© by PSP Volume 30– No. 02/2021 pages 869-875 Fresenius Environmental Bulletin

BIOCHEMICAL COMPOSITION AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF ENDEMIC *HELICHRYSUM ARENARIUM* SSP. *AUCHERI* ETHANOL EXTRACT

Mustafa Eray Bozyel[1,*],Kerem Canli[2], Atakan Benek[3], Ali Yetgin[4], Ergin Murat Altuner[3]

[1]Department of Biology, Faculty of Arts and Science, Canakkale Onsekiz Mart University, Canakkale, Turkey
[2]Department of Biology, Faculty of Science, Dokuz Eylul University, Buca, Izmir, Turkey
[3]Department of Biology, Faculty of Science and Arts, Kastamonu University, Kastamonu, Turkey
[4]Toros Tarım A.S., Mersin, Turkey

ABSTRACT

The phenomenon of using plants for therapeutic purposes dates back to the earlier times in the history of humanity. Even though there have been remarkable developments in the modern medicine, pharmaceutical, and chemical industries, the therapeutic use of medicinal plants is still up to date. Due to the emergence of antibiotic-resistant strains and the proliferation of side effects in synthetic drugs, the importance of research on the antimicrobial potential and biochemical composition of plant extracts has increased. The main purpose of this present study is to screen both the antibacterial and antifungal activity of endemic *Helichrysum arenarium* ssp. *aucheri* ethanol extract by disk diffusion on some Gram-negative and Gram-positive bacteria and *Candida albicans*, and to identify the major compounds found in the ethanol extract by GC/MS. According to the results obtained from the study, it can be proposed that the extract showed antibacterial and antifungal activity for all microorganisms excluding *Salmonella typhimurium* SL1344, *Salmonella infantis*, *E. coli* (food isolate), *Escherichia coli* ATCC 25922 and *Enterobacter aerogenes* ATCC 13048, and some chemical compounds were detected, which may be responsible for the antimicrobial activity.

KEYWORDS:
Helichrysum arenarium ssp. *aucheri*, ethanol extract, GC/MS, biochemical composition, disk diffusion method, antimicrobial activity.

INTRODUCTION

The belief in the ability of plants to cure diseases extends almost to the period in which man exists. Throughout human history, different forms of plants have been used as a poultice for the treatment of wounds, as a decoction for the treatment of diseases or as direct food. There is countless historical evidence that plants are used on all continents around the world. Even the Neanderthals, who lived sixty thousand years ago, were discovered to use the Hollyhock (*Alcea* sp.) plant in the Iraqi region. One of the oldest references for modern pharmacology was the book of Hippocrates, which contains 300-400 different medicinal plants in the late 5th century BC, and the other was Dioscorides' De Materia Medica in the first century AD [1]. Nowadays, these plants and new species are used in traditional treatments. It is estimated that 10% of all plants are used in traditional medicine in the world. Scientific curiosity about the plants used in traditional treatments all over the world and their extracts, components, and effects has also led to the emergence of ethnopharmacology [2]. In the world, approximately half of the deaths in tropical countries are caused by infections. In Africa, 300,000 children die each year from infections caused by microorganisms related to *E. coli*, *Shigella*, and *Salmonella* species. Perhaps this is not surprising when looking at the socio-economic situation of the countries, but even in developed countries, infection-related diseases and deaths are observed to be increased every day. For example, research completed in the US proposed that the 5th reason for deaths, which were caused as a reason for infections in 1981, with a 58% increase in 1992 climbed to 3rd place [3, 4]. Thus, developing novel strategies to prevent and treat infectious diseases are required. Therefore, it is quite natural for pharmacologists and especially microbiologists to resort to plants in search of antimicrobial agents. Over time, microorganisms gain resistance to drugs and transfer them to new generations. This limits the lifetime of the drugs and makes antimicrobial need constant. Thus, plants are always inevitable for clinical microbiologists in their search for new antimicrobials. Also, based on the advantage that the plants have been experienced throughout history, the information obtained about the plant contents and their uses are scientifically researched in the laboratories. As a result, the effects of the plants used on microorganisms will be revealed. Thus, the incorrect use of the public will be prevented [1].

The genus *Helichrysum* (Asteraceae) is presented by 600 species all over the world. *Helichrysum* species are reported to have a high degree of

polymorphism. It has been reported that some of them show significant pharmacological properties and find wide use in perfumery [5, 6]. It is represented in Turkish flora by 30 taxa, of which 17 are endemic [7].

Some of the *Helichrysum* taxa have traditionally been used in folk medicine in their geography for centuries. *Helichrysum* teas are known to be used against gallbladder disorder since they have bile regulating and diuretic effects [8]. The genus *Helichrysum* is used to treat several health problems in Europe and Africa [9-13]. *Helichrysum* species have traditionally been used in the treatment of infections, wounds, and respiratory disorders [10, 11, 13, 14, 15]. It has also been reported that *Helichrysum* species are used as incense and in the treatment of colds [13].

Many species of *Helichrysum* distributed in Turkey is used as a diuretic and an antiurolithiatic by the public. The bile regulation and diuretic activities of these species are as a result of their flavonoid content [8, 16]. *H. arenarium* (L.) Moench, called dwarf everlast, is a well-known remedial plant in most parts of the world. For example, *H. arenarium* is commonly consumed as herbal tea against asthma, diarrhea, jaundice, stomach pain, urogenital disorders and to treat kidney stone problems [17, 18]. It is known that a group of flavonoids isolated from *H. arenarium* inflorescence have diuretic properties and are used in kidney and urinary tract disorders. The diuretic and choleretic properties of this plant extract have been confirmed by pharmacological investigations [19]. Besides, *H. arenarium* was identified by Czinner et al. [9, 20] as having cholagogue and choleretic, hepatoprotective, and detoxifying activities. *H. arenarium* ssp. *aucheri* (Boiss.) P.H.Davis & Kupicha subspecies is endemic to Turkey. It is a perennial herbaceous plant. It blossoms between May and August. It is an element of the Irano-Turanian floristic region. It is spread over terrestrial Anatolia and neighboring northern Anatolia. It is an aromatic and medicinal plant, which is commonly growing on sandy soils or dry lime, coasts, steppes, and distributed at an altitude between 250 and 3200 meters [21]. Previous studies are present in the literature about the antimicrobial activity of *H. arenarium* against yeasts, Gram-negative and Gram-positive bacteria strains [22, 23], however to the best of our knowledge the antibacterial and antifungal potential of *H. arenarium* ssp. *aucheri* ethanol extract hasn't been analyzed yet.

The medicinal use of plants is a tradition that has continued for centuries in Anatolia. These uses, which have survived to the present day, should be continued traditionally and the structures and medicinal properties of plants should be revealed using modern techniques. In recent years, the importance given to medicinal plants and studies examining their antimicrobial activities has increased in Turkey [24, 25, 26, 27, 28, 29, 30, 31, 32].

In the present study, we report on the antibacterial activity of *H. arenarium* ssp. *aucheri* ethanol extract by disk diffusion method against several Gram-negative and Gram-positive bacteria strains and *Candida albicans* and its biochemical composition by Gas Chromatography-Mass Spectrometry.

MATERIALS AND METHODS

Endemic plant samples. *H. arenarium* ssp. *aucheri* were collected from Kayseri/TURKEY and identified by Dr. Mustafa Eray BOZYEL. The plant samples were placed in sample bags and kept in room conditions.

Active compound extraction. Dried *H. arenarium* ssp. *aucheri* aerial part samples were ground to obtain a fine powder, to increase the surface area for extraction. The active compounds were extracted by ethanol (Sigma Aldrich) through shaking at room temperature for two days [33]. After filtering through Whatman No. 1 filter paper, the ethanol in the extract was evaporated at 45°C under vacuum by using a rotary evaporator (Heidolph Hei-Vap Value HL/HB-G1) [34]. The remnant was weighed and an extract stock was prepared by using a defined volume of ethanol, and 50 µL and 100 µL of the extracted stock were transferred on empty sterile antibiotic disks to load 2.72 and 5.45 mg extracts on disks respectively.

Microorganisms. *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans* (food isolate), *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* (food isolate), *Escherichia coli* ATCC 25922, *Escherichia coli* (food isolate), *Klebsiella pneumoniae* (food isolate), *Listeria innocua* (food isolate), *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescence* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis* (food isolate), *Salmonella kentucky* (food isolate), *Salmonella typhimurium* SL1344, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (clinical isolate) and *Staphylococcus epidermidis* DSMZ 20044 were used in the study [35].

Inocula preparation. The incubation conditions for microorganisms excluding *C. albicans* were 37°C for 24 hours, but 27°C for 48 hours for *C. albicans*. Inoculum for each microorganism was prepared in 0.9% sterile saline solution and the turbidity of all inocula was adjusted by comparing with 0.5 McFarland standard [36].

Testing antimicrobial activity. The disk diffusion test, which was described previously in detail by Canli et al. was used for testing the antimicrobial

activity of *H. arenarium* ssp. *aucheri* ethanol extract. The Petri dishes containing disks, on which the ethanol extract was loaded, incubated according to the suitable time-temperature combinations mentioned above, and the inhibition zones were observed and recorded in millimeters [37].

Negative control. Empty antibiotic disk and ethanol loaded disk were used as negative controls.

GC-MS Analysis. The composition of *H. arenarium* ssp. *aucheri* ethanol extract was determined according to the protocol given in previous studies by GC-MS analysis [38].

Statistics. All tests were applied as triplicates. One-way analysis of variance (ANOVA), which is a parametric method was performed (P = 0.05) [39]. Pearson correlation coefficient was determined for any possible correlation between the intensity of antimicrobial activity and concentration. R Studio, version 3.3.2 was used for statistical analysis [40].

RESULTS AND DISCUSSION

Antimicrobial activity. The data obtained from the study about the inhibition zone diameters are shown in Table 1. According to the results, negative controls show no activity [41]. Additionally, statistical analysis verified that the differences between the results of three replicates of each extract volume were statistically non-significant (p>0.05). Also, obtaining a Pearson correlation coefficient of 0.2913 presented a very weak positive correlation between the antimicrobial activity and the volumes of extracts used.

Table 1 clearly shows that 50 µL ethanol extract of *H. arenarium* ssp. *aucheri* was presented antimicrobial activity against *B. subtilis*, *C. albicans*, *E. durans*, *E. faecalis*, *E. faecium*, *K. pneumoniae*, *L. monocytogenes*, *P. fluorescens*, *S. aureus* (clinical isolate), *S. aureus*, *S. epidermidis* and *S. enteritidis* with inhibition zones between 7 and 20 mm. 100 µL ethanol extract of *H. arenarium* ssp. *aucheri* was presented antimicrobial activity against all microorganisms observed in 50 µL besides with *L. innocua*, *P. aeruginosa*, and *S. kentucky* with inhibition zones ranging between 7 and 30 mm.

Previously, *E. faecium* was thought to be a normal member of the gastrointestinal microflora, but *E. faecium* has become one of the main pathogens of nosocomial infections due to its resistance to a great number of antibiotics. Moreover, the treatment of these infections is quite difficult [42]. Canli et al. [43] showed that *Rheum rhabarbarum* (Polygonaceae) caused 28 mm of inhibition zones for 100 µL ethanol extract against *E. faecium* (food isolate), whereas Ozcan and Acet [44] presented that 200 µg ethanol extract of *Helichrysum chionophilum* (Asteraceae) showed an inhibition zone of 15 mm against *E. faecium* (clinical isolate). The results of our study presented 30 mm of inhibition zones for 100 µL ethanol extract, which contains 5.45 mg extract, against *E. faecium* (food isolate).

S. aureus is one of the microorganisms that cause nosocomial infections commonly seen in intensive care units in hospitals [45]. Several researchers have been conducting various investigations to identify certain plant extracts that exhibit antimicrobial activity on *S. aureus* strains. Canlı et al. [46] observed 21 mm of inhibition zones for 120 µL ethanol extract of *Anacyclus pyrethrum* (Asteraceae) against *S. aureus* ATCC 25923, whereas Albayrak et al. [23] identified that *H. arenarium* ssp. *aucheri* (Asteraceae) presented 21 mm of inhibition zone for 10% methanolic extract against *S. aureus* ATCC 29213. In this study, it was observed that different concentrations of *H. arenarium* ssp. *aucheri* methanolic extract showed no antimicrobial effect against *S. aureus* ATCC 25923 [23]. The reason for this difference can be explained by the use of two different *S. aureus* strains in these studies, namely *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923. Previous it was proved that *S. aureus* ATCC 25923 presents higher resistance against the standard antibiotics than *S. aureus* ATCC 29213. A report about the antimicrobial resistance of Enterococci, Staphylococci and *E. coli* proposed that the MIC range of *S. aureus* ATCC 25923 against 11 standard antimicrobials (Cefoxitin, Chloramphenicol, Ciprofloxacin, Erythromycin, Florfenicol, Gentamicin, Penicillin, Streptomycin, Sulfisoxazole, Tetracycline, and Trimethoprim) was between 14 and 37 µg/mL, where the MIC range of *S. aureus* ATCC 29213 against the same antimicrobials are mostly between 0.125 and 8 µg/mL, which shows that *S. aureus* ATCC 25923 presents higher resistance against the standard antibiotics than *S. aureus* ATCC 29213 [47]. On the other hand, the results of our study presented 22 mm of inhibition zones for 100 µL ethanol extract, which contains 5.45 mg extract, against *S. aureus* (clinical isolate).

Also, an inhibition zone over 22 mm for *E. faecium* against Quinupristin-dalfopristin, which is a combination of two antibiotics used to treat Staphylococci infections, is accepted to be susceptible [48]. Our extract presented a 30 mm inhibition zone for 100 µL extract, which clearly shows that the *E. faecium* strain used in our study is susceptible to 100 µL extract.

Besides, an inhibition zone over 22 mm, 21 mm, and 20 mm for *S. aureus* against Cefoxitin/Levofloxacin, Ciprofloxacin, and Ceftaroline/Ofloxacin respectively are accepted to be susceptible [48]. 100 µL of our extract presented a 22 mm inhibition zone against *S. aureus* (clinical isolate) and a 20 mm inhibition zone against *S. aureus*

TABLE 1
Antimicrobial activity results for *H. arenarium* ssp. *aucheri*
(As the diameter of inhibition zones (mm))

Microorganisms	50 µL*	100 µL*
B. subtilis	13,00 ± 0,00	15,00 ± 0,00
C. albicans	10,00 ± 0,00	13,00 ± 0,71
E. aerogenes	-	-
E. coli (food isolate)	-	-
E. coli	-	-
E. durans	12,00 ± 0,00	15,00 ± 0,71
E. faecalis	11,00 ± 0,71	14,00 ± 0,00
E. faecium	20,00 ± 0,71	30,00 ± 0,00
K. pneumoniae	7,00 ± 0,00	7,00 ± 0,00
L. innocua	-	13,00 ± 0,00
L. monocytogenes	16,00 ± 0,00	20,00 ± 0,00
P. aeruginosa	-	12,00 ± 0,71
P. fluorescens	8,00 ± 0,00	14,00 ± 0,00
S. aureus (clinical isolate)	8,00 ± 0,00	22,00 ± 0,00
S. aureus	15,00 ± 0,71	20,00 ± 0,00
S. enteritidis	7,00 ± 0,00	8,00 ± 0,00
S. epidermidis	16,00 ± 0,00	19,00 ± 0,00
S. infantis	-	-
S. kentucky	-	7,00 ± 0,00
S. typhimurium	-	-

"-": No inhibition; *: The data is given as the mean values of three replicates with standard errors.

TABLE 2
The major chemical components of *H. arenarium* ssp. *aucheri* according to the GC-MS analysis

No	Retention Time	Components	Formula	Molecular Weight (g/mol)	Area (%)
1	13.059	Butanoic acid, 2-methyl-3-oxo-, ethyl ester	$C_7H_{12}O_3$	144.168	1.41
2	31.239	D-Allose	$C_6H_{12}O_6$	180.156	4.12
3	35.027	Quinic acid	$C_7H_{12}O_6$	192.167	2.72
4	48.005	Palmitic acid	$C_{16}H_{32}O_2$	256.424	4.57
5	54.213	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.445	2.85
6	54.410	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.430	1.71
7	57.284	UNKNOWN	-	-	1.62
8	57.783	Coumarin-6-ol, 3,4-dihydro-4,4,5,7-tetramethyl-, methylsulfate(ester)	$C_{14}H_{18}O_5S$	298.355	1.35
9	59.855	UNKNOWN	-	-	1.84
10	62.730	UNKNOWN	-	-	6.27
11	63.293	UNKNOWN	-	-	1.03
12	64.743	EUPULONE			3.16
13	65.210	Dotriacontane	$C_{32}H_{66}$	450.866	5.29
14	67.118	UNKNOWN	-	-	1.09
15	68.926	UNKNOWN	-	-	1.05
16	70.418	Tetratetracontane	$C_{44}H_{90}$	619.185	2.76
17	72.462	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.583	1.19
18	74.495	Naringenin	$C_{15}H_{12}O_5$	272.253	22.81
19	75.567	Tetrapentacontane	$C_{44}H_{90}$	619.185	6.51
20	82.903	Tetrapentacontane	$C_{44}H_{90}$	619.185	6.03
21	89.193	Stigmasterol	$C_{29}H_{48}O$	412.691	1.26
22	91.393	Stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414.707	2.54

"-": No information

ATCC 25923, which also show that both *S. aureus* strains used in our study can be accepted to be susceptible against 100 µL of *H. arenarium* ssp. *aucheri* ethanol extract.

Biochemical composition of ethanol extract. The GC-MS analysis of *H. arenarium* ssp. *aucheri* ethanol extract with its major components, which were observed higher than 1%, and their composition percentages are given in Table 2. The GC-MS chromatogram of *H. arenarium* ssp. *aucheri* ethanol extract is given in Figure 1.

According to Table 2, Naringenin (22.81%), Tetrapentacontane (12.54%), and Dotriacontane (5.29%) are observed mainly in *H. arenarium* ssp. *aucheri* ethanol extract composition.

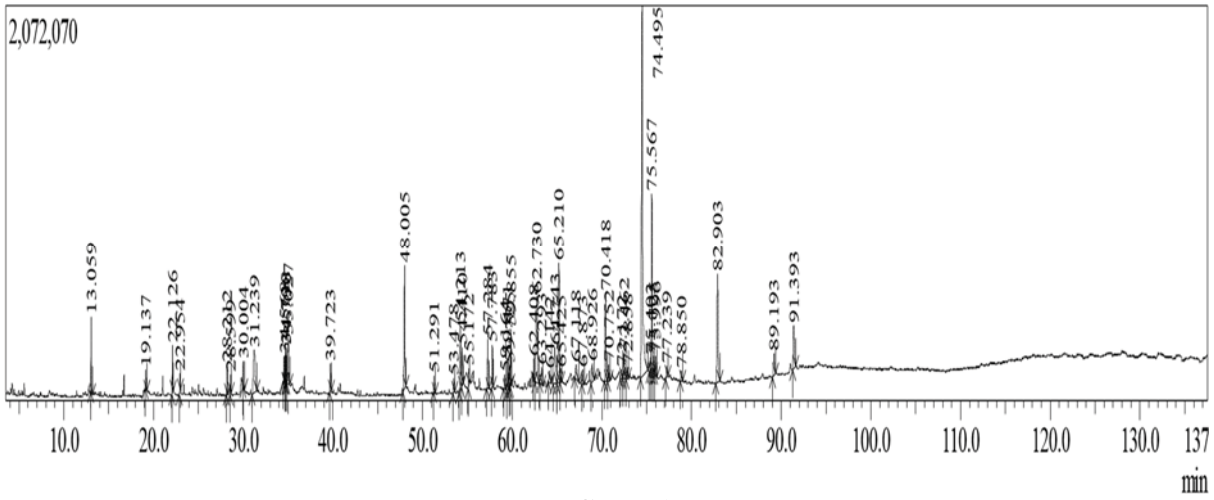

FIGURE 1
GC-MS chromatogram of *H. arenarium* ssp. *aucheri*

CONCLUSIONS

H. arenarium ssp. *aucheri* has antimicrobial activity against a large range of tested strains. Some compounds found in the extract is not matching with the library. As a reason for that, this medicinal plant is proposed to contain some unknown molecules and they should be identified and their 3D structure should also be determined. The unknown compound, which consists of 6.27% should be analyzed in detail. Also, the mode of action(s) of the extract should be determined in further studies.

ACKNOWLEDGEMENTS

Disclosure. An earlier version of this study was presented as an abstract in the Second Japan-Turkey International Symposium on Pharmaceutical and Biomedical Sciences, 2017.

Conflicts Of Interest. The authors declare that they have no conflicts of interest.

REFERENCES

[1] Cowan, M.M. (1999) Plant Products as Antimicrobial Agent. Clin. Microbiol. Rev. 12(4), 564-582.

[2] Raza, M. (2006) A Role for Physicians in Ethnopharmacology and Drug Discovery. Journal of Ethnopharmacology. 104, 297-301.

[3] Karou, D., Nadembega, W.M.C., Ouattara, L., Ilboudo, D.P, Canini, A., Nikiema, J.B., Simpore, J., Colizzi, V. and Traore, A.S. (2007) African Ethnopharmacology and New Drug Discovery. Medicinal and Aromatic Plant Science and Biotech. 1(1), 61-69.

[4] Iwu, M.M., Duncan, A.R. and Okunji, C.O. (1999) New Antimicrobials of Plant Origin. J.Janick (ed.), ASHS Press, Alexandria, VA., 457-462.

[5] Bianchini, A., Tomi, P., Costa, J., and Bernardini, A.F. (2001) Composition of Helichrysum italicum (Roth.) G. Don fil. subsp. italicum Essential Oils from Corsica (France). Flavour and Fragnance Journal. 16, 30-34.

[6] Elkıran, Ö. (2012). The Biosystematics of Some Taxa Belongs to Genus Helichrysum Mill., Fırat University Graduate School Of Science, Elazığ.

[7] Güner, A., Aslan, S., Ekim, T., Vural, M. and Babaç, M.T. (2012) Turkey Plant List (Vascular Plants)]. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul.

[8] Süzgeç, S., Meriçli, A.H., Houghton, P.J. and Çubukçu, B. (2005). Flavonoids of Helichrysum compactum and Their Antioxidant and Antibacterial Activity. Fitoterapia. 76(2), 269-272.

[9] Czinner, E., Hagymasi, K., Blazovics, A., Kery, A., Szőke, É. and Lemberkovics, E. (2000). In Vitro Antioxidant Properties of Helichrysum arenarium (L.) Moench. Journal of Ethnopharmacology. 73(3), 437-443.

[10] Watt, J.M. and Breyer-Brandwijk, M.G. (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa: Being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. 2nd Ed., E & S Livingstone, Edinburg.

[11] Hutchings, A. (1996). Zulu Medicinal Plants: An Inventory. University of Natal Press, Kwa-Zulu-Natal.

[12] Hutchings, A. and Van Staden, J. (1994) Plants Used for Stress-Related Ailments in Traditional Zulu, Xhosa and Sotho Medicine, Part 1: Plants Used for Headaches. Journal of Ethnopharmacology. 43, 89-124.

[13] Van Wyk, B.E., Van Oudtshoorn, B. and Gericke, N. (1997) Medicinal Plants of South Africa. Briza Publications, Pretoria.
[14] Meyer, J.J.M. and Dilika, F. (1996) Antibacterial Activity of Helichrysum pedunculatum Used in Circumcision Rites. Journal of Ethnopharmacology. 53, 51-54.
[15] Stafford, G.I., Jäger, A.K. and Van Staden, J. (2005) Effect of Storage on the Chemical Composition and Biological Activity of Several Popular South African Medicinal Plants. Journal of Ethnopharmacology. 97, 107-115.
[16] Bozyel, M.E. and Merdamert, E. (2018) Antiurolithiatic Activity of Medicinal Plants in Turkey. In: Science, Ecology and Engineering Research in the Globalizing World. St. Kliment Ohridski University Press, Sofia, 152-167.
[17] Baytop, T. (1997) A Dictionary of Vernacular Names of Wild Plants of Turkey. Türk Dil Kurumu Yayınları, Ankara.
[18] Tabata, M., Honda, G. and Sezik, E. (1998) A Report on Traditional Medicine and Medicinal Plants in Turkey. Kyoto University Faculty of Pharmaceutical Sciences, Kyoto.
[19] Szadowska, A. (1962) Pharmacology of Galenic Preparations and Flavonoids from Helichrysum arenarium. Acta Poloniae Pharmaceutica. 19, 465-479.
[20] Czinner, E., Hagymasi, K., Blazovics, A., Kery, A., Szőke, É. and Lemberkovics, E. (2001) The In Vitro Effect of Helichrysi flos on Microsomal Lipid Peroxidation. Journal of Ethnopharmacology. 77(1), 31-35.
[21] Davis, P.H. (1975). Flora of Turkey and the East Aegean Islands, Vol. 5, Edinburgh Univ. Pres, Edinburgh.
[22] Albayrak, S., Aksoy, A., Sagdic, O. and Budak, U. (2010) Phenolic Compounds, Antioxidant and Antimicrobial Properties of Helichrysum species Collected from Eastern Anatolia, Turkey. Turk J Biol. 34, 463-473.
[23] Albayrak, S., Aksoy, A., Sagdic, O. and Hamzaoglu, E. (2010) Compositions, Antioxidant and Antimicrobial Activities of Helichrysum (Asteraceae) Species Collected from Turkey. Food Chem. 119, 114-122.
[24] Yetgin, A., Canlı, K. and Altuner, E.M. (2018) Comparison of Antimicrobial Activity of Allium sativum Cloves from China and Taşköprü, Turkey. Advances in Pharmacological Sciences. 2018.
[25] Canlı, K., Yetgin, A. and Altuner, E.M. (2017) Antimicrobial Activity Screening of Allium tuncelianum. European Journal of Biomedical and Pharmaceutical Sciences. 4(4), 33-36.
[26] Canlı, K., Yetgin, A., Şimşek, Ö. and Altuner, E.M. (2017). Antimicrobial Screening of Alchemilla vulgaris Herbs and Flowers. International Journal of Pharmaceutical Science Invention. 6(2), 13-16.
[27] Canlı, K., Yetgin, A., Akata, I. and Altuner, E.M. (2016) In Vitro Antimicrobial Screening of Aquilaria agallocha Roots. African Journal of Traditional, Complementary and Alternative Medicines. 13(5), 178-181.
[28] Canli, K., Yetgin, A., Akata, I. and Altuner, E.M. (2016) In Vitro Antimicrobial Activity of Angelica sylvestris Roots. International Journal of Biological Sciences. 1(1), 1-7.
[29] Ataslar, E., Guloren, O.T., Filik-Iscen, C. and Ilhan, S. (2019) In Vitro Antimicrobial and Antioxidant Activities of Four Gypsophila L. Species Plant Extracts. Fresen. Environ. Bull. 28, 1841-1851.
[30] Iscan, G.S., Acikara, O.B., Eryilmaz, M., Ozbilgin, S., Ergenel, B. and Yildiz, H.Y. (2019) Phytochemical Analysis of Beach-Cast Seaweed, Zostera spp. Collected from Izmir Coasts, Turkey and Evaluation of In Vitro Antimicrobial Activity. Fresen. Environ. Bull. 28, 3333-3341.
[31] Yildirim, M.U. (2019) In Vitro Plant Regeneration and Antibacterial Activities of Gentiana lutea–An Endangered Medicinal Plant Species–. Fresen. Environ. Bull. 28(7), 5070-5074.
[32] Sevindik, E., Aydin, S., Apaydin, E., Okan, K. and Efe, F. (2019) Antibacterial and Antifungal Activities of Essential Oils from Laurus nobilis L. Flowers and Leaves Grown in the West Anatolian Area. Fresen. Environ. Bull. 28, 6555-6559.
[33] Altuner, E.M. and Canlı, K. (2012) In Vitro Antimicrobial Screening of Hypnum andoi A.J.E. Sm. Kastamonu Üniversitesi Orman Fakültesi Dergisi. 12(1), 97-101.
[34] Altuner, E.M., Canli, K. and Akata, I. (2014) Antimicrobial Screening of Calliergonella cuspidata, Dicranum polysetum and Hypnum cupressiforme. Journal of Pure and Applied Microbiology. 8(1), 539-545.
[35] Canli, K., Simsek, O., Yetgin, A. and Altuner, E.M. (2017) Determination of the chemical composition and antimicrobial activity of Frankenia hirsuta. Bangladesh Journal of Pharmacology. 12(4), 463-469.
[36] Canli, K., Akata, I. and Altuner, E.M. (2016) In vitro Antimicrobial Activity Screening of Xylaria hypoxylon. African Journal of Traditional, Complementary and Alternative Medicines. 13(4), 42-46.
[37] Canli, K., Bozyel, M.E. and Altuner, E.M. (2017) In vitro antimicrobial activity screening of Maclura pomifera fruits against wide range of microorganisms. International Journal of Pharmaceutical Sciences Invention. 6(8), 19-22.

[38] Canlı, K., Yetgin, A., Benek, A., Bozyel, M.E. and Altuner, E.M. (2019) In Vitro Antimicrobial Activity Screening of Ethanol Extract of Lavandula stoechas and Investigation of Its Biochemical Composition. Advances in Pharmacological Sciences, 2019.
[39] Canli, K., Yetgin, A., Akata, I. and Altuner, E.M. (2017) Antimicrobial Activity and Chemical Composition Screening of Epilobium montanum Root. Indian Journal of Pharmaceutical Education and Research. 51(3s), 239-243.
[40] Core R Team (2016). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
[41] Bozyel, M.E., Şenturan, M., Benek, A., Merdamert Bozyel, E., Canli, K. and Altuner, E.M. (2019) In Vitro Antimicrobial Activity Screening of Heliotropium europaeum Against Wide Range of Microorganisms and Multi Drug Resistant (Mdr) Bacteria. European Journal of Biomedical and Pharmaceutical Sciences. 6(3), 113-117.
[42] Willems, R.J. and Van Schaik, W. (2009) Transition of Enterococcus faecium from Commensal Organism to Nosocomial Pathogen. Future Microbiology. 4(9), 1125-1135.
[43] Canli, K., Yetgin, A., Akata, I. and Altuner, E.M. (2016) In vitro Antimicrobial Activity Screening of Rheum rhabarbarum Roots. International Journal of Pharmaceutical Sciences Invention. 5(2), 1-4.
[44] Ozcan, K. and Acet, T. (2018) Antimicrobial and Antioxidant Screening, Synergy Studies of Helichrysum chionophilum Extracts Against to Resistant Microbial Strains. Fresen. Environ. Bull. 27, 5045-5052.
[45] Richards, M.J., Edwards, J.R., Culver, D.H. and Gaynes, R.P. (1999) Nosocomial Infections in Medical Intensive Care Units in the United States: National Nosocomial Infections Surveillance System. Crit Care Med. 27, 887-892.
[46] Canli, K., Yetgin, A., Akata, I. and Altuner, E.M. (2017) Antimicrobial Activity and Chemical Composition Screening of Anacyclus pyrethrum Root. Indian Journal of Pharmaceutical Education and Research. 51(3s), 244-248.
[47] Bortolaia, V., Hendriksen, R., Karlsmose, S. and Aarestrup, F. (2012) The 10th EURL-AR Proficiency Test Enterococci, Staphylococci and E. coli 2011.: European Union Reference Laboratory–Antimicrobial Resistance.
[48] Eucast (2019). European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. Version 9, 1-100.

Received **12.12.2019**
Accepted **28.10.2020**

CORRESONDING AUTHOR

Mustafa Eray Bozyel
Department of Biology,
Faculty of Arts and Science,
Canakkale Onsekiz Mart University,
Canakkale – Turkey

e-mail: m.eraybozyel@gmail.com