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Abstract

Ethnopharmacological relevance

Helichrysum italicum (Roth) G. Don fil. (family *Asteraceae*) has been used for its medicinal properties for a long time and, even nowadays, continues to play an important role in the traditional medicine of Mediterranean countries. Based on this traditional knowledge, its different pharmacological activities have been the focus of active research.

Aim of the review

To provide an overview of the current state of knowledge of the pharmacological activities of *H. italicum*, as well as its traditional uses, toxicity, drug interactions and safety.

Materials and methods

The selection of relevant data was made through a search using the keywords "*Helichrysum italicum"* and "*H. italicum*" in "Directory of Open Access Journals", "Google Scholar", "ISI Web of Knowledge", "PubMed", "ScienceDirect" and "Wiley Online Library". Information obtained in local and foreign books and other sources was also included.

Results

There are reports on the traditional use of *H. italicum* in European countries, particularly Italy, Spain, Portugal and Bosnia and Herzegovina. In these countries, its flowers and leaves are the most used parts in the treatment of health disorders such as allergies, colds, cough, skin, liver and gallbladder disorders, inflammation, infections and sleeplessness. In order to validate some of the traditional uses of *H. italicum* and highlight other potential applications for its extracts and isolated compounds, several scientific studies have been conducted in the last decades. *In vitro* studies characterized *H. italicum* as an antimicrobial and anti-inflammatory agent. Its flavonoids and terpenes were effective against bacteria

(e.g. *Staphylococcus aureus*), its acetophenones, phloroglucinols and terpenoids displayed antifungal action against *Candida albicans* and its flavonoids and phloroglucinols inhibited HSV and HIV, respectively. *H. italicum* acetophenones, flavonoids and phloroglucinols demonstrated inhibitory action in different pathways of arachidonic acid metabolism and other pro-inflammatory mediators. Regarding *H. italicum in vivo* activity, the highlight goes to the anti-erythematous and photoprotective activities of its flavonoids, demonstrated both in animals and humans, and to the anti-inflammatory properties exhibited by its flavonoids, acetophenones and phloroglucinols, as seen in animal models. Concerning its safety and adverse effects, while *H. italicum* does not display significant levels of cytotoxicity or genotoxicity, it should be noticed that one of its flavonoids inhibited some CYP isoforms and a case has been reported of an allergic reaction to its extracts.

Conclusions

H. italicum is a medicinal plant with promising pharmacological activities. However, most of its traditionally claimed applications are not yet scientifically proven. Clinical trials are needed to further confirm these data and promote *H. italicum* as an important tool in the treatment of several diseases.

Keywords: *Helichrysum italicum*; traditional medicine; everlasting; anti-inflammatory; antimicrobial.

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1. Introduction

Medicinal plants play an important role in the discovery and isolation of new drugs, as has been the case for morphine, digitoxin, quinine, reserpine and pilocarpine (Balunas and Kinghorn, 2005; Gurib-Fakim, 2006). Consequently, there is a clear indication that this is a viable path of clinical innovation, as

evidenced by some plant species from the *Helichrysum* Miller genus (family *Asteraceae*). This genus includes more than a thousand taxa that have a higher occurrence in the Mediterranean areas of Europe (Facino et al., 1988; Morone-Fortunato et al., 2010; Perrini et al., 2009). The name of the genus is derived from the Greek words "helios" and "chryos", which mean, respectively, "sun" and "gold", in direct relation to the fact that the plant species of this genus typically have inflorescences of a bright yellow color (Perrini et al., 2009).

One of the earlier mentions of the medicinal uses of plants from the *Helichrysum* genus appears in the work of the Greek Theophrastus of Eresos "Historia Plantarum" (3^{rd}-2^{nd} century B.C.). There, he reports that "*Heleiochrysos*" may be used in the treatment of burns (mixed with honey) and stings/bites of venomous animals (Scarborough, 1978). Another example of an ancient report of *Helichrysum* medicinal properties comes in book four of "De Materia Medica" (1^{st} century A.D.), written by the Greek Pedanius Dioscorides, where the decoction of the filaments of *Helichrysum* flowers macerated in wine is described as possessing diuretic properties and being useful in the treatment of urinary disorders, snake bites, sciatica and hernias (Quer, 1993). Concerning the Renaissance period, the first written record of the medicinal uses of *Helichrysum* species in South Africa is attributed to the Dutch botanist Herman Boerhaave, who reported their use in the treatment of nervousness and hysteria in 1727 (Lourens et al., 2008). Other authors from the same period have cited *Helichrysum* sp., as is the case of Robert Morison who named the species *Helichrysum chrysocome angustifolia vulgaris* (now *H. stoechas* (L.) Moench) (Morison, 1699).

In the early descriptions of the medicinal uses of plants from this genus, *Helichrysum* is frequently addressed as a whole, without a clear indication of the specific species to which the information pertains. The fact that *Helichrysum* is considered a very complex genus, with great similarities between some species (Sala, 2001) may justify historical and popular difficulties in the correct identification of the plants.

In recent decades, some of the most studied species of this genus are *Helichrysum arenarium* (L.) Moench (Czinner et al., 2000), *Helichrysum stoechas* (L.) Moench (Carini et al., 2001), *Helichrysum graveolens* (M.Bieb.) Sweet (Aslan et al., 2007) and *Helichrysum italicum* (Roth) G. Don (Facino et al., 1988). The interest in these species has been motivated by their traditional therapeutic applications: *H. arenarium* inflorescences use in Central Europe has been reported for its antiseptic, coleretic and

spasmolytic properties (Sala, 2001), while *H. graveolens* traditional applications in controlling the symptoms of diabetes mellitus, wound healing and as a diuretic have been reported in Turkey (Aslan et al., 2007). *H. stoechas* is particularly referred in Spanish folk medicine for its anti-inflammatory and wound healing properties as well as uses for toothache, urologic conditions (Mulet, 1991; Rivera et al., 2008) and digestive disorders (González-Tejero, 1989; Peris et al., 2001). *H. italicum* use has also been reported in inflammatory and allergy conditions such as those related with the respiratory tract, as well as skin conditions (Peris et al., 1995; Peris et al., 2001), among others. For *H. italicum* essential oil in particular, wound healing and other skin conditions (such as hematoma and scars) have been pointed out as interesting aromatherapy applications being stated that «its effects are so convincing that it has never met with any kind of criticism despite the absence of data on its effectiveness» (Schnaubelt, 1999).

Since *H. italicum* pharmacological data are rather dispersed in the literature, this review aims to describe the traditional use and the available scientific data on *H. italicum* pharmacological activity and establish the relationship between them. Available safety and toxicity data are also addressed. This knowledge allows a discussion of the existing gaps, highlighting the need and interest for scientific validation of specific traditional uses and may be important in the identification of potential therapeutic applications not yet fully clinically explored for *H. italicum* plant or extracts.

The first scientific studies on the medicinal properties of *H. italicum* are attributed to Leonardo Santini, whose clinical research in patients with psoriasis was conducted in the 40s and 50s of the 20th century. However, his findings were published in journals of very little importance and were largely ignored after his death (Appendino et al., 2007; Bauer et al., 2011; Campanini, 2004). Consequently, the search of the keywords "*Helichrysum italicum*" or "*H. italicum*" in a scientific database such as PubMed reveals that the majority of research work related to this plant has been published after the 90s and up to now. However, considering the important role that *H. italicum* plays in the traditional medicinal practice of Mediterranean countries, it is surprising that review articles on its traditional uses, pharmacological activity and therapeutic interest are so scarce (Ríos, 2008) and do not include the most recent studies. As a result, in this paper we review the current state of knowledge of the traditional uses, pharmacological activities, toxicity, drug interactions and safety of *H. italicum*.

2. Taxonomic classification and general characteristics

H. italicum (synonyms: *Gnaphalium glutinosum* Ten.; *G. italicum* Roth; *H. angustifolium* var. *numidicum* (Pomel) Maire; *H. italicum* var. *numidicum* (Pomel) Quézel & Santa; *H. italicum* var. *serotinum* (Boiss.) O. Bolòs & Vigo; *H. numidicum* Pomel; *H. rupestre* subsp. *glutinosum* (Ten.) Nyman; *H. stoechas* subsp. *numidicum* (Pomel) Batt.), also known as "perpétuas-das-areias", "perpétuas-de-Itália", "immortelle" or "everlasting" (Ivanovic et al., 2011), is an aromatic shrub 30-70 cm high (Galbany-Casals et al., 2011). It exhibits a strong and persistent smell similar to curry (Appendino et al., 2007), has yellow flowers and blossoms between May and June (Bianchini et al., 2009).

This species has the ability to grow naturally in the dry, sandy and stony areas of the Mediterranean regions due to the fact that it is a xerophyte plant (i.e. it has adapted in order to be able to survive in environments that lack water). This characteristic also allows *H. italicum* to grow at a wide range of altitudes, between the sea level and 2200 m (Galbany-Casals et al., 2011; Nostro et al., 2001; Perrini et al., 2009), and assumes particular relevance under an economic perspective.

H. italicum can be further divided into six subspecies which are distributed in different regions of the Mediterranean basin (Table 1).

Table 1. *H. italicum* subspecies and distribution (Biondi, 2007; Galbany-Casals et al., 2011; Paolini et al., 2006; Proença da Cunha et al., 2012).

Taxa	Distribution
Helichrysum italicum (Roth) G. Don subsp. *italicum*	Mediterranean basin
Helichrysum italicum subsp. *microphyllum* (Willd.) Nyman	Balearic Islands (Majorca and Dragonera), Sardinia, Corsica, Crete and Cyprus
Helichrysum italicum subsp. *picardii* Franco	France, Italy, Portugal and Spain
Helichrysum italicum subsp. *pseudolitoreum* (Fiori) Bacch. & al.	Argentario, Gargano and Mount Conero
Helichrysum italicum subsp. *serotinum* (Boiss.) P.Fourn.	Iberian Peninsula
Helichrysum italicum subsp. *siculum* (Jord. & Fourr.) Galbany & al.	Sicily

3. Traditional uses

In the last few decades some ethnopharmacological surveys have been carried out in different regions in order to gather knowledge on the traditional uses of a large variety of plants, among which *H. italicum* (Table 2).

Table 2. Ethnopharmacological studies of *H. italicum* in different regions of Europe, with indication of its medicinal uses, used plant parts and type of preparation.

Year	Region	Plant name	Medicinal Uses	Plant Part	Preparation	Reference
1989	Granada, Spain	*H. italicum* (Roth) G. Don	Toothache	Flower	Infusion (mouth rinsing)	(González-Tejero, 1989)
1991	Castellón, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Digestive disorders	Flower	Infusion	(Mulet, 1991)
1993	Múrcia, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Analgesic, anti-odontalgic, astringent, antiemetic and dermatologic tonic	_	_	(Rivera and Obón, 1993)
1997	Campidano and Urzulei, Sardinia, Italy	*H. italicum* subsp. *microphyllum* (Willd.) Nyman	Allergy	Whole plant	Infusion	(Bruni et al., 1997)
1998	Córdoba, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Stomach cleanser	-	Decoction	(Luque et al., 1998)
1999	Giglio, Tuscany Archipelago, Italy	*H. italicum* (Roth) G. Don	Cough, colds, tracheitis and	Leaf and flower tip	Infusion and vapor	(Unicini Manganelli

			laryngitis		s	and Tomei, 1999)
2000	Garfagnana, Lucca Province, Italy	*H. italicum* (Roth) G. Don	Colds	Aerial parts	Infusion and fumes	(Pieroni, 2000)
2001	Fluminimaggiore, Sardinia, Italy	*H. italicum* subsp. *microphyllum* (Willd.) Nyman	Skin diseases (alopecia)	Whole plant	Decoction	(Ballero et al., 2001)
2005	Jaén, Spain	*H. italicum* (Roth) G. Don	Digestive disorders and catarrh	-	-	(Pardo de Santayana et al., 2005)
2005	Ibi, Alicante, Spain	*H. italicum* (Roth) G. Don	Toothache and mouth antiseptic	Flower	Infusion (mouth rinsing)	(Barber et al., 2005)
2007	Alt Empordà, Catalunya, Spain	*H. italicum* (Roth) G. Don	Digestive disorders	Flower	Infusion	(Parada, 2007)
2007	Bosnia and Herzegovina	*H. italicum* (Roth) G. Don	Liver and gall disorders, cough	Flower	Infusion	(Redzic, 2007)
2007	Calabria, Italy	*H. italicum* (Roth) G. Don	Brochitis and pharyngitis	Flowery tops	Infusion or powder mixe	(Passalacqua et al., 2007)

					d with hone y	
20 08	Sannio, Benevento, Campania, Italy	*H. italicum* (Roth) G. Don	Cough	Flower	Infusi on or decoc tion	(Guari no et al., 2008)
20 08	La Coruña, Spain	*H. italicum* (Roth) G. Don	Skin inflamm ation	Flower	Infusi on (exter nal use)	(Lator re, 2008)
20 08	Valencia, Spain	*H. italicum* (Roth) G. Don	Intestina l parasitic infection s	-	-	(Segar ra i Durà, 2008 cited by Latorr e, 2008)
20 08	Jumilla-Yecla, Murcia, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Wound healing	Flower , leaf and stem	Powd er	(River a et al., 2008)
20 09	Baixo Alentejo; Barlavento Algarvio, Portugal	*H. italicum* (Roth) G. Don	Dermato logic disorder s	Aerial parts	Essen tial oil	(Proen ça da Cunha et al., 2007)
20 09	Riviera spezzina, Liguria, Italy	*H. italicum* (Roth) G. Don subsp. *italicum*	Sleeples sness, headach e, sniffles and cough	Flower and leaf	Fume s Infusi on	(Corn ara et al., 2009)

			Inflammation and cough Stomach ache Helmintic infections	Flower Flower and leaf Young leaves and apical part Flower	Decoction Juice Decoction	
2010	Western Granada, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Digestive disorders, gastralgia Cough, mouth ailments, liver disease, herpes	Inflorescence Flowery plant	Infusion Infusion	(Benitez et al., 2010)
2012	Portugal	*H. italicum* subsp. *picardi* Franco	Dermatomycosis	Aerial parts	Essential oil	(Proença da Cunha et al. 2012)
2013	National Park of Cilento and Vallo di Diano, Campania, Italy	*H. italicum* (Roth) G. Don	Asthma	Flowering tops	Decoction	(Di Novella et al.,

						2013)

These data show that the most frequently reported traditional uses of *H. italicum* are related to respiratory, digestive and skin inflammatory conditions. Other therapeutic applications include antimicrobial uses and wound healing, as well as gall and bladder disorders and analgesic uses. Scientific validation of this knowledge relies on *in vitro* and *in vivo* studies. Available studies on *H. italicum* pharmacological activities are reviewed in section 5.

There are reports of the traditional use of other species from the *Helichrysum* genus, as highlighted in Table 3. Among these, one of the species with more reported traditional uses is *H. stoechas*, which is closely related to *H. italicum* (Proença da Cunha et al., 2007),

Table 3. Examples of ethnopharmacological uses of *Helichrysum* sp. (other than *H. italicum*) in different regions, with indication of its medicinal uses, used plant parts and type of preparation.

Plant name	Year	Region	Medicinal Uses	Plant Part	Preparation	Reference
H. arenarium (L.) Moench	1998	Europe	Diuretic	Flower	Herbal tea	(Cañigueral et al., 1998)
H. foetidum var. *foetidum* (L.) Moench	1999	Eastern Cape Province, South Africa	Infected sores	Leaves	Poultice	(Grierson and Afolayan, 1999)
H. melaleucum Rchb.	1995	Madeira e Porto Santo Islands, Archipelago of Madeira, Portugal	Bronchitis, cough and pharingitis. Cardiotonic	Flower heads and leaves	Infusion	(Rivera and Obon, 1995)
H. obconicum DC	1995	Madeira e Porto Santo Islands, Archipelago of Madeira, Portugal	Stomach and intestinal disorders	Flower and leaves	Infusion	(Rivera and Obon, 1995)

H. orientale (L.) Vaill	1995	Madeira e Porto Santo Islands, Archipelago of Madeira, Portugal	Asthma and cough	Flower heads	Tea	(River a and Obon, 1995)
	2013	Marmaris, Anatolia, Turquey	Sore throat, dyspnea, cough and cold Nephritis, icterus, dysuria and kidney stone	Aerial parts Capitulum	Infusion Infusion	(Gurdal and Kultur, 2013)
H. peduncu latum Hilliard & B.L.Burtt	1995	Transkei, South Africa	Inflammation and wounds	Leaves	-	(Bhat and Jacobs, 1995)
H. plicatum DC	1995	Taurus Mountains, Anatolia, Turquey	Kidney stones Jaundice Dysurea	Flower Flower +herb Flower +herb	Infusion Decoction Infusion	(Yesilada et al., 1995)
	2013	Malatya, Anatolia, Turquey	Wounds	Flower	Pomade	(Tetik et al., 2013)
	2013	Solhan, Anatolia, Turquey	Diabetes, hepatitis and kidney stones	Flower	Infusion	(Polat et al., 2013)
H. stoechas (L.) Moench	1989	Granada, Spain	Digestive disorders	Flower	Infusion	(González-Tejero, 1989)
	1991	Castellón, Spain	Conjuctivitis and ocular	Flower and	Decoction	(Mulet, 1991)

			infections Fever Digestive disorders Hypertension Intestinal inflammation Intestinal spasms Pharyngitis and tonsillitis Wounds	stem Flower Flower Flower	Infusion Decoction Ointment	
	2001	Iberian Penisula and Balearic Islands	Digestive and respiratory inflammation, hepatic disorders, headaches and hypercholesterolemia	Flowery tops	Decoction	(Peris et al., 2001)
	2002	Girona, Catalonia, Spain	Constipation	Whole plant	Infusion	(Latorre, 2008)
	2003	Rute, Cordoba, Spain	Digestive disorders	-	-	(Sanchez-Romero, 2003)
	2006	Beja, Alentejo, Portugal	Colds, digestive disorders, fever,	Flower	Decoction/ infusion	(Carvalho, 2006)

			measles and pain.			
	2011	Serra da Estrela Natural Park, Portugal	Antipyretic and decongestant	Flower and stem	Infusion	(Silva et al., 2011)
	2012	Mallorca Island, Balearic Islands	Hypertension	Flower	Tisane	(Carrió and Valles, 2012)
H. stoechas subsps. *stoechas* (L.) Moench	2008	Jumilla-Yecla, Murcia, Spain	Hemorrhoids Intestinal parasitic infections and wounds Kidney disorders Toothache	Flower, leaf and stem	Infusion (soaking cotton in a bag) Powder Infusion Infusion (rinses)	(Rivera et al., 2008)

4. Plant extracts and chemical composition

A large variety of extracts of *H. italicum* can be prepared, and the resulting products differ in their chemical composition (Table 4).

The most analyzed extract of *H. italicum* is the essential oil, which can be obtained from all the green parts of the plant (Leonardi et al., 2013). Consequently, studies reporting its composition are numerous (Angioni et al., 2003; Bertoli et al., 2012; Bianchini et al., 2009; Bianchini et al., 2004; Bianchini et al., 2003; Bianchini et al., 2001; Conti et al., 2010; Leonardi et al., 2013; Mancini et al., 2011; Mastelic et al., 2008; Mastelic et al., 2005; Morone-Fortunato et al., 2010; Paolini et al., 2006; Perrini et al., 2009; Roussis et al., 2000; Satta et al., 1999; Usai et al., 2010). Distinct essential oil chemotypes have been obtained from the two main subspecies of *H. italicum*. More specifically, at least three from *H. italicum*

subsp. *italicum* (Morone-Fortunato et al., 2010): one characterized by an elevated percentage of monoterpenes such as neryl acetate, neryl propanoate and α-pinene (Bianchini et al., 2001; Paolini et al., 2006), another constituted by a high amount of geraniol and geranyl acetate (Bianchini et al., 2001; Morone-Fortunato et al., 2010), and a third one with a large proportion of sesquiterpenes (Bianchini et al., 2001; Morone-Fortunato et al., 2010). Regarding *H. italicum* subsp. *microphyllum,* two main essential oil chemotypes are described: one rich in nerol, neryl acetate, neryl propionate, linalool and limonene, and another characterized by a high quantity of *ar*-curcumene, γ-curcumene and rosifoliol (Angioni et al., 2003). Finally, it is relevant to underline that the chemical composition of *H. italicum* subsp. *italicum* essential oil demonstrates an elevated level of intraspecific differences in response to environmental factors, particularly soil properties (Bianchini et al., 2009).

Table 4. Main types of chemical compounds present in extracts obtained from different parts of *H. italicum.*

Taxa	Plant part	Extract	Main types of compounds	Reference
H. italicum subsp. *microphyllum* (Willd.) Nyman	Leaves and flowerheads	Acetone	Acetophenones, phloroglucinols, pyrones and sesquiterpenes	(Rosa et al., 2007)
H. italicum (Roth) G. Don	Flowers	Diethyl ether	Flavonoids, terpenes, coumarins and steroids	(Nostro et al., 2000)
H. italicum (Roth) G. Don	Flowers	Essential oil	Monoterpenes and sesquiterpenes	(Ivanovic et al., 2011)
H. italicum (Roth) G. Don	Flowering tops	Ethanol	Flavonoids	(Nostro et al., 2004)
H. italicum (Roth) G. Don	Aerial parts	Methanol	Flavonoids, acetophenones and triterpenes	(Sala et al., 2001)
H. italicum (Roth) G. Don	Flowers	Supercritical CO_2	Sesquiterpenes and waxes	(Ivanovic et al., 2011)

5. Pharmacological activities

5.1. In vitro studies

5.1.1. Anti-inflammatory activity

Acetophenones isolated from the CH_2Cl_2, EtOAc and BuOH fractions of the methanolic extract of *H. italicum* were tested for their ability to inhibit arachidonic acid metabolism in two different *in vitro* models. In the first one, both 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone and 4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone (Fig. 1; **1,2**) were able to reduce the production of leukotriene B_4 (Table 5). In the second assay, only 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (100 μM) had an inhibitory effect on the activity of cyclooxygenase-1 (COX-1) in human platelets stimulated by Ca^{2+} and calcium ionophore A23187, as measured by a 59 % reduction of the production of 12-hydroxyheptadecatrienoic acid (Sala et al., 2003b).

The flavonoids gnaphaliin and pinocembrin (Fig. 1; **3,4**) isolated from the methanolic extract of *H. italicum* were also able to inhibit the production of leukotriene B_4 (Table 5) (Sala et al., 2003a).

Table 5. Inhibition of leukotriene B_4 production by 100 μM of acetophenones and flavonoids isolated from *H. italicum* in an *in vitro* model of rat polymorphonuclear leukocytes stimulated by calcium A23187.

Compound	Inhibition (%)	IC_{50} (μM)	Reference
4-hydroxy-3-(3-methyl-2-butenyl)acetophenone	95	24	(Sala et al., 2003b)
4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone	44	111	(Sala et al., 2003b)
Gnaphaliin	94	-	(Sala et al., 2003a)
Pinocembrin	96	-	(Sala et al., 2003a)

Both the whole acetone extract and arzanol (Fig. 1; **5**) obtained from *H. italicum* subsp. *microphyllum* displayed a potent inhibitory effect upon Nuclear Factor Kappa B (NF-κB) activity, with IC_{50} values of 25 and 5 $\mu g.mL^{-1}$, respectively. Moreover, arzanol inhibited the production of IL-1β, TNFα, IL-6, IL-8 and PGE_2 in human peripheral monocytes stimulated by lipopolysaccharides (LPS), with IC_{50} values of 5.6, 9.2, 13.3, 21.8 and 18.7 μM, respectively (Appendino et al., 2007) as well as the biosynthesis of PGE_2 in whole blood (Bauer et al., 2011).

Arzanol was also able to inhibit the activity of 5-Lipoxygenase (5-LO) in a cell free assay (IC_{50}=3.1 μM), the formation of leukotrienes in human neutrophils (IC_{50}=2.9-8.1 μM) and the activity of COX-1 and the formation of prostaglandin PGE_2 derived from COX-2 (IC_{50}=2.3-2.9 μM). This latter effect was found to

result from arzanol's interference with microsomal PGE_2 synthase (mPGES) (IC_{50}=0.4 μM) rather than with COX-2 (Bauer et al., 2011). These combined effects of arzanol are particularly remarkable as they are similar to other dual COX/5-LO inhibitors such as licofelone, a novel and very potent anti-inflammatory that also acts by inhibiting COX-1, mPGES-1 and 5-LO pathways (Koeberle et al., 2008).

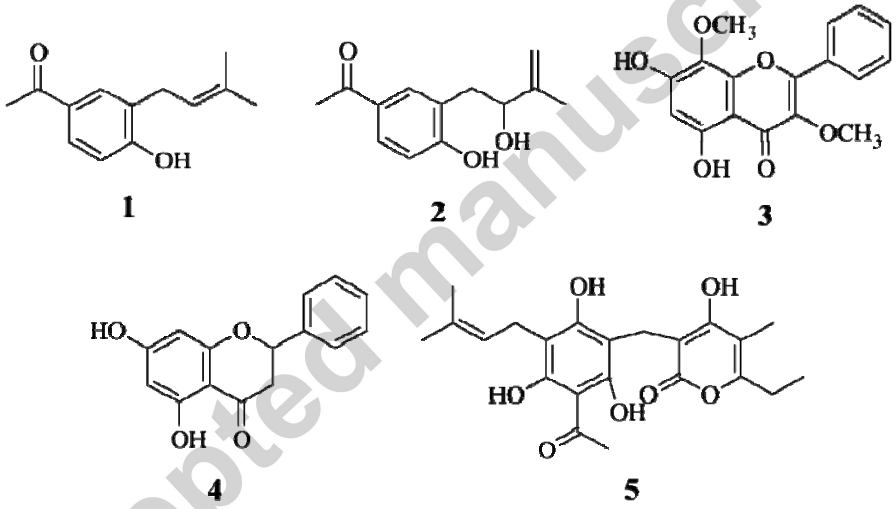

Figure 1. Chemical structures of compounds with *in vitro* anti-inflammatory activity isolated from *H. italicum*: 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (1); 4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone (2); Gnaphaliin (3); Pinocembrin (4) and Arzanol (5).

5.1.2. Antimicrobial activity

Several extracts of *H. italicum* exhibited an inhibitory effect on Gram-positive bacteria growth and/or virulence factors (Table 6), while the results against Gram-negative bacteria were less evident.

It was demonstrated that both the essential oil (Chao et al., 2008; Mastelic et al., 2005; Rossi et al., 2007) and the diethyl ether extract (Nostro et al., 2001; Nostro et al., 2002) of *H. italicum* had the ability to inhibit the growth of *Staphylococcus aureus* in a concentration dependent manner, with no difference in

sensitivity between methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus* strains (Nostro et al., 2001). Furthermore, it was also showed that the diethyl ether extract at sub-minimum inhibitory concentrations (sub-MIC) reduced the activity of *S. aureus* enzymes, specifically DNAse, lipase, thermonuclease and coagulase (Nostro et al., 2001). Sub-MIC concentrations of the extract also compromised *S. aureus* ability to produce the enterotoxins B and C (Nostro et al., 2002).

There is some controversy regarding which components of the extracts are responsible for the antibacterial activity of *H. italicum* against *S. aureus*. Some studies highlight the terpenoid fraction (Mastelic et al., 2005) while others suggest that these activities might be due to both terpenes and flavonoids (Nostro et al., 2001; Nostro et al., 2002). However, the ability of terpenes and flavonoids to interact with the cytoplasmatic membrane of *S. aureus* and induce its structural and functional destabilization highlights their prominent role in the antibacterial activity demonstrated by *H. italicum* (Nostro et al., 2001; Nostro et al., 2000).

Moreover, *H. italicum* ethanolic extract inhibited the growth and interfered with the cariogenic effects of *Streptococcus mutans* (Nostro et al., 2004), one of the main microorganisms responsible for dental caries (Giacaman et al., 2010). Sub-MIC concentrations of the ethanolic extract reduced the cell-surface hydrophobicity, cellular aggregation and adherence of *S. mutans.* The authors inferred that these beneficial effects on the cariogenic action of *S. mutans* may occur due to the flavonoidic content of the *H. italicum* ethanolic extract, since several members of this class of compounds exhibit anti-cariogenic activities against this microorganism (Ferrazzano et al., 2011).

Table 6. MIC of different *H. italicum* extracts against Gram-positive bacteria.

Microorganism	Extract	MIC	Reference
Bacillus subtilis	Diethyl ether	125 $\mu g.mL^{-1}$	(Nostro et al., 2000)
Micrococcus luteus	Methanol	50 $\mu g.mL^{-1}$	(Tundis et al., 2005)
Staphylococcus aureus	Essential oil	5 $\mu L.mL^{-1}$	(Mastelic et al., 2005)
	Diethyl ether	125-500 $\mu g.mL^{-1}$	(Nostro et al., 2001)
Streptococcus mutans	Ethanol	62.50 $\mu g.mL^{-1}$	(Nostro et al., 2004)

It should also be noticed that the essential oil of *H. italicum* and one of its components, geraniol (Fig. 2), displayed an elevated capacity to restore the antibiotic activities of several drugs against multidrug-resistant Gram negative bacteria. More specifically, Lorenzi *et al.* (2009) reported that *H. italicum* essential oil significantly increased the efficacy of chloramphenicol against multidrug-resistant strains of *Enterobacter aerogenes*, *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Furthermore, geraniol, besides restoring the activity of chloramphenicol against *E. aerogenes*, also increased its susceptibility to the β-lactams penicillin and ampicillin, and the fluoroquinolone norfloxacin. *H. italicum* essential oil and geraniol acted by efflux pump inhibition, which is important since most bacteria are resistant to antibiotics due to the use of efflux pumps (Van Bambeke et al., 2000). These findings are particularly relevant due to the increase of multidrug-resistant bacterial strains, among which Gram-negatives are the most problematic because there is a lack of effective therapeutic alternatives to the conventional antibiotics (Giamarellou, 2010).

Figure 2. Chemical structure of Geraniol.

H. italicum essential oil was effective against *Candida albicans* (Mastelic et al., 2005), a very important pathogen that is the causal agent of conditions that range from trivial oral and genital infections to fatal systemic infections in immunocompromised patients (McCullough et al., 1996). The anti-candida activity of the essential oil (MIC of 5 $\mu g.mL^{-1}$) was attributed to the terpenoid fraction and its oxygen-containing compounds (Mastelic et al., 2005), which, typically, are the most active (Palmeira-de-Oliveira et al., 2009).

Phloroglucinol and acetophenone derivatives extracted from the aerial parts of *H. italicum* were found active against different species of *Penicillium* (Tomás-Barberán et al., 1990).

Both the whole acetone extract and its most active compound, arzanol (Fig. 1; **5**), isolated from *H. italicum* subsp. *microphyllum* inhibited the TNFα-induced HIV-1-LTR transactivation in a T cell line in a concentration-dependent manner (IC_{50} of 25 $\mu g.mL^{-1}$ and 5 μM, respectively). Furthermore, it was also

shown that pre-treatment with arzanol of Jurkat cells infected with HIV-1 reduced the viral replication (Appendino et al., 2007).

The high resistance level of *Herpes Simplex Virus* (HSV) to classic antiviral drugs (Morfin and Thouvenot, 2003) stresses the need for new, less expensive and toxic treatments. As such, a diethyl ether extract obtained from the flowering tops of *H. italicum* was studied for its anti-HSV-1 activity, and it was effective in concentrations ranging from 100 to 400 $\mu g.mL^{-1}$ (Nostro et al., 2003). The authors suggested that this activity might be due to the presence of the flavonoids apigenin and luteolin (Fig. 3; **1,2**) in the composition of *H. italicum*, as these compounds have already showed anti-HSV activity in other studies (Mucsi et al., 1992; Wleklik et al., 1988).

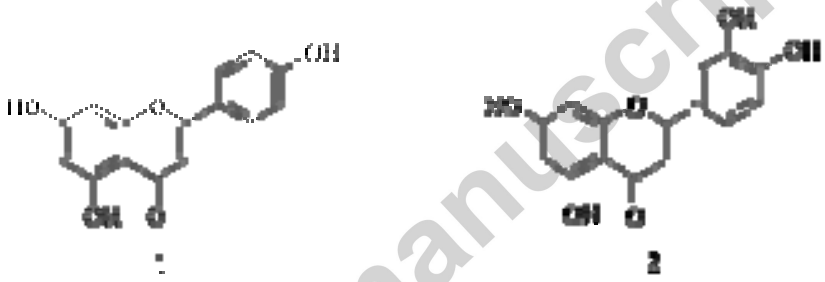

Figure 3. Chemical structures of antiviral compounds isolated from *H. italicum*: Apigenin (1) and Luteolin (2).

5.1.3. Insecticidal and repellent activity

The *Aedes albopictus* and *Aedes aegypti* mosquitoes are the main vectors of epidemic diseases like dengue and yellow fever (Vontas et al., 2012). Since several essential oils have already showed insecticidal and/or larvicidal activity against mosquitoes from the *Aedes* genus (Araujo et al., 2003; Carvalho et al., 2003; Cheng et al., 2003), Conti *et al.* (2010) tested the efficacy of *H. italicum* essential oil against *A. albopictus* larvae. The results showed that the essential oil exhibited a high level of toxicity to the larvae because when it was tested at a concentration of 300 ppm, the mortality rate was 100%, with the LC_{50} being determined as 178.1 ppm.

Additionally, the essential oil repellent activity against *A. aegypti* was shown to be independent of the tested concentration (0.1-10%), and it was able to repeal about 30 % of the mosquitoes. The authors

suggested that *H. italicum* essential oil might be an interesting agent to be included in mosquito repellent formulations in combination with other active compounds (Drapeau et al., 2009).

5.2 In vivo studies

An 8 % alcoholic solution of crude extract of the flowering tops of *H. italicum* and a 2 % alcoholic solution of a flavonoid fraction isolated from it were topically applied to guinea pigs while only the flavonoid fraction was applied to humans 10 minutes before or after exposure to UVB radiation to evaluate their photoprotective and anti-erythematous activities, respectively. Both the crude extract and the flavonoid fraction completely prevented the onset of the erythematous response in guinea pigs and humans. When tested in humans, the flavonoid fraction provided a sun protection factor of approximately 5. The study confirmed that the flavonoids are the active compounds, as their fraction reduced the UVB induced erythema to a similar extent to the whole extract (Facino et al., 1988). The proposed mechanism of action of the flavonoid fraction might include the inhibition of the local production of prostaglandins in the irradiated skin, particularly by luteolin influence (Wolfle et al., 2011), and the inhibition of histamine release and radical scavenging activity mediated by apigenin (Hirano et al., 2001; Middleton and Drzewiecki, 1984). The authors proposed that *H. italicum* flavonoids might be useful in the formulation of products for burn treatment, radioprotection and sunscreen effect.

H. italicum methanolic extract and all its fractions (hexane, CH_2Cl_2, EtOAc and BuOH) were able to reduce the edema induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in mice ears, being the BuOH fraction the most active, followed by the methanolic extract, EtOAc, hexane and CH_2Cl_2 fractions, respectively. When the edema was induced by ethylphenylpropiolate, only the EtOAc and BuOH fractions were active. In another assay where phospholipase A_2 (PLA_2) obtained from the venom of *Naja mossambica* and serotonin were used to induce paw edema in mice, the methanol extract and the BuOH fraction were the most effective in the first case, whereas in the second case, all the fractions were active, with the EtOAc being the most potent. Finally, when chronic inflammation was induced by multiple applications of 2 μg of TPA, the ear edema was reduced by 65, 44 and 48 % with 200 $mg.kg^{-1}$ of the methanolic extract, hexane and CH_2Cl_2 fraction, respectively, whereas the leukocyte infiltration was reduced by all the fractions (40-66 %) and the methanolic extract (58 %). The authors concluded that the anti-inflammatory activity of these extracts might be due to pro-inflammatory enzyme inhibition, free radical scavenging activity or effects similar to the ones induced by corticoids (Sala et al., 2002).

Based on these results, the authors tested several compounds isolated from the CH_2Cl_2, EtOAc and BuOH fractions of the methanolic extract of *H. italicum* for their anti-inflammatory activity in the assay involving the topical application of 2.5 µg of TPA in mice ears. The 4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone (Fig. 1; **2**) isolated from the CH_2Cl_2 fraction was found to be the most effective and exhibited a ID_{50} of 0.63 µmol (Sala et al., 2001).

On the model of chronic inflammation induced by multiple topical applications of 2 µg of TPA in mice ears, both 0.5 mg of 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (Fig. 1; **1**) and 12-hydroxytremetone (Fig. 4; **1**) reduced myeloperoxidase activity by 57 and 71 %, respectively. When the compounds (80 $mg.kg^{-1}$) were tested against the paw edema induced by PLA_2, the most active compounds 1 hour after the injection were 12-hydroxytremetone-12-*O*-β-D-glucopyranoside, 3-(2-hydroxyethyl) acetophenone-4-*O*-β-D-glucopyranoside and maltol β-D-*O*-glucopyranoside (Fig. 4; **2,3,4**), which reduced the edema by 65, 57 and 52 %, respectively. Finally, when edema was induced in the mice paws by subplantar injection of carrageenan (3 % w/v), the orally administered 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (150 $mg.kg^{-1}$) reduced the edema by 51, 71 and 66 % at 1, 3 and 5 h after the injection, respectively (Sala et al., 2003b).

The flavonoids (gnaphaliin, pinocembrin (Fig. 1; **3,4**) and tiliroside (Fig. 4; **5**) isolated from the methanolic extract of *H. italicum* and injected at a dosage of 80 $mg.kg^{-1}$ were able to reduce over 50 % of the edema in the paws of mice, 60 minutes after being induced by PLA_2. However, when the edema was induced by subcutaneous injection of serotonin (3 % w/v), all the flavonoids, administered by the same route at a dose of 50 $mg.kg^{-1}$, reduced the edema formation but to a lower extent (less than 40 %). Furthermore, 0.5 mg of all flavonoids reduced the edema induced by the topical application of 2.5 µg of TPA in the mice ears, with values of inhibition of 72 (ID_{50}=210 µg), 81 (ID_{50}=61 µg) and 80 % (ID_{50}=357 µg) for gnaphaliin, pinocembrin and tiliroside, respectively. Finally, when the flavonoids were tested against the model of chronic inflammation induced by multiple applications of TPA, tiliroside (0.5 mg) was the most effective compound as it diminished the edema formation by almost 50 % and reduced the neutrophil infiltration by 88 % (Sala et al., 2003a).

Bauer *et al.* (2011) tested arzanol (Fig. 1; **5**) for its anti-inflammatory activity against pleurisy induced by the injection of carrageenan into the pleural cavity of rats: when arzanol was administered intraperitoneally at a dose of 3.6 $mg.kg^{-1}$, it diminished the inflammatory response as measured by the

reduction of exudate formation (59 %), cell infiltration (48 %), and the levels of PGE_2 (47 %), 6-krto $PGF_1\alpha$ (27 %) and LTB_4 (31 %).

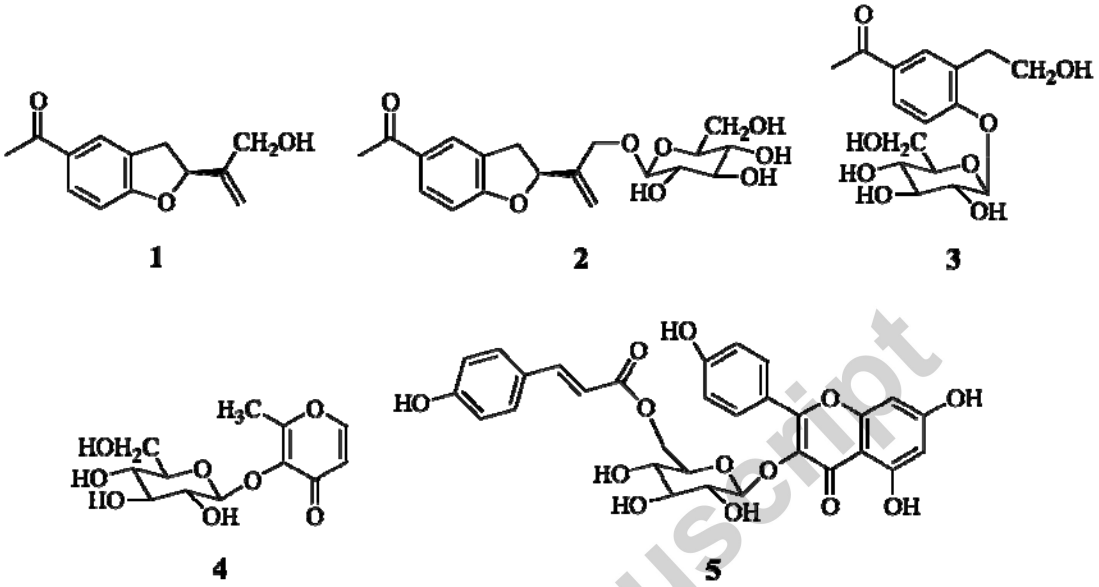

Figure 4. Chemical structures of compounds with *in vivo* anti-inflammatory activity isolated from *H. italicum*: 12-hydroxytremetone (1); 12-hydroxytremetone-12-*O*-β-D-glucopyranoside (2); 3-(2-hydroxyethyl) acetophenone-4-*O*-β-D-glucopyranoside (3), maltol β-D-*O*-glucopyranoside (4) and tiliroside (5).

In contrast to animal studies, there is a severe lack of human clinical trials of the effects of the extracts and isolated compounds of *H. italicum*, which undermines the possibility of confirming the results obtained in both *in vitro* and *in vivo* animal studies and ultimately validating the traditional uses of this plant.

When two drops of *H. italicum* subsp. *serotinum* essential oil were administered orally two times a day during ten days, followed by the topical application of the essential oil (diluted to 10 % in *Rosa rubiginosa* vegetal oil) for a period of 2-3 months in the post-operative scars of patients submitted to a plastic surgery of the thorax, a reduction of local inflammation, edema, bruises and hematomas was seen (Voinchet and Giraud-Robert, 2007).

The development and study of adequate dosage forms to potentiate the efficacy and safety of the extracts of *H. italicum* is also being taken into account, as can be exemplified by the medication sticks containing *H. italicum* essential oil previously developed by our research group (Palmeira-de-Oliveira et al., 2011).

6. Toxicity, drug interactions and adverse effects

6.1. Cytotoxicity, genotoxicity and antigenotoxicity

The cytotoxicity of *H. italicum* essential oil was studied using the yeast *Sacchoromyces cerevisiae*, and it was shown that it had a minimal effect on the survival of the yeast cells in the stationary and exponential phase, up to the tested concentration of 5 $\mu L.mL^{-1}$ (Bakkali et al., 2005).

For the study of the genotoxicity of the essential oil, both the *Sacchoromyces cerevisiae* (Bakkali et al., 2005) and *Drosophila melanogaster* (Idaomar et al., 2002) models were used. In both cases, the results indicated that this essential oil did not exhibit any kind of significant genotoxicity, when used up to a maximum concentration of 0.3 % (Idaomar et al., 2002).

When the essential oil was mixed with the promutagen urethane, it was able to reduce the number of somatic mutations induced by urethane in *D. melanogaster* wings between 54 and 57 % for concentrations up to 0.3 % (Idaomar et al., 2002). The authors proposed that the antigenotoxicity of *H. italicum* might occur due to the interaction of some of its compounds with the cytochrome P450 enzymes (CYP), as it is known that urethane uses this metabolic pathway to originate its ultimate metabolites with mutagenic activity (Hoffler et al., 2005).

Concerning the cytotoxity of arzanol (Fig. 1; **5**), the MTT assay and the measurement of lactate dehydrogenase release were performed in Vero cells cultures and the results showed that arzanol did not exhibit toxicity at any of the tested concentrations (0.5-40 μM) (Rosa et al., 2007).

The diethyl ether extract of *H. italicum* was tested for cytotoxicity and genotoxicity in Vero cells and by the *Bacillus subtilis* rec-assay (Mazza, 1982), respectively, and it was shown that only concentrations of 800 $\mu g.mL^{-1}$ displayed cytotoxicity, whereas there was a complete lack of genotoxicity (Nostro et al., 2003).

6.2. Inhibition of cytochrome P450 enzymes

In a study conducted by Sun *et al.* (2010), tiliroside (Fig. 4; **5**) (100 μM) was incubated with human liver microssomes and strongly inhibited, in a competitive manner, the isoforms CYP3A4 (71.6 %), CYP2C9 (85 %) and CYP2C8 (82.3 %), with values of IC_{50} of 9.0±1.7, 10.2±0.9 and 12.1±0.9 μM, respectively. Considering that CYP enzymes are the main catalysts of the metabolism of drugs (Guengerich, 2006), and that, specifically, CYP3A4, CYP2C9 and CYP2C8 are involved in the metabolism of several clinically important drugs (Lai et al., 2009; Thorn et al., 2011), these results highlight the possible drug-herb interactions when using plants that contain tiliroside (Sun et al., 2010). However, the majority of flavonoids have a low oral bioavailability and can be degraded by the bacteria present in the gut (Moon et al., 2006) and consequently, the concentrations that are achievable *in vivo* may not be sufficient to cause medical important interactions (Sun et al., 2010). Furthermore, these interactions are not expected to pose significant safety problems when topical administration is required, due to the reduced serum concentrations obtained through this route.

6.3. Tolerance

Using the previously described protocol (section 5.2), Voinchet *et al.* reported a remarkable level of tolerance by the patients exposed to *H. italicum* essential oil. This can be concluded from the fact that no patient displayed any adverse effects related to the utilization of the essential oil, which conveys that it was well tolerated even after prolonged use (Voinchet and Giraud-Robert, 2007). On the other hand, a recent case report described the occurrence of allergic contact dermatitis in a 69-year-old non-atopic woman caused by the hydrophilic and lipophilic fractions of the flowering tops of *H. italicum* contained in an emollient cream that she applied to treat a moderate case of xerosis. The positive reactions were detected by a patch test and confirmed with the isolated fractions of *H. italicum* extract. However, further tests performed by the authors with both fractions in ten healthy volunteers provided negative results (Foti et al., 2013). In fact, *H. italicum* has even been shown to inhibit contact dermatitis in different animal models (Ríos et al., 2005; Sala, 2001), suggesting that the former may correspond to an isolated hypersensitivity reaction report.

7. Critical perspective

Critical analysis of the traditional data and scientific studies presented in this review reveals that the traditional uses of *H. italicum* are much wider in application than those confirmed by experimental data.

Among the claimed medicinal effects, the ability to reduce or modulate inflammation is the most studied property of *H. italicum* extracts or isolated compounds. Moreover, wound healing and skin protective properties seem to be the best documented therapeutic effects of *H. italicum* as shown by *in vivo* studies performed with topical application of *H. italicum* extracts.

Most of the cited research works were performed with organic extracts obtained from *H. italicum*. However, since traditional uses are, mainly, the result of infusion or decoction of parts or the whole plant, the study of aqueous extracts would be of remarkable importance to validate this knowledge. In fact, the type and concentration of herbal components and, consequently, their therapeutic effects is highly dependent on the method of preparation of the extracts. On the other hand, one of the major limitation of the available scientific data concerning *H. italicum* is the frequent absence of indication of the subspecies used in each study, which hinders the comparison between them.

Other traditionally claimed properties of *H. italicum* extracts have been explored in marketed products such as cosmetics and food supplements. However, efficacy data obtained through clinical trials are not generally available. As these products are not proposed as treatment of diseases, demonstration of their clinical profile is not legally required. Body hygiene cosmetic products (including the genital area) claim the calming and antimicrobial properties of *H. italicum* essential oil incorporated in their formulas, while oral supplements developed to favour venous circulation or cough treatment highlight the calming and protective properties from different lyophillized *H. italicum* influorescences extracts (Aboca, 2013; Rottapharm|Madaus, 2011).

It is interesting to note that although some research works have highlighted the insecticidal effect of *H. italicum,* ethnobotanical data report its use as flea (parasite) repellent for animal use (Barber et al., 2005; Rivera et al., 2008) but not as insecticidal or insect repellent.

According to the available scientific studies, some of the traditional uses of this plant still lack validation. These include the analgesic effect (toothache, headache, stomach ache) and application on sleeplessness, digestive non-inflammatory disorders, alopecia and helmintic infections. Therefore, these properties stand as open research fields for *H. italicum*.

In vitro toxicity evaluation studies of *H. italicum* are rather scarce and only include its essential oil and diethyl ether extract. Nonetheless, they seemingly indicate a favorable safety profile. However, caution

must be taken due to the reported effects of *Helichrysum* spp in human *in vitro* lymphocytes (*H. sanguineum, H. pamphylicum, H. orientale, H. noeanum)* (Eroglu et al., 2010) and even animal poisoning (*H. blandoskianum*) (McAuliffe and White, 1978) (*H. argyrosphaerum)* (van der Lugt et al., 1996). Although not related with *H. italicum,* animal poisoning shall call attention to the oral modifications that plant components may suffer during digestion, absorption and distribution by the blood or lymph stream.

Topical use of undiluted *H. italicum* essential oil has been referred in aromatherapy literature (Schnaubelt, 1999) while it has been pointed out as neurotoxic by other references (Peris et al., 1995). As for other drugs, toxicity may be dependent on the applied dose and concentration, justifying the high tolerance observed after the 2-3 months treatment with the diluted essential oil in the study of Voinchet et al. mentioned in section 6.3.

8. Conclusions and study perspectives

In this review we aimed to highlight the meaningful traditional uses and the most important data regarding *H. italicum* pharmacological activities, of which the anti-inflammatory and antimicrobial are the best studied.

Comparing the results obtained in the pharmacological studies of *H. italicum* with its traditional uses, it becomes clear that only a few of the latter have already been scientifically validated. Particularly, the importance of *H. italicum* extracts and isolated compounds as anti-inflammatory and antimicrobial agents has already been confirmed. However, there is still room for further studies of other of its frequently reported traditional uses, such as the treatment of digestive non-inflammatory disorders, alopecia, helmintic infections, sleeplessness and its analgesic effect.

H. italicum bioactivity depends on the chemical composition of its different extracts, from which most of the main active compounds have already been isolated. Regarding these active compounds, the most important ones are acetophenones, flavonoids and pholoroglucinol derivatives. Extra attention should be given to the acetophenones 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone, 4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone, the flavonoids tiliroside, gnaphaliin, apigenin and luteolin and the prenylated α-pyrone-pholorglucinol etherodimer arzanol, due to their diverse and important properties. Also, the

study of pharmacological properties of aqueous extracts is essential to confirm data from traditional use of infusions and decoctions.

Other than the scientific identification of mechanism of action pathways, the pressure for a commercial product also explains the search for the most active components of *H. italicum* extracts. However, it should be stressed that under a classical phytotherapeutic point of view, corroborated by aromacology, whole extracts should be used, based on the theory that side effects are less frequent and that synergistic or at least additive effects will result.

The literature described profile seems to point to concentrate future studies on skin/mucosa inflammatory erythematous diseases, for which much investment shall be made, with particular attention to preparations and final dosage forms.

Although the studies of *H. italicum* show great promise, most of its pharmacological activities have only been demonstrated in *in vitro* models. Consequently, it is of utmost importance that the investigation of *H. italicum* extracts and their compounds continues to follow the proper phases of efficacy and safety testing in more *in vivo* studies.

Finally, clinical trials must be conducted in order to verify if the promising pharmacological activities of *H. italicum* can be translated into clinical usefulness in a safe and effective manner and to fully validate its recognized use in the traditional medicine of Mediterranean countries.

Abbreviations

5-LO = 5-Lipoxygenase

COX = Cyclooxygenase

IC = Inhibitory Concentration

IL = Interleukin

TNFα = Tumor Necrosis Factor α

PGE_2 = Prostaglandin E2

LPS = Lipopolysaccharides

MIC = Minimum Inhibitory Concentration

HIV = Human Immunodeficiency Virus

LTR = Long Terminal Repeat

HSV = Herpes Simplex Virus

LDL = Low-density Lipoprotein

NADPH = Nicotinamide Adenine Dinucleotide Phosphate

DPPH = 2,2-diphenyl-1-picrylhydrazyl

EDTA = Ethylenediaminetetraacetic Acid

LC = Lethal Concentration

UVB = Ultraviolet radiation B

TPA = 12-*O*-tetradecanoylphorbol-13-acetate

PLA_2 = Phospolipase A_2

ID = Inhibitory Dose

LTB_4 = Leukotriene B4

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

CYP = Cytochrome P450

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References

Table 3. *Helichyrsum italicum* subspecies and distribution (Biondi, 2007; Galbany-Casals et al., 2011; Paolini et al., 2006; Proença da Cunha et al., 2012).

Taxa	Distribution
Helichrysum italicum (Roth) G. Don subsp. *italicum*	Mediterranean basin
Helichrysum italicum subsp. *microphyllum* (Willd.) Nyman	Balearic Islands (Majorca and Dragonera), Sardinia, Corsica, Crete and Cyprus
Helichrysum italicum subsp. *picardii* Franco	France, Italy, Portugal and Spain
Helichrysum italicum subsp. *pseudolitoreum* (Fiori) Bacch. & al.	Argentario, Gargano and Mount Conero
Helichrysum italicum subsp. *serotinum* (Boiss.) P.Fourn.	Iberian Peninsula
Helichrysum italicum subsp. *siculum* (Jord. & Fourr.) Galbany & al.	Sicily

Table 4. Ethnopharmacological studies of *H. italicum* in different regions of Europe, with indication of its medicinal uses, used plant parts and type of preparation.

Year	Region	Plant name	Medicinal Uses	Plant Part	Preparation	Reference
1989	Granada, Spain	*H. italicum* (Roth) G. Don	Toothache	Flower	Infusion (mouth rinsing)	(González-Tejero, 1989)
1991	Castellón, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Digestive disorders	Flower	Infusion	(Mulet, 1991)
1993	Múrcia, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Analgesic, anti-odontalgic, astringent, antiemetic and dermatologic tonic	_	_	(Rivera and Obón, 1993)
1997	Campidano and Urzulei, Sardinia, Italy	*H. italicum* subsp. *microphyllum* (Willd.) Nyman	Allergy	Whole plant	Infusion	(Bruni et al., 1997)
1998	Córdoba, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Stomach cleanser	-	Decoction	(Luque et al., 1998)
1999	Giglio, Tuscany Archipelago, Italy	*H. italicum* (Roth) G. Don	Cough, colds,	Leaf and	Infusion	(Uncini

			tracheitis and laryngitis	flower tip	and vapors	Manganelli and Tomei, 1999)
2000	Garfagnana, Lucca Province, Italy	*H. italicum* (Roth) G. Don	Colds	Aerial parts	Infusion and fumes	(Pieroni, 2000)
2001	Fluminimaggiore, Sardinia, Italy	*H. italicum* subsp. *microphyllum* (Willd.) Nyman	Skin diseases (alopecia)	Whole plant	Decoction	(Ballero et al., 2001)
2005	Jaén, Spain	*H. italicum* (Roth) G. Don	Digestive disorders and catarrh	-	-	(Pardo de Santayana et al., 2005)
2005	Ibi, Alicante, Spain	*H. italicum* (Roth) G. Don	Toothache and mouth antiseptic	Flower	Infusion (mouth rinsing)	(Barber et al., 2005)
2007	Alt Empordà, Catalunya, Spain	*H. italicum* (Roth) G. Don	Digestive disorders	Flower	Infusion	(Parada, 2007)
2007	Bosnia and Herzegovina	*H. italicum* (Roth) G. Don	Liver and gall disorders, cough	Flower	Infusion	(Redzic, 2007)
2007	Calabria, Italy	*H. italicum* (Roth) G. Don	Brochitis and pharyngi	Flowery tops	Infusion or powd	(Passalacqua et al.,

			tis		er mixed with honey	2007)
2008	Sannio, Benevento, Campania, Italy	*H. italicum* (Roth) G. Don	Cough	Flower	Infusion or decoction	(Guarino et al., 2008)
2008	La Coruña, Spain	*H. italicum* (Roth) G. Don	Skin inflammation	Flower	Infusion (external use)	(Latorre, 2008)
2008	Valencia, Spain	*H. italicum* (Roth) G. Don	Intestinal parasitic infections	-	-	(Segarra i Durà, 2008 cited by Latorre, 2008)
2008	Jumilla-Yecla, Murcia, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Wound healing	Flower, leaf and stem	Powder	(Rivera et al., 2008)
2009	Baixo Alentejo; Barlavento Algarvio, Portugal	*H. italicum* (Roth) G. Don	Dermatologic disorders	Aerial parts	Essential oil	(Proença da Cunha et al., 2007)
2009	Riviera spezzina, Liguria, Italy	*H. italicum* (Roth) G. Don subsp. *italicum*	Sleeplessness, headache, sniffles	Flower and leaf	Fumes	(Cornara et al., 2009)

			and cough Inflammation and cough Stomach ache Helmintic infections	Flower Flower and leaf Young leaves and apical parts Flower	Infusion Decoction Juice Decoction	
2010	Western Granada, Spain	*H. italicum* subsp. *serotinum* (Boiss.) P.Fourn.	Digestive disorders, gastralgia Cough, mouth ailments, liver disease, herpes	Inflorescence Flowery plant	Infusion Infusion	(Benitez et al., 2010)
2012	Portugal	*H. italicum* subsp. *picardi* Franco	Dermatomycosis	Aerial parts	Essential oil	(Proença da Cunha et al. 2012)
2013	National Park of Cilento and Vallo di Diano, Campania, Italy	*H. italicum* (Roth) G. Don	Asthma	Flowering	Decoction	(Di Novell

				tops		a et al., 2013)

Table 3. Examples of ethnopharmacological uses of *Helichrysum* sp. (other than *H. italicum*) in different regions, with indication of its medicinal uses, used plant parts and type of preparation.

Plant name	Year	Region	Medicinal Uses	Plant Part	Preparation	Reference
H. arenarium (L.) Moench	1998	Europe	Diuretic	Flower	Herbal tea	(Cañigueral et al., 1998)
H. foetidum var. *foetidum* (L.) Moench	1999	Eastern Cape Province, South Africa	Infected sores	Leaves	Poultice	(Grierson and Afolayan, 1999)
H. melaleucum Rchb.	1995	Madeira e Porto Santo Islands, Archipelago of Madeira, Portugal	Bronchitis, cough and pharingitis. Cardiotonic	Flower heads and leaves	Infusion	(Rivera and Obon, 1995)
H. obconicum DC	1995	Madeira e Porto Santo Islands, Archipelago of Madeira, Portugal	Stomach and intestinal disorders	Flower and leaves	Infusion	(Rivera and Obon, 1995)
H. orientale (L.) Vaill	1995	Madeira e Porto Santo Islands, Archipelago of Madeira, Portugal	Asthma and cough	Flower heads	Tea	(Rivera and Obon, 1995)
	2013	Marmaris, Anatolia, Turquey	Sore throat, dyspnea, cough and cold Nephritis, icterus,	Aerial parts Capitulum	Infusion Infusion	(Gurdal and Kultur, 2013)

			dysuria and kidney stone			
H. peduncu latum Hilliard & B.L.Bur tt	19 95	Transkei, South Africa	Inflammatio n and wounds	Leaves	-	(Bhat and Jacobs , 1995)
H. plicatu m DC	19 95	Taurus Mountains, Anatolia, Turquey	Kidney stones Jaundice Dysurea	Flower Flower +herb Flower +herb	Infusion Decoction Infusion	(Yesil ada et al., 1995)
	20 13	Malatya, Anatolia, Turquey	Wounds	Flower	Pomade	(Tetik et al., 2013)
	20 13	Solhan, Anatolia, Turquey	Diabetes, hepatitis and kidney stones	Flower	Infusion	(Polat et al., 2013)
H. stoecha s (L.) Moench	19 89	Granada, Spain	Digestive disorders	Flower	Infusion	(Gonz ález-Tejero , 1989)
	19 91	Castellón, Spain	Conjuctivitis and ocular infections Fever Digestive disorders Hypertensio n Intestinal inflammatio n	Flower and stem Flower Flower	Decoction Infusion Decoction	(Mulet , 1991)

			Intestinal spasms Pharyngitis and tonsillitis Wounds	Flower	Ointment	
	2001	Iberian Penisula and Balearic Islands	Digestive and respiratory inflammation, hepatic disorders, headaches and hypercholesterolemia	Flowery tops	Decoction	(Peris et al., 2001)
	2002	Girona, Catalonia, Spain	Constipation	Whole plant	Infusion	(Latorre, 2008)
	2003	Rute, Cordoba, Spain	Digestive disorders	-	-	(Sanchez-Romero, 2003)
	2006	Beja, Alentejo, Portugal	Colds, digestive disorders, fever, measles and pain.	Flower	Decoction/ infusion	(Carvalho, 2006)
	2011	Serra da Estrela Natural Park, Portugal	Antipyretic and decongestant	Flower and stem	Infusion	(Silva et al., 2011)
	2012	Mallorca Island, Balearic Islands	Hypertension	Flower	Tisane	(Carrió and Valles,

						2012)
H. stoechas subsps. *stoechas* (L.) Moench	2008	Jumilla-Yecla, Murcia, Spain	Hemorrhoids Intestinal parasitic infections and wounds Kidney disorders Toothache	Flower, leaf and stem	Infusion (soaking cotton in a bag) Powder Infusion Infusion (rinses)	(Rivera et al., 2008)

Table 4. Main types of chemical compounds present in extracts obtained from different parts of *H. italicum.*

Taxa	Plant part	Extract	Main types of compounds	Reference
H. italicum subsp. *microphyllum* (Willd.) Nyman	Leaves and flowerheads	Acetone	Acetophenones, phloroglucinols, pyrones and sesquiterpenes	(Rosa et al., 2007)
H. italicum (Roth) G. Don	Flowers	Diethyl ether	Flavonoids, terpenes, coumarins and steroids	(Nostro et al., 2000)
H. italicum (Roth) G. Don	Flowers	Essential oil	Monoterpenes and sesquiterpenes	(Ivanovic et al., 2011)
H. italicum (Roth) G. Don	Flowering tops	Ethanol	Flavonoids	(Nostro et al., 2004)
H. italicum (Roth) G. Don	Aerial parts	Methanol	Flavonoids, acetophenones and triterpenes	(Sala et al., 2001)
H. italicum (Roth) G. Don	Flowers	Supercritical CO_2	Sesquiterpenes and waxes	(Ivanovic et al., 2011)

Table 5. Inhibition of leukotriene B_4 production by 100 μM of acetophenones and flavonoids isolated from *H. italicum* in an *in vitro* model of rat polymorphonuclear leukocytes stimulated by calcium A23187.

Compound	Inhibition (%)	IC_{50} (μM)	Reference
4-hydroxy-3-(3-methyl-2-butenyl)acetophenone	95	24	(Sala et al., 2003b)
4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone	44	111	(Sala et al., 2003b)
Gnaphaliin	94	-	(Sala et al., 2003a)
Pinocembrin	96	-	(Sala et al., 2003a)

Table 6. MIC of different *H. italicum* extracts against Gram-positive bacteria.

Microorganism	Extract	MIC	Reference
Bacillus subtilis	Diethyl ether	125 $\mu g.mL^{-1}$	(Nostro et al., 2000)
Micrococcus luteus	Methanol	50 $\mu g.mL^{-1}$	(Tundis et al., 2005)
Staphylococcus aureus	Essential oil	5 $\mu L.mL^{-1}$	(Mastelic et al., 2005)
	Diethyl ether	125-500 $\mu g.mL^{-1}$	(Nostro et al., 2001)
Streptococcus mutans	Ethanol	62.50 $\mu g.mL^{-1}$	(Nostro et al., 2004)

Traditional uses

- Analgesic
- Antiallergic
- Antiasthmatic
- Anti-inflammatory
- Antimicrobial
- Antitussive
- Sedative

Helichrysum italicum

Scientifically validated uses

- Anti-inflammatory
- Antimicrobial
- Insecticidal/Repellent
- Photoprotective

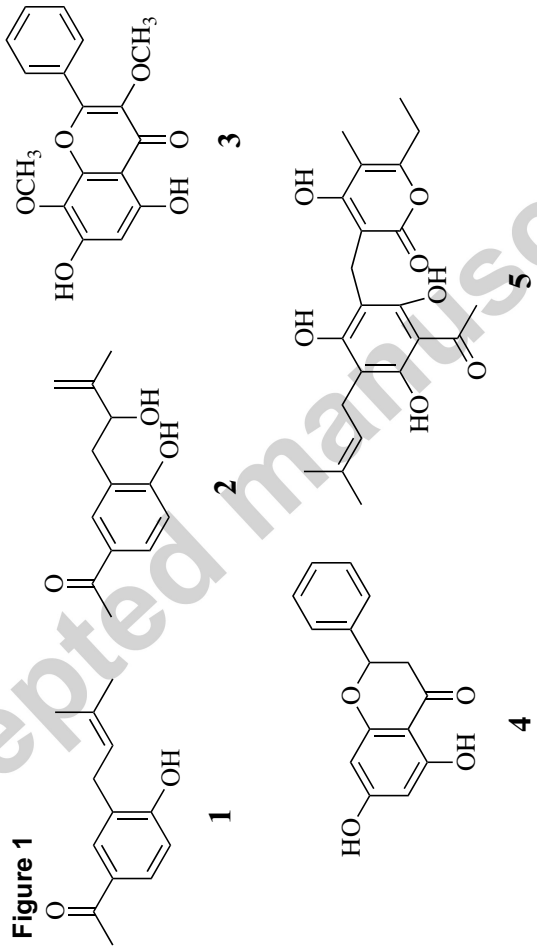

Figure 1

1 **2** **3** **4** **5**

Figure 2

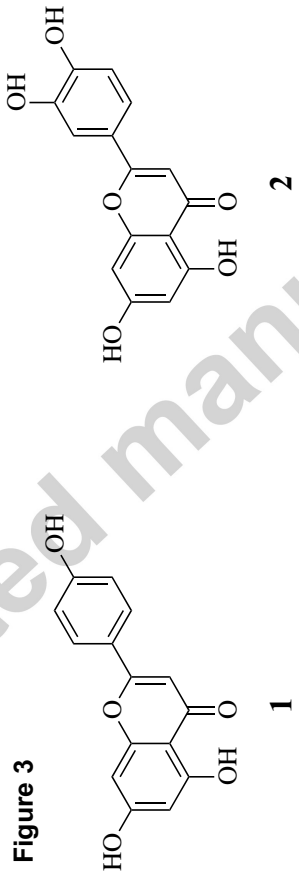

Figure 3

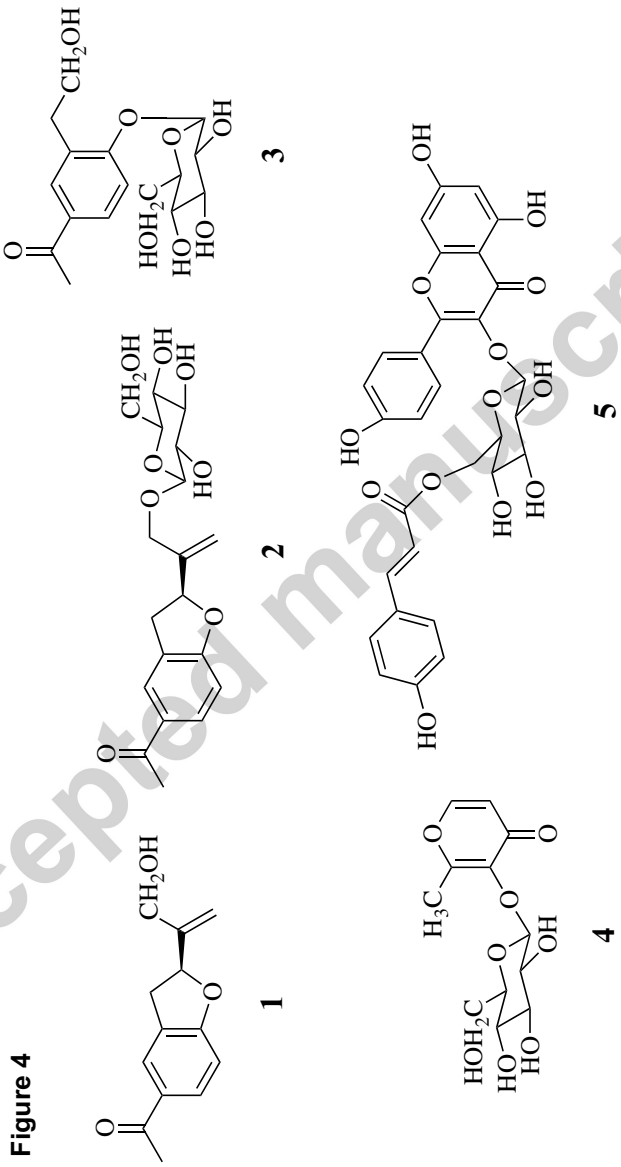

Figure 4