Karadeniz Fen Bilimleri Dergisi, 13(2), 680-689, 2023. DOI: 10.31466/kfbd.1271087

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377 https://dergipark.org.tr/tr/pub/kfbd

Araştırma Makalesi / Research Article

Antioxidant and antimicrobial activities of the essential oil of *Helichrysum chionophilum*: an endemic plant of Türkiye

Tuba ACET[1*], Kadriye ÖZCAN[2]

Abstract

Diseases that develop due to microorganisms have become increasingly dangerous today. Especially in the fight against resistant microorganisms, there is a need for cheaper, natural and effective methods. Plant materials containing biologically active compounds come to the fore in this sense. Therefore, in the present study, the antioxidant and antimicrobial activities of the essential oil obtained from *H. chionophilum* collected from Gümüşhane were investigated. The essential oil was obtained by hydro-distillation using a Clevenger-type apparatus. Free radical scavenging activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazil) method. Antimicrobial activity was examined using the microdilution method using 17 bacteria and 2 yeasts. According to the findings, IC_{50} value of the radical scavenging activity of the essential oil of *H. chionophilum* plant was found to be 260.0±0.2 µg/mL. Also, the essential oil of the plant showed outstanding antimicrobial activity against *Y. pseudotuberculosis* (MIC: 16 µg/mL), *S. epidermidis* (MIC: 16 µg/mL), and *C. albicans* (MIC: 16 µg/mL) and *C. tropicalis* (MIC: 32 µg/mL). The results indicate that *H. chionophilum*, which is an endemic species, and its essential oils can be used as a new and alternative natural agent thanks to its antioxidant and antimicrobial properties.

Keywords: Antioxidant, antimicrobial, *Helichrysum chionophilum*

Helichrysum chionophilum'un uçucu yağının antioksidan ve antimikrobiyal aktiviteleri: Türkiye'nin endemik bir bitkisi

Öz

Mikroorganizmalara bağlı olarak gelişen rahatsızlıklar günümüzde giderek tehlikeli bir hal almıştır. Özellikle, dirençli organizmalar ile mücadele noktasında, daha ucuz, doğal ve etkili yöntemlere ihtiyaç duyulmaktadır. Biyolojik olarak aktif bileşikleri barındıran bitki materyalleri bu manada ön plana çıkmaktadır. Bu nedenle, mevcut çalışmada, Gümüşhane'den toplanan *Helichrysum chionophilum*'dan elde edilen uçucu yağın antioksidan ve antimikrobiyal aktiviteleri araştırılmıştır. Uçucu yağ, Clevenger tipi bir aparat kullanılarak hidro-damıtma yönteminden elde edildi. Serbest radikal yakalama aktivitesi, DPPH (2,2-difenil-1-pikrilhidrazil) yöntemi kullanılarak belirlendi. Antimikrobiyal aktivite mikro dilüsyon yöntemi kullanılarak 17 bakteri ve 2 maya kullanılarak incelendi. Elde edilen bulgulara göre, *H. chionophilum* bitkisi radikal süpürme aktivitesinin IC_{50} değeri 260.0±0.2 µg/mL olarak bulunmuştur. Ayrıca, bitkinin uçucu yağı, *Y. pseudotuberculosis* (MIC: 16 µg/mL), *S. epidermidis* (MIC: 16 µg/mL) ve *C. albicans* (MIC: 16 µg/mL) ve *C. tropicalis'e* (MIC: 32 µg/mL) karşı olağanüstü antimikrobiyal aktivite göstermiştir. Elde edilen sonuçlar, endemik bir tür olan *H. chionophilum* ve uçucu yağlarının antioksidan ve antimikrobiyal özellikleri sayesinde yeni ve alternatif doğal bir ajan olarak kullanılabileceğini işaret etmektedir.

Anahtar Kelimeler: Antioksidan, antimikrobiyal, *Helichrysum chionophilum*

[1]Department of Occupational Health and Safety, Gümüşhane University, Gümüşhane, Turkey, tubaacet@hotmail.com
[2]Department of Genetic and Bioengineering, Giresun University, Giresun, Turkey, kadriye.ozcangiresun.edu.tr

[1]https://orcid.org/0000-0002-0981-9413 [2]https://orcid.org/0000-0002-4913-6035

[*]**Sorumlu Yazar/Corresponding Author**

Geliş/Received: 26.03.2023 **Kabul/Accepted:** 22.05.2023

1. Introduction

With its 600 varieties, *Helichrysum* Mill. (Asteraceae) is widely found throughout the World (Lourens et al., 2008; Giovanelli et al., 2018). Sixteen of these plants are endemic to Türkiye and generally known as "ölmez çiçek (immortal flower)", "altınotu (golden herb)" or "kovanotu" in Anatolia (Baytop, 1997; Güner et al., 2012). The members of *Helichrysum* species are generally perennial and scrub plants and have aromatic properties (Giovanelli et al., 2018; Akinfenwa et al., 2022). In Anatolia and the rest of the world, it is consumed herbal tea (Ebrahimzadeh and Tavassoli, 2015; Acet and Özcan, 2022) (and for coughing and respiratory problems, digestive system problems, dermal inflammations, wound healing, and painkiller/anti-inflammatory (Lourens et al., 2004; van Vuuren et al., 2006; Viagas et al., 2004), diarrhea (Benelli et al., 2018), antimicrobial and sedative (Kurti et al., 2019), anti-diabetic (Aslan et al., 2017), diuretic and passing the kidney stone (Yeşilada et al., 1995; Süzgeç-Selçuk and Birteksöz, 2011). In order to confirm the traditional uses of *Helichrysum* species and to emphasize the potential uses, many studies have been carried out on the extracts and essential oils of the plants (biological activities) (Albayrak et al., 2010; Kürkçüoğlu et al., 2019; Viegas et al., 2014; El-Olemy et al., 2005; Giuliani, et al., 2016; Afoulous et al., 2011; Judzentiene and Butkiene, 2006; Jokić et al., 2016; Özcan and Acet, 2018b).

The essential oils are defined as the natural herbal products consisting of volatile secondary metabolites characterized with complex ingredients and strong odor (Kurti et al., 2019). Some *Helichrysum* species and the essential/volatile oils gaining them pharmacological properties were reported to show chemical differences depending on the vegetation, environment, and geographical origins (Bianchini et al., 2009; Furlan and Bren, 2023). For this reason, it is thought that the plants in Gümüşhane region, which have different climates and heights, might be rich in secondary metabolites.

In literature, there are few studies on the biological activities of *Helichrysum chionophilum*, which is an endemic species. Tepe et al. (2005) investigated the antioxidant activities of methanol extracts of *H. chionophilum* collected from Kayseri region at different times, whereas Albayrak et al. (2010) analyzed the in vitro antioxidant, radical scavenging, and antimicrobial activities of this plant's methanol extract. In a study carried out recently, the composition of the oil obtained from *H. chionophilum* collected in Kayseri was determined (Kürkçüoğlu et al., 2019). Besides that, in our previous studies, the antioxidant and antimicrobial characteristics of different solvent extracts of this plant collected from Gümüşhane with certain antibiotics were reported, as well as its synergic activity. And, the phenolic profiles, fatty acid compositions and biological activities of *H. chionophilum* were evaluated (Özcan and Acet, 2018b; Acet et al., 2019). The fact that some extracts showed more effective biological activity when compared to the extracts obtained from other regions suggests that

the essential oil of the plant should be investigated. In the present study, antioxidant and antimicrobial activities of essential oil obtained from the endemic plant collected from Gümüşhane region were investigated. In this study, the radical scavenging effect and antimicrobial activity of the oil of this plant are reported for the first time.

2. Material and Method

2.1. Plant Material

H. chionophilum plants were collected from the heights of 2800-2900 m from Artabel/Gümüşhane in August 2018 during the flowering period. The identification of the plant was performed according to "Flora of Turkey and the East Aegean Islands" (Davis et al., 1988). The voucher specimen was prepared (TA1601) and kept at Gümüşhane University.

2.2. Oil Extraction

After drying and powdering the *H. chionophilum*, the essential oil was obtained from 200 g of the sample with the hydro-distillation method by using a Clevenger-type apparatus for 3 hours. Then, the water content of this oil was removed by using Na_2SO_4 and then kept at -20 °C until analysis.

2.3. DPPH·-Scavenging Activity

The radical scavenging activity of the essential oil was determined by using DPPH method (Özcan and Acet, 2018b). The results were expressed as IC_{50}, which refers to the oil concentration scavenging 50% of DPPH radical, and Trolox that is a standard antioxidant.

2.4. Antimicrobial Activity

2.4.1. Test organisms

17 bacteria [8 Gram (-) and 9 Gram (+)] and 2 yeasts were used in antimicrobial activity experiments. The standard strains used are; *Enterococcus faecium* DSMZ 13590, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* RSKK 709, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 29998, *Listeria monocytogenes*,

Klebsiella pneumoniae ATCC 13883, *Listeria innocua* ATCC 33090, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445, *Vibrio parahaemolyticus* ATCC 17802, *Yersinia enterocolitica* ATCC 27729, *Yersinia pseudotuberculosis* ATCC 911, *Proteus vulgaris* ATCC 13315, *Candida albicans* DSMZ 5817, *Candida tropicalis* NRRLL YB-366.

2.4.2. Minimum inhibition concentration (MIC)

The minimum inhibition concentrations of the samples were determined by using the microdilution method (CLSI, 2017) on 96-well microplates. The oil was diluted with DMSO and the obtained serial dilution concentration range (512-1 µg/mL) was used for determining the MIC values. In sum, the samples were added into the wells at predetermined concentrations and the test microorganism solutions (0.5 MacFarland) were inoculated. The microbial growth was determined after the incubation for 48 hours at 37 °C. The MIC value was specified as the lowest oil concentration preventing the microbial growth. The chloramphenicol, novobiocin, nalidixic acid, and nystatin were used as positive controls.

2.4.3. Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration values were determined by taking 10µL from the wells, in which the MIC test was performed, and then inoculating them into fresh agar for 1 day at 37 °C. The concentration, at which no growth was observed, was determined to be MBC. The MBC value was expressed as the lowest oil concentration killing the microorganisms (Özcan and Acet, 2018b).

3. Findings and Discussion

The radical scavenging activity of the oil was determined using DPPH method and expressed as IC_{50} value and Trolox-equivalent (Table 1). DPPH method is one of the stable, easy, fast, and accurate methods used in order to determine the radical scavenging activities of the plants (Ebrahimzadeh et al., 2010; Özcan and Acet, 2022). Although the antioxidant activities of the oil of this plant were examined in the present study for the first time, the radical scavenging activities of oils of different *Helichrysum* species were also investigated by using DPPH method. The IC_{50} value of the oil of *Helichrysum microphyllum* subsp. *tyrrhenicum* collected from Sardinia was reported to be 2.060 mg/mL (Ornano eta l., 2015). Kladar et al. (2015) investigated the radical scavenging activity of both ethanol extract and essential oil of *Helichrysum italicum* plant, which they have collected

from Montenegro, by using DPPH method and they reported the radical scavenging effectiveness (IC_{50}: 1.37 mg/mL) of the oil to be lower than that of ethanol extract (IC_{50}: 0.99 µg/mL). Afoulous et al. (2011) used the DPPH method and reported the radical scavenging efficiency of the essential oil of *Helichrysum gymnocephalum* species to be IC_{50}>126 mg/mL. The radical scavenging activity (IC_{50}: 0.26 mg/mL) of *H. chionophilum* oil, which we analyzed here, seems to be clearly stronger than the oils of similar species. Moreover, the antioxidant activity of the *H. chionophilum* extracts collected from different regions were investigated by the researchers and, by using DPPH method, the antioxidant activity was reported to be IC_{50} values of 40.5 µg/mL (Tepe et al., 2005), 53.10 µg/mL (Albayrak et al., 2010), and 67.44 µg/mL (Özcan and Acet, 2018b).

Table 1. Antioxidant activity of *H. chionophilum* essential oil

Sample	Radical Scavenging activity	
	DPPH (IC_{50} value, µg/mL)	Trolox Equivalent (mg TE/g oil)
Essential oil	260.0±0.2	34.82±0.03
Trolox	9.5±0.01	-

*TE: Trolox equivalent

The antimicrobial activity of the oil on Gram (-) (Table 2), Gram (+) bacteria (Table 3), and yeasts (Table 4) were determined. The MBC values are presented in Table 5. The MIC values against all the microorganisms, which were used for investigating the antimicrobial activity of the oil, were at a remarkable level (16-64 µg/mL) (Tables 2-4). The lowest MIC value (16 µg/mL) was found in *Y. pseudotuberculosis, S. epidermidis,* and *C. albicans*. While the oil showed activity against *Y. enterocolitica, S. epidermidis, S. aureus,* and MRSA similar to the positive control (chloramphenicol), the activity against *C. albicans* was equivalent to the positive control chloramphenicol and nystatin. The results obtained here were found to be similar to or higher than the antimicrobial activity of the *H. chionophilum* extracts reported in the previous study (Özcan and Acet, 2018b). However, although no anticandidal activity was reported for the extracts (Özcan and Acet, 2018b; Albayrak et al., 2010), a high level of anticandidal activity was detected in oil in the present study (Table 4).

When compared to Gram (-) bacteria, Gram (+) bacteria were reported to be more sensitive to the essential oils obtained from the plants (Djihane et al., 2017; Cantore et al., 2004; Kladar et al., 2015). Moreover, it was reported in many studies that the oils have a high level of antifungal activity (González, et al., 2012; Djihane et al., 2017).

Table 2. Antimicrobial activity of *H. chionophilum* essential oil against Gram (-) bacteria

Test organisms	MIC value (µg/mL)			
	Essential oil	Chloramphenicol	Novobiocin	Nalidixic acid
S. typhimurium	64	8	1	256
V. parahaemolyticus	64	2	1	4
P. aeruginosa	64	16	4	4
Y. enterocolitica	64	64	128	32
K. pneumoniae	64	32	1	64
E. coli	32	4	1	256
Y. pseudotuberculosis	16	8	4	128
P. vulgaris	32	16	2	256

Table 3. Antimicrobial activity of *H. chionophilum* essential oil against Gram (+) bacteria

Test organisms	MIC value (µg/mL)			
	Essential oil	Chloramphenicol	Novobiocin	Nalidixic acid
E. hirae	32	8	1	256
B. cereus	32	2	1	4
S. epidermidis	16	16	4	4
S. aureus	64	64	128	32
MRSA	64	32	1	64
E. faecium	32	4	1	256
E. faecalis	64	8	4	128
L. monocytogenes	64	16	2	256
L. innocua	32	32	2	128

Table 4. Antimicrobial activity of *H. chionophilum* essential oil against yeasts

Yeasts	MIC value (µg/mL)	
	Essential oil	Nystatin
C. albicans	16	16
C. tropicalis	32	16

Table 5. MBC value of the essential oil against test microorganisms

Test organisms	MBC value (µg/mL)	Test organisms	MBC value (µg/mL)
S. typhimurium	128	*E. hirae*	64
V. parahaemolyticus	64	*B. cereus*	64
P. aeruginosa	64	*S. epidermidis*	32
Y. enterocolitica	128	*S. aureus*	128
K. pneumoniae	64	*MRSA*	64
E. coli	64	*E. faecium*	64
Y. pseudotuberculosis	16	*E. faecalis*	128
P. vulgaris	64	*L. monocytogenes*	128
C. albicans	32	*L. innocua*	64
C. tropicalis	32	*E. hirae*	64

4. Conclusions and Recommendations

In conclusion, the essential oil components of *H. chionophilum*, which is endemic to Türkiye, were identified and the antioxidant and antimicrobial activities were analyzed. The results clearly revealed that the essential oil of this plant showed significant inhibition activity against various microorganisms (especially *S. epidermidis*, *Y. pseudotuberculosis*, and *C. albicans*). Furthermore, it can be stated that the antioxidant activity of the essential oil was at a higher level when compared to those of extracts. It is thought that the biological activity that was detected might be explained with the presence of some component of the essential oil. In recent years, the awareness of the risks of synthetic additives and medications on the organisms has arisen throughout the world and the interest in natural sources has also increased. For this reason, benefiting from the herbs used in folk medicine is thought as an alternative method. From this aspect, the results obtained in the present study showed that the essential oil of *H. chionophilum*, which is widely used in folk medicine, might be used as a natural agent thanks to its antioxidant and antimicrobial properties. Besides that, it would be beneficial to carry out further analyses on the plants collected at different seasons and from different regions, which have different ecological characteristics, and to determine the most effective season for this plant. Moreover, in order for this plant to be used as a food additive and to be evaluated from the pharmacological aspect, the biological activities should be validated by using further tests in *in-vivo* settings.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

References

Acet, T., and Özcan, K. (2022). *Heracleum pastinacifolium*'nın antioksidan ve antimikrobiyal özellikleri ile fenolik içeriğinin aydınlatılması. *Karadeniz Fen Bilimleri Dergisi*, 12(1), 22-32.

Acet, T., Ozcan, K., and Zengin, G., (2020). An assessment of phenolic profiles, fatty acid compositions, and biological activities of two *Helichrysum* species: *H. plicatum* and *H. chionophilum. Journal of Food Biochemisrty*. 44, e13128.

Afoulous, S., Ferhout, H., Raoelison, E.G., Valentin, A., Moukarzel, B., Couderc, F., and Bouajila, J., (2011). *Helichrysum gymnocephalum* Essential oil: chemical composition and cytotoxic, antimalarial and antioxidant activities, attribution of the activity origin by correlations. *Molecules*, 16(10), 8273-8291.

Akinfenwa, A.O., Sagbo, I.J., Makhaba, M., Mabusela, W.T., and Hussein, A.A., (2022). *Helichrysum* genus and compound activities in the management of diabetes mellitus. *Plants (Basel)*, 23, 11(10), 1386.

Albayrak, S., Aksoy, A., Sağdiç, O., and Budak, Ü., (2010). Phenolic compounds and antioxidant and antimicrobial properties of *Helichrysum* species collected from eastern Anatolia, Turkey. *Turkish Journal of Biology*, 34, 463-473.

Aslan, M., Orhan, D.D., Orhan, N., Sezik, E., and Yesilada, E., (2007). In vivo antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. *plicatum* capitulums in streptozotocin-induced-diabetic rats. *Journal of Ethnopharmacology*, 109(1), 54-59.

Başer, K.H.C., Demirci, B., and Kirimer, N., (2002). Compositions of the essential oils of four Helichrysum species from Madagascar. Journal of Essential Oil Research, 14(1), 53-55.

Baytop, T., (1997). Türkçe bitki adiarı sözlüğü (A dictionary ofvernacular names of wild plants of Turkey) (p. 578). Ankara: Publication of the Turk Dil Kurumu (The Turkish Language Society).

Benelli, G., Pavela, R., Rakotosaona, R., Randrianarivo, E., Nicoletti, M., and Maggi, F., (2018). Chemical composition and insecticidal activity of the essential oil from *Helichrysum faradifani* endemic to Madagascar. *Natural Product Research*, 32(14), 1690-1698.

Bianchini, A., Santoni, F., Paolini, J., Bernardini, A.F., Mouillot, D., and Costa, J., (2009). Partitioning the relative contributions of inorganic plant composition and soil characteristics to the quality of *Helichrysum italicum* subsp. *italicum* (Roth) G. Don fil. essential oil. *Chemistry and Biodiversity*, 6(7), 1014-33.

Cantore, L.P., Iacobellis, S.N., Marco, D.A., Capasso, F., and Senatore, F., (2004). Antibacterial activity of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller var. (MILLER) Essential oil. *Journal of Agricultural and Food Chemistry*, 7862-7866.

Chalchat J.C., and Özcan, M.M., (2006). Composition of the essential oil of *Helichrysum chasmolycicum* growing wild in Turkey. *Journal of Medicinal Food*, 9(2), 287-289.

Chinou, L.B., Bougatsos, C., and Perdetzoglou, D., (2004). Chemical composition and antimicrobial activities of *Helichrysum amorginum* cultivated in Greece. *Journal of Essential Oil Research*, 16(3), 243-245.

Clinical and Laboratory Standards Institute (CLSI), (2017). Performance Standards for Antimicrobial Susceptibility Testing; 27th Informational Supplement. CLSI/NCCLS, 27th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA.

Davis, P.H., Mill R.R. and Tan, K., (1988). Flora of Turkey and the East Aegean Islands. Edinburgh University Press. Edinburgh, UK.

Djihane, B., Wafa, N., Elkhamssa, S., Pedro, D.H.J., Maria, A.E., and Mihoub, Z.M., (2017). Chemical constituents of *Helichrysum italicum* (Roth) G. Don essential oil and their antimicrobial activity against Gram-positive and Gram-negative bacteria, filamentous fungi and *Candida albicans. Saudi Pharmaceutical Journal*, 25, 780-787.

Ebrahimzadeh, M.A., and Tavassoli, A., (2015). Antioxidant properties of *Helichrysum pseudoplicatum* Nab. *Pharmaceutical and Biomedical Research*, 1(1), 37.

Ebrahimzadeh, M.A., Nabavi, S.F., Nabavi, S.M., and Eslami, B., (2010). Antihemolytic and antioxidant activities of *Allium paradoxum*. *Central European Journal of Biology*, 5, 338-345.

El-Olemy, M.M., Al-Rehaily, A.J., Albishi, O.A., Mossa, J.S., Demirci B., and Başer, K.H.C., (2005). Composition of the Essential Oil of *Helichrysum forsskahlii (*Gmel) Hilliard et Burt. *Journal of Essential Oil Research*, 17(1), 112-116.

Furlan, V., and Bren, U., (2023). *Helichrysum italicum*: From extraction, distillation, and encapsulation techniques to beneficial health effects. *Foods*, 12(4), 802.

Giovanelli, S., De Leo, M., Cervelli, C., Ruffoni, B., Ciccarelli, D., and Pistelli, L., (2018). Essential oil composition and volatile profile of seven *Helichrysum* species grown in Italy. *Chemistry and Biodiversity*, 15, e1700545.

Giuliani, C., Lazzaro, L., Calamassi, R., Calamai, L., Romoli, R., Fico, G., Foggi, B., and Lippi, M.M., (2016). A volatolomic approach for studying plant variability: the case of selected *Helichrysum* species (Asteraceae). *Phytochemistry*, 130, 128-143.

González, A.M., Tracanna, M.I., Amani, S.M., Schuff, C., Poch, M.J., Bach, H., and Can, C., (2012). Chemical composition, antimicrobial and antioxidant properties of the volatile oil and methanol extract of *Xenophyllum poposum*. *Natural Product Communications*, 7(12), 1663-1666.

Güner, A., S. Aslan, T. Ekim, M. Vural ve M. T. Babaç, 2012. Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul.

Javidnia, K., Miri, R., Soltani M., and Khosravi, A.R., (2009). Essential oil composition of two Iranian endemic *Helichrysum* Miller. species (*H. leucocephalum* Boiss. and *H. artemisioides* Boiss. et Hausskn.). *Journal of Essential Oil Research*, 21(1), 54-56.

Jokić, S., Rajić, M., Bilić, B., and Molnar, M., (2016). Supercritical extraction of scopoletin from *Helichrysum italicum* (Roth) G. Don flowers. *Phytochemical Analysis*, 27, 290-295.

Judzentiene, A., and Butkiene, R., (2006). Chemical composition of the essential oils of wild *Helichrysum arenarium* (L.) with differently colored inflorescences from Eastern Lithuania. *Journal of Essential Oil Research*, 18(1), 80-83.

Judžentienė, A., Charkova, T., and Misiūnas, A., (2019). Chemical composition of the essential oils from *Helichrysum arenarium* (L.) plants growing in Lithuanian forests. *Journal of Essential Oil Research*, 31(4), 305-311.

Kladar, N.V., Anačkov, G.T., Rat, M.M., Srđenović, B.U., Grujić, N.N., Šefer, E.L., and Božin, B.N., (2015). Biochemical characterization of *Helichrysum italicum* (Roth) G.Don subsp. *italicum* (Asteraceae) from Montenegro: phytochemical screening, chemotaxonomy, and antioxidant properties. *Chemistry and Biodiversity*, 12(3), 419-31.

Kürkçüoğlu, M., Ağalar, H.G., Aksoy A., and Başer, K.H.C., (2019). Composition of the essential oils of two endemic Helichrysum species in Turkey. *Records of Natural Products*, 13(3), 236-242.

Kurti, F., Giorgi, A., Beretta, G., Mustafa, B., Gelmini, F., Testa, C., Angioletti, S., Giupponi, L., Zilio, E., Pentimalli, D., and Hajdari, A., (2019). Chemical composition, antioxidant and antimicrobial activities of essential oils of different *Pinus* species from Kosovo. *Journal of Essential Oil Research*, 31(4), 263-275.

Lourens, A.C.U., Reddy, D., Baser, K.H.C., Viljoen, A.M., and Van Vuuren, S.F., (2004). In vitro biological activity and essential oil composition of four indigenous South African *Helichrysum* Species. *Journal of Ethnopharmacology*, 95, 253-58.

Lourens, A.C.U., Viljoen, A.M., and van Heerden, F.R., (2008). South African *Helichrysum* species: A review of the traditional uses, biological activity and phytochemistry. *Journal of Ethnopharmacology*, 119(3), 630-652.

Ornano, L., Venditti, A., Sanna, C., Ballero, M., Maggi, F., Lupidi, G., Bramucci, M., Quassinti, L., and Bianco, A.C., (2015). Chemical composition and biological activity of the essential oil from *Helichrysum microphyllum* Cambess. ssp. tyrrhenicum Bacch., Brulloe Giusso growing in La Maddalena Archipelago. *Sardinia, Journal of Oleo Science*, 64(1), 19-26.

Özcan, K., and Acet, T., (2018b). Antimicrobial and antioxidant screening, synergy studies of *Helichrysum chionophilum* extracts against to resistant microbial strains. *Fresenius Environmental Bulletin*. 27(7), 5045-5052.

Özcan, K., and Acet, T., (2022). *Stachys macrantha* (K.Koch) Stearn'ın biyolojik aktivitesinin belirlenmesi. *Bitlis Eren Üniversitesi Fen Bilimleri Dergisi*, 11(1), 156-163.

Öztürk, B., Özek, G., Özek, T., and Baser, K.H.C., (2014). Chemical Diversity in Volatiles of *Helichrysum plicatum* DC. Subspecies in Turkey. *Records of Natural Products*, 8(4), 373-384.

Ralijerson, L.B., Rabehaja, D.J.R., Rajaonarison, J.F., Urverg, S.R., Hérent, M.F. Mavar-Manga, H., and Tilquin, B., (2005). Comparison between the fresh and dry essential oil of *Helichrysum faradifani* Scott Elliot from Madagascar. *Journal of Essential Oil Research*, 17(6), 597-600.

Rančić A., Soković, M., Vukojević, J., Simić, A., Marin, P., Duletić-Laušević, S., and Djoković, D., (2005). Chemical composition and antimicrobial activities of essential oils of *Myrrhis odorata* (L.) Scop, *Hypericum perforatum* L and *Helichrysum arenarium* (L.) Moench. *Journal of Essential Oil Research*, 17(3), 341-345.

Rossi, P.G., Berti, L., Panighi, J., Luciani, A., Maury, J., Muselli, A., De Rocca Serra, D., Gonny, M., and Bolla, J.M., (2007). Antibacterial action of essential oils from Corsica. *Journal of Essential Oil Research*, 19, 176-182.

Roussis, V., Tsoukatou, M., Chinou L.B., and Harvala, C., (2002). Composition and antibacterial activity of the essential oils of two *Helichrysum stoechas* varieties growing in the Island of Crete. *Journal of Essential Oil Research*, 14(6), 459-461.

Süzgeç-Selçuk, S., and Birteksöz, A.S., (2011). Flavonoids of *Helichrysum chasmolycicum* and its antioxidant and antimicrobial activities. *South African Journal of Botany*, 77(1), 170-174.

Tepe, B., Sokmen, M., Akpulat H.A., and Sokmen, A., (2005). In vitro antioxidant activities of the methanol extracts of four *Helichrysum* species from Turkey. *Food Chemistry*, 90(4), 685-689.

van Vuuren, S.F., Viljoen, A.M., van Zyl, R.L., van Heerden, F.R., and Baser, K.H.C., (2006). The antimicrobial, antimalarial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don subsp. *cymosum*. *South African Journal of Botany*, 72, 287-290.

Viegas, D.A., Palmeira-de-Oliveira, A., Salgueiro, L., Martinez-de-Oliveira, J., and Palmeira-de-Oliveira, R., (2014). *Helichrysum italicum*: From traditional use to scientific data. *Journal of Ethnopharmacology*, 151(1), 54-65.

Yeşilada, E., Honda, G., Sezik, E., Tabata, M., Fujita, T., Tanaka, T., Takeda, Y., and Takaishi, Y., (1995).Traditional medicine in Turkey. V. Folk medicine in the inner Taurus Mountains. *Journal of Ethnopharmacology*, 46(3), 133-152.