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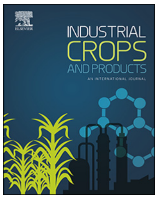

Helichrysum italicum (Roth) G. Don: Taxonomy, biological activity, biochemical and genetic diversity

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ABSTRACT

Helichrysum italicum (Roth) G. Don is a thermophilus perennial from family Asteraceae. Due to versatile biological activities and applications, the interest in *Helichrysum italicum* scientific research and use has increased in the last couple of decades. Consequently, due to the increased industrial demand its plantation farming experienced a huge expansion in Eastern European Mediterranean countries. The aim of this review article was to present the available data on *Helichrysum italicum* and to identify the gaps in the knowledge, and openings for future potential investigations and contributions to the topic. The review of the Scopus database was performed and scientific studies from 1990 to the most recent publications in 2019 were summarized. The article includes the last reviewed taxonomy, detailed description of morphological characteristics, geographical distribution, uses, chemical diversity, biological activity (antimicrobial, antiviral, antioxidant, anti-inflammatory, repellent, and insecticidal activity), and genetic diversity. Additionally, some other potential uses are described. The majority of the available scientific data is focused on the chemical composition of the essential oil, however, little is known about the cultivation management of *Helichrysum italicum* that includes sowing requirements, seedling production, plant density, fertilization and nutrition requirements, harvesting, diseases, insects, and weed control. Genetic information on *Helichrysum italicum* is also scarce, but needed for future breeding programs of *Helichrysum italicum* and selection of new cultivars.

1. Introduction

Helichrysum italicum (Roth) G. Don is a perennial subshrub of the genus *Helichrysum* from the family Asteraceae. Genus *Helichrysum* comprises of more than 600 species spread all over the world, while species *H. italicum* is distributed in the Mediterranean basin. It grows on alkaline, dry, sandy and poor soil (Fig. 1) at the altitude from the sea level up to 2200 m (Galbany-Casals et al., 2011). *Helichrysum italicum* is a valuable medicinal plant that has been used in the folk medicine for decades as choleretic, diuretic and expectorant (Chinou et al., 1996). Nowadays, among the wider population it is best known for its ability to regenerate skin and it is present in many high brands of cosmetic industry as well as in perfume industry due to its characteristic scent (Hellivan, 2009). Also, numerous researches are devoted to investigations of its properties that are potentially responsible for human health benefit. As result of that, authors proved its strong anti-inflammatory, antioxidant, antimicrobial and antiviral, as well as antiproliferative activity. Many industries increased demands for *H. italicum* which prompted new research on the topic. In the Eastern European Mediterranean countries (Croatia and Bosnia and Herzegovina) there was a significant increase of the commercial exploitation of the wild *H. italicum* populations. A great demand for the *H. italicum* essential oil (EO) resulted in the huge expansion of its plantation cultivation (Fig. 2) in: France, Croatia, Bosnia and Herzegovina, Italy, Bulgaria and Serbia.

The aim of this review article was to present the available data on *H. italicum* and to identify the gaps in the knowledge and to guide future investigations and contributions to the topic. Scopus database was reviewed and scientific publications from 1990 up to the latest in 2019 were summarized. This review paper summarizes the available literature on novel taxonomy data, morphological characteristics, biological activity, and chemical diversity of *H. italicum*. Authors determined large variability of the EO that was attributed to the influence of geographical origin, ecological condition, the plant growth stage and genotype, which is more closely discussed in this review paper. Also,

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Fig. 1. Wild *Helichrysum italicum* populations on Island of Cres, Croatia (Photo: Tonka Ninčević).

Fig. 2. Cultivation of *Helichrysum italicum* (Photo: Tonka Ninčević).

the activity of new phenolic compounds discovered in the *H. italicum* is described in the paper. Furthermore, the review summarizes the genetic structure and diversity data that is of a great importance for the conservation of natural populations and the development of breeding programs now that *H. italicum* has become economically recognized plant.

2. Taxonomy and geographical distribution

Helichrysum italicum (Roth) G. Don in Loudon, Hort. Brit. 342 (1830).

The synonyms for *H. italicum* are:

Gnaphalium italicum Roth in Bot. Mag. (Römer& Usteri) 4 (10): 19 (1790) (basionym)

Helichrysum italicum (Roth) Guss., Fl. Sicul. Syn. 2: 469 (1844), *comb. superfl.*

H. angustifolium subsp. *italicum* (Roth) Briq. & Cavill. in Burnat, Fl. Alpes Marit. 6 (2): 265 (1917).

Helichrysum italicum (Roth) G. Don belongs to the family Asteraceae, subfamily Asteroideae, tribe Gnaphalieae and genus *Helichrysum*. The Genus *Helichrysum* Mill. comprises of *ca.* 600 species that are native in Africa, Madagascar, Mediterranean Basin, Macronesia, central Asia and India (Anderberg, 1991). In the Mediterranean region there are nearly 25 native species of the genus *Helichrysum* (Morone-Fortunato et al., 2010). The genus name originates from two Greek words '*helios*' meaning sun and '*chryos*' meaning gold, which refers to the inflorescence color of the most species from the genus, including *H. italicum* (Perrini et al., 2009).

Clapham (1976) classified the Mediterranean, European, western Asian and central Asian *Helichrysum* species into two sections: *Helichrysum* sect. *Helichrysum* and *H.* sect. *Virginea* (DC.) Gren. & Godr, while in Flora Europea (2006) *Helichrysum* species are classified in three sections: *Helichrysum* sect. *Helichrysum, H.* sect. *Virginea,* and Sect. *Xerochlaena* (DC.) Bentham (Table 1). *H. italicum* is native in the central Mediterranean area, and isolated localities in North Africa in the west and Cyprus in the east (Galbany-Casals et al., 2006).

The classification within the species *H. italicum* differs from an author to author. Due to a strong polymorphism in *H. italicum* morphology, subspecies sometimes overlap, and it is difficult to make a strong distinction between the two. Clapham (1976) divided *H. italicum* into three subspecies: 1) *H. italicum* ssp. *italicum*; 2) *H. italicum* ssp. *microphyllum* and 3) *H. italicum* ssp. *serotinum*.

Galbany-Casals et al. (2006) suggest these three subspecies of *H. italicum*: 1) *H. italicum* ssp. *italicum* (widespread in Italy and Croatia, eastern Mediterranean coast of France and Corsica, Bosnia and Herzegovina, Serbia, Montenegro, Slovenia, Greece, mainly Aegean islands and Cyprus and in dispersed localities in Algeria, Morocco and Tunisia); 2) *H. italicum* ssp. *siculum* (native in Sicily), and 3) *H. italicum* ssp. *microphyllum* (dispersed in the Mediterranean islands of Majorca, Corsica, Sardinia, Crete, and Cyprus).

By applying novel morphological and genetic analysis Galbany-Casals et al. (2011) found that there is a spatial distribution of genetic diversity and proposed new division into the Western and Eastern Mediterranean groups that is different from the traditional division into subspecies ssp. *italicum* i ssp. *microphyllum.*

Herrando-Moraira et al. (2016) performed a detailed multivariate analysis of morphological characters using an exhaustive sampling of *H. italicum* and provided a revised taxonomic treatment for the whole *H. italicum* complex. The authors proposed a new combination of the *H. italicum* subspecies: 1) *H. italicum* ssp. *italicum* (widespread in Italy, Croatia, eastern Mediterranean coast of France and Corsica, Bosnia and Herzegovina, Greece – mainly Aegan islands and Cyprus), 2) *H. italicum* ssp. *microphyllum* (endemic to Crete), 3) *H. italicum* ssp. *siculum* (endemic to Sicily), and 4) *H. italicum* ssp. *tyrrhenicum* (disjunct distribution area between islands Corsica, Sardinia, Majorca, and Dragonera Islet).

3. Morphological characteristics

H. italicum is an aromatic perennial subshrub up to 70 cm high (Fig. 3). It has several vegetative stems that are erect, leafy and hairy along the whole length and sometimes bearing axillary leaf fascicles. Flowering stems are erect, ascendant and leafy along the length. Stems are 20 to 50 cm long. Lower and medial cauline leaves of vegetative and flowering stems are 2–37 × 0.4–1.8 mm, elongated, linear, obtuse, hairy and on upper side greenish and on lower silvery. On adaxial surface leaves are subglabrous to tomentose and eglandular to rarely glandular, while on abaxial surface are densely tomentose and glandular. Leaves on the floral stems are erecto-patent and decreasing in size to the synflorescence. The lower leaves are collected in the rosette and others are alternately distributed by the stem. Synflorescence is corymbose, 4.5–62 × 3–80 mm, with 2–120 capitula (Fig. 4). The size of capitula is 4–6.5 × 2–4(5) mm, cylindrical to narrowly campanulate, heterogamous or rarely homogamous with pistillate florets (1–10) and hermaphroditic florets (8–31). The bracts are imbricate, the inner ones are longer than the outer, linear and scarious while the outer ones are broadly rounded, coriaceous and tomentose (Clapham, 1976; Galbany-Casals et al., 2006; Herrando-Moraira et al., 2016). The number of chromosomes in *H. italicum* is $2n = 28$ (D’Amato, 1971). Glandular hairs secreting essential oils are of one single morphological type and for all *H. italicum* genotypes formed by 12 cells. Glandular hairs are formed in three zones: basal zone, median zone and apical zone, and are located on the flower petals, sepals, bracts and stem leaves. In *H. italicum* ssp. *italicum* on the tubular corolla of each flower, density of glandular trichomes is high, but density decreases on the bracts, while in *H. italicum* ssp. *microphyllum,* density of glandular trichomes is even on all plant organs (Perrini et al., 2009). The fruit is achene dark brown color (Domac, 1994), 0.6–1.1 × 0.2–0.7 mm, cylindrical with dispersed shining white glands or eglandular (Clapham, 1976). The mass of a thousand grain amounts approximately 0.03 g (Stepanović et al., 2009). *H. italicum* flowers from (May) June to August (September) (Fig. 5) (Galbany-Casals et al., 2006).

Table 1
Genus *Helichrysum* Mill. – botanical division of European taxa (Flora Europea, 2006).

	Genus *Helichrysum* Mill	Distribution
Sect. VIRGINEA (DC) Fiori		
	H. amorginum Boiss. and Oprh.	Kikladhes (Amorgos) Greece
	H. sibthropii Rouy	north-east Greece (Athos)
	H. doerfleri Rech. fil	east Crete
	H. frigidum (Labill.) Wild.	mountains of Corsica and Sardinia
Sect. HELICHRYSUM		
	H. stoechas[a]	entire Mediterranean
	H. rupestre (Rafin.) DC.[a]	south Spain and Balearic islands, Sardinia, Sicily
	H. heldreichii Boiss.[a]	southwestern Crete
	H. ambiguum (Pers)[a]	islands of Baleares
	H. saxatile[a]	Sardinia and Pantelleria (Sicily)
	H. italicum (Roth)[a]	central Mediterranean and isolated localities in North Africa, and Cyprus
	H. orientale (L.)	Greece and Aegean region
	H. plicatum DC. Prodr.	south part of Balkan peninsula
	H. arenarium (L.) Moench	from Netherlands, south Sweden and Estonia southwards to south Germany, south Bulgaria and west Kazahstan
	H. graveolens (Bieb.)	Krym and south west Asia
Sect. XEROCHLAENA (DC.) Bentham		
	H. foetidum (L.) Cass	west Europe (naturalized) (from south Africa)
	H. bracteatum (Vent.) Andrews	cultivated for ornament and locally

[a] Species are classified in *H. stoechas* group as woody perennials.

Fig. 3. *Helichrysum italicum* subshrub (Photo: Tonka Ninčević).

Fig. 4. *Helichrysum italicum* synflorescence (full flowering stage) (Photo: Tonka Ninčević).

Fig. 5. *Helichrysum italicum* in post flowering stage (Photo: Tonka Ninčević).

4. Chemical compounds of the *Helichrysum italicum*

H. italicum is both harvested from the wild and cultivated for the production of EO and extracts.

Various authors explored the composition of the EO (e. g. Bianchini et al., 2001; Angioni et al., 2003; Paolini et al., 2006; Mastelic et al., 2008; Mancini et al., 2011; Maksimovic et al., 2013; Costa et al., 2015; Zeljkovic et al., 2015; Jerkovic et al., 2016; Mouahid et al., 2017; Tzanova et al., 2018; Oliva et al., 2019) and found a great variability in the chemical composition. The variability was found to be dependent of the following factors: geographical origin, plant growth stage and environment conditions.

The authors suggested that there are more types of the *H. italicum* EO with specific chemical composition according to the geographical origin:

1) Corsican oils are characterized by the prevalence of oxygenated compounds (neryl acetate, neryl propionate, aliphatic ketones and β-diketones) and low percentage of hydrocarbons (limonene, γ-curcumene, α-curcumene) (Bianchini et al., 2001);
2) essential oils from former Yugoslavia are characterized by a high content of α-pinene (22%), then y-curcumene (10%), β-selinene (6%), neryl acetate (6%) and β-caryophyllene (5%), while along the Adriatic Coast main compounds are: α-curcumene (15–29%) or γ-curcumene or α-pinene (25–30%), and neryl acetate (4–14%) (Weyerstahl et al., 1986);
3) in oils from Greece the main components are geraniol (36%), geranil acetat (15%) and nerolidol (12%) (Chinou et al., 1996); while
4) in Tuscan oils, depending on the sampling location, the main compound is α-pinen (33–53%), or neryl acetate (10–22%) and high content of sesquiterpene hydrocarbons (23–39%) with a significant share of β-selinene, β-cariophylene and α-selinene (Bianchini et al., 2003).

The essential oils from Croatia have similar composition as oils from Italy (Bianchini et al., 2003). In Croatia a few authors studied indigenous populations of *H. italicum* (Blažević et al., 1995; Mastelic et al., 2005, 2008; Zeljkovic et al., 2015). Blažević et al. (1995) examined the composition of the essential oil in nine natural populations from Croatia, and the main compounds were α-pinen, neryl acetate, α-cedren, nerol, α-curcumen, γ-curcumen and geranyl acetate. Qualitative composition of the oil was similar in all nine populations but differed in quantity of particular components. The oil yield ranged from 0.08 to 0.32%. Mastelic et al. (2008) researched the *H. italicum* essential oil of Croatian origin and its chemical composition showed numerous monoterpenes, sesquiterpene and nonterpene compounds (hydrocarbons, alcohols, carbonyls, esters, ethers and phenols). Authors hydrodistilled samples of *H. italicum* collected near Split and identified 44 compounds. The main compounds were: α-pinene (12.8%), 2-methyl-cyclo-hexyl pentanoate (11.1%), neryl acetate (10.4%), 1,7-di-epi-α-cedrene (6.8%) and thymol (5.4%). Seven esters were identified, representing 29.0% of the total oil. Ivanovic et al. (2011) studied dried flowers of *H. italicum* collected during September and October in flowering stage from Konavle (Croatia). The most abundant compounds were *y*-curcumene (12.4%), β-selinene (9.9%), trans-β-caryophyllene (6.9%), α-selinene (5.9%), italicene (4.6%) and α-curcumene (4.0%).

Genotypes of 20 native *H. italicum* ssp. *italicum* from different locations in Italy and Corsica were analyzed and different chemotypes were determined, which confirmed that the variability of the EO composition is linked to its geographical origin. Three EO profiles were determined: 1) genotypes with high amounts of nerol and its esters; 2) genotypes with major compounds β and α-selinene and 3) genotypes with major compound γ-curcumene. However, the authors pointed out that the origin of the plant cannot be determined only by the type of the oil because *H. microphylum* has the same chemical composition as the chemotype 1) of *H. italicum* ssp. *italicum* (Morone-Fortunato et al., 2010). Oliva et al. (2019) analyzed the composition of *H. italicum* EO from the Montenegro. Samples of EO were analyzed in liquid and vapor phase on the headspace gas chromatography-mass spectrometry (HS/GC–MS). Results showed that EO from liquid phase had the highest amounts of sesquiterpenes: b-eudesmene (21.65%), b-bisabolene (19.90%) and monoterpenes a-pinene (16.90%) and neryl acetate (10.66%). In the vapor phase monoterpene hydrocarbons fraction was dominant and represented by a-pinene (78.76%).

Furthermore, different plant growth stages affect the composition of the *H. italicum* EO. Blažević et al. (1995) examined the variations in the *H. italicum* essential oil composition during six different plant growth stages: early shoots, corymb forming, before flowering, in flowering, after flowering and late summer shoots. Compound α-pinene was high in the stages of early shoots (24.58%) and corymb forming (28.86%) and it was relatively low in the stages of flowering and after flowering. Compound α-curcumene was low in the stage of early shoots (0.44%) and high in the stage of flowering (28.06%). Compound ɣ-curcumene was the highest in the stage of early shoots (16.65%) and in flowering stage was 12.03%. Neryl acetate was the highest in the stage of plant shoots (9.02%) and it was the lowest after the flowering (5.57%) that indicates that neryl acetate is the highest in the youngest plant parts (shoots) and the lowest in the old parts (after flowering). It can be concluded that in the early stages of plant growth monoterpenes are dominant, while sesquiterpenes are dominant during the flowering and after flowering. Bianchini et al. (2001) have done similar research on *H. italicum* ssp. *italicum* in the different plant growth stages on the Corsican coast. In all growth stages neryl acetate was the major component, ranging from 20.3 to 35.3%, and the maximum value was obtained just before flowering. Compound γ-curcumen was also higher in period before flowering and in the flowering period with maximal amount 6.6%. Non-terpenic ketones: pentan-3-one, 2-methylpentan-3-one and 4-methylhexan-3-one showed variations in amount during different vegetation stages and had the highest values in the stages of early shoots. Wild Sardinian *H. italicum* ssp. *microphyllum* was the subject of Usai et al. (2010) research. The samples were collected from three different elevations and weather conditions in northwestern Sardinia. Four different plant growth stages were studied: vegetative, pre-flowering, flowering and post flowering in the period from February to July. In all plant growth stages the major compounds were neryl acetate (17.6–35.6%) and 5-eudesmen-11-ol (6.4–23.5%). The content of neryl acetate increased from vegetative period to the flowering stage, and after it decreased. It has been shown that growth and decline of 5-eudesmen-11-ol and neryl acetate is associated, when one decreased the other increased. Research has pointed out that the maximum amount of EO and the best composition (with high values of aldehydes) had *H. italicum* ssp. *microphyllum* harvested in July. The authors also compared the EO of dried and fresh plants to assess if there is a change in the composition due to the drying process, but results showed that there was no significant difference. Tzanova et al. (2018) analyzed chemical composition of the *H. italicum* introduced from Corsica and cultivated in South Bulgaria. Authors compared composition of EO from different growth stages: just before flowering and in full flowering. Sesquiterpenes and oxygenated monoterpens showed higher amounts in the stage just before flowering compared with the stage of full flowering.

Besides determining the optimal harvesting period, Angioni et al. (2003) also assessed the influence of different plant parts (flowers and stems) on the composition and the yield of the *H. italicum* ssp. *microphyllum* EO. The highest yields were obtained at the end of the flowering stage where flowering tops yielded 0.18% (v/w) and the stems yielded 0.04% (v/w). The major compounds (nerol, neryl acetate, neryl propionate, limonene and linalool) were the highest during the flowering stage in both observed plant parts but higher in the flowers than in the stems.

Many authors pointed out the influence of the elevation on the EO composition. Usai et al. (2010) suggested collecting plants at the altitude between 500–700 m above the sea level to obtain high oil yield and oil rich in aldehydes that are responsible for the preferable aroma of the plant. Tundis et al. (2005) discovered that altitude impacts the content of trans-caryophyllene in the *H. italicum* essential oil. In the study samples collected at 490 m above the sea level had the highest amount of trans-caryophyllene, while those collected at 800 m showed lower quantities. Melito et al. (2016) also proved influence of altitude and climate on the EO composition. Authors examined 146 *H. italicum* ssp. *microphyllum* genotypes from the seaside (0–60 m above the sea level) and mountains (600–1250 m above the sea level) in Sardinia. The obtained results showed that there is a correlation between the habitat type and the composition of the EO based on significantly different GC oil profiles of both habitats. Quantity of nerolidol was correlated to the average winter temperature and positively correlated to spring and summer precipitation were italicene, bergamotene, nerol and curcumene. Zeljkovic et al. (2015) also confirmed, on four populations of *H. italicum* in Dalmatia (Croatia), that different altitudes and sun exposures have impact on the chemical composition of EOs. Three samples had high amounts of sesquiterpene hydrocarbons and one sample (collected near the sea) had high amounts of oxygenated sesquiterpenes. The main compounds of sample from the island of Brač were α-*trans*-bergamotene (10.2%), β-acoradiene (10.1%) and α-curcumene (8.7%); from sample of mountain Biokovo were α-humulene (7.3%) and β-acoradiene (6.9%), and from the sample collected in Dalmatian hinder land main compound was β-acoradiene (6.7%). In the sample collected near the sea, the major constituent was rosifoliol (8.5%).

Bianchini et al. (2009) researched the effect of ecological factors and different soil properties on the composition of *H. italicum* EO. For the experiment authors analyzed the samples of *H. italicum* in different growth stages from 48 locations in Corsica. The results showed great variability in chemical composition of EO, but mostly with high share of oxygenated monoterpenes (11.7–70.0%) and neryl acetate as main component. The results of principal component analysis (PCA) and partial redundancy analysis (RDA) revealed correlations between the EO composition and the texture and acidity of soils. On the other hand, inorganic composition of the plant and soil and the plant growth stages had lesser influence on the chemical composition of the EO. Main component neryl acetate was correlated with high percentages of coarse sand and fine silt, which is knowledge of high value in the process of manipulating the conditions to obtain the desired composition of the EO.

Different phenolic compounds were identified in *H. italicum.* Flavonoids such as apigenin, glycosyl-apigenin, luteolin, gnaphaliin, naringenin, glycosil-naringenin and glycosyl-chalcone were isolated by Facino et al. (1988) and showed photoprotective activity. Sala et al. (2003) studied properties of gnaphaliin, pinocembrin and tiliroside. Many other authors also isolated and studied flavonoids (*e.g.* Pietta et al., 1991; Karasartov et al., 1992; Wollenweber et al., 2005; Schinella et al., 2007; De la Garza et al., 2013; Mari et al., 2014). Coumarins (esculetin, scopoletin, and isoscopoletin) were found by Karasartov et al. (1992) in the chloroform extract of *H. italicum* flowers while phenolic acids were isolated and studied by Zapesochnaya et al. (1990, 1992), Mari et al. (2014) and Kladar et al. (2015). Generalić Mekinić et al. (2016) studied stilbenes that are produced by limited numbers of plants. The authors in evaluation of *H. italicum* ethanol extract, detected high amount of resvetrol (3,5,4′-trihydroxy stilbene) (6.63 mg/g) and its derivatives piceid, astringin and isorhopotin (0.40 mg/g).

5. Isolation methods for *Helichrysum italicum* chemical compounds

In addition to the above-mentioned parameters such as geographical origin, plant growth stage and environmental conditions, that have been shown to affect the composition and amount of EO, now more researches focus on the methods of isolation and extraction of essential oil that also have great impact on the EO composition. Maksimovic et al. (2017) in the study compiled all different isolation methods of *H. italicum* and obtained compounds. The EO and interesting components are extracted from plants using hydrodistillation, steam distillation, organic solvent methods and supercritical CO_2 (SC-CO_2). It was found that distillation and supercritical CO_2 extraction were convenient for the extraction of terpenes, while phenolic compounds are most commonly isolated with organic solvent extraction. Most commonly used organic solvents are acetone, methanol or ethanol. Hydrodistilation is mostly used, but it has certain disadvantages due to the use of high temperatures that can affect the quality. Organic solvent methods also have same disadvantages such as need for separation and organic solvent traces are found in the extracts. Therefore, other methods of separation are applied based on the use of supercritical fluids. These methods are non-toxic, produce solvent-free extracts at low temperatures, and give the highest extraction yield and a higher selectivity of the extracted components. Many authors have conducted research on *H. italicum* using supercritical CO_2 extraction, whether it is about optimization of supercritical CO_2 extraction (Ivanovic et al., 2011; Jerkovic et al., 2016) or comparison of *H. italicum* composition obtained by hydrodistillation and supercritical CO_2 extraction (Maksimovic et al., 2013; Costa et al., 2015; Jerkovic et al., 2016; Mouahid et al., 2017). It was reported that chemical profile of supercritical extract is dominated with sesquiterpenes followed by waxes (Ivanovic et al., 2011). Jerkovic et al. (2016) performed supercritical CO_2 extraction of *H. italicum* flowers at various process parameters. The aim was to set optimal parameters regarding to the yield that was accomplished by response surface methodology where yield was 4.09% at 20 MPa and 52 °C. Costa et al. (2015) also tested different conditions of supercritical CO_2 extraction (SFE-CO_2) (pressure 9 and 12 MPa, and particle size from < 4 mm and 4 cm) on *H. italicum* from Portugal. The goal was to determine the impact on yield, chemical composition and organoleptic properties. Hydrodistillation of *H. italicum* was also performed in order to compare possible differences between two methods of extraction. Different conditions of SFE-CO_2 (pressure and particle size) influenced the yield of supercritical CO_2 extraction. Chemical composition of analyzed extracts showed that monoterpene hydrocarbons were dominant in the EO and sesquiterpene hydrocarbons in the SFE-CO_2 extracts. Regarding organoleptic properties, SFE-CO_2 extract kept the original pleasant fragrance of the living plant. Andreani et al. (2019) using gas chromatography-olfactometry-aroma extract dilution analysis (GC-O AEDA) attempted to determine the compounds responsible for the scent of the *H. italicum* (commercial sample of EO produced industrially). The most odor-active constituents perceived by all the panelists and showing the highest mean flavor dilution factor were 4,6-dimethyloctan-3,5-dione, 1,8-cineole and nerol. Other compounds also influenced the aroma of *H. italicum* EO but to a lesser extend.

In recent studies, the authors attempted to prove that the use of modern methods of *H. italicum* EO analysis can help in the genotype differentiation (Giuliani et al., 2016 and Schipilliti et al., 2016). Giuliani et al. (2016) have made characterization of the volatile profiles of *Helichrysum* populations (from Tuscany) and compared the differences between populations. Volatile organic components were analyzed with headspace solid phase microextraction HS-SPME equipped with GC/MS. Authors have concluded that the applied method could be used to help determine the taxonomic affiliation of species and subspecies. Schipilliti et al. (2016) analyzed EO of *H. italicum* from Sicily and Corsica by GC/MS, GC/FID and gas chromatography carbon isotope ratio mass spectrometry (GC-C-IRMS). The aim of the work was attempt to differentiate the genotypes of *H. italicum* plants of different geographic origin, investigating the carbon isotope ratio of the volatile terpenes (from the plant flowers). The authors stated that GC-C-IRMS analyses, with the use of the i-std, could act as a preliminary and rapid method of genotype differentiation.

6. Biological activity

In the Mediterranean region, *H. italicum* has been used in the folk medicine for centuries. The review of ethnopharmacological studies of *H. italicum* in different regions of Europe by Viegas et al. (2014) showed that *H. italicum* was used as cure for many ailments and diseases (toothache, digestive disorders, cough, colds, laryngitis, skin diseases, alopecia, dermatomycosis, liver and gall disorders, bronchitis, insomnia, headache, and herpes). Scientists proved many of those activities: strong anti-inflammatory (Sala et al., 2002), antioxidant (Sala et al., 2003; Rosa et al., 2007; Kladar et al., 2015; Generalić Mekinić et al., 2014), antimicrobial (Nostro et al., 2001, 2002; Mastelic et al., 2005; Rossi et al., 2007; Chao et al., 2008; Generalić Mekinić et al., 2014; Stupar et al., 2014; Djihane et al., 2017; Malenica Staver et al., 2018), antiviral (Wleklik et al., 1988; Mucsi et al., 1992; Appendino et al., 2007) as well as antiproliferative (Han et al., 2017; Malenica Staver et al., 2018).

Thanks to its numerous beneficial effects on human health *H. italicum* has wide application in the various industries such as pharmaceutical, alimentary, perfume, and cosmetic. Maksimovic et al. (2018) aimed at creating acceptable form for the oral intake of *H. italicum* bioactive compounds, with corn starch xerogels as the carriers for the oral intake.

Many world-leading cosmetic companies have developed cosmetic lines with *H. italicum* due to its anti-aging effect (Guinoiseau et al., 2013), which is most likely due to the high content of polyphenols as flavonoids that are known to protect against oxidants (Facino et al., 1990). Mixture of the *H. italicum* essential oil and macerated oil of musk rose was tested on skin after cosmetic and reconstructive surgery. Application of the mixture helped to reduce edema, bruising and inflammation, and most probably italidiones were responsible for that while component neryl acetate was responsible for the pain relief (Voinchet and Giraud-Robert, 2007). *Helichrysum italicum* is also used in aromatherapy due to its ability to heal red veins, thrombosis, hematoma and bruises (Peace Rhind, 2012). In support of this thesis, Han et al. (2017) tested biological activity of *H. italicum* EO on human dermal fibroblast system. The EO had significant anti-proliferative activity: it inhibited some tissue-remodeling, e.i. the production of collagen I and III and proved great wound healing potential. Biological activites are described in detail below.

6.1. *Antimicrobial activity*

Numerous studies were carried out to test the effect of the *H.*

italicum EO and extract on Gram-positive and Gram-negative bacteria and various fungi. Chinou et al. (1996) detected bacteriostatic activities of two *Helichrysum* (*italicum* ssp. *italicum* and ssp. *amorginum*) against Gram-positive bacteria: *Staphylococcus aureus* and *Staphylococcus epidermidis*; and Gram-negative bacteria: *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Both oils showed strong activity against *S. aureus* and *S. epidermis* and weaker against *E. cloacae, P. aeruginosa,* and *K. pneumoniae,* while *E. coli* was the most resistant. The authors pointed out that the bacteriostatic activity of the oils is associated with oxygenated monoterpenes geraniol, geranyl, and neryl acetate. Mastelić et al. (2005) compared antimicrobial activity of the Croatian *H. italicum* oil and its fractions of hydrocarbons and oxygen containing compounds. The authors determined the minimum inhibitory concentration against *S. aureus*, *E. coli*, *P. aeruginosa,* and *Candida albicans*. The most effective activities against *S. aureus* and *C. albicans* showed EO and its terpenoid fraction that has higher antimicrobial activity than terpene fraction, which confirms findings of Chinou et al. (1996) that oxygenated monoterpenes (geraniol, geranyl, and neryl acetate) are most probably responsible for antimicrobial activity. Lorenzi et al. (2009) described that the essential oil of *H. italicum* significantly reduces the resistance of *Enterobacter aerogenes, E. coli, P. aeruginosa, and Acinetobacter baumannii* on multiple antimicrobial drugs. It was found that geraniol from the *H. italicum* essential oil acts as an inhibitor of the efflux pump. It restored the activity of chloramphenicol against *E. aerogenes* and also increased its susceptibility to the B-lactams and to the fluoroquinolone norfloxacin that are very important clinical antibiotics. These findings are very valuable providing the possibility to develop new drugs that bacteria are not yet resistant to. The results obtained by Nostro et al. (2001) demonstrated that diethyl ether *H. italicum* extract inhibits the growth of some *S. aureus* strains (*Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC 6538 P), methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA) isolates) and inhibits enzymes that are considered as virulence factors (coagulase, DNAse, thermonuclease, and lipase). The authors suggest that probably the most responsible components for the antimicrobial activity are flavonoids and terpenes, which was also confirmed in other studies (Cosar and Cubukcu, 1990; Tomas-Barberan et al., 1990; Nostro et al., 2000). Tundis et al. (2005) studied antibacterial activity of *H. italicum* from Calabria and Sardinia. Methanolic extracts from both origins showed the best effect on the Gram-positive bacteria, especially *Micrococcus luteus*. Furthermore, antifungal activity of Sardinian extracts against phytopathogen fungus *Pythium ultimum* was more active probably due to the difference in the chemical composition of Calabrian and Sardinian *H. italicum*. Tagliatela-Scafati et al. (2013) evaluated the antibacterial activity of arzanol, coumarates, benzofurans, pyrones and heterodimeric phloroglucinols isolated from *H. italicum* subsp. *microphyllum.* Only heterodimers of arzanol were efficient against multidrug-resistant *S. aureus* isolates. In the newer study, the antibacterial activity was tested on *Compylobacter coli*, *E. coli*, *Salmonella infantis*, *Bacillus cereus*, *Listeria monocytogenes* and *S. aureus* giving promising results, *i.e.* strong antibacterial activity against Gram-positive bacteria (Generalić Mekinić et al., 2014). Similar results have been obtained by Djihane et al. (2017). The authors isolated EO from *H. italicum* by hydrodistillation and predominate compounds were oxygenated sesquiterpenes (61.42%). The EO was tested against 12 bacteria (Gram-positive and Gram-negative) as well as two yeasts and four fungi. The results pointed out that *H. italicum* from Algeria has potent antimicrobial activity and significant antifungal activity. The moderate antifungal activity of *H. italicum* EO on the fungi isolated from cultural heritage objects was reported by Stupar et al. (2014). The main components of the tested EO were γ-curcumene (22.45%), α-pinene (15.91%), and neryl acetate (7.85%). The strongest effect was observed on *Epicoccum niger* and *Penicillium* sp., while the most resistant was *Trichoderma viride*. D'Abrosca et al. (2016) isolated and characterized from *H. italicum* three new amino-phloroglucinol derivatives: helicristalicines A–C that are unusual diamino-phloglucinols. Helicristalinine B showed high bactericidal activity against *S. epidermidis* and was able to inhibit *S. epidermidis* biofilm formation. These two abilities make helicristalinine B promising candidate for antibacterial drug. Werner et al. (2019) also isolated and characterized from aerial parts of *H. italicum* two new arzanol derivatives: helitalone A that is a rare dimer of substituted α- and γ-pyrone units, and helitalone B, which is identical to arzanol except for isopropyl group that replaced ethyl group. Isolated compounds were tested for antibacterial activities against various Gram-positive and Gram-negative bacteria but did not have any significant antibacterial activities at initial dose of 20 μg/ml. Malenica Staver et al. (2018) published the evaluation of antimicrobial activity of *H. italicum* EO against several Gram-positive and Gram-negative bacteria. The tests showed that *H. italicum* EO had weak to moderate antimicrobial potential with *S. aureus* and *S. epidermidis* as the most sensitive bacterial strains.

Cui et al. (2015) evaluated the antimicrobial effect of *H. italicum* EO against seven microorganisms; *S. aureus* ATCC25923, *Bacillus subtilis* ATCC 6633, *Bacillus pumilus* ATCC 27142 (Gram-positive) and Gram-negative bacteria; *Salmonella typhimurium* ATCC 19659, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 13883. The antibacterial activity was evaluated *in vitro* and on fresh raw vegetables: water spinach, greens and lettuce to determine whether it has a potential to be use for vegetable preservation. Both sets of experiments showed that *H. italicum* inhibits the growth of *E. coli* and *S. aureus*. Longer exposure time resulted in higher reduction of the evaluated strains. The authors conclusion is that EO of *H. italicum* could be used in preservation of vegetables. In the newer research, the authors Cui et al. (2016) studied the antibacterial potential of *H. italicum* in protection of food. The authors investigated the ability of *H. italicum* to combat the bacterial contamination with *S. aureus* and obtained promising results. The authors note that by forming biofilms on the solid surfaces the bacteria are more resistant to the application of antibiotics and that the biofilms in many cases are the cause of persistent infections. To test the antibiofilm effect of *S. aureus* EO was applied solely, as well as cold nitrogen plasma (CNP) and finally the combination of the EO and CNP was evaluated. Different concentration of EO were tested (0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1.0 mg/mL, 2.0 mg/mL, and 4.0 mg/mL), various incubation times (10, 20, 30, 40, 50, and 60 min) and surface types (96-well plates and stainless steel). With the increase of EO concentration the decrease in *S. aureus* biofilms was obtained as measured with biofilm colony-counting assay. On the stainless steel at the exposure time of 40 min the concentration of 1.0 mg/mL reduced the number of bacteria by 5.07 logs and at higher concentrations (2 and 4 mg/mL) no viable bacteria was detected. On 96-well plates *S. aureus* was reduced from 7.22 logs to 2.98 logs at dosage of 1 mg/mL and exposure time of 40 min. The effect of 0.5% concentration increased with duration of the treatment. In the combined treatment the evaluated concentration of the EO was 0.5 mg/mL, treatment time equaled 40 min, CNP power was set to 400 W and the treatment was performed for 1 min, resulting in the reduction of bacterial population by 5.50 on stainless steel and from 7.60 logs to 1.34 logs on 96-well plates.

6.2. Anti-viral activity

Besides the antimicrobial activity, anti-viral properties of the *H. italicum* extracts have also been revealed. Activity of the *H. italicum* extracts against the herpes simplex virus type 1 (HSV-1) was reported by Nostro et al. (2003). Effective concentrations were those from 100 to 400 μg/mL and concentrations up to 200 μg per disk did not show genotoxic effect. Anti HSV activity might be attributed to flavonoids apigenin and luteolin present in *H. italicum*, for which anti-HSV activity has been proven in some other investigations (e. g. Wleklik et al., 1988; Mucsi et al., 1992). The antiviral activity of a polyphenol compound arzanol extracted from *H. italicum* ssp. *microphyllum* was investigated by Appendino et al. (2007), and the authors suggested that it inhibits the

HIV-1 replication in T lymphocytes. Results suggest that it has potential as an antiviral agent.

6.3. Antioxidant and anti-inflammatory activity

When referring to the anti-inflammatory effect of *H. italicum*, it is inevitable to mention arzanol. Arzanol is a prenylated heterodimeric phloroglucinol α-pyrone firstly identified in *H. italicum* ssp. *microphillum* and determined as leading anti-inflammatory compound (Kothavade et al., 2013). Appendino et al. (2007) developed a method of extraction and isolation of arzanol from the acetone extract of aerial parts of *H. italicum* ssp. *microphyllum*. Arzanol showed strong NF-$_\kappa$B (nuclear factor kappa-light-chain-enhancer of activated B cells) inhibiting activity ($IC_{50} = 5\,\mu g/mL$) that is one of the key regulators of genes involved in the immune/inflammatory response and is implicated in the regulation of several factors (cytokines, chemokines, adhesion molecules, acute phase proteins, inducible effectors enzymes) involved in inflammatory conditions.

Bauer et al. (2011) conducted a study in which the results showed that arzanol acts as inhibitor of the biosynthesis of pro-inflammatory mediators such as prostaglandin E_2 (PGE_2), thromboxane B2 (TXB_2), and leukotrienes (LTs). These findings were also proved in the *in vivo* test on carrageenan induced pleurisy in rats where arzanol reduced the inflammatory reaction significantly. Arzanol also acts as an antioxidant that is reported in study of Rosa et al. (2011). Arzanol was used in the *in vitro* experiment as pretreatment for low density lipoprotein (LDL) where after 2 h of oxidation it preserved lipoproteins from oxidative damage caused by Cu2+ ions. Furthermore, at non-cytotoxic concentrations, arzanol acted protective on tertiary-butyl hydroperoxide (TBH) -induced oxidative damage in a Vero cells and human intestinal epithelial cells (Caco-2). Sala et al. (2003) isolated from aerial parts of *H. italicum* three flavonoids: gnaphaliin, pinocembrin and tiliroside with the aim of testing its antioxidant effect *in vitro* and *in vivo* experiments. Flavonoid tiliroside showed the strongest effect by significantly inhibiting enzymatic and non-enzymatic lipid peroxidation, also showing strong scavenger properties and powerful antioxidant activity in the DPPH test. *In vivo*, tiliroside significantly inhibited the mouse paw oedema induced by phospholipase A2 as well as the mouse ear inflammation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Poli et al. (2003) studied the antioxidant activity of supercritical CO_2 extracts of the *H. italicum*. Four different samples of *H. italicum* dried flowers were used (from plants native on the coast of the north-east Italy, plants cultivated in experimental fields, plants grown in the nursery and from plants commercially packed). Using the DPPH method and β-carotene bleaching test the antioxidant activity was analyzed. All extracts showed, with more or less efficiency, an antioxidant activity for both used methods. The authors pointed out that the *H. italicum* supercritical extracts can be valuable natural antioxidant for food and cosmetic industries. Molnar et al. (2017) investigated different medicinal plant extracts obtained by various methods and analyzed 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and total phenolic content. *H. italicum* had high total phenol content (132.1 mg gallic acid equivalents (GAE)/g) in 96% EtOH extract that was highly correlated with DPPH scavenging activity (93.5%). Rigano et al. (2014) from flowers of *H. italicum* subsp. *italicum,* for the first time, isolated gnaphaliol 9-O-propanoate among five other already known acetophenones. The new one and other two isolates (acetotrixymetone and 1-[2-[1-[(acetyloxy) methyl]ethenyl]-2,3-dihydro-3-hydroxy-5-benzofuranyl]-ethanone) were tested for cytotoxicity, anti-inflammatory and antioxidant properties. Cytotoxicity assay showed safe biological profile, while these three compounds had no anti-inflammatory properties. Only acetotrixymetone acted as an antioxidant. Goncalves et al. (2017) revealed that methanol extract of *H. italicum* was abundant with phenolic compounds - caffeoylquinic and dicaffeoylquinic acids and pinocembrin. Regarding to them, *H. italicum* showed high inhibitory potential against acetylcholinesterase (AChE) (78.29%), tyrosinase (74.13%) and a-glucosidase (96.65%), enzymes linked to Alzheimer disease. Inhibition of enzymes AChE and tyrosinase by *H. italicum* extract was reported for the first time in this research.

In the paper of Generalić Mekinić et al. (2014) the profile of phenolic acids was reported along with antioxidant activities of *H. italicum* extract in comparison to chamomile (*Matricaria recutita* L.), and common yarrow extracts (*Achillea millefolium* L.). Chemical analysis of ethanolic extracts of all three species has shown variability in phenolics content. *H. italicum* extract contained the highest amount of flavonoid fraction, and was especially abundant in caffeic acid. Antioxidant properties were evaluated by five different methods (ferric reducing antioxidant power (FRAP), DPPH, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), chelating activity, and Briggs-Raucher reaction), whereas *H. italicum* extract exhibited the lowest antioxidant activity when compared chamomile and common yarrow extracts. A week antioxidant activity of *H. italicum* extract from Montenegro was also reported by Kladar et al. (2015).

In the folk medicine herbal tea from *H. italicum* is frequently mentioned as traditional cure for gastrointestinal diseases. To justify this traditional use Rigano et al. (2013) tested the influence of the *H. italicum* ssp. *italicum* ethanolic extract on the intestinal motility using *in vivo* and *in vitro* experiments. The results showed that the ethanolic (EtOH) extract of the *H. italicum* flowers induced antispasmodic actions in the isolated mouse ileum and inhibited transit preferentially in the inflamed gut that confirmed the traditional use of the *H. italicum* ssp. *italicum* flowers for intestinal diseases. The authors stated that these findings can be of potential clinical interest for normalizing intestinal motility in inflammatory bowel disease since they do not cause side effects as existing medications.

Recently, the evaluation of *H. italicum* EO antiproliferative activity was published by Malenica Staver et al. (2018). To evaluate antiproliferative activity, cell death and apoptosis induction, cancer cell lines were used (HeLa, MCF-7, SW620, CFPAC-1 and MiaPaCa-2). The EO contained manly γ-curcumene, α-pinene and neryl acetate and exhibited modest antiproliferative activity on MCF-7 and HeLa cell lines and caused the induction of apoptosis. The EO had no significant effect on cell cycle in examined cell lines, excluding MiaPaCa-2 cells. In this cell line the number of cells in sub G1 phase of cell cycle was increased and multiple cell death mechanisms were involved, such as apoptosis, senescence or necrosis. The obtained results are promising as they point out the possibility of using EO as natural antibiotics or anticancer agents. This is also supported by the findings of Ornano et al. (2015) who reported strong cytotoxic effect of *H. italicum* subsp. *microphyllum* on human malignant melanoma cells (A375).

6.4. Insecticidal and repellent activity

Aside from the previously presented and more known activities of *H. italicum,* there are also those less known such as insecticidal and repellent. Epidemic diseases transmitted by mosquitoes are a growing worldwide problem that is the reason why scientists are constantly looking for the new solutions. Finding alternatives to synthetic insecticides and repellents puts EO and extracts into focus as less harmful for people and environment. It is known that many compounds isolated and identified from different plant extracts can cause toxic activity against mosquito species. Conti et al. (2010) researched EO of *H. italicum* from Elba Island against the tiger mosquito *Aedes albopictus.* The results showed high toxicity at the highest dosage of 300 ppm, with mortality rates up to 100% ($LC_{50} = 178.1$ and $LC_{90} = 288.6$). Benelli et al. (2014) also evaluated activity of *H. italicum* EO against the tiger mosquito which showed larvicidal effect. Furthermore, Drapeau et al. (2009) evaluated repellent potential of the *H. italicum* essential oil from Corsica against the mosquito *Aedes aegypti.* Space repellent properties were evaluated in a Y-tube olfactometer device. The *H. italicum* oil showed that the activity of the test mosquitoes and the attractivity of the finger were reduced to 30% while testing these oils and compared with the finger alone as a control stimulus. The results also displayed that an increase in the concentration (0.1–10%) of the oil did not lead to a stronger effect. Given results indicate that the *H. italicum* EO might be considered as a secondary factor to extend the protection of stronger repellent molecules. Other investigations have also shown promising insecticidal and repellent activity of *H. italicum* EO against stored products pest maise weevil (*Sitophilus zeamais)* (Bertoli et al., 2012) as well as disease vectors southern house mosquito *(Culex quinquefasciatus)* and housefly (*Musca domestica)* (Benelli et al., 2018).

6.5. Other activities

Apart from the above mentioned uses of *H. italicum*, other versatile applications have been recorded by numerous authors. For example, the use of *H. italicum* in the landscape architecture has been evaluated. As a xerophyte *H. italicum* has proved to be a great candidate to be grown on urban green roofs system in semi-arid or arid climatic conditions of the Mediterranean region (Monteiro et al., 2017a,b; Papafotiou et al., 2018).

Water extracts of *H. italicum* and three other species of the Mediterranean flora were investigated as dye agents of natural fabrics, such as cotton and flax with the addition of mordants. The aim was to test their ability to protect from the UV radiation (Grifoni et al., 2014). Flax mordanted with alum and dyed with *H. italicum* extract showed satisfying protection level.

Biodegradable edible films as packing materials are of great interest in present day as they reduce the amount of waste and environmental pollution. The applicability of *H. italicum* secondary metabolites in creating pectin- and alginate-based edible films containing *H. italicum* extract has been explored by Karača et al. (2019). According to the authors, *H. italicum* is a promising material with great application potential as it is rich in polyphenols with high antioxidant activity and thereby has the ability to protect the stored food from oxidation and microbial degradation.

A study on phytoremediation potential of *H. italicum* was conducted by Brunetti et al. (2018). The aim of the study was to determine its ability to absorb heavy metals (Cd, Co, Cr, Cu, Ni, Pb, and Zn) from the contaminated soil. Two types of plant material were used, nonmycorrhized plants and plants mycorrhized with *Septoglomus viscosum*. The results of the research have shown that *H. italicum* plants reduce the soil heavy metals content and that nonmycorrhized plants absorb higher amounts. The authors pointed out that the plants grown on contaminated soil are safe to be used for cosmetic and medicinal purposes, considering that heavy metals accumulate in the root system and not in the above-ground parts of the plants from which the EO are isolated.

Performance of *H. italicum* EO as corrosion inhibitor of steel in 1 M HCl (Hydrochloric Acid) was evaluated by Cristofari et al. (2012). The effect of different EO concentrations was tested as well as different temperatures. The results were obtained with weight loss measurements. The major component of the EO was neryl acetate (31%), followed by γ-curcumene (10.7%). The inhibition efficiency of 82.33% at 2 g/L was recorded. Higher concentrations resulted in higher efficiency. On the contrary, higher temperatures decreased the EO activity as corrosion inhibitor.

7. Genetic diversity

There are numerous researches on *H. italicum* biochemical diversity, but very few related to genetic diversity of *H. italicum* and the relationship between genotypes and chemotypes.

The genetic variation in the *H. italicum,* was analyzed with amplified fragment length polymorphism (AFLP), chloroplast DNA (cpDNA) sequences and morphological characters by Galbany-Casals et al. (2011). The goal was to research whether ssp. *microphyllum* has a common origin, constituting an independent gene pool from ssp. *italicum*, or if the morphological differences between ssp. *microphyllum* and ssp. *italicum* have arisen independently in different locations from a common wider gene pool. The authors have determined that there is a geographic structure to the genetic variation within *H. italicum*, with eastern and western Mediterranean groups that differ from an actual division into subspecies *microphyllum* and *italicum,* and that within the western Mediterranean group of the species there is a considerable polymorphism in chloroplast DNA sequences among and within some populations.

The correlations between the chemical profiles of the essential oil and genotype have also been studied by several authors. Angioni et al. (2003) used random amplified polymorphic DNA (RAPD) markers to study the genetic diversity of Sardinian *H. italicum* ssp. *microphyllum.* The samples of DNA were obtained from the leaves and flowers collected from the wild plants of the two types and from the plants grown at the same altitudes (40 m) in an experimental field. The chemical composition of the EO was also evaluated and revealed two types of oil: one with high concentrations of nerol and its esters, linalool and limonene and other with high amounts of rosifoliol. The genetic analysis confirmed the existence of two chemotypes. Moreover, Melito et al. (2013) described 50 populations of *H. italicum* collected from the different habitats in Sardinia, using AFLP markers to investigate genetic diversity and GC—MS for the EO composition analyses. The relationships between the genetic structure of the population, soil, habitat and climate variables and essential oil chemotypes were evaluated. The results showed a correlation between chemotype, genetic diversity and sampling site. Morone-Fortunato et al. (2010) studied the chemical composition and its relationship with genotype in 20 indigenous populations of *H. italicum* ssp. *italicum* collected at different locations in Italy and in Corsica. AFLP markers were used to examine the level of genetic variability among the genotypes with the aim to reveal a possible correlation between the genetic and chemical data. Based on the major compounds of the essential oil three different chemotypes have been defined, those groups were also confirmed by the genetic analysis.

Among the most recent publication is that of Hladnik et al. (2019) that revealed the complete chloroplast genome of *H. italicum* sampled in the North Adriatic Region. Length of the chloroplast genome was 152,431 bp and it contained 131 genes (85 protein-coding genes, 36 transfer RNA genes, 8 ribosomal RNA genes, and 2 partial genes). According to authors these findings will be used for the development of molecular markers to be used in future genetic studies of *H. italicum.*

8. Conclusion

The research on *H. italicum* has been a topic of interest for many scientists. Many of them confirmed its traditional use and found even new possibilities for its potential use. Scientists confirmed its strong anti-inflammatory, antioxidant, antimicrobial, antiproliferative, and antiviral activities, which is the reason for its use in cosmetic, pharmaceutical, perfume, and alimentary industries. A high variability in the essential oil composition has been determined and various investigations confirmed the fact that environmental factors influence their content and composition. A relatively small number of available literature is focused on genetics and genetic diversity of *H. italicum.* Some work has been done by employing different molecular markers (RAPD, AFLP, cpDNA), and determined correlation between genotypes and chemical composition of the essential oils. The most available scientific work on biological activities is focused on *H. italicum* antimicrobial activity. *Helichrysum italicum* has shown to be effective on numerous Gram-negative and Gram-positive bacteria. Couple of studies have addressed its potential use in the preservation of food and elimination of its bacterial contaminations. Also, some articles evaluate other possible uses of *H. italicum.* For example, the possible utilization of *H. italicum* in urban green roofs, creation of edible biofilms with *H. italium* extracts, and *H. italicum* as steel anticorrosive agent. The phytoremediational effect of *H. italicum* was also investigated and demonstrated its ability to absorb heavy metals without contaminating the EO to be extracted. Repellent and insecticidal activity has also been investigated, implicating its possible use in plant and human's protection. The comprehensive analysis of the available data showed the increased number of studies on *H. italicum*. However, some major gaps have been encountered. The producers are faced with many challenges considering the fact that *H. italicum* is relatively new in cultivation and in the past only wild populations were harvested. The under-explored area is the cultivation management of the species. These include sowing requirements, plant density, irrigation, fertilization, harvesting, and drying methods, and understanding of the impact of each factor on the EO yield, quality, and composition. As it has been mentioned, there is a relatively small number of genetic studies which are a prerequisite for future breeding programs and development of commercial cultivars of *H. italicum* which are not available at this time. The identified gaps give openings for contributing new research.

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