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ANTIOXIDANT ACTIVITIES AND TOTAL PHENOLIC COMPOUNDS AMOUNT OF SOME ASTERACEAE SPECIES

Ufuk Özgen[1*], Ahmet Mavi[2], Zeynep Terzi[1], Maksut Coşkun[3], Ali Yıldırım[2]

[1]Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, 25240, Erzurum, TURKEY

[2]Atatürk University, Kazım Karabekir Education Faculty, Department of Chemistry, 25240, Erzurum, TURKEY

[3]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100, Tandoğan-Ankara, TURKEY

Abstract

Antioxidant and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities, reducing powers and the amount of total phenolic compounds of some medicinal Asteraceae species used in folk medicine in Eastern Anatolia were studied. These species are Achillea biebersteinii, Achillea wilhelmsii, Artemisia absinthium, Artemisia austriaca, Cichorium intybus, Helichrysum arenarium subsp. rubicundum, Tripleurospermum oreades var. oreades. The highest antioxidant activity is shown by methanol extract of A. austriaca followed by water extract of A. austriaca, methanol extract of A. wilhelmsii, water extract of H. arenarium, water extract of A. biebersteinii, water extract of A. absinthium, water extract of C. intybus, water extract of A. wilhelmsii, water extract of T. oreades. The highest DPPH radical scavenging activity is also shown by methanol extract of A. austriaca followed by water extract of A. austriaca, water extract of H. arenarium, water extract of A. absinthium, water extract of A. biebersteinii, water extract of C. intybus, water extract of A. wilhelmsii, water extract of T. oreades, methanol extract of A. wilhelmsii.

Key Words: *Antioxidant, radical scavenging, reducing power, phenolic compound, Asteraceae*

Bazı Asteraceae Türlerinin Antioksidan Aktiviteleri ve Total Fenolik Bileşik Miktarları

Doğu Anadolu'da halk arasında tedavi amacıyla kullanılan bazı Asteraceae türlerinin antioksidan, 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürücü aktiviteleri, indirgeme güçleri ve total fenolik bileşik miktarları araştırılmıştır. Bu türler, Achillea biebersteinii, Achillea wilhelmsii, Artemisia absinthium, Artemisia austriaca, Cichorium intybus, Helichrysum arenarium subsp. rubicundum ve Tripleurospermum oreades var. oreades'den oluşmaktadır. En yüksek antioksidan aktivite A. austriaca'nın methanol ekstresinde gözlenmiştir; bunu sırasıyla A. austriaca su ekstresi, A. wilhelmsii metanol ekstresi, H. arenarium su ekstresi, A. biebersteinii su ekstresi, A. absinthium su ekstresi, C. intybus su ekstresi, A. wilhelmsii sulu ekstresi ve T. oreades sulu ekstresi izlemektedir. En yüksek DPPH radikal süpürücü aktivite A. austriaca'nın methanol ekstresinde gözlenmiştir; bunu sırasıyla A. austriaca su ekstresi, H. arenarium su ekstresi, A. absinthium su ekstresi, A. biebersteinii su ekstresi, C. intybus su ekstresi, A. wilhelmsii su ekstresi, T. oreades su ekstresi ve A. wilhelmsii metanol ekstresi izlemektedir.

Anahtar Kelimeler: *Antioksidan, radikal süpürücü, indirgeme gücü, fenolik bileşik, Asteraceae*

* Corresponding author Phone: +90 442 231 24 37 Fax: +90 442 236 09 62

e-mail: uozgen@atauni.edu.tr

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are various forms of activated oxygen and nitrogen, which include free radicals such as superoxide ions ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$) and nitric oxide radicals ($NO^{\cdot}$), as well as non-free-radical species such as hydrogen peroxide (H_2O_2) and nitrous acid (HNO_2) (1-3). In living organisms ROS and RNS can form in different ways. Normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells (4-6). Some exogenous sources of free radicals are tobacco smoke, ionizing radiation, organic solvents and pesticides (7-10). Free radicals can cause lipid peroxidation in foods that leads to their deterioration (11).

Oxidation does not affect only lipids. ROS and RNS may cause DNA damage that could lead to mutation (12, 13). In addition, ROS and RNS have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (6, 14-16). When produced in excess, ROS can cause tissue injury, whilst, tissue injury can itself cause ROS generation (12). Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damage and numerous damage removal and repair enzymes to remove or repair damaged molecules (8, 17-19). However, the natural antioxidant mechanisms can be inefficient, hence dietary intake of antioxidant compounds becomes important (5, 16, 20, 21). Although there are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods, it has been reported that these compounds may have side effects (22-28). In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of a number of human diseases (29, 30). Therefore, research into the determination of natural antioxidant sources is important.

This study is aimed to determine the antioxidant and DPPH radical scavenging acti-vities (AA and DPPH-RS), reducing powers (RP) and amount of total phenolic compounds (APC) of seven medicinal Asteraceae plants that have been used commonly in Eastern Turkey. These plants are *Achillea biebersteinii*, *Achillea wilhelmsii*, *Artemisia absinthium*, *Artemisia austriaca*, *Cichorium intybus*, *Helichrysum arenarium* subsp. *rubicundum* and *Tripleurospermum oreades* var. *oreades*. Parts used, uses/ailments treated and preparations of the plants are given in Table 1. Localities, parts and extraction solvents of the plants used in activity studies are given in Table 2.

TABLE 1. Local names, parts used, uses/ailments treated and Asteraceae plants used in the village of Ilıca District, Erzurum, Turkey.

Botanical Name and Voucher Specimen	Local Names	Part Used	Use/Ailment Treated	Preparation
Achillea biebersteinii Afan. (AEF 21170)	*Paz›ma, Pazvanat, Pazvana, Paspanos*	*Herb*	*Against dyspnea, gynecological diseases, urinary system infections*	*Decoction*
Achillea wilhelmsii C. Koch. (AEF 21169)	*Pazvat, Pesvana*	*Flower*	*For wound healing*	*Powder (spilled to the wounds)*
			To have an abortion	*Decoction*
Artemisia absinthium L. (AEF 21140)	*Pire otu*	*Herb*	*Against urinary system infections*	*Decoction*
Artemisia austriaca Jacq. (AEF 21139)	*Yavflan*	*Herb*	*To alleviate abdominal pain As emetic*	*Eaten fresh*
			Against dyspnea	*Decoction or powder (as a cigarette)*
			For hemorroids	*Decoction*
			For wound healing and eczema	*Powder*
Cichorium intybus L. (AEF 21144)	*Çatlangoz, Çatlangufl*	*Herb*	*For eczema and hemorrhoids*	*Decoction*
Helichrysum *arenarium (L.) Moench subsp. rubicundum (C. Koch.) Davis & Kupicha (AEF 21145)*	*Sar› çiçek*	*Flower*	*Against dyspnea, kidney stones, internal diseases, pruritis and diabetes; as antifungal*	*Decoction*
Tripleurospermum *oreades (Boiss.) Rech var. oreades (AEF 21190)*	*Papatya, Oflofl*	*Herb*	*Against alopecia, digestion and urinary system infections, stomachache, headache, abdominal pain, external infections, hemorrhoids, eczema, rheumatism, hypertension, dyspnea, cough; for mouth wounds and hair bleaching*	*Decoction*

All species were collected from some villages of Ilıca District in Erzurum Province (Turkey). They were authenticated by Dr. Ufuk Özgen and Prof. Dr. Maksut Coşkun. Voucher specimens were deposited in Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu (AEF): Tripleurospermum oreades (Boiss.) Rech var. oreades (AEF 21190), Artemisia absinthium L. (AEF 21140), Cichorium intybus L. (AEF 21144), Helichrysum arenarium (L.) subsp. rubicundum (C. Koch) Davis & Kupicha (AEF 21145), Artemisia austriaca Jacq. (AEF 21139), Achillea wilhelmsii C. Koch. (AEF 21169), Achillea biebersteinii Afan. (AEF 21170).

Experimental

Material

TABLE 2. Localities, parts and extraction solvents of plants used in activity studies

Species	Village	Collection Date	Altitude (m)	Part Used	Extraction Solvent
A. biebersteinii	Dilimli	10.06.2000	1800	Herb	Water
A. wilhelmsii	Söğütlü	12.06.2000	1800	Flower	Water and Methanol
A. absinthium	Çavuşoğlu	13.06.2000	1900	Herb	Water
A. austriaca	Söğütlü	12.06.2000	1800	Herb	Water and Methanol
C. intybus	Halilkaya	03.09.2000	2000	Herb	Water
H. arenarium	Karakale	08.08.2000	2100	Flower	Water
T. oreades	Kayapa	11.06.2000	1770	Herb	Water

Extraction

Taking consideration of traditional usage in general, the most suitable parts of plants and extraction solvents were chosen. Plants that have been used generally as decoction by public were extracted with water. Plants that have been used generally for other usages (eating, powdering for treatment) were also extracted with methanol. All plants were dried and powdered using a mill before extraction.

For extraction, 20 g powdered sample was extracted with 400 ml water or methanol by reflux about half an hour, and then filtered. Extract was evaporated and then lyophilized.

Aerial parts of *T. oreades*, *A. absinthium*, *C. intybus*, *A. austriaca*, *A. biebersteinii* and flowers of *H. arenarium* and *A. wilhelmsii* were used for extraction. Water extracts of all plants and also methanol extracts of *A. austriaca* and *A. wilhelmsii* were tested. In the AA and DPPH-RS studies, various concentrations, 50, 100, 250 and 500 µg/ml, were studied.

Determination of Antioxidant Properties

Antioxidant Activity (Thiobarbituric acid test -TBA test-)

The in vitro antioxidant activity tests were carried out using the lipid peroxidation of liposomes assay where the TBA test has been applied to assess the efficacy of the compounds to protect liposomes from lipid peroxidation (31). The TBA reaction is based on the fact that peroxidation of most membrane systems leads to formation of small amounts of free malondialdehydes (MDA). One molecule of MDA reacts with two molecules of TBA (Sigma) to yield a colored product, which in an acidic environment absorbs light at 532 nm and it is readily extractable into organic solvents (31). It can, thus, be measured and quantified spectrophotometrically. The intensity of color is a measure of MDA concentration. To eliminate the solvent effect, control was the test solution containing the extraction solvent. Absorbance at 532 nm was determined on a Helios β UV/VIS spectrophotometer.

The incorporation of any antioxidant compound in the lipid peroxidation assay reaction mixture will lead to a reduction of the extent of peroxidation. The methanolic and/or aqueous extracts from investigated plants were tested for their antioxidant activity against peroxidation of liposomes which were prepared from bovine brain extract in phosphate buffered saline (5 mg/ml) in the laboratory. Peroxidation was started by adding $FeCl_3$ (Riedel-de Haen) and ascorbic acid (Merck) followed by incubation at 37 °C for 20 min. Ascorbic acid is a well known anti-oxidant but also pro-oxidant property in the presence of certain transition metal ions, such as Fe or Cu (7). BHT (Sigma) in ethanol was added to prevent lipid peroxidation during the TBA test itself. Propyl gallate (Sigma) was used as a positive control at 2 µg/ml concentration. Data are given as % peroxidation inhibition-concentration (Table 4) and IC_{50} (µg/ml extract concentration required for 50% peroxidation inhibition) (Figure 1)

DPPH Radical-Scavenging

This was carried according to Blois method with a slight modification (32). Briefly, 1 mM solution of DPPH (Sigma) radical solution in methanol was prepared and then, 1 ml of this solution was mixed with 3 ml of extract solution in ethanol. After 30 minutes incubation in dark, absorbance was measured at 517 nm. This activity is given as IC_{50}RS (µg/ml extract concentration required for 50 % inhibition of the DPPH radical absorbance at 517 nm) and % DPPH radical scavenging that is calculated in equation; % DPPH Radical Scavenging = ((Control Absorbance - Extract Absorbance)/(Control Absorbance)) x100

BHT (butylated hydroxy toluene) was used as a positive control at 40 µg/ml concentration. Control was the test solution without extract.

Reducing Power

This was carried out as described previously (33). Briefly, extract solution were mixed with 0.2 M, pH 6.6 phosphate buffer (final volume 3.5 ml). 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (Fluka) ((1%), then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 ml of trichloroacetic acid (10%) (Sigma) was added to the mixture that was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (Riedel-de Haen) (0.1%), and the absorbance was measured at 700 nm. The same procedure was performed for ascorbic acid at different concentrations. Thus, reducing powers of the extracts were expressed as ascorbic acid equivalent using calibration curve.

Amount of Total Phenolic Compounds

This was carried out as described previously (34). Briefly, extract solution was transferred into test tube then final volume was adjusted to 4 ml by addition of distilled water. Afterwards,

0.25 ml of Folin-Ciocalteu Reactive (FCR) (Fluka) was added into this mixture and after 3 minutes 0.75 ml of Na_2CO_3 (20%) was added into. Subsequently, mixture was shaken on a shaker for 2 hours at room temperature and then absorbance was measured at 760 nm. Gallic acid (Sigma) was used as a standard phenolic compound. Thus, the phenolic compound content was determined as gallic acid equivalent using ca-libration curve.

Statistical Analyzes

In all cases three measurements were performed. The results shown are the means of these measurements (Table 4). Data were analyzed with SPSS software to determine whether there is any correlation between antioxidant properties in an extract. Pearson parametric correlation analyzes was carried out with SPSS software.

Result and Discussion

All extracts have antioxidant and DPPH radical scavenging activities, reducing po-wers and phenolic compounds. In addition, these antioxidant properties are concentration dependent at studied range (Table 4). Especially, methanolic extracts of *A. austriaca* has more antioxidant potential than others and its aqueous extract (IC_{50}: 88µg/ml, IC_{50}RS: 146 µg/ml in Figure 1). Methanolic extract of *A. austriaca* has also the highest reducing power and amount of phenolic compounds. Aqueous extract of *A. austriaca* has lower antioxidant potential than its methanolic extract but more than the other plants. The lowest antioxidant potential was shown by aqueous extracts of *T. oreades* and *A. wilhelmsii* whose antioxidant and DPPH radical scavenging activities, reducing powers and amount of phenolic compounds are relatively lower than the others (Figure 1 and Table 4).

Data were analyzed with SPSS software to determine whether there is any correlation between antioxidant properties and the extracts. In the aqueous extracts of *T. oreades*, *A. absinthium* and *A. wilhelmsii,* there is a statistically significant correlation between all antioxidant properties ($p<0.05$) (Table 3). In the other extracts, statistically significant correlation is observed between only some antioxidant properties. For example, *A. biebersteinii* in which there is not a statistically significant correlation between AA and DPPH-RS but among the others (Table 3). From these results, we can suggest that AA may be affected by different parameters, such as DPPH-RS, RP, and APC. In contrast, there is a statistically significant correlation between RP and APC in all extracts. In the light of this, it could be speculated that phenolic compounds may mainly cause RP of the extracts.

TABLE 3. *p* values of Pearson parametric correlation analyzes carried out with SPSS software.

	DPPH-RS	RP	APC	DPPH-RS	RP	APC	DPPH-RS	RP	APC
AA	*0.024*	*0.051*	*0.08*	*0.079*	*0.085*	*0.093*	*0.076*	*0.063*	*0.055*
DPPH-RS		*0.037*	*0.068*		*0.039*	*0.051*		*0.001*	*0.003*
RP			*0.005*			*0.001*			*0.001*
	*A. austriaca**			*A. austriaca*			*H. arenarium*		
AA	*0.063*	*0.029*	*0.017*	*0.069*	*0.033*	*0.042*	*0.005*	*0.000*	*0.000*
DPPH-RS		*0.015*	*0.020*		*0.009*	*0.004*		*0.006*	*0.007*
RP			*0.002*			*0.002*			*0.000*
	*A. wilhelmsii**			*A. biebersteinii*			*A. absinthium*		
AA	*0.099*	*0.047*	*0.059*	*0.036*	*0.012*	*0.006*	*0.004*	*0.011*	*0.016*
DPPH-RS		*0.015*	*0.008*		*0.007*	*0.013*		*0.002*	*0.006*
RP			*0.001*			*0.001*			*0.001*
	C. intybus			*T. oreades*			*A. wilhelmsii*		

*Methanol extract; If asterisk is not present, it indicates water extracts

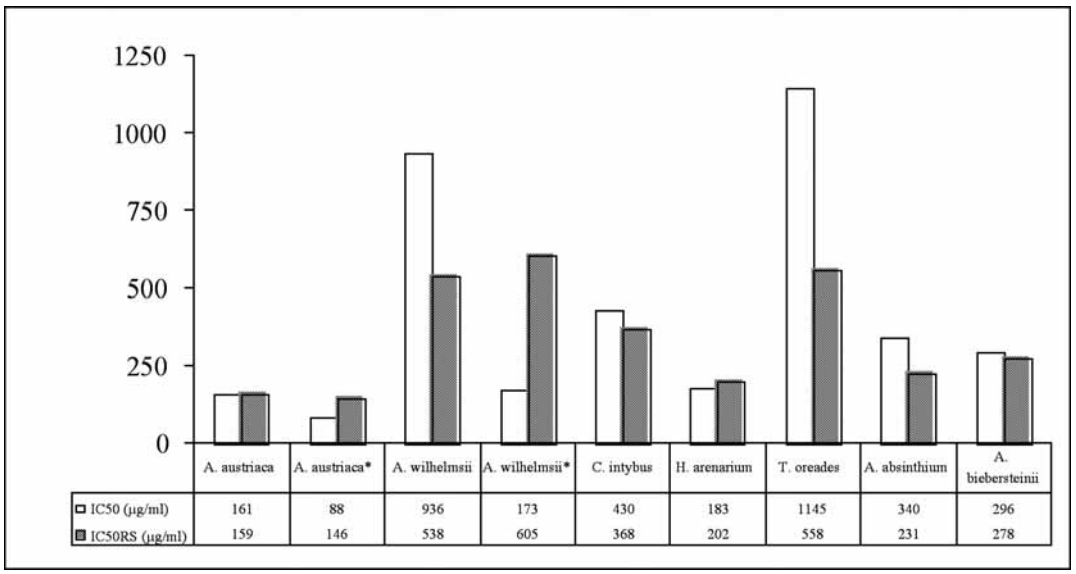

FIGURE 1. IC_{50} and $IC_{50}RS$ values of extracts.

*Methanol extract; If asterisk is not present, it indicates water extracts

IC_{50}: µg/ml extract concentration required for 50% peroxidation inhibition

$IC_{50}RS$: µg/ml extract concentration required for 50 % inhibition of the DPPH radical

TABLE 4. Antioxidant activities and total phenolic compounds amount of plants.

	AA (Inhibition %)				DPPH-RS (Inhibition %)				RP (μg/ml Ascorbic Acid Equivalent)				APC (μg/ml Gallic Acid Equivalent)			
Concentration (μg/ml)	**50**	**100**	**250**	**500**	**50**	**100**	**250**	**500**	**50**	**100**	**250**	**500**	**50**	**100**	**250**	**500**
*A. austriaca**	41.6±8.8	52.4±1.7	65.3±4.2	71.5±3.3	19.9±4.6	30.0±3.1	76.5±8.0	91.5±0	3.8±0.1	7.0±0.2	15.8±0.2	26.7±0.5	4.7±0.1	9.0±1.2	18.8±0.9	37.2±7.7
A. austriaca[Δ]	7.1±4.5	45.3±3.4	63.4±0	81.6±2.2	17.0±0	27.5±0.6	73.7±0.5	87.8±0.2	2.7±0.2	5.5±0.2	12.9±0.4	22.1±0.4	2.7±0.1	4.9±0.2	10.3±0.2	18.2±1.2
H. arenarium[Δ]	0.7±4.1	38.7±4.0	60.3±0	80.6±1.2	20.6±0	28.3±0.8	58.6±0.8	89.0±0.2	3.8±0.1	6.0±0.1	11.9±0.4	18.2±1	5.2±0.1	9.5±0.2	17.9±0.7	28.2±0.7
*A. wilhelmsii**	31.4±2.5	43.5±1.9	53.5±2.1	66.4±2.5	7.9±1.4	10.3±0	19.6±1.6	41.1±0.5	2.1±0.3	3.4±0.1	7.8±0.8	13.1±1.2	2.5±0.4	5.4±0.2	11.3±1	18.8±0.9
A. biebersteinii[Δ]	9.8±6.3	30.3±1.7	46.5±0	62.4±3.9	17.0±0	22.4±1.8	47.7±1.7	87.1±0.8	3.2±0.2	5.5±0.3	11.3±0.3	17.7±1.2	3.1±0	5.6±0.1	11.5±0.3	19.8±1.1
A. absinthium[Δ]	8.7±0	16.9±0	40.1±1.1	65.7±9.9	7.8±0	16.7±1.1	57.9±1.9	86.5±0.3	4.1±0.2	7.0±0.2	14.5±0.2	22.9±0.8	3.8±0.2	7.2±0.1	14.9±0.1	24.1±0.9
C. intybus[Δ]	7.9±5.8	26.7±8.6	40.0±0	51.6±4.6	3.1±0	10.4±2.5	35.1±1.8	84.9±0.7	2.4±0.1	5.1±0.2	11.8±0.1	19.3±0.2	4.2±0.2	7.1±0.1	14.9±0.4	25.2±0.9
T. oreades[Δ]	12.4±3.1	16.7±0.6	24.4±2.6	31.7±0.6	0±0	2.8±1.6	18.5±0	49.0±1.7	2.1±0.2	3.3±0.1	7.0±0	12.1±0.3	2.3±0.1	4.1±0.1	8.6±0.1	14.3±0.5
A. wilhelmsii[Δ]	0.9±2.1	3.4±3.0	10.0±0	28.4±1.6	10.1±2.4	12.9±0	25.3±0	47.3±0.7	2.5±0.2	3.4±0.3	6.4±0.2	10.8±0.5	2.5±0	4.5±0.1	9.3±0.2	16.0±0.2
Propyl gallate (2μg/ml)	95±1.0				-				-				-			
BHT (40 μg/ml)	-				75.7±1.9				-				-			

[Δ]Water extract *Methanolic extract

Asteraceae (Compositae) is the largest family of flowering plants and contains about 900 genera and some 13,000 species. The Asteraceae contains a wide variety of chemical constituents. Already mentioned are the latex of the Liguliflorae and the inulin, which is often present in very large amounts (e.g. in dahlia tubers). Some of the volatile oils found in the Tubuliflorae contain acetylenic compounds and the sesquiterpenes known as azulenes. Many sesquiterpene lactones occur and are of varing types, including eudesmanolides (e.g. santonin), germacranolides and pseudoguaianolides. Pyrrolizidine, pyridine, quinoline and diterpenoid type alkaloids occur in the family. Other constituents include the insecticidal esters of pyrethrum, triterpenoid saponins of grindelia, cycliyols, coumarins and flavonoids (35).

Artemisia austriaca contains flavonoids and sesquiterpene lactone (36, 37), *A. absinthium* has sesquiterpene lactones (38), *Achillea biebersteinii* contains flavonoids and other constituents (39), *A. wilhelmsii* contains flavonoids and sesquiterpene lactones (40). *Cichorium intybus* contains coumarins and sesquiterpene lactones (41, 42). *Helichrysum arenarium* has flavonoids and phenolic compounds (43, 44). It has not been found any phytochemical study on *Tripleurospermum oreades,* but there are some studies a few *Tripleurospermum* species (e.g. *T. maritimum, T. perforatum*). These two species contain flavone and flavonol glycosides (45). It has been reported that polar subfraction of the methanol extract of *Achillea biebersteinii* shown antioxidant activity (46).

As mentioned above, these plants contain phenolic compounds, especially flavonoids. Flavonoids have antioxidant potential (47). Therefore, their antioxidant and radical scavenging activities, reducing powers could be caused by these flavonoids. However, antioxidant activities of extracts are not higher than propyl gallate (Sigma).

Lipid peroxidation is a chain reaction. This reaction can be initiated by a reactive radical abstracting an electron from a nonradical. Thus, radical is transformed a nonradical. However, simultaneously a new radical can be formed and so reaction can continue. In presence of phenolic compounds hydroxyl hydrogen with an electron can be donated, thus radical can be scavenged. Because of the resonance stability, newly formed phenoxy radical is more stable than firstly formed radical. Thus, chain reactions can be retarded (7). However, as mentioned above, we could not find statistically significant correlation between antioxidant activity and amount of phenolic compounds in some extracts. The same situation was seen between DPPH radical scavenging activity and amount of phenolic compounds. Especially, aqueous extract of *A. austriaca* has relatively lower phenolic compound, its antioxidant activity relatively higher. At first glance, it can be thought as a contradiction. Nevertheless, it should be kept in mind that antioxidant activity is the consequence of cooperative behaviors of all antioxidant properties (e.g. radical scavenging, reducing power, decomposition of peroxides) (32) and phenolic compounds do not have same antioxidant activities. In addition some of them can be act as prooxidant. Therefore, amount of phenolic compounds could not be major criteria in the assessment of antioxidant activity. In

addition, solubility of a phenolic compound in the peroxidation environment can affect its activity.

At 50 µg/ml concentration, methanol extracts are more effective antioxidants than water extracts, although it contains relatively lower amount of phenolic compounds. As it can be seen in table 4, aqueous and methanolic extracts of *A. wilhelmsii* has the same amount of phenolic compounds at 50 µg/ml concentration, while antioxidant activity of methanolic extracts is markedly higher than water extract. In addition, at the same concentration, although *H. arenarium* has higher amount of phenolic compound (5.2 µg/ml Gallic Acid Equivalent) than methanolic extract of *A. austriaca* (4.7 µg/ml Gallic Acid Equivalent), antioxidant activity of methanolic extracts is markedly higher than water extract. In the light of these results, it can be speculated that at 50 µg/ml concentration, methanol extracts are more effective antioxidants. Solubility of water extracts, which contain relatively more polar compounds in the water phase, may be more than that of apolar peroxidation environment. In contrast, solubility of methanol extracts, which contains relatively more apolar compounds in the peroxidation environment, may be more than that of polar water phase. Therefore, methanol extracts could be more effective antioxidant at 50 µg/ml concentration in this test system.

Determinations of compounds, which are responsible for antioxidant activity of A. austriaca, are the aim of the further studies.

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