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Chemical Composition and the Pharmacological Activities of the Essential oils and Extracts from *Helichrysum Arenarium*

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ABSTRACT

The plant Helichrysum belongs to the Asteraceae family and in the literature can be found as everlasting flowers. This plant has therapeutic effect, and it is used in traditional medicine to cure different disease such as biliary tract diseases, infections of the skin, gallbladder, respiratory and digestive tracts disorders, wounds, some of them in modern medicine are confirmed as antimicrobial activity. Different investigations about the phytochemical composition and pharmacological activities have revealed the potential of Helichrysum arenarium for drug discovery. The pharmcological analyses have shown different activities such as chlorotic, hepatoprotective, anti-inflammatory, antioxidant, antiviral, detoxifying and antimicrobial activities. Phytochemical investigations have shown that Helichrysum plants are rich with different compounds such as flavonoids, fatty acids, carotenoids, vitamins, phthalides, phenolic acids and essential oils. The aim of this review is to present a concise and coherent overview of the chemical composition and the pharmacological activities of the essential oil, the main classes that are present in the composition of essential oils and activities of some of the bioactive constituents and extracts of Helichrysum arenarium. Scientific databases have been used such as PubMed, Scopus, Google Scholar, and Research Gate to collect the data for this review.

Keywords: *Helichrysum Arenarium*; Phytochemical; Pharmacological; Essential Oils; Bioactive Constituents

Introduction

Helichrysum arenarium belongs to Asteracea family and it is aslo known as Sandy Everlasting and Immortelle [1]. The height of this plant is 15-40 cm, flowers in June-September [2]. It can be found in different geographical areas, Europe, north Balkans, western Siberia, and Asia. It grows in sandy soils, more rarely in open rocky places, and is found in dry pine forests [2,3]. *H. arenarium* can be found in different colours from lemon, yellow and orange [2]. This plat has therapeutic uses in traditional medicine, and it has been used to cure various ailments such as liver, biliary tract diseases [4], infections of the skin, gallbladder, respiratory and digestive tracts disorders, kidney stones [5] and cholecystitis [6]. The pharmcological analyses has shown its chloroetic, hepatoprotective [1,3], hypolipidemic [3], hepatotropic [3], anti-inflammatory [4] antioxidant [4], antiviral [3], detoxifying [4] and antimicrobial activities [1]. *Helichrysum arenarium* is composed of different groups of compounds but the most imoprtant are flavonoids, which can be found in different forms. Other characteristic compounds of the inflorescence of *Helichrysum arenarium* are fatty acids, carotenoids, vitamins, phthalides, coumarins, phytosterols, phenolic acids, essential oils and inorganic compounds (such as macro- and micronutrients) [4,7-9]. Jia, et al. [6] isolated a new phthalide from *H. arenarium* named arenophthalide D.

In literature were reported different flavonoid compounds in the inflorescence of Helichrysum areanrium which are naringenin, naringenin-5-glucoside [(+)-naringenin-5-β-D-glucoside = helichrysin A, (-)-naringenin-5-β-D-glucoside = helichrysin B = salipurposide] and naringenin-5-diglucoside [10]. The flavone and flavonol compounds present in *Helichrysum arenarium* are reported to be apigenin, apigenin-7-glucoside, luteolin, luteolin-7-g1ucoside, kaempferol, kaempferol-3-glucoside, kaempferol-3-diglucoside, quercetin, quercetin-3-glucoside and galangin (3,5,7-trihydroxyflavone), 3,5-dihydroxy6,7,8- tri methoxy flavone [10]. Essential oils (EO) are natural products that are used as natural preservative agents alone or in combination with other preservatives [11]. In the literature it is reported that essential oils isolated from the inflorescence of *H. arenarium* have strong antimicrobial activity [12]. The most used method for isolation of EO from the Helichrysum is hydrodestilation method, but it should be mentioned that except for advantages this method also has some disadvantages. The disadvantages are low efficiency process, due to the Hight temperature during the treatment occurs degradation and isomeration and some of the bioactive compounds can be loss [11].

There are much research about the Helichrysium plant, but the number of scientific research on the essential oils of *H. arenarium* plants is very limited [12-17]. The research of the chemical composition of the essential oils and extracts of H. arenarium was done by different scientists [2,14-18] and they possess strong pharmacological properties [15]. Also, different research were published for the antioxidant activity of the isolated extract from *H. Arenarium* [13,17]. The aim of the study was to collect information from the published literature about the composition of the essential oils from *H. Arenarium* and their pharmacological activities as a source of bioactive components used for the food, cosmetics, and pharmaceutical industries.

Search Strategy

The selection of relevant data was made through different scientific databases such as ScienceDirect, Google Scholar, PubMed, Wiley Online Library, Research Gate, Scopus. The limited number of scientific articles about the essential oils obtained from *H. arenarium* makes it difficult to compare the data with the literature.

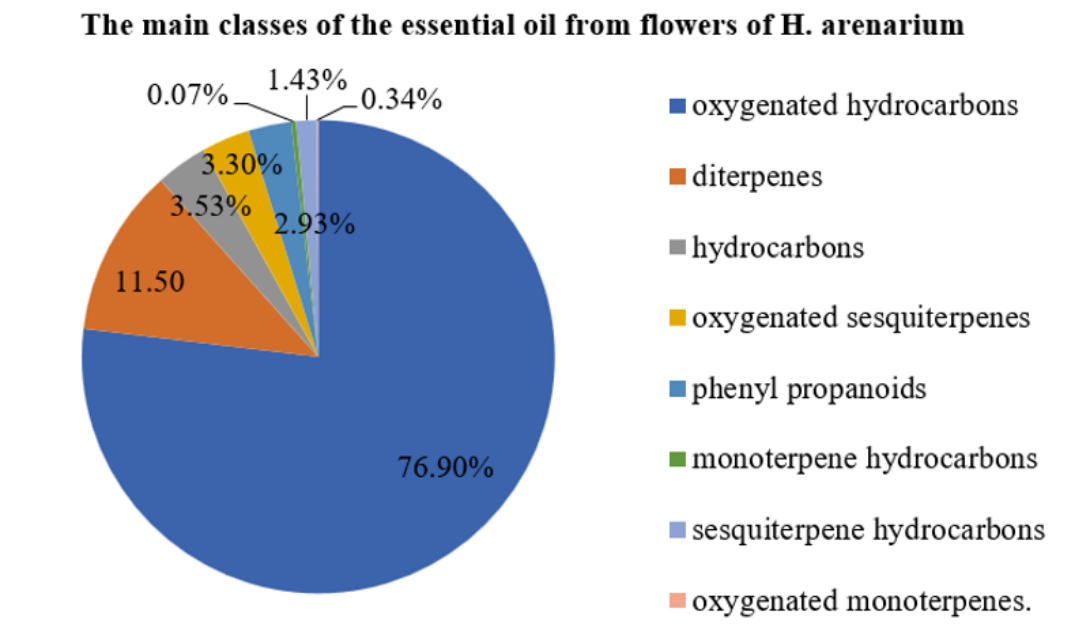

Figure 1: Percentage of the different chemical classes of components present in *H. arenarium* essential oil.

Chemical Composition of the Essential Oils from H.arenarium

In the published literature, the essential oils from the *Helichrysum arenarium* were in different amounts and different chemical composition, which may be the result of different environmental factors such as the type of soil where it grows, the origin, precipitation level, the intensity of the heat and the climate, as well as the genetic characteristics [2]. The yield of essential oil from *H. arenarium* analyzed by Stankov, et al. [19] was 0.07%. In the study 42 components were identified that represent 97.89% of the total content of essential oils. Some of the compounds had concentrations above 1% and most

of the compounds had concentrations below 1%. The dominant components of the essential oil were oleic acid (30.28%), ethyl hexa decanoate (20.19%), linoleic acid (18.89%) and sclareol (4.22%). The most abundant group of compounds were oxygenated hydrocarbons (76.90%), diterpenes (11.50%), followed by hydrocarbons (3.53%), oxygenated sesquiterpenes (3.30%), phenyl propanoids (2.93%), monoterpene hydrocarbons (0.34%), sesquiterpene hydrocarbons (1.43%), and oxygenated monoterpenes (0.07%). In Figure 1 are presented the main classes of the composition of the essential oils from Helichrysum areanrium [19].

The report of chemical composition of the inflorescence and leaf of the essential oils from 16 samples of *Helichrysum arenarium* that were collected in Lithuanian forests reported fifty-six compounds of the essential oils that made up 80.1-98.8% of total oil content. In the oils it was found that the percentage of monoterpene hydrocarbons was from 0.8% to 6.7%, the quantity of oxygenated monoterpenes was 2.0-13.9%, the amount of sesquiterpene hydrocarbons varied from 29.0% to 70.1% and the percentage of oxygenated sesquiterpenes was 2.0-12.2% [2]. The major terpenoid constituents were 1,8-cineole (8.9%, one sample), β-caryophyllene (5.8-36.2%), and γ- and δ-cadinene (5.8% and 9.0 %, respectively). However, the essential oils also contain aliphatic hydrocarbons such as octadecane and heneicosan in significant quantities. Octadecane dominated with its amount ranging from 7.1-22.3%, while the percentage of heneicosan varied from 7.9% to 20.0% [2]. Another report about the composition of the *Helichrysum arenarium* oil obtained by hydrodistillation and analyzed by GC and GC/MS showed that twenty-four volatile components were present in the essential oil, including: linalool (1.7%), anethole (3.2%) cawacrol (3.6%) and a-muurolol (1.3%). Also in the composition of the oil were aliphatic acids (34.6%) and their esters (28.5%) and significant amounts of other aromatic compounds (10.2%) [13].

Essential oils obtained from Hungarian and Polish commercial samples and a plant sample cultivated in Hungary were analyzed. The results showed different isolated compounds: linalool, α-terpine, anethol, anisaldehyde, thymol, varvacol, eugenol, α-humule, caryophyllenol, β-caryophyllene, pelargonic, caprinic, laurinic acids and methyl palmitate. The authors conclude that the major components of the essential oil of the samples was methyl palmitate with quantity of 28.5% and 21.7% respectively, but in the cultivated sample is capric acid with amount of 19.8%. It should be mentioned that the authors found that alkyl carbonic acids were the dominant components of the essential oils of *Helichrysum arenarium* [14]. Chemical composition analysis of the essential oil reported 75 compounds divided in different classes such as monoterpenes that are represented with the quantity of 65.72-73.99%, sesquiterpenes with amount of 16.08-19.41%, diterpenoids had the percentage of (3.25-4.27 %) and long-chain alkane with amount of 4.33-5.69 %, all those classes made 90.82-94.4% of the total oil. It is shown that the dominant components of the essential oils of *H. arenarium* are α-pinene (34.64-44.35%), sabinene (10.63-11.1%), germacrene D (3.56-4.86%), β-gurjunene (3.61%), β-pinene, trans-verbenol and D-limonene [18].

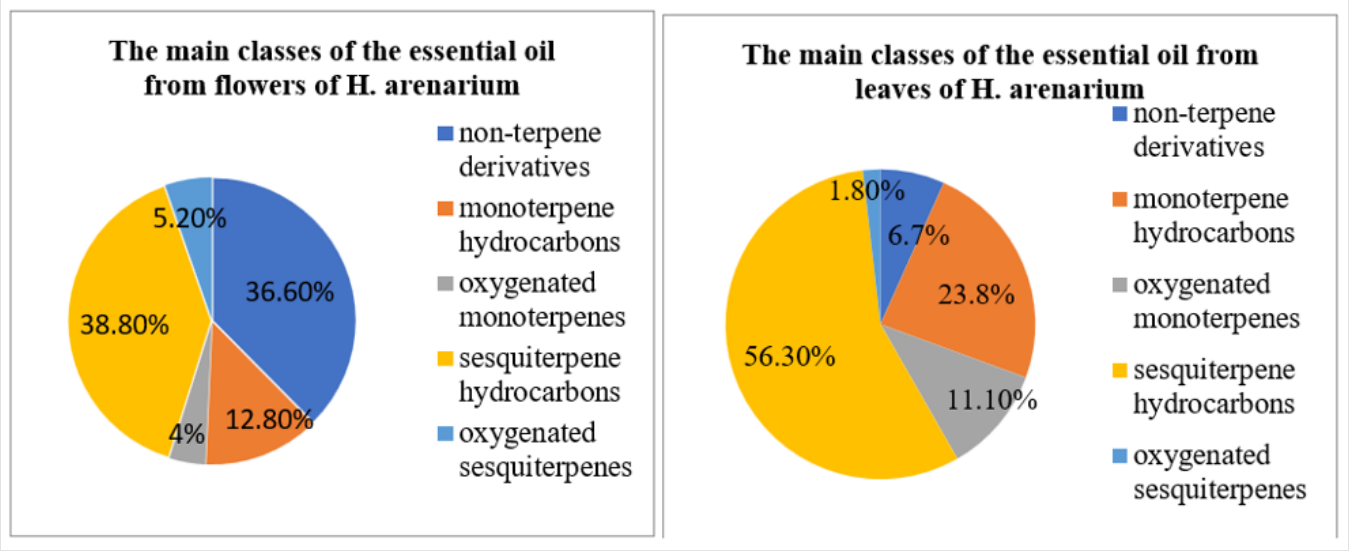

Figure 2: Percentage of the different chemical classes of components present in *H. arenarium* essential oil.

Essential oils from flowers and leaves of *H. arenarium* were analysed by GC-MS method, the total oil composition was represented by 96.8-99.7%. The main classes that were in the composition of the essential oil from flowers and leaves were non-terpene derivatives with high amount of 36.6% and 6.7%, the quantity of monoterpene hydrocarbons was 12.8% and 23.8%, oxygenated monoterpenes with quantity of 4.0% and 11.1%, sesquiterpene hydrocarbons with percentage of 38.8% and 56.3% and oxygenated sesquiterpenes with amount of 5.2% and 1.8%, Figure 2 show the percentage of the main classes of the essential oils from *H. arenarium* [20]. The most important groups that make up the essential oils of *H. arenarium* flowers are monoterpene hydrocarbons such as β-pinene (55.2%) and α-pinene (15.8%), while other compounds that were identified in the essential oils flowers and leaves from *H. arenarium* were β-caryophyllene, 1,8-cineole, pentadecanoic acid, 6,10,14-trimethyl-2 pentadecanone and bis-(2-methylpropylester)-1,2-benzendicarboxylic acid [20].

According to the published data β-caryophyllene was found to be the major constituent followed by δ-cadinene, octadecane and heneicosan. The main groups of the essential oils were monoterpene hydrocarbons with quantity of 0.8% to 6.7%, the quantity of oxygenated monoterpenes was 2.0-13.9%, the amount of sesquiterpene hydrocarbons was from 29.0% to 70.1% and the percentage of oxygenated sesquiterpenes was 2.0-12.2% [15]. Furthemore, in chemical composition analysis of the essential oil were identified different groups such as sesquiterpene hydrocarbons with amount of 20.6-41.2% of total essential oil, monoterpene fraction with quantity of 6.7-14.8% contained mostly of oxygenated monoterpenes 5.5-13.6%. Another group in the composition of the essential oil were aliphatic hydrocarbons with percentage of 20.5% the oxygenated sesquiterpenes were identified with quantity of 10.3-12.2% and oxygenated aliphatics with percentage of 5.3-11.4% [16]. This study of essential oils was developed around the morphological differences that exhibit diversity of flower colour. Trans-caryophyllene, δ-cadinene and heneicosan were identified as the main components in yellow lemon, orange, and brown-orange flowers. 1,8-cineole was identified as an additional component from the above-mentioned compounds in the chemical profile from the citrus flower. Tetradecanoic acid was the main component of yellow flower oils, while nonadecane was the main component of yellow-brown flower oils [16].

Table 1: Chemical composition of the essential oils from *Helichrysum arenarium* reported from the published literature.

Part analyzed	Compound name	Reference
Inflorescence and leaves	1,8-cineole, β-caryophyllene, γ- and δ-cadinene, octadecane and heneicosan	[2]
Inflorescence	Linalool, anethole, cavacrol, α-muurolol, octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid, anethole and methyl palmitate	[13]
Flowers	α-Pinene, p-Cymene, γ-Terpinene, β-Linalool, Borneol, n-Dodecane, Thymol, Carvacrol, Aromadendrene, α-Caryophyllene, β-Caryophyllene Germacrene, γ-Cadinene, δ-Cadinene, Linoleic acid, Oleic acid, Carpilic acid	[19]
Inflorescence	linalool, α-terpine, anethol, anisaldehyde, thymol, varvacol, eugenol, α-humule, caryophyllenol, β-caryophyllene, pelargonic, caprinic, laurinic acids and methyl palmitate	[14]
Aerial parts of the flower	α-pinene, sabinene, germacrene D, β-gurjunene, β-pinene, transverbenol and D-limonene	[18]
Leaves and flowers	β-pinene and α-pinene, β-caryophyllene, β-pinene, 1,8-cineole, pentadecanoic acid, and bis-(2-methyl propylester)-1,2-benzendicarboxylic acid.	[20]
Leaves and inflorescences	β-caryophyllene, δ-cadinene, octadecane and heneicosan	[15]
Inflorescences	Trans-caryophyllene, δ-cadinene, heneicosane, 1,8-cineole, tetradecanoic acid, nonadecane.	[16]
Leaves and inflorescences	Palmitic, myristic and lauric acids, n-nonanal	[8]
Inflorescences	β-spathulenol, ledol and α-bisabolol	[11]

Another investigation about the chemical composition of the essential oils from *H. arenarium* inflorescence was done by Judzentiene, et al., [8], GC-MS method was used for analysis of the EOs where the main components were palmitic (hexadecanoic 23.8%), myristic (etradecanoic 14.9%) and lauric (dodecanoic 6.1%) acids, while other constituents presented in remarkable amount were terpenoids trans-β-caryophyllene (5.4 %) and phytone (hexahydrofarnesyl acetone) (4.4 %). Moreover, the predominant constituents of *H. arenarium* leaf EO were palmitic acid (18.8 %), n-nonanal (10.4 %), and myristic acid (8.7 %). This oil contained appreciable amounts of trans-β-caryophyllene (6.5 %), α-pinene (4.2 %), and 1,8-cineole (3.9 %) [8]. In the study done by Rančić et al., [12] the chemical composition of the oils from *H. arenarium* is composed by diepi-α-cedrene (17.9%), α-ylangene (13.9%), cyclosativene (11.9%) and limonene (11.4%). The chemical profile of essential oils, concerning *Helichrysum arenarium* showed three main components were found

using the GC-MS analysis: β-spathulenol, ledol and α-bisabolol [11]. Other studies concerning the chemical composition of essential oils of this species were performed and the main components were α-pinene (32%), 1,8-cineole (16%), α-humulene (15%), α-caryophyllene (8%) [21]. However, the results present in this review were used from the published literature [2,8,11-16]. Knowing the constituents of essential oils from several Helichrysum species that have pharmaceutical and cosmetic uses can help identify useful species and chemotypes for various industrial applications [22]. Table 1 [2,8,11,13-16,18-20] presents the predominant constituents of the essential oils and analyzed parts from *Helichrysum arenarium*.

Pharmacology of the Essential Oils and Extracts from *Helichrysum arenarium*

In literature are published a variety of pharmacological activities for the bioactive compounds and the most important are flavonoids which are responsible for biological activities [23]. The isosalipurposide chalcone derivative has been reported to be responsible for the yellow color of this plant as well as for the hepatoprotective activity and at the same time it is one of the derivatives with the highest percentage of flavonoids [9,24]. In the report investigated by Judzentiene, et al. [8] the antioxidant activity and scavenging activity of essential oils and extracts of *H. arenarium* was analysed. From the results *H. arenarium* extracts show higher scavenging activity than essential oils. Antioxidant activity macht up with total polyphenol content in extracts and radical scavenging properties of EOs and extracts. The toxic activity of the essential oils of *Helichrysum arenarium* was valued using a brine shrimp bioassay where it was proven to be toxic. According to Tepe, et al. [7] the extracts from *Helichrysum arenarium* obtained by Soxhlet extraction were screened for their possible antioxidant and radical-scavenging activity. The results showed that the water extracts of *Helichrysum arenarium* exhibited antioxidant and weak radical-scavenging activities. Measured by the DPPH assay, *H. arenarium* extract showed free radical scavenging activity [7]. Also, in the study done by Albayrak, et al. [25] the methanolic extract of *H. arenarium* showed radical scavenging activity. Albayrak, et al. [25] investigated the antimicrobial activities of the methanolic extracts of some Helichrysum spp. including the methanolic extract of *H. arenarium* (L.) Moench subsp. aucheri (Boiss). The results showed that *H. Arenarium* extract has broad antimicrobial activity against all microorganisms in the agar diffusion test. Moreover, in another study the residues from the menthol extract of *H. Arenarium* exhibited antiradical activity and the main constituent was caffeic acid [17].

In the agar diffusion assay, the extract of Helichrysum showed high antimicrobial activity against all tested clinical isolates, responsible for lower respiratory tract pathogens [5]. From the results reported by Moghadam, et al. [10] it can see that the essential oil from *H. arenarium* showed wide antimicrobial activity against the microorganisms tested. In the same report with the microdilution method, was determined the antibacterial activity. The result showed that essential oil indicated important bacterial activity against tested bacteria. In addition, the EO isolated from the *H. arenarium* has antimicrobial activity [11] against all bacterial and yeast species tested, also showed antifungal activity against all the fungal species tested [12]. The essential oil of *Helichrysum arenarium* L. had antimicrobial activity against microorganisms tested with the microwell dilution method [21]. Moreover, the result from Babotă, et al. [26] showed that the antifungal activity of the samples Penicillium fumiculosum showed the highest reactivity to ethanolic extracts and 70% (v/v) ethanolic extracts of *H. arenarium*. The 70% (v/v) ethanolic extract of Helichrysum flowers showed the highest antioxidant activity [27]. Ethanol extracts obtained from *H. arenarium* from Turkey showed strong antioxidant properties based on high levels of flavonoids, poly phenols and porphyrin pigments, while the samples with 70% ethanol showed higher anti-radical activity than samples with 95% ethanol [19]. Kéry, et al. [27] reported that the bioactive components of *Helichrysum Arenarium* inhibit lipid peroxidation because they act as a primary and secondary antioxidant and scavenging free radicals.

Using an HPLC-DAD-TOF method Judzentiene, et al. [8] in the composition of *H. arenarium* methanolic extracts identified: luteolin-7-Oglucoside, naringenin and its glucoside, apigenin, chlorogenic acid, arenol, and arzanol. The obtained extracts showed higher radical scavenging activities. The antioxidant activity of EOs and extracts was also tested by two methods. Regarding the H-donor activity, which were determined spectrophotometrically and by chemiluminescence, was described by the lyophilizing antioxidant properties. The lyophylitic agents were able to transmit protons for the free radicals, neutralizing them and inhibiting lipid peroxidation, the lyophylitic agents are also excellent antioxidants [1]. Table 2 [1,5,8,17,28,29] presents the biological properties of the predominant *H. arenarium* constituents.

Table 2: Activity of the predominant *H.arenarium* compounds published in literature.

Compound	Activity	References
Caffeic acid quercetin	Antiradical	[17]
chlorogenic acid and dicaffeoylquinic acids and flavonoids (apigenin, naringenin, apigenin-7-O-glucoside and naringenin-O-hexosides	Antibacterial	[5]
luteolin-7-Oglucoside, naringenin and its glucoside, apigenin, chlorogenic acid, arenol, and arzanol	Higher radical scavenging	[8]
apigenin, apigenin-7 -glucoside, luteolin, luteolin-7-g1ucoside, kaempferol, kaempferol-3-glucoside, kaempferol-3-diglucoside, quercetin, quercetin-3-glucoside and galangin (3,5,7-Trihydroxy flavones) 3,5-Dihydroxy-6,7,8-tri methoxyflavone 2′,4,4′,6′-tetrahydroxychalkon-6′-O-glucoside	Antibacterial and antiviral Antifungal Liver-protective	[1]
Narirutin, Naringin, Eriodictyol, Luteolin, Galuteolin, Astragalin, Kaempferol	Anti-atherosclerotic	[28]
Spathulenol and β-pinene	Antimicrobial Antibacterial	[29]

Conclusions and Study Perspectives

In this review we aimed to highlight the most important data regarding chemical composition of the essential oils from *Helichrysum arenarium*, the main classes and compounds that take place in the composition of the essential oils. The most abundant group of compounds present in essential oils were oxygenated hydrocarbons, diterpenes, followed by hydrocarbons, oxygenated sesquiterpenes, phenyl propanoids, monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated monoterpenes. About the bioactive constituents the most important are α-pinene, β-pinene, sabinene, germacrene D, oleic acid, ethyl hexadecanoate, linoleic acid, 1,8-cineole, β-caryophyllene, γ- and δ-cadinene. It should be mentioned that the classes and the constituents differ by the way of investigation, conditions, and the places from where it is the plant.

In this review were discussed pharmacological activities, of which the antioxidant, antimicrobial, antibacterial, and antiradical are the most studied. *Helichrysum arenarium* bioactivity depends on the chemical composition of the essential oils, from which the major bioactive constituents have already been isolated. Although the studies of the essential oils from *Helichrysum arenarium* show great promise, they should continue to be tested for the bioactive constituents and pharmacological activities and to be seen if they can be applied into clinical usefulness in a safe and productive manner.

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