Chapter 6
Antimicrobial Properties of Selected Native Greek Aromatic Plants: An Ethnopharmacological Overview

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Abstract Aromatic plants and their essential oils have been used therapeutically for centuries. Many published scientific studies have described their remarkable healing properties. The antimicrobial activity of plant species, such as *Origanum dictamnus*, commonly known as "cretan dittany", *Sideritis* sp. (Lamiaceae), *Cistus creticus* (Cistaceae), and *Pistacia lentiscus* (Anacardiaceae), has been reported by several researchers. These plants are used since antiquity and in this chapter, the authors attempt to present comprehensive information of their ethnopharmacological uses and chemical composition, together with data extracted from published antimicrobial studies (*in vitro*, *in vivo*, and clinical ones). Novel information and reports of medicinal uses not previously described in relevant ethnobotanical and pharmacological literature are highlighted. It is also noteworthy, that except *Cistus*, all selected species have been approved as traditional herbal medicines, based on their longstanding medicinal use in European Union, by the European Medicines Agency, and Herbal Monographs on them have been developed recently by Herbal Medicinal Products Committee (HMPC).

Keywords Lamiaceae · *Origanum dictamnus*; cretan dittany · *Sideritis* species · Cistaceae *Cistus creticus* · Anacardiaceae *Pistacia lentiscus* · Mastic

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Abbreviations

CAPeo	Cretan Aromatic Plant Essential Oil
CIM	Complementary and Integrative Medicine
CMG	Chios Mastic Gum
EDQM	European Directorate of Quality of Medicines
EMA	European Medicines Agency
EO	Essential Oil
EU	European Union
HMPC	Herbal Medicinal Products Committee
MBC	Minimum Bactericidal Concentration
MIC	Minimal Inhibitory Concentration
PDO	Product of Protected Designation of Origin
THMP	Traditional Herbal Medicinal Product

1 Introduction

Ethnopharmacology, as a specifically designated field of research, has a relatively short history. The term was first described in 1967 as the title of a book on hallucinogens. It considers the pharmacological activity of plants, fungi, and other organisms used in traditional medicine used locally or traditionally as a medicine or to improve health. It applies a unique approach in pharmacology: it also considers the traditional, and therefore anthropological, context of the drug's origin. Ethnopharmacology represents a multidimensional approach, shaped by tradition and science that improves knowledge of plant uses and local meaning of health and disease (Heinrich 2000; World Health Organization [WHO] 2002).

The Balkan Peninsula and regions around the South East Mediterranean Sea are characterized by high biodiversity endemism and long tradition in folk medicine. Parts of the areas belong to one of the thirty-six world's biodiversity hotspots due to geology, climate, and geographical location (high mountains and water near the coast). Moreover, the inhabitation and organization of societies, the development of trade among autochthonous populations along with the constant observation of nature and human health, have led to the established use of various natural products as therapeutics. Especially Greece is noted for its high plant species diversity (5800 species and 1893 subspecies) and endemism (22.2% of all species present with 1278 species and 452 subspecies) (Strid 1986, 2016; Strid and Tan 1991, 1997, 2002; Kougioumoutzis et al. 2021).

The ethnopharmacological knowledge found in the historical texts of these regions (from the fifth century BC to the nineteenth century AD) can be considered as the basis of Western pharmacopoeias. Traces for this development can be found in ancient Greece in the Corpus of Hippocrates "*The Father of Medicine*," in Roman empire in the book of "*De Materia Medica*" of Dioscorides, in Byzantine manuscripts, and in several manuscripts of the early modern periods found in monasteries and libraries. Besides historical texts, the significant ethnopharmacological knowledge and the experiences of people traditionally using folk medicinal practices, especially in remote places of these regions, are transmitted through generations orally. However, oral traditions on which much of this medical knowledge rests are imperiled, may eventually fade away and hence need to be recorded, assessed, and preserved (Hanlidou et al. 2004).

Aromatic plants and their essential oils have a long-standing tradition of medicinal use dating back to ancient times. Thus, essential oils are commonly applied to the skin; inhaled; gargled and ingested as well as being used as bath additives. Essential oils have also been proved to exert antimicrobial activity against a large number of bacteria and fungi. Pharmacology, medical and clinical microbiology, dermatology, veterinary medicine, phytopathology, food industry, food preservation, and cosmetology are some fields in which essential oils can be applied effectively (Koutelidakis et al. 2016).

In light of the recent growth in antibiotic resistance, the usage of plant-derived antimicrobial agents could serve as an effective alternative treatment against infections from human-, zoo-, and phyto-pathogenic microorganisms and/or as safe natural food preservatives, effective in the storage process. The antimicrobial activity of certain Greek endemic aromatic plants has played a very important role ethnopharmacologically, in the Mediterranean basin as well as in Greece since antiquity (Hanlidou et al. 2004; Baser and Buchbauer 2010; Tisserand and Young 2014; Koutelidakis et al. 2016).

This chapter aims to perform a comprehensive assessment of the antimicrobial potential of selected Greek native aromatic plants (the aerial parts of *Origanum dictamnus* L., the four most widely cultivated and used *Sideritis* species, *Cistus creticus* as well as *Pistacia lentiscus,* resin so called mastic). This chapter is based on information collected from scientific journals, books, and electronic search. These sources include Scopus, Pubmed, Web of Science, and Google scholar as well as local books on ethnopharmacology, and botany of these plants. The reported data on phytochemical studies, biological activity, and traditional uses have been reviewed. Issues discussed comprise the following:

1. Ethnopharmacological uses of the plants through an extensive review of relevant literature.
2. Systematic analysis of their phytochemical constituents.
3. An evaluation of these species' antimicrobial effects as a basis for supporting potential therapeutic applications.

2 *Origanum dictamnus* EMA/HMPC/200429/2012 Corr

2.1 History and Ethnobotany

Origanum dictamnus (Lamiaceae) known as Dittany of Crete consists of the dried flowering aerial parts of the herb (Liolios et al. 2010). The plant is native and endemic to Crete, widely distributed along the Cretan island. Dittany is a short green-white lanate shrub with a pink corolla. It grows wild inside fissures of calcareous cliffs – up to 1900 m above sea level. The name of the genus "*Origanum,*" according to researchers, has its roots in the Greek words "oros" (mountain) + "ganos" (which means "becoming bright"). This name probably originates and is closely associated with the high mountains, which form the natural growing environment of *Origanum* plants in the Mediterranean (Liolios et al. 2010). The species name *dictamnos* probably derived from "*Dicti*" + "*thamnos*" [*Dicti*" is the name of Cretan mountain where Zeus (Jupiter) was raised up by the goat Amalthia, dedicated to him, and *"thamnos"* means shrub in Greek] (Liolios et al. 2010).

Seeds of the plant have been excavated in the Minoic palaces Knossos, and Zacros. Since antiquity, dittany was considered as a drug against many illnesses such as gastric ulcers, to facilitate childbirth and against stomach and gynecological disorders. Hippocrates, father of Medicine, has used it on Cos island, against gastric complaints, tuberculosis, and in poultices for wounds. The most famous reference to the medicinal potential of dittany comes from Aristotle, who claimed that when wild goats living on the Cretan Mountains were struck by poisoned arrows, they ate aerial parts of *O. dictamnus.* This had the effect of causing the arrows to leave their bodies and heal their wounds (Historia Animalium 9.16.1). Theophrastus (Liolios et al. 2010) has repeated the above statement of his mentor. The Latin writers, Cicero, Virgil, followed by Pliny and Celsus and later Dioscorides in "De Materia Medica," have also attributed multiple therapeutic effects to *O. dictamnus* (Liolios et al. 2010). During the middle ages, the plant was recorded in the Codex of Charlemagne in about 795 A.D. *"…dictamnum, sinape, satureiam, sisimbrium, mentam, mentastrum...*" (chapter LXX.). Around this time, the plant started being used in monasteries, as a constituent of the renowned liqueurs Benedictine and Trappistine by Benedictines and Trappistines monks, respectively. Even in our day, dittany is being used in distilleries. Vermouth, for example, is flavored with this highly aromatic species with significant commercial interest for herbal teas (Liolios et al. 2010). Since the last century, dittany has been cultivated widely in Crete reaching an annual production of 50 tons/year: 85% of the product is exported (mainly to EU and Japan), while 15% of the total production is absorbed by the Greek market (Liolios et al. 2010).

2.2 Chemical Constituents

The lipid composition and a variety of nonpolar components (fatty acids, lipids, sterols and essential oil) have been fully identified (EMA 2012). Major components of *O. dictamnus* essential oil are monoterpenes: carvacrol (major constituent) and its isomer thymol, γ-terpinene, and p-cymene (Liolios et al. 2009, 2010). Although the composition of EOs can differ between harvesting seasons and between geographical sources, the sum of the amounts of these four compounds present has been found to be comparable in almost all the specimens.

Polyphenolic components, flavonoids, and coumarins have also been isolated and identified from methanol and water extracts of the aerial parts of the plant (Liolios et al. 2010; EMA 2012; Varsani et al. 2017) such as: coumaric acid, ferulic acid, hydrated catechin, or catechin (Proestos et al. 2006). Depsides have been isolated from polar extracts of the aerial parts of *O. dictamnus* such as salvianolic acid P, rosmarinic acid and rosmarinic acid methyl ester, along with monoterpenes (thymoquinone and thymoquinol 2-*O*-*β*-glucopyranoside), phenolic acids (oresbiusin A and caffeic acid), flavonoids (apigenin, kaempferol, quercetin, eriodictyol, taxifolin, narigenin), and alicyclic derivatives (12-hydroxy jasmonic acid and its 12-O-*β*-D-glucoside) (Chatzopoulou et al. 2010). Triterpenes, such as oleanolic acid, the rare 21α-OH oleanolic acid, ursolic acid, 21α-OH ursolic acid, and ouvaol, have also been structurally determined (Fig. 6.1).

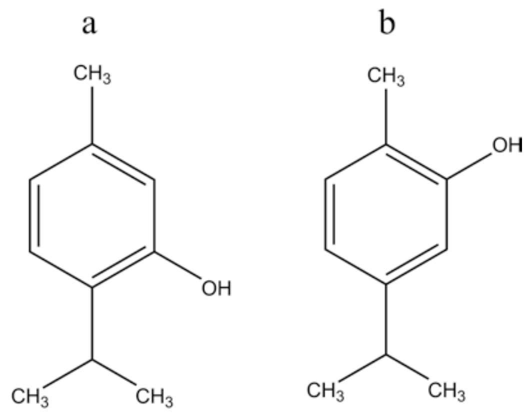


Fig. 6.1 (**a**) Thymol and (**b**) carvacrol

2.3 Antimicrobial Activity

In Vitro

The water extract of *O. dictamnus* has been tested against the yeast *Yarrowia lipolytica*. Dittany extracts (concentration of 5 g/L – presented the greater lag time and the greater inhibition activity against *Y. lipolytica* of all tested extracts (Karanika et al. 2001). The traditionally known use of the plant against gastric ulcers was supported *in vitro* as an aqueous 70% methanol extract was tested against one reference strain of *Helicobacter pylori.* Additionally, 15 clinical isolates of *H. pylori* (from clinical biopsies) and *O. dictamnus* proved very active against *Helicobacter* strains (MIC approximately 2.50 mg/ml) (Stamatis et al. 2003). The methanol extract and isolated polar compounds from *O. dictamnus* (salvianolic acid, rosmarinic acid methyl ester, thymoquinone, thymoquinol 2-O-β-glucopyranoside; oresbiusin A, caffeic acid; eriodictyol, taxifolin, naringenin, and 12-hydroxyjasmonic acid) have showed significant MIC values of 0.012–0.22 mmol/ml, showing activity against Gram-negative clinical strains *Acinetobacter haemolyticus*, *Empedobacter brevis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Chatzopoulou et al. 2010). The EOs from the leaves and bracts of dittany of Crete have exerted strong antimicrobial effect versus *Staphylococcus aureus, S. epidermidis,* and *S. hominis*, (Economakis et al. 2002).

Moreover, the essential oil of dittany exerted antimicrobial action against the phytopathogenic bacteria for potato tubers *Erwinia carotovora,* also due to carvacrol (Vokou et al. 1993).

A dose-dependent inhibition against the mycelial growth of *P. digitatum* and conidial germination was observed by *O. dictamnus*, EO. Mycelial growth was totally inhibited at 300 μg/mL, while complete inhibition of germination was observed at the concentration of 250 μg/mL. In another study (Liolios et al. 2009), the antimicrobial activities of the oils from the plant as well as the pure substances carvacrol, thymol, and their mixtures carvacrol/thymol (6:1) carvacrol/c-terpinene, have been determined before and after liposomal encapsulation. The tests were conducted against the following: four Gram-positive bacteria – *S. aureus*, *S. epidermidis*, *S. mutans*, and *S. viridans*; four Gram-negative bacteria – *P. aeruginosa*, *E. coli*, *E. cloacae,* and *K. pneumoniae*; three human pathogenic fungi – *Candida albicans*, *C. tropicalis*, and *C. glabrata*; as well as against the food-pathogen *Listeria monocytogenes*. The EOs showed comparable activities against all tested microbial strains. Pure compounds (carvacrol, thymol) are proved to be more active than oil, and their antimicrobial activities significantly increased after their encapsulation in liposomes.

Carvacrol and thymol, the isomeric phenol of carvacrol, seem to have strong antimicrobial activities, whereas their biosynthetic precursors, γ-terpinene and p-cymene, are inactive. Carvacrol in particular seems to possess strong antibacterial activity against *Bacillus cereus*, a spore-forming food-borne pathogenic bacteria, and it has also shown antifungal activity versus *Rhizopus* (9 species), *Mucor*

(4 species), and *Aspergillus* (7 species). A dose-dependent inhibition of the mycelial growth of *Penicillium digitatum* was observed for carvacrol and thymol with MIC of 125 μg/mL and 160 μg/mL, respectively (Liolios et al. 2010).

Furthermore, dittany decoctions have also been revealed to exert promising antibacterial effects against Gram-positive and Gram-negative human pathogens, as well as against a panel of isolated clinical *Malassezia* strains (Varsani et al. 2017; Paloukopoulou et al. 2021).

2.4 Antiviral Activity

Clinical Data

An EO combination based on three aromatic plants (*Coridothymus capitatus*= syn *Thymbra capitata*. *O dictamnus* and *Salvia fruticosa*), in extra-virgin olive oil, denoted as CAPeo (Cretan Aromatic Plant essential oil) has been shown to reduce the duration and severity of symptoms of patients with upper respiratory tract viral infections. Pretreatment with the EO combination demonstrated that CAPeo exerts its antiviral activity after A/H1N1 or HRV14 entry in host cells, whereas it confers a preventive reactivity against RSV. The combination further resulted in a defective trafficking of influenza A Nucleoprotein (NP), suggesting NP as a valid target of this mixture. Moreover, the combination has been concluded to possess antiviral effects and has potential as an herbal agent against influenza viruses and rhinovirus (Tseliou et al. 2019), confirming the longstanding traditional use of Cretan dittany against cough and cold (Pirintsos et al. 2020).

2.5 Food Preservative Action

A range of fungal species are associated with postharvest spoilage of grapes. However, *Aspergillus carbonarius* is the primary fungus responsible for the contamination of grapes with ochratoxin A, a mycotoxin causing several confirmed negative health effects in humans and animals. Aiming to find a method, safe for consumers, to prevent postharvest decay and ochratoxin A contamination of grapes, the potential use of essential oils as preservatives was investigated. EOs of dittany, together with other oreganos' EOs, were tested after they have been studied by GC-MS and the results showed that EO of dittany was most effective in causing total inhibition of fungal growth with a minimum concentration of 100 μL L-1, after having been tested on Sultana grapes during postharvest storage. The exhibited activity has been attributed mostly to high carvacrol content (Kontaxakis et al. 2020).

Among *Origanum* species, *O. dictamnus, known as* Cretan dittany, is a well-known Greek plant characterized by strong antimicrobial activities, while it is also reported to be effective against fungal pathogens of humans and plants. The above effects are supported by limited *in vitro* and clinical data as well as a long-standing traditional use in the Mediterranean region. Based to these data the medicinal use of the infusion/decoction of the plant has been approved, as a Traditional Herbal Medicine (THM) by EMA against cough and cold. Moreover, the plant could be further used in food area as a potential natural preservative.

3 Greek Mountain Tea Sideritis sps. (*S. scardica*, *S. raeseri*, *S. clandestina*, *S. Syriaca*) (EMA/HMPC/39455/2017)

3.1 History and Ethnobotany

Sideritis (Lamiaceae), also known as iron wort and shepherd's tea, is a genus of flowering plants known for their traditional use as aromatic herbal teas. *Sideritis* plants are abundant in the Mediterranean, the Balkans, and the Iberian Peninsula. The *Sideritis* genus comprises of certain Greek species (*S. scardica*, *S. raeseri*, *S. clandestina*, *S. syriaca*) commonly known as "Greek mountain tea" or "iron wort" and has been widely used in traditional medicine of the region, for their anti-microbial and anti-inflammatory properties.

Sideritis plants are hardy flowering perennials, found on rocky slopes at elevations over 1000 m. Only some of the species are cultivated, among which *Sideritis scardica* Griseb., *Sideritis clandestina* (Bory & Chaub) Hayek, *Sideritis raeseri* Boiss & Heldr., and *Sideritis syriaca* L. are cultivated mainly in Greece and Bulgaria.

Sideritis is known to ancient Greeks as described by Dioscorides in "*De Materia Medica"*. Since antiquity, *Sideritis* was typically referenced for being capable of healing wounds caused by iron weapons during battle. The name "sideritis" (iron wort) derives from the Greek word for iron, "σίδηρος" literally translated as "he who is or has the iron," because it has been considered a great "remedy against trauma from iron weapons," that is to say wounds of war in ancient times. Dioscorides recommended its herbal infusion to soldiers as a rejuvenating, regenerating aid to help them heal quicker (González-Burgos et al. 2011).

In Crete, under Venetian rule, iron wort (*Sideritis syriaca*) earned another name, popular to this day on the island and throughout the world: *malotira* (μαλοτήρα). This name derives from the Italian: *male* means ailment/illness, while *tirare* means to pull, to draw out. Malotira draws out the illness.

The most common English name other than Mountain Tea is "Shepherd's Tea," because Greek shepherds would use the plant to brew tea while tending their flocks high in the mountains. Indeed, *sideritis* (or malotira) is a pleasant herbal remedy for sore throat, a great aid for diseases of the respiratory system, possessing soothing and healing properties,

3.2 Chemical Constituents

Terpenes, flavonoids, essential oils, iridoids, coumarins, lignans, and sterols have been identified in the *Sideritis* genus. Differences in chemical composition are observed between the *Sideritis* sp. and between the geographic regions where they grow (Bojovic et al. 2011). According to existing references, the following secondary metabolites have been isolated from Mediterranean *Sideritis* species: (Petreska et al. 2011; Todorova and Trendafilova 2014; Papaefstathiou et al. 2014; Vassilopoulou et al. 2013).

Monoterpenes, Diterpenes Many diterpenes (ent- kaurene derivatives) have been identified (Fraga 2012) such as isolinearol, leucanthol 18-monoacetate, siderol, sideroxol, epoxysiderol, and eubol.

Sesquiterpenes Verbascoside, leucoscweptoside, martynoside and lavandulifolioside, ajugol, ajugoside, and melittoside have been isolated from *S. scardica* (Fraga 2012).

Flavonoids Flavonoid 7-O-diglycosides, two types of flavones, 8-OH (hypolaetin and isoscutellarein and their methoxy derivatives) and 5,7-OH (apigenin and luteolin), 8-OH (hypolaetin and isoscutellarein and their methoxy derivatives) and 5,7-OH isomers of apigenin 7-O-(coumaroyl) glucopyranoside together with apigenin 7-O-acetylcoumaroyl- allosylglucoside (Petreska et al. 2011; Bojovic et al. 2011; Yaneva and Balabanski 2013; Fraga 2012; Vassilopoulou et al. 2013; Papaefstathiou et al. 2014)**. Triterpenes, Coumarins, Sterols,** campesterol (7.6%), stigmasterol (28.4%) and β-sitosterol**, Phenylpropanoids** hydroxycinnamic acids, phenylethanoid glycosides.

Essential Oil of Sideritis Species
The essential oil of *S. scardica* (0.03%) consists mainly of beta-pinene (17.9%), carvacrol (14.8%), and a-pinene (Fraga 2012). Another study (Kostadinova et al. 2008) has reported that the EOs of *S. scardica* and *S. raeseri* from Bulgaria and North Macedonia contain mainly diterpenes, while the EO of *S. raeseri* had higher concentrations of sesquiterpenes with germacrone (25%) and elemol acetate (15.9%) as main constituents (Bankova et al. 1996; Kostadinova et al. 2008; Bojovic et al. 2011).

3.3 Antimicrobial Activity

Sideritis scardica, *Sideritis clandestina*, *S. rae*seri, and *Sideritis syriaca* L. are botanically very closely related species of the same genus and have been described as endemic species of the Balkan Peninsula growing wild and also cultivated since the last century in Central Balkan and has been traditionally used as a healing aromatic herbal tea in folk medicine of this geographic area since centuries. “Mountain tea” species (“Pirin tea” or “Mursalski tea”) (*S. scardica*; *S. clandestina*, *S. raeseri* and *S. syriaca*) are widely used mainly for the relief of cough associated with cold and mild gastrointestinal disorder.

Todorova and Trendafilova (2014) have summarized the available data on the antimicrobial activity of *S. scardica* (ethanol extract and its ethyl-ether, ethyl-acetate, and n-butanol fractions). Antimicrobial activity of varying degrees against *S. epidermidis, Micrococcus luteus, S. aureus, E. coli, K. pneumoniae, P. aeruginosa,* and the yeast *C. albicans* has been demonstrated. Stronger activity was observed against *S. epidermidis*, *M. luteus, E. coli*, and *P. aeruginosa,* with moderate activity against *K. pneumoniae*. The MIC values of the tested extracts ranged from 40 μg/mL. The investigators concluded that the different types of terpenoids could contribute to this antibacterial activity (Tadic et al. 2012). Extracts from *S. scardica*, *S. syriaca*, and *S. montana*, /extracted with organic solvents, have exhibited strong activity against *S. aureus*, and butanol extract of *S. syriaca* exhibited antiyeast activity toward *C. albicans* (Yaneva and Balabanski 2013). Very recently, the activity of the essential oil from *Sideritis raeseri* susp *raeseri* was studied against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli, Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Pseudomonas fragi*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. Growth inhibition of all microorganisms tested was documented, although it was significantly lower compared to gentamycin, ciproxin, and voriconazole, which were used as positive controls (Mitropoulou et al. 2020).

Traditionally, Iron wort has widely been used for wound healing and the treatment of respiratory conditions. These alleged effects are supported mainly by its essential oil’s antimicrobial effects, based on multiple *in vitro* studies. *Sideritis* species have been used by local people as herbal tea and in traditional medicine since centuries and people are taking advantage of the high species diversity and are aware of their useful properties through their traditional uses demonstrated biological activities and pharmacological properties (Aneva et al. 2019).

4 *Cistus creticus* L. Pink Rock-Rose

Cistus L. (from the Greek word kistos-κίστος) or rock-rose is a genus of perennial herbaceous plants among the Cistaceae family, that have hard leaves and grow in open areas of stony, infertile soils and they are indigenous to the Mediterranean region. *C. creticus* (pink rock-rose) is a small, woody plant, distributed along the coast of the Central-Eastern Mediterranean including the islands of Corsica, Sardinia, and Crete, as well as N Africa, and W Asia. Its distribution extends from sea level to 800 m in arid and warm areas. The plant has five violet petals on its flowers and numerous stamens; its fruits are capsules containing small seeds, covered by a hard water-impermeable coating (Papaefthimiou et al. 2014). *C creticus* has been traditionally known as a natural remedy due to its pharmacological properties. It comprises of three identified subspecies, subsp. *corsicus* limited to the islands of Corsica (France) and Sardinia (Italy), subsp. *Creticus*, which is endemic to the coastal areas of Crete (Greece), while subsp. *eriocephalu*s is exclusively located on all three mentioned islands (Corsica, Sardinia, and Crete) (Demetzos et al. 1997).

4.1 History: Ethnopharmacology

Most *Cistus* species have aromatic foliage while *C. creticus,* in particular, also exudes a highly aromatic gum or resin, called "*ladano*" "*ladanum"* or "*labdanum*,*"* which has been used in incenses since ancient times and is now a valuable ingredient of perfumes.

The oleoresin 'ladano' has been mentioned by Dioscorides, Plinius the younger, and Herodotus (Papaefthimiou et al. 2014) and has been established as an anti-infective agent since antiquity in Mediterranean traditional medicine.

C. creticus has been used during the Middle Ages, as an expectorant against catarrh, externally in plasters for treating ulcerating wounds and as an ingredient for wound ointments. Traditionally, the herbal tea of the plant is drunk in cases of bronchitis or colds. Available literature suggests that Cistus plants are grown and collected mainly in the island of Crete, in Turkey, Spain, and Italy. Therefore, it is mostly used and preferred in these countries.

4.2 Chemical Constituents

Labdane-Type Diterpenes

In *C. creticus*, several terpenes (monoterpenes, sesquiterpenes, labdane-type diterpenes) have been reported (Papaefthimiou et al. 2014; Chinou et al. 1994; Demetzos et al. 1997). The labdane derivatives labd-13(E)-ene, 8a-15-diol, labd-7,13-(E)-dien-15-ol, labd-13(E)-en-8a-ol-15-yl acetate, labd-7,13(E)-dien-15-yl acetate, and ent-manoyl oxide mixture of the isomers (ent-13-epi-manoyl oxide; ent-manoyl oxide; ent-8-epi-manoyl oxide) were identified and characterized from nonpolar extracts and of its essential oils (leaves, fruits and resin) (Demetzos et al. 1990) (Fig. 6.2).

Polyphenols, Catechins

Aerial parts: catechin, gallocatechin, protocatechuic acid, shikimic acid, and gallocatechin-3-gallate, as well as trimeric and dimeric proanthocyanins have been isolated (Demetzos et al. 1990; Danne et al. 1993; Petereit et al. 1991). Several

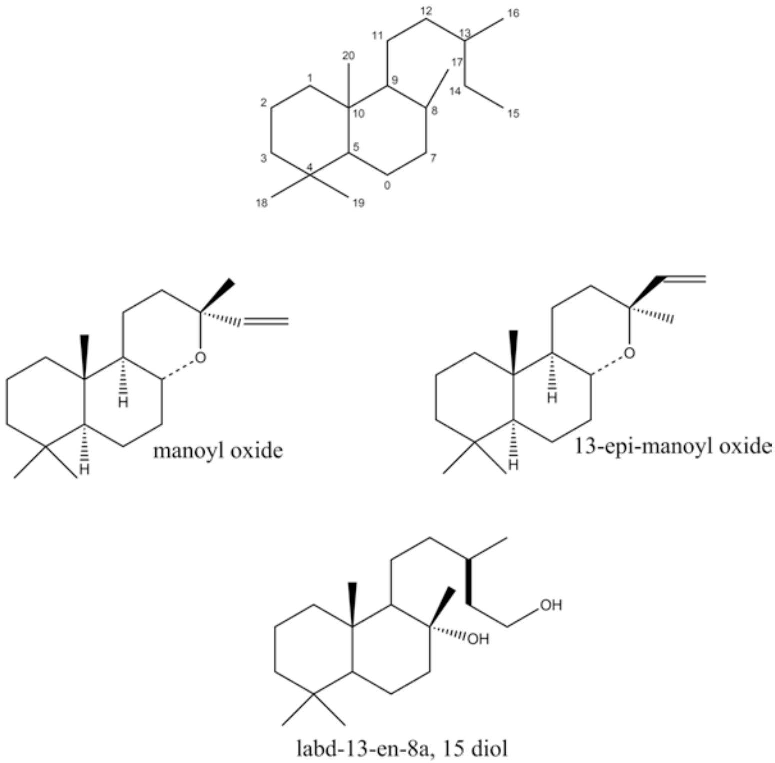


Fig. 6.2 Basic structure of labdane-type diterpene (**a**), (**b**) manoyl oxide, 13-epi-manoyl oxide, and (**c**) labd-13-en-8a,15 diol, in *C. creticus*

flavonoid derivatives have been identified including kaempferol, quercetin, apigenin, naringenin myricetin, coumarins (aesculin, scopoletin (6-O-methyl-7-hydroxycoumarin)) (Demetzos et al. 1990; Danne et al. 1993; Petereit et al. 1991). The polyphenolic composition of aqueous extracts from *C. creticus* has been performed using HPLC-DAD-ESI-MS/MS methodology. Major compounds of three main groups have been identified, i.e., ellagitannins, flavonoids, and phenolic acids derivatives (Barrajón-Catalán et al. 2011). Moreover, **t**he polyphenols from four different commercial *C. creticus* (*C. incanus*) herbal teas have been extracted and all extracts have been characterized qualitatively and quantitatively by HPLC. Twenty nine polyphenols, including ellagitannins, flavanols, and glycosylated flavonols, were identified (Wittpahl et al. 2015) (Fig. 6.3).

Essential Oil

Monoterpenes, sesquiterpenes, diterpene esters, and alcohols are the main components identified (α-cadinene, δ-cadinene, viridiflorol, bulnesol, ledol, α-copaene, β-selinene, cubenene, manoyloxide and 13-epi-manoyloxide) (Demetzos et al.

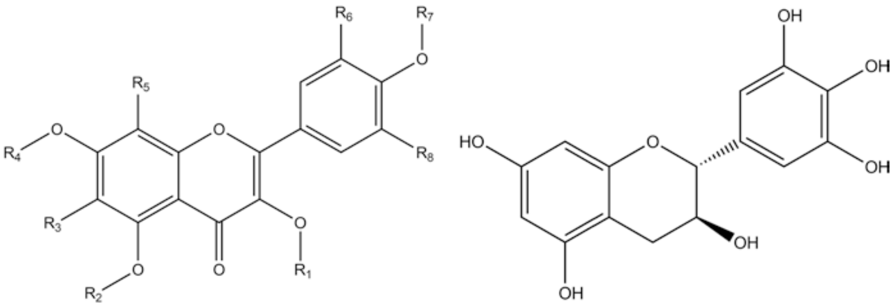


Fig. 6.3 (**a**) Flavonols, (**b**) gallocatechin from *Cistus creticus* L

1997). A comparison between the essential oil composition of *C. creticus* subsp. *corsicus* against *riocephalus* has been reported (Paolini et al. 2009). It has been documented that the two species are differentiated by botanical characteristics linked with essential oil production and composition of the volatile fraction (limonene or 13-epi-manoyl oxide, respectively), as the major components. Recently, it has been also affirmed that some differences are evident between the chemical profile of polyphenols in subsp. *creticus* and the other two subspecies (*eorsicus* and *eriocephallus*). However, it appears to be clear that the secondary metabolites are similarly comparable (quantitative than qualitative differences) (Mastino et al. 2018).

4.3 Antimicrobial and Antifungal Activity

In Vitro

The antimicrobial and antifungal activity of seven labdane-type diterpenoids isolated from the leaves of *Cistus incanus subsp. creticus* was assessed *in vitro* (Chinou et al. 1994) showing strong antimicrobial effects against *S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans*.

Bouamama et al. (2006) examined the antimicrobial activity of leaf extracts obtained from *C. villosus* (= *C. incanus*) and *C. monspeliensis* L. against ten strains of bacteria and fungi. All extracts showed inhibitory activity against most of them (*S. aureus*, *P. aeruginosa*, *C. albicans, C. krusei, C. glabrata* and *Aspergillus fumigatus* were investigated). The most susceptible ones were *S. aureus* and *C. glabrata* (MIC values 0.78 mg/ml and 0.19 mg/ml respectively).

Diterpenes of the plant have proved to be the driving force behind the antimicrobial activity of pink rock-rose infusions.

In situ studies revealed that *C. creticus (*syn *C. incanus)* infusions reduce the initial bacterial adhesion in the oral cavity, potentially due to contained polyphenols. Furthermore, the *in vitro* antibacterial activity of the methanolic extracts against *Streptococcus mutans* has been examined using a live/dead assay (BacLight®). With this approach, it has yielded antibacterial properties. Antibacterial studies of the essential oil of *C. creticus subsp. eriocephalus* leaves have been carried out against Gram-positive, Gram-negative organisms and fungi *B. cereus*, *B. subtilis, C. albicans, S. faecalis, S. aureus, S. epidermidis, P. aeruginosa,* and *E coli.* Gram-negative bacteria (*P. aeruginosa, E. coli*) were more resistant than the Gram-positive bacteria and the yeast (Wittpahl et al. 2015).

The aqueous extracts of *C. creticus* (= *C. incanus*) has revealed antibacterial activities more effective against Gram-positive bacteria, particularly *S. aureus* (MIC values 0.5–32 mg/mL) and *S. epidermidis* (MIC 0.25–8 mg/mL) than Gram-negative bacteria. They were also weak inhibitors of *C. albicans* and *C. glabrata* growth (MIC values over 8 mg/mL) (Viapiana et al. 2017).

Moreover, the antibacterial activities of phenolics derived from 14 *C. incanus* samples of different origin (Turkey, Albania, Greece, etc.), obtained as herbal teas, were compared. The antimicrobial activity was assessed with the use of thin-layer chromatography–direct bioautography (TLC-DB) against the Gram-negative naturally luminescent marine bacterium *Aliivibrio fischeri* and the Gram-positive soil bacterium *Bacillus subtilis*. It was established that in spite of the different origin of the investigated herbal samples, in qualitative terms, their antibacterial activity was closely comparable and more strongly pronounced against the Gram-positive than the Gram-negative bacteria (Szeremeta et al. 2018). Moreover, the antibacterial profile from 11 among previously referred commercial *C. incanus* herbal teas against the same microorganisms (*A. fischeri* and *B subtilis*) proved apigenin, kaempferide, and acylated kaempferol glycosides (cis- and trans-tiliroside and their conjugates with p-coumaric acid) to be most effective antibacterial components (Móricz et al. 2018).

Very recently, the essential oil from *Cistus leaves and crude extracts from Tafraout, Morocco, has also been examined for antibacterial and antifungal activities (Ait Lahcen et al. 2020).*

4.4 Antiviral Activity

In Vitro

A special branded plant extract from *Cistus incanus,* rich in polyphenols (CYSTUS052), exhibited antiviral activity against the avian influenza A virus (H7N7) in cell (Droebner et al. 2007). In MDCK cells, a 90% reduction of plaque numbers on cells preincubated with the plant extract was achieved. The same extract demonstrated to exert an anti-influenza virus activity in A549 or MDCK cell cultures infected with prototype avian and human influenza strains of different subtypes (Ehrhardt et al. 2007). Viruses did not develop resistance to the extract when compared to amantadine that resulted in the generation of resistant variants after only a few passages. The authors have suggested that the effect appears to be mainly due to binding of the polymeric polyphenol components of the extract to the virus surface, thereby inhibiting binding of the haemagglutinin to cellular receptors.

Cistus creticus (= *C. incanus*) had also been shown to inhibit human immunodeficiency virus (HIV) infections *in vitro*. Antiviral activity seems highly selective for virus particles, preventing primary attachment of the virus to the cell surface and viral envelope proteins from binding to heparin. Rockrose extracts also inhibited infection by virus particles pseudotyped with Ebola and Marburg virus envelope proteins, indicating that antiviral activity of the extract extends to emerging viral pathogens showing potent and broad *in vitro* antiviral activity against viruses that cause life-threatening diseases in humans as promising sources of antiviral agents targeting virus particles (Rebensburg et al. 2016).

The hemorrhagic dengue fever affects up to 500 million patients, annually causing 20.000 deaths, with no chemotherapeutic agent available. Several extracts and fractions of *Cistus creticus* resin (labdanum) – standardized on labdane-type diterpenes via GC-MS – have been studied on their activity against the dengue virus (DENV-2 strain 00st-22A) using *in vitro* Vero cell cultures. Preliminary experiments with a labdanum diethyl ether raw extract have not yielded measurable results due to cytotoxic effects against Vero cells; cell viability was constantly checked using the MTT-test. In the most active fraction at 30 μg/ml, dengue virus proliferation was 100% suppressed and cell viability was over 90%. Such antiviral activity of a diethyl ether extract of labdanum against a virulent hemorrhagic fever like dengue is described for the first time (Kuchta et al. 2020).

In Vitro Effects

The same branded *Cistus* extract as mentioned before (Droebner et al. 2007) in a form of aerosol has been assayed for the treatment of mice infected with a mouse-adapted highly pathogenic avian influenza virus (FPV, H7N7). Inbred female Balb/c and C57Bl/6 mice were infected. The treatment was performed for 5 days and monitored for 15 days after infection. In conclusion, the mice treated with the extract did not develop disease, showed neither differences in their body temperature nor in their gross motor activity, and exhibited no histological alterations of the bronchioles epithelial cells (Droebner et al. 2007). From the reported data, a potential mechanism of action was proposed. This suggests that polyphenolic ingredients of the extract block virus infection by a direct (physical) interaction with the virus particles.

Clinical Studies

A double-blind, placebo-controlled clinical study (Kalus et al. 2009) was developed, where 160 patients (7–81 years old), suffering from an infection of the upper respiratory tract, participated. The product (in lozenges) investigated consisted of a special branded aqueous *Cistus creticus* extract as explained before (Droebner et al. 2007). Of the 160 participants in this study (56 men and 104 women), 129 patients completed the study, 82.5% of the *Cistus* group, and 80.0% of the placebo group.

Based on the findings 92 (57.5%), volunteers had a viral infection (11 patients with Influenza A and 7 patients with Influenza B) and 67 (41.8%) a bacterial infection. Patients were observed and treated up to 7 days. All participants of the trial were asked to judge the maximum severity of the following symptoms on a questionnaire by their physician: pain, cough intensity, cough frequency, sputum and sniffles. Each symptom was determined as follows: 0 = not present, 2 = slight, 4 = mild, and 6 = severe. The total score was calculated by adding the five symptom scores and therefore ranged from 0 to 30. The score of subjective symptoms decreased significantly during observation ($p < 0.001$). The decrease was more pronounced in the group treated with *Cistus* compared to placebo ($p < 0.001$). The time period, in which the drug achieved clinical symptom improvement, was shorter for *Cistus* than for placebo. It is still unclear how *Cistus* extracts in the mouth help to reduce the spread of influenza virus in the lung via a binding effect. According to previous studies (both *in vitro* and *in vivo*), nonspecific pharmacological interactions could be involved (Ehrhardt et al. 2007; Droebner et al. 2007) Many different metabolites (mostly polyphenols) may be responsible for the antiviral and antibacterial properties of this medicinal plant. In conclusion, *Cistus* was more effective in reducing the average duration and severity of symptoms in patients with infection of the upper respiratory tract than placebo.

In a second trial (Kalus et al. 2010), three hundred volunteers (age 5–85 years) were recruited in this randomized, open trial study. All suffered from an infection of the upper respiratory tract: 131 (43.7%) volunteers had a viral infection, 163 (54.3%) a bacterial infection, and six patients (2%) both types or a mixed infection. Two hundred and seventy-seven (277) patients completed the study. One hundred and forty-one (141) patients were randomly placed into the *Cistus* group, and 136 into the green tea placebo group. Patients were observed and treated for 7 days. The subjects were asked to judge the maximum severity of their symptoms as previously described (Kalus et al. 2009). An improvement of the symptoms in the *Cistus* group was observed in 57.8% (160/277) of the patients after an average of 3.2 days. A comparison of the single parameters shows that in the *Cistus* branded group, the severity level decreased in all the parameters [pain cough (strength), cough (frequency), rhinorrhoea, and sputum] for the 277 examined patients with the exception of rhinorrhoea, whereas in the green tea group, the pain intensity only slightly decreased. Approximately 24.9% (69/277) of the patients complained of adverse effects, observed more significantly in the green tea group with 35.3% (48/136) than in the *Cistus* group with 14.9% (21/141) ($p < 0.001$). More frequent adverse effects were nausea and dizziness.

4.5 Activities Against SARS-CoV-2

During the COVID-19 pandemic, people are facing risks of adverse health effects due to the restrictions implemented such as quarantine measures, reduced social contact, and self-isolation. In several review articles, data has been collected on potential preventive and therapeutic health benefits of Complementary and Integrative Medicine (CIM) that might be useful during the COVID-19 pandemic. In several among them, the safe and supportive use of *Cistus creticus* herbal teas has been proposed due to its potentially beneficial antivirus effects (Seifert et al. 2020; Şener 2020; Stange and Uehleke 2020).

4.6 Food Supplementation

In the search for new functional foods and novel products to develop new prohealth high-quality foods, *C. creticus* has been proposed as an innovative functional supplement, added in wheat as a flour supplement. Supplementation of bread or pasta with 3% water extract yielded products with desirable characteristics, which were favored by consumers (Cacak-Pietrzak et al. 2019; Lisiecka et al. 2019). The use of such water extracts in low concentrations (up to 3%) as flavoring agents could be helpful, while further safety assessment studies must be conducted in order to approve the use of pink rockrose for food preservation, brought about through its expressed antimicrobial activities (Barrajón-Catalán et al. 2011).

Conclusively, *Cistus* genus is widespread in the Mediterranean regions with several species traditionally known as natural remedies, among which *C. creticus* (= *Cistus* × *incanus* L.) (pink rock-rose), rich in bioactive metabolites, has been used by the pharmaceutical and food industries. The aerial parts of the plant have been used as a healing herbal tea, and consumption has been proposed mainly for the relief of cough associated with cold. *Cistus* water leaf extracts have showed *in vitro* antimicrobial activity against a range of human pathogenic bacteria and fungi, while a special branded extract of *Cistus* rich in polyphenols has exhibited antiviral activity against the avian influenza A virus (H7N7) *in vitro* and *In vivo* (Ehrhardt et al. 2007; Droebner et al. 2007), while in COVID-19 pandemic, it had been also proposed due to its beneficial antivirus effects. All scientific data are strongly supportive to the ethnopharmacological relevance of the wide continuous use of pink rock-rose establishing it as an anti-infective agent since antiquity in Mediterranean ethnopharmacology.

5 *Pistacia lentiscus* (EMA/HMPC/46756/2015)

Pistacia lentiscus (Anacardiaceae), cultivated in the island of Chios in Greece, is the source of unique resin called “mastic” – a well-known natural antimicrobial and healing agent all around the world. The mastic tree (*Plentiscus* L.) is naturally distributed in coastal regions of the Mediterranean, (Gruenwald et al. 2007). It is an evergreen shrub, 2–3 meters high that grows slowly, and becomes ready for cultivation after 40–50 years. The plant is renowned for its production of a resin, known as Mastic gum, which is obtained as an exudate after carving the trunk and branches

(Paraschos et al. 2007, 2012). The Southern area (Mastihohoria) of the Greek island of Chios is among the major global commercial sources of mastic resin [Chios Mastic Gum (CMG)], while, since 1997, it has been registered as a Product of Protected Designation of Origin (PDO) in the EU (EMA 2015a, b).

A well-documented botanical study by Browicz has proposed that instead of *Pistacia lentiscus* var. *Chia* (Desf. Ex Poiret) DC, the name *Pistacia lentiscus* cv. *Chia* should be used as cv, means cultivated clone. Recently, botanical classification of *Pistacia lentiscus* L. without any further variety and/or cultivar has been accepted in the monograph of European Pharmacopoeia (Browicz 1987; EMA 2015a, b).

5.1 History and Ethnopharmacology

Preparations of *P. lentiscus* resin have been used traditionally for stomach disorders like dyspepsia and peptic ulcer, as well as for dermatological problems and oral hygiene for more than 2500 years. A series of reports in international medical journals refer to these historically recorded properties of mastic, which are based on the results of scientific studies as well as on clinical trials carried out by researchers, and have revealed that metabolites of the mastic tree possess interesting bioactive properties (Al-Habbal et al. 1986; Huwez and Al-Habbal 1986; Huwez et al. 1998; EMA 2015a, b).

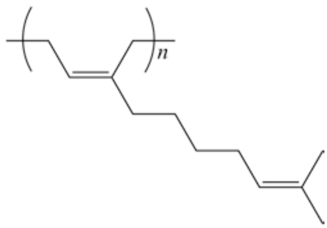
Ancient Greek physicians, such as Hippocrates, Dioscorides, Theophrastus, and Galen, first mentioned its properties and recommended its use for its distinctive flavor and its therapeutic properties (Paraschos et al. 2007). In the last century, mastic gum is being used as a flavoring agent in Mediterranean cuisine, in the production of chewing gum, in the cosmetics industry, in dentistry and traditionally, by the local population of Chios Island, for the relief of epigastric pain and protection against peptic ulcer disease. Mastic gum has been demonstrated to possess anti-inflammatory, antioxidant as well as antimicrobial activities.

5.2 Chemical Constituents

The resin of the tree appears to consist of a variety of secondary metabolites, some of which have been isolated and determined in nature for the first time in this plant. A major of these components is the natural polymer, which was identified as *cis*-1,4-poly-β-myrcene (EMA 2015a, b) (Fig. 6.4).

The resin is also rich in nonvolatile natural products such as triterpenes, phytosterols, and polyphenolic molecules. Resin's triterpenes are mainly composed of the tetracyclic euphane and dammarane skeleton type and of the pentacyclic oleanane and lupane skeleton type such as mastic acid, isomastic acid, oleanolic acid, and tirucallol etc. (Gruenwald et al. 2007; EMA 2015a, b) (Fig. 6.5).

Fig. 6.4 Natural polymer of *P. lentiscus* resin, *cis*-1,4-poly-β-myrcene



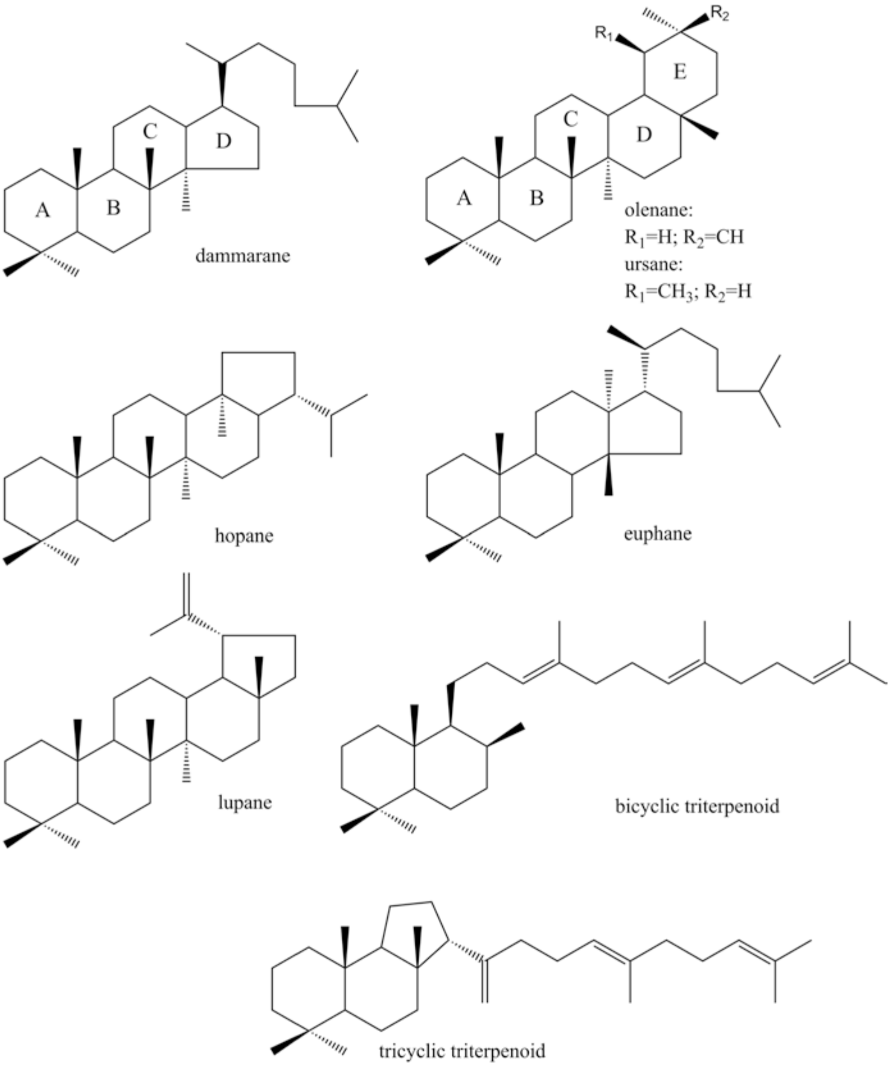


Fig. 6.5 Triterpenoid types in mastic

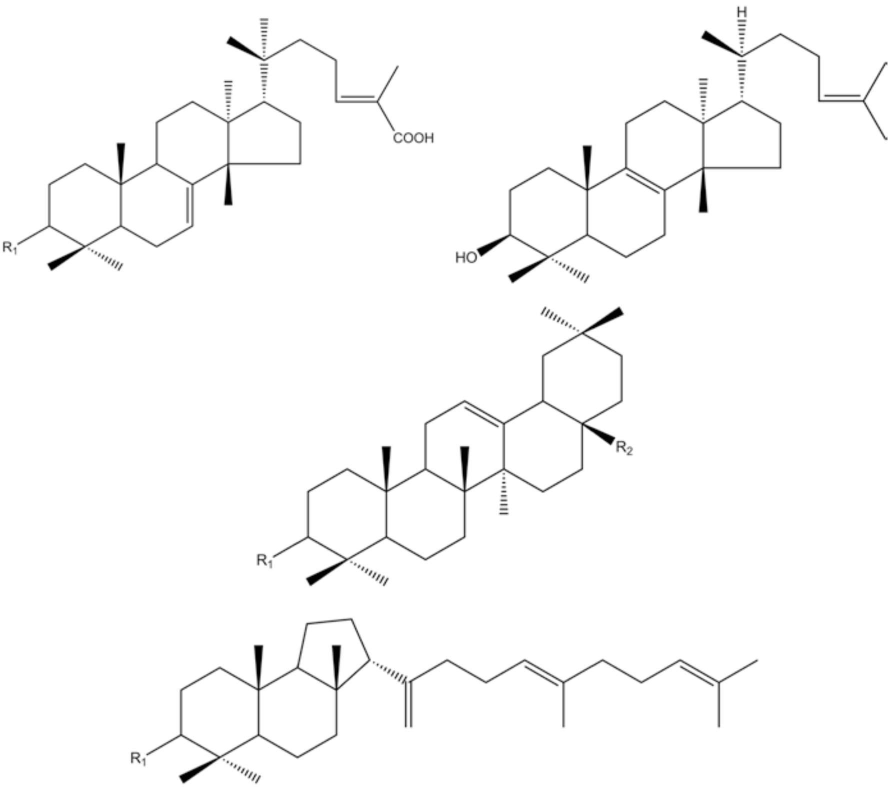


Fig. 6.6 (**a**) Masticadienonic acid, (**b**) tirucallol, (**c**) oleanolic acid, and (**d**) 3β-hydroxymalabarica-14 (26),17E,21-triene

The main such nonvolatile natural products reported in the literature are masticadienonic acid, tirucallol, oleanolic acid, masticadienonic acid, 3-oxo-dammara-20(21),24-diene, 3β-hydroxymalabarica-14(26),17E,21-triene, 3-oxo-malabarica-14(26),17E,21-triene, etc., together with 1,4-poly-β-myrcene (EMA 2015a, b; Paraschos et al. 2007) (Fig. 6.6).

Mastic gum also contains a small fraction (approximately 2%) of essential oil, thoroughly analyzed (Magiatis et al. 1999; EMA 2015a, b). This essential oil contains volatile and aromatic ingredients (monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes). The chemical composition of essential oils of resin, leaves and unripe and ripe fruits of mastic has been studied comparatively, identifying a total of 90 components (50% monoterpene hydrocarbons, 20% oxygenated monoterpenes, and 25% sesquiterpenes) with α-pinene (79%) and the myrcene (3%) being the major components (EMA 2015a, b). In a very recent study, *α*-pinene and *β*-myrcene were found in abundance in the fresh oils of mastic gum; however, in the oil of the aged collection, oxygenated monoterpenes and benzenoids, such as verbenone, pinocarveol, and *α*-campholenal, were found at the highest rates (Pachi et al. 2021).

5.3 Mastic Gum: Antimicrobial Activities

In Vitro

Several studies have been conducted to investigate mastic antimicrobial properties. Different *P. lentiscus* L. leaves preparations (10% decoction, petroleum ether extract, ethanol extract, infusion, and maceration) have tested against selected Gram-positive (+) and Gram-negative (−) bacteria, but only the leaves decoction showed activity (MIC 312 mg/ml), while all other preparations were practically inactive against *S. lutea*, *S. aureus* and *E. coli* (Aksoy et al. 2006; EMA 2015a, b).

Daifas et al. (2004) have investigated the effect of mastic and its essential oil, alone and in conjunction with ethanol, on the growth of proteolytic strains of *Clostridium botulinum*. They proved that mastic and mastic oil in ethanol can effectively be used as factors against the appearance of *botulinum* neurotoxin in nutrition goods. More specifically, the results of the laboratory tests showed that the addition of only 0.3% mastic oil was required for the inhibition of proteolytic strains of *Clostridium botulinum*. The authors concluded that mastic and mastic oil could potentially be used as natural preservative in bakery products.

The antibacterial activity of mastic oil can be attributed to the combination of several components rather than to one particular compound. It is also interesting to note that different bacteria are susceptible or not to different compounds of the essential oil (Magiatis et al. 1999; EMA 2015a, b).

A number of studies have shown that mastic and mastic oil exhibit actions on gastrointestinal lesions. After discovery of *Helicobacter pylori* and correlation with gastrointestinal disease in 1983, the interest for the determination of mechanism of action of mastic and mastic oil for these disorders focused on the exploration and eventual finding of anti-*H. pylori* properties.

The first study that proves mastic anti-*H. pylori* activity is published in the New England Journal of Medicine in 1998 (Huwez et al. 1998). Mastic proved to kill the *H. pylori* NCTC 11637 strain and the six clinical isolates. This action was due to the fact that mastic exterminated *H. pylori*, which is liable for the majority of the digestive ulcer cases. In the specific study, fresh samples were used with the presence of *H-pylori*, which were isolated from patients and the minimum bactericidal concentration (MBC) of mastic was searched, which means the minimum concentration required in order to exterminate 99.9% of the bacterium within 24 hours. Mastic exterminated the bacterium in all the examined samples, regardless of the size of the population. The MBC of mastic was 60 μg/ml, but even in smaller concentrations, the antibacterial action has been observed.

Marone et al. (2001) and Bona et al. (2001) assessed the antibacterial effect of mastic on the clinical isolates of *H. pylori* at concentrations of 2000 to 1.9 μg/ml. Specifically, the inhibition of growth of *H. pylori* in the presence of aqueous mastic extracts was studied. The results showed that the extracts of at least 1.4 g resin affect the viability of bacterium, preventing cell growth. (Bona et al. 2001).

Paraschos et al. (2007) utilized an established *H. pylori* infection model to evaluate the potential therapeutic effect of total mastic extract administration on *H. pylori* colonization and development of associated gastritis. The antimicrobial activities of different fractions as well as that of mastic total extract, against a panel of 10 clinical isolates of *H. pylori* and the CCUG 38771 reference strain, were tested in a period of 3 months. Mastic extracts exhibited concentration- and strain-dependent bactericidal activities. The experiments showed that the mastic total extract could moderately reduce *H. pylori* colonization in the antrum and corpus of the stomach. According to the authors, the results also suggest that habitual long-term mastic consumption may be effective in moderating *H. pylori* colonization.

A recent study (Miyamoto et al. 2014) examined the components of mastic responsible for anti-*H. pylori* activity. GC–MS analysis of the essential oil of mastic led to the identification of 20 components among which α-pinene (82.26%) was the most abundant. Then, the authors examined which component inhibits the growth of *H. pylori* testing commercially available compounds against *H. pylori* strains that were established from patients with gastritis, gastric ulcer, and gastric cancer. Some of them showed antibacterial activity against clarithromycin- resistant and/or metronidazole-resistant strains. α-Terpineol and (E)-methyl isoeugenol showed anti *H-pylori* activity not only against drug-sensitive strains but also against drug-resistant strains. These 10 compounds also showed antibacterial activity against three different strains (*Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*). The authors have concluded that these components could be useful to overcome the drug-resistance *H. pylori* growth in stomach.

Mastic has been also used widely, as a traditional remedy, since antiquity for oral malodour, and oral hygiene and this knowledge has been assessed also in recent studies. Mastic has showed selective antibacterial action against oral bacteria *Porphyromonas gingivalis* and *Prevotella melaninogenica* (EMA 2015a, b). Additionally, the antimicrobial activity of Chios Mastic Gums with their respective Chios Mastic Oils was evaluated, with growth tests against the fungi *Aspergillus nidulans*, *Aspergillus fumigatus*, *Candida albicans*, *Mucor circinelloides*, and *Rhizopus oryzae*, and the bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, with the samples exhibiting a moderate activity (Pachi et al. 2021).

Clinical Data

Sixty volunteers (60) with symptoms and endoscopic confirmation of duodenal ulcer participated in a first clinical study (Al-Habbal et al. 1986). The results showed that in the group that consumed CMG, there was an alleviation of the symptoms in 80% of the cases, while the endoscopic examination has confirmed that duodenal ulcer was cured in 70% of the cases. The authors have concluded that mastic was more active than placebo for the alleviation and the treatment of ulcer symptoms, with no undesirable side effects. The same research team has published (Huwez and Al-Habbal 1986) the findings of another clinical study in patients who suffered from gastric ulcers but of benign nature. CMG was also administered in the dosage of 2 g per day for 4 weeks to 6 patients with diagnosed gastric ulcer by means of gastroscopy. The results of the study have shown that the administration of CMG caused symptom relief in all patients.

The *in vitro* studies showed antimicrobial activity of mastic and mastic fractions and preparations against a panel of Gram-positive and Gram-negative bacteria as well as its particular strong activity against *H. pylori*, with MBC, at 60 μg/ml. These findings gave a positive signal to the therapeutic indication of mastic against mild dyspeptic disorders. Overall, the results of this study signify the prospects of *Pistacia lentiscus* resin for the development of herbal formulations against infections as well as for its applications in oral hygiene products.

6 Conclusions

The chemistry of aromatic plants exhibits an amazing assortment of biologically active compounds such as essential oils mixtures, terpenoids, phenolic acids, and flavonoids that are widely employed as bioactive sources. This chapter aims to provide a critical evaluation of current knowledge about four selected aromatic plant genera (*O. dictamnus*, *Sideritis* species, *C. creticus* and *P. lentiscus* resin), all of them native and/or endemic in Greece, to be used as potential source of natural antimicrobials. All collected information sourced from ethnobotanical literature together with the results of existing pharmacological data have been approved by EMA. Following this extensive literature search, it can be concluded that many traditional uses of them are supported by modern *in vitro* or *in vivo* pharmacological studies. As discovery of pronounced natural antimicrobials, among aromatic plants, for future experimental studies, are still of high growing interest, well-designed studies are required to establish links between their traditional /ethnopharmacological uses and evaluated bioactivities. Moreover, their safe uses have to be fully ensured, before any potential exploitation in pharmaceutical and/or agricultural industries.

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