Helichrysum arenarium

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Helichrysum arenarium (L.) Moench, belonging to the *Asteraceae* family, is known in traditional medicine for its diuretic, choleretic, and anti-inflammatory properties. *Helichrysum arenarium* (sandy everlasting) is a source of active pharmacological compounds used in complementary medicine to prevent digestive and hepatobiliary illnesses.

Helichrysum arenarium | antimicrobial | antioxidant | anti-inflammatory

1. Introduction

Systematic framing. *Helichrysum arenarium* (L.) Moench ssp. *arenarium*, ssp. *Ponticum (Velen)* E.I. Nyarady, *Asteraceae*/Composite DC, synonym *Gnaphalium Arenarium* L *(Composite)* [1], is a herbaceous perennial plant in the order *Asterales.* The *Asteraceae* family is the biggest family of flowering plants, with over 1600 genera and over 23,000 species spread over various climates and areas around the world [2][3]. It is a diverse and heterogeneous family that includes important species used as sources of food, spices, or for medicinal purposes. The *Asteraceae* family presents several compounds that can be studied and tested as having medicinal potential with various bioactivities.

Biological peculiarities. *Helichrysum arenarium* (L.) Moench is a plant that belongs to the *Helichrysum* section and normally grows up to 20 cm high but can reach heights of 10 to 30/50 cm [4][5]. It has lanceolate leaves that are whitish-green due to the numerous white hairs that cover them, giving a felt-like appearance [5]. The appearance of the habitus of the plants can be observed, with young shoots on which the lanceolate leaves are inserted and apically the inflorescence in the early stages of flowering.

The stem is usually branched at the top, with terminal inflorescence grouped in globular heads with golden-yellow flowers, which have a diameter of 3 to 6/9 mm [4]. The flowering period takes place between the months of June and October [WHO, 2010] [6]. Ligulate flowers, citrine yellow and glossy, cover the hermaphrodite central flowers that are small in size, and thereby determine a nest appearance. The peduncles of the flowers, as well as the leaves, are covered with fine, pubescent hairs [5][7][8].

The *Helichrysi flos* product, represented by the heads of the inflorescence, is also found in the literature under the names of *Flores Stoechados citrinae* or *Flores Gnaphalii arenariae* and is harvested before the full flowering of the capitula, which are grouped in false terminal umbels, with approximately 10–30 (100) flowers [4][5][7][8]. The taste of the flower is spicy, aromatic, and slightly bitter.

2. Area of Spread, Cultivation Techniques, and Applications

H. arenarium is found in Europe and Central Asia [4][9]. According to Maznev N.I. [10], this species is named after the Greek words helios (sun) and chrysos (gold), referring to the bright golden color of the inflorescence.

H. arenarium is totally protected in Sweden and Serbia and is listed in state-run reserves in Denmark and Estonia [11][12]. The species obtained some legal protection in Poland in the 1970s, which stopped the overexploitation of this natural resource [13]. It is found throughout Eastern Europe and Central Asia, including China and Western Siberia [4]. It grows in sandy soils in the Netherlands, Sweden, and Estonia, and further south in Germany, Bulgaria, and Kazakhstan [14], as well as in dry pine forests. *H. arenarium* has been found from southern Scandinavia to the northern part of the Balkan Peninsula and from the Bay of Biscay to the Ural Mountains according to Euro and Med Plantbase reports [15].

It was growing in the wild flora of the plains in Romania in grassy, sandy, or rocky areas. According to Dihoru G. and Negrean G. [16], *Helichrysum arenarium* does not appear on the red list of Romanian vascular plant species and subspecies. Dihoru G. and Boruz V., 2014 [17] indicated harvesting recommendations for the main spontaneous medicinal plants in Romania established on the basis of the frequency of the species in the national flora and the plant parts collected. Based on the field research, they recommended that *H. arenarium* be classified as a level 5 species. In this case, picking should be strictly prohibited, and the authors suggested that this spontaneous medicinal plant is in danger [17].

The neglect of the clonal character and ignorance of mycorrhizal associations were the main problems in the previous experiments that tried to cultivate the plant. Sawilska and collaborators [18] took a step forward in explaining the necessity of mycorrhizal associations of arbuscular fungi *Glomus* intraradices with plant roots, but inoculation of soil with mycorrhizal inoculum did not significantly influence the *H. arenarium* growth or the flowering of single shoots. However, it has been proved that the presence of arbuscular fungi in the soil supports plant growth and development at the early growing stages [18]. These plants had a better-developed root system and significantly increased photosynthetic parameters. Similar findings were found in another study that describes the associations between arbuscular mycorrhiza (*Glomeromycota*) as a colonization model and the ornamental plant *Iris germanica* [19]. Moreover, the authors suggest that the plant metabolic state controls the plant–fungi interaction, and a depletion of the plant carbon flux decreases the sporulation rate [19]. In vitro techniques guarantee high genetic stability and an increased probability to obtain a sterile culture [20]. Figas et al., 2016 have improved the in vitro propagation using sandy everlasting explants of apical buds and have shown the highest number of shoots (24.7) were obtained on Murashige–Skoog (MS) medium with 5 mg/L kinetin and 0.5 mg/L indole-3-acetic acid [21]. Another study that used root explants reported a significant increase in shoot proliferation (25.77 shoots per explant) in a medium with 1 mg/L 6-benzyladenine [22]. Using a different micropropagation method on MS and Gamborg media with 2,4-dichlorophenoxyacetic acid, primary and secondary calluses were obtained [23].

Acclimatization techniques are another issue for the growth of sandy everlasting under ex vitro conditions. Figas et al., 2016 increased the efficiency of plant acclimatization from 56 to 75% using MS water solution (25%) for

irrigation which enables a higher survival rate for plantlets transferred to the greenhouse environment and later to field conditions [21]. Sawilska et al. [18] performed a large experiment during growing seasons (from 2003 to 2005) to evaluate the response capacity of *H. arenarium* cultures in the conditions of a fallow field on barren soil. They concluded that several factors associated with the presence of fungi mycorrhiza decisively influence potential and actual fertility as well as the development of the amount of biomass. In addition, it was proved that pluviothermal conditions during the blooming period influence the reproduction processes, and they are more important than population age and the level of fungi root colonization [18].

3. Bioactive Compounds

A group of researchers [24] demonstrated in 1998 that flavonoids are responsible for the cholagogue activity of extracts from sandy everlasting flowers. In general, the inflorescence of *H. arenarium* contains three types of flavonoids: flavonols, flavones, and flavanones [25][26] including 39 compounds (**Figure 3**). The main compounds in sandy everlasting are the chalcone isosalipurposide and the flavanones naringenin and naringenin-5-O-glucoside [25][27]. Flavonoids are one of the most abundant and widely spread groups of phenolics in plants, with many biological and pharmacological effects [28], some of them because of their phenolic structure, including antioxidant properties and the inhibition of processes mediated by free radicals [29]

Naringenin and naringenin-5-O-glucoside are two flavanone derivatives, compounds: (+)-naringenin-5-D-glucoside named helichrysin A and (-)-naringenin-5-D-glucoside is calledhelichrysin B orsalipurposide. Other substances are naringenin-4′-O-glucoside, and naringenin-5-O-diglucoside. *Helichrysi flos* flavones and flavonolic compounds include apigenin-7-O-glucoside, apigenin, luteolin, luteolin-7-O-glucoside, kaempferol, kaempferol-3-O-glucoside, kaempferol-3-O-diglucoside, quercetin-3-O-glucoside, and 3, 5-dihydroxy-6, 7, 8-trimethoxiflavone. Izosalipurposide (2, 4, 4, 6-tetrahydrochalcon-6'-O-glucoside) is a distinctive and dominating compound of inflorescence [26][30]. Since 1999, Czinner and colleagues [30] have been searching for the flavonoid complex in everlasting flowers in comparison to silibinin, a flavonoid derived from *Silybum marianum* L.

4. Extraction Products

In terms of the extraction methods utilized to acquire the flavonoid complex, the studies cited in the literature cover a wide range of plant species (**Table 1**) [29][31][32][33][34][35][36][37][38][39][40][41][42][43]. The information on *H. arenarium*, on the other hand, is restricted to hypoalcoholic extracts used for research purposes regarding the actions of the components. The ultrasound-assisted approach, which is commonly used for the extraction of important compounds, has been tested in recent extraction procedures. These extracts have the potential to be a rich source of active biological compounds [32].

Table 1. Methods for obtaining bioactive compounds from *Helichrysum arenarium* (L.) Moench.

Extraction Technique	Solvent	Active Constituents	References
Distillation	Water	Monoterpenoids, sesquiterpenoids, phenolic compounds	[29][33][37]
Maceration	Alcohol	Alkaloids, carotenes, flavonoids, tannins	[31][37]
Solvent extraction or enfleurage	Solvent organic	Monoterpenes, sesquiterpenes, monoterpenoids, phenolic compounds, carotenes	[33][37][38][40][42]
Ultrasonic-assisted extraction (UAE) or sonication	Ethanol aqueous solution	Phenolic acids and flavonoids	[32][35][37][41][43]
Supercritical fluid extraction (SFE)	Supercritical carbon dioxide	Nonpolar natural products such as lipid and volatile oil.	[34][35][36][37]
Microwave-assisted extraction (MAE): two types of methods: 1. solvent-free extraction; 2. solvent extraction	1. usually for volatile compounds; 2. usually for nonvolatile compounds	Essential oils: - Monoterpene hydrocarbons - Sesquiterpene hydrocarbons - Oxygenated monoterpenes - Oxygenated sesquiterpenes	[33][37][39]

Recently, an efficient microwave liquid–liquid extraction technique was developed to extract essential oil and nonvolatile flavonoids (astragalin, apigenin, luteolin, kaempferol, and quercetin) from *H. arenarium* inflorescences with a small amount of solvent, effectively reducing the organic content of wastewater [33]. Based on the technical results, the method of ionic liquid-mediated microwave-assisted hydrodistillation concatenated liquid–liquid extraction (IL-MHDE) is an alternative solution for the simultaneous distillation of essential oils and nonvolatile compounds from medicinal plants [33].

Supercritical fluid extraction (SFE) for substance isolation plays an essential role in bioactive compound extraction technologies. These substances include flavonoids, carotenoids, other phenolic compounds, essential oils, lipids and fatty acids, alkaloids, and other bioactive phytochemicals [34]. Because flavonoids are polar molecules, there has been little research on their separation with pure CO_2. The supercritical CO_2 extraction method (SC-CO_2) of moderately polar chamomile apigenin, for example, where research has demonstrated that it is faster than other standard methods [35]. Considering the polarity of flavonoids, polar modifiers must be added to SC-CO_2 to increase solubility. Studies on the influence of modifiers have made numerous contributions to increasing the efficiency of bioactive compound extraction [34]. These investigations have proven that the addition of a modifier improves extraction efficiency by boosting yields. However, using too much co-solvent is not cost-effective because it requires more energy to remove it. Although CO_2 is the most commonly used liquid for SFE, any other liquid could be used.

5. Pharmacological Properties

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5.1. Choleretic and Cholagogue Activities

In 1962, Szadowska et al. [44] discovered mild choleretic and antispasmodic effects of this plant in rat models by intravenous administration of flavonoids extracted from sandy everlasting. After 15 min, the increase in bile secretion was 180, 185, and 160% higher than the initial value (100%) [4][44]. Apigenin from *H. arenarium* ether extract was found to have the most potent antispastic activity on isolated smooth muscle and gallbladder ex vivo. That result suggested that *H. arenarium* extract could be used as an adjuvant in the treatment of cholecystitis and cramp-like gallbladder disorders [45]. Because of these properties, the extract is used in therapeutic applications in Europe, such as the treatment of arthritis, rheumatism, gout, and cystitis as well as the stimulation of gastric secretion and the treatment of gall bladder disorders [45][46][47][48].

5.2. Antioxidant Activities

The choleretic and hepatoprotective activities of *Helichrysum arenarium* (L.) Moench, flos inflorescence could be attributed to the antioxidant properties of its phenolic compounds and flavonoids [25]. The identification of phenolic compounds and flavonoids can be accomplished using HPLC equipment as well as gas chromatography.

The antioxidant properties of freeze-dried extracts of the sandy everlasting inflorescence were investigated, as well as the total polyphenol and flavonoid contents of *Helichrysi flos* aqueous and freeze-dried extracts. Czinner et al. have demonstrated that *Helichrysi flos* lyophilisate is more effective than silibinin in terms of antioxidant action, measured using H-donor activity [25][49]. However, in analyzing the reducing power property and total scavenger capacity, silibinin proved to be more effective than the flavonoids present in the lyophilisate of the inflorescence of *H. arenarium* (*Helichrysi flos*) [25].

5.3. Anti-Inflammatory Activities

The presence of flavonoids (narirutin, naringin, eriodictyol, luteolin, galuteolin, astragalin, and kaempferol) in *H. arenarium* flower extracts has sparked interest in antiatherosclerotic activity research [50][51]. It has been proved that plant extracts have anti-inflammatory properties, specifically by the reduction in C-reactive protein (CRP) expression, inhibition of the activities of c-Jun NH2-terminal kinases (JNK2) and p38, and mitogen-activated protein kinase (MAPK) pathway suppression [50].

5.4. Antimicrobial Activities

Antibacterial, antiviral, and antifungal properties of the *Helichrysum* species have been investigated in several Euroasia countries. Moreover, the European Medicine Agency published a report in 2015 on the pharmacological effects, clinical efficacy and safety, and antimicrobial properties of *H. arenarium* (L.) Moench [14]. The first investigation of the antibacterial activity of the everlasting flower flavonoid compounds was performed in the former Soviet Union by Khristenko LA and concluded that the preparations in the concentration of 20–40 μg/mL were active against two important Gram-positive species (*Staphylococcus* sp. and *Streptococcus* sp.) [52]. Later, aerial

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parts of the plant or the whole overground plant were used to prepare infusions, decoctions, essential oils, and extracts with different qualitative content.

The antimicrobial activity of essential oils of *H. arenarium* has been investigated on different test microorganisms, clinical strains, and food contamination microbes. Rancic et al., 2005 tested the antibacterial activity of 1–5 µL of everlasting flower essential oil on *Escherichia coli* ATCC 35,218, *Micrococcus luteus* ATCC 9341, *Pseudomonas tolaasii*, isolated from *Agaricus bisporus*, *Salmonella enteritidis* ATCC 13,076, *S. Typhimurium* ATCC 13,311, *Staphylococcus aureus* ATCC 6538, and *S. epidermidis* ATCC 12,228 and concluded that at the minimum volume (1 µL) the oil had activity against all bacterial species tested [53].

Helichrysum arenarium extracts and herbal teas have been used traditionally in European countries. The antioxidant and antimicrobial activities of two subspecies of *H. arenarium* (L.) Moench, erzincanicum Davis and Kupicha, Erzican and rubicundum (C.Koch.) Davis and Kupicha, Erzurum, collected from different regions of Turkey, were analyzed. Methanolic extracts from the whole dried plants were screened against 15 strains of bacteria and fungi using the agar-well diffusion method, and the results were compared with standard antibiotics [54][55]. Statistical differences were found among the chemical compositions and the antimicrobial and antioxidant activities of these subspecies. Additionally, extracts were active against *Aeromonas hydrophila*, *B. brevis*, *B. cereus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *S. aureus* ATCC 29,213, but no activity was detected against tested strains of *E. coli*, *Morganella morganii*, *Proteus mirabilis*, *Mycobacterium smegmatis*, *Yersinia enterocolitica*, or yeast *S. cerevisiae* [54][55]. However, at the highest concentration (100,000 µg/mL), methanolic extracts were similar to or less effective than the standard antibiotics. Dried flowers collected from northeastern Romania were used to prepare extracts and to analyze their phenolic content and antimicrobial activity [3][56].

5.5. Pharmacoeconomic Benefits

Helichrysum arenarium is well known among the plant species used in conventional medicine. The plant is valuable as a cholagogic, choleretic, and antimicrobial agent and moderate spasmolytic agent, as presented in **Figure 1** [30][57][58][59][60]. Infusions of *Helichrysi flos* have effects on liver disease, gallbladder issues, and gastric secretion [31]. As a result, treating hepatobiliary illnesses with "Flamin" tablets containing pure flavonoids at a dose of 50 mg three times a day for 40 days is recommended [61]. The effects of lavender oil and dry *Helichrysum arenarium* concentrate (Flamin) on cholagogic activity, liver detoxification, antibacterial activity, and inflammatory response were investigated while researching the creation of the medication "Lavaflam". Due to its pharmacoeconomic benefits over its analogs, the drug was produced and introduced to the Ukrainian pharmaceutical market [62].

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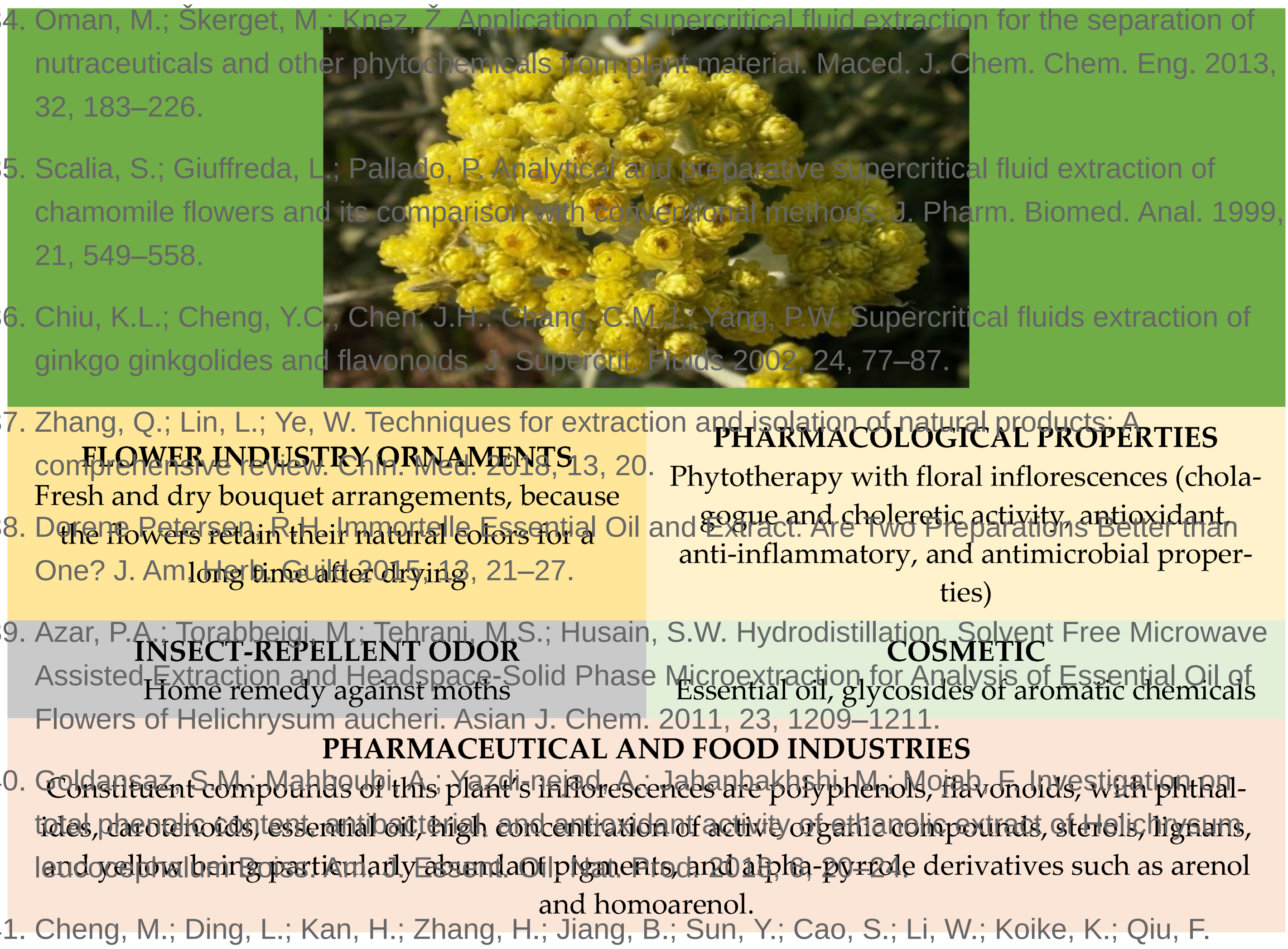

Figure 1. Pharmacoeconomic benefits of *Helichrysum arenarium* (L.) Moench.

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