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Review

Helichrysum arenarium: From Cultivation to Application

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Abstract: *Helichrysum arenarium* (L.) Moench, belonging to the *Asteraceae* family, is known in traditional medicine for its diuretic, choleretic, and anti-inflammatory properties. This review focuses on the superiority of *Helichrysum arenarium* (sandy everlasting) over other known plants as a source of active pharmacological compounds used in complementary medicine to prevent digestive and hepatobiliary illnesses. Because the species exists both in spontaneous flora and in crops, this article highlights the development of a controlled culture of *H. arenarium*, following the reproducible quality of the plant as a biological material. The diversity of phytocompounds, especially well-characterized flavonoid extracts, and the differences between extraction procedures are discussed. Antiviral, antibacterial, and antifungal activities against human pathogens proved by different plant extracts and their mechanisms of action are analyzed. This study aims to contribute to the insufficient knowledge regarding the effects of *Helichrysum* species and to reveal that their extracts can be a valuable source for new active pharmaceutical ingredients.

Keywords: *Helichrysum arenarium*; cultivation methods; traditional uses; phytochemistry; choleretic and cholagogue activities; anti-inflammatory; antioxidant and antimicrobial activities

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1. Introduction

The international scientific community has shown the need to find new remedies for diseases caused by digestive and hepatobiliary dysfunctions by searching new plant raw materials and bioactive substances., At the same time, the biological effects of *Helichrysum arenarium* that allow for extensive phytotherapeutic applications of this plant have been highlighted.

The pharmacological profile of *Helichrysum arenarium* (also known as "Sandy everlasting", "Dwarf everlasting", "Immortelle", and "Siminoc" in popular Romanian folklore) has recently been improved by new research. This has pointed out supplementary effects besides stimulating the production and circulation of bile secretion by identifying the primary phytochemical ingredients [1]. The antioxidant, antidiabetic, and neuroprotective properties of this plant species were investigated in order to obtain novel therapeutic products. *Helichrysi arenarii flos* was not included in the European Pharmacopoeia, although it was listed as having cholagogue and choleretic activity by the state Russian Pharmacopoeia [2], Pharmacopoeia Helvetica, and the Polish Pharmacopoeia [3–6]. *H. arenarium* is protected in Sweden and Serbia; however, it is classed as "care demanding" in Denmark and Estonia [7].

In the 1970s, Poland granted the species partial legal protection, putting an end to the overexploitation of this natural resource [8]. The protection regulations have prompted the development of new technologies for efficient plant growing capable of restoring the species for use in phytotherapy. Small farms in Latvia cultivate *H. arenarium* for local consumption, with an estimated production area of less than 2 hectares [7]. Nowadays, the former countries of the Soviet Union as well as Poland and Turkey are the main suppliers of *Helichrysi arenarii flos* on the market [9].

H. arenarium is well known in phytotherapy for its potential in the treatment of gallbladder disorders and is classified as a species in danger of extinction in several European countries; however, few data about its cultivation are available in the literature [10]. The first research on the cultivation of *H. arenarium* was carried out by Clasquin and Henry [11] in France using in vitro micropropagation by direct organogenesis induction with somatic tissue fragments. Later, more results were obtained by researchers from Poland and the Czech Republic [12,13] on in vitro cultivation to capitalize on the obtained plants' ornamental uses.

Significant research has been dedicated lately to the production of relevant compounds by plant tissue cultures. As a result, remarkable technological progress has been made, and nowadays research based on cell and tissue cultures is performed both at a fundamental level and in the form of applicative research. This uses cultures on a large industrial scale for the commercial production of secondary metabolites. "In vitro" techniques make it easy to stimulate the full expression of the metabolic sequences that results in the synthesis of the desired metabolite. This provides important economic benefits through the possibility of industrial-scale synthesis, as well as by the selection of certain biosynthetic pathways according to the desired purpose [14–16]. The main biologically active compounds of sandy everlasting flowers are flavonoids, so in 1998 a group of researchers [17] showed that flavonoids represent the cholagogue activity of extracts of these flowers.

Various studies at the national and international levels regarding the composition of *Helichrysum arenarium* have shown that the presence of phenolic and flavonoid compounds provides antibacterial and antioxidant properties against pathogens [8,18]. For instance, the antibacterial activity of the methanolic extract of sandy everlasting flowers, alone and in combination with ciprofloxacin, was tested against methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae* isolates from patients with lower respiratory tract infections [18]. This research reveals a potential use of *Helichrysi arenarii flos* extract in combined therapy of pathogenic infection. Another recent study has proved the antibacterial effect of *H. arenarium* essential oil on the growth of food-contaminating microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Staphylococcus aureus*. The latest study results revealed that essential oils have a significant antimicrobial activity; therefore, they could be used as a natural preservative to increase the shelf life of food products [19].

We performed a literature search on the following databases: Google Scholar, Scopus, and PubMed using Boolean operators of original and review articles in English, French, and Romanian as our research method. Typical search terms were novel targets in *Helichrysum arenarium* to find suitable papers for this manuscript (especially between 2000 and 2022). For the search, the following keywords were used: *H. arenarium*, sandy everlasting, cultivation methods, phytochemistry, bioactive compounds extraction, choleretic and cholagogue activities, antioxidant activity, anti-inflammatory activity, antimicrobial activity, and applications.

The goal of this paper is to bridge the knowledge gap about the effects of *Helichrysum* species and to demonstrate how the extracts can serve as sources for novel and potentially valuable medicinal components.

2. Morphology, Taxonomy: Photo, Flowers

Systematic framing. *Helichrysum arenarium* (L.) Moench ssp. *arenarium*, ssp. *Ponticum (Velen)* E.I. Nyarady, *Asteraceae*/Composite DC, synonym *Gnaphalium Arenarium* L *(Composite)* [20], 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 [21,22]. 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 [10,23]. It has lanceolate leaves that are whitish-green due to the numerous white hairs that cover them, giving a felt-like appearance [23]. Figure 1 shows *H. arenarium* plants that were cultivated in Romania at the "Dimitrie Brândză" Botanical Garden of the University of Bucharest in 2013 [24]. 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.

Figure 1. *Helichrysum arenarium* plants in the early stage of blooming (July 2013) at the "Dimitrie Brândză" Botanical Garden of the University of Bucharest, Romania (used with permission from Ref. [24]).

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 [10]. The flowering period takes place between the months of June and October [WHO, 2010] [9]. Ligulate flowers, citrine yellow and glossy, cover the hermaphrodite central flowers that are small in size, and thereby determine a nest appearance (Figure 2). The peduncles of the flowers, as well as the leaves, are covered with fine, pubescent hairs [23,25,26].

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 [10,23,25,26]. The taste of the flower is spicy, aromatic, and slightly bitter.

Figure 2. *Helichrysum arenarium* plants in the stage of full flowering inflorescence (August 2013) at the "Dimitrie Brândză" Botanical Garden of the University of Bucharest, Romania (used with permission from Ref. [24]).

3. Area of Spread, Cultivation Techniques, and Applications

H. arenarium is found in Europe and Central Asia [10,27]. According to Maznev N.I. [28], 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 [7,29]. The species obtained some legal protection in Poland in the 1970s, which stopped the overexploitation of this natural resource [30]. It is found throughout Eastern Europe and Central Asia, including China and Western Siberia [10]. It grows in sandy soils in the Netherlands, Sweden, and Estonia, and further south in Germany, Bulgaria, and Kazakhstan [19], 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 [31].

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. [32], *Helichrysum arenarium* does not appear on the red list of Romanian vascular plant species and subspecies. Dihoru G. and Boruz V., 2014 [33] 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 [33].

Although the plant is particularly attractive to the pharmaceutical industry and was listed as an endangered species in several European countries, there is little information on its cultivation in the literature. The first attempts to cultivate *H. arenarium* date back to the mid-1970s [34,35], but none was completely successful in terms of survival ratio and development [36]. According to Sawilska and Jendrzejczak, in a 2009 investigation [37], a commercially available medium for growing chrysanthemums, garden peat supplemented with perlite, and sandy clay from an arable field proved to be the best substrates for the adaptation of *H. arenarium* seedlings. However, most of the authors concluded that this plant is difficult to cultivate because laboratory research produced slightly more inflorescence biomass than in natural environments. Tyszynska-Kownacka [38] speculated that this species might be unsuitable for breeding. Later research revealed why these attempts failed [39–41].

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 [35] 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 [35]. 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* [42]. 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 [42]. In vitro techniques guarantee high genetic stability and an increased probability to obtain a sterile culture [14]. 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 [12]. 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 [13]. Using a different micropropagation method on MS and Gamborg media with 2,4-dichlorophenoxyacetic acid, primary and secondary calluses were obtained [11].

Compared to other plants, the *H. arenarium* in vitro propagation techniques have not received considerable attention. Therefore, more studies are required, particularly in countries where this plant is no longer available in the wild or is subject to legal limitations. For instance, the commercial cultivation of *H. arenarium* using traditional methods is illegal in Poland and only plant tissue and cell culture techniques are allowed to obtain clonal propagation material [8,13]. French researchers [11] took the same strategy that encouraged the use of biotechnologies to save this species from extinction.

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 [12]. Sawilska et al. [35] 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 [35].

The former Soviet Union countries, Poland, and Turkey are the market's primary suppliers of "sandy plants" [43]. Plants of this species are used as flower industry ornaments, particularly in natural gardens or for fresh and dry bouquet arrangements because the flowers retain their natural colors for a long time after drying. The plant was previously used as a home remedy against moths due to its distinct, insect-repellent odor [44]. However, the most important applications of the sandy everlasting plants are in the pharmaceutical industry and that is related to the main constituents of the plant inflorescence—polyphenols, especially flavonoids in the form of glycosides. The antioxidant, anti-inflammatory, and antimicrobial properties are linked to the phenolic content. Other studies have focused on volatile compounds, such as the essential oil of *H. arenarium*, which has antimicrobial properties. Because of the high concentration of active organic compounds in *Helichrysi arenarii flos* (*Helichrysum arenarium* inflorescence), the plant is used in phytotherapy. Rančić and collaborators [45] have highlighted that various plant compounds, such as polyphenols and flavonoids, are important in the pharmaceutical, cosmetic, and food industries.

4. Bioactive Compounds

A group of researchers [17] 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 [46,47] including 39 compounds (Figure 3). The main compounds in sandy everlasting are the chalcone isosalipurposide and the flavanones naringenin and naringenin-5-O-glucoside [46,48]. Flavonoids are one of the most abundant and widely spread groups of phenolics in plants, with many biological and pharmacological effects [49], some of them because of their phenolic structure, including antioxidant properties and the inhibition of processes mediated by free radicals [18].

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 [47,50]. Since 1999, Czinner and colleagues [50] have been searching for the flavonoid complex in everlasting flowers in comparison to silibinin, a flavonoid derived from *Silybum marianum* L.

In addition to flavones, everlasting flowers (*H. arenarium*) include polyphenols, sterols, lignans, and glycosides of aromatic chemicals, with phthalides, carotenoids and essential oil. Other compounds found are alpha-pyrrole derivatives, such as arenol and homoarenol [10], and the yellow pigment is particularly abundant.

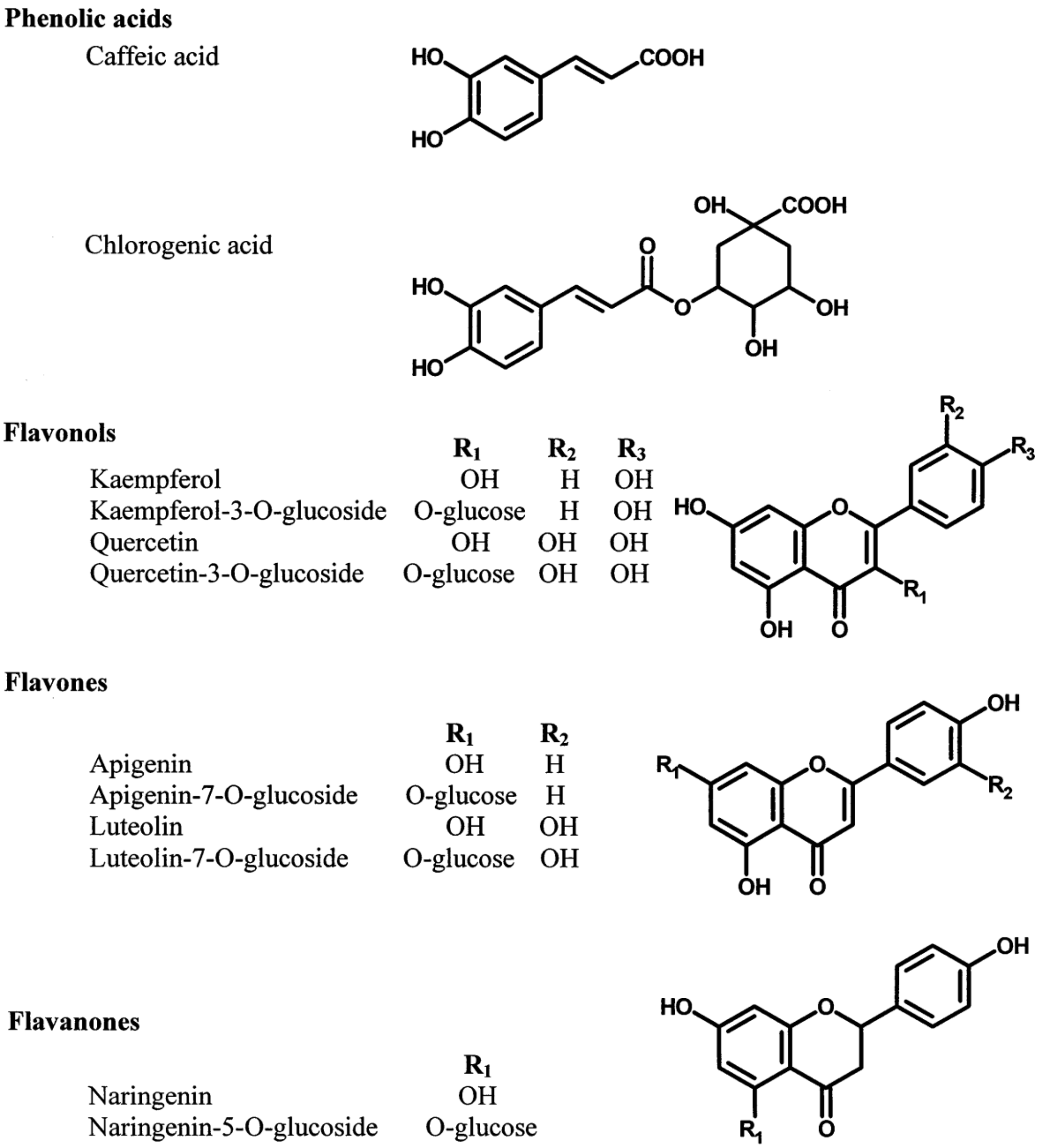

Figure 3. Chemical structures of the compounds identified by TLC (reprinted with permission from Ref. [18], Czinner, E. et al., *J. Ethnopharm*, 2000).

5. 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) [1,18,51–62]. 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 [51].

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

Extraction Technique	Solvent	Active Constituents	References
Distillation	Water	Monoterpenoids, sesquiterpenoids, phenolic compounds	[18,52,56]
Maceration	Alcohol	Alkaloids, carotenes, flavonoids, tannins	[1,56]
Solvent extraction or enfleurage	Solvent organic	Monoterpenes, sesquiterpenes, monoterpenoids, phenolic compounds, carotenes	[52,56,57,59,61]
Ultrasonic-assisted extraction (UAE) or sonication	Ethanol aqueous solution	Phenolic acids and flavonoids	[51,54,56,60,62]
Supercritical fluid extraction (SFE)	Supercritical carbon dioxide	Nonpolar natural products such as lipid and volatile oil.	[53–56]
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	[52,56,58]

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 [52]. Based on the technical results, the method of ionic liquid-mediated microwave-assisted hydrodistillation concatenated liquid–liquid extraction (ILMHDE) is an alternative solution for the simultaneous distillation of essential oils and nonvolatile components from medicinal plants [52].

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 [53]. 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 is one such example, where research has demonstrated that it is faster than other standard methods [54]. 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 [53]. 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. Chiu and colleagues [55] investigated flavonoid extraction from ginkgo using CO_2, N_2O, and Freon 1, 1, 1, 2-tetrafluoroethane (R134a). In this circumstance, no supercritical media could extract flavonoids from dried Ginkgo leaves. The addition of ethanol as a modifier resulted in a successful extraction.

6. Pharmacological Properties

H. arenarium inflorescence was used in traditional medicine for its choleretic, diuretic, anti-inflammatory, and detoxifying properties. Different parts of the plant have been used in infusions to treat cystitis, arthritis, rheumatism, and gout, to stimulate gastric secretion, and to treat gallbladder disorders [2]. In Europe, *Helichrysi arenarii flos* has long been known to have choleretic and cholagogue action, which is mentioned in the pharmacopeia as the only application of these flowers [2,63–65]. In the last years, studies have proved the antioxidant properties of the sandy everlasting flowers and suggested that this plant could be a reliable source of potentially bioactive compounds capable of preventing metabolic or neurodegenerative diseases [1]. Several studies have been conducted to identify *H. arenarium* compounds with specific effects on living organisms.

6.1. Choleretic and Cholagogue Activities

In 1962, Szadowska et al. [64] 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%) [10,64]. 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 [66]. 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 [2,66–68]

H. arenarium can be taken as a decoction (1:20, 100 mL, 2–3 times per day) or as "Flamin" tablets (purified flavonoids) at a therapeutic dose of 50 mg, 3 times per day for 40 days. Prolonged administration, on the other hand, may cause biliary congestion [69]. *Helichrysi flos* is a cholagogic and choleretic agent with the ability to stimulate gastric juice secretion [70,71]. Flamin tablets are used in cases of cholecystopathy to change the chemical composition of bile (by increasing the cholesterol collate coefficient), regulate gastrointestinal functions, and increase diuresis [72].

6.2. 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 [46]. 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 inflorescences 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 [46,73]. 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*) [46]. In another study, the sandy everlasting lyophilized flavonoid content was determined to be 0.47%, and the authors hypothesized that this concentration of flavonoids could have a therapeutic effect [18,46]. According to recent results [74], using the spontaneous medicinal plant species *Cnicus benedictus* L. (blessed thistle, *Asteraceae* family), it was revealed that in vitro induced callus had higher amounts of flavonoids and polyphenols. This is another reason to justify the development of new protocols to improve the in vitro techniques in the case of *Helichrysum arenarium*.

6.3. 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 [75,76]. 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 [75].

H. arenarium flower extracts were studied due to their anti-inflammatory and antioxidant effects. An acetophenone derivative (a flavonoid) demonstrated in vivo (topical) anti-inflammatory efficacy comparable to that of an indomethacin standard [77]. Acetophenone glucosides, flavonoids, and other isolated compounds also demonstrated in vivo and in vitro anti-inflammatory action [78–81]. The positive results imply that more studies on the anti-inflammatory effects should be performed.

6.4. Antimicrobial Activities

Since prehistoric times, different parts of plants have been used in traditional medicine to prevent or cure infectious diseases. Recently, the crisis of antimicrobial resistance has become an important reason for the discovery of new antimicrobials of plant origin, with compounds different from synthetic therapeutic substances that could act alone or improve the efficiency of drugs. A number of these phytocompounds seem to have structures and modes of action distinct from those of currently used antibiotics, and therefore the search for potentially new and useful substances is important [82,83].

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 [19]. 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.) [84]. Later, aerial parts of the plant or the whole overground plant were used to prepare infusions, decoctions, essential oils, and extracts with different qualitative content.

It is well known in the literature that variability in the chemical composition of the plants is linked to the part of the plant used (roots, stems, leaves, flowers) and the extraction method, but genetic, geographic, and climatic factors also play important roles. Major antimicrobial constituents in *Helichrysum* species are polyphenols, especially flavonoids and volatile compounds in essential oils [22,76,85]. Studies on the antimicrobial properties of *H. arenarium* strongly suggested that essential oils or extracts from the whole dried plant have different effects than when inflorescence was used.

The antimicrobial activity of essential oils of *H. arenarium* has been investigated on different test microorganisms, clinical isolates, and food contamination microbes. Rančić 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 [45]. Moreover, the inhibition zones (mm) of 5 μL essential oil preparation were higher than the results obtained for antibiotic streptomycin used as a control. Clinical isolates of *Candida albicans* and fungi *Aspergillus niger* ATCC 6275, *A. flavus* ATCC 9170, *Cladosporium cladosporioides* ATCC 13,276, *Penicillium funiculosum* ATCC 10,509, and *Trichoderma viride* IAM 5061 were tested, and the minimum inhibitory concentration (MIC) detected was between 10 and 30 μg/mL, while the minimum fungicidal concentration (MFC) ranged from 15 to 60 μg/mL [45]. Similar antimicrobial activities were obtained in experiments that tested seven species of food contamination microbes and a different method to prepare steam-distilled essential oil [84]. In this case, *Bacillus subtilis* ATCC 6633 (MIC = 781.25 μg/mL) showed more resistance than *E. coli* 0157 NTCC 12,900 and *S. aureus* ATCC 6538, both with MIC = 97.65 μg/mL. Yeast *Saccharomyces cerevisiae* 5052 PTCC, fungi *Candida albicans* ATCC 10,231, and two *Aspergillus* species (*A. flavus* PTCC and *A. parasiticus* PTCC 5018) were tested, and minimal bactericidal concentrations (MBC) and MFC were in a range of 390.625–6250 μg/mL [86]. In both studies, sandy everlasting essential oils inhibited the growth of the most dangerous pathogens *S. aureus* and *C. albicans,* but the results are difficult to compare as the preparations were obtained using different methods.

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 [87,88]. 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* [87,88]. 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 [22,85]. Gradinaru tested the antibacterial activity of the methanolic extract of *H. arenarium* subsp. *arenarium* and noticed that another *S. aureus* ATCC 25,923 was more susceptible to the plant extract than *Streptococcus pneumoniae* ATCC 49,619 with an MIC of 0.62 compared to 1.25 mg/mL [85]. Moreover, the extract showed antibacterial effects against clinical isolates methicillin-resistant *S. aureus,* penicillin-resistant *S. pneumoniae* (MIC = 2.5 mg/mL), and 16 times higher activity against the ampicillin-resistant *Moraxella catarrhalis* strain (MIC = 0.15 mg/mL). Again, the extract was less active than antibiotic ciprofloxacine and the extract–antibiotic interaction was analyzed [72].

In another experiment, the antibacterial and antifungal activities of methanolic, ethanolic, and 70% (*v/v*) ethanolic extract were compared based on the obtained MIC values, and *S. aureus* ATCC 49,444 and *E. coli* ATCC 25,922 proved to be the most sensitive strain [22]. In the case of the Gram-negative bacteria, methanolic and ethanolic extracts had similar activities (MIC = 7.81 mg/mL and MBC = 15.62 mg/mL), while the least effective on Gram-positive bacteria was 70% (*v/v*) ethanolic extract (MIC = 15.62 mg/mL and MBC = 31.25 mg/mL). The authors concluded that phenolic compounds, especially the higher amount of chlorogenic acid detected in the plant extracts could be responsible for the antibacterial effect [22]. Antifungal activity was tested against five strains, including *Penicillium fumiculosum* and *C. albicans*, with the highest sensitivity for 70% (*v/v*) ethanolic extracts. Still, compared to standard controls, streptomycin and fluconazole, the antimicrobial activities were modest.

The antiviral effects of Turkey *H. arenarium* plants were revealed for the first time in recent research that used aqueous and ethanol 80% extracts [89]. Viruses Herpes simplex Type-1 (HSV-1) and Parainflfluenza-3 (PI-3), both important human pathogens, were tested using Madin–Darby bovine kidney and the Vero cell line. In particular, the ethanolic extract at the maximum nontoxic concentration (32 μg/mL for HSV-1 and 64 μg/mL for PI-3) were found with antiviral potential on both DNA and RNA viruses, with higher inhibitory action than acyclovir. In the same study, both extracts proved to be more potent against Gram-positive (*S. Aureus*, *E. Faecalis*) than Gram-negative pathogens (*E. coli*, *P. aeruginosa*, *P. mirabilis*, *A. baumanni*, *K. pneumoniae*) and showed considerable antifungal activity at 8 μg/mL concentration against *Candida albicans* and *C. parapsilosis* [89].

These studies provide practical information on the antimicrobial potential of *H. arenarium* preparations and illustrate their promising potential for use in cosmetics or pharmaceutical products to treat wound infections. Another interesting application of everlasting plant water extract has been reported, namely, the synthesis of antimicrobial silver nanoparticles (AgNPs) where the extract was used as a reducing and capping agent [90].

6.5. 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 4 [50,91–94]. Infusions of *Helichrysi flos* have effects on liver disease, gallbladder issues, and gastric secretion [7]. 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 [69]. 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 [95].

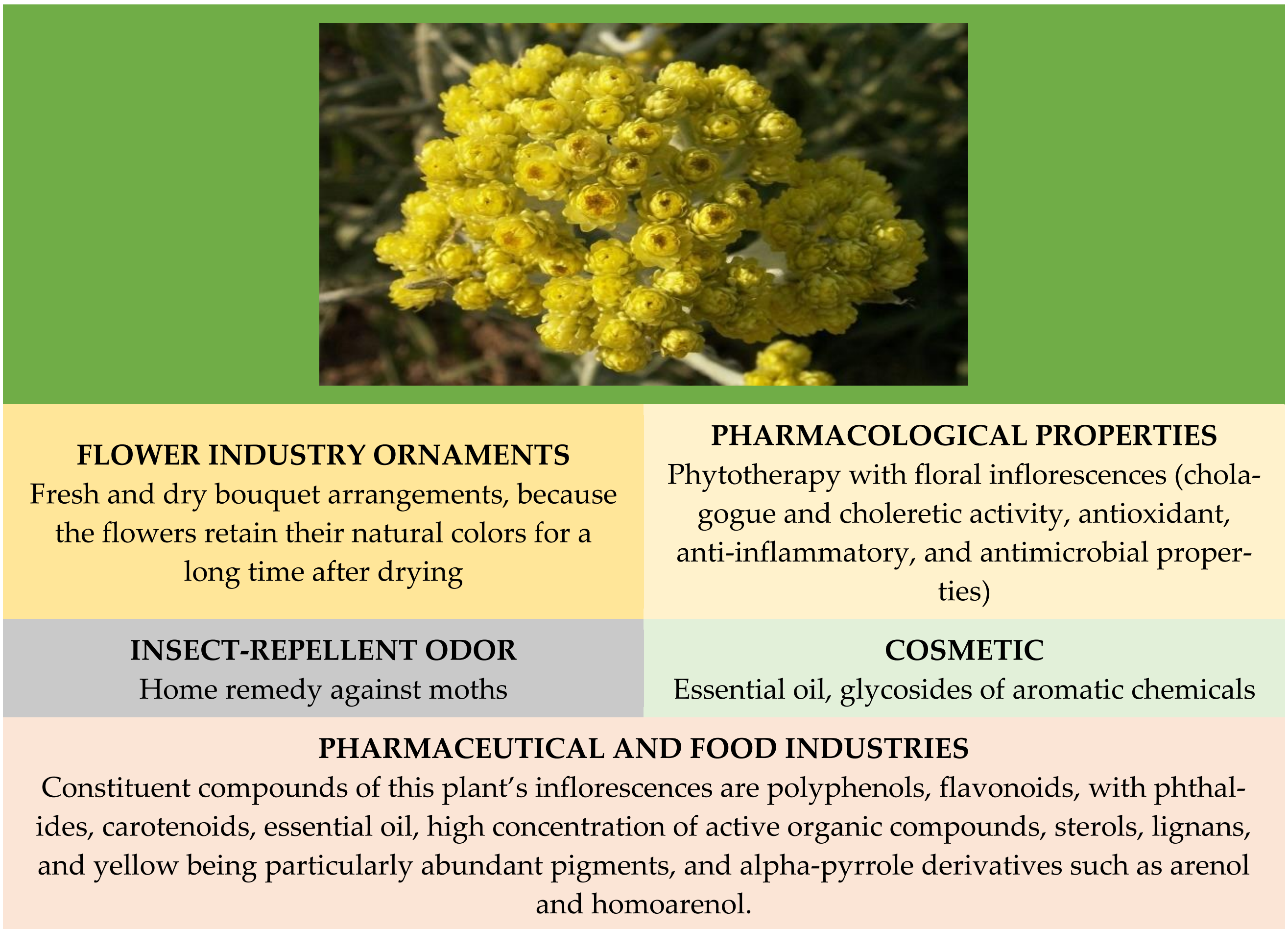

Figure 4. Pharmacoeconomic benefits of *Helichrysum arenarium* (L.) Moench.

The European Medicines Agency (EMA) advises using *Helichrysi flos* in the form of tea blends made in pharmacies because there are currently no clinical data on the therapy with preparations based on *H. arenarium*.

Helichrysi flowers retain their natural color even after drying, which makes this plant useful in aromatherapy and horticulture for designing floral ornaments (Figure 4). Moreover, the flowers were used for dying home-made fiber fabrics to obtain a bright and durable yellow color [23].

7. Conclusions and Perspectives

In 2010, the WHO monographs [9] on commonly used medicinal plants included a special monograph on *Helichrysi arenarii flos*. However, publications on the chemistry and pharmacological effects of this plant are sparse, and no special publication on its safety is available.

H. arenarium is a very important species for the pharmaceutical industry and is listed as an endangered species in some European nations; therefore, additional efforts have been made to improve cultivation techniques. Traditional cultivation was refined using inoculation with a variety of mycorrhizal fungi. It is also necessary to continue the development of alternative methodologies for biopreservation, resynthesis, and obtaining biomass with a high content of bioactive substances that could be exploited biotechnologically by taking into account the advantages offered by "in vitro" systems. Further studies should focus on the characteristics of cell proliferation, the intensity of callus biomass accumulation, and seedling regeneration. In order to obtain varieties with sustainable agricultural production from this crop, the extension of biotechnological applications of clonal multiplication through in vitro techniques should be continued.

New studies on *Helichrysum arenarium* species will shed additional light on the valorization of plant raw materials and the extraction of bioactive components in order to highlight the biological effects that will lead to extensive phytotherapeutic applications.

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