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Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey

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ABSTRACT

The methanolic extracts of 16 *Helichrysum* species were investigated for their *in vitro* antioxidant, radical scavenging and antimicrobial activities. All the extracts showed strong antioxidant and radical scavenging activity. The highest total antioxidant capacity as ascorbic acid equivalents (AAE) of 194.64 mg/g dry extract was obtained for *Helichrysum noeanum* in the phosphomolybdenum assay. The highest IC_{50} value (7.95 μg/ml) was observed for the extract of *Helichrysum stoechas* subsp. *barellieri* in the DPPH assay. The total phenolic contents of the extracts ranged from 66.74 to 160.63 mg gallic acid equivalents (GAE)/g dry extract. The major component present in the extracts was identified as chlorogenic acid followed by apigenin-7-glucoside and apigenin by HPLC analysis. All the extracts showed significant antimicrobial activity against microorganisms containing 13 bacteria and two yeasts in the agar diffusion method.

1. Introduction

Lipid peroxidation is one of the major reasons for deterioration of food products during processing and storage. Radicals are known to take part in lipid peroxidation and play an important role in the progression of a great number of pathological disturbances, such as atherosclerosis, brain dysfunction, cancer promotion, heart diseases, immune system decline and neurodegenerative diseases (Czinner et al., 2001). Antioxidants are the compounds that, when added to food products, act as radical scavengers, prevent the radical chain reactions of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the process of lipid peroxidation (Young & Woodside, 2001).

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used as antioxidants in foods to prevent or retard lipid oxidation. However, restrictions on the use of these compounds are being imposed because of their carcinogenicity and some side effects (Velioglu, Mazza, Gao, & Oomah, 1998). Thus, evaluation of the antioxidative activity of naturally occurring substances has been the focus of interest in recent years (Jayaprakasha, Rao, & Sakariah, 2004).

Consumers are becoming more conscious of the nutritional value and safety of their food and ingredients. The preference for natural foods and food ingredients that are believed to be safer, healthier and less subject to hazards is increasing compared to their synthetic counterparts (Farag, Badei, Heweij, & El-Baroty, 1986). Thus, the evaluation of antioxidative activity of naturally occurring substances has been focus of interest in recent years (Jayaprakasha et al., 2004). The use of plants, herbs as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants (Madsen & Bertelsen, 1995).

Antimicrobial agents, including food preservatives, have been used to inhibit food borne bacteria and extend the shelf life of processed food. Many naturally occurring extracts from plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Bagamboula, Uyttendaele, & Debevere, 2003).

Plants contain a diverse group of phenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavonoids. All the phenolic classes have received considerable attention because of their physiological functions, including free radical scavenging, antioxidants (Bandoniene & Murkovic, 2002) and antimicrobial activity (Nychas, Tassou, & Skandamis, 2003). The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metal chelating potential (Pietta, 2000).

Helichrysum Mill. includes about 500 species, widespread throughout the world. This genus is represented, in Turkish flora,

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by 27 taxa, 15 of which are endemic (Davis, 1975; Davis, Mill, & Tan, 1988; Guner, Ozhatay, Ekim, & Baser, 2000; Sumbul, Gokturk, & Dusen, 2003). The *Helichrysum* species commonly used in folk medicine as a herbal tea for various biological properties including anti-inflammatory (Sala et al., 2003) antioxidant (Czinner et al., 2001; Tepe, Sokmen, Akpulat, & Sokmen, 2005) and antimicrobial activity (Sagdic, Karahan, Ozcan, & Ozkan, 2003) in Turkey and various parts of the world. *Helichrysum* species are generally known under the names "ölmez çiçek or altınotu" and are widely used as herbal teas in Turkey (Baytop, 1997). *Helichrysum* species have been used in folk medicine for at least 2000 years against gall bladder disorders, because of their bile regulatory and diuretic effects. These effects of *Helichrysum* species are due to the flavonoids that they contain. In Turkey, several *Helichrysum* species are used in folk medicine for removing the kidney stones and as diuretics (Suzgec, Mericli, Houghton, & Cubukcu, 2005).

Although biological activities of many *Helichrysum* species have been investigated in different countries, there are only a few reports of the *Helichrysum* species belonging to Turkish flora (Ozkan, Sagdic, & Ozcelik, 2004; Tepe et al., 2005).

The aim of the present works is to study phenolic content, antioxidant and antimicrobial activities of methanol extracts of some *Helichrysum* species collected from Turkey.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, gallic acid, ascorbic acid, Mueller–Hinton agar, Mueller–Hinton broth, Malt extract agar and Malt extract broth were purchased from Merck. The other chemicals and solvents used in this experiment were of analytical grade, purchased from Merck.

2.2. Plant material

The collection information of the *Helichrysum* species, which are individually numbered, is listed below:

1. *Helichrysum arenarium* (L.) Moench subsp. *aucheri* (Boiss) Davis & Kupicha, 1100 m, Kırsehir, Turkey, 18 July 2007. (E)
2. *Helichrysum armenium* DC. subsp. *armenium*, 1200 m, Sivas, Turkey, 13 June 2006.
3. *Helichrysum artvinense* Davis & Kupicha, 645 m, Artvin, Turkey, 19 August 2006. (E)
4. *Helichrysum chionophilum* Boiss. & Bal., 2030 m, Kayseri, Turkey, 14 August 2007. (E)
5. *Helichrysum compactum* Boiss, 1480 m, Antalya, Turkey, 12 June 2007. (E)
6. *Helichrysum goulandriorum* E. Georgiadou, 915 m, Kahramanmaras, Turkey, 07 July 2007. (E)
7. *Helichrysum graveolens* (Bieb.) Sweet, 1840–1900, Bursa, Turkey, 29 July 2006.
8. *Helichrysum heywoodianum* Davis, 820 m, Aydın, Turkey, 25 Jue 2006. (E)
9. *Helichrysum kitianum* Yıldız, 2030 m, Sivas, Turkey, 19 July 2006. (E)
10. *Helichrysum noeanum* Boiss., 1240 m, Ankara, Turkey, 30 June 2005. (E)
11. *Helichrysum orientale* (L.) DC., 17 m, Aydın, Turkey, 14 April 2006.
12. *Helichrysum pallasii* (Sprengel) Ledeb., 2450–2500 m, Bayburt, Turkey, 18 July, 2006.
13. *Helichrysum peshmenianum* S. Erik, 2700 m, Nigde, Turkey, 17 July 2007. (E)
14. *Helichrysum plicatum* DC. subsp. *plicatum,* 1950 m, Yozgat, Turkey, 11 July 2006.
15. *H. plicatum* DC. subsp. *polyphyllum* (Ledeb.) Davis & Kupicha, 1950 m, Adana, 07 July 2007.
16. *Helichrysum stoechas* (L.) Moench subsp. *barrelieri* (Ten) Nyman, 51 m, Aydın, Turkey, 14 April 2006.

Voucher specimens were identified by Dr. Ahmet Aksoy and have been deposited at the Herbarium of the Department of Biology, Erciyes University, Kayseri, Turkey. Nine of *Helichrysum* species collected is endemic (E) to Turkish flora (Davis, 1975; Davis et al., 1988; Guner et al., 2000).

2.3. Preparation of plant extractions

Dried aerial parts of the plant at room temperature were ground to a fine powder with a grinder. Then the powdered plant material (10 g) was extracted using a Soxhlet type extractor with 100 ml methanol (MeOH) at 60 °C for 6 h. Thereafter, the extract was filtered and evaporated to dryness under vacuum at 40 °C with a rotary evaporator. After determining the yield, the extract was dissolved in methanol for further study.

2.4. HPLC analysis of phenolic compounds in the extracts

The extracts were dissolved in methanol at a concentration of 10 mg/ml. A high performance liquid chromatograph (Shimadzu) was equipped with HPLC pumps (LC-10ADvp) and a DAD detector (278 nm). Eclipse XDB-C18 (5 μm) column (250 × 4, 60 mm) (Agilent) was used. The flow rate was 0.8 ml/min and the injection volume 20 μl. The analyses of the phenolic compounds were carried out at 30 °C using two linear gradients of methanol. Acacetin, apigenin, apigenin-7-glucoside, caffeic acid, catechin, chlorogenic acid, epicatechin, eriodictyol, ferulic acid, gallic acid, hesperidin, luteolin, naringenin, *p*-coumaric acid, *p*-hydroxybenzoic acid, quercetin, rosmarinic acid, resveratrol, rutin and syringic acid were used as standard. Identification and quantitative analysis were done by comparison with standards.

2.5. Determination of total phenolic content

The total phenolic content of the extracts was determined using the Folin–Ciocalteau reagent (Singleton & Rossi, 1965). The reduction of the Folin–Ciocalteau reagent by phenolic compounds under alkaline conditions, which resulted in the development of a blue colour, was recorded at an absorbance of 765 nm. Briefly, 40 μl of the methanol solution of the extract (1 mg/ml) was mixed with 2.4 ml of distilled water. 200 μl of Folin–Ciocalteau reagent was added and the contents of the flask mixed thoroughly. After 1 min, 600 μl of sodium carbonate (20% Na_2CO_3) was added and the volume was made up to 4.0 ml with distilled water. After 2 h incubation at room temperature, the absorbance was measured at 765 nm with spectrophotometer (Shimadzu) and compared to a gallic acid calibration curve. The data are presented as the average of triplicate analyses. Results were expressed as mg of gallic acid (GAE) equivalents/g extract.

2.6. Determination of antioxidant activity

2.6.1. Phosphomolybdenum assay

The antioxidant activity of *Helichrysum* methanolic extracts was determined by the phosphomolybdenum method of Prieto, Pineda, and Aguilar (1999). 0.4 ml of the methanolic extract (1 mg/ml) was mixed with 4 ml of reagent solution (0.6 M sulphuric acid, 28 mM

sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm. In the case of the blank, 0.4 ml of methanol was used in place of sample. The antioxidant activity was determined using a standard curve with ascorbic acid solutions as the standard. The mean of three readings was used and the reducing capacity of the extracts was expressed as mg of ascorbic acid equivalents (AAE)/g extract.

2.6.2. DPPH radical–scavenging activity

The capacity of methanol extracts to scavenge the lipid-soluble DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, which results in the bleaching of the purple colour exhibited by the stable DPPH radical, is monitored at an absorbance of 517 nm. The ability of the extracts to scavenge DPPH radical was assessed spectrophotometrically (Lee et al., 1998). 50 μl aliquots of the proper methanolic extract dilution at a concentration range of 0.1–2 mg/ml was mixed with 450 μl Tris–HCl buffer (pH = 7.4) and 1 ml of the methanolic DPPH solution (0.1 mM). Methanol was used as a control instead of extract. The mixtures were left for 30 min at room temperature in the dark and the absorbance at 517 nm measured using methanol as blank. IC_{50} (concentration causing 50% inhibition) values of methanolic extracts were determined graphically. The same procedure was repeated with BHT as a positive control. The measurements were performed in triplicate and the results were averaged.

Radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by following equation:

$$\text{Percentage inhibition } (I\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.7. Determination of antimicrobial activity

The microorganism strains used in this study were *Aeromonas hydrophila* ATCC 7965, *Bacillus* brevis FMC 3, *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Morganella morganii, Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 (A), *S. aureus* ATCC 25923 (B), *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223 and *Saccharomyces cerevisiae* BC 5461.

The agar-well diffusion method was employed for the determination of antimicrobial activities of extracts (Sagdic et al., 2003). Each microorganism was suspended in sterile nutrient broth. Test yeasts (*C. albicans*, *S. cerevisiae*) were suspended in malt extract broth and each microorganism was diluted at ca. 10^6–10^7 colony-forming units (cfu)/ml. 250 μl of each microorganism was added into a flask containing 25 ml sterile Mueller–Hinton agar or malt extract agar at 45 °C and poured into Petri dishes (9 cm in diameter). Then the agars were allowed to solidify at 4 °C for 1 h. Four equidistant wells (4 mm in diameter) were cut from the agar. The extracts were prepared at 1%, 2.5%, 5% and 10% concentrations in absolute methanol and 40 μl of extract solutions were applied to the wells. Absolute methanol without herb extract was used as a control. *Y. enterocolitica, C. albicans* and *S. cerevisiae* was incubated at 25 °C for 24–48 h in the inverted position. The other microorganisms were incubated at 37 °C for 18–24 h. At the end of the period, all plates were examined for any zones of growth inhibition and the diameters of these zones were measured in millimetres. All the tests were performed in duplicate and the results were presented as averages.

2.8. Statistical analysis

Data from the experiments were subjected to analysis of variance (ANOVA) using SPSS (2001) for Windows. Percentage data were transformed using arcsine Ox before ANOVA. Means were separated at the 5% significance level by the least significant difference (LSD) test. Bivariate correlations were analysed by Pearson's test using SPSS 10.0 on Windows.

3. Results and discussion

In this study, total extract yield, total phenolic content, antioxidant and antimicrobial activities of methanolic extracts of *Helichrysum* species collected from Turkey were determined. The extract yields ranged from 8.00% to 33.36% (w/w) (Table 1). Among the tested *Helichrysum* species; *H. kitianum* had highest extract yields while *H. chionophilum* had lowest extract yield.

The total phenolic content of the extracts, as estimated by the Folin–Ciocalteau reagent method, ranged from 66.74 ± 1.3 to 160.63 ± 1.2 mg GAE/g dry extract (Table 1). Statistical differences among the total phenolic contents of *Helicrysum* extracts were important ($p < 0.05$). The highest level of phenolics was found in *H. noeanum*, while the lowest was in *H. peshmenianum* (Table 1). No references concerning the total phenolic content of *Helichrysum* species could be found despite the thorough literature survey except Ozkan et al. (2004) reported that the total phenolic content of methanolic extract of *Helicrysum chasmolycicum* was 108.33 ± 0.88 mg GAE/g. Also, at our previous study (Albayrak, Sag-

Table 1
The yields, total phenolic contents, total antioxidant activities and IC_{50} values of *Helichrysum* methanolic extracts.

Plant no.	*Helichrysum* species	Yield (%)	Total phenolic content (mg GAE/g extract)	Total antioxidant activity (mg AAE/g extract)	DPPH IC_{50} (μg/ml)
H1	*H. arenarium* subsp. *aucheri*	12.60	115.76 ± 1.2^{c}	147. 68 ± 0.3^{k}	37.52^{b}
H2	*H. armenium* subsp. *armenium*	25.55	89.02 ± 0.8^{f}	157.29 ± 0.2^{i}	16.61^{h}
H3	*H. artvinense*	13.85	83.98 ± 1.0^{h}	171.02 ± 0.5^{e}	21.23^{f}
H4	*H. chionophilum*	8.00	106.97 ± 0.6^{d}	140.43 ± 0.5^{l}	53.10^{a}
H5	*H. compactum*	13.55	79.59 ± 0.6^{i}	165.00 ± 0.3^{f}	27.32^{d}
H6	*H. goulandriorum*	23.10	114.41 ± 1.0^{c}	124.86 ± 0.2^{m}	23.92^{e}
H7	*H. graveolens*	29.37	92.77 ± 0.6^{e}	160.34 ± 0.9^{h}	15.28^{h}
H8	*H. heywoodianum*	28.90	93.85 ± 0.4^{e}	191.97 ± 0.4^{b}	22.23^{ef}
H9	*H. kitianum*	33.36	75.16 ± 0.6^{j}	172.17 ± 0.5^{d}	26.37^{d}
H10	*H. noeanum*	18.42	160.63 ± 1.2^{a}	194.64 ± 0.4^{a}	18.46^{g}
H11	*H. orientale*	32.53	73.70 ± 0.3^{k}	110.03 ± 0.3^{o}	29.53^{c}
H12	*H. pallasii*	16.57	94.13 ± 0.1^{e}	118.99 ± 0.5^{n}	26.23^{d}
H13	*H. peshmenianum*	13.62	66.74 ± 1.3^{l}	125. 47 ± 0.3^{m}	35.55^{b}
H14	*H. plicatum* subsp. *plicatum*	14.79	87.36 ± 0.6^{g}	163.47 ± 0.5^{g}	23.48^{e}
H15	*H. plicatum* subsp. *polyphyllum*	19.70	154.64 ± 0.6^{b}	152.64 ± 0.3^{j}	13.23^{i}
H16	*H. stoechas* subsp. *barellieri*	16.87	94.16 ± 0.5^{e}	188.26 ± 0.5^{c}	7.95^{j}

In each column, means of three independent experiments (± standard deviation) with different superscript letters are significantly different ($p < 0.05$).
Total phenolic activity expressed as gallic acid equivalent (GAE), total antioxidant activity expressed as ascorbic acid equivalent (AAE).

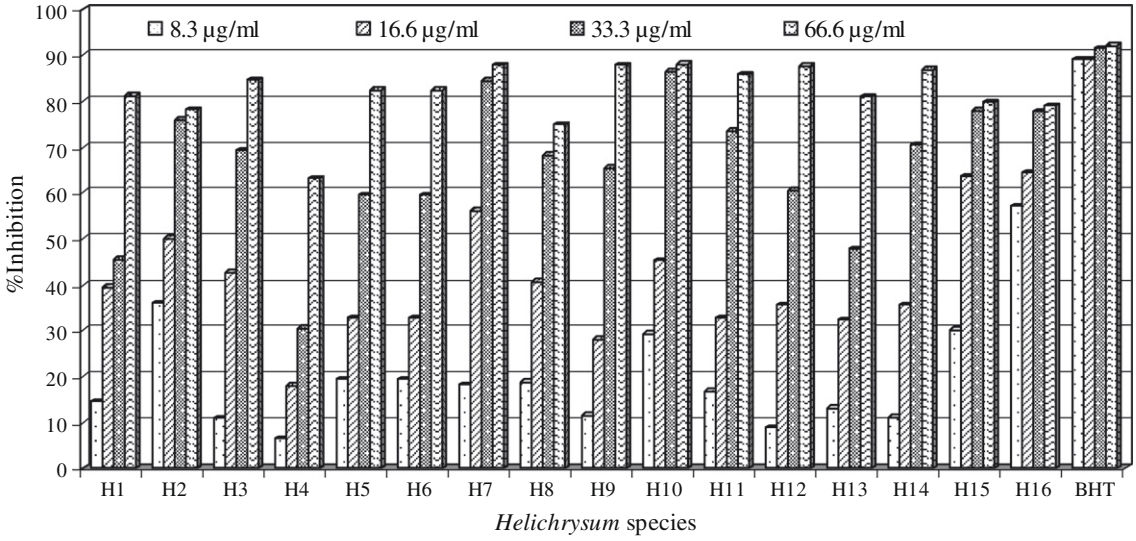
dic, Aksoy, & Hamzaoglu, 2008) the total phenolic contents of methanolic extracts of *Helicrysum pamphylicum*, *Helicrysum sanguineum* and *H. chasmolycicum* collected from Turkey were found to be 119.85 ± 2.0, 63.8 ± 0.6 and 71.51 ± 0.5 mg GAE/g dry extract, respectively. The differences in the total phenolic contents of *Helichrysum* species may be due to the difference in their chemical composition, collection time, collection area and season.

The phosphomolybdenum assay has been introduced for the measurement of antioxidant activity of the methanolic extracts. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes with a maximal absorption at 695 nm (Prieto et al., 1999). The results indicated that the methanolic extracts of *Helichrysum* tested had strong total antioxidant activity (Table 1). There were statistical differences in the total antioxidant activity of the extracts tested ($p < 0.05$). The methanolic extract of *H. noeanum* had the highest antioxidant activity with a value of 194.64 mg AAE/g dry extract. The lowest antioxidant activity was found in the methanolic extract of *H. orientale* with a value of 110.03 mg AAE/g dry extract (Table 1).

Bivariate correlations were analysed by Pearson's test using SPSS 10.0 on Windows. It was observed that there is no correlation between the antioxidant activities and total phenolic contents of the methanolic extract of *Helichrysum* tested (Pearson's correlation coefficient = 0.242). Also, the correlation was not observed between the total phenolic contents and free radical scavenging activities of these extracts (Pearson's correlation coefficient = 0.201). It is important to examine the correlation between the total phenolic content and the total antioxidant activity. Because some authors have shown that a high total phenol content increases the antioxidant activity (Kumaran & Karunakaran, 2007). But, the correlation between the total phenolic content and antioxidant activity was not observed in present study. Also, the correlation between the total phenolic content and free radical scavenging activity was not observed in present study. These results are in accordance with other reports, which have reported that there is no correlation between the content of these main antioxidant compounds and the radical scavenging capacity (Yu et al., 2002).

The free radical scavenging activity of the methanolic extracts of *Helichrysum* tested were determined through the DPPH method and results are presented in Table 1 and Fig. 1. DPPH is a useful reagent for investigating the free radical scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow coloured diphenylpricrylhydrazine. The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction (Shon, Kim, & Sung, 2003). The free radical scavenging activities of the *Helichrysum* extracts tested at 8.3–66.6 μg/ml concentrations were compared with BHT (Fig. 1). The extracts exhibited concentration dependent DPPH radical scavenging activity. The free radical scavenging activity of *H. noeanum* was similar to that of the positive control, BHT at 66.6 μg/ml concentration. The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure antioxidant activity. As the IC_{50} value of the extract decreases, the free radical scavenging activity increases. Among 16 extracts tested in this study, *H. stoechas* subsp. *barellieri* showed the highest scavenging activity (IC_{50} = 7.95 μg/ml) while *H. chionophilum* showed the lowest scavenging activity (IC_{50} = 53.10 μg/ml) ($p < 0.05$) (Table 1). The antioxidant activity shown by the extracts may be due to the presence of various flavonoids and phenolic acid. Variations in the free radical scavenging activity of different extracts may be attributed to differences in their chemical composition. It is clear that all the extracts showed an increase in free radical scavenging activity with an increase in the dose of extract.

Although there are many reports dealing with antioxidant activity of different *Helichrysum* species, data about antioxidant and free radical scavenging activity of *Helichrysum* species belonging to Turkish flora is not sufficient (Ozkan et al., 2004; Tepe et al., 2005). Antioxidant and antiradical activities of the methanolic ex-



H1: H. *arenarium* subsp. *aucheri*, H2: *H. armenium* subsp. *armenium*, H3: *H. artvinense*, H4: *H. chionophilum*, H5: *H. compactum*, H6: *H. goulandriorum*, H7: *H. graveolens*, H8: *H. heywoodianum*, H9: *H. kitianum*, H10: *H. noeanum*, H11: *H. orientale*, H12: *H. pallasii*, H13: *H. peshmenianum*, H14: *H. plicatum* subsp. *plicatum*, H15: *H. plicatum* subsp. *polyphyllum*, H16: *H. stoechas* subsp. *barellieri*.

Fig. 1. % Inhibition values of the methanolic extracts of *Helichrysum* species tested by DPPH assay.

tracts from *H. pamphylicum*, *H. sanguineum* and *H. chasmolycicum* collected from different regions of Turkey have already been determined in our previous study (Albayrak et al., 2008). In our previous paper (Albayrak et al., 2008), we reported the antioxidant activities of *H. pamphylicum*, *H. sanguineum* and *H. chasmolycicum* were 173.58 ± 1.1, 159.94 ± 0.3 and 147.88 ± 0.9 mg AAE/g extract in the phosphomolybdenum assay. Also, IC_{50} values of those species were found to be 15.21, 12.90 and 25.33 μg/ml, respectively in the DPPH radical scavenging activity assay. Total antioxidant activities of the methanolic extracts of *H. noeanum*, *H. heywoodianum* and *H. stoechas* subsp. *barellieri* were observed to be higher than that of the *H. pamphylicum*, *H. sanguineum* and *H. chasmolycicum* extracts. The free radical scavenging activity of *H. stoechas* subsp. *barellieri* (IC_{50} = 7.95 μg/ml) was higher than that of the *H. pamphylicum*, *H. sanguineum* and *H. chasmolycicum* extracts, as well. Total antioxidant activity values of *Helichrysum* extracts tested in this study were consistent with that reported by Ozkan et al. (2004) in that total antioxidant activity of *H. chasmolycicum* collected from Turkey was found to be 246.83 ± 1.23 mg AAE/g. In addition, a study conducted by Tepe et al. (2005) indicated that *H. chionophilum*, *H. plicatum*, *H. arenarium* and *H. noeanum* showed free radical scavenging activity in the DPPH assay. When results of our present study compared with those of a previous study by Tepe et al. (2005), all methanolic extracts tested in this study showed better radical scavenging activity. Lourens, Reddy, Baser, Viljoen, and Van Vuuren (2004) reported that the IC_{50} values of *H. dasyanthum*, *H. excisum*, *H. felinum* and *H. petiolare* methanolic extracts were 12.33, 13.67, 20.71 and 28.70 μg/ml, respectively. According to our findings, free radical scavenging activity of *H. stoechas* subsp. *barellieri* was stronger than that of those methanolic extracts.

It is obvious that the total phenolic content measured by the Folin–Ciocalteau procedure does not give a full picture of the quality or quantity of the phenolic constituents in the extracts (Wojdylo, Oszmianski, & Czemerys, 2007). Therefore, the phenolic acids and flavonoids of *Helichrysum* species tested were determined by the HPLC method. The data from the qualitative and quantitative analysis of the extracts made using HPLC coupled with photodiode array DAD detection, is presented in Table 2. The amount of each compound is demonstrated as μg/g dry residue. Phenolic compounds could not be identified in the extracts not shown in the table. Chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, apigenin, apigenin-7-glucoside, epicatechin, eriodictyol, hesperidin, luteolin, naringenin, quercetin and resveratrol were identified by comparison with the retention times and UV spectra of authentic standards analysed under identical analytical conditions, while the quantitative data was calculated from their respective calibration curves. The major component present in the extracts was identified as chlorogenic acid (1603.2 μg/g) followed by apigenin-7-glucoside (876.8 μg/g) and apigenin (351 μg/g). The least abundant compound present in the extracts was resveratrol (2.57 μg/g). Catechin, gallic acid, rosmarinic acid, rutin and acacetin could not be identified in the all extracts tested.

The phenolic compositions of *H. plicatum* subsp. *plicatum* (Cubukcu, Mericli, Bingol, Yuksel, & Damadyan, 1986), *H. compactum* (Suzgec et al., 2005), *Helichrysum italicum* (Sala et al., 2003) and *H. stoechas* (Carini, Aldini, Furlanetto, Stefani, & Facino, 2001) have been determined previously. From the compounds identified, apigenin and naringenin have already been reported to be present in *H. plicatum* subsp. *plicatum* growing in Anatolia by Cubukcu et al. (1986). Also, several flavonoids, isoastragalin, isosalipurposide, helichrysin A and B were isolated as the major constituents from the capitulums of *H. plicatum* subsp. *plicatum* (Aslan, Orhan, Orhan, Sezik, & Yesilada, 2007). Our results are in agreement with those reported by Suzgec et al. (2005) in that apigenin, apigenin-7-glucoside, luteolin, naringenin and quercetin have been isolated from the capitulums of *H. compactum* and were shown to possess antioxidant activity. Chlorogenic acid, naringenin, quercetin and apigenin glucosides have been detected previously from the capitulums of *H. stoechas* and were shown to possess antioxidant activity (Carini et al., 2001). The remaining phenolic components including caffeic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, epicatechin, eriodictyol, hesperidin and resveratrol were reported for first time in this study. The differences in the quality and quantity of phenolic compounds may be due to a variety of reasons ranging from climate and geography to a difference in the specificity of the extraction procedures used. The amount of total phenolics calculated from the data obtained by HPLC analysis was different from that estimated by Folin–Ciocalteau method which can be explained due to the limitation of phenolic substances used as standard (Dastmalchi et al., 2008). Literature surveys indicate that the plant phenolics constitute

Table 2
The quantity of some phenolic compounds determined in methanolic extracts by HPLC.

Helichrysum taxa	Compounds															Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
H. arenarium subsp. *aucheri*	89.9	2.03	–	1.36	–	–	27.5	99.0	–	–	–	4.54	9.16	–	0.20	233.69
H. armenium subsp. *armenium*	148.4	1.57	–	–	–	–	11.5	37.0	2.18	–	5.44	11.45	11.45	–	–	228.99
H. artvinense	53.0	1.56	–	2.23	–	1.93	11.8	6.4	2.55	–	1.85	10.93	12.05	–	–	47.51
H. chionophilum	32.3	2.50	–	2.10	–	0.58	21.2	155.5	5.98	–	–	4.98	2.35	15.03	0.21	242.73
H. compactum	114.3	2.39	–	–	–	–	23.1	70.5	–	1.27	–	2.55	1.92	6.91	0.11	223.05
H. goulandriorum	114.3	2.21	1.40	–	–	–	22.7	33.8	1.13	4.73	–	7.20	11.44	–	0.14	199.05
H. graveolens	107.2	1.30	0.98	–	–	2.87	30.5	45.6	–	–	4.14	8.99	5.87	2.19	–	209.64
H. heywoodianum	30.9	1.07	0.54	–	–	–	–	17.1	–	1.82	2.72	6.54	0.76	3.16	0.13	64.74
H. kitianum	92.0	1.45	0.16	–	–	–	19.6	32.4	–	–	4.15	8.49	4.59	–	0.32	163.16
H. noeanum	81.5	2.03	0.16	–	0.53	–	31.0	26.3	–	–	4.93	–	5.42	–	0.31	152.18
H. orientale	91.3	1.45	0.70	–	0.17	1.25	6.3	44.0	–	–	–	–	6.30	–	0.37	151.84
H. pallasii	90.5	1.84	1.27	1.54	1.77	1.86	10.2	54.1	–	–	1.84	–	0.75	–	–	165.67
H. peshmenianum	152.1	1.77	0.22	–	–	–	51.0	45.8	–	–	1.46	4.44	1.47	–	0.21	258.47
H. plicatum subsp. *plicatum*	156.2	1.72	–	0.35	0.76	–	37.9	85.8	–	–	3.37	6.95	9.97	–	–	303.02
H. plicatum subsp. *polyphyllum*	136.5	1.16	0.86	0.61	1.63	4.31	36.3	52.8	2.12	–	4.26	7.23	13.20	–	–	260.98
H. stoechas subsp. *barrelieri*	112.8	2.70	0.26	1.26	–	–	10.4	70.7	10.59	–	5.26	–	–	–	0.57	214.54
Total	1603.2	28.75	6.55	9.45	4.86	12.8	351	876.8	24.55	7.82	39.42	84.29	96.7	27.29	2.57	

Compounds; 1: chlorogenic acid, 2: caffeic acid, 3: ferulic acid, 4: *p*-coumaric acid, 5: *p*-hydroxybenzoic acid, 6: syringic acid, 7: apigenin, 8: apigenin-7-glucoside, 9: epicatechin, 10: eriodictyol, 11: hesperidin; 12: luteolin, 13: naringenin, 14: quercetin, 15: resveratrol.
–: not detected.

Table 3
Antibacterial activity against Gram (−) bacteria of *Helichrysum* species (10%, 5%, 2.5% and 1% concentration).

Plant names (%)		*A. hydrophila*	*E. coli*	*K. pneumoniae*	*M. morganii*	*P. mirabilis*	*P. aeruginosa*	*Y. enterocolitica*
H1	10	20.0 ± 0.0[a]	7.0 ± 0.0	15.0 ± 0.0	20.0 ± 0.0	–	27.0 ± 1.4	–
	5	18.5 ± 0.7	7.0 ± 0.0	13.0 ± 0.0	18.5 ± 0.7	–	25.0 ± 1.4	–
	2.5	17.0 ± 0.0	–	12.0 ± 0.0	17.5 ± 0.7	–	23.0 ± 1.4	–
	1	15.5 ± 0.7	–	10.5 ± 0.7	15.5 ± 0.7	–	20.5 ± 0.7	–
H2	10	19. ± 0.7[a]	–	23.0 ± 1.4	–	6.0 ± 0.0	20.0 ± 2.1	7.0 ± 0.0
	5	18.0 ± 0.7	–	18.0 ± 0.7	–	–	18.0 ± 1.4	6.0 ± 0.0
	2.5	16.0 ± 1.4	–	15.0 ± 1.4	–	–	16.0 ± 0.0	–
	1	15.0 ± 2.1	–	14.0 ± 0.0	–	–	12.0 ± 0.0	–
H3	10	15.5 ± 0.7	7.0 ± 0.0	9.0 ± 0.0	–	–	12.0 ± 0.0	–
	5	14.5 ± 0.7	–	8.0 ± 0.0	–	–	11.0 ± 0.0	–
	2.5	13.0 ± 0.0	–	7.0 ± 0.0	–	–	10.0 ± 0.0	–
	1	8.0 ± 0.0	–	–	–	–	8.5 ± 0.0	–
H4	10	16.0 ± 0.0[a]	7.0 ± 0.0	11.0 ± 0.0	15.0 ± 0.0	–	23.5 ± 0.7	–
	5	15.0 ± 0.0	6.0 ± 0.0	9.0 ± 0.0	13.0 ± 0.0	–	22.5 ± 0.7	–
	2.5	13.0 ± 0.0	–	7.0 ± 0.0	11.5 ± 0.7	–	20.5 ± 0.7	–
	1	10.0 ± 0.0	–	6.0 ± 0.0	10.5 ± 0.7	–	19.0 ± 0.0	–
H5	10	26.0 ± 0.0	9.5 ± 0.7	14.0 ± 1.4	–	–	22.0 ± 1.4	–
	5	24.0 ± 1.4	8.0 ± 1.4	12.0 ± 1.4	–	–	20.5 ± 0.7	–
	2.5	22.5 ± 0.7	–	11.0 ± 1.4	–	–	20.0 ± 0.0	–
	1	19.0 ± 1.4	–	10.0 ± 1.4	–	–	16.0 ± 1.4	–
H6	10	31.0 ± 0.0	8.0 ± 0.0	19.5 ± 0.7	–	–	31.0 ± 1.4	–
	5	30.0 ± 0.0	7.0 ± 0.0	16.5 ± 0.7	–	–	30.0 ± 1.4	–
	2.5	29.0 ± 0.0	7.0 ± 0.0	14.5 ± 0.7	–	–	29.0 ± 1.4	–
	1	26.5 ± 0.7	–	13.0 ± 1.4	–	–	27.0 ± 1.4	–
H7	10	30.0 ± 0.0	7.0 ± 0.0	19.0 ± 1.4	–	–	26.5 ± 0.7	–
	5	28.0 ± 0.0	–	18.0 ± 1.4	–	–	25.5 ± 0.7	–
	2.5	26.5 ± 0.7	–	16.0 ± 0.0	–	–	24.5 ± 0.7	–
	1	24.0 ± 0.0	–	14.5 ± 0.7	–	–	23.5 ± 0.7	–
H8	10	32.0 ± 1.4	–	26.5 ± 0.7	–	–	30.0 ± 0.0	–
	5	28.5 ± 0.7	–	24.5 ± 0.7	–	–	28.5 ± 0.7	–
	2.5	24.5 ± 0.7	–	22.5 ± 0.7	–	–	24.5 ± 0.7	–
	1	23.0 ± 1.4	–	17.5 ± 0.7	–	–	19.5 ± 0.7	–
H9	10	27.0 ± 1.4	7.0 ± 0.0	18.0 ± 0.0	–	–	21.0 ± 0.0	–
	5	24.0 ± 1.4	–	15.0 ± 0.7	–	–	20.0 ± 0.0	–
	2.5	22.0 ± 0.7	–	14.0 ± 0.7	–	–	19.0 ± 0.0	–
	1	20.0 ± 0.0	–	13.0 ± 0.7	–	–	17.0 ± 0.0	–
H10	10	12.5 ± 0.7	–	11.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	12.5 ± 0.7	–
	5	11.5 ± 0.7	–	9.0 ± 0.0	6.0 ± 0.0	–	11.0 ± 0.0	–
	2.5	10.0 ± 1.4	–	8.0 ± 0.0	–	–	10.0 ± 0.0	–
	1	8.0 ± 0.0	–	8.0 ± 0.0	–	–	6.5 ± 0.7	–
H11	10	18.0 ± 1.4	–	23.0 ± 2.1	7.0 ± 0.0	7.5 ± 0.7	20.0 ± 0.0	–
	5	16.0 ± 0.7	–	17.0 ± 1.4	–	–	18.0 ± 0.0	–
	2.5	14.0 ± 1.4	–	16.0 ± 1.4	–	–	16.0 ± 0.7	–
	1	11.0 ± 0.7	–	14.0 ± 0.7	–	–	12.0 ± 0.0	–
H12	10	16.0 ± 0.0	–	8.0 ± 0.0	–	–	12.0 ± 0.0	–
	5	14.5 ± 0.7	–	7.0 ± 0.0	–	–	10.5 ± 0.7	–
	2.5	10.0 ± 0.0	–	–	–	–	9.0 ± 0.0	–
	1	8.5 ± 0.7	–	–	–	–	6.5 ± 0.7	–
H13	10	15.5 ± 0.7	10.0 ± 0.0	7.0 ± 0.0	16.0 ± 0.7	–	21.0 ± 1.4	–
	5	13.0 ± 0.0	9.0 ± 0.0	–	12.5 ± 1.4	–	16.0 ± 0.0	–
	2.5	12.0 ± 0.0	–	–	12.0 ± 0.7	–	15.0 ± 0.0	–
	1	8.0 ± 0.0	–	–	10.5 ± 1.4	–	11.0 ± 1.4	–
H14	10	20.5 ± 0.7	–	14.5 ± 0.7	–	–	26.0 ± 0.7	–
	5	19.5 ± 0.7	–	13.5 ± 0.7	–	–	21.5 ± 1.4	–
	2.5	16.5 ± 0.7	–	12.5 ± 0.7	–	–	19.0 ± 0.7	–
	1	12.5 ± 0.7	–	11.0 ± 1.4	–	–	14.5 ± 1.4	–
H15	10	23.5 ± 0.7	15.0 ± 1.4	16.0 ± 0.0	–	–	26.0 ± 0.0	–
	5	22.5 ± 0.7	13.0 ± 1.4	13.5 ± 0.7	–	–	25.0 ± 0.0	–
	2.5	20.5 ± 0.7	12.0 ± 1.4	13.0 ± 0.0	–	–	24.5 ± 0.7	–
	1	18.5 ± 0.7	10.5 ± 0.7	12.0 ± 0.0	–	–	22.0 ± 1.4	–
H16	10	14.0 ± 0.0	–	17.0 ± 0.0	11.5 ± 2.1	–	20.5 ± 0.7	–
	5	12.0 ± 0.0	–	13.0 ± 0.0	9.5 ± 0.7	–	19.0 ± 1.4	–
	2.5	11.0 ± 0.0	–	12.0 ± 0.0	8.0 ± 1.4	–	13.5 ± 0.7	–
	1	9.5 ± 0.7	–	10.0 ± 0.0	–	–	12.0 ± 0.0	–

–: not detected.
[a] Values expressed are mean ± standard deviation of two experiments, inhibition zones include diameter of hole (4 mm).

Table 4
Antibacterial activity against Gram (+) bacteria of *Helichrysum* species.

Plant names (%)		*B. brevis*	*B. cereus*	*B. subtilis*	*M. smegmatis*	*S. aureus* (A)	*S. aureus* (B)
H1	10	23.5 ± 0.7[a]	26.0 ± 0.0	–	–	21.0 ± 0.0	–
	5	21.5 ± 0.7	25.0 ± 0.0	–	–	20.0 ± 0.0	–
	2.5	20.5 ± 0.7	22.0 ± 0.0	–	–	19.5 ± 0.7	
	1	18.0 ± 1.4	20.0 ± 0.0	–	–	18.5 ± 0.7	–
H2	10	20.0 ± 0.0	19.0 ± 0.0	29.0 ± 1.4	21.0 ± 0.7	19.0 ± 0.7	29.0 ± 2.1
	5	18.5 ± 0.7	16.0 ± 1.4	21.0 ± 1.4	17.0 ± 0.7	17.0 ± 0.7	23.0 ± 0.0
	2.5	17.5 ± 0.7	16.0 ± 0.7	17.0 ± 0.7	15.0 ± 0.7	15.0 ± 0.7	20.0 ± 0.0
	1	14.5 ± 0.7	14.0 ± 0.7	13.0 ± 0.7	13.0 ± 0.7	11.0 ± 0.7	11.0 ± 1.4
H3	10	21.0 ± 0.0	21.0 ± 1.4	17.5 ± 0.7	–	10.0 ± 0.0	13.0 ± 0.0
	5	17.5 ± 0.7	16.0 ± 1.4	14.0 ± 1.4	–	8.0 ± 0.0	12.0 ± 0.0
	2.5	16.5 ± 0.7	13.0 ± 0.0	9.5 ± 0.7	–	7.0 ± 0.0	7.5 ± 0.7
	1	15.0 ± 0.0	11.5 ± 0.7	8.5 ± 0.7	–	6.0 ± 0.0	6.0 ± 0.0
H4	10	17.0 ± 1.4	22.5 ± 0.7	–	–	15.5 ± 0.7	–
	5	13.5 ± 0.7	21.0 ± 1.4	–	–	14.0 ± 1.4	–
	2.5	12.5 ± 0.7	19.5 ± 0.7	–	–	13.0 ± 1.4	–
	1	11.0 ± 0.0	18.0 ± 1.4	–	–	11.0 ± 0.0	–
H5	10	23.5 ± 0.7	22.0 ± 1.4	–	–	15.5 ± 0.7	–
	5	21.0 ± 1.4	20.0 ± 0.0	–	–	14.0 ± 1.4	–
	2.5	18.5 ± 0.7	19.5 ± 0.7	–	–	13.0 ± 1.4	–
	1	16.5 ± 0.7	17.5 ± 0.7	–	–	12.0 ± 1.4	–
H6	10	28.5 ± 0.7	26.0 ± 0.0	–	–	24.5 ± 0.7	–
	5	27.5 ± 0.7	25.5 ± 0.7	–	–	23.5 ± 0.7	–
	2.5	24.0 ± 1.4	24.5 ± 0.7	–	–	21.0 ± 1.4	–
	1	23.0 ± 1.4	22.0 ± 1.4	–	–	19.0 ± 1.4	–
H7	10	30.0 ± 0.0	25.5 ± 0.7	34.5 ± 0.7	–	21.5 ± 0.7	29.5 ± 0.7
	5	29.0 ± 0.0	24.5 ± 0.7	33.5 ± 0.7	–	19.0 ± 0.0	26.5 ± 0.7
	2.5	27.0 ± 0.0	23.0 ± 1.4	30.5 ± 0.7	–	17.5 ± 0.7	25.0 ± 1.4
	1	25.0 ± 0.0	20.5 ± 0.7	28.5 ± 0.7	–	15.5 ± 0.7	20.5 ± 0.7
H8	10	20.5 ± 0.7	24.5 ± 0.7	27.0 ± 1.4	24.5 ± 0.7	25.5 ± 0.7	29.5 ± 0.7
	5	19.5 ± 0.7	23.0 ± 0.7	22.5 ± 0.7	22.0 ± 1.4	23.5 ± 2.1	27.0 ± 1.4
	2.5	17.5 ± 0.7	20.5 ± 0.7	20.0 ± 0.0	20.5 ± 0.7	21.5 ± 2.1	25.0 ± 1.4
	1	16.5 ± 0.7	19.0 ± 1.4	19.5 ± 0.7	19.5 ± 0.7	19.0 ± 0.0	22.0 ± 1.4
H9	10	27.0 ± 0.0	24.0 ± 1.4	27.0 ± 1.4	–	18.0 ± 0.0	21.0 ± 0.0
	5	25.0 ± 0.0	20.0 ± 0.0	24.0 ± 0.7	–	17.0 ± 0.0	20.0 ± 0.0
	2.5	23.0 ± 1.4	19.0 ± 0.0	22.0 ± 0.7	–	15.0 ± 0.0	19.0 ± 0.7
	1	18.0 ± 1.4	18.0 ± 0.0	20.0 ± 0.0	–	14.0 ± 0.0	17.0 ± 0.0
H10	10	16.5 ± 0.7	21.5 ± 2.1	21.5 ± 2.1	16.0 ± 1.4	9.0 ± 0.0	9.5 ± 0.7
	5	15.5 ± 0.7	16.5 ± 0.7	16.5 ± 2.1	13.o ± 1.4	8.0 ± 0.0	8.5 ± 0.7
	2.5	14.5 ± 0.7	10.5 ± 0.7	13.0 ± 0.0	11.5 ± 0.7	7.0 ± 0.0	7.5 ± 0.7
	1	8.0 ± 0.0	8.0 ± 0.0	10.0 ± 0.0	8.5 ± 0.7	–	–
H11	10	21.0 ± 0.7	29.0 ± 0.7	26.0 ± 0.7	21.0 ± 0.7	10.0 ± 0.0	21.0 ± 2.1
	5	15.0 ± 0.7	23.0 ± 2.1	20.0 ± 0.0	19.0 ± 1.4	9.0 ± 0.0	18.0 ± 0.7
	2.5	13.0 ± 0.7	20.0 ± 0.7	15.0 ± 1.4	17.0 ± 2.1	7.5 ± 0.0	16.0 ± 0.7
	1	11.0 ± 0.7	16.0 ± 0.7	14.0 ± 0.7	12.0 ± 0.7	–	13.0 ± 1.4
H12	10	21.0 ± 0.0	19.5 ± 0.7	10.0 ± 0.0	–	10.5 ± 0.7	12.5 ± 0.7
	5	20.0 ± 0.0	14.5 ± 0.7	7.5 ± 0.7	–	8.0 ± 0.0	10.0 ± 0.0
	2.5	19.0 ± 0.0	13.0 ± 0.0	6.5 ± 0.7	–	6.5 ± 0.7	8.0 ± 0.0
	1	11.5 ± 0.7	10.5 ± 0.7	–	–	–	7.0 ± 0.0
H13	10	22.0 ± 0.7	15.5 ± 0.7	–	–	16.5 ± 0.7	–
	5	16.0 ± 0.0	13.0 ± 1.4	–	–	14.5 ± 0.7	–
	2.5	15.0 ± 0.0	12.5 ± 0.7	–	–	12.0 ± 1.4	–
	1	9.5 ± 1.4	11.0 ± 0.0	–	–	7.0 ± 0.0	–
H14	10	22.0 ± 0.0	23.5 ± 0.7	23.5 ± 0.7	–	13.0 ± 0.0	28.5 ± 0.7
	5	21.0 ± 0.0	21.5 ± 0.7	22.0 ± 1.4	–	12.0 ± 0.0	26.0 ± 0.7
	2.5	19.0 ± 0.0	19 ± 0.0	16.0 ± 1.4	–	8.5 ± 0.7	24.5 ± 1.4
	1	13.0 ± 0.0	14 ± 1.4	11.0 ± 1.4	–	–	14.5 ± 0.7
H15	10	25.5 ± 0.7	24.0 ± 1.4	–	–	21.5 ± 0.7	–
	5	21.5 ± 0.7	22.5 ± 0.7	–	–	20.0 ± 0.0	–
	2.5	21.0 ± 0.0	21.5 ± 0.7	–	–	18.5 ± 0.7	–
	1	19.5 ± 0.7	20.0 ± 0.0	–	–	17.0 ± 1.4	–
H16	10	15.5 ± 0.7	14.5 ± 0.7	25.0 ± 1.4	17.5 ± 0.7	11.0 ± 1.4	14.0 ± 0.0
	5	14.5 ± 0.7	12.5 ± 0.7	16.5 ± 0.7	16.0 ± 1.4	10.0 ± 1.4	10.5 ± 0.7
	2.5	12.5 ± 0.7	11.0 ± 1.4	12.5 ± 0.7	12.0 ± 1.4	8.5 ± 0.7	9.5 ± 0.7
	1	10.0 ± 0.0	8.5 ± 0.7	10.0 ± 0.0	10.5 ± 0.7	7.0 ± 0.0	8.5 ± 0.7

H1: H. *arenarium* subsp. *aucheri*, H2: *H. armenium* subsp. *armenium*, H3: *H. artvinense*, H4: *H. chionophilum*, H5: *H. compactum*, H6: *H. goulandriorum*, H7: *H. graveolens*, H8: *H. heywoodianum*, H9: *H. kitianum*, H10: *H. noeanum*, H11: *H. orientale*, H12: *H. pallasii*, H13: *H. peshmenianum*, H14: *H. plicatum* subsp. *plicatum*, H15: *H. plicatum* subsp. *polyphyllum*, H16: *H. stoechas* subsp. *barellieri*.
–: not detected.
[a] Values expressed are mean ± standard deviation of two experiments, inhibition zones include diameter of hole (4 mm).

one of the major groups of compounds acting as primary antioxidants (Pietta, 2000). Therefore, it is worthwhile to determine phenolic compounds of the *Helichrysum* species tested.

At 1%, 2.5%, 5% and 10% concentrations, the antimicrobial activity of methanolic extracts of *Helichrysum* species tested were determined by the agar-well diffusion method using 13 bacteria and two yeasts. The results of *in vitro* antimicrobial activity assay showed that the extracts possessed broad antimicrobial activity against the microorganisms tested. The extracts caused different inhibition zones on the tested microorganisms. The methanolic extracts at the lowest concentration was also the least effective. Pure methanol (control) used as solvent had no inhibitory effects on the fifteen microorganism tested.

The antibacterial effect of extracts against seven Gram (−) bacteria were shown in Table 3. Among the Gram (−) bacteria tested, the most sensitive bacteria were *A. hydrophila* and *P. aeruginosa* while the most resistant bacteria were *Y. enterocolitica*, *P. mirabilis* and *M. morganii*. Among the extracts tested, *H. armenium* subsp. *armenium* only inhibited the growth of *Y. enterocolitica* at concentrations of 5% and 10%. As clearly seen in Table 4, all of the extracts tested had activity against Gram (+) bacteria tested. *M. smegmatis* was the most resistant bacterium while *B. brevis* and *B. cereus* were the most sensitive bacteria compared to other Gram (+) bacteria. Gram (+) bacteria are more sensitive to the methanolic extracts tested than Gram (−) bacteria. The effect of extracts against *C. albicans* and *S. cerevisiae* are shown in Table 5. Among the extracts tested *H. armenium* subsp. *armenium* and *H. heywoodianum* were only effective against *S. cerevisiae*. *H. heywoodianum* was more effective than *H. armenium* subsp. *armenium* against *S. cerevisiae*. Only 8 out of 16 extracts tested inhibited the growth of *C. albicans*. These were obtained from *H. armenium* subsp. *armenium*, *H. artvinense*, *H. graveolens*, *H. heywoodianum*, *H. kitianum*, *H. orientale*, *H. pallasii* and *H. plicatum* subsp. *plicatum*. Among these species, *H. plicatum* subsp. *plicatum* was most effective against *C. albicans*.

Table 5
Antimicrobial activity against yeast of *Helichrysum* species.

Plant names (%)		*C. albicans*	*S. cerevisiae*
H. armenium subsp. *armenium*	10	7.0 ± 0.0[a]	8.0 ± 0.0
	5	6.0 ± 0.0	7.0 ± 0.0
	2.5	–	7.0 ± 0.0
	1	–	7.0 ± 0.0
H. artvinense	10	22.0 ± 0.0	–
	5	20.0 ± 0.0	–
	2.5	19.0 ± 0.0	–
	1	16.0 ± 0.0	–
H. graveolens	10	29.0 ± 0.0	–
	5	27.5 ± 0.7	–
	2.5	25.5 ± 0.7	–
	1	24.5 ± 0.7	–
H. heywoodianum	10	9.0 ± 0.0	12.5 ± 0.7
	5	8.0 ± 0.0	10.0 ± 0.0
	2.5	8.0 ± 0.0	8.0 ± 0.0
	1	8.0 ± 0.0	8.0 ± 0.0
H. kitianum	10	28.0 ± 0.7	–
	5	25.0 ± 0.0	–
	2.5	25.0 ± 0.0	–
	1	24.0 ± 0.7	–
H. orientale	10	6.0 ± 0.0	–
	5	–	–
	2.5	–	–
	1	–	–
H. pallasii	10	23.0 ± 1.4	–
	5	20.0 ± 0.0	–
	2.5	19.0 ± 0.0	–
	1	17.0 ± 1.4	–
H. plicatum subsp. *plicatum*	10	30.5 ± 0.7	–
	5	26.5 ± 0.7	–
	2.5	25.5 ± 0.7	–
	1	24.5 ± 0.7	–

–: not detected.
[a] Values expressed are mean ± standard deviation of two experiments, inhibition zones include diameter of hole (4 mm).

The antimicrobial activities of different *Helichrysum* species have been studied by different researchers. But, there is limited data on the antimicrobial activity of the *Helichrysum* species growing in Turkish flora. For example, Ozkan et al. (2004) determined that the *H. chasmolycicum* methanolic extract had antibacterial activity and *Y. enterocolitica* was the most resistant bacteria among the bacteria tested by the agar diffusion method. It has previously been reported that *H. compactum* exhibited antibacterial activity against six different *S. aureus* strains, *E. coli* and *Y. enterocolitica* (Sagdic et al., 2003). In our previous study (Albayrak et al., 2008) the antimicrobial effect of methanolic extracts of *H. pamphylicum*, *H. sanguineum* and *H. chasmolycicum* against the same microorganisms as mentioned in the present study were determined and it was reported that these extracts were inactive against *E. coli* and *P. mirabilis*.

The antimicrobial activity of methanolic extract of *H. aureonitens* against *B. cereus* was showed by Meyer and Afolayan (1995). Also, Cushine and Lamb (2005) showed that *H. aureonitens* had antibacterial activity against *S. aureus*. It was determined that the methanolic extracts of *H. dasyanthum*, *H. felinum*, *H. excisum* and *H. petiolare* were active against *B. cereus* and *S. aureus* (Lourens et al., 2004). Steenkamp, Mathivha, Gouws, and Van Rensburg (2004) reported that the methanol extract of *H. foetidum* inhibited the growth of *S. aureus*, *Streptococcus pyogenes*, *E. coli* and *P. aeruginosa*. Van Vuuren, Viljoen, van Zyl, van Heerden, and Baser (2006) showed that the acetone extract of *H. cymosum* had antimicrobial activities against *Enterococcus faecalis*, *B. cereus*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Y. enterocolitica*, *Cryptococcus neoformans* and *C. albicans* (Minimum inhibition concentration = 0.078–0.313 mg/ml). In this study, the findings were similar to the observations of previous studies. But, it is difficult to compare the results of different studies because of the different *Helichrysum* species and/or different methods used for evaluation of antimicrobial activities.

4. Conclusions

The results of this study reveal that the 16 selected species of *Helichrysum* contain a considerable amount of phenolic compounds, and had significant antioxidant and antimicrobial activity. It is believed that the results of this study will contribute to the recent increase in research on using natural products in many areas such as food, pharmacy, alternative medicine and natural therapy. Further studies should be carried out for the evaluation of the *in vivo* potential of these extracts in animal models and, isolation and identification of individual phenolic compounds, as well.

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