Pharmacological Properties of Essential Oil Constituents and their Mechanisms of Action

18

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Contents

Abstract

Since time immemorial, essential oils (EOs) have been utilized for their therapeutic properties. However, during recent decades, a renewed interest has been experienced in EOs and their individual constituents due to their remarkable pharmacological potentials evidenced by various experimental studies. For instance, they are broadly acknowledged for their antimicrobial, antidiabetic, anti-inflammatory, antispasmodic and anticancer properties. Also, EOs have demonstrated to improve conditions associated with brain and cardiovascular diseases amongst others. Multiple studies have identified and isolated EOs phytocomponents possessing therapeutic activities. In addition, studies have elucidated their underlying mechanisms of actions involved in the treatment and management of several ailments.

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Thus, given the wide spectrum of biological activities exhibited by these active compounds, this chapter endeavours to provide a mechanistic overview of some common EOs constituents with regard to their pharmacological properties.

18.1 Introduction

Essential oils (EOs) are volatile aromatic, hydrophobic and oily liquids obtained from plants. They may be produced from cells or groups of cells specialized within the specific parts of plants, such as leaves, bark or wood, foliage, fruits, seeds, stems and rhizomes (Miguel 2010). EOs play a significant role in protecting the plants against bacterial, viral, fungal and insect attacks, and also herbivores by decreasing their appetite for the plants. Besides, they serve in attracting some insects for seeds and pollen dispersal or help to keep away unwanted insects (Bakkali et al. 2008).

EOs can be obtained by a number of different techniques, including fermentation, expression or enfleurage, although hydrodistillation is the most commonly used one (Speranza and Corbo 2010). A large number of herbal plants have been studied for their EOs and exploited for commercial applications (Mohammadreza 2008), particularly in cosmetics, perfumery, agriculture and food industries (Burt 2004).

EO constituents can be categorized into two distinct classes of chemicals, namely terpenes and phenylpropanoids. Even though terpenes and their derived oxygenated compounds (terpenoids) are more common and rich in EOs, certain species are composed of high amounts of shikimates, notably phenylpropanoids, which confer a specific flavour and odour to the plant (Tisserand and Young 2013; Baser and Buchbauer 2015; Zuzarte and Salgueiro 2015). However, it is important to note that EOs composition can vary depending on factors, such as variety of plants, parts of the plant, growth area, time of harvest, climatic changes, conditions of storage as well as the chemotype of each constituent (Pauli and Schilche 2009).

EOs and their active components have been extensively studied for their vast range of biological activities along with their underlying mechanisms of actions. For instance, they have been reported to act as natural antimicrobials (Edris and Farrag 2003; Burt 2004; Swamy et al. 2016) and have even been evaluated for their potential uses as substitute antidotes for the treatment of several infectious diseases (Freires et al. 2015). Additionally, they are regarded as potent antioxidants, anti-inflammatory (Miguel 2010; Pérez et al. 2011) and antidiabetic agents (Tahir et al. 2016; Ya'ni et al. 2018), amongst others. EOs have also demonstrated anticarcinogenic effects through a range of mechanisms, including acting directly on the tumour cells or by interaction with the microenvironment (Edris 2007;

Sitarek et al. 2017). Moreover, their importance in aromatherapy has been widely documented. For instance, in medical aromatherapy, EOs have been reported to help in health promotion and treatment of clinically diagnosed medical diseases (Maeda et al. 2012).

Even though EOs major constituents are crucial for their bioactivities, minor components can play a significant role as well, since they can increase the effects of the major constituents. However, some of these EOs compounds have been shown to exhibit both additive and antagonistic effects (Bassolé and Juliani 2012). Therefore, EOs bioactivity can be regarded as the sum of its components acting either synergistically or antagonistically (Baser and Buchbauer 2010; Elshafie et al. 2015). This chapter aims to discuss different mechanisms of actions demonstrated by EOs constituents (in synergism, as single agents or as potentiating agents in tandem with conventional drugs) in relation to their pharmacological properties.

18.2 Essential Oils (EOs) Major Compounds and Their Mechanisms of Actions

18.2.1 Antimicrobial Property

The antimicrobial potentials of EOs and their components have been extensively studied in the search for more effective antimicrobials to combat antibiotic-resistant pathogenic microorganisms (Swamy et al. 2016). In that aspect, EOs that are abundant with phenols or aldehydes, such as cinnamaldehyde, citral, thymol, eugenol or carvacrol (Fig. 18.1) were found to exhibit the highest antibacterial potential, followed by those composed of terpene alcohols like terpineol, fenchyl alcohol, and borneol. On the other hand, volatile oils consisting of esters or ketones like geranyl acetate, α-thujone or β-myrcene demonstrated much weaker activity, while EOs containing terpene hydrocarbons were generally not active (Davidson 1997; Dorman and Deans 2000). Besides, EOs rich in phenolic compounds like eugenol, carvacrol and thymol (Fig. 18.1) are usually characterized by significant antibacterial activities (Knobloch et al. 1986; Dorman and Deans 2000; Lambert et al. 2001; Swamy et al. 2016). In addition, these compounds have been held accountable for the disruption of plasma membrane, protons driving force, active transport, electron flow as well as the coagulation of cellular contents (Sikkema et al. 1994; Denyer and Hugo 1991; Pauli 2001; Swamy et al. 2016). Thus, the antimicrobial activity of EO constituents is determined by the lipophilicity of their hydrocarbon skeleton as well as the hydrophilicity of their major functional groups, and accordingly it has been classified in the following order: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons (Kalemba and Kunicka 2003).

A major compound of EOs, eugenol (Fig. 18.1) has been evaluated for its antibacterial effect and its mechanism of action against *Salmonella typhi* has been elucidated (Devi et al. 2010). According to them, eugenol was observed to decrease the viability of cells and cause absolute inhibition of the bacterium with the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of
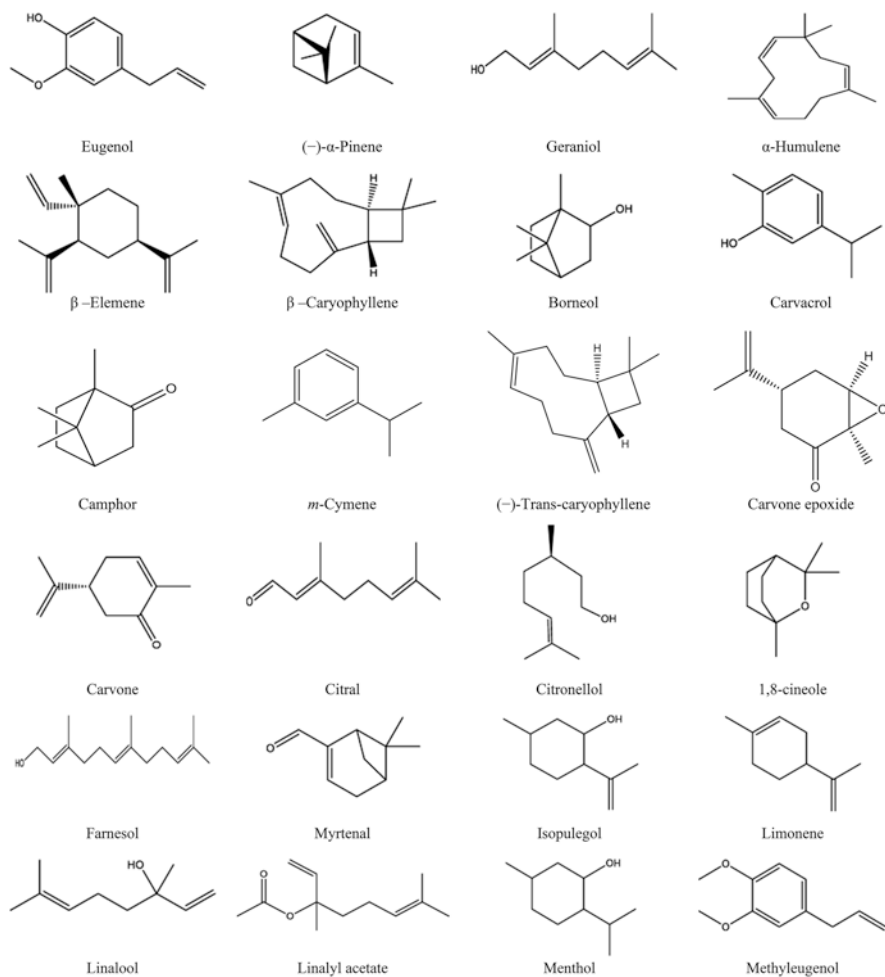
Fig. 18.1 Chemical structures of EO constituents discussed in book chapter

0.0125% and 0.025%, respectively. Additionally, inactivation of *S. typhi* was induced within 60 min of exposure to eugenol. Eugenol's chemoattractant property along with the observed strong antibacterial activity at alkaline pH suggests that this compound can act more effectively when given in vivo. Furthermore, the interaction of eugenol on *S. typhi* cell membrane was found to be responsible for its antibacterial action. In this regard, eugenol was noted to cause increased membrane permeability, disruption of bacterial cell membrane and distortion of membrane macromolecules. Likewise, other studies have confirmed the antibacterial activity of eugenol to be indicative of its disruptive action on cytoplasmic membrane, thus enhancing its non-specific permeability (Gill and Holley 2006; Swamy et al. 2016). It has also been
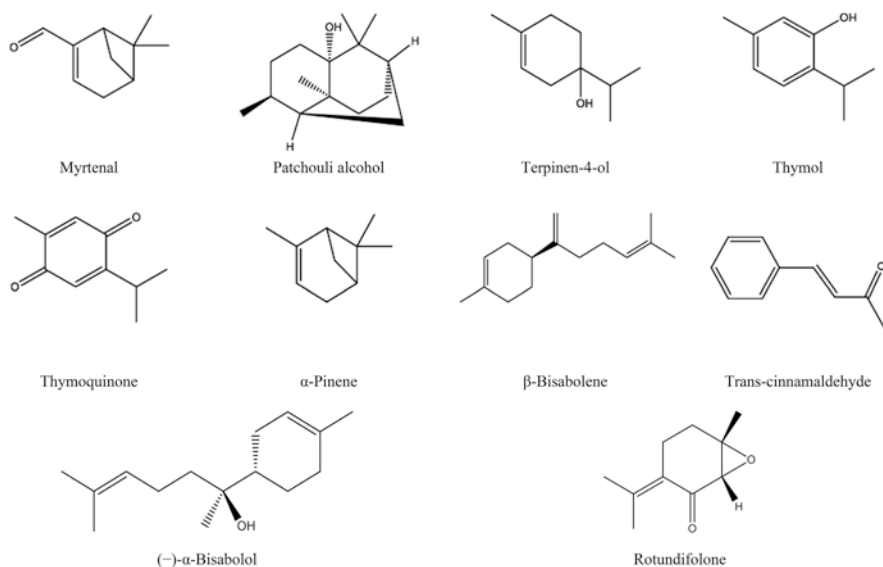
Myrtenal   Patchouli alcohol   Terpinen-4-ol   Thymol

Thymoquinone   α-Pinene   β-Bisabolene   Trans-cinnamaldehyde

(−)-α-Bisabolol   Rotundifolone

Fig. 18.1   (continued)

highlighted that the hydrophobic characteristic of eugenol permits its penetration in the lipopolysaccharide of the gram-negative bacterial cell membrane, hence modifying the structure of the cell and resulting into the escape of intracellular components (Burt 2004). Also, the hydroxyl group of eugenol is believed to attach to proteins, which then prevents enzyme action in *Enterobacter aerogenes* (Burt 2004).

The mechanism of action of (−)-α-pinene (Fig. 18.1) in relation to its modulation of antibiotic resistance in *Campylobacter jejuni* has also been reported by Kovač et al. (2015). Remarkably, (−)-α-pinene was found to effectively modulate antibiotic resistance in *C. jejuni* by reducing the MIC of erythromycin, triclosan and ciprofloxacin by up to 512-fold. Further, they used insertion mutagenesis method using ethidium bromide to identify the target antimicrobial efflux systems, and DNA microarrays were used to evaluate *C. jejuni* adaptability to (−)-α-pinene. The results showed that (−)-α-pinene promoted the expression of cmeABC as well as Cj1687, a putative antimicrobial efflux gene. The accumulation of ethidium bromide was found higher in the wild-type strain as compared to the antimicrobial efflux mutant strains. Thus, it confirmed that these antimicrobial efflux systems were the target of action of (−)-α-pinene. Furthermore, a decrease in membrane integrity induced by (−)-α-pinene implied that the enhanced microbial influx was the secondary mode of action of (−)-α-pinene. It was also revealed from the findings that (−)-α-pinene caused the disruption of several metabolic pathways, in particular those implicated in heat-shock retorts. Therefore, the inhibition of microbial efflux and reduced membrane integrity including metabolic disruption were amongst the mechanisms of (−)-α-pinene involved in the modulation of antibiotic resistance in *C. jejuni*.

Kannappan and co-authors (2019) demonstrated the combinatorial antibiofilm efficacy of geraniol (Fig. 18.1) and cefotaxime against *S. epidermidis* (ATCC 35984) and MRSA (ATCC 33591). Cefotaxime, a broad-spectrum third-generation cephalosporin antibiotic, acts by binding to one or more of the penicillin-binding proteins and hence causes inhibition of bacterial cell wall formation (Luthy et al. 1979). Significant diminution in biofilm biomass and slime formation was observed following treatment with geraniol and cefotaxime combination (GCC), yielding a minimal biofilm inhibitory concentration of 100 μg/mL and 2 μg/mL of geraniol and cefotaxime respectively. The results also revealed that GCC targeted the initial attachment of the cells associated with biofilm formation. Moreover, treatment of the test pathogens to GCC reduced the production of staphyloxanthin pigment in MRSA and consequently rendered the pathogenic cells vulnerable to the host immune responses. In addition to inhibiting biofilm formation, GCC was found to suppress the production of extracellular polymeric substance (EPS) and slime. GCC treatment was also found to induce reduced expression of surface adhesin genes, and in MRSA, the gene responsible for the production of virulence factor such as staphylococcal enterotoxin A was downregulated. Microscopic analysis made on GCC-treated EPS corroborated with the findings of the in vitro biofilm inhibition assay, thus establishing the destructive effect of GCC on the evaluated pathogens' biofilm formation. Besides, GCC considerably increased the vulnerability of the test pathogens towards human blood. In vivo assay conducted on *Caenorhabditis elegans* also revealed the antibiofilm potential of GCC in the control of biofilm-associated infections caused by *Staphylococcus* species. For instance, while the nematodes exposed to the test pathogens demonstrated an enhanced colonization together with deformed pharynx and internal hatching of eggs, those treated with GCC and test pathogens cells displayed reduced internal colonization as well as healthy pharynx (Kannappan et al. 2019).

Moreover, Yuan and Yuk (2019) investigated the adaptive responses of *E. coli* O157:H7 with regard to its virulence gene expression and virulence properties at the sublethal concentrations of trans-cinnamaldehyde (TC) (Fig. 18.1), carvacrol (Car) (Fig. 18.1) and thymol (Thy) (Fig. 18.1). *E. coli* O157:H7 grown to the early stationary phase in the presence of sublethal EOs demonstrated notable reduction in motility (reversible subsequent to stress elimination), biofilm formation capacity and efflux pump activities, without inducing antibiotic resistance or infection, since no marked changes were noted with regard to the invasive and adhesive capacity of the test pathogens on human colon adenocarcinoma (Caco-2) cells. Reduced expression of related virulence genes, together with those encoding biosynthesis and functioning of flagella, biofilm formation regulators and multidrug efflux pumps, in addition to type III secretion system components, was reported. Hence, TC, Thy and Car at sublethal doses did not seem to cause potentiation of virulence in adapted *E. coli* O157:H7 (Yuan and Yuk 2019).

Further, Guimarães et al. (2019) investigated the antibacterial activity of several terpenoids and terpenes found in EOs. In general, the results showed that oxygenated functional groups in terpenes showed better antibacterial action compared to hydrocarbons. Out of 16 compounds identified to possess antibacterial activity,

carvacrol, eugenol and thymol were found to be the most potent; even greater than sulfanilamide against the four strains tested (*B. cereus, E. coli, S. aureus* and *S. typhimurium*). Contrastingly, compounds such as borneol, camphor, *m*-cymene, (±)-linalool and (+)- and *R*-(+)-limonene (Fig. 18.1) displayed the least antibacterial activity. Nonetheless, only six of the 16 compounds that showed antimicrobial action were seen to be bactericidal (absence of growth) in the study. For instance, none of compounds tested were found to be bactericidal against *B. cereus*, while only terpineol and thymol demonstrated bactericidal activity against *S. aureus*. However, the lowest MBC value against *S. typhimurium* (0.06 mg/mL) was presented by eugenol. Moreover, although thymol exhibited the lowest MIC values against the tested strains, it did not show any bactericidal action at concentrations of MIC, 2× MIC as well as 4× MIC, but rather at concentration 0.12 mg/mL. Eugenol and thymol were observed to cause inhibition (IC_{100}) of *S. aureus* and *S. typhimurium* growth. The morphological changes detected in *E. coli, S. aureus* and *S. typhimurium* that were treated with β-citronellol, L-carveol and trans-geraniol were also analysed. The treated *E. coli* cells were of irregular sizes with the presence of debris, probably due to interrupted cell division or cell membrane dysfunction compared to the control cells which had smooth surfaces and bacillary shapes. Besides, cells treated with geraniol and citronellol were smaller, had considerably rough surfaces and the cells were adhered to one another. On the other hand, *S. aureus* treatment with terpineol disrupted cell division and were found to have a distorted "grape bunch" shape, a typical morphology of the colonies. As for *S. typhimurium*, terpineol and eugenol treatment revealed the loss of cell membrane integrity or function as the death mechanisms involved, whereby the cell membrane was completely destroyed along with the presence cell debris. Further, carveol, citronellol, eugenol, geraniol and terpineol were found to be fast-acting compounds, given that they caused *E. coli* and *S. typhimurium* inactivation within 2 h. The authors also pointed out that the compounds that exhibited the best activity in relation to their MIC and time-kill kinetics had low molecular weights as well as polar functional groups. Such features could enhance antimicrobial potential by facilitating permeation via the outer cell membrane as confirmed by eugenol action, a low-molecular-weight phenolic compound that displayed fast time-kill kinetics leading to the death of *S. typhimurium* at all concentrations in only 2 h (Guimarães et al. 2019).

Carvacrol (Fig. 18.1), a major monoterpene of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) volatile oils, has been reported to possess a strong antifungal activity against *Candida albicans* (Chaillot et al. 2015). In the attempt of understanding the underlying mechanism of action, they demonstrated that fungal cells need the UPR (unfolded protein response) signalling path to be able to counterattack carvacrol. Moreover, carvacrol was found to act as an ER (endoplasmic reticulum) stress inducer, disturbing the morphology as well as the integrity and protein-folding capacity of the ER leading UPR activation. Carvacrol also enhanced the antifungal action of fluconazole, echinocandin and caspofungin (antifungal drugs) as well as UPR inducers, such as tunicamycin and dithiothreitol against *C. albicans*.

Furthermore, Xia et al. (1999) demonstrated in their study that α-pinene (Fig. 18.1) exerts a significant antifungal activity against *C. albicans*. The

antifungal mechanisms that were put forward included the rupture of *C. albicans* cell wall and cytoplasmic membrane, the release of intracellular components and fusion of cell residues into irregular masses. Besides, there was the inhibition of RNA, DNA, cell wall polysaccharide and cell membrane ergosterol synthesis.

Linalool and geraniol (Fig. 18.1), two major oxygenated monoterpenes present in EOs of various medicinal plants such as *Pelargonium graveolens*, *Peperomia pellucida* and *Acorus calamus* (Souza et al. 2016; Okoh et al. 2017; Parki et al. 2017), have also been tested for their anticandidal potential against five *Candida* species of ATCC strains (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. tropicalis*) (Singulani et al. 2018). Although both linalool and geraniol were found to inhibit candidal growth in vitro, geraniol was observed to be more effective. Furthermore, while *C. albicans* was mostly resistant to the compounds (MIC ≥ 1000 μg/mL), *C. parapsilosis* was susceptible (MIC 37.5 and 125 μg/mL for geraniol and linalool, respectively) (Singulani et al. 2018).

18.2.2 Antidiabetic Property

Citronellol (Fig. 18.1), which is a monoterpene alcohol commonly found in citrus species, such as oranges, lemons, and pomelos, has been evaluated for its antidiabetic effect in streptozotocin (STZ)-induced diabetic rats (40 mg/kg body weight (b.w.)) (Srinivasan and Muruganathan 2016). According to them, citronellol administered orally for 30 days (50 mg/kg) was observed to improve the insulin, hepatic glycogen and haemoglobin levels along with a substantial decline in the levels of glucose and glycated haemoglobin (HbA1c). Additionally, changes in enzyme activities involved in carbohydrate metabolism, kidney and hepatic biomarkers were brought to near-normal levels in citronellol-treated rats. Hypoplasia and extensive damages of the islets of langerhans in STZ-induced diabetic rats were decreased in groups treated with citronellol. Moreover, citronellol supplement enhanced insulin immunoreactivity and augmented the number of immunoreactive β-cells in the treated group. Thus, citronellol demonstrated the capacity to improve the secretion of insulin or regeneration of the β-cells in the diabetic animals (Srinivasan and Muruganathan 2016).

The effect of carvone (Fig. 18.1), a monoterpene ketone, on carbohydrate metabolic enzymes in the liver of STZ-induced diabetic rats (40 mg/kg b.w.) was also studied in a similar way by Muruganathan and Srinivasan (2016). Carvone (50 mg/kg b.w.) daily oral administration in diabetic rats for 30 days resulted into a significant decrease in the levels of HbA1c and plasma glucose, while a considerable amelioration in haemoglobin and insulin levels was noted. Carvone administration also caused a reversal in the activities of the carbohydrate metabolic enzymes, enzymatic antioxidants and hepatic marker enzymes that were eventually restored close to normal levels in the diabetic rats. Gliclazide was also used as a standard oral hypoglycaemia drug for comparison. Moreover, carvone treatment was found to reduce the STZ-induced damage in hepatic and pancreas β-cells (Muruganathan and Srinivasan 2016).

The anti-hyperglycaemic property of eugenol was also determined by assessing the activities of key enzymes of glucose metabolism in STZ-induced diabetic rats (40 mg/

kg b.w.) (Srinivasan et al. 2014). Eugenol was intragastrically administered in diabetic rats for 30 days at 2.5, 5 and 10 mg/kg b.w. At an effective dose of 10 mg/kg b.w., remarkable decrease in blood glucose and HbA1c levels and increase in plasma insulin level were reported. Eugenol also caused altered activities of key enzymes involved in carbohydrate metabolism, for instance, pyruvate kinase, glucose-6-phosphate dehydrogenase, hexokinase, fructose-1,6-bisphosphatase, glucose-6-phosphatase and liver marker enzymes (serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) and creatine kinase including blood urea nitrogen in serum and blood to significantly reverse to near-normal levels in diabetic rats. In furtherance, eugenol improved body weight and hepatic glycogen level in the treated group, thus demonstrating the potential of eugenol as an anti-hyperglycaemic agent in STZ-induced diabetic rats (Srinivasan et al. 2014).

Likewise results were obtained by myrtenal (Fig. 18.1), a natural monoterpene that has been evaluated for its efficacy as an anti-hyperglycaemic agent as well as its β-cell protective properties in STZ-induced diabetic rats (Rathinam et al. 2014). Myrtenal was orally administered to diabetic rats for 28 days resulting into a significant rise in insulin and haemoglobin levels and reduction in the levels of HbA1c and plasma glucose. The diabetic rats subjected to myrtenal treatment were also protected from loss of body weight. The altered activities of the key metabolic enzymes implicated in carbohydrate metabolism and hepatic enzymes in the diabetic rats were considerably improved by myrtenal administration. Myrtenal also caused an improvement in hepatic and muscle glycogen content in diabetic rats. Further, histopathological analysis unveiled the restoration of reduced islet cells to near-normal conditions, and alteration in the liver architecture was also averted following myrtenal treatment (Rathinam et al. 2014).

The acyclic monoterpene alcohol, geraniol occurring in several medicinal plants exhibits numerous medicinal properties including antidiabetic effect. According to a study by Babukumar et al. (2017), the administration of geraniol at its effective dose of 200 mg/kg b.w. for 45 days relatively enhanced the levels of insulin, haemoglobin and reduced HbA1c and plasma glucose in diabetic-treated rats. Geraniol ameliorated the key enzymes involved in glucose metabolism. Further, the content of hepatic glycogen was improved in diabetic rats that were treated with geraniol. This suggests that geraniol possessed anti-hyperglycaemic potential.

Several EO constituents have also been evidenced to exhibit enhanced antidiabetic effects in combination with antidiabetic drugs. For instance, carvacrol, was evaluated for its anti-hyperglycaemic activity and potential to improve dysregulated carbohydrate metabolism in combination with rosiglitazone in high-fat diet (HFD)-induced type 2 diabetic C57BL/6 J mice (Ezhumalai et al. 2014). The post-oral administration of carvacrol and rosiglitazone was given at 20 mg/kg b.w. and 4 mg/kg b.w., respectively, for 35 days. The HFD mice demonstrated increased levels of insulin, glycosylated haemoglobin and plasma glucose and a declined level of haemoglobin. Besides, there were higher activities of enzymes involved in carbohydrate metabolism in the liver of HFD mice. However, the treatment of diabetic mice with carvacrol and rosiglitazone resulted in increased glucokinase activity due to improved sensitivity of insulin. Likewise, glucose-6-phosphate dehydrogenase

activity was augmented causing enhanced utilization of glucose. In addition, activities of gluconeogenic enzymes (fructose-1,6-bisphosphatase and glucose-6-phosphatase) were significantly reduced in carvacrol- and rosiglitazone-treated mice resulting into reduced levels of blood glucose. Furthermore, the activities of hepatic marker enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase), which were in elevated levels in HFD mice, were restored to normal levels through improved insulin resistance. These results were also supported by histopathological analysis of pancreas, which was in line with the biochemical findings.

18.2.3   Cardioprotective Property

The cardioprotective activities of EOs and their derived components have been widely documented. Included amongst them is 1,8-cineole (Fig. 18.1) a major monoterpenic oxide present in many EOs, which has been evaluated for its effects on systolic blood pressure (SBP) as well as oxidative stress in rats that have been constantly exposed to nicotine via intraperitoneal injection (Moon et al. 2014). A remarkable decline in SBP was observed along with a significant increase in the levels of plasma nitrite in 1,8-cineole-treated rats compared to those exposed to nicotine alone. Besides, a significant rise in the levels of lipid peroxidation and plasma corticosterone was detected in untreated rats. The elevated levels of plasma corticosterone were not reduced by 1,8-cineole; however the increase in lipid peroxidation levels was considerably antagonized in 1,8-cineole-treated rats (0.01 and 0.1 mg/kg). Thus, the results were indicative that the antihypertensive effect of 1,8-cineole led to reduced SBP, which may be linked to the control of nitric oxide levels and oxidative stress in rats that were chronically exposed to nicotine (Moon et al. 2014).

The cardiovascular effects of 1,8-cineole have also been reported by other authors in both in vitro and in vivo experimental models. For instance, Lahlou et al. (2002) demonstrated that 1,8-cineole administration via bolus injections (0.3–10 mg/kg, i.v.) in both conscious and anaesthetized rats induced comparable and dose-dependent declines in mean aortic pressure. Additionally, at the highest dose, i.e., 10 mg/kg, 1,8-cineole caused a significant reduction in heart rate. Moreover, 1,8-cineole-induced hypotension (10 mg/kg) was found to be related to significant bradycardia in both conscious and anaesthetized rats. This resulting effect seemed to be from vagal origin, given that it was significantly decreased with methylatropine pretreatment (i.v.) or by bilateral vagotomy. Furthermore, in the same study, in vitro experiments performed using isolated rat aorta preparations revealed that 1,8-cineole at 0.006–2.6 mM elicited a concentration-dependent decrease in potassium-induced contractions (60 mM). Hence, this study showed that 1,8-cineole displayed hypotensive effects in conscious and anaesthetized rats, which appeared to be associated with active vascular relaxation.

Furthermore, El-Bassossy et al. (2017) showed that geraniol provided effective protection against cardiac dysfunction caused by diabetes. Oral administration of geraniol

(150 mg kg^{-1} day^{-1}) in STZ-injected rats considerably caused the alleviation of the attenuated cardiac systolic function associated with diabetes indicated by hindering the reduction in the rate of rise (dP/dt_{max}) in ventricular pressure and the rise in systolic duration detected in diabetic rats. Additionally, geraniol alleviated impaired diastolic function as demonstrated by restraining the decrease in the rate of fall (dP/dt_{min}) in ventricular pressure and isovolumic relaxation constant (*Tau*) increase in diabetic rats. Additionally, geraniol averted any boost in QTc and T-peak-T-end intervals, left ventricular (LV) ischaemia and arrhythmogenesis markers in diabetic rats. Geraniol also suppressed the exaggerated oxidative stress by preventing 8-isoprotane increase. Moreover, geraniol was able to prevent the inhibition in catalase (CAT) activity although it did not affect the superoxide dismutase (SOD) activity in the heart. Besides, geraniol was observed to partly reduce hyperglycaemia and prevent hypercholesterolemia, but did not have any effect on the adiponectin serum level in diabetic rats.

Moreover, Menezes et al. (2010) investigated the effects of five terpenes, namely, (−)-β-pinene, (+)-α-pinene, (±)-linalool, (±)-citronellol (monoterpenes) and (−)-α-bisabolol (a sesquiterpene) on the blood pressure and heart rate in non-anaesthetized normotensive rats. The monoterpenes were observed to display hypotension associated with tachycardia that could be indicative of an effect on the peripheral vascular resistance with resultant baroreflex response. Alternatively, (−)-α-bisabolol (Fig. 18.1) induced hypotension linked to intense bradycardia, probably as a result of reduced cardiac output. In addition, although all terpenes demonstrated hypotensive effects, terpene hydrocarbons were less efficient as compared to the terpene alcohols.

Linalyl acetate (Fig. 18.1) has also been evaluated for its possible cardiovascular effects on adolescent rats acutely exposed to nicotine (Kim et al. 2017). The levels of nitric oxide, heart rate, systolic blood pressure, vascular contractility and lactate dehydrogenase (LDH) activity were the parameters measured in this study. Significant reductions in both heart rate and LDH activities were noted in linalyl acetate-treated rats. Furthermore, acute nicotine exposure resulted into a minor relaxation effect that was followed by a sustained recontraction stage in contracted mouse aortic rings, while nicotine and linalyl acetate demonstrated a steady relaxation effect. Additionally, treatment with linalyl acetate was found to reduce elevated nitrite levels induced by nicotine. Other authors have also reported the cardiovascular activities of linalyl acetate. For instance, Koto et al. (2006) showed in their study that linalyl acetate as the main component of lavender EO caused vascular smooth muscle relaxation in rabbit carotid arteries, which was partly due to the activation of the nitric oxide/cGMP pathway. Furthermore, linalyl acetate-rich lavender (linalyl acetate 43.73%) aromatherapy was established to cause a rise in coronary flow velocity reserve, hence enhancing coronary circulation in healthy men (Shiina et al. 2008).

Carvacrol and thymol have also been observed to induce vasodilatory effects in isolated rat thoracic aorta preparations (Peixoto-Neves et al. 2010). Both terpenoids caused relaxation of the KCl- and phenylephrine (PHE)-induced contractions of the aortic rings in a concentration-dependent manner. Moreover, carvacrol and thymol were found to totally terminate the phasic component of PHE-provoked

endothelium-containing ring contractions in Ca^{2+}-free medium with 2 mM ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid. In Ca^{2+}-free medium, carvacrol and thymol considerably decreased the $CaCl_2$-induced contractions at 400 μM. In addition, both carvacrol (1000 μM) and thymol (300 μM) significantly diminished the contraction evoked by phorbol dibutyrate (an activator of protein kinase C) at 1 μM. Furthermore, an enhancement was observed in the magnitude of the inhibitory activity in the presence of the thapsigargin, Ca^{2+} pump inhibitor (1 μM). However, none of the two terpenoids were found to modify the resting potential of vascular smooth muscle cells at 1000 μM. Hence, relaxation in rat isolated aorta induced by carvacrol and thymol was endothelium independent, an effect apparently mediated through some mechanisms involving a transduction pathway between release of Ca^{2+} from sarcoplasmic reticulum and by regulating Ca^{2+} sensitivity of the contractile system. It was also proposed that thymol and carvacrol caused blockage of Ca^{2+} influx through the membrane, at low concentrations.

The cardioprotective effects of carvacrol and thymol have also been highlighted by other studies. For instance, Chen et al. (2017) demonstrated in their study that carvacrol provided significant protection of the heart function as well as reduced the myocardial infarct size in myocardial ischaemia/reperfusion (I/R) injured rats. In addition, the levels of SOD and CAT were increased, while the malondialdehyde (MDA) level and cardiomyocytes apoptosis were decreased. Carvacrol treatment also caused the upregulation of phosphorylated ERK without having any effect on p38 mitogen-activated protein kinase (p38MAPK) and c-Jun N-terminal kinase (JNK). Besides, the protective efficiency of carvacrol on cardiomyocytes hypoxic reperfusion (H/R) injury was observed in vitro. Furthermore, carvacrol pretreatment markedly increased the activation of Akt/eNOS pathway in cardiomyocytes subjected to H/R, and carvacrol protective effects were terminated in the presence of the Akt inhibitor LY294002. Hence, the cardioprotective effect of carvacrol was attributable to its anti-apoptotic and antioxidant activities through the activations of the Akt/eNOS and MAPK/ERK signalling pathways. El-Sayed et al. (2016) as well demonstrated that carvacrol and thymol exerted protective effects against doxorubicin (DOX)-induced cardiotoxicity in rats (10 mg/kg i.v.). Administration of carvacrol and thymol (25 and 20 mg/kg p.o., respectively) was found to ameliorate the heart function and oxidative stress parameters. However, thymol was more cardioprotective than carvacrol. A synergistic cardioprotective effect was achieved when carvacrol and thymol were combined together, which might have resulted from its anti-inflammatory including antioxidant and anti-apoptotic activities.

18.2.4 Anticancer Property

In the search for novel anticancer agents derived from plant sources, EOs and their active constituents have been found to be a promising alternative to surmount chemotherapy drug resistance as well as cancer recalcitrance. For instance, thymoquinone (TQ) (Fig. 18.1), a bioactive component obtained from *Nigella sativa* L. seeds and other EOs, has demonstrated notable anticancer potential (Arafa et al. 2011).

Interestingly, TQ was found to significantly restrain doxorubicin-resistant human breast cancer (MCF-7/DOX) cell proliferation. Amongst the different mechanisms proposed, TQ was reported to increase cellular levels of phosphatase and tensin homolog (PTEN) proteins that led to a considerable decline of the phosphorylated Akt cell survival protein. Expression of PTEN was accompanied with increase in PTEN mRNA. Additionally, TQ caused G2/M phase arrest in MCF-7/DOX cells as well as augmented the cellular levels of p53 and p21 proteins. TQ-induced apoptosis was allied with the disruption of mitochondrial membrane potential, caspases activation and cleavage of poly-(ADP-ribose) polymerase-1 (PARP). Upregulation of Bax and downregulation of Bcl2 proteins, resulting in an increase in Bax/Bcl2 ratio, were also observed (Arafa et al. 2011). Mauro et al. (2013) also elucidated the mechanism of action involved in the chemotherapeutic property of D-limonene which was found to be cytotoxic to V79 Chinese hamster cells. It was revealed that D-limonene affected the dividing cells by preventing the assemblage of mitotic spindle microtubules caused by tubulin depolymerization in the early phase of mitosis. Furthermore, both chromosomal segregation and cytokinesis were affected leading to aneuploidy, which consequently resulted into the death of the cell or even genomic instability.

Thymol was also investigated for its chemoprotective effect against genotoxicity induced by bleomycin in human normal lymphocytes as well as anti-proliferative effect on human ovarian cancer cells (SKOV-3) (Arab et al. 2015). Bleomycin (BLM) is an anticancer agent that causes tissue toxicities through DNA damage and death of cells. Samples of blood were treated with BLM following 2 h incubation with thymol at different concentrations (50, 100 and 150 μM). In order to determine the frequency of micronuclei in cytokinesis-blocked binucleated lymphocytes, lymphocytes were cultured with a mitogenic stimulation. A marked decline in the frequency of micronuclei in lymphocytes treated with thymol and BLM was noted compared to blood samples incubated with only BLM (frequency of micronuclei in BLM-treated lymphocytes: 7.79 ± 2.00). Thymol was observed to significantly mitigate micronuclei frequency at doses 50 and 100 μM in BLM-treated lymphocytes (frequency: 2.81 ± 0.99, 2.42 ± 0.16, respectively). Thymol did not demonstrate any genotoxicity in cultured lymphocytes at 150 μM without BLM treatment. Moreover, neither cell protective effect nor improved cell death was observed with thymol pretreatment of SKOV-3 cells. Therefore, this study suggested that thymol provided selective protection to human lymphocytes against DNA damage that was BLM induced without any protection on the killing effect of BLM on cancerous cells (Arab et al. 2015). The effects of thymol on several other cancer lines have been studied as well, whereby their mechanisms responsible for their anticancer or chemopreventive properties have been elucidated (Table 18.1). Likewise, farnesol (Fig. 18.1), an acyclic sesquiterpene alcohol found in the EOs of a variety of plants such as citronella, lemon grass, rose, balsam and neroli (Goossens and Merckx 1997; Azanchi et al. 2014; Krupčík et al. 2015), has been studied for their anticancer effect on different cancer cell lines (Table 18.1).

The anticancer property of patchouli alcohol (PA) (Fig. 18.1), a component isolated from the *Pogostemon cablin* EO, has been tested by Jeong et al. (2013) against

Table 18.1 Effects of thymol and farnesol on various cancer cell lines and anti-cancer mechanisms involved

Cancer type	Cancer cell lines	Anti-cancer mechanisms involved	References
Thymol			
Acute promyelocytic leukaemia	HL-60	Induction of cell cycle arrest at sub-G0/G1 phase, ↑production of reactive oxygen species (ROS) and mitochondrial H_2O_2, depolarization of mitochondrial membrane potential, ↑Bax protein level, ↓expression of Bcl-2 protein, activation of caspase-dependent (caspase-9, -8 and -3 and PARP cleavage) and caspase-independent apoptosis pathways (induction of apoptosis inducing factor (AIF) translocation from mitochondria to cytosol and nucleus)	Deb et al. (2011)
Human colon carcinoma	HCT-116	Induction of oxidative stress through ROS production, DNA and mitochondrial damage, upregulated expression of PARP-1, p-JNK, cytochrome-C and caspase-3 proteins and mitoptotic cell death	Chauhan et al. (2017)
Human glioblastoma	DBTRG-05MG	↑Intracellular Ca^{2+}, release of phospholipase C- and protein kinase C-dependent Ca^{2+} from endoplasmic reticulum, apoptosis and cell death	Hsu et al. (2011)
Human gastric carcinoma	AGS	Cell growth inhibition, apoptosis, production of intracellular ROS, depolarized mitochondrial membrane potential, activation of the proapoptotic mitochondrial proteins (Bax), caspases and PARP in AGS cells	Kang et al. (2016)
Acute T lymphoblastic leukaemia	CEM	Cell cycle arrest at G0/G1 phase	Jaafari et al. (2012)
Mastocytoma	P-815		
Human chronic myelogenous leukaemia	K-562		
Human breast adenocarcinoma	MCF-7		
Human osteosarcoma	MG63	↑ROS, phospholipase C-dependent Ca^{2+} release from endoplasmic reticulum, entry of Ca^{2+} through protein kinase C-sensitive store-operated Ca^{2+} channels, ↑intracellular Ca^{2+}, apoptosis via mitochondrial pathway	Chang et al. (2011)
Farnesol			
Cervical	HeLa	Anti-proliferative, induction of apoptotic cell death, mitochondrial membrane potential loss, downregulated expression of the PI3K and Akt proteins	Wang et al. (2018a)
Lung carcinoma	H460	Induction of endoplasmic reticulum stress and unfolded protein response, activation of p38, ERK, and JNK, induction of apoptosis, activation of caspase-3, -9 and PARP cleavage	Joo et al. (2007)

(continued)

Table 18.1 (continued)

Cancer type	Cancer cell lines	Anti-cancer mechanisms involved	References
Meningiovma	IOMM-Lee	Induction of apoptosis, increase in active caspase-3 and cleaved PARP1, ↓viability	Pfister et al. (2013)
Multiple myeloma	U266	Inhibition of the activation of transcription factor STAT3 (signal transducer and activator of transcription 3) and STAT3-DNA binding activity, inhibition of cell proliferation, ↑accumulation of cells in the sub-G1 phase, induction of apoptotic cell death, activation of caspase-3 and cleavage of PARP, downregulated expression of proteins involved in tumourigenesis including cell cycle regulator protein Cyclin D1 and anti-apoptotic proteins	Lee et al. (2015)
Prostate	DU145	↓Cell proliferation, induction of apoptosis, ↑expression of p53, p-c-Jun N-terminal kinase, cleaved-caspase-3, Bax, and cleaved-caspase-9, ↓expression of p-phosphatidylinositol-3-kinase (PI3K), p-Akt, p-p38, Bcl-2 and p-extracellular signal-regulated protein kinase	Park et al. (2014)

↑Increased, ↓decreased

several cancer lines such as prostate cancer cells (PC3), breast cancer cells (MCF7), human umbilical vein endothelial cells (HUVEC) and pancreatic cancer cells (BxPC3) including human colorectal cancer cells (SW480 and HCT116). PA was noted to exhibit a suppressive activity on cell growth and promote apoptosis dose dependently in the cancer cells. With regard to human colorectal cancer, exposure of PA to SW480 and HCT116 cells caused the increased expression of p21 and downregulated the expression of cell cycle regulatory proteins (cyclin D1 and cyclin-dependent kinase 4 (CDK4)) in a dose-dependent way. Additionally, PA inhibited the expressions of c-myc, HDAC2 (histone deacetylase 2) and HDAC enzyme activity. c-Myc, a transcription factor that mediates progression of cancer, is greatly overexpressed in 60% of colorectal cancer (Smith and Goh 1996), while HDACs are involved in the regulation of cell signalling and gene expression, and their overexpression can result into several diseases including cancers (Haberland et al. 2009). PA also induced activation of the transcriptional activity of NF-κB by increasing the translocation of p65 into the nucleus in human colorectal cancer cells. NF-κB has been reported to cause sensitization of cells to apoptosis as well as promote proapoptotic response (Gibson et al. 2000; Baud and Karin 2009). Moreover, the potent antitumor properties of sesquiterpenes such as β-elemene (Fig. 18.1), has been extensively studied. In this context, it was found to exert anti-glioblastoma effect by restraining cell proliferation and arresting cells in the G0/G1 mediated by the mutually compensatory activation of mitogen-activated protein kinase kinase-3 and -6 (MKK3 and MKK6) (Zhu et al. 2011). Similarly, Liang et al. (2012) showed that β-elemene inhibited viability osteosarcoma cells as a result of apoptosis. However, since treatment with β-elemene also caused upregulation of

hypoxia-inducible factor 1α (HIF-1α) protein inducing partial inhibition of apoptosis, expression of HF-1α was reduced with tiny interfering RNA or co-treatment with an HIF-1α inhibitor (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole). This in turn was observed to greatly improve the antitumor potential of β-elemene. Additionally, more recently Wang et al. (Wang et al. 2018b) demonstrated that β-elemene was able to suppress human cervical cancer SiHa cell proliferation, migration and invasion, as well as promote apoptosis by reducing the Wnt/β-catenin signalling pathway which is associated with tumour formation, invasion and metastasis of different kinds of cancer (Polakis 2000; Hoffmeyer et al. 2012).

EO constituents have also been shown to improve the cytotoxicity of chemotherapy drugs in several cancer cell lines, resulting in the use of reduced concentration of the drug to provide same effect (Legault and Pichette 2007; Rabi and Bishayee 2009). Such synergism has been observed in the case of β-caryophyllene (Fig. 18.1) which, although did not exert any cytotoxic effect alone at the tested concentration, was reported to clearly increase cytotoxicity in combination with paclitaxel in numerous cancer cell lines. It also was pointed out that accumulation of β-caryophyllene in the lipid bilayer resulted into altered permeability that eventually contributed to enhance the permeability of cell membrane for uptake of paclitaxel (Legault and Pichette 2007).

Another such example is a monoterpene alcohol, linalool (Fig. 18.1), which has been tested both individually and combined with doxorubicin, for its effects on two breast cancer adenocarcinoma cell lines (MCF7 WT and MCF7 AdrR). While linalool was found to inhibit cell proliferation only moderately, subtoxic levels of linalool were able potentiate doxorubicin-induced cytotoxic and proapoptotic effects in both cell lines. Strikingly, a considerable synergistic interaction could be observed in MCF7 AdrR (adriamycin-resistant) cells (IC50 of doxorubicin, 16.16 ± 0.94 μM; IC50 of doxorubicin + 50 μM linalool, 1.24 ± 0.26 μM), which could be at least partly attributed to the capacity of linalool to boost doxorubicin buildup and to induce a decline in Bcl-xL levels (Ravizza et al. 2008).

Furthermore, geraniol has been reported to cause sensitization of cancer cells to the 5-fluorouracil, a conventional chemotherapeutic drug, including an increase uptake of the drug (Carnesecchi et al. 2002, 2004). Geraniol also demonstrated to exhibit a chemoprotective action towards rat normal colon cells when a potent carcinogen dimethylhydrazine was administered; this effect was thought to be mediated by reducing DNA damage in contrast with the control where no EO was used (Vieira et al. 2011).

β-Bisabolene (Fig. 18.1), a sesquiterpene present in EO of plants such as *Commiphora guidotti*, was tested for their selective cytotoxicity in both human and murine mammary tumour cells (Yeo et al. 2016). Treatment with β-bisabolene was found to lead to loss of viability of mammary cancer cells as a result of apoptosis. β-Bisabolene was also efficient in decreasing the growth of transplanted 4 T1 mammary tumours in female Balb/C mice in vivo (37.5% decrease). In addition, histological studies performed on the β-bisabolene-treated tumours showed an apparent increase in cell death. Conversely, the number of proliferating cells was decreased in β-bisabolene-treated tumours (Yeo et al. 2016).

18.2.5 Anticonvulsant Activity

Anticonvulsant effects of EOs components have also been reported. As example, Quintans-Júnior et al. (2010) investigated the anticonvulsant effect of three EO components, namely, (-)-borneol, citral and carvacrol in rodents using two animal models of convulsion, i.e., pentylenetetrazole (PTZ)-induced convulsion and maximal electroshock (MES) tests. In their study, the mice were pretreated with the EO components, followed by injection of PTZ at 60 mg/kg after 30 min. Similarly, for MES test, the mice received electroconvulsive shock (130 V, 150 pulses/s, 0.5 s) 30 min after the monoterpenes were injected. The latency for developing convulsions including the percentage protection were then noted. While (-)-borneol and citral were able to stimulate an increase of latency for the development of convulsions by PTZ induction in all doses, carvacrol was effective in only high dose. Besides, these monoterpenes were observed to be efficient in the prevention of tonic convulsions caused by MES. Flumazenil (FLU), an explicit antagonist of the benzodiazepine (BZD) site in the γ-aminobutyric acid (GABA$_A$)-BZD receptor complex (File and Pellow 1986), was employed to examine the role of the participation of GABA$_A$-BZD receptors in the monoterpene-prompted anticonvulsant properties. Interestingly, the presence of FLU was not capable to inverse the anticonvulsant effects of the two monoterpenes, carvacrol and citral, thus implying that there was no direct activation of the BZD site of the GABA$_A$-BZD receptor involved as the mechanism of action. On the contrary, (-)-borneol produced significant antagonistic effect indicating the possible modulation of the GABAergic system via the improvement of GABA$_A$-BZD receptor (Quintans-Júnior et al. 2010).

The effect of isopulegol (a monoterpene alcohol) (Fig. 18.1), in PTZ-encouraged convulsions, was also examined and its possible mechanism of action elucidated (Silva et al. 2009). Intraperitoneal injection of saline, diazepam or isopulegol was administered 30 min before PTZ. The latency for developing convulsions and mortality rate were recorded in mice. Moreover, the antioxidant activity of catalase and the concentrations of lipid peroxidation and reduced glutathione in brain hippocampus were determined. Isopulegol was found to significantly extend mortality and the latency for convulsions in mice in the same way to diazepam. In addition, high dose of isopulegol was observed to induce protection to all animals. FLU was also used in order to investigate on the participation of GABAergic system. The pretreatment of FLU resulted in reduced seizure prolongation latency elicited by both isopulegol and diazepam, even if it was unable to reverse the latency and mortality percentage protection. Also, the monoterpene alcohol significantly averted PTZ-induced rise in lipid peroxidation and maintained normal levels of catalase activity along with the prevention of the PTZ-induced loss of reduced glutathione in the treated mice hippocampus. These findings were indicative that the anticonvulsant and bioprotective activities of isopulegol against convulsions induced by PTZ were probably associated with positive modulation of BZD-sensitive GABA$_A$ receptors together with antioxidative properties.

Terpinen-4-ol (4TRP) (Fig. 18.1), a constituent of EOs obtained from several aromatic plants including *Melaleuca alternifolia* (Carson et al. 2006), was examined for

its anticonvulsant potential by studying the electrophysiological as well as the behavioural activities in rats and mice models (Nóbrega et al. 2014). For this purpose, 4TRP was administered intracerebroventricularly (i.c.v.) (10, 20 and 40 ng/2 μL) and intraperitoneally (i.p.) (25–200 mg/kg) in the animals, while for in vitro experiments, 4TRP was used at 0.1 and 1.0 mM. Based on the results, 4TRP (i.p.) was found to inhibit PTZ-evoked seizures, thus demonstrating anticonvulsant effects. In addition, the protection provided by 4TRP (i.c.v.) against PTZ-induced seizures were found to corroborate with the behavioural results. 3-Mercapto-propionic acid-induced convulsions were used to confirm the involvement of the GABAergic neurotransmission in 4TRP anticonvulsant activity. Moreover, since FLU was not able to reverse the anticonvulsant effect, it could be concluded that 4TRP did not bind to the BZD-binding site although its action was directly or indirectly related to the GABAergic system. Besides, 4TRP inhibited sodium current through voltage-dependent sodium channels and hence its anticonvulsant activity may be associated with changes in neuronal excitability as a result of regulation of these channels (Nóbrega et al. 2014).

As per the study of Sancheti et al. (2014), thymol was found to exhibit potent anticonvulsant as well as antiepileptogenic effects in several experimental models. For instance, the anticonvulsant potential of thymol at a dose of 5–100 mg/kg i.p. was studied using MES-, PTZ-, 4-aminopyridine- (4-AP) and strychnine-induced seizures in rats/mice models. Thymol treatment demonstrated anticonvulsant effect against MES (66.66% death protection at both 50 and 100 mg/kg) and PTZ models (66.66 and 83.33% death protection at 50 and 100 mg/kg, respectively) unlike strychnine and 4-AP models. Thymol capacity to obstruct neuronal voltage-gated Na^+ channels and its positive allosteric modulation of $GABA_A$ receptor could possibly be responsible for thymol anticonvulsant effect in PTZ and MES models (Haeseler et al. 2002; García et al. 2006). Furthermore, thymol was also found to induce marked decrease in locomotor activity (16–80% reduction in locomotion at the doses 25–100 mg/kg, respectively). PTZ-induced kindling model and measurement of MDA and glutathione levels were employed to assess the antiepileptogenic activity of thymol (5–25 mg/kg). Thymol (25 mg/kg, i.p.) was observed to cause significant decrease in the seizure score and MDA level and increase in glutathione level in PTZ-induced kindling animal model (Sancheti et al. 2014).

18.2.6 Antispasmodic Property

EOs and their individual components have also been investigated for their antispasmodic potential. For example, Magalhães and co-workers (1998) showed that *Croton nepetaefolius* EO as well as its components, namely, methyl eugenol (Fig. 18.1), terpineol and cineole, possessed myorelaxant and antispasmodic effects. Both in vivo (mice) and in vitro (guinea pig isolated ileum, cardiac, pyloric and ileocaecal sphincters) models were used to investigate their effects on intestinal motility and mechanical action of intestinal smooth muscle, respectively. The EO was found to be potent modulator of intestinal smooth muscle. Additionally, EO-induced modulation of gastrointestinal smooth muscle activity was indicative

that it was able to assist digestion via its relaxant activities without causing gut stasis. More specifically, the EO was found to increase the intestinal transit of charcoal marker delivered to the mice stomach, while it preferentially reduced basal tonus compared with the amplitude of spontaneous contractions in segments of guinea pig ileum and the sphincters. Similar to the EO, methyleugenol, cineole and terpineol induced concentration-linked relaxation activity of basal tonus and also caused obstruction of 60 mM [K^+]-induced contraction when applied individually (Magalhães et al. 2004). The major compound of *C. nepetaefolius* EO, methyleugenol seemed to play a significant role with better relaxant potency (EC50 for reduction of basal tonus, 9.4 ± 3.3 μg/mL; IC50 for the inhibition of potassium contraction, 12.3 ± 2.7 μg/mL) in comparison with *C. nepetaefolius* EO (EC50, 15.7 ± 4.4 μg/mL; IC50, 18.2 ± 2.3 μg/mL) in isolated intestinal smooth muscle. Cineole (25.37%), the major component found in the EO of *C. nepetaefolius*, comparatively appeared to provide the least contribution (EC50, 322.1 ± 29.8 μg/mL; IC50, 418.9 ± 58.7 μg/mL) as it had only weak relaxant and antispasmodic effect to that of the EO. On the other hand, terpineol by itself induced a higher maximal relaxation of the isolated ileum than the other two constituents (EC50, 70.7 ± 10.7 μg/mL; IC50, 95.4 ± 7.5 μg/mL). However, its presence in the EO did not affect the maximal relaxant response due to its limited amount (4.96%) in the EO, or probably the presence of other constituent(s), which might have inhibited terpineol-induced depressant effect of the basal intestinal tonus.

Likewise, Lima et al. (2000) investigated the effects of methyleugenol (Fig. 18.1) on guinea pig isolated ileum, whereby it was found to reversibly cause relaxation of the basal tonus (EC50: 52.2 ± 18.3 μM), which remained unchanged even by hexamethonium (0.5 mM) or tetrodotoxin (0.5 μM). In addition, relaxation of the ileum pre-contracted with 60 mM KCl was reported. Besides, even though a slight hyperpolarization of the ileum was induced by methyleugenol, it did not have any effect on the depolarized tissues. Moreover, contractions elicited by acetylcholine, KCl and histamine were inhibited by methyleugenol (IC50: 82, 65 and 124 μM, respectively). Thus, it was deduced that methyleugenol caused ileum relaxation by acting directly on the smooth muscle via mechanism mostly independent of membrane potential modifications.

Furthermore, the spasmolytic activity of several monoterpenes present in *Mentha x villosa* leaves EO has been studied by De Sousa et al. (2008). Limonene oxide, pulegone oxide and carvone epoxide, pulegone, (−)-carvone, (+)-carvone, (+)-limonene and rotundifolone were amongst the monoterpenes investigated. Rotundifolone (Fig. 18.1), a component present in many EOs, is known to be spasmolytic. The relationship between the structure and spasmolytic action of rotundifolone and its monoterpene analogues in ileum obtained from guinea pig was examined. With the exception of (+)-limonene and pulegone, all the other analogues were observed to exert spasmolytic effect that was stronger than rotundifolone. The results also revealed that the functional groups and their respective position at the ring of rotundifolone contributed to the ileum relaxation activity and the absence of the oxygenated molecular structure did not really determine the molecule bioactivity. Carvone epoxide (Fig. 18.1) was observed to exhibit significantly stronger spasmolytic behaviour compared to rotundifolone.

Eugenol present in the EOs of many aromatic plants, has also been studied by Leal-Cardoso et al. (2002) for their inhibitory activities on isolated rat ileum. The findings of this study revealed that eugenol was able to cause relaxation of the isolated ileum and inhibit contractions caused by both receptor-independent and -dependent mechanisms. Moreover, eugenol was able to hinder responses of different contractile stimuli, for instance, elevated [K$^+$] (depolarizing stimulus) and acetylcholine (neurotransmitter) in the absence and presence of a concentration of nifedipine that prevents [K$^+$]-induced contractions. As a result, a complete relaxation of the tonus increased by 60 mM K$^+$ with a maximal effect comparable to nifedipine was noted by eugenol. Also, the contraction caused by acetylcholine agonists in solution with nifedipine and Ca^{2+}-free solutions was blocked by eugenol, thus demonstrating its capacity to depress the contraction of the ileal smooth muscle at a certain stage of the signal transduction cascade away from receptors of sarcoplasmic membrane. Therefore, given that the blockage of contractions elicited by 60 mM K$^+$ and acetylcholine did not cause significant changes to the membrane potential of muscles in normal nutrient solution and complete relaxation of contractions induced by high K$^+$ was brought about by eugenol without modifying the membrane potential, it was implied that eugenol had a depressant effect on isolated ileum independent of the participation of membrane potential and voltage-dependent Ca^{2+} channels.

The mechanism responsible for the antispasmodic action of menthol (Fig. 18.1), the major constituent in peppermint oil, on the human distal colon has also been evaluated in vitro by Amato et al. (2014). In a concentration-dependent manner, menthol (0.1 mM–30 mM) was found to reduce the amplitude of the spontaneous contractions without having an effect on the frequency and the resting basal tone. Tetrodotoxin (a neural blocker), tetraethylammonium (a blocker of potassium (K+)-channels), 5-benzyloxytryptamine (a transient receptor potential-melastatin8 channel antagonist) or 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (inhibitor of nitric oxide (NO)-sensitive soluble guanylyl cyclase) at concentrations 1 μM, 10 mM, 1 μM and 10 μM, respectively, showed no effect on the inhibitory action of menthol. In contrast, significant reduction was obtained in menthol inhibitory actions in the presence of 3 nM nifedipine (a voltage-activated L-type Ca^{2+} channel blocker). Menthol also decreased the contractile responses resulting from the exogenous application of Ca^{2+} (75–375 μM) in a Ca^{2+}-free solution, or caused by KCl (40 mM) in a concentration-dependent manner. Besides, menthol (1–3 mM) potently reduced both the electrical field stimulation (EFS)-induced atropine-sensitive contractions and contractile responses induced by carbachol. Therefore, menthol was observed to exhibit spasmolytic effects in circular muscle of human colon by the direct inhibition of contractility of the gastrointestinal smooth muscle, via the blockage of Ca^{2+} influx through the sarcolemma L-type Ca^{2+} channels (Amato et al. 2014).

18.2.7 Anti-Inflammatory Property

Various plants and their active components utilized in traditional medicine have been evidenced to be valuable in the treatment of inflammatory disorders (Bernstein et al.

2018; Oguntibeju 2018). Likewise, a series of in vivo, in vitro and in silico studies have been conducted on the anti-inflammatory properties of EOs and their compounds (Pérez et al. 2011; Yadav et al. 2013; Maurya et al. 2014; Andrade 2015; Yang et al. 2016). For instance, Hirota et al. (2010) assessed the anti-inflammatory potential of limonene (Fig. 18.1) obtained from *Citrus junos* Tanaka peel in the treatment of bronchial asthma. For this purpose, the level of monocyte chemoattractant protein-1 (MCP-1), p38 mitogen-activated protein kinase (MAPK), nuclear factor (NF) kappa B as well as the reactive oxygen species (ROS) on human eosinophilic leukaemia HL-60 clone 15 cells was measured. Interestingly, at low concentration (7.34 mM), limonene was found to be effective in hindering the production of ROS for eotaxin-stimulated HL-60 clone 15 cells. Furthermore, a significant reduction was observed in the production of MCP-1 at 14.68 mM of limonene. This was achieved by activating NF-kappa B similar to when the proteasomal inhibitor MG132 was added. Inhibition of cell chemotaxis in a p38 MAPK-dependent manner comparable to the addition of SB203580 (inhibitor of p38 MAPK) was also noted at a concentration of 14.68 mM of limonene.

Two sesquiterpenic constituents, namely, α-humulene and (−)-trans-caryophyllene (Fig. 18.1) isolated from *Cordia verbenacea* EO, were assessed by Fernandes et al. (2007) for their anti-inflammatory properties. Several inflammatory experimental models involving mice and rats and different inflammation inducers were used. For instance, paw oedema caused by carrageenan in mice was strikingly repressed by these two sesquiterpenes (50 mg/kg, oral administration). Besides, both active compounds were found to effectively decrease bradykinin-, ovalbumin- and platelet-activating factor-induced mouse paw oedema. Similarly, the formation of prostaglandin E_2, expression of inducible cyclooxygenase (COX) and nitric oxide synthase caused by carrageenan intraplantar injection in rats were also diminished by α-humulene and (−)-trans-caryophyllene. However, oedema induced by histamine injection was reduced solely by α-humulene. Furthermore, while (−)-trans-caryophyllene was able to reduce only the release of TNFα (tumour necrosis factor-α), α-humulene mostly averted both TNFα and IL-1β (interleukin-1β) production in carrageenan-injected rats upon systemic treatment. The results also revealed that the two sesquiterpenes demonstrated anti-inflammatory activities comparable to those experienced by dexamethasone-treated rats (positive control drug).

Moreover, thymol isolated from EO obtained from the leaves of *Lippia gracilis* (32.68%) is thought to be a chief phytoconstituent accountable for its antinociceptive as well as its anti-inflammatory effects (Mendes et al. 2010). Thymol has been demonstrated to hinder the discharge of arachidonic acid, COX and prostaglandins biosynthesis like prostaglandin E2 in visceral pain model (Mendes et al. 2010). Likewise, thymol at 100 mg/kg was observed to minimize inflammation and encourage wound healing in rodent models by inhibiting leucocytes influx to the wounded regions and as a result prevented oedema (Riella et al. 2012).

Liang et al. (2014) also evaluated the anti-inflammatory property of thymol (10, 20, 40 μg/mL) in lipopolysaccharide (LPS)-stimulated mouse mammary epithelial cells (mMECs). Thymol was seen to significantly hold back the production of IL-6 and TNF-α in LPS-stimulated mMECs. Thymol was also found to suppress the expression of COX-2 and iNOS dose dependently. In addition, the phosphorylation

of NF-κB p65, IκBα, JNK, ERK and p38 mitogen-activated protein kinases (MAPKs) in LPS-stimulated mMECs was blocked by thymol. These findings thus pointed out that thymol anti-inflammatory activity in LPS-stimulated mMECs was brought about by hindering the activation of MAPK and NF-κB signalling pathways (Liang et al. 2014).

Furthermore, Xia et al. (2019) investigated the anti-inflammatory potential of trans-cinnamaldehyde (TCA) (Fig. 18.1), a compound present in *Cinnamomum cassia* Presl on cartilage chondrocytes in vitro and in rat models of osteoarthritis (OA). SW1353 cells and human primary chondrocytes were exposed to varying concentrations of TCA (2–20 μg/mL) for 2 h followed by stimulation of IL-1β, an inflammatory cytokine. TCA showed insignificant effect on cell viability at 10 μg/mL. TCA treatment at concentration 2–10 μg/mL was observed to reduce the levels of expression of matrix metalloproteinases (MMP-1, MMP-3, MMP-13) as well as a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-4 and ADAMTS-5) dose dependently in IL-1β-stimulated SW1353 cells. TCA-treated IL-1β-stimulated SW1353 cells at concentration 20 μg/mL also showed reduction in matrix metalloproteinases expression levels, significantly lower than that of 10 μg/mL TCA. Similarly, increase in TCA up to 20 μg/mL also reduced ADAMTS-5, involved in the degradation of cartilaginous matrix, while contrarily ADAMTS-4 showed a significant increase in expression level at 20 μg/mL in IL-1β-stimulated SW1353 cells. Pretreatment of IL-1β-stimulated SW1353 cells with TCA inhibited NF-κB activation, IκBα degradation and increased p-IκBα expression, suggesting that nuclear factor-kappa B (NF-κB) inactivation was caused by TCA. NF-κB activation by stimulation of IL-1β can lead to the expression of proinflammatory mediators thereby causing inflammation (Marcu et al. 2010). Interestingly, TCA was also found to suppress the activation of p-p38 and p-JNK1/2, while p-ERK levels were not considerably affected. IL-1β-Stimulated human primary chondrocytes treated with TCA at 10 μg/mL, considered to be the effective concentration, also demonstrated comparable results to IL-1β-stimulated SW1353 cells. In vivo evaluation to examine the TCA chondrocyte protective effects involved the intraperitoneal injection of TCA (50 mg/kg) in monosodium iodoacetate-induced OA rats. Based on the results, TCA was established to reduce OA progression and the Osteoarthritis Research Society International (OARSI) scores, therefore confirming its cartilage protecting effect and anti-inflammatory property vis-à-vis OA.

18.3 Conclusion and Future Prospects

Plant-derived EOs are complex mixtures of bioactive components responsible for a wide spectrum of biological activities. Several studies have been conducted on EOs, including their individual constituents to evaluate their pharmacological properties. Moreover, studies have been focusing on their underlying mechanisms of actions involved in showing those effects. However, their isolated active compounds, occurring at high amounts have been of particular interests as they are believed to play a major role in the pharmacological actions demonstrated by the EOs.

Nevertheless, it has been reported that EOs minor components can also exert synergistic or antagonistic effects, and as a result contribute to the EOs bioactivities. Therefore, the evaluation of the isolated EO constituents and a better understanding of their corresponding mechanistic actions can aid in more specific and targeted approach in the treatment of several diseases. In addition to acting as a substitute for synthetic drugs with low toxicity, many of the EO constituents have been seen to have drug potentiating effect, resulting into more enhanced effects and concomitantly reducing the concentration of drug being used as well as their possible adverse effects. Thus, this is a very interesting feature that can be further explored by the scientific community given the vast panoply of bioactive components present in volatile oils, and in this way they can be used in combination with synthetic drugs for better treatment and management of diseases.

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