# Anti-Infectious Activity in the Cistaceae Family in the Iberian Peninsula

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**Abstract:** Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. New antimicrobials are needed in medicine due to the rapid emergence of new resistant and opportunistic microbes and the increasing number of patients suffering from immunosuppressive situations, e.g., acquired immunodeficiency syndrome, transplantation, cancer, etc… Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. Plants have been a source of therapeutic agents from more than 5000 years. Approximately 25% of modern medications are developed from plants. In the area of infectious diseases, 75% of new drugs originated from natural sources between 1981 and 2002. As less than 10% of the world's biodiversity has been tested for biological activity, many more useful natural lead compounds are awaiting discovery. The Cistaceae family comprises a large number of species, growing in the warm temperate regions of the Mediterranean area, that have been and are still used as medicinal plants, particularly in folk medicine. In the present review, we analyse the past, present and future of medicinal plants of the Cistaceae family present in the Iberian Peninsula, both as potential antimicrobial crude drugs as well as a source of natural compounds that act as new anti-infectious agents.

## INTRODUCTION

The introduction of sulphonamide antibiotics in the 1930s and penicillin in the 1940s revolutionised medicinal practice by dramatically decreasing the fatality rates associated with bacterial infections. These discoveries led to a concerted search for new antimicrobial drugs during the following 30 years and resulted in the discovery of most of the antimicrobial drug classes known today. However, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite this tremendous progress in human medicine. The rapid onset of resistance to most antimicrobial drugs due to the emergence of new resistant and opportunistic microbes, and the increasing number of patients suffering from immunosuppressive situations, e.g., acquired immunodeficiency syndrome, transplantation, cancer,…, has diminished their effectiveness and, as a consequence, a continual search for novel antimicrobials needs to be undertaken.

Drugs derived from plant sources have been empirically used in the treatment of various human disorders for thousands of years. Since antiquity, man has used plants to treat common infectious diseases. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterise as antimicrobial principles, was well accepted. The prevalence of natural product-derived antimicrobial drugs may be due to the evolution of secondary metabolites as biologically active chemicals that conferred selectional advantages to the producing organisms. Natural products are also likely to have evolved to penetrate cell membranes and interact with specific protein targets. In addition, natural products have an element of structural complexity which is required for the inhibition of many antimicrobial protein targets. Relevant reviews on the role of natural products in antimicrobial drug discovery have been published recently [1-7]. It is estimated that there are 250000 to 500000 species of plants on Earth. A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes. As less than 10% of the world's biodiversity has been tested for biological activity, many more useful natural lead compounds are awaiting discovery.

The Cistaceae (rock-rose) family is a rather small family of plants known for its beautiful shrubs, covered by abundant flowers. This family consists of about 170-200 species in eight genera, growing in the warm temperate regions of the Mediterranean area, that have been and are still used as medicinal plants, particularly in folk medicine. The Cistaceae family is represented by five genera in the Iberian Peninsula: *Cistus* L., *Helianthemum* Miller, *Tuberaria* (Dunal) Spach, *Halimium* (Dunal) Spach and *Fumana* (Dunal) Spach [8, 9]. In the present review, we analyse the past, present and future of medicinal plants of the Cistaceae family present in the Iberian Peninsula and growing in different locations, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infectious agents.

## *Cistus* L. GENUS

The genus *Cistus* comprises a group of about 20 shrub species found widespread throughout the whole Mediterranean region to the Caucasus [8, 9]. *Cistus* is one of the most characteristic genera of the Mediterranean flora. Shrubby species primarily occur as woodland understory and others are dominant in evergreen scrub. Being one of the main constituents of the Mediterranean-type maquis shrubland, this

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**Table 1. Medicinal Plants of the Cistaceae Family Present in the Iberian Peninsula Containing Antimicrobial Activity**

| Scientific Name | Compound | Activity[a] | References |
|---|---|---|---|
| *C. creticus* | - | Bacteria | 13 |
| *C. creticus* ssp. *creticus* | Terpenes | General | 19,20,27,28 |
| *C. creticus* ssp. *eriocephalus* | Essential oil | Bacteria | 30 |
| *C. incanus* ssp. *creticus* | Diterpenes | Bacteria, fungi | 23 |
| *C. ladaniferus* | Essential oil | Bacteria, fungi | 32,34 |
| *C. laurifolius* | Essential oil, polyphenols | Bacteria, fungi | 13,14,34,36 |
| *C. monspeliensis* | Essential oil, terpenes, polyphenols | General | 13,15-17,26,27,34,37,38,44 |
| *C. parviflorus* | Essential oil | Bacteria | 13,33 |
| *C. populifolius* | Diterpenes | General | 24,25,45 |
| *C. salviifolius* | Essential oil | Bacteria | 13,31 |
| *C. tauricus* | Essential oil | Bacteria, fungi | 34 |
| *C. villosus* | Essential oil, polyphenols | Bacteria, fungi, viruses | 16-18,34,38,40,41 |
| *H. voldii* ssp. *nova* | Essential oil | Bacteria | 57 |
| *H. glomeratum* | Polyphenols | General | 46-53 |
| *T. lignosa* | - | Viruses | 45,56 |

[a]"General" denotes activity against multiple types of microorganisms (e.g., bacteria, fungi and protozoa) and "bacteria" denotes activity against Gram (+) and Gram (-) bacteria.

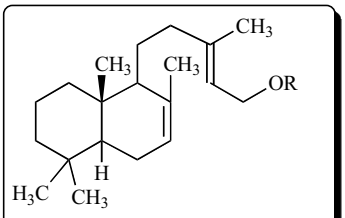
plant genus is peculiar in that it has developed a range of specific adaptations to resist summer drought and frequent disturbance events, such as fire and grazing [10, 11].

Various *Cistus* species are used (in Italy, Greece, Spain, Morocco and Turkey) for the treatment of diarrhoea and peptic ulcers, as general remedies for several skin diseases and as anti-inflammatory and antispasmodic agents [12]. Pharmacological studies on *Cistus* extracts from different geographical locations reported them to have antibacterial, antifungal and antiviral activities. Guvenc and coworkers [13] investigated the antimicrobial activity of Turkish *Cistus* species. The genus *Cistus* is represented by five species in Turkey: *Cistus creticus* L., *Cistus laurifolius* L., *Cistus monspeliensis* L., *Cistus parviflorus* Lam. and *Cistus salviifolius* L. In Turkish folk medicine, these five *Cistus* species are reported to be effective against a broad range of disorders either internally or externally. All of the extracts of these plants showed some activity against *Bacilus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*. Butanol extracts of the leaves and fruits of *C. creticus* showed the highest activity. Additionally, in a screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity, Yesilada and coworkers [14] reported the inhibitory properties of one *Cistus* species, *C. laurifolius*.

Some medicinal plants from Tunisia were also investigated for antimicrobial activities [15]. Antibacterial activity was demonstrated from one *Cistus* species, *C. monspeliensis*, especially against Gram (+) bacteria. This species from Morocco, together with *Cistus villosus* L. (= *C. incanus*), plants used in traditional medicine, were also investigated against the following microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida krusei*, *Candida glabrata* and *Aspergillus fumigatus* [16, 17]. Results showed that the various extracts differed clearly in their antimicrobial activities. *C. villosus* extracts exhibited more interesting activity than *C. monspeliensis* when used against *Staphylococcus aureus* (minimal concentration of inhibition MIC 0.78 mg/ml) and *Candida glabrata* (MIC 0.19 mg/ml), which are the most susceptible microorganisms. On the other hand, *Candida krusei* and *Aspergillus fumigatus* were the least susceptible microorganisms to all *Cistus* extracts. One of these Moroccan plants, *C. villosus*, totally inhibited the growth of the citrus fruit pathogens: *Penicillum digitatum*, *Penicillium italicum* and *Geotrichum candidum* [18]. This plant may be an alternative to the chemical fungicides currently used in the control of postharvest citrus fruit diseases.

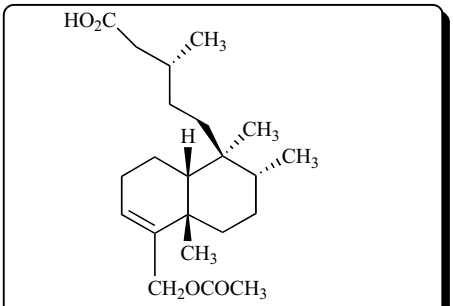
Phytochemical studies on different *Cistus* species have been undertaken in order to reveal the presence of compounds with an antimicrobial activity. These reports mainly concerned diterpenoids and essential oils. *Cistus creticus* L. ssp. *creticus* is an indigenous shrub of the Mediterranean area, found in all kinds of soil. The glandular trichomes covering its leaf surfaces secrete a resin called "ladano", which has been renowned since ancient times for its aromatic and pharmaceutical properties. The resin, among others, contains a number of specific labdane-type diterpenes that exhibit antibacterial and antifungal action. Several labdane-type diterpenes were isolated from the resin of the plant [19, 20]. Manolyl oxides, such as 13(*E*)-labd-13-ene,8α-ol-15yl malo-

nic acid are new natural products. Some of these labdane diterpenes are being chemi-synthesised in the laboratory and evaluated for antimicrobial activity [21]. Chloroethyl carbamidic esters (**1**) showed the strongest antibacterial activity against Gram (+) and Gram (-) bacteria, and some pathogenic fungi. Some of these compounds have been patented for cosmetic use. For example, labdenoic acid and related compounds such as melanin formation inhibitors, cell activators and antimicrobial agents for manufacturing cosmetics are claimed [22]. From another related *Cistus* species, *Cistus incanus* L. ssp. *creticus* (L.) Heywood, several labdane-type diterpenoids were also isolated as antimicrobial agents [23]. Their antibacterial and antifungal activities were tested against *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Torulopsis glabrata*, *Saccharomyces cerevisiae* and *Candida albicans*.




**1:** Chloroethyl carbamidic esters
R = $COH_2CO_2H$
R = $CONHCH_2CH_2Cl$

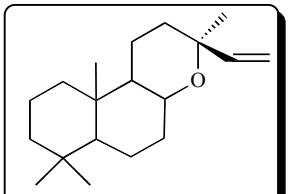
Reports on the antimicrobial activity of clerodane-type diterpenoids isolated from *Cistus* species have also been found in the literature. From *Cistus populifolius* L., several *neo*-clerodane diterpenoids with antifeedant activity were isolated and also semi-synthesised [24, 25]. These works are an approach to obtaining highly functionalised diterpenoids from readily available natural products, that could serve as a starting point for a future synthesis of new antifeedant agents. Examples of other antimicrobial clerodane diterpenoids from *Cistus* species also included (+)-19-acetoxy-*cis*-clerodan-3-ene-15-oic acid (**2**) isolated from *C. monspeliensis* leaves, which was found to exhibit significant antibacterial activity against *Staphylococcus aureus* [26].



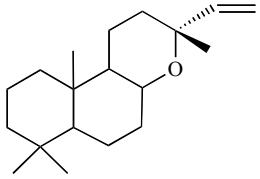

**2:** (+)-19-acetoxy-*cis*-clerodan-3-ene-15-oic acid

Fokialakis and coworkers [27] evaluated eleven *cis*-clerodane diterpenes, seven labdane-type diterpenes and one triterpene isolated from *C. monspeliensis* and the resin "ladano" from *C. creticus* ssp. *creticus* against *Leishmania donovani* promastigotes, the causative agent for visceral leishmaniasis. Acetoxy-*cis*-clerod-3-en-15-ol, 15,18-diacetoxy-*cis*-clerod-3-ene and 13-(*E*)-8α-hydroxylated-13-en-15-ol 2-chloroethylcarbamate exhibited the most potent and selective leishmanicidal activity.

Other antimicrobial compounds from the *Cistus* genus are those present in their essential oils. Demetzos and coworkers [28] reported the antimicrobial activity of the essential oil of the resin "ladano" from *C. creticus* ssp. *creticus* against *Staphylococcus aureus*, *Staphylococcus epidermis* and *Staphylococcus homini*. Fractionation of the resin and susceptibility testing showed that its anti-staphylococcal activity was mainly due to the diterpene sclareol. Additionally, the composition of this essential oil was investigated by gas chromatography/mass spectrometry [29]. Among the 49 components identified (representing 76.56% of the oil composition), diterpenes (59.59%) predominated. The major components were manoyl oxide (**3**) and 13-*epi*-manoyl oxide (**4**). From another related *C. creticus* species, *Cistus creticus* ssp. *eriocephalus* (Viv.) Greuter and Burdet, Demetzos and coworkers [30] reported antibacterial studies *in vitro* against Gram (+) and Gram (-) microorganisms. Thirty-nine components were identified, representing 73.9% of the oil composition. The main components of the oil were α-cadinene, δ-cadinene, viridiflorol, bulnesol, ledol, α-copaene, β-selinene, rubenene, manoyl oxide (**3**) and 13-*epi*-manoyl oxide (**4**).




**3:** Manoyl oxide

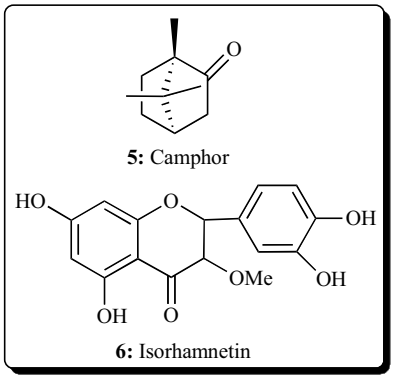



**4:** 13-*epi*-manoyl oxide

The composition of the essential oils of fifteen populations of *C. salviifolius* from Crete, Greece, with antimicrobial activity against Gram (+) and Gram (-) bacteria was also investigated [31]. Labdane diterpenes were detected and identified in the essential oils of this species for the first time. Camphor (**5**), viridiflorol, longiborneol, phyllocladene, abietatriene and *cis*-feruginol are the main constituents, while the group of oxygenated sesquiterpenes has the highest percentage composition. More recently, the antimicrobial activity of 28 essential oil samples isolated from local plants cultivated in Corsica was evaluated against a large panel of human pathogenic bacteria [32]. Among them, the essential oil from *Cistus ladaniferus* (L.) showed compositions without components known as active, suggesting the presence of compounds not previously described as antibacterial agents. Examples of other antimicrobial essential oils from *Cistus* species also included the essential oil from the leaves of *C.*

*parviflorus* [33], and the essential oils from some species of the *Cistus* genus cultivated in the Crimea, which inhibited the growth of *Candida albicans* and *Mycobacterium* B5 [34].

Reports of other antimicrobial compounds isolated from the *Cistus* genus also included polyphenols. *Cistus* species is a tea rich in polyphenols which are expected to have antibacterial properties. Hannig and coworkers [35] reported that *Cistus* tea may be used to reduce the initial bacterial adhesion in the oral cavity since the amount of bacteria detected was reduced significantly with *Cistus* tea. Ustün and coworkers [36] isolated several flavonoids from *C. laurifolius*, a plant used traditionally in folk medicine against gastric ailments. It is known that there is a causal relationship between peptic ulcer and *Helicobacter pylori* infection. These isolated flavonoids, mainly quercetin 3-methylether (isorhamnetin) (**6**) (MIC 3.9 μg/ml) had activity against *Helicobacter pylori*. Another flavonoid isolated from *C. monspeliensis*, 3,7,4',5'-tetramethylether of myricetin, showed antimicrobial activity against Gram (+) bacteria [37]. This species, together with *C. villosus*, also yielded other polyphenolic compounds, catechins and gallocatechins, with antifungal activity [38].




**5:** Camphor

**6:** Isorhamnetin

A review of the literature on the evaluation of *Cistus* species extracts shows that many studies into their antiviral activities have been carried out in recent years. The common cold disease comprises a primary infection caused by rhinoviruses, adenoviruses or coronaviruses. Pandalis [39] patented the antiviral activity of *Cistus* extracts in infections with human rhinovirus type 14 cells, indeed, they may be useful for the prophylaxis and/or treatment of common cold diseases. Influenza, a respiratory disease caused by influenza viruses, is still a worldwide threat with a high potential to cause a pandemic. The occurrence of highly pathogenic avian influenza viruses of the H5N1 subtype capable of infecting and killing humans, highlights the urgent need for new and efficient countermeasures against this viral disease. Ehrhardt and coworkers [40] demonstrated that a polyphenol-rich extract, CYSTUS052, from the Mediterranean plant *C. villosus*, exerts a potent anti-influenza virus activity in A549 or MDCK cell cultures infected with prototype avian and human influenza strains of different subtypes. Additionally, CYSTUS052 exhibits antiviral activity against a highly pathogenic avian influenza virus H7N7 in a mouse infection model *in vivo* [41]. These results are consistent with the fact that these plant extracts have been used in traditional medicine in southern Europe for centuries without any reported complications, and perhaps may be used against a possible influenza pandemic [42, 43].

In searching for natural products as potential antiviral agents, various research groups have initiated antiviral screening programmes for plants used all over the world as anti-infectious agents in traditional medicine. Some of these reports concern species from the *Cistus* genus. Sassi and coworkers [44] investigated the antiviral activity of some Tunisian medicinal plants against herpes simplex virus type I (HSV-1). Extracts of eight plants among 15 tested showed some degree of antiviral activity, including *C. monspeliensis*. Similarly, dichloromethane and ethanol extracts of 12 plants with a history of use in traditional medicine were tested against HSV-1, vesicular stomatitis virus (VSV) and poliovirus type 1 [45]. It was found that the ethanol extract of *C. populifolius* inhibited the replication of HSV-1, but had no effect on VSV and poliovirus replication.

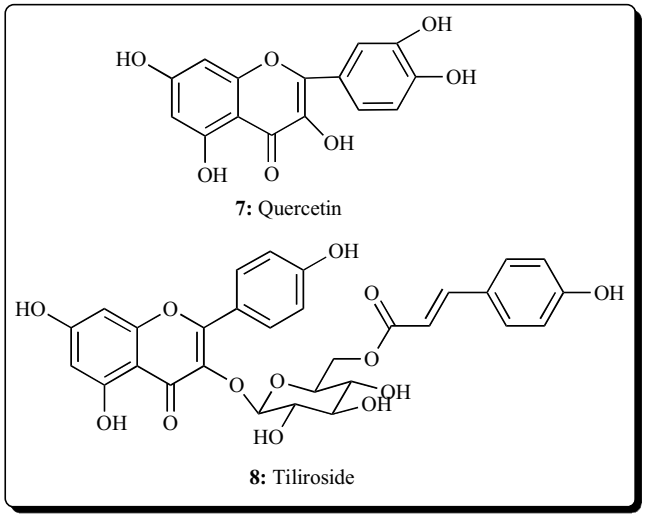
## *Helianthemum* MILLER. GENUS

*Helianthemum* (rockrose, sunrose, rushrose) is a genus of about 110 species of evergreen or semi-evergreen subshrubs [8, 9]. They are widely distributed in the Americas, Europe and from North Africa to Asia Minor and Central Asia, with the centre of diversity in the Mediterranean region. Various *Helianthemum* species are used in traditional medicine to treat diseases now known to be of microbial origin [12]. In this genus, reports on the antimicrobial activity concern mainly the species *Helianthemum glomeratum* Lag.

Diarrhoeal infection diseases are a great problem throughout the world and responsible for considerable morbidity and mortality, especially in developing countries. Ethnobotanical studies indicate that *H. glomeratum* is a plant widely used in Maya communities to treat diarrhoea. A possible mechanism to counteract infectious diarrhoea would be by inhibiting the growth of the enteropathogen causing the disease. Meckes and coworkers [46] investigated the antimicrobial activity of *H. glomeratum* against bacterial enteropathogens isolated from faeces of children with acute diarrhoea or dysentery. A methanol extract obtained from the leaves and stems was highly active against *Shigella* spp. and *Vibrio cholerae*. Activity against *Salmonella* spp. and *Escherichia coli* isolates was also present. The plant is also active against *Entamoeba histolytica* and *Giardia lamblia* trophozoites [47]. Some of these results were also demonstrated *in vivo*. Barbosa and coworkers [48] reported the antigiardial activity of crude methanol extract from *H. glomeratum* using experimental infections of *Giardia lamblia* in suckling female CD-1 mice, which hold out the prospect of using the extracts of this plant as an option for developing novel antigiardial phytodrugs.

Structural characterisation and biological evaluation of the compounds isolated from *H. glomeratum*, particularly that of polyphenols, has been the aim of a series of studies carried out to define the further potential use of this plant in the treatment of infectious diarrhoea and dysentery in children. The flavan-3-ols, (-)-epigallocatechin and (-)-epigallocatechin gallate isolated from *H. glomeratum* roots, were tested for their antiamoebic and antigiardial effects *in vitro* [49]. Compared with the activity detected with the leaf and the root methanol extracts, the effect of (-)-epigallocatechin

against *Entamoeba histolytica* was of a similar potency. It also suppressed the growth of *Giardia lamblia* in axenic cultures. Apparently, the biological properties detected are due to the presence of (-)-epigallocatechin in the plant, although several flavonoids isolated from leaves could account for the antiprotozoal properties of this herbal resource. For example, Calzada and coworkers [50] isolated the flavonoids kaempferol and quercetin (**7**) as the major amoebicidal compounds. These flavonoids were also active *in vivo* using an experimental infection of *Giardia lamblia* in suckling female CD-1 mice [51]. More recently, bioassay-guided fractionation of the methanol extract of aerial parts from *H. glomeratum* afforded 5 flavonol glycosides: tiliroside (**8**), kaempferol-3-*O*-(3'',6'' di-*O*-*E*-*p*-coumaroyl)-β-glucopyranoside, astragalin, quercitrin and isoquercitrin [52]. The *in vitro* antiprotozoal assay showed that tiliroside was the most potent antiamoebic and antigiardial compound. In a microbiological evaluation of medicinal plants used by the Maya people of southern Mexico, it was demonstrated that the plant *H. glomeratum* was also active against *Escherichia coli* and the fungus *Candida albicans* [53].




**7:** Quercetin



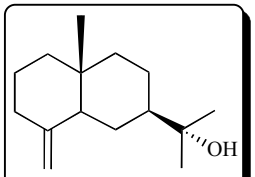

**8:** Tiliroside

## *Tuberaria* (DUNAL) SPACH GENUS

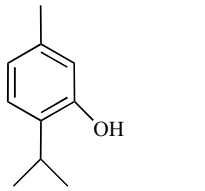
*Tuberaria* (rockrose) is a genus of about 112 species of annual or perennial plants in the rockrose family Cistaceae, native to western and southern Europe [8, 9]. They occur on dry, stony sites, often close to the sea. *Tuberaria* species are used as food plants by the larvae of some Lepidoptera species. Only a few studies have been reported on the antimicrobial activity of *Tuberaria* species, and these concerned mainly the species *Tuberaria lignosa* (Sweet) Sampaio. *T. lignosa* is a medicinal herb widely distributed in the Iberian Peninsula. In Spanish traditional folk medicine, different parts of the plants are used for treating various diseases and ailments, such as gastrointestinal disorders, wounds, skin infections and warts, which could be related to viral origin [54, 55]. In searching for natural products as potential antiviral agents, various research groups have initiated antiviral screening programmes for plants used all over the world as anti-infectious agents in traditional medicine. Some of these reports concern the species *T. lignosa*. Dichloromethane and ethanol extracts of 27 plants with a history of use in traditional medicine were tested against HSV-1, VSV, poliovirus type 1 and human immunodeficiency virus (HIV) [45, 56]. It was found that the ethanol and aqueous extracts of *T. lignosa* inhibited the replication of HSV-1 and HIV, respectively. The aqueous extract of this plant showed inhibitory effects against HIV-induced infectious in MT-2 cells at concentrations ranging from 12.5 to 50 μg/ml, without showing appreciable cytotoxicity. These results indicate that the aqueous extract of *T. lignosa* possess anti-HIV properties of therapeutic interest. Although the extract has not yet been further analysed chemically, the active components appear to be mainly water-soluble polar substances.

## *Halimium* (Dunal) Spach GENUS

In another Cistaceae genus present in the Iberian Peninsula, *Halimium* genus, the only reports in the literature concern the antimicrobial activity of the essential oil and hexane extract of *Halimium voldii* Kit Tan Perdetzoglou & Raus ssp *nova* against Gram (+) and Gram (-) bacteria [57]. Thirty compounds were identified in the essential oil of this plant, representing 88.7% of the oil composition. The main components were nonanal, dodecane, *Z*-caryophyllene, γ-muurolene, δ-cadinene, caryophyllene oxide, β-eudesmol (**9**) and manoyl oxide (**3**). Thymol (**10**) was identified in the hexane extract as the main compound.




**9:** β-eudesmol




**10:** Thymol

## *Fumana* (Dunal) Spach GENUS

*Fumana* (needle sunrose) is a small genus close to sunrose (*Helianthemum* ssp.). They are evergreen perennials or rarely annual species, native to rocky to stony sites in full sun in Europe and the Mediterranean region, including the Iberian Peninsula [8, 9]. Like other Cistaceae species, various *Fumana* species are used in traditional folk medicine to treat diseases now known to be of microbiological origin. However, there are no reports in the literature about the antimicrobial activity of the *Fumana* genus.

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