Biological properties and resistance reversal effect of *Helichrysum italicum* (Roth) G. Don

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Helichrysum italicum, which belongs to the family *Asteraceae*, is an evergreen plant native to the Mediterranean area. Since older times, extracts and essential oils (EOs) from the aerial parts (leaves, flowering tops) of the plant are used in traditional medicine for herbal remedies. They are known to possess several biological properties, including antimicrobial, anti-inflammatory, antioxidant and anti-viral activities, as well as preventive effects against insects. The chemical variability exhibited by *H. italicum* extracts and EOs could explain all these health promoting activities. This review summaries the present state of knowledge on chemical constituents of *H. italicum* and its biological properties.

Keywords *Helichrysum italicum* sp; biological activities; chemical constituents; essential oils; extracts.

1. Introduction

The genus *Helichrysum* (Miller) belongs to the *Asteraceae* family and is a very large genus including approximately 600 species widespread all over the world. *Helichrysum* species are distributed from the lower-meso-Mediterranean to the lower-sub-humid bioclimatic environments, growing at a wide range of altitudes from the sea level up to 1700 m, preferably on sandy or loamy soils [1]. Almost 25 species are native of Mediterranean area and the most widespread species is *Helichrysum italicum* (Roth) G. Don (syn. *H. augustifolium* DC). It is a small aromatic shrub, up to 40-50 cm high, with yellow flowers growing on dry cliffs and sandy soil (Figure 1).

Fig. 1 photography of the yellow flowering tops of the plant.

H. italicum subsp. *italicum* and *H. italicum* subsp. *microphyllum* (Willd.) Nyman are the most investigated subspecies [2].

H. italicum has some fairly unusual and very useful properties. In Europe, the plant is used over the years to refresh the air, repel insects and for medicinal purposes [3]. For instance, dried inflorescences of this plant are used as a moth antifeedant whereas flowering tops find application in folk medicine for their anti-inflammatory and anti-allergic properties and in cosmetics for treatment of skin sunburn and erythema [4-5]. Decoctions of flowering tops are also used for fumigations in the treatment of asthma [6].

Besides its ornamental value, the success of this plant is also due to several activities related to the essential oils (EOs) produced by the glandular hairs present on their leaves and flower heads. Only the EO extracted from the plants belonging to *H. italicum* species is used in aromatherapy practice. Its cicatrizant properties suggest that the EO can be used to aid skin regeneration and help with wound healing. Voinchet and Giraud-Robert [7] investigated the therapeutic effects and potential clinical applications of *H. italicum* EO and a macerated oil of musk rose (*Rosa rubiginosa*) after cosmetic and reconstructive surgery. The objectives of reducing inflammation, oedema and bruising were well

achieved. The authors highlighted that neryl acetate, a main component of the EO, contributed to pain relief. -They also attributed the observed effects to the occurrence of italidiones in *H. italicum* EO. This class of molecules is also reputed to have anti-haematomal properties so that *H. italicum* EO is often called the 'super arnica of aromatherapy'. That is why this useful phlebotonic is indicated for couperose skin (red veins), haematoma (even old haematomas), thrombosis and the prevention of bruises [8]. When *H. italicum* EO is mixed with some other specific EOs, the mixtures are thought to be anti-allergenic. So, these aromatherapy prescriptions could be helpful in cases of asthma, hay fever or eczema (Table 1).

Table 1 *H. italicum* essential oil in aromatherapy prescriptions

System	*H. italicum* in mixture with
Integumentary (eczema, inflammation, wound healing) Trauma	Lavender, Roman chamomile, geranium, yarrow, vetiver, patchouli, sandalwood, cypress, rose Can be used neat or diluted (10-50%)
Immune (allergic responses)	Rose, sandalwood, German chamomile

Due to its skin regenerative properties, *H. italicum* EO is known to prevent skin aging. That is why it is widely used in the formulation of anti-aging creams and cosmetics based on *H. italicum* EO that are now flooding the market.

The EO of *H. italicum* is obtained by steam distillation of flowering tops. The flowering tops are cut by hand, in wild places, and the cut is from mid-June to mid-July, early flowering. The flowering tops are processed after harvesting. The yield of production is about 0.9 to 1.5: *ie* a ton of flowering tops produces about 900 g to 1.5 kg of EO. This EO is increasingly sought after, but unfortunately many sites are endangered (fires, advanced buildings...) and its price becomes higher due to its rarity.

2. Components of *H. Italicum* Extracts and Essential Oils

Since the end of the fifties, phytochemicals belonging to different families of compounds have been identified in solvent extracts of *H. italicum* and when necessary their structure elucidated. Representative components are listed below:

- acids: acetic acid, caprilic acid [9], fatty acids [10];
- angeloylated glycerides, constituting an unusual class of lipids named santinols [11];
- phenolic compounds: caffeic, *p*-coumaric, ferulic and chlorogenic acids [9, 12] as well as phenolics that includes coumarates, benzofurans, pyrones [11] and 7-hydroxy-5-methoxyphthalide and 12-hydroxytremetone (bitalin A) [12];
- triterpenes: β- sitosterol and ursolic acid [10], α-amyrin, uvaol and ursolic acid lactone [13]
- flavonoids : apigenin, glycosyl-apigenin, luteolin, gnafalin, naringenin, glycosyl-naringenin [4], kaempferol-3-glucoside and naringenin-glycoside [5], B-ring deoxyflavonoids [14];
- chalcones: glycosyl-chalcone [4] 4,2',4',6'-tetrahydroxychalcone-2'-glucoside [14];
- acetophenone glucosides and a benzo-γ-pyrone glucoside [15];
- arzanol, a prenylated heterodimeric phloroglucinyl α-pyrone and helipyrone, a dimeric pyrone [15,16,17].

Identification of individual components of *H. italicum* EO has been investigated since a long time. However, our attention will be focused on papers that report on chemical analyses carried out with modern analytical techniques. *H. italicum* EO exhibited various compositions depending of the sub-species, the location of harvest, the physiological stage of the plant, etc. They are summarized on the table 2.

In short, *H. italicum* EOs contained numerous monoterpenes and sesquiterpenes usually found in EOs. The structure of new compounds has been elucidated, various acyclic 1,3-diones [18, 19], italicene and isoitalicene, helifolene and iso-helifolene, various bisabolane diols [32] eudesm-5-en-11-ol [20].

H. italicum of the Adriatic coast (sub-species not specified) produces EO with α-pinene, α- and γ-curcumene as major components [33, 34, 35]. EO (subsp *italicum*) from Tuscany contained mainly α-pinene and neryl acetate [25] while an oil sample from Southern Italy was dominated by *iso*-italicene epoxide [26]. Other Italian EOs contained mainly γ-curcumene, β-selinene and α-selinene [27].

H. italicum EOs from Mediterranean islands exhibited various compositions. For instance, oil from the Greek island of Amorgos (ssp *italicum*) was dominated by geraniol [28] while plants from Crete (ssp *microphyllum*) produced oil containing mainly sequiterpene hydrocarbons [21]. *H. italicum* ssp *microphyllum* from Corsica and Sardinia is rich in neryl acetate [1, 22, 23] while some samples contained appreciable amounts of eudesm-5-en-11-ol [24]. Some Sardinian oil samples contained rosifoliol and γ-curcumene as main components [22].

The composition of *H. italicum* ssp *italicum* EO from Corsica and Tuscan Archipelago Islands was dominated by neryl acetate [25, 29, 30]. Correlations between the EO composition and various parameters were shown: texture and acidity of soils, inorganic composition of plant and soil, vegetative stage of development [37]. Some oil samples from

Tuscan Archipelago Islands contained unusually high amounts of β-diketones [30]. Oils from Elba Island (Italy) were characterized by a high content of oxygenated monoterpenes while monoterpene hydrocarbons and sesquiterpene hydrocarbons reached appreciable in some samples [31, 35, 36].

Table 2 Components of *H. italicum* essential oils.

origin	Major components	Other components	Ref
Helichrysum italicum* ssp *microphyllum			
Crete	β-selinene (17.1/16.7%), γ-curcumene (13.7/6.6%)	α-selinene (3.8/5.4%), Italicene (5.1/1.4%).	[21]
Sardinia	neryl acetate (28.9%), neryl propionate (11.4%)	γ-curcumene (11.4%), nerol (10.7%)	[22]
Sardinia	rosifoliol (20.2%), γ-curcumene (18.2%)	linalool (14.9%)	[22]
Sardinia	neryl acetate (21.4/16.9%), dihydro-occidentalol (12.2/7.6%)	nerol (7.3/5.4%), neryl propionate (5.6/4.6%)	[23]
Sardinia	neryl acetate (17.6-56.1%), eudesmen-5-en-11-ol (3.7-23.5%)	nerol (3.7-14.4%)	[24]
Corsica	neryl acetate (55.7/41.5%)	neryl propionate (12.7/5.6%)	[1]
Helichrysum italicum ssp italicum			
Tuscany (Italy)	α-pinene (4.1-53.5%), neryl acetate (0.3-22.0%)	β-selinene (7.2-12.5%), β-caryophyllene (5.7-11.0%)	[25]
Cilento (Italy)	*iso*-italicene epoxide (16.8%)	hexadecene (9.8%), β-costol (7.5%)	[26]
Italy	γ-curcumene (0-41.0%), β-selinene (0-38.0%), α-selinene (0-26.5%), γ-eudesmol (0-20.4%)	nerol (0.4-18.8%), (E)-β-caryophyllene (0-18.6%), neryl acetate (0.4-15.1%)	[27]
Amorgos (Greece)	geraniol (35.6%)	geranyl acetate (14.7 %), (E)-nerolidol (11.9%).	[28]
Corsica	neryl acetate (15.8-42.5%) γ-curcumene (0.8-13.6%)	limonene (1.9-7.3%), neryl propionate (1.5-6.7%),	[29] [25]
Corsica	neryl acetate (32.0%), ar-curcumene (6.4%)	4,6,9-trimethyldec-8-en-3,5-dione (11.0%)	[27]
Tuscan Archipelago (Italy)	neryl acetate (14.9–44.5%), neryl propionate (3.0–16.4%)	γ-curcumene (5.4-13.7%), nerol (1.4-7.6%), eudesm-5-en-11-ol (1.1-7.6%)	[30]
Elba Island (Italy)	neryl acetate (5.6-45.9%), α-pinene (0.8 –32.9%), 1,8-cineole (up to 18.2%)	eudesm-5-en-11-ol (1.8-17.2%), nerol (up to 12.8%), limonene (up to 12.9%)	[31]
Elba Island (Italy)	neryl acetate (11.4%), γ–eudesmol (8.5%)	(E)–β–caryophyllene (7.8%), γ-curcumene (7.7%)	[27]
Sub-species not specified			
Ex-Yugoslavia	α-pinene (21.7%), γ-curcumene (10.4%)	neryl acetate (6.1%), β-selinene (6.0%),	[32]
Croatian Adriatic coast	α-pinene (0.1-29.9%), α-curcumene (1.0-28.6%), γ-curcumene (0-22.0%)	α-cedrene (0.2-16.7%), neryl acetate (4.1-13.5%), spathulenol (up to 13.2%)	[33]
Croatia	α-pinene (10.2%), neryl acetate (11.5%)	α-cedrene (9.6%)	[34]
Elba island (Italy)	neryl acetate (25.3% ± 2.9), α-pinene (14.5% ± 2.1)	limonene (12.3% ± 2.8), γ-curcumene (8.7% ± 1.4).	[35] [36]

It is likely that the variability of components present in *H. italicum* extracts and EOs has a strong influence on their biological activity.

3. Biological Activities of *H. Italicum* Essential Oil and Extracts

Metabolites isolated from *H. italicum*, and especially its volatile fraction, have been found to display many biological properties, such as antimicrobial, anti-inflammatory, anti-viral antioxidant activities. The insecticidal effects of the EO have also been described. Until now, no reports on the possible phytotoxic activity of the secondary metabolites of the plant have been reported.

3.1. Antimicrobial activity

Of all the properties claimed for *H. italicum*, the antibacterial effect of extracts, EOs and their constituents has received a main attention. Several data report the effectiveness of *H. italicum* extracts against Gram positive bacteria. Nostro and coworkers [38] demonstrated that diethyl ether extracts of *H. italicum* has inhibitory effect on *Staphylococcus aureus* strains reducing both their growth and some of the enzymes considered as virulence factors. With minimum inhibitory concentration (MIC) values ranging from 125 to 500 mg/L, this extract is so effective on methicillin sensitive *S. aureus* strains (MSSA) as on methicillin resistant *S. aureus* isolates (MRSA). It also inhibits the enzymatic activity of these strains with a more pronounced effect on the coagulase than on the DNAse, lipase and thermonuclease. Other works have been done to evaluate the effects of *H. italicum* extracts on different bacterial virulence factors, such as toxins production or cell aggregation. It has been shown that low concentrations of *H. italicum* diethyl ether extract reduce the enterotoxins B and C production by *S. aureus* [39]. In the same way, it was highlighted that subminimum inhibitory concentrations (7.81 to 31.25 µg/mL) of *H. italicum* ethanolic extracts inhibit *in vitro* adherence and cellular aggregation of the cariogenic *Streptococcus mutans* bacterium [40] extracts seem to be able to interfere with bacterial virulence and thereby show considerable interest to control undesirable and pathogenic bacteria.

Two main studies carried out in our laboratories report the antibacterial properties of *H. italicum* EO and its related constituents. Rossi and coworkers [41] demonstrated that the EO, obtained from endemic plants of Corsica, is more effective on the Gram positive bacterium *S. aureus* than on the Gram negative strains *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*. It is commonly known that Gram negative bacteria are less susceptible to EOs than Gram positive bacteria, and this is directly connected to the bacterial cell wall structure. In Gram negative bacteria, the cell wall is a complex envelope constituted by the cytoplasmic membrane, the periplasm and the outer membrane. The latter one restricts diffusion of hydrophobic molecules through its lipopolysaccharide covering, thus acting as a strong permeability barrier [42].

So, these bacteria are particularly difficult to eradicate, especially as they have also developed effective mechanisms of resistance such as efflux pumps overexpression. Efflux pumps play a key role in the bacterial resistance to antibiotics and contribute to the spread of multidrug resistant pathogens (MDR phenotype). These protein carriers are able to expel from the cells structurally diverse drugs, including antibiotics, rendering them therapeutically ineffective. By blocking this mechanism with efflux pump inhibitors (EPIs), it is possible to restore the effectiveness of antibiotics. Lorenzi and coworkers [43] have shown that *H. italicum* EO significantly reduces the MDR resistance of several Gram negative strains of *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. The highest activity was obtained for the MDR clinical isolate of *E. aerogenes* EA27, which overexpresses the AcrAB-TolC efflux pump and are thus resistant against the last resort antibiotic used in intensive care units, *i.e* chloramphenicol. At a concentration of 2.5%, *H. italicum* EO reduces eightfold the MIC (from 1,024 to 128 mg/L) of chloramphenicol for EA27 strain. Moreover, *H. italicum* EO restores the chloramphenicol susceptibility of EA27 to a level that is close to that of the control phenylalanine arginine ß-naphthylamide (PAßN), *i.e* MIC of 64 mg/mL. It is clear from these data that *H. italicum* EO contains one or more compounds that have EPI activity. Therefore, a chromatographic fractionation assay was made to isolate that or these agents. Then, the EPI's activity of the main fractions recovered was evaluated against the derivative mutant of *E. aerogenes* EA27, *i.e* EAEP289 strain. It has been shown that combinations of the two most active fractions (italidiones, F2 and alcohols, F3) can reduce chloramphenicol resistance from an initial MIC of 1,024 to 128 mg/L. Reduction of resistance was also achieved when either the F2 or F3 fraction was combined with PAßN. Combination of the latter produced the strongest effect comparable to a complete reversal of chloramphenicol resistance (MIC of less than 0.25 mg/L). Due to the high activity of the F3 fraction, several chloramphenicol susceptibility assays were performed with commercially available constituents of this fraction. Among the compounds tested, geraniol produces significant restoration of susceptibility of the MDR strain EAEP289 to chloramphenicol by as much as 16-fold. When combined with PAßN, it rendered the bacterium fully susceptible to chloramphenicol, *i.e*., it completely reversed initial resistance. Geraniol (3,7-dimethylocta-2(*E*),6-dien-1-ol) is an acyclic monoterpenic compound which presents a stereochemistry (*E*). Its hydrocarbon backbone is constituted of two isoprene units and functionalized with an hydroxyl group (Figure 2).

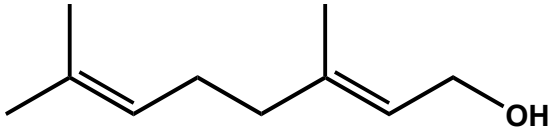

Fig. 2 Chemical structure of geraniol

Geraniol is soluble in dimethylsulfoxide (DMSO) and methanol. These solvents complicate experiments and cause reproducibility difficulties, related to the lack of homogeneity of the solutions. So, complementary tests were carried out with several derivatives compounds of geraniol in order to improve its solubility while retaining its efficiency. It was found that hydrochloride geranyl amine salt (Figure 3), a water soluble compound, is as effective as geraniol since it reduces the MIC of chloramphenicol to the same extent as PAßN.

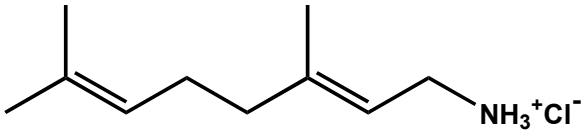

Fig. 3 Chemical structure of hydrochloride geranyl amine

It was thus concluded that the replacement of the primary alcohol group of geraniol by an amino-hydrochloride function has improved the solubility of the molecule.

If the antibacterial properties of *H. italicum* are widely described in the literature, less is known about its effect against yeasts and fungi. However, it has been reported that the EO of *H. italicum* from Croatia and, more precisely, its oxygenated fraction are active against *Candida albicans*. The terpenoid components of this fraction inhibit the growth of the yeast by producing an inhibition zone of 10 mm and a MIC of 5 µg/mL [37].

3.2. Anti-inflammatory activity

H. italicum is known to contain ketones that contribute to reduce the inflammation process. Arzanol, a prenylated heterodimeric phloroglucinyl α-pyrone (Figure 4), was identified as the major anti-inflammatory component [44].

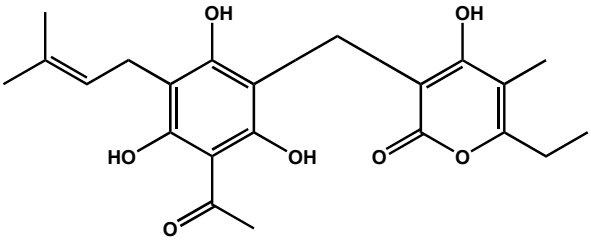

Fig. 4 Chemical structure of arzanol

Inflammation is a complex biological response that involves several enzymatic reactions. The prostanoids and leukotrienes (LTs) formed from arachidonic acid *via* the cyclooxygenase (COX)-1/2 and 5-lipoxygenase (5-LO) pathway, respectively mediate inflammation, chronic tissue modeling, cancer, asthma and autoimmune disorders. The non-steroidal anti-inflammatory drugs administered for therapeutic purposes act by blocking formation of all the prostanoids but their clinical use is hampered by severe side effects including gastrointestinal injuries, renal irritations and cardiovascular risks [19]. It has been shown that arzanol potently inhibits the nuclear transcription factor NFκβ activation in T cells as well as the release of pro-inflammatory mediators such as interleukin (IL)-1β, Il-6, Il-8, tumor necrosis factor (TNF)α and in lipopolysaccharide stimulated monocytes [18]. More recently, Bauer and coworkers [44] have investigated the effects of arzanol on the biosynthesis of prostanoids and LTs and have evaluted its anti-inflammatory efficacy *in vitro* and *in vivo*. They have shown that this molecule potently inhibits the inducible microsomal prostaglandin (PG)E_2 synthase (EC 5.3.99.3), COX-1(EC 1.14.99) and 5-LO (EC 7.13.11.34) *in vitro* with IC_{50} values ranging from 0.4 to 9 µM. *In vivo*, arzaanol suppresses the inflammatory response of the carrageenan-induced pleurisy in rats (3.6 mg/kg, intraperitoneal) with significantly reduced levels of PGE_2 (2.27 ng/rat) in the pleural exudates. Taken together, all these findings show that arzanol act as potent dual-inhibitor of pro-inflammatory mediators and inflammatory enzymes, providing a mechanistic rationale for the well-known anti-inflammatory activity of *H. italicum*. Moreover, this compound displays a large spectrum of properties including anti-oxidant and anti-viral activities (see sections 3.3 and 3.4).

3.3. Anti-viral activity

A few studies of the anti-viral properties of *H. italicum* extracts and their constituents have been published. It has been pointed out that a diethyl ether extract, obtained from the flowering tops of *H. italicum*, possesses significant activity against the herpes simplex virus type 1 (HSV-1) at concentrations ranging from 100 to 400 µg/mL. Moreover, this extract has no genotoxic effect since it does not provoke DNA damages at concentrations up to 200 µg per disk [45]. In a more recent study, Appendino and coworkers [18] have shown that arzanol inhibits the HIV-1 replication in T cells. This anti-HIV activity was further investigate by infecting Jurkat cells with the pNL4-3 HIV-1 clone pseudotyped with the VSV envelope, which can supports a robust HIV-1 replication. Upon integration into host chromosomes, this recombinant virus expresses the firefly luciferase gene, and therefore, luciferase activity in infected cells correlates with the rate of viral replication. A pretreatment of Jurkat cells 30 min prior to infection with increasing doses of arzanol resulted in a dose-dependent inhibition of luciferase activity.

3.4. Antioxidant activity

Some flavonoids constituents of *H. italicum* exhibit antioxidant activities, which are closely related to their anti-inflammatory effects. In a study investigated by Sala and coworkers [46], whose purpose was to assess the antioxidant properties of three flavonoids (gnaphaliin, pinocembrin and tiliroside) isolated from the aerial parts of *H. italicum*, the tiliroside was identified as the most active compound. More precisely, the scavenger properties of these flavonoids were tested first *in vitro* and then *in vivo* by means of different models of inflammation. Tiliroside shows significant inhibition of enzymatic and non-enzymatic lipid peroxidation (IC_{50} values: 12.6 and 28 µM respectively). It has scavenger properties (IC_{50} = 21.3 µM) and very potent antioxidant activity in the reduction of stable radical 1,1-diphenyl-2-pycryl-hydrazyl (DPPH) test (IC_{50} = 6 µM). *In vivo*, it significantly reduces the mouse paw oedema induced by phospholipase A_2 (ID_{50} = 35.6 mg/kg) and the mouse ear inflammation induced by TPA (ID_{50} = 0.357 mg/ear). Recently, the protective effect in lipid peroxidation of azarnol was highlighted. Its antioxidant activity was assessed against the oxidative modification of lipid components induced by Cu^{2+} ions in human low density lipoprotein (LDL) and by *tert*-butyl hydroperoxide (TBH) in cell membranes. *In vitro*, LDL pretreatment with azarnol significantly preserves lipoproteins from oxidative damage at 2 h of oxidation and exerts a remarkable reduction of polyunsaturated fatty acids and cholesterol levels. At non-cytotoxic concentrations, it also protects VERO cells against TBH induced oxidative stress [47]. So, azarnol can be qualified as a potent natural antioxidant with a protective effect against lipid oxidation in biological systems.

3.5. Anti-larvicidal activity

Only a few reports describe the effects of *H. italicum* against insects. It has been recently shown that the EO isolated from the leaves of *H. italicum*, growing on Elba Island, induces larval mortality of the *Culicidae* mosquito *Aedes albopictus* at 300 ppm with mortality rates ranging from 98.3% to 100% [38]. Use of botanical derivatives in mosquito control, instead of synthetic insecticides, is thought to be harmless to humans and other nontarget organisms. So, further investigations are needed to identify natural mosquitocidal compounds, which could be utilized in commercial formulations.

4. Conclusion

In conclusion, this survey of the literature showed that *H. italicum* sp exhibits interesting biological activities that seem to be due to the large diversity of its chemical contents. These remarkable properties explain the enthusiasm that exists around *H. italicum* sp essential oil and extracts. However, care should be taken to the species or species derivatives, maturation state, part of the plant used, and to the extraction procedures that are undertaken to produce bio-active extracts.

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