levels of other biochemical parameters were not statistically changed (Table 28.2). Our results showed that the placebo-mediated reduction of PLT level was higher than MgSO4 ($P < 0.001$). Changes in serum levels of other biochemical parameters were not statistically different between groups ($P > 0.05$) (Table 28.3).

### 28.3.3 Anti-inflammatory Effect of MgSO4 on Brain Function

Using of MgSO4 significantly reduced the serum concentrations of IL-6 ($P < 0.001$), IL-8 ($P < 0.001$), SNSE ($P = 0.011$), S100B ($P = 0.007$), EGF ($P = 0.002$), PAF ($P = 0.005$) and CRP ($P = 0.001$). However, reduction in TGF and PAI levels were not statistically significant in MgSO4 group ($P > 0.05$). In placebo group, except of significant reduction in PAF level ($P = 0.045$), the levels of other inflammatory parameters were not statistically changed ($P > 0.05$) (Table 28.2). Our results demonstrated that MgSO4-mediated reductions of IL-6 ($P = 0.001$), IL-8 ($P < 0.001$), SNSE ($P = 0.016$), S100B ($P = 0.003$) and EGF ($P = 0.037$) levels were significantly higher when compared with placebo, while changes in serum levels of PAF and CRP were not statistically different between groups ($P > 0.05$) (Table 28.3).

### 28.3.4 Effect of MgSO4 on the Amiodarone-Induced Immunological Indicators

Using of MgSO4 significantly reduced the serum concentrations of IgG ($P = 0.022$), but IgA level ($P > 0.05$) was not altered after MgSO4 treatment. In placebo group the levels of both IgG and IgA were not statistically changed ($P > 0.05$) (Table 28.2). In addition, changes in serum levels of both IgG and IgA were not statistically different between groups ($P > 0.05$) (Table 28.3).

**Table 28.1** Baseline comparison between MgSO4 group and placebo group

| | | MgSO4 (N = 18) | Placebo (N = 18) | P value |
|---|---|---|---|---|
| Age (y ± SD) | | 65.78 ± 12.13 | 66.5 ± 12.58 | 0.862 |
| Sex | Female | 9 (50%) | 9 (50%) | 1.000 |
| | Male | 9 (50%) | 9 (50%) | |
| Hypertension (no. (%)) | | 10 (56%) | 6 (33.3%) | 0.180 |
| Hyperlipidemia (no. (%)) | | 3 (16.7%) | 2 (11.1%) | 0.500 |
| Diabetes (no. (%)) | | 5 (27.8%) | 4 (22.2%) | 0.500 |
| After 3-month mortality (no. (%)) | | 2 (11.1%) | 3 (16.7%) | 0.500 |
| Weight (kg) | | 83.78 ± 9.26 | 76.22 ± 9.32 | 0.020* |
| Height (cm) | | 170.83 ± 9.78 | 174.61 ± 6.72 | 0.186 |
| BMI (kg/m$^2$) | | 29.07 ± 5.35 | 25.11 ± 3.81 | 0.015* |
| IL-6 (pg/ml) | | 9.89 ± 2.78 | 8.33 ± 1.33 | 0.043* |
| IL-8 (pg/ml) | | 25.06 ± 4.39 | 13 ± 1.53 | 0.000* |
| FBS (mg/dl) | | 188.78 ± 70.06 | 172.78 ± 93.95 | 0.566 |
| SNSE (μg/L) | | 12 ± 2.52 | 8.39 ± 1.2 | 0.000* |
| S100B (pg/ml) | | 11.83 ± 2.15 | 12.67 ± 1.37 | 0.175 |
| PAF (ng/ml) | | 95.11 ± 24.79 | 43.39 ± 14.79 | 0.000* |
| PAI (ng/ml) | | 67.39 ± 26.79 | 12.11 ± 5.5 | 0.000* |
| NGAL (ng/ml) | | 212.39 ± 53.49 | 207.22 ± 52.83 | 0.772 |
| EGF (pg/ml) | | 87.56 ± 36.41 | 88.56 ± 36.77 | 0.935 |
| TGF(pg/ml) | | 10.72 ± 5.68 | 18.67 ± 8.98 | 0.003* |
| IgA (mg/dl) | | 7.56 ± 1.65 | 7 ± 1.46 | 0.292 |
| IgG (mg/dl) | | 58.06 ± 15.74 | 28.33 ± 6 | 0.000* |
| WBC (cells//μl) | | 7.78 ± 2.29 | 7.78 ± 1.44 | 1.000 |
| PLT (Plt/μl) | | 158.11 ± 32.3 | 237.73 ± 60.39 | 0.000* |
| Hb (g/L) | | 10.28 ± 1.49 | 10.89 ± 1.41 | 0.214 |
| CRP (mg/L) | | 10.5 ± 2.07 | 11.67 ± 2.25 | 0.114 |
| ALT (mg/dl) | | 28.78 ± 4.7 | 26.95 ± 11.02 | 0.521 |
| AST (mg/dl) | | 23.61 ± 4.23 | 19.06 ± 5.33 | 0.008* |
| BUN (mg/dl) | | 23.22 ± 3.39 | 18 ± 1.46 | 0.000* |
| Scr (mg/dl) | | 1.56 ± 0.57 | 1.44 ± 0.35 | 0.486 |
| T.Bill (mg/dl) | | 1.20 [1–1.7] [a] | 1 [1–2][a] | 0.772 |
| Na (mEq/L) | | 141.95 ± 4.67 | 143.11 ± 12.1 | 0.705 |
| K (mEq/L) | | 4.72 ± 0.81 | 4.37 ± 0.73 | 0.181 |
| PH | | 7.31 ± 0.33 | 7.24 ± 0.41 | 0.571 |
| Lactate (mg/dl) | | 1.59 ± 0.61 | 2.06 ± 0.87 | 0.072 |
| RASS | | (−3) [(−4) – (−2)] [a] | (−3) [(−4) – (−2)][a] | 0.300 |
| APACHE | | 22.5 [21–24][a] | 24 [21–25][a] | 0.347 |
| SOFA | | 13 [11.75–14][a] | 12.5 [11–14][a] | 0.923 |
| Candida | | 2.5 [2–3][a] | 2 [2–2.25][a] | 0.066 |
| GCS | | 7 [6–8][a] | 7 [5.75–8][a] | 0.536 |
| NIHSS | | 37 [35–39][a] | 37 [34.75–39][a] | 0.911 |

*ALT* Alanine aminotransferase, *APACHE* Acute Physiology and Chronic Health Evaluation, *AST* Aspartate aminotransferase, *BMI* Body mass index, *BUN* Blood urea nitrogen, *CRP* C-reactive protein, *EGF* Epidermal growth factor, *FBS* Fasting blood sugar, *GCS* Glasgow Coma Scale, *Hb* Hemoglobin, *IgA* Immunoglobulin A, *IgG* Immunoglobulin G, *IL-6* Interleukin 6, *IL-8* Interleukin 8, *K* Potassium, *kg* kilogram, *MgSO4* Magnesium sulfate, *ml* milliliter, *Na* Sodium, *NGAL* Neutrophil gelatinase-associated lipocalin, *NIHSS* National Institutes of Health Stroke Scale, *PAF* Platelet- activating factor, *PAI* Plasminogen activator inhibitor, *pg* Picogram, *PLT* Platelet, *RASS* Richmond Agitation-Sedation Scale, *S100B* S100 calcium binding protein B, *Scr* Serum creatinine, *SNSE* Serum neuron-specific enolase, *Sofa* Sequential Organ Failure Assessment, *T.Bill* Total bilirubin, *TGF* Transforming growth factor, *WBC* White blood cell, *Y* Years, Values are expressed as mean ± SD, * = statistically significant, a = Values which expressed as Median [IQR]

**Table 28.2** Pre vs. Post comparison in each group

| | MgSO4 (N = 18) | | | Placebo (N = 18) | | |
|---|---|---|---|---|---|---|
| | Before | After | P value | Before | After | P value |
| IL-6 (pg/ml) | 9.89 ± 2.79 | 6.94 ± 2.39 | 0.000* | 8.33 ± 1.33 | 7.67 ± 1.411 | 0.131 |
| IL-8 (pg/ml) | 25.06 ± 4.39 | 16.89 ± 4.92 | 0.000* | 13 ± 1.53 | 13.17 ± 2.92 | 0.810 |
| FBS (mg/dl) | 188.78 ± 70.06 | 180.17 ± 59.97 | 0.703 | 172.78 ± 93.95 | 174.89 ± 66.52 | 0.933 |
| SNSE (µg/L) | 12 ± 2.52 | 9.89 ± 2.93 | 0.011* | 8.39 ± 1.2 | 8 ± 0.97 | 0.130 |
| S100B (pg/ml) | 11.83 ± 2.15 | 9 ± 2.87 | 0.007* | 12.67 ± 1.37 | 12.56 ± 1.76 | 0.749 |
| PAF (ng/ml) | 95.11 ± 24.79 | 81.83 ± 25.05 | 0.005* | 43.39 ± 14.79 | 38.22 ± 18.67 | 0.045* |
| PAI (ng/ml) | 67.39 ± 26.79 | 64.78 ± 22.75 | 0.627 | 12.11 ± 5.50 | 13.67 ± 4 | 0.167 |
| NGAL (ng/ml) | 212.39 ± 53.49 | 193.11 ± 39.32 | 0.034* | 207.22 ± 52.83 | 211.72 ± 55.13 | 0.627 |
| EGF (pg/ml) | 87.56 ± 36.41 | 67.28 ± 31.76 | 0.002* | 88.56 ± 36.77 | 88.61 ± 39.7 | 0.994 |
| TGF(pg/ml) | 10.72 ± 5.68 | 10.56 ± 5.97 | 0.929 | 18.67 ± 8.98 | 19.56 ± 6.45 | 0.537 |
| IgA (mg/dl) | 7.56 ± 1.65 | 7.56 ± 1.2 | 1.000 | 7 ± 1.46 | 6.89 ± 1.41 | 0.726 |
| IgG (mg/dl) | 58.06 ± 15.74 | 52.67 ± 13.89 | 0.022* | 28.33 ± 6 | 27.06 ± 5.33 | 0.221 |
| WBC (cells//µl) | 7.78 ± 2.29 | 7.06 ± 1.59 | 0.283 | 7.78 ± 1.44 | 8.06 ± 2.34 | 0.598 |
| PLT (Plt/µl) | 158.11 ± 32.30 | 167.11 ± 48.75 | 0.263 | 237.72 ± 60.39 | 185.83 ± 54.65 | 0.000 * |
| Hb (g/L) | 10.28 ± 1.49 | 9.44 ± 1.5 | 0.031* | 10.89 ± 1.41 | 10.83 ± 2.01 | 0.917 |
| CRP (mg/L) | 10.5 ± 2.07 | 8.83 ± 1.76 | 0.001* | 11.67 ± 2.25 | 11.22 ± 3.06 | 0.631 |
| ALT (mg/dl) | 28.78 ± 4.7 | 27.94 ± 3.52 | 0.570 | 26.94 ± 11.02 | 26.61 ± 10.43 | 0.402 |
| AST (mg/dl) | 23.61 ± 4.23 | 24.61 ± 3.68 | 0.502 | 19.06 ± 5.33 | 18.83 ± 4.13 | 0.756 |
| BUN (mg/dl) | 23.22 ± 3.39 | 22.94 ± 3.08 | 0.749 | 18 ± 1.46 | 18.17 ± 1.25 | 0.660 |
| Scr (mg/dl) | 1.56 ± 0.57 | 1.4 ± 0.52 | 0.204 | 1.44 ± 0.35 | 1.28 ± 0.33 | 0.143 |
| T.Bill (mg/dl) | 1.20 [1–1.7] [a] | 1 [0.9–1.28] [a] | 0.021* | 1 [1–2] [a] | 1 [1–2][a] | 0.848 |
| Na (mEq/L) | 141.94 ± 4.67 | 142.44 ± 5.09 | 0.760 | 143.11 ± 12.1 | 141.11 ± 6.2 | 0.522 |
| K (mEq/L) | 4.72 ± 0.81 | 4.58 ± 1.08 | 0.639 | 4.37 ± 0.73 | 4 ± 0.47 | 0.038 |
| Lactate (mg/dl) | 1.59 ± 0.61 | 1.61 ± 0.61 | 0.890 | 2 [1–3][a] | 2 [1–2][a] | 0.166 |
| RASS | (−3) [(−4) – (−2)][a] | (−2) [(−3) – (−1.75)][a] | 0.021* | (−3) [(−4) – (−2)] [a] | (−3) [(−3) – (−2)][a] | 0.765 |
| APACHE | 22.5 [21–24][a] | 19.5 [16.75–20][a] | 0.000* | 24 [21–25][a] | 23 [21.75–25][a] | 0.982 |
| SOFA | 13 [11.75–14][a] | 11 [10.75–12][a] | 0.005* | 12.5 [11–14][a] | 12 [11–13][a] | 0.025* |
| Candida | 2.5 [2–3][a] | 2 [2–2][a] | 0.013* | 2 [2–2.25][a] | 2 [1–2.25][a] | 0.132 |
| GCS | 7 [6–8][a] | 6 [5–7][a] | 0.024* | 7 [5.75–8][a] | 6.5 [5–7.25] [a] | 0.258 |
| NIHSS | 37 [35–39][a] | 30.5 [29.75–37][a] | 0.001* | 37 [34.75–39][a] | 37 [35–38.25] | 0.877 |

*ALT* Alanine aminotransferase, *APACHE* Acute Physiology and Chronic Health Evaluation, *AST* Aspartate aminotransferase, *BUN* Blood urea nitrogen, *CRP* C-reactive protein, *EGF* Epidermal growth factor, *FBS* Fasting blood sugar, *GCS* Glasgow Coma Scale, *Hb* Hemoglobin, *IgA* Immunoglobulin A, *IgG* Immunoglobulin G, *IL-6* Interleukin 6, *IL-8* Interleukin 8, *K* Potassium, *kg* kilogram, *MgSO4* Magnesium sulfate, *ml* milliliter, *Na* Sodium, *NGAL* Neutrophil gelatinase-associated lipocalin, *NIHSS* National Institutes of Health Stroke Scale, *PAF* Platelet- activating factor, *PAI* Plasminogen activator inhibitor, *pg* Picogram, *PLT* Platelet, *RASS* Richmond Agitation-Sedation Scale, *S100B* S100 calcium binding protein B, *Scr* Serum creatinine, *SNSE* Serum neuron-specific enolase, *Sofa* Sequential Organ Failure Assessment, *T.Bill* Total bilirubin, *TGF* Transforming growth factor, *WBC* White blood cell, *Y* Years, Values are expressed as mean ± SD, * = statistically significant, a = Values which expressed as Median [IQR]

**Table 28.3** Mean changes between MgSO4 group and placebo group

| | Mean change | | |
|---|---|---|---|
| | MgSO4 | Placebo | P value |
| IL-6 (pg/ml) | −2.94 ± 2.04 | −0.67 ± 1.78 | 0.001* |
| IL-8 (pg/ml) | −8.17 ± 6.83 | 0.17 ± 2.9 | 0.000* |
| FBS (mg/dl) | −8.61 ± 94.12 | 2.11 ± 105.09 | 0.749 |
| SNSE (μg/L) | (−3) [(−4) – (1.25)][a] | (−1) [(−1) – (1)][a] | 0.016* |
| S100B (pg/ml) | (−4) [(−6.5) – (−1)][a] | (−1) [(−1) – (1)][a] | 0.003* |
| PAF (ng/ml) | −13.28 ± 17.62 | −5.17 ± 10.11 | 0.099 |
| PAI (ng/ml) | −2.61 ± 22.37 | 1.56 ± 4.57 | 0.449 |
| NGAL (ng/ml) | −19.28 ± 35.54 | 4.5 ± 38.56 | 0.063 |
| EGF (pg/ml) | −20.28 ± 23.17 | 0.06 ± 32.4 | 0.037* |
| TGF(pg/ml) | −0.17 ± 7.79 | 0.89 ± 5.99 | 0.651 |
| IgA (mg/dl) | 0 [0–0.25][a] | 0 [(−1) – (1)][a] | 0.857 |
| IgG (mg/dl) | −5.39 ± 9.06 | −1.28 ± 4.27 | 0.094 |
| WBC (cells//μl) | −0.72 ± 2.76 | 0.28 ± 2.19 | 0.237 |
| PLT (PLT/μl) | 9 ± 32.95 | −51.89 ± 45 | 0.000* |
| Hb (g/L) | (−1) [(−2) – (−0.5)][a] | 0.5 [(−1.25) – (1)][a] | 0.213 |
| CRP (mg/L) | −1.67 ± 1.71 | −0.44 ± 3.85 | 0.231 |
| ALT (mg/dl) | −0.83 ± 6.1 | −0.33 ± 1.64 | 0.741 |
| AST (mg/dl) | 1 ± 6.18 | −0.22 ± 2.98 | 0.457 |
| BUN (mg/dl) | −0.28 ± 3.63 | 0.17 ± 1.58 | 0.638 |
| Scr (mg/dl) | −0.16 ± 0.5 | −0.16 ± 0.45 | 0.972 |
| T.Bill (mg/dl) | (−0.2) [(−0.63) – (−0.03)][a] | 0 [(−1) – (1)][a] | 0.252 |
| Na (mEq/L) | 0.5 ± 6.84 | −2 ± 12.98 | 0.474 |
| K (mEq/L) | −0.14 ± 1.28 | −0.37 ± 0.7 | 0.515 |
| Lactate (mg/dl) | 0.02 ± 0.67 | −0.28 ± 0.83 | 0.240 |
| RASS | 1 [0–2] [a] | 0 [(−1) – (1)][a] | 0.038* |
| APACHE | (−3.5) [(−6) – (−2)][a] | 0 [(−2) – (2)][a] | 0.000* |
| SOFA | (−2) [(−3) – (−1)][a] | (−1) [(−1.25) – (−0.5)][a] | 0.054 |
| Candida | (−1) [(−1) – (0)][a] | 0 [(−1) – (0)][a] | 0.335 |
| GCS | (−1) [(−1.25) – (−0.5)][a] | (−1) [(−1) – (1)][a] | 0.348 |
| NIHSS | (−4.5) [(−8.25) – (−1.75)][a] | (−1) [(−2) – (2)][a] | 0.002* |

*ALT* Alanine aminotransferase, *APACHE* Acute Physiology and Chronic Health Evaluation, *AST* Aspartate aminotransferase, *BUN* Blood urea nitrogen, *CRP* C-reactive protein, *EGF* Epidermal growth factor, *FBS* Fasting blood sugar, *GCS* Glasgow Coma Scale, *Hb* Hemoglobin, *IgA* Immunoglobulin A, *IgG* Immunoglobulin G, *IL-6* Interleukin 6, *IL-8* Interleukin 8, *K* Potassium, *kg* kilogram, *MgSO4* Magnesium sulfate, *ml* milliliter, *Na* Sodium, *NGAL* Neutrophil gelatinase-associated lipocalin, *NIHSS* National Institutes of Health Stroke Scale, *PAF* Platelet- activating factor, *PAI* Plasminogen activator inhibitor, *pg* Picogram, *PLT* Platelet, *RASS* Richmond Agitation-Sedation Scale, *S100B* S100 calcium binding protein B, *Scr* Serum creatinine, *SNSE* Serum neuron-specific enolase, *Sofa* Sequential Organ Failure Assessment, *T.Bill* Total bilirubin, *TGF* Transforming growth factor, *WBC* White blood cell, *Y* Years, Values are expressed as mean ± SD, * = statistically significant, a = Values which expressed as Median [IQR]

### 28.3.5 Effect of MgSO4 on Prevention of Organ Failure

Using of MgSO4 can significantly improve the RASS (P = 0.021), Candida (P = 0.013) and Sofa (P = 0.005) scores of patients, though Sofa score (P = 0.025) was significantly improved in placebo group as well (Table 28.2). MgSO4-mediated elevation of RASS was just significantly higher when compared with placebo (P = 0.038) (Table 28.3).

### 28.3.6 Effect of MgSO4 on Neurological Improvement

Using of MgSO4 could significantly improve the NIHSS (P = 0.001), while significantly reduced the GCS (P = 0.024) (Table 28.2). MgSO4-mediated reduction of NIHSS was just significantly higher when compared with placebo (P = 0.002) (Table 28.3).

### 28.3.7 Effect of MgSO4 on Morbidity and Mortality in Stroke Patients

Using of MgSO4 may significantly improve the APACHE score (P < 0.001), which was not statistically changed in placebo group (P > 0.05) (Table 28.2), and MgSO4-mediated reduction of APACHE score was significantly higher when compared with placebo (P < 0.001) (Table 28.3). Although there was no death during the study, but 3 months after completion of the study, 2 (11.1%) patients of MgSO4 group and 3 (16.7%) patients of placebo group died, which was not statistically significant between groups (P > 0.05) (Table 28.1).

## 28.4 Discussion

Significant positive effect of intravenous (IV) administrated MgSO4 on the outcome in patients with acute stroke was shown [21], though supportive effect of it in amiodarone-induced complications in these patients was not determined. Our results demonstrated the beneficial effects of MgSO4 in patients with stroke who were administered with amiodarone when compared with placebo. The present trial illustrated significant reduction in NGAL, Hb and T.Bill levels in MgSO4 group that were not different from placebo group, but failed to show significance of the observed reduction in levels of FBS, WBC, ALT, BUN, Scr, and K and observed elevation in the levels of PLT, AST, NA and lactate in MgSO4 group. It was probably due to the different baseline levels of them in MgSO4 group compared with placebo, especially AST, BUN and PLT. Such that, in placebo group because of higher baseline level of PLT, higher reduction of its level was occurred compared with MgSO4 group.

Since immunological pulmonary complications and adverse neurological effects were reported in amiodarone-treated patients [12], we investigated the efficacy of MgSO4 on amiodarone-induced inflammatory and immunological indicators in stroke patients. Our results showed a significant reduction of IgG levels in MgSO4 group, which was initially higher compared with placebo group, while having no statistical impact on the concentration of IgA. In addition, we demonstrated that treatment with MgSO4 results in a significant decline in serum levels inflammatory markers including IL-6, IL-8, SNSE, S100B**,** EGF, PAF and CRP while having no statistical impact on the concentrations of PAI as the main inhibitor of tPA and anti-inflammatory marker like TGF, which was initially higher in placebo group than MgSO4 group. It was shown that SNSE level, a marker for neuronal damage whose reduction was significantly higher in MgSO4 group compared with placebo in our study, was associated with obesity and BMI > 25 [22], as our results showed that weight and BMI (>25) were significantly higher in MgSO4 group than placebo. The reduction in SNSE suggested to be due to impaired glucose metabolism and neuronal differentiation [22], though our results failed to show significance of the observed reduction in levels of FBS.

Since the expected outcome in human clinical stroke trials is neurological and functional improvements [23], we also investigated the efficacy of MgSO4 on prevention of organ failure and neurological improvement. Our results revealed that using of MgSO4 may improve the RASS, Candida and Sofa scores of patients, though just RASS score was shown better recovery in MgSO4 group when compared with placebo. In this regard, one previous study showed that IV MgSO4 did not reduce disablement of patients at 3 months when given within 12 h of clinically diagnosed stroke [24]. In addition, Sofa score was significantly improved in placebo

group as well. Furthermore, treatment with MgSO4 resulted in an improvement of NIHSS and subsequently neurologic deficit improvement after 5 days compared with placebo. In this sense, Lampl et al. also illustrated the neurological improvement of AIS patients in MgSO4 group compared with placebo [21]. However, our results failed to show improvement in GCS, which was significantly reduced in MgSO4 group, but this reduction was not statistically different between groups.

The results of our trial illustrated that IV administration of MgSO4 improved APACHE score in stroke patients who were administered with amiodarone when compared with placebo. Although the effects of the MgSO4 on reduction in the early death number of patients compared with placebo was shown [25], we demonstrated that the number of patients who died 3 months after completion of study was not statistically different between groups and that might be due to the amiodarone administration of both groups in our study. Indeed, it was shown that IV MgSO4 did not reduce death at 3 months when given within 12 h of clinically diagnosed stroke [24]. Furthermore, Sleeswijk et al. reported that in critically ill patients with new-onset AF who were administered with MgSO4 and then with amiodarone, APACHE score and hospital mortality were both higher in Mg non-responders group than responders group, though this was not significant. The authors proposed that pretreatment with MgSO4 had beneficial effects to reduce APACHE score and restore sinus rhythm in these patients [18].

In conclusion, MgSO4 treatment in AIS patients, who were treated with amiodarone, showed improvement of inflammatory, immunological and neurological indicators and reduction of disability in AIS patients, and this therapeutic strategy may be considered as a promising therapy for these patients in order to reduce amiodarone-induced complications.

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# 29

# *In Silico* Identification of Novel Interactions for FABP5 (Fatty Acid-Binding Protein 5) with Nutraceuticals: Possible Repurposing Approach

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**Abstract**

Fatty Acid Binding-Protein 5 (FABP5) is a cytoplasmic protein, which binds long-chain fatty acids and other hydrophobic ligands. This protein is implicated in several physiological processes including mitochondrial β-oxidation and transport of fatty acids, membrane phospholipid synthesis, lipid metabolism, inflammation and pain. In the present study, we used molecular docking tools to determine the possible interaction of FABP5 with six selected compounds retrieved form Drugbank. Our results showed that FABP5 binding pocket included 31 polar and non-polar amino acids, and these residues may be related to phosphorylation, acetylation, ubiquitylation, and mono-methylation. Docking results showed that the most energetically favorable compounds are NADH (−9.12 kcal/mol), 5′-O-({[(Phosphonatooxy)phosphinato]oxy}phosphinato)adenosine (−8.62 kcal/mol), lutein (−8.25 kcal/mol), (2S)-2-[(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)methyl]amino}benzoyl)amino]pentanedioate (−7.17 kcal/mol), Pteroyl-L-

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glutamate (−6.86 kcal/mol) and (1S,3R,5E,7Z)-9,10-Secocholesta-5,7,10-triene-1,3,25-triol (−6.79 kcal/mol). Common interacting residues of FABP5 with nutraceuticals included SER16, LYS24, LYS34, LYS40 and LYS17. Further, we used the SwissADME server to determine the physicochemical and pharmacokinetic characteristics and to predict the ADME parameters of the selected nutraceuticals after molecular analysis by docking with the FABP5 protein. Amongst all compounds, pteroyl-L-glutamate is the only one meeting the Lipinski's rule of five criteria, demonstrating its potential pharmacological use. Finally, our results also suggest the importance of FABP5 in mediating the anti-inflammatory activity of the nutraceutical compounds.

**Keywords**

FABP5 · Molecular docking · Nutraceuticals · ADME · Inflammation

## 29.1 Introduction

Fatty Acid-Binding Protein 5 is a cytoplasmic protein belonging to the family Fatty acid-binding proteins (FABPs). These proteins are structurally conserved cytosolic proteins with a broad specificity for ligands including eicosanoids, peroxisome proliferators, bile salts, long-chain fatty acids and cannabinoids [11, 26]. To date, at least 12 of *FABP* different genes have been identified [33]. FABPs have differential expression in different tissues and organs, including liver (FABP1), intestine (FABP2), heart (e.g FABP3), adipocytes and macrophages (FABP4), epidermis (FABP5), Ileum (FABP6) and brain (FABP7) among others [14, 22, 33].

FABP5 is also known as psoriasis-associated fatty acid-binding protein, epidermal, or cutaneous fatty acid-binding protein (PA-, E-, or C-FABP), has been shown to be present or expressed in several tissues including epidermis, nociceptive dorsal root ganglia, spinal cord and liver [22]. It is a small protein of 135 amino acids, with a structural conformation of ten β-strands (β1-β10), two α-helices (α1-α2) and ten loops (L1-L10, [6]). FABP5 binds to long-chain fatty acids and other hydrophobic ligands such as saturated FAs, MUFAs (monounsaturated FAs), *n*-3, and *n*-6 PUFAs (polyunsaturated FAs) through its binding pocket, which includes ARG109, ARG129 and TYR131 [3]. Its main functions include fatty acid uptake, transport, and metabolism. Moreover, this protein may modulate the actions of the PPAR β/δ (nuclear receptor peroxisome proliferator-activated receptor), and promote cell proliferation, survival and migration by exhibiting pro-oncogenic activities in colorectal, ovarian, breast and prostate cancers [1, 12, 22, 29]. Finally, FABP5 has been explored as a potential pharmacological target for inflammation, pain and amelioration of drug withdrawal symptoms [4, 17, 34].

Previous computational studies have been developed to understand the regulation of FABP5 and other FABPs by different ligands [24, 31]. For example, Yan et al. [31] used a bioinformatic approach based on molecular dynamics in combination with molecular mechanics generalized Born surface area (MM-GBSA) to determine the binding selectivity of three FABP4/FABP5 inhibitors with therapeutic potential for arteriosclerosis and inflammation [31]. Similarly, Shinoda et al. [24] reported the affinity of ten polyphenolic ligands for FABP3, FABP4 and FABP5. Using computational docking simulations and experimental methods, the potential anti-inflammatory and protective effects of FABP ligands in neurodegenerative disorders and peripheral ischemic injury were explored [24]. Briefly, nutraceuticals are naturally occurring compounds present in food with possible medical benefits, which include amino acids like N-acetylcysteine, carotenoids, polyphenols, vitamins, minerals and fatty acids [27]. Nutraceuticals have been used to improve health and prevent chronic diseases including diabetes, cardio and cerebrovascular diseases, cancer, and the neuroinflammatory diseases Alzheimer and Parkinson [2, 5, 15, 16, 18, 20, 21, 25, 28, 32]. At this point, targeting FABP5 using pharmacological approaches could be a

potential way to mitigate inflammation and lipid metabolism abnormalities in different diseases. Therefore, in the present study, we used molecular docking tools to explore the possible interaction of FABP5 with six selected nutraceutical compounds. Identification of the residues important for this interaction could pave the way for drug design against lipid dysfunction and neuroinflammatory diseases.

## 29.2 Materials and Methods

### 29.2.1 Ligand Preparation

A list with a total of 60 naturally occurring ligands compounds database (version 5.1.7) was retrieved from DrugBank (www.drugbank.ca) in SDF format and then converted into.mol using Molecular Operating Environment (MOE 2015.10, Chemical Computing Group). Polar hydrogens and charges were added before docking. The molecular structure of all compounds was built using the ligand builder plugin in MOE.

### 29.2.2 Structure Preparation

The 3D structure of the human protein Fatty acid-binding protein 5 (FABP5, entry: 5HZ5) were downloaded from Protein Data Bank (https://www.rcsb.org/). The protein structure was prepared using the "Structure preparation" plugin in MOE for protonation and energy minimization, the protein-associated ligands were removed, and the missing hydrogen atoms were added. To determine the protein's binding pocket we used the CASTp server (http://sts.bioe.uic.edu/castp).

### 29.2.3 Molecular Docking of FABP5 with Nutraceuticals

For docking, chain A of the FABP5 protein was used. Initially we performed a blinded docking to determine the possible interaction site of the ligands to the FABP5 protein using MOE. Based on the interaction energy and best conformations, we selected 6 compounds (Fig. 29.1) for a more exhaustive study. A second docking analysis was carried out, now with exhaustiveness of 100 different poses using the GBVI/WSA dG and London dG scores as parameters. The analyzed docking parameters were: Root-mean-square deviation (RMSD), water accessible surface area (ASA), potential energy (E), electrostatic potential energy (E_ele), electrostatic interaction energy (E_rele), van der Waals interaction energy (E_rvdw), van der Waals potential energy (E_vdw), total SCF energy (kcal/mol) calculated using the MNDO Hamiltonian (MNDO_E), energy of the highest occupied molecular orbital (HOMO), energy of the lowest unoccupied molecular orbital (LUMO) and radius of gyration (rgyr). Validation of docking results were performed by running the same experiment using Autock Vina on PyRx.

### 29.2.4 Studies of Toxicity/ADMET of Nutraceutical Compounds

The SwissADME server (http://www.swissadme.ch/) was used to determine the physicochemical and pharmacokinetic characteristics and to predict the ADME parameters of the selected nutraceuticals after molecular analysis by docking with the FABP5 protein.

## 29.3 Results and Discussion

### 29.3.1 Binding Pocket of FABP5

The use of molecular docking for screening possible compounds for drug repurposing has been rapidly expanding. In a straightforward manner, it generates a great amount of data, making it feasible to identify possible sites and/or domains of interaction between ligands and target proteins. This makes this approach very useful as to selection of drug candidates and druggable targets. Initially, in the present study, using the CASTp server we determined that the FABP5 binding pocket included the amino acid residues PHE19, TYR22, MET23, LEU26, VAL28, LEU32,

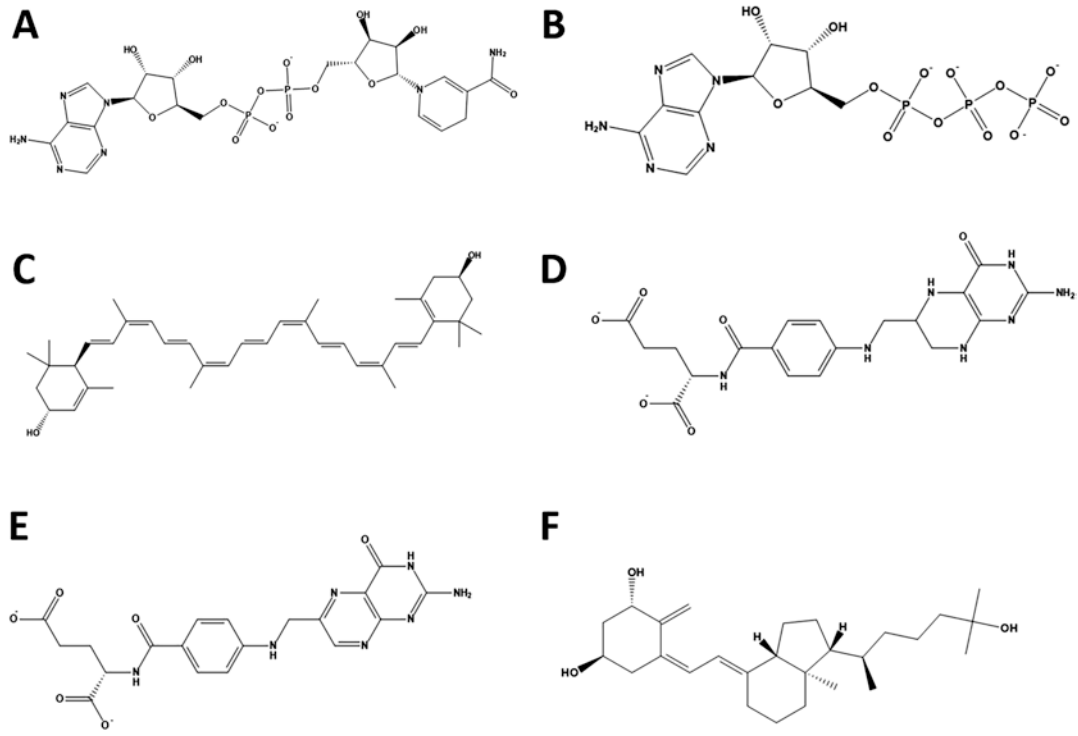


**Fig. 29.1** Molecular structure of naturally occurring compounds. (**a**) NADH(2-); (**b**) 5′-O-({[(Phosphonatooxy) phosphinato]oxy}phosphinato)adenosine; (**c**) lutein; (**d**) (2S)-2-[(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)methyl]amino}benzoyl)amino]pentanedioate; (**e**) Pteroyl-L-glutamate; and (**f**) (1S,3R,5E,7Z)-9,10-Secocholesta-5,7,10-triene-1,3,25-triol

MET35, GLY36, ALA39, PRO41, CYS43, ILE54, THR56, SER5, LYS61, THR62, GLN64, PHE65, GLU75, THR76, THR77, ALA78, ASP79, ARG81, GLN98, ILE107, ARG109, VAL118, CYS120, ARG129, and TYR131. Previous structural studies have shown that FABP5 binding pocket for linoleic acid consists of ARG129, TYR131 (hydroxyl moiety) and ARG109 [3], which is consistent with our results. Moreover, our results are also in line with previous studies reporting that the binding site of both FABP4 and 5 is within the β-barrel, containing the loops β3–β4 and β5–β6, in combination with α1-loop-α2 domain forming a sort of like controlling gate to allow the entrance and exit of ligands involved in the interaction [13, 31]. This study also described that the residues ARG126, ARG106 and TYR128 interact in the barrel's cavity through electrostatic interactions [31]. Finally, a recent study using molecular dynamics approaches showed the energy contributions of key residues such as F19, Y22, M23, P41, T56 and L60 from K61, R109 and R129 to be important in the interaction with studied ligands [6].

### 29.3.2 Molecular Docking of Nutraceuticals and FABP5

We started from an initial list of 60 different ligands of natural origin obtained from the DrugBank database, from which by blinded docking we selected a few for a more detailed analysis. Interestingly, some of the residues that make part of the FABP5 binding pocket are directly involved in the interaction with some ligands, which suggests that they can interact with the protein through their active site. In this first approximation, the 10 best binding poses

from each ligand were analyzed, from which we selected a total of 6 ligands (Fig. 29.1) that presented the best interaction energy. After this, we performed a more exhaustive docking, now with 100 different poses and found that NADH (2-) (−9.12 kcal/mol) presents the greatest interaction energy, followed by 5′-O - ({[(Phosphonatooxy) phosphinato] oxy} phosphinato) adenosine (−8.62 kcal/mol), lutein (−8.25 kcal/mol), (2S) -2 - [(4 - {[(2-Amino-4-oxo-1,4,5, 6,7,8-hexahydro-6-pteridinyl) methyl] amino} benzoyl) amino] pentanedioate (−7.17 kcal/mol), Pteroyl-L-glutamate (−6.86 kcal/mol) and (1S, 3R, 5E, 7Z) -9,10-Secocholesta-5,7,10-triene-1,3,25-triol (−6.79 kcal/mol) (Table 29.1). Interestingly, lutein, which is a xanthophyll-type carotenoid found in leafy vegetables and yellow fruits, has been shown to exert neuroprotective and anti-inflammatory effects in animal models of ocular diseases [23] through the suppression of reactive oxygen species (ROS) and inflammatory signaling. Moreover, pteroyl-L-glutamate (folic acid) is important in the metabolism of amino acids and nucleic acids, and has been used as an adjuvant to cytotoxic agents in cancer treatment [10], and in the modulation of inflammatory response in microglia [8]. Similarly, 9,10-Secocholesta-5,7,10-triene-1,3,25-triol (the active metabolite of vitamin D-3) has potential benefits against carcinogenic cells proliferation, and anti-inflammatory effects through the inhibition of NF-κB signaling, and the suppression of prostaglandin metabolism [30]. Finally, both NADH and ATP (5′-O - ({[(Phosphonatooxy) phosphinato] oxy} phosphinato) adenosine) have shown to be important in the regulation of pro-inflammatory cytokines, the inflammatory related kinases IKKβ, JNK and ERK and enzymes such as Sirt1, Sirt6, PARP-1, ART-1 [7, 19]. These newly presented results further support the importance of FABP5 in the regulation of inflammatory processes. However, further research is needed in order to establish the physiological and molecular mechanisms of this regulatory process.

The FABP5 residues that interact through H-bonds with NADH (2-) are GLU21, VAL14, ASP15, ASP20, SER16, LYS24, LYS34, ARG33, and ionically with LYS34 Y LYS24; through H-bonds, 5′-O - ({[((Phosphonatooxy) phosphinato] oxy} phosphinato) adenosine interacts with GLY18, SER16, PH19, LYS17, LYS40, SER16, ALA39, ARG129, and by ionic bonds with LYS17, LYS40, ARG129. For the ligand (2S) -2 -

**Table 29.1** Docking results showing the binding and solvation energies (in kcal/mol) and interacting residues in FABP5-nutraceuticals complex

| Ligand | Binding energy (kcal/mol) | Solvation energy (kcal/mol) | Interacting residues | | |
|---|---|---|---|---|---|
| | | | H-bonds | Ionic | pi-H |
| NADH(2-) | −9.12 | −73.18 | GLU21, VAL14, ASP15, ASP20, **SER16**, **LYS24**, **LYS34**, ARG33 | **LYS34**, **LYS24** | |
| 5′-O-({[(Phosphonatooxy)phosphinato] oxy}phosphinato)adenosine | −8.62 | −73.76 | GLY18, SER16, PH19, **LYS17**, **LYS40**, **SER16**, ALA39, ARG129 | **LYS17**, **LYS40**, ARG129 | |
| Lutein | −8.25 | −49.38 | GLU71 | | |
| (2S)-2-[(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl) methyl]amino}benzoyl)amino] pentanedioate | −7.17 | −54.8 | ASP15, MET38, **SER16**, PHE19, ASP20, **LYS17**, GLY18, **LYS34**, **LYS40** | **LYS34** | **SER16** |
| Pteroyl-L-glutamate | −6.86 | −48.23 | ASP15, **SER16**, PHE19, GLY18, **LYS34**, **LYS17** | **LYS34** | |
| (1S,3R,5E,7Z)-9,10-Secocholesta-5,7,10-triene-1,3,25-triol | −6.79 | −42.28 | GLU21, **LYS40** | | |

1. In bold are sites of post-translational modifications

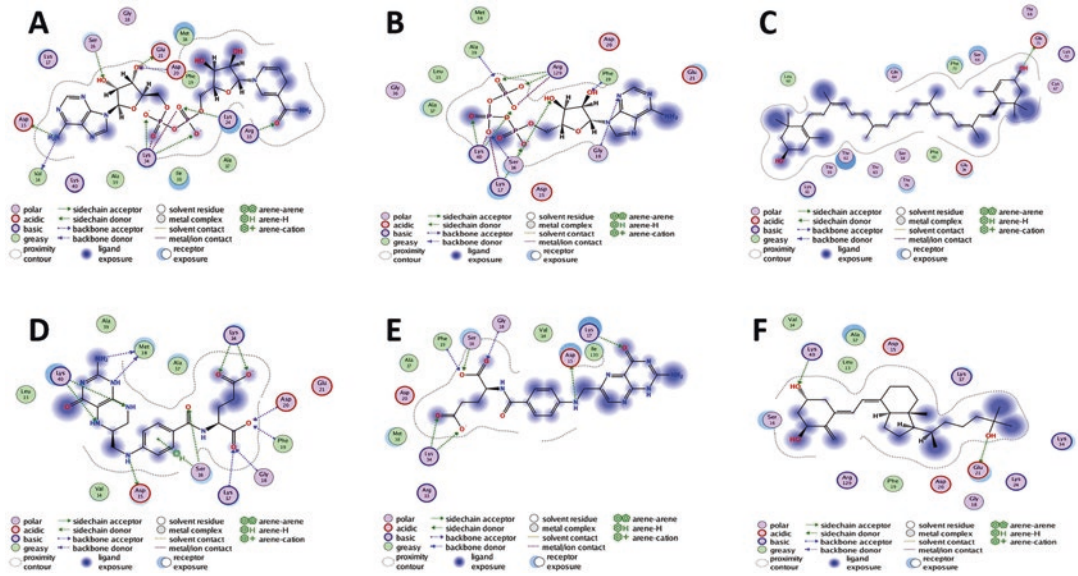


**Fig. 29.2** 2D representation of the binding interaction of FABP5 with selected nutraceuticals. (**a**) NADH(2-); (**b**) 5′-O-({[(Phosphonatooxy)phosphinato]oxy}phosphinato)adenosine; (**c**) lutein; (**d**) (2S)-2-[(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)methyl]amino}benzoyl)amino]pentanedioate; (**e**) Pteroyl-L-glutamate; and (**f**) (1S,3R,5E,7Z)-9,10-Secocholesta-5,7,10-triene-1,3,25-triol

[(4 - {[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl) methyl] amino} benzoyl) amino] pentanedioate the interaction by H-bonds includes the residues ASP15, MET38, SER16, PHE19, ASP20, LYS17, GLY18, LYS34, LYS40, and by ionic with LYS34 and pi-H with SER16. The Pteroyl-L-glutamate forms H-bonds with ASP15, SER16, PHE19, GLY18, LYS34, LYS17) and ionic with LYS34. Finally, the ligand (1S, 3R, 5E, 7Z) -9,10-Secocholesta-5,7,10-triene-1,3,25-triol forms H-bonds with GLU21 and LYS40 (Figs. 29.2, and 29.3 and Table 29.1). Binding sites of a ligand onto a protein can induce post-translational modifications, potentially changing the functionality and conformation of protein. Interestingly, some of the residues through which the ligands interact with FABP5 are sites of these modifications, suggesting that in addition to binding to them, the selected nutraceuticals can modulate their response depending on the type of modification. We found that the residue SER16 may be related to phosphorylation, while LYS17 (site for acetylation, ubiquitylation and succinylation), LYS24 (site for ubiquitylation and mono-methylation), LYS34 (ubiquitylation) and finally LYS40 might be implicated in acetylation and ubiquitylation (Table 29.1).

Based on a more detailed analysis of the interaction of six nutraceuticals with FABP5, we calculated different docking scores (Table 29.2), noting that 5′-O - ({[((Phosphonatooxy) phosphinato] oxy} phosphinato) adenosine has a lower RMSD with a major electrostatic potential energy, van der Waals potential energy and overall potential energy (E). For the radius of gyration, a measure that determines the conformation of a protein in terms of its stability and folding, we observed that 5′-O - ({[(Phosphonatooxy) phosphinato] oxy} phosphinato) adenosine is the one with the lowest value, while lutein shows the highest value compared with other ligands. ASA of a protein, the parameter that denotes the water accessible surface, suggests that lutein followed by NADH (2-) are the ones that present the highest values when compared to the others.

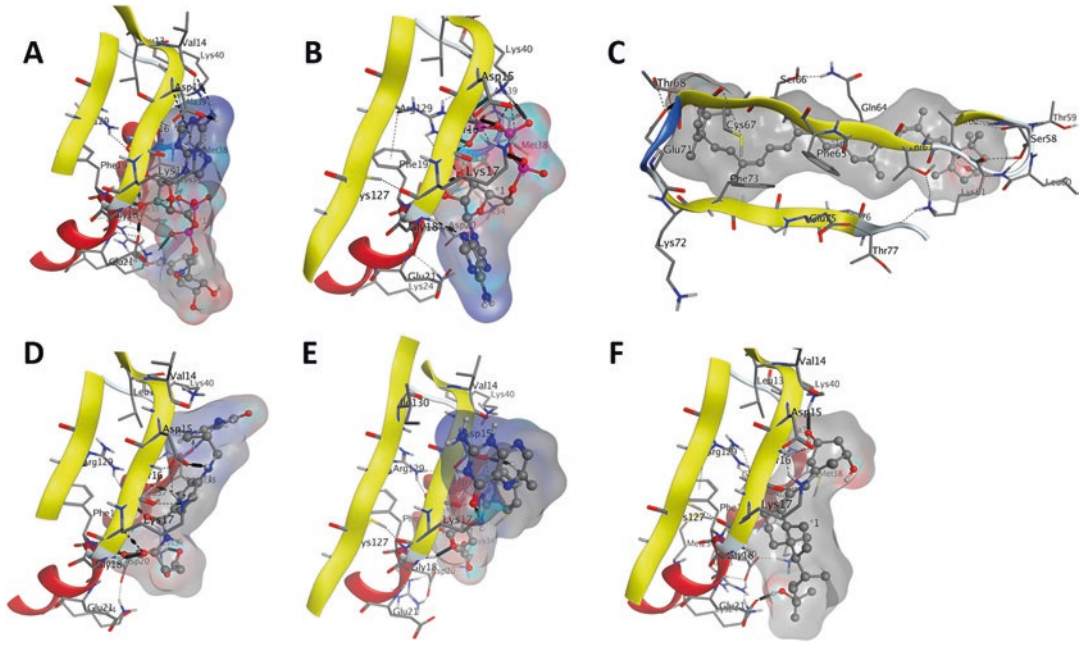


**Fig. 29.3** 3D representation of the binding interacting site of FBP5 with nutraceuticals. (**a**) NADH(2-); (**b**) 5′-O-({[(Phosphonatooxy)phosphinato]oxy}phosphinato)adenosine; (**c**) lutein; (**d**) (2S)-2-[(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)methyl]amino}benzoyl)amino]pentanedioate; (**e**) Pteroyl-L-glutamate; and (**f**) (1S,3R,5E,7Z)-9,10-Secocholesta-5,7,10-triene-1,3,25-triol

### 29.3.3 ADME Properties of All Nutraceuticals

ADME serves as a score to determine the ligand's physicochemical and pharmacokinetic characteristics, being useful to determine whether a drug could be considered as a potential therapeutic agent. The properties that ADME determines include absorption, bioavailability, hepatic metabolism, and excretion. According to the Lipinski's rule of five, a potential drug must meet the following criteria: no more than 5 h-bond donors, no more than 10 h-bond acceptors, a molecular weight of less than 500 daltons, a logP not exceeding 5 (high lipophilicity) and molar refractivity between 40 and 130. Within the selected ligands, only pteroyl-L-glutamate fulfilled the criteria, which suggests its potential for pharmacological therapy (Table 29.3). Importantly, ADME parameters (Absorption, Distribution, Metabolism and Excretion) are essential in the discovery phase of potential drugs as they increase the success rate in the search of novel compounds that can pass to clinical phases [9].

## 29.4 Conclusions

In conclusion, the present study evaluated *in silico* interactions of FABP5 with 6 putative ligands with nutraceutical properties. Our results suggest that NADH (2-) presents the greatest interaction energy, followed by 5′-O - ({[(Phosphonatooxy) phosphinato] oxy} phosphinato) adenosine, lutein, (2S) -2 - [(4 - {[(2-Amino-4-oxo-1,4,5, 6,7,8-hexahydro-6-pteridinyl) methyl] amino} benzoyl) amino] pentanedioate, pteroyl-L-glutamate and (1S, 3R, 5E, 7Z) -9,10-Secocholesta-5,7,10-triene-1,3,25-triol. We further evaluated the physicochemical and pharmacokinetic characteristics of the ligands and found that only pteroyl-L-glutamate fulfilled

**Table 29.2** Docking parameters of the interaction between FABP5 and nutraceuticals

| Ligand | rmsd | ASA | E | E_ele | E_rele | E_rvdw | E_vdw | MNDO_E | HOMO | LUMO | rgyr |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NADH(2-) | 2.82291 | 839.5574 | −35.4412 | −108.171 | −40.8406 | −37.8426 | −0.57688 | −215462 | −3.41347105 | 3.46229598 | 5.534173 |
| 5′-O-({[(Phosphonatooxy)phosphinato]oxy}phosphinato)adenosine | 1.938272 | 605.9523 | −49.9683 | −137.942 | −72.2703 | −29.9188 | −2.2277 | −164470 | 4.75841895 | 7.14563996 | 4.278326 |
| Lutein | 2.880526 | 1057.088 | 18.5405 | −47.9968 | 3.435218 | 3.07E+11 | 27.81394 | −151039 | −8.677676188 | −0.38554375 | 8.874208 |
| (2S)-2-[(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)methyl]amino}benzoyl)amino]pentanedioate | 2.663734 | 694.2721 | 44.12733 | 2.329971 | −40.9342 | −30.1765 | 11.17686 | −140888 | −1.830259 | 2.40383797 | 5.411572 |
| Pteroyl-L-glutamate | 2.111345 | 700.5408 | 34.00002 | −14.7785 | −44.5371 | 80275.5 | 14.16352 | −139570 | −1.678346 | 1.39721699 | 5.648998 |
| (1S,3R,5E,7Z)-9,10-Secocholesta-5,7,10-triene-1,3,25-triol | 2.164886 | 719.0398 | 32.38068 | −49.6568 | 8.136407 | 1.21E+11 | 18.28136 | −116296 | −9.1618599 | −0.072299003 | 5.045943 |

Abbreviations: *RMSD* Root-mean-square deviation, *ASA* water accessible surface area, *E* potential energy, *E_ele* electrostatic potential energy, *E_rele* electrostatic interaction energy, *E_rvdw* van der Waals interaction energy, *E_vdw* van der Waals potential energy, *MNDO_E* total SCF energy (kcal/mol) calculated using the MNDO Hamiltonian, *HOMO* energy of the highest occupied molecular orbital, *LUMO* energy of the lowest unoccupied molecular orbital, rgyr radius of gyration

**Table 29.3** ADME properties and characteristics of selected nutraceutical ligands

| Ligand | Molecular weight | H-bond donor | H-bond acceptor | Rotatable bonds | Molecular Refractivity | LogP | GI solubility | BBB solubility | Pgp substrate |
|---|---|---|---|---|---|---|---|---|---|
| NADH(2-) | 663.43 | 6 | 17 | 11 | 142.18 | −4.15 | Low | No | Yes |
| 5′-O-({[(Phosphonatooxy)phosphinato]oxy}phosphinato)adenosine | 503.15 | 3 | 16 | 8 | 89.28 | −3.67 | Low | No | Yes |
| Lutein | 568.87 | 2 | 2 | 10 | 186.76 | 9.25 | Low | No | Yes |
| (2S)-2-[(4-{[(2-Amino-4-oxo-1,4,5,6,7,8-hexahydro-6-pteridinyl)methyl]amino}benzoyl)amino]pentanedioate | 443.41 | 6 | 7 | 10 | 117.15 | −1.2 | Low | No | No |
| Pteroyl-L-glutamate | 439.38 | 4 | 9 | 10 | 108.03 | −0.82 | Low | No | No |
| (1S,3R,5E,7Z)-9,10-Secocholesta-5,7,10-triene-1,3,25-triol | 416.64 | 3 | 3 | 6 | 127.4 | 5.08 | High | No | No |

the Lipinski's criteria, suggesting its potential application for pharmacological uses. However, additional computational and experimental validation studies are needed to establish the *in vivo* and *in vitro* interactions of FABP5 and its regulatory mechanisms against anti-inflammatory processes.

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**Conflict of Interests** None of the authors has a competing interest directly related to the content of this study.

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# Index





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