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Antimicrobial and Antioxidant Activities of Different Extracts of *Helichrysum arenarium* subsp. (L.) Moench *aucheri*

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Abstract: *Helichrysum arenarium* (L.) Moench subsp. *aucheri* is a herbaceous perennial herb belonging to the Asteraceae. This plant has biological activities such as antibacterial, antiviral, anti-inflammatory, antifungal, antiproliferative, antioxidant, and antiradical. In this study, antimicrobial and antioxidant activities of methanol and ethanol extracts of aerial parts of *H. arenarium* subsp. *aucheri* were investigated. To determine the antimicrobial activity pathogenic microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus megaterium*, *Candida glabrata*, *Candida albicans* and *Trichophyton* sp. Antioxidant activity was determined with total antioxidant value (TAS), total oxidant value (TOS) and 2.2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging capacity. In the results obtained, it was determined that the methanol extract had an antimicrobial effect (9.3 mm) only against *C. albicans*. It was found that the ethanol extract showed antimicrobial activity at different rates (8.8-20.4 mm) against *S. aureus*, *B. megaterium*, *C. glabrata*, *C. albicans* and *Trichophyton* sp. The TAS value of the methanol extract was 3.00 mmol, and the TAS value of the ethanol extract was 3.15 mmol. The TOS value of the methanol extract of the same species was calculated as 6.81 μmol, and the TOS value of the ethanol extract was calculated as 12.64 μmol. The DPPH radical scavenging effects of extracts of goldengrass was found to increase depend on concentrations.

Key words: *Helichrysum arenarium* subsp. *aucheri*, goldengrass, antimicrobial, antioxidant.

Helichrysum arenarium subsp. (L.) Moench *aucheri*'nin Farklı Ekstraktlarının Antimikrobiyal ve Antioksidan Aktivitesi

Öz: *Helichrysum arenarium* (L.) Moench subsp. *aucheri*, Asteraceae ait otsu çok yıllık bir bitkidir. Bu bitki, antibakteriyel, antiviral, antiinflamatuar, antifungal, antiproliferatif, antioksidan, antiradikal gibi biyolojik aktivitelere sahiptir. Bu çalışmada, *H. arenarium* subsp. *aucheri*'nin toprak üstü kısımlarının metanol ve etanol ekstraktlarının antimikrobiyal ve atioksidan aktiviteleri araştırılmıştır. Antimikrobiyal aktivitenin belirlenebilmesi için *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus megaterium*, *Candida glabrata*, *Candida albicans*, *Trichophyton* sp. patojenik mikroorganizmalar kullanılmıştır. Antioksidan aktivite toplam antioksidan değeri (TAS), toplam oksidan değeri (TOS) ve 2.2-diphenyl-1-picrilhydrazyl (DPPH) radikal süpürme kapasitesi ile belirlenmiştir. Elde edilen sonuçlarda metanol ekstresinin sadece *C. albicans*'a karşı antimikrobiyal etkisinin (9.3 mm) olduğu tespit edilmiştir. Etanol ekstresinin ise *S. aureus*, *B. megaterium*, *C. glabrata*, *C. albicans*, ve *Trichophyton* sp.'ye karşı farklı oranlarda antimikrobiyal etki (8.8-20.4 mm) gösterdiği bulunmuştur. Metanol ekstresinin TAS değeri 3.00 mmol, etanol ekstresinin TAS değeri 3.15 mmol olarak tespit edilmiştir. Aynı türün metanol ekstresinin TOS değeri 6.81 μmol, etanol ekstresinin TOS değeri 12.64 μmol olarak hesaplanmıştır. Altın otun'un ekstrelerinin DPPH radikalini süpürücü etkilerinin artan konsantrasyonlara bağlı olarak arttığı belirlenmiştir.

Anahtar kelimeler: *Helichrysum arenarium* subsp. *aucheri*, Altın otu, antimikrobiyal, antioksidan.

1. Introduction

There are around 600 species of *Helichrysum* in the Asteraceae family. The members of this genus are native kto Africa (South Africa has 244 species), Madagascar, Australasia, and Eurasia. The inflorescences of plant species belonging to this genus are usually bright yellow [1,2]. Researchers have reported that some *Helichrysum* species are used in traditional medicine to treat various ailments such as skin infections, gallbladder, respiratory and digestive system disorders, and kidney stones [3-6]. It has also been used in folk medicine for the treatment

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of urogenital disorders, asthma, jaundice, stomach ailments and various ailments such as diarrhea, gallbladder, arthritis and cystitis [7-9]. It has been used for many years in the cosmetic industry for its fragrance [10].
In Central Europe, this strain is used to make antiseptic medications, while in South Africa, it is used to cure tuberculosis and its symptoms [11-15]. This plant, which grows wild in Anatolia, is used in herbal tea [7]. Recent years, some species have been reported to have antimicrobial and antioxidant effects [16]. The best known and studied species of this genus are *Helichrysum italicum*, *Helichrysum* stoechas and *Helichrysum arenarium* [2]. *Helichrysum arenarium* (L.) Moench subsp. *aucheri* is a species of Asteraceae family, commonly known as 'immortal flower, golden herb or mantuvar' in Turkey [17]. Essential oils, polyphenols, fatty acids, carotenoids, bitter substances, mineral salts, vitamins, steroids, polysaccharides, glycosides, coumarins, and other compounds may be found in *H. arenarium* flowers. It has been approved to contain a high concentration of phenolic compounds [7,10,15,18-20]. It is also known that this species has different medicinal effects antioxidant, hepatoprotective, antibacterial, antiviral, antifungal, anti-inflammatory and antiproliferative [2,21-22]. In particular, it is known that the most important group of compounds responsible for biological effects are phenolics [23]. Recent studies have focused on the essential oils of this species. Because the essential oils obtained are known to have antimicrobial and antioxidant effects. Especially the height at which the plant is collected and which parts of the plant are used are important in terms of evaluating these results [20,24-25]. Volatile compounds such as trans-caryophyllene, α-humulene, α-pinene, dl-limonene, trans-caryophyllene, β-pinene, limonene were detected in *H. arenarium* subsp *aucheri* [17].

Due to the fact that this species grows naturally in our country, its bioactive components and the fact that it has been little studied in the literature, in this study, it was aimed to evaluate the antimicrobial and antioxidant properties of methanol and ethanol extracts of aerial parts of *H. arenarium* subsp. *aucheri*.

2. Material and Methods

2.1. Obtaining of Plant Material

H. arenarium subsp. *aucheri* samples were collected around the Nemrut crater lake of Bitlis (north-38°37'10″; east-42°14'28″; 2628 m) in August 2020 (Figure 1). Taxonomic description of plant material was carried out by the systematics-botany expert Prof. Dr. Şemsettin Civelek of Fırat University using the book Flora of Turkey [26]. The powdered plant material weighed 0.5 g. 100 mL of solvent 96% methanol (MetOH) and ethanol (EtOH) was added to the weighed plant. It was then mixed on a rotary shaker and filtered using Whatman filter paper (pore size 11µ).

Figure 1. Golden Grass (*H. arenarium* subsp. *aucheri)*.

2.2. Extraction Process

The drying process of the plant was carried out in a dark and moisture-free environment. Then 0.5 g of the powdered aerial parts was weighed. 100 mL of 96% methanol (MetOH) and ethanol (EtOH) were added to the weighed plant. It was then stirred on a rotary shaker (Gerhardt RO500/Germany) in a dark environment at room temperature for 72 hours (Shaker speed 60) and filtered using Whatman filter paper. The prepared extracts were stored at +4 ºC.

2.3. Analaysis Method

In this study; *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* DMS 50071, *Klebsiella pneumoniae* ATCC700603, *Bacillus megaterium* DSM32, *Staphylococcus aureus* COWAN1, *Candida glabrata* ATCC66032, *Candida albicans* FMC17, *Trichophyton* sp. microorganisms were used. Antimicrobial activities of the extracts of aerial parts of *H. arenarium* subsp. *aucheri* were determined according to the disk diffusion method [27]. Prepared broth cultures yeast (*C. glabrata* and *C. albicans*), dermatophyte fungi (*Trichophyton* sp.) and bacterial (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *B. megaterium*, *S. aureus*) were cultured on Sabouraud Dextrose Agar, Glucose Sabouroud Buyyon (Difco) and Müeller Hinton Agar, respectively inoculated at 1% (10^4 yeast/ml, 10^4 yeast/ml and 10^6 bacteria/ml) and placed in sterile petri dishes. Antimicrobial discs (6 mm diameter), each impregnated with 100 µl (500 µg) of different extracts, were gently transferred on agar medium. Following incubation for 1.5-2 hours at 4°C, the yeast, dermatophyte fungi and bacteria were transferred onto plates and incubated for for 72 hours at 25 ± 0.1°C, for 72 hours at 25 ± 0.1°C and 24 hours at 37 ± 0.1°C, respectively. Nystatin (30 µg/disc) (for yeast) and Streptomycin sulfate (10 µg/disc) (for bacteria) were used as standard disc. The zones (mm) were then measured. Total oxidant and total antioxidant effects of methanol and ethanol extracts of were determined using Rel Assay kits (Rel Assay Kit Diagnostics, Turkey). TOS and TAS values were expressed as µmol H_2O_2 equivalent/L and mmol Trolox equivalent/L, respectively [28-29]. The antioxidant activity was carried out by the 2.2-diphenyl-1-picrilhydrazyl (DPPH) (the absorbances of each mixture were read at 570 nm in the Elisa reader) radical scavenging capacity method [30-31].

2.4. Statistical Analysis

SPSS Statistics (version 22) was used to perform the statistical analysis and generate the figures. Analysis of variance (ANOVA) and Student's t-test were performed, and $p < 0.01$ was considered significant.

3. Result and Discussion

3.1. Antimicrobial Effect

The antimicrobial effect of the methanol and ethanol extracts of the plant is as seen in Table 1.

Table 1. Results of the disk diffusion method of plant extracts against the tested microorganisms (Inhibition zones measured in mm).

Microorganisms	Methanol	Ethanol	Standard antibiotics
S. aureus	-	9.6± 0.7	19.5± 0.11
E. coli	-	-	19.8± 0.15
K. pneumoniae	-	-	17.5± 0.13
B. megaterium	-	20.4 ± 0.2	21.6± 0.13
P. aeruginosa	-	-	20.5± 0.19
C. glabrata	-	9.5± 0.9	21.5± 0.16
C. albicans	9.3± 0.13	8.8± 0.15	23.7± 0.17
***Trichophyton* sp.**	-	13.8± 0.8	22.8± 0.18

No significant differences were found in the means with the '-' symbol in the same column ($p > 0.01$)

MetOH extract of plant created 9.3 mm zone of inhibition against *C. albicans*. EtOH extract of *H. arenarium* subsp. *aucheri* showed inhibition zone on *B. megaterium, S. aureus*, *C. glabrata*, *C. albicans*, *Trichophyton* sp. (8.8-20.4 mm), but it did not show inhibition zone against *K. pneumoniae, E. coli, P. aeruginosa* (Table 1). The comparison of the ethanol and methanol extracts of *H. arenarium* subsp. *aucheri* in terms of antimicrobial activity against *B. megaterium* showed that the ethanol extract was the most effective (20.4 mm) (Table 1). Lourens et al. [32] showed that the antibacterial antimicrobial effects of *Helichrysum excisum* and *Helichrysum dasyanthum* acetone extracts against *S. aureus* were 312.5 and 15.63 µg/mL, respectively. Furthermore, minimum inhibitory concentration (MIC) results on tested bacteria treated with *Helichrysum* extract revealed that *S. aureus* was more susceptible than *Streptococcus pneumoniae* as 0.62 and 1.25 mg/mL, respectively [11]. The antimicrobial effects of *H. arenarium* L. essential oil aginst *S. aureus, E. coli, Bacillus subtilis*, *Saccharomyces cereviciae*, *C. albicans*, *Aspergillus parasiticus* and *Aspergillus flavus* were investigated. As a result, *B. subtilis* was found to be more

resistant than the other two bacterial species (MIC=781.25 and MBC=6250 µg/ml). Among the tested yeasts the sensitive of *S. cerevisiae* (MIC=97.65 and MFC=781.25 µg/ml) was more sensitive than *C. albicans* [12].

Bigović et al. [33] reported that the antimicrobial effects of *H. plicatum* ethanol extracts against various microorganisms including *B. subtilis, E. coli*, *Listeria monocytogenes, Micrococcus flavus, Micrococcus luteus, Proteus mirabilis, P. aeruginosa, Salmonella typhimurium*, *Salmonella enteritidis* and *S. aureus* were between (0.01 and 0.055 mg/mL). In a previous study, by using the methanol and water extracts of *H. foetidum,* the MIC values were higher than 4 mg/ml against the test bacteria such as *E. coli*, *P. aeruginosa, S. aqureus* and *Streptococcus pyogenes* [34]. There have been more studies using different *Helichrysum* species for their antimicrobial effects, particularly of methanol extracts against a wide variety of test microorganisms (*Bacillus brevis, Aeromonas hydrophila*, *B. cereus, P. aeruginosa, E. coli, K. pneumoniae*, *C. albicans* and *S. aureus)*. Inhibition zones ranged from 6.5 mm to 28 mm, but no activity was detected against *E. coli* [35]. A recent study by Babotă et al. [16] showed that both *S. aureus* and *E. coli* were similarly affected by ethanolic extracts of *H. arenarium*, with a MIC value of 7.81 mg/mL. On the other hand, *H. arenarium* ethanol extract at concentrations of 20 and 50 mg/mL caused an inhibitory effect on *S. aureus* as zones of 25 mm and 28 mm, respectively [14].

Bozyel et al. [36] Most recently reported that *H. arenarium* spp. *aucheri* ethanol extract as 50 µL presented an antimicrobial activity with inhibition against *C. albicans* (10 mm), *K. pneumoniae* (7 mm), *S. aureus* (15 mm), while antimicrobial activity by 100 µL of ethanol extract of the same species was against *P. aeruginosa* was found to be 12 ± 0,71 mm. Noori et al. [37] A chemical analysis of the essential oil of *H. arenarium* L. found a total of 38 components. A-pinene, 1,8-cineole, â-humulene, and â-caryophyllene were the main components of essential oil. Less than 29% of the oil was made up of the other separated components. The antimicrobial effect of *Helichrysum arenarium* L. essential oil was found against *Streptococcus agalactiae, S. aureus* and *Serratiamarcescens* with MIC rate of ml respectively (812 ,812 and 406 µg). Djihane et al. [38] The essential oil of *H. italicum* (Roth) G. Don has been found to have antimicrobial activity against various microorganisms (*S. aureus, E. coli*, *Micrococcus luteus*, *Enterococcus cereus, K. pneumonia*, *Bacillus cereus*, *B. subtilis, Staphylococcus epidermidis*, *Enterococcus faecalis, P. aeruginosa*, *Proteus mirabilis*, *Listeria monocytogenes* and yeasts *C. albicans*, *Saccharomyces cerevisiae*, *Fusarium solani* var. coeruleum, *Alternaria alternata, Aspergillus niger*, *Ascochyta rabi*). *H. italicum* inhibited the growth of all microorganisms tested except *E. coli*, *K. pneumonia* and *L.monocytogenes*. The most sensitive bacterium is *E. cereus* with bactericidal (MBC) and minimal inhibitory (MIC) value of 0.79 µg ml^{-1}. Vujic et al. [39], reported that different (ethanol, dichloromethane and acetonitrile oil) extracts of *H. plicatum* have antimicrobial effects against three Gram-positive bacteria (*B. subtilis*, *S. aureus*, *Clostridium sporogenes*) and five Gram-negative bacteria (*P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Salmonella enterica* subsp. enterica, *Proteus hauseri*) two yeasts (*S. cerevisiae*, *C. albicans*), and *Aspergillus brasiliensis*. All extracts (ethanol, dichloromethane and acetonitrile oil) were found to have significant antibacterial activity at concentrations of 0.157-2.5 mg/mL. Zheljazkov et al. [24] It was determined the antimicrobial effect of *H. italicum* EO against nine microorganisms by using the disk diffusion method. Microorganisms antimicrobial activity was found to range of 2.33-14.67 mm. The EO of *H. italicum* against *S. aureus* was found to be 9.33 to 14.67 mm. Duran et al. [40] The antimicrobial effect results showed that *H. plicatum* extracts had stronger antibacterial activity against *Salmonella enteritidis* (24.13±1.15 and 156 µg/mL) among gram-negative bacteria. Additionally, it was found to have inhibitory activities for *B. cereus* (16.66 ±1.52 and 312µg/mL).

The comparisons of results obtained from different studies in the literature clearly show the differences depending on the species and microorganisms tested. The reason for that is most likely to be due to the bioactive contents of the plants, the place of collection, the solvent used and the extraction methods used.

3.2. Antioxidant Effect

The TAS value of the MetOH extract of the plant at 1mg/mL concentration was calculated as 3.00 mmol, and TAS value of the EtOH extract was calculated as 3.15 mmol. The TOS value of the MetOH extract of the same species was calculated as 6.81 µmol, and the TOS value of the EtOH extract was calculated as 12.64 µmol (Table 2).

Table 2. TAS and TOS values of *H. arenarium* subsp. *aucheri*.

	TAS (mmol Trolox equiv./L)	**TOS (µmol H_2O_2 equiv./L)**
***H. arenarium* subsp. *aucheri*-MetOH**	3.00± 0.11	6.81± 0.9
***H. arenarium* subsp. *aucheri*-EtOH**	3.15± 0.17	12.64± 0.16

Table 3. Percent inhibition of the DPPH radical of *H. arenarium* subsp. *aucheri*.

Concentrations	***H.arenarium* subsp. *aucheri*-MetOH**	***H.arenarium* subsp. *aucheri*-EtOH**
1000 µg/mL	34.55± 0.17	34.45± 0.19
500 µg/mL	29.48± 0.21	26.22± 0.23
250 µg/mL	17.58± 0.27	17.32± 0.32
125 µg/mL	10.05± 0.11	12.35± 0.14

It has been determined that the scavenging effects of DPPH radicals of MetOH and EtOH extracts of *plant* increased with increasing concentrations (Table 3). The antioxidant levels of various plant members have been studied extensively in the literature. Antioxidant level of *H. chasmolycicum* aerial parts followed by methanol extract was measured as IC_{50} 0.92 mg/mL by using DPPH method [5]. Moreover, Albayrak et al. [35], investigated antioxidant properties of four different subspecies belong to *H. arenarium* which were subsp. *erzincanicum*, *rubicundum*, *araxinum* and *pseudoplicatum* evaluated by DPPH IC_{50} (µg/mL) values as 23.03 µg/mL, 47.64 µg/mL, 27.32 µg/mL and 38.82 µg/mL, respectively. The antioxidant activities of the extracts of *Helichrysium* species including *H. chionophilum, H. chasmolycicum*, *H. arenarium* subsp. *aucheri* and *H. plicatum* subsp. plicatum were also reported where the IC_{50} values were found as 40.5 µg/mL, 246.83 ± 1.23 mg AAE/g, 47.6 and 48.0µg/mL, respectively [41,42]. The DPPH radikal scavenging effects of *H. arenarium* methanol and ethanol extracts were 4.91 ± 1.90 and 7.21 ± 2.81 mg TE/mL, respectively. In the same study, it is emphasized that the antioxidant effect of *H. arenarium* may be related to the phenolic compounds it contains [16]. Further research has also shown that aqueous alcoholic extracts of *H. İtalicum* have high antioxidant properties, so that different extracts (MeOH, EtOH, 60% EtOH and 70% MeOH) of *H. italicum* led to the TEAC values of 73.18 ± 3.51, 58.35 ± 5.25, 132.38 ± 1.15 and 144.36 ± 7.01 mM TE/g DW, respectively. On the other hand, the ethyl acetate extract caused low antioxidant activity of 24.58 ± 2.00 mM TE/g DW [43]. More recently, antioxidant properties of various species including *Helichrysum pandurifolium, Helichrysum foetidum, Helichrysum petiolare* and *Helichrysum cymocum* have been studied. The IC50 values of the radical scavenging activity for all plants studied ranged from 20.81-36.19 µg/mL (NO), 11.85-41.13 µg/mL (DPPH) and 0.505-0.636 µg/mL (FRAP). Among all these, *H. petiolare* had highest total phenolic content (54.69 ± 0.23 mg/g), highest total flavonoid content (56.19 ± 1.01 mg/g) and thus the highest total antioxidant capacity (48.50 ± 1.55 mg/g), in comparison to other species studied [44]. Kherbache et al. [45] found that the radical scavenging activity of the ethyl acetate extract (IC50 = 54.82 ± 1.50 µg/mL) of *Helichrysum stoechas* was significantly higher than that of the butanolic extract (IC50 = 83.66 ± 1.02 µg/mL). Stankov et al. [46] determined that the total polyphenol and flavonoid contents in the ethanol extract of *Helichrysum arenarium* varied. They reported that the antioxidant effect of ethanol extract is related to these components. A more recent study on Sandy everlasting extracts of *H. italicum* (Roth) and *H. arenarium* (L.) Moench showed that these plants possessed significantly higher radical scavenging activities (for inflorescences from 1.96 to 6.13 mmol/L and for leaves ranged from 11.18 to 19.13 TROLOX equivalent) revealed by comparison to those of all tested EOs (0.25 to 0.46 mmol/L TROLOX equivalent) [20].

The results of the present study compared with those obtained in the literature in terms of antioxidant properties of different plant extracts clearly showed that, there is a large variability, depending on the plant collection site, the plant species, plant parts, its biochemical contents, methods, solvents and concentrations used.

4. Conclusion

In this study, antimicrobial and antioxidant effects of aerial parts of *H.arenarium* subsp. *aucheri* extracts on some tested microorganisms were investigated. The EtOH extract of *H. arenarium* subsp. *aucheri* showed the best antimicrobial effect against *B. megaterium*. Moreover, the total antioxidant level of the ethanol extract of the same species was also high, but differently the total oxidant level of the ethanol extract was interestingly found high. These differences might be due to the presence or absence of oxidant/antioxidant compounds produced by the plant in sufficient amounts depending on the solvent. It is clear that the biological effects of *H. arenarium* subsp. *aucheri* determined in this study may well be important and thus need further study.

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