Beneficial Biological Effects of *Helichrysum italicum*

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Helichrysum italicum (family Asteraceae), due to its various beneficial biological effects, represents an important plant in the traditional medicine of Mediterranean countries. There is a renewed interest in this medicinal plant, especially in investigations involving the isolation and identification of its bioactive compounds from extracts and essential oils, as well as in experimental validation of their pharmacological activities. The research is focused on the beneficial biological effects of *Helichrysum italicum* extracts, essential oils, and their major bioactive polyphenolic compounds, ranging from antioxidative, anti-inflammatory, and anticarcinogenic activities to their antiviral, antimicrobial, insecticidal, and antiparasitic effects.

Helichrysum italicum | beneficial biological effects | polyphenolic compounds

1. Introduction

The interest in natural phytochemicals concerning their therapeutic and beneficial health properties has gradually increased in recent years. Mediterranean plants are a rich source of bioactive compounds important to human health [1][2]. The genus *Helichrysum (Miller)* belongs to the Asteraceae family and includes more than a thousand taxa that have a high occurrence in the Mediterranean areas of Europe [3][4][5]. *Helichrysum (Miller)* grows at a wide range of altitudes from the sea level up to 1700 m, preferably on sandy or loamy soils [6]. The name of the genus is derived from the Greek words “helios” (sun) and “chryos” (gold) and is directly related to the typical bright yellow-colored inflorescences [6]. *Helichrysum italicum* in full blossom is shown in Figure 1.

Figure 1. *Helichrysum italicum* in full blossom (photo taken by Dr. Veronika Furlan).

Helichrysum italicum, belonging to the *Helichrysum (Miller)* genus, is an evergreen plant native to the Mediterranean area. *Helichrysum italicum*, due to its various beneficial biological effects, represents an important everlasting plant in the traditional medicine of Mediterranean countries [5]. The interest in *Helichrysum italicum,* also known as immortelle or everlasting, has been motivated by its traditional therapeutic applications in inflammatory and allergy conditions, such as asthma and skin inflammatory conditions [7]. The use of *Helichrysum italicum* essential oils has also been reported in aromatherapy applications, wound healing, and skin conditions such as hematoma and sunburn [8]. Voinchet et al. [9] showed that the application (for 2–3 months) of *Helichrysum italicum* subsp. *serotinum* essential oil diluted to 10% in *Rosa rubiginosa* vegan oil reduced local inflammation, edema, bruises, and hematomas in the post-operative scars. In addition, its therapeutic use, related to antioxidant and antimicrobial properties [10][11][12][13] has long been recognized. In the agri-food sector, *Helichrysum italicum* flowers can be used for seasoning and flavoring food, such as bakery products and soft drinks, and as natural food additives or preservatives due to their antibacterial (against *Micrococcus luteus*, *Bacillus cereus*, and *Pseudomonas aeruginosa*) [14], antifungal (against *Aspergillus niger* and *Alternaria alternata*) [14] and insecticidal properties (against mosquito *Aedes albopictus* (Diptera: Culicidae)) [15]. In a very recent study, the consumption of *Helichrysum italicum* infusion was reported to significantly reduce serum levels of proinflammatory interleukine 1β (IL-1β) alongside Proteobacteria reduction. According to the authors, *Helichrysum italicum* infusion possesses prebiotic activities and can improve gut microbiota [16].

2. Biological Effects of *Helichrysum italicum* Extracts

2.1. Biological Effects of Major Bioactive Compounds from *Helichrysum italicum* Extracts

Helichrysum italicum extracts contain mainly non-volatile polyphenolic compounds that possess various beneficial biological effects, namely antioxidative, anti-inflammatory, antimicrobial, and anticarcinogenic effects, with cytoprotective activity towards normal cells and cytotoxic effects against cancer cells [17]. Polyphenols are a large group of at least 10.000 known compounds which contain one or more aromatic rings with at least one phenolic hydroxyl group. They are secondary plant metabolites that protect the plants against reactive oxygen and nitrogen species, UV light, pathogens, and parasites [18][19]. The quality of *Helichrysum italicum* extracts is correlated mainly with the content of flavonoids (e.g., gnaphaliin and tiliroside), and a prenylated α-pyrone–phloroglucinol heterodimer arzanol, as well as with the content of polyphenolic acids (e.g., chlorogenic acid), acetophenones (e.g., 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone), and triterpenes (e.g., ursolic acid).

2.1.1. Phenolic Acids

Phenolic acids, containing a phenolic ring and a carboxylic acid functional group, can be divided into two groups, namely hydroxycinnamic and hydroxybenzoic acids with their respective derivatives [20]. Chlorogenic acid, an ester of caffeic and quinic acid, is the most abundant hydroxycinnamic acid from *Helichrysum italicum* methanolic extracts (up to 0.77% of the extraction yield) [21][22]. In vitro and in vivo studies have reported several pharmacological effects of chlorogenic acid, namely antioxidant, anti-inflammatory, anticancer, antibacterial, and antiviral effects.

Vanucci-Bacqué et al. [23] demonstrated the antioxidant activity of chlorogenic acid (10 µM), which was assessed as superoxide anion radical scavenging activity (35.5%). DPPH free radical scavenging activity of chlorogenic acid was also reported (IC_{50} 20 µg/mL) [24]. Moreover, Luyen et al. [25] reported anti-inflammatory activity of chlorogenic acid (10 uM) in mouse macrophage RAW264.7 cells, which was assessed as inhibition of lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF-α) production (24.73%). Chlorogenic acid (100 uM) also inhibited cyclooxygenase 2 (COX2) by 30% [26]. The inhibition of the proliferation of human glioma U251 cancer cells (56.63%) and rat glioma C6 cancer cells (77.37%) by 100 uM chlorogenic acid was also observed [27]. Furthermore, D'Abrosca et al. [28] reported that chlorogenic acid (128 µg/mL), isolated from the methanol extract of *Helichrysum italicum,* inhibited biofilm formation of *Pseudomonas aeruginosa* by 45%. Konstantinopoulou et al. [29] also demonstrated the antimicrobial activity of chlorogenic acid against *Helicobacter pylori* (MIC 6.25 µg/mL). The antifungal activity of chlorogenic acid against *Candida krusei* and *Candida albicans* (MIC > 64 µg/mL) was observed as well [30]. In addition, it was reported that chlorogenic acid (25 µM) inhibited human immunodeficiency virus type 1 integrase (HIV-1 IN) by 59.7% [31].

Caffeic acid is a very common hydroxycinnamic acid with many beneficial biological effects, which is present in *Helichrysum italicum* methanolic extracts up to 0.015% [21][22][32]. In the study of Georgiev et al. [33], caffeic acid (3.6 mM) demonstrated 88.04% DPPH radical scavenging activity. Similarly, Digiacomo et al. [34] reported 90.27% DPPH radical scavenging activity caffeic acid (30 uM). Bora-Tatar et al. [35] identified caffeic acid (500 µM) as a

potent histone deacetylase (HDAC) inhibitor due to its 80% inhibition of HDAC in human immortal Hela cells. Yu et al. [36] also reported significant inhibition of potato 5-lipoxygenase (5-LOX) by caffeic acid (4 µg/mL), indicating its anti-inflammatory activity. The authors also reported significant anti-inflammatory activity of caffeic acid (30 mg/kg) against carrageenan-induced paw edema in a rat model. The anti-inflammatory activity of caffeic acid was also assessed as inhibition of LPS-induced TNF-α (IC_{50} > 50 µg/mL), IL-12 (IC_{50} > 50 µg/mL), and IL-6 (IC_{50} > 50 µg/mL) production in wild-type embryonic C57BL/6 mouse bone marrow dendritic cells [37]. Moreover, the MTT assay of Chen et al. [38] confirmed the cytoprotective activity of caffeic acid against H_2O_2-induced cytotoxicity in human endothelial Ea.hy926 cancer cells (EC_{50} 12.6 µM). Miamaye et al. [39] also demonstrated inhibition of human amyloid beta (A42) aggregation by caffeic acid (IC_{50} 32.8 µg/mL), which indicates it has potential in the treatment of Alzheimer's disease. Furthermore, caffeic acid (50 µg/mL) demonstrated antibacterial activity against *Fusarium graminearum* (63%) [40] and *Staphylococcus epidermidis* (EC_{50} 2.78 µg/mL) [41]. The MTT assay of Fu et al. [42] also showed its antifungal activity against *Candida albicans* (MIC > 50 µg/mL) as well as antibacterial activity against *Pseudomonas fluorescens* (MIC > 50 µg/mL), *Staphylococcus aureus* (MIC > 50 µg/mL) and *Bacillus subtilis* (MIC > 50 µg/mL). In addition, it was observed that caffeic acid inhibits HIV1 integrase strand transfer activity (IC_{50} 24 µg/mL) and, therefore, possesses antiviral activity [43].

2.1.2. Flavonoids

Flavonoids are the largest group of dietary polyphenols. They possess a 15-carbon structure consisting of two phenyl rings and a heterocycle. Due to their structural diversity, they are further divided into seven subclasses; namely flavanols (catechins), flavanones, flavones, flavonols, isoflavones, anthocyanins, and chalcones. According to several studies, polyphenols from the flavonoid class possess antioxidant, anti-inflammatory, antiproliferative, anticarcinogenic, and antimicrobial activities [44]. Flavonols gnaphaliin and tiliroside, as well as the flavanone naringenin, are the most common flavonoids, present in *Helichrysum italicum* methanolic extracts up to 0.03%, 0.0063%, and 0.023%, respectively [45]. The flavonols quercetin and kaempferol, as well as their glucosides, were also identified in *Helichrysum italicum* methanolic extracts (up to 0.015% and 0.0026%, respectively) [46]. The presence of flavones luteolin and apigenin in *Helichrysum italicum* ethanolic extracts, as well as the flavanone pinocembrin in methanolic extracts, was also reported; however, their extraction yields were not specified [3][47].

The flavonol gnaphaliin and flavanone pinocembrin, isolated from the methanolic extract of *Helichrysum italicum*, were able to inhibit the production of inflammatory leukotriene B4 in an in vitro model of calcium ionophore A23187-stimulated rat polymorphonuclear leukocytes by 94% and 96%, respectively, in comparison with the untreated control [48]. According to the authors, gnaphaliin, tiliroside, and pinocembrin (0.5 g) also reduced TPA-induced edema in the mice ears by 72, 80, and 81%, respectively (ID50 values of 210 µg/ear, 357 µg/ear, and 61 µg/ear, respectively). Tiliroside also diminished neutrophil infiltration by 88% [48]. An anti-inflammatory activity of naringenin (0.3 µM) in CD1 mice, assessed as 43% inhibition of croton oil-induced ear edema relative to untreated control, was also observed [49]. Moreover, Shin et al. [50] observed inhibition of nuclear factor kappa B (NF-κB) activation by naringenin (10 uM) in colon HCT116 cells, which was assessed as inhibition of TNF-α-induced transcriptional activation.

Sala et al. [48] investigated the antioxidant properties of three flavonoids, gnaphalin, pinocembrin, and tiliroside, isolated from the aerial parts of *Helichrysum italicum*. Tiliroside exhibited the best DPPH scavenging potential (IC_{50} value of 6 μM), as well as significant inhibition of enzymatic and non-enzymatic lipid peroxidation (IC_{50} values of 12.6 and 28 μM, respectively). Tiliroside also exhibited superoxide-scavenging activity with an IC_{50} value > 100 μM. The superoxide-scavenging activity of naringenin was reported as well (IC_{50} value > 50 μM) [51].

In the study of Sun et al. [52], tiliroside significantly inhibited the main cytochrome P450 (CYP) enzymes present in the metabolism of clinically important drugs, in comparison with positive CYP inhibitors. Tiliroside was the most effective inhibitor of CYP2C9 (85%) with an IC_{50} of 10.2 ± 0.9 μM, followed by CYP2C8 (82.3%) with an IC_{50} value 12.1 ± 0.9 μM, and CYP3A4 (71.6%) with an IC_{50} value of 9.0 ± 1.7 μM. Takemura et al. [53] reported that naringenin also inhibited human CYP1A1, CYP1A2, and CYP1B1 enzymes (IC_{50} values of 15.17, 26.34, and 3.66 μM, respectively). Furthermore, Chen et al. [54] reported the antifungal activity of tiliroside (100 ug/disc) against *Ceratocystis paradoxa*, *Athelia rolfsii*, and *Alternaria mali* assessed as mycelial growth inhibition (GI) of 27.6, 22.4, and 55.6%, respectively. The same authors also reported cytotoxicity of tiliroside (20 mg/L) against cotton leafworm *Spodoptera litura* cells (GI 65%). In addition, the antiparasitic activity of tiliroside against *Entamoeba histolytica* (IC_{50} 17.45 μM) was observed [55]. Freitas et al. [56] reported the antileishmanial activity of tiliroside (841 uM) against *Leishmania amazonensis amastigote* (67.8%) and *Trypanosoma cruzi amastigote* (45%) as well. Tan et al. [57] also observed weak inhibition of HIV1 by tiliroside (IC_{50} < 200 μg/mL). On the other hand, Li et al. [58] reported that naringenin strongly inhibited His6-tagged HIV-1 integrase with an IC_{50} value of 1.7 μM. Moreover, the antifungal activity of naringenin against *Candida albicans* and *Cryptococcus neoformans* ATCC 90113 was reported at IC_{50} values of >50 μg/mL [59].

2.1.3. Acetophenones and Tremetones

Acetophenones or methyl phenyl ketones are aromatic compounds that were first isolated in hydroxylated form from *Helichrysum italicum* methanolic extracts by Sala et al. [60]. Tremetones, also identified in *Helichrysum italicum* methanolic extracts in hydroxylated form, can be classified as benzofurans. Specifically, in the study of Sala et al. [60], six acetophenones and 12-hydroxytremetone (bitalin A) were isolated from the methanolic extract of *Helichrysum italicum* and then tested in two in vitro models and one in vivo model for their ability to inhibit arachidonic acid metabolism, and for evaluation of their antioxidative and anti-inflammatory potential. In the first in vitro model of calcium ionophore A23187-stimulated rat polymorphonuclear leukocytes, 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (100 μM) was able to reduce the production of leukotriene B4 by 95% (IC_{50} 24 μM) and 4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone (100 μM) reduced the production of leukotriene B4 by 44% (IC_{50} 111 μM). In the second in vitro model, only 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (100 μM) inhibited the activity of cyclooxygenase-1 (COX1) in calcium ionophore A23187-stimulated human platelets by 59%. Interestingly, none of the compounds exhibited scavenging activity against superoxide radicals. In the in vivo model, orally administered 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (150 mg/kg) reduced the carrageenan-induced edema in the mice paws by 51% after 1 h, by 71% after 3 h, and by 66% after 5 h. When the edema was induced by multiple injections of 2 μg TPA in mice ears, 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (0.5 mg) and 12-hydroxytremetone reduced the edema formation by 57%, and 71%, respectively [60]. The most effective

compounds against PLA2-induced paw edema were 12-hydroxytremetone-12-O-β-D-glucopyranoside, 3-(2-hydroxyethyl)acetophenone-4-O-β-D-glucopyranoside and maltol β-D-O-glucopyranoside, which reduced the edema by 65, 57, and 52%, respectively [60].

Sala et al. [61] tested the anti-inflammatory activity of several acetophenones from dichloromethane, ethyl acetate, and butanol fractions of *Helichrysum italicum* methanolic extract. According to the results, 4-hydroxy-3-(2-hydroxy-3-isopentenyl)acetophenone isolated from the dichloromethane fraction proved to be the most active inhibitor of TPA-induced inflammation in mice ears with ID50 of 0.63 µmol/ear. Rigano et al. [62] first isolated a new acetophenone derivative gnaphaliol 9-O-propanoate together with known acetophenones, such as 1-[2-[1-[(acetyloxy) methyl]ethenyl]-2,3-dihydro-3-hydroxy-5-benzofuranyl]-ethanone and acetotrixymetone, from flowers of *Helichrysum italicum* subsp. *italicum*. A safe toxicological profile was confirmed for all three acetophenones, while only acetotrixymetone exhibited antioxidative activity. Interestingly, none of the compounds (1–30 µM) exhibited anti-inflammatory activity, since the LPS-induced increase in nitrite levels was not significantly modified.

2.1.4. Pyrones

Arzanol, a prenylated phloroglucinyl α-pyrone heterodimer, was identified as the major anti-inflammatory compound in acetone extracts of aerial parts of *Helichrysum italicum* subsp. *microphyllum,* representing 0.32% of extraction yield [13]. According to Appendino et al. [10] arzanol represents a potent inhibitor of nuclear transcription factor NF-*κ*B activation with an IC_{50} value of 5 µM. Moreover, it was proven to inhibit the release of proinflammatory mediators in human peripheral monocytes such as IL-1*β* (IC_{50} 5.6 µM) and TNF-*α* (IC_{50} 9.2 µM), as well as IL-6, prostaglandin E2 (PGE2), and IL-8 with the IC_{50} values of 13.3, 18.7, and 21.8 µM, respectively. Bauer et al. [11] also investigated the effects of arzanol on the biosynthesis of prostaglandins and leukotrienes in vitro and in vivo. According to the authors, arzanol can inhibit the inducible microsomal prostaglandin E2 synthase (mPGE2), the formation of leukotriens in human neutrophilis, COX1 and 5-lipoxygenase (5-LOX) in vitro, with IC_{50} values ranging from 0.4 µM to 9 µM. It was also reported that the inhibition of PGE2 biosynthesis resulted from arzanol's interference with mPGES rather than COX2. In vivo, arzanol (3.6 mg/kg) suppressed the carrageenan-induced inflammatory response in the pleural cavity of rats and significantly reduced exudate formation (59%), cell infiltration (48%), and levels of PGE2, leukotriene B4 (LTB4) and 6-keto prostaglandin F1 alpha (PGF1α) by 47, 31, and 27%, respectively. According to Rosa et al. [63], arzanol, isolated from *Helichrysum italicum* also possesses cytotoxic potential, as it selectively reduced viability of colon Caco-2 cells (55%) at a concentration of 100 µg/mL as well as in immortal HeLa (36%) and melanoma B16F10 (95%) cancer cell lines at the highest tested concentration of 200 µg/mL. Moreover, Appendino et al. [10] reported that arzanol inhibits the TNFα-induced HIV-1 replication in a T cell line in a concentration-dependent manner. Anti-HIV activity was further investigated by infecting Jurkat (T lymphocyte) cells with a pNL4-3 HIV-1 clone pseudotyped with the vesicular stomatitis virus (VSV) envelope, which can support HIV-1 replication. A pretreatment of Jurkat cells with increasing concentration of arzanol (5–25 µM) resulted in a concentration-dependent inhibition of viral replication (35–65%). Furthermore, in the study of Rosa et al. [64] the protective effect of arzanol in lipid peroxidation was investigated. Its antioxidant activity was tested against the Cu^{2+} ions-induced oxidative modification of lipid components in human low-density lipoprotein (LDL) and tert-butyl hydroperoxide (TBH)-induced oxidative damage in cell membranes. In vitro, LDL pretreatment with

arzanol (50 µM) significantly protected lipoproteins from oxidative damage and exerted a remarkable reduction of polyunsaturated fatty acid and cholesterol levels ($p < 0.001$ versus oxidized control). At non-cytotoxic concentrations (25 µM and 50 µM), it also significantly protected kidney Vero cells and Caco-2 epithelial cells against TBH-induced oxidative stress. Rosa et al. [12] also confirmed that arzanol from *Helichrysum italicum* subsp. *microphyllum* did not exhibit toxicity in Vero cell cultures at any tested concentrations (0.5–40 µM). Tagliatela-Scafati et al. [13] evaluated the antibacterial activity of arzanol, coumarates, benzofurans, pyrones, and heterodimeric phloroglucinols isolated from *Helichrysum italicum* subsp. *microphyllum*. Only heterodimeric phloroglucinyl pyrone arzanol was efficient against multidrug-resistant *Staphylococcus aureus* strains, with MIC values of 1–4 µg/mL. In addition, Werner et al. [65] isolated and characterized two new arzanol derivatives from aerial parts of *Helichrysum italicum*, namely helitalone A, a dimer of substituted α- and γ-pyrone units, and helitalone B, a compound similar to arzanol with the isopropyl group replaced by an ethyl group. Antibacterial activities of isolated pyrone derivatives were tested against various Gram-positive and Gram-negative bacteria, but they did not exhibit any significant antibacterial effects at tested concentration of 20 µg/mL.

Arzanol can, therefore, act as a potential inhibitor of proinflammatory mediators, inflammatory enzymes, and HIV replication in T cells. Arzanol is also a potent natural antibacterial agent and antioxidant with a protective effect against lipid peroxidation in biological systems, and its diversity of action may well be utilized in cancer therapy.

2.1.5. Triterpenes

Terpenes are a diverse class of aromatic organic compounds with a skeleton built from isoprene units, e.g., carbon atoms in the multiples of five (C5n). The most important terpenes from *Helichrysum italicum* extracts and essential oils can be divided into mono (C10), sesqui- (C15), and triterpenes (C30) based on the number of isopnene subunits. Ursolic acid is the only triterpene identified in acetone extracts of *Helichrysum italicum* in higher quantities (up to 0.40%) [13].

Liobikas et al. [66] reported the antioxidant activity of ursolic acid (1.6 ng/mL) in Wistar rat heart mitochondria, which was assessed as a reduction in H_2O_2 production by 55.6%. Anti-inflammatory activity of ursolic acid (10 mg/kg) against carrageenan-induced paw edema in Wistar albino rat model, after 4 h (75.17%) was also observed [67]. Ghosh et al. [68] reported antinociceptive activity (reduced sensitivity to pain) of ursolic acid (10 mg/kg) in Swiss albino *Mus musculus* model, which was assessed as 61.44% inhibition of formalin-induced paw licking, relative to untreated control, after 30 min. The antibacterial activity of ursolic acid against *Enterococcus faecalis* (MIC 16 µg/mL) was also reported [69]. Nguyen et al. [70] observed weak antiviral activity of ursolic acid (2.7 uM) against HIV1 3B-infected human leukemia CEM-SS cells, which was assessed as 22% inhibition of virus-induced cytopathic effect after 6 days. De Brum Vieira et al. [71] also reported the antiparasitic activity of ursolic acid against metronidazole-sensitive *Trichomonas vaginalis* (MIC 50 µM), while Freitas et al. [56] observed the antiparasitic activity of ursolic acid against *Trypanosoma cruzi* (IC_{50} 4 µM).

Kwon et al. [72] reported induction of apoptosis by ursolic acid (40 µM) in human prostate RC-58T/h/SA#4 cells, which was assessed as an increase in sub-G1 DNA content by 58.6% after 24 h. Ursolic acid (20.6 uM) also

induced cell cycle arrest in human gastric AGS cells at sub-G0/G1 phase and G0/G1 phase by 86.53% and 33.2%, respectively, after 48 h [73]. Yang et al. [74] also observed weak antiproliferative activity of ursolic acid (100 μM) against rat liver HSC-T6 cells after 48 hrs (14.8%). Cytotoxicity of ursolic acid (50 μM) against human immortal HeLa cells and vaginal malignant melanoma HMVII cells were assessed as a reduction in cell viability by 50% and 60%, respectively, after 24 h [71]. In addition, ursolic acid (50 μM) demonstrated cytotoxicity against vaginal malignant melanoma HMVII cells by a 90% reduction in cell viability after 48 h. Wiemann et al. [75] reported cytotoxicity of ursolic acid against various human cancer cell lines, especially against colon HT-29 cancer cells (EC_{50} 10.6 μM) and human ovarian A2780 cancer cells (EC_{50} 11.7 μg/mL).

3. Biological Effects of *Helichrysum italicum* Essential Oils

3.1. Biological Effects of Major Bioactive Compounds from *Helichrysum italicum* Essential Oils

The main chemical compounds present in *Helichrysum italicum* essential oils can be divided into monoterpenes (C10) and sesquiterpenes (C15). The monoterpenes are formed from the coupling of two isoprene units (C10) and are the most representative terpenes, constituting 90% of the essential oils. The sesquiterpenes are formed from the assembly of three isoprene units (C15), and their structure and function are similar to those of the monoterpenes [76].

Various *Helichrysum italicum* essential oils from two main subspecies of *Helichrysum italicum*, namely *italicum* and *microphyllum,* have been intensively studied. Morone-Fortunano et al. [4] analyzed 20 *Helichrysum italicum* subsp. *italicum* genotypes from different locations in Italy and Corsica (France) and revealed that the essential oils contained mainly γ-curcumene (up to 41%), β-selinene (up to 38%), α-selinene (up to 26.5%), and neryl acetate (up to 32%). The concentrations of nerol and γ-eudesmol also reached appreciable amounts in some samples (up to 18.8% and 20.6%, respectively). Furthermore, Leonardi et al. [77] studied the composition of 21 *Helichrysum italicum* essential oil samples of subsp. *italicum* from seven locations of Elba Island (Tuscany, Italy). Monoterpene and sesquiterpene hydrocarbons accounted for 2.3–41.9% and 5.1–20.1% of the identified compounds, respectively. Essential oils from Elba Island (Italy) subsp. *italicum* were dominated by neryl acetate (up to 45.9%), followed by α-pinene (up to 32.9%), eudesm-5-en-11-ol (up to 17.2%), limonene (up to 12.9%) and nerol (up to 12.8%) [77]. Tuscan Archipelago Islands *Helichrysum italicum* essential oil subsp. *italicum* was also dominated by neryl acetate (up to 44.5%), followed by neryl propionate (up to 16.4%), γ-curcumene (up to 13.7%), and nerol (up to 7.6%) [78]. On the other hand, *Helichrysum italicum* subsp. *italicum* essential oil sample from Cilento (Italy) was dominated by iso-italicene epoxide (16.8%) [79]. According to Bianchini et al. [80] subsp. *italicum* essential oil samples from Tuscany contained mainly α-pinene (up to 53.5%) and neryl acetate (up to 22%), followed by β-selinene (up to 12.5%) and β-caryopyllene (up to 11%), while the sample from Corsica was dominated by neryl acetate (up to 38.9%) followed by neryl propionate (up to 5.9%) [80]. In another study of Bianchini et al. [81], the characterization of Corsican essential oils subsp. *italicum* also identified neryl acetate as a predominant compound, with amounts from 15.8% (from plants in the stage of early shoots) to 42.5% (in full flowering period). Interestingly,

Helichrysum italicum essential oil subsp. *italicum* from Greek island of Amorgos was characterized by a high content of geraniol (35.59%) and a significant amount of geranyl acetate (20.76%) and nerolidol (11.86%) [82].

According to Morone-Fortunato et al. [4], three different chemotypes were observed in subsp. *italicum*:

(a) genotypes rich in nerol and its esters;

(b) genotypes with a dominance of β and α-selinene;

(c) genotypes with high amounts of γ-curcumene.

Furthermore, essential oils subsp. *microphyllum* (Willd.) Nyman from Sardinia were mostly dominated by neryl acetate (26–35.6%) and nerol (9.1–14.4%) [83][84][85], while neryl propionate (up to 11.4%), γ-curcumene (up to 18.2%), and eudesm-5-en-11-ol (up to 23.5%) were also present in significant amounts. Melito et al. [86] examined 146 *Helichrysum Italicum* subsp. *microphyllum* genotypes from the seaside (0–60 m above the sea level) and mountains (600–1250 m above the sea level) in Sardinia to prove the influence of altitude and climate on the *Helichrysum italicum* essential oil composition. The results showed that there is a correlation between the habitat type and the secondary metabolite production based on significantly ($p < 0.0001$) different essential oil compositions between both habitats. Considering the importance of climatic factors on the chemical composition of the essential oil, the quantity of nerolidol was correlated with the mean winter temperature, while italicene, bergamotene, nerol, and curcumene were positively correlated with spring and summer percipitation. Similarly, two studied genotypes of *Helichrysum italicum* subsp. *microphyllum* from Corsica were rich in neryl acetate (up to 55.7%), and also contained appreciable amounts of neryl propionate (up to 12.7%) [6]. On the other hand, *Helichrysum italicum* subsp. *microphyllum* essential oil from Crete contained mainly sesquiterpenes β-selinene (up to 17.2%) and γ-curcumene (up to 13.7%) followed by α-selinene (up to 5.39%) [87].

It must be noted that many authors did not specify the subspecies of *Helichrysum italicum* from which the studied essential oils were obtained. For example, Croatian oil samples (subsp. not specified) were dominated by neryl acetate as a major compound (11.5%) [88], while a surprisingly lower content of neryl acetate (up to 9.02%) was present in *Helichrysum italicum* essential oils from the Croatian Adriatic coast (subsp. not specified), where α-pinene (up to 29.9%), and α-curcumene (up to 28.64%) were determined as major compounds [89]. In a recent study, Oliva et al. [90] analyzed the composition of *Helichrysum Italicum* essential oil (subsp. not specified) from Montenegro. According to the results, essential oil from the liquid phase possessed high amounts of sesquiterpenes β-eudesmene (21.65%), and β-bisabolene (19.90%), as well as monoterpenes α-pinene (16.90%) and neryl acetate (10.66%). On the other hand, the vapor phase was enriched with monoterpene hydrocarbons fraction with α-pinene (78.76%) as the major compound.

It can be concluded that *Helichrysum italicum* essential oils exhibit various compositions depending on the geographical location where the plant grows, the sub-species, acidity, and type of soil, as well as the developmental stage of the plant. Due to different chemical compositions, essential oils from various sub-species

and geographical locations may possess distinct biological effects. Hladnik et al. [91] revealed the complete chloroplast genome of *Helichrysum italicum* subsp. *italicum* sampled in the North Adriatic Region. The chloroplast genome contained 131 genes (85 protein-coding genes, 36 transfer RNA genes, 8 ribosomal RNA genes, and 2 partial genes) and its length was 152,431 bp. According to the authors, these findings could be used for the development of reliable molecular markers for future genetic studies of *Helichrysum italicum*. There are numerous research articles on *Helichrysum italicum* biochemical diversity, however, only a few are related to its genetic diversity and the relationship between genotypes and chemotypes [92].

3.1.1. Monoterpenes

Based on the number of isoprene subunits, the most important terpenes from *Helichrysum italicum* essential oils belong to monoterpenes (C10) and sesquiterpenes (C15). Monoterpenes and sesquiterpenes from *Helichrysum italicum* essential oils also contain different functional groups and can be predominantly classified as alcohols (e.g., nerol, eudesm-5-en-11-ol) and esters (e.g., neryl acetate, neryl propionate).

Nerol and its derivatives are largely employed as cosmetic ingredients due to their sweet rose fragrance. The richest natural sources of monoterpene nerol include rose, palmarosa, and citronella as well as *Helichrysum italicum* essential oils. Its esters (nerol acetate in particular as well as nerol propionate) are also commonly encountered as major compounds in *Helichrysum italicum* essential oils from Italy and France (up to 18.8%, 55.7%, and 16.4%, respectively) [4][6][77][78][84]. In the study of Cordali et al. [93], nerol (10 µL) showed insecticidal activity against the first, second, and third-instar larval stage of *Leptinotarsa decemlineata*-infested potato leaves assessed as mortality relative to untreated control after 96 h (56.7%, 56.7%, and 80%, respectively). Ramos Alvarenga et al. [94] reported that nerol also possesses antimicrobial activity against *Mycobacterium tuberculosis* H37Rv at a MIC value of 128 µg/mL. Moreover, nerol was reported to possess acaricidal activity against *Psoroptes* cuniculi, which was observed at inhalation of 3 µL (83.3%) and 6 µL (100%) of nerol after 24 h [95]. The same authors also conducted a direct contact assay where nerol showed 100% acaricidal activity against *Psoroptes cuniculi* at 0.125, 0.25, and 1% dilution in physiological saline after 48 h. The repellent activity of nerol (0.2 uL/cm^2) against *Tribolium castaneum* (red flour beetle) was also assessed as induction of repellency measured 2 h and 4 h after exposure (98% and 95%, respectively) [96]. In the study of Kordali et al. [93] neryl acetate (20 µL) showed lower insecticidal activity than nerol against the first, second, and third-instar larval stage of *Leptinotarsa decemlineata*-infested potato leaves, which was assessed as mortality relative to untreated control after 96 h (10, 6.7, and 46.7%, respectively). According to Ortar et al., [97] neryl acetate also has agonist activity against rat transient receptor potential cation channel, subfamily A, member 1 (TRPA1) expressed in human embryonic kidney HEK293 cells, which was assessed as inhibition of the increase in intracellular Ca^{2+} concentration (IC_{50} 21.2 µM).

α-pinene is the most abundant terpene in nature, which occurs in the essential oils of *Pinus palustris* Mill. at concentrations of up to 65%, *Pinus caribaea* at concentrations up to 70% [98] and *Helichrysum italicum* at concentrations up to 53.5% [77][80][88][89]. Nowadays, α-pinene is used in the production of gin [99]. Burits et al. [100] reported the potent antioxidative activity of pure α-pinene in the DPPH assay (IC_{50} value of 0.78 µL/mL) as well as emphasized its potential to inhibit lipid peroxidation (IC_{50} value of 0.51 µL/mL). De-Oliveira et al. [101] demonstrated

that (–)-α-pinene and (+)-α-pinene modulate hepatic mono-oxygenase activity CYP2B1, which catalyzes biotransformation of promutagens or procarcinogens into genotoxic chemical carcinogens (IC_{50} value of 0.087 µM and 0.089 µM, respectively). Lorente et al. [102] demonstrated the anti-inflammatory activity of α-pinene (80 mg/kg) against carrageenan-induced plantar edema in Wistar rat paw (26.2% edema reduction). Rufino et al. [103] showed the anti-inflammatory activity of α-pinene (200 µg/mL) against human primary chondrocytes, which was determined as 40.6% inhibition of IL-1β-induced NO production relative to a IL-1β-treated control. α-pinene showed weak antimicrobial activity against other tested strains, namely *Candida albicans*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* (MIC > 900 µL/mL) [104].

Limonene, the main constituent of the citrus essential oil of sweet orange peel oil (*Citrus sinensis, Rutaceae*), is frequently present in considerable amounts in the *Helichrysum italicum* essential oil as well (up to 12.9%) [77][80][81]. Monocyclic monoterpene (+)- and (−)-limonene enantiomers are extensively used as fragrances in household cleaning products, in the cosmetic industry in creams, perfumes, and soaps, in the food industry as flavor additives for food, and as industrial solvents. According to Schnuch et al. [105], limonene belongs to the third group (Group III) of substances that are considered extremely rare sensitizers, and may even be considered as non-sensitizers (upper confidence interval (CI) of less than 0.5%). However, it must be noted that limonene can become an allergen after substantial air oxidation [106]. In the study of Souza et al. [107] the anti-inflammatory activity of limonene in the LPS-induced pleurisy mouse model was investigated. After oral administration of pure limonene, a significant reduction of LPS-induced cell migration was observed. Pure limonene also reduced the production of NO by 50% and inhibited γ-interferon by 86% at a dose of 25 µg/well. De-Oliveira et al. [101] demonstrated that d-limonene modulates hepatic monooxygenase activity of CYP2B1 enzyme (IC_{50} value of 0.19 µM), which catalyzes the biotransformation of procarcinogens. Wilkins et al. [108] identified d-limonene as effective in the treatment of gastroesophageal reflux disorder. A double-blind, placebo-controlled trial was conducted with 13 patients. After 14 days 86% of patients who took d-limonene were asymptomatic. In the placebo group, only 29% of patients reported relief of symptoms after 14 days.

3.1.2. Sesquiterpenes

α and β-selinene are ubiquitous sesquiterpene hydrocarbons present as the major compounds in *Helichrysum italicum* subsp. *italicum* essential oil from Italy and Corsica (up to 26.5% and 16.7%, respectively) [4][87]. They possess sweet woody and herbaceous fragrances, which play an important role in chemical ecology as pheromones [99]. Moreover, γ-curcumene and eudesm-5-en-11-ol are sesquiterpenes, which have been identified as major compounds in the essential oils of *Helichrysum italicum* subsp. *italicum* from Italy and Corsica (up to 41% and 17.2%, respectively) [87]. The biological activities of individual major sesquiterpenes from *Helichrysum italicum* essential oils currently remain unexplored. Sesquiterpenes, therefore, represent interesting candidates for further research.

4. Encapsulation of *Helichrysum italicum* Extracts, Essential Oils and Individual Bioactive Compounds

Low absorption and bioavailability represent the main obstacles to the successful delivery of natural polyphenols from *Helichrysum italicum* extracts and essential oils from the gastrointestinal tract to the targeted tissues in vivo. To improve bioavailability, absorption, solubility, and rapid metabolic degradability of polyphenols, various drug delivery systems, such as nanoparticles, emulsions, and liposomes have been intensively studied [109][110][111]. Encapsulation (microencapsulation, nanoencapsulation) is a simple and cost-effective method in which bioactive compounds are coated or entrapped into cell wall material. Polysaccharides, derived from animals (chitosan), algae (alginate, carrageenan), plants (pectin, starch, cellulose, hyaluronate), and bacteria (dextran and xanthan gum) are commonly used for bioactive compound encapsulation. *Helichrysum italicum* extract was successfully encapsulated into various alginate-protein matrices, which served as carriers for the formulation of biodegradable edible films of immortelle [112]. Chitosan is also considered as an effective delivery system for polyphenolic compounds [113][114] and is often combined with natural polysaccharides, such as alginate, to form complexes [115][116][117].

Nowadays, liposomes are receiving increasing attention as one of the most promising carriers of various bioactive polyphenolic compounds, as they exhibit exceptional biocompatibility, biodegradability, non-toxicity, non-immunogenicity, improved targeted delivery, and successfully protect polyphenolic compounds from light and degradation processes [118]. Liposomes, vesicles that consist of one or more phospholipid bilayers, possess significant potential in the cosmetic and food industries due to minimal adverse effects [118]. Successful encapsulation of biologically active polyphenolic compounds [119], extracts [120][121], and essential oils [122] obtained from different natural materials into liposomes was recently reported in several studies. Pharmaceutical and cosmetic formulations with liposomes incorporating bioactive compounds allow better bioavailability of bioactive compounds, thereby increasing their efficacy [123]. Liposomes with encapsulated extracts of various herbs and spices exhibited excellent inhibitory effects against various tested bacterial strains, which was even higher than in the case of tested pure extracts [124]. Liposomes can also protect natural polyphenols from *Helichrysum italicum* against metabolic degradation, enhance their beneficial effects in the target tissues, and amplify their antioxidative, anti-inflammatory, antibacterial, and anticarcinogenic effects, which is vitally important in the treatment of various diseases. In addition, nanoparticle drug delivery systems using liposomes as well as natural polysaccharides (such as chitosan, alginate, pectin, cellulose, and xanthan gum) represent promising alternatives to magnetic metal-based nanoparticles due to their reduced toxicity, higher biocompatibility, and improved targeted delivery. Future studies should, therefore, focus on the incorporation of bioactive compounds from *Helichrysum italicum* into liposomes and polysaccharides. This will represent an important novelty for cosmetic formulations and dietary supplements.

5. Future Perspectives

It can be concluded that *Helichrysum italicum* possesses various beneficial biological effects, and has the potential for applications in the cosmetic, pharmaceutical, and food industries, as well as in the development of novel antimicrobial, antiviral, and insecticidal agents. Moreover, mechanistic insights into *Helichrysum italicum* polyphenol interactions with human, bacterial, fungal, and viral proteins, which are crucial for the design and

optimization of novel drugs, can be revealed in house developed inverse molecular docking protocol [125][126] as well as by extensive molecular dynamics simulations coupled with free-energy calculations [127][128]. In silico quantum-mechanical simulations performed by the research group [129][130] could also reveal cancer-preventive mechanisms of bioactive polyphenols, such as arzanol from *Helichrysum italicum*, against various ultimate chemical carcinogens to which people are frequently exposed.

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