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Screening of Total Flavonoid, Phenol Contents and Antioxidant Capacities of Some *Achillea* L. species growing in Turkey

Türkiye'de Yayılış Gösteren Bazı *Achillea* L. Türlerinin Total Flavonoid, Fenol İçerikleri ve Antioksidan Kapasitelerinin İncelenmesi

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Abstract

The aim of the present study was to investigate the antioxidant capacities of the infusions prepared from 15 *Achillea* L. (Asteraceae) species growing in Turkey. The antioxidant capacities of these species were evaluated by using different methods (total antioxidant capacity, free radical and $OH^{\bullet}$ radical scavenging capacity, H_2O_2 reducing power). Total flavonoid content was determined by using the aluminium-chloride method. Total phenol content was determined by the modified colorometric method using the Folin-Ciocalteu Reagent. Our results clearly demonstrate that all infusions have antioxidant capacity. *A. millefolium* ssp. *pannonica* has the highest antioxidant activity with higher value than 10 mM α-tocopherol/100 ml. Moreover, *A. grandifolia, A biebersteinii, A. schisckinii, A. nobilis* ssp. *neilrechii* has ability as 8-8.7 mM α-tocopherol/100 ml. These results are consistent with total flavonoid and phenol contents.

Key words: *Achillea*, flavonoid, phenol, antioxidant, scavenging capacity.

Introduction

In recent years, a worldwide trend towards the use of natural phytochemicals present in plants with an antioxidant role has been proposed for use in foods. Natural antioxidants may have applications in the food industry and there is some evidence that these substances may carry over an antioxidant effect within the human body after consumption (Lim *et al.*, 2001).
Achillea L. (Asteraceae) is represented by 42 species in the flora of Turkey and the rate of endemism is 50% (Huber-Morath *et. al.*, 1975; Duman *et. al.*, 2000). Infusions prepared from *A. millefolium* and related species are frequently used as diuretic, appetizing, emmenagog, for wound healing, abdominal pain and against diarrhea in Turkish traditional medicine (Yeşilada *et. al.*, 1993; Fujita *et. al.*, 1995; Honda *et. al.*, 1996; Baytop, 1999). Many papers about secondary metabolites (Marchart *et. al.*, 2003; Glasl *et. al.*, 2001; Kubelka *et. al.*, 1999; Kastner *et. al.*, 1995; Chandler *et. al.*, 1982) and bioactivities (Karamenderes *et. al.*, 2003; Ünlü *et. al.*, 2002; Rezaeipoor *et. al.*, 1999; Montanari *et. al.*, 1998) of *Achillea* species can be found in the literature, but only essential oil of *A. millefolium* was investigated for *in vitro* antioxidant features (Candan *et. al.*, 2003). The aim of the present study was to investigate antioxidant

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activity of infusions of 15 species of *Achillea* including *A. clypeolata* Sm., *A. schischkinii* Sosn., *A. teretifolia* Willd., *A. coarctata* Poir., *A. phyrigia* Boiss.&Bal., *A. crithmifolia* Waldst.&Kit., *A. nobilis* L. ssp. *neilrechii* (Kerner) Formánek, *A. millefolium* L. ssp. *millefolium, A. biebersteinii* Afan., *A. kotschyi* Boiss. ssp. *kotschyi, A. nobilis* L. ssp. *sipylea* (O. Schwarz) Bässler, *A. setacea* Waldst.&Kit., *A. falcata* L., *A. grandifolia* Friv. and *A.multifida* (DC.) Boiss.

Materials and Methods

Chemicals: Hydrogen peroxide (3% solution), methanol, glacial acetic acid, aluminium chloride, ammonium molybdate, ethyl acetate, quercetin, gallic acid, Folin-Ciocalteu Reagent obtained from Merck. Thiobarbituric acid and 2,2-diphenyl-2-picrylhydrazyl hydrate obtained from Sigma-Aldrich. Other chemicals used were of analytical grade.
Plant Material: Achillea species were collected at the time of full flowering from various locations of Turkey in the years 2000 and 2001 (Table 1). Voucher specimens were identified and deposited in the Herbarium of Ege University, Faculty of Pharmacy, Izmir (IZEF), Turkey

Table 1. Localities of collected *Achillea* species.

Plant name	**Locality**	**IZEF Number**
A. clypeolata Sm.	Kırklareli, Vize, 500m	5479
A. schischkinii Sosn.	Sivas, Karacaören, 1800m	5503 **E**
A. teretifolia Waldst&Kit.	Niğde, Altunhisar, Melediz Mountain, 1700m	5497
A. coarctata Poir.	Tekirdağ, Ganos Mountain, 200m	5473
A. phyrigia Boiss&Bal.	Kırşehir, Mucur, Seyfe Lake, 1000m	5498 **E**
A. crithmifolia Waldst&Kit.	Kırklareli, Kıyıköy, 220m	5477
A. nobilis L. ssp. *neilrechii* (Kerner) Formănek	Burdur, Elmalıyurt, 1350m	5510
A. millefolium L. ssp. *pannonica* (Scheele) Hayek	Kırklareli, İğneada, 450m	5481
A. biebersteinii Afan	Konya, Aksaray, Hasan Mountain, 1600m	5501
A. kotschyi Boiss. ssp. *kotschyi*	Erzurum, Oltu Kaleboğazı Village, 1250m	5505
A. nobilis L. ssp. *sipylea* (O.Schwarz) Bässler	Manisa, Spil Mountain, 1350m	5511 **E**
A. setacea Waldst&Kit.	Kırklareli, Saray, 210m	5476
A. falcata L.	Burdur, Elmalıyurt, 1600m	5509
A. grandifolia Friv.	İzmir, Kemalpaşa, Nif Mountain, 1100m	5514
A. multifida (DC) Boiss.	Bursa, Uludağ, 1865m	5598 **E**

E: Endemic

Preparation of infusions: Dried and pulverized flower heads of plants were boiled in distilled water (1:1; mg/ml) for 2 min, then centrifuged at 4000 rpm for 5 min and the supernatants were used for the experiments (Auddy *et. al.*, 2002).

Total Antioxidant Capacity (TAC): The spectrophotometric assay for the quantative determination of antioxidant capacity was carried out (Prieto *et al.*, 1999). The assay is based on reduction of Mo (VI) to Mo (V) by the sample analyst and subsequent formation of a green phosphate Mo (V) complex at acidic pH. The amount of TAC were expressed for samples in mM α-tocopherol/100 ml infusion.

Free Radical Scavenging Capacity (DPPH-RSC): Free radical scavenging capacity of plant infusions against stable DPPH$^{\bullet}$ (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically (Nagaia *et. al.*, 2002). When DPPH$^{\bullet}$ reacts with an antioxidant compound, that can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light-yellow) were measured at 515 nm on a UV/visible light spectrophotometer.

OH$^{\bullet}$ Radical Scavenging Capacity (OH$^{\bullet}$-RSC): Hydroxyl radical scavenging capacity was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{+3}/ascorbate/EDTA/H_2O_2 system. The attack of the hydroxyl radical to deoxyribose leads to TBARS formation. The absorbance of formed TBARS were measured at 520 nm. OH$^{\bullet}$ radical scavenging ability was evaluated as the inhibition rate of 2-deoxyribose oxidation by OH$^{\bullet}$ (Wettasinghe *et al.*, 1999).

H_2O_2 Reducing Power (H_2O_2-RP): 0.1 ml infusion sample (1mg/ml) was prepared in 3.3 ml of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µl of 43 mM solution of hydrogen peroxide (prepared in the same buffer). The absorbance value (at 230 nm) of the reaction mixture was recorded at 0 min and then at 5.min and 10.min. For each concentration, a separate blank sample (devoid of hydrogen peroxide) was used for background subtraction (Wettasinghe, *et. al.* 1999). The reduction in H_2O_2 concentration in assay media was expressed as %.

Flavonoid Content Determination: Total flavonoid contents were determined by aluminium chloride method (Deutsches Arzneibuch, 1996) measuring flavonoids in $AlCl_3$-complex form of purified ethyl acetate phase obtained after acid hydrolysis. The amount of flavonoid were expressed as %.

Total Phenol Content Determination: The adapted method used for the determination of total phenols by using Folin-Ciocalteus Reagent (Mc Donald *et al.*, 2001). Total phenol values are expressed as gallic acid equivalents (mg/L infusion samples) which is a common reference compound.

Results

Total flavonoid and phenol contents of the *Achillea* infusions (1mg/ml) are expressed as mean ± SD of 5 separate experiments (Table 2).

Total antioxidant capacity, free radical (DPPH) and OH$^{\bullet}$ radical scavenging capacity and H_2O_2 reducing power of the plant infusions (1mg/ml) are given as mean ± SD of 3 separate experiments (Table 3).

Discussion

Much attention has been focused on the protective biochemical function of naturally occuring antioxidants in biological systems and on the mechanism of their action. Despite much interest in the antioxidant activity of *Achillea* species, it is uncertain which of the phenols and flavonoids exhibit the greatest antioxidant effect. The TAC method based on reduction of Mo (VI) to Mo (V) by the sample analyst was used to measure the amount of total antioxidant capacity. As a result, the high ability was detected in *Achillea* species. Especially, *A. millefolium* ssp. *pannonica* has the highest antioxidant activity with higher value than 10 mM

α-tocopherol/100 ml. Moreover, *A. grandifolia, A biebersteinii, A. schisckinii, A. nobilis* ssp. *neilrechii* was ability as 8-8.7 mM. α-tocopherol/100 ml. Thus it was found that the *Achillea* species possesed the antioxidative substances having high activity.

Table 2. Total flavonoid and phenol contents of the *Achillea* infusions.

Plant	Total Flavonoid (%)	Total Phenol (mg/L)
A. clypeolata	0.131±0.0086	109.090± 1.818
A. schischkinii	0.248±0.0081	135.757±1.050
A. teretifolia	0.151±0.0334	100.000±1.830
A. coarctata	0.075±0.0087	120.605±1.139
A. phyrigia	0.366±0.014	110.908±3.149
A. crithmifolia	0.233±0.0086	158,182±4.810
A. nobilis ssp. *neilrechii*	0.136±0.0016	119.395±2.778
A. millefolium ssp. *pannonica*	0.329±0.0015	181.212±2.417
A. biebersteinii	0.232±0.0016	145.757±2.288
A. kotschyi ssp. *kotschyi*	0.108±0.0012	134.242±1.388
A. nobilis ssp. sipylea	0.255±0.0015	147.877±2.776
A. setacea	0.096±0.0084	121.818±1.818
A. falcata	0.250±0.0080	157.787±1.136
A. grandifolia	0.179±0.0021	119.395±2.778
A. multifida	0.218±0.0013	151.212±1.388

Table 3. TAC, DPPH-RSC, OH -RSC and H_2O_2-RP of the *Achillea* infusions.

Plant	TAC (mMαTocopherol/100ml)	DPPH-%RSC	$OH^{\bullet}$-%RSC	H_2O_2-%RP
A. clypeolata	7.056±0.040	32.624±0.287	40.879±0.589	50.150±0.250
A. schischkinii	8.514±0.080	33.586±0.435	45.453±0698	55.652±0.395
A. teretifolia	6.928±0.101	28.700±0.554	39.036±0.145	47.100±0.420
A. coarctata	4.671±0.141	23.886±1.304	35.012±0.478	40.915±0.517
A. phyrigia	7.242±0.140	34.809±0.857	39.709±0.985	49.350±0.499
A. crithmifolia	6.485±0.121	26.941±0.520	38.954±1.471	45.257±0.722
A. nobilis ssp. *neilrechii*	8.086±0.114	36.427±0.435	47.789±1.250	55.758±0.818
A. millefolium ssp. *pannonica*	10.128±0.181	41.574±1.71	56.471±0.987	70.300±0.920
A biebersteinii	8.419±0.129	33.456±0.920	46.478±0.147	55.820±0.815
A. kotschyi ssp. *kotschiyi*	5.599±0.161	27.381±0.756	37.895±0.874	44.150±0.620
A. nobilis ssp. *sipylea*	5.485±0.120	26.691±0.561	36.236±0.168	45.138±0.515
A. setacea	6.999±0.201	27.429±0.895	38.148±0.582	44.280±0.289
A. falcata	6.742±0.242	23.739±0.689	39.447±0.921	46.300±0.395
A. grandifolia	8.742±0.242	36.654±0.852	46.456±0.685	54.841±0.427
A. multifida	6.285±0.195	32.579±0.654	39.590±0.967	46.259±0.639

From DPPH radical scavenging test, it was found that *Achillea* species functioned effectively and lastingly among these samples. The capacity of prepared infusions to scavenge the 'stable' free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was monitored with some slight modifications (Nagai *et al.*, 2002). DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples (Lim *et al.*, 2001; Kweon *et al.*, 2001; Imark *et al.*, 2001; Nagai 2002). It is reported that DPPH radical scavenging activities of nine berries were associated with the contents of the total phenolics and flavonoids (Amakura *et al.*, 2000). It suggests that *Achillea* species having stronger DPPH radical scavenging activity seems to include high contents of the total phenolics because of easily extraction of phenolic compounds with water. According to free radical scavenging (DPPH) test, each of the *Achillea* infusions showed 23-41% DPPH$^{\bullet}$ radical scavenging ability. It was suggested that *Achillea* infusions had DPPH$^{\bullet}$ radical scavenging-like activities. Among these tested samples, *A. millefolium* ssp. *pannonica* showed the highest ability against DPPH radical.

Flavonoids were reported as hydroxyl radical scavengers (Husain *et al.*,1987; Nagai *et al.*, 2002). It is also noted that effectiveness of such compounds increases with increasing number of hydroxyl groups attached to the aromatic B-ring. As is the case for many other free radicals, OH$^{\bullet}$ can be neutralized if it is provided with a hydrogen atom. The highest hydroxyl radical scavenging activity was observed in *A. millefolium* ssp. *pannonica.* Moreover, highly hydroxyl radical scavenging activity was found in *A. grandifolia, A biebersteinii, A. schischkinii* and *A. nobilis* ssp. *neilrechii*. Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from membrane and bring about peroxidic reactions of lipids (Nagai *et al.*, 2002). From this point, it is expected that *Achillea* species demonstrate the antioxidant effects against lipid peroxidation to scavenge the hydroxyl radicals at the stage of initiation and termination of peroxyl radicals.

The concentration of H_2O_2 in the systems containing infusion dropped during the initial 10 min period of the assay. During this period, the reduction in H_2O_2 concentration in the systems containing 0.1 ml infusion samples was 44-70% of the initial concentration. The decomposition of H_2O_2 into water may occur according to the following reaction:

$$H_2O_2 + 2H^+ + 2e \longrightarrow 2\,H_2O$$

Since phenolic compounds present in the plant are good electron doners, they may accelerate the conversion of H_2O_2 into H_2O. There is well supported evidence that the phenolic compounds found in various plant materials posses free radical scavenging properties (Shi, *et al.*, 1991; Husain *et al.*, 1987). In addition, it was reported that flavonoids were OH$^{\bullet}$ scavengers (Nagai, *et al.*, 2002). The highest total phenol, flavonoid contents and antioxidant capacities were found in *A. millefolium* ssp. *pannonica.*

In conclusion, we demonstrated that *Achillea* infusions, used traditionally, are good scavengers of active oxygen species (including hydroxyl radical, H_2O_2) and DPPH$^{\bullet}$ (free radical 2,2-diphenyl-1-picrylhydrazyl). Antioxidant capacity results are consistent with total flavonoid and phenol contents.

Özet

Bu çalışmanın amacı Türkiye'de yayılış gösteren 15 *Achillea* L. (Asteraceae) türünden hazırlanan infüzyonların antioksidan kapasitelerinin araştırılmasıdır. Bu türlerin antioksidan kapasiteleri farklı yöntemler kullanılarak incelendi (total antioksidan kapasite, serbest radikal ve OH$^{\bullet}$ radikal temizleme kapasitesi, H_2O_2 azaltma gücü). Total flavonoid içeriği alüminyum klorid yöntemi kullanılarak belirlendi. Total fenol içeriği Folin-Ciocalteus belirteci kullanılan modifiye kolorimetrik bir yöntemle saptandı.

Sonuçlarımız *Achillea* türlerinden hazırlanan tüm infüzyonların antioksidan etkiye sahip olduğunu açıkça göstermektedir. *A. millefolium* ssp. *pannonica* 10 mM α-tokoferol /100 ml'den daha yüksek bir değerle, en fazla antioksidan kapasiteye sahiptir. Ayrıca, *A. grandifolia, A biebersteinii, A. schisckinii, A. nobilis* ssp. *neilrechii* 8-8.7 mM α-tokoferol/100 ml antioksidan kapasitededir. Bu sonuçlar, total flavonoid ve fenol içerikleri ile uyumludur.

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References

Amakura, Y., Umino, Y., Tsuji, S., Tonogai, Y. (2000). Influence of jam processing on the radical scavenging activity and phenolic content in berries. *J. Agric. Food Chem.* 48: 6292-97.

Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F., Tripathi, P.C., Seal, T., Mukherjee, B. (2002). Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenarative diseases. *J. Ethnopharmacology* 84: 131-138.

Baytop, T. (1999). Türkiye'de Bitkiler ile Tedavi. 2. ed., Nobel Tıp Kitabevleri, Ltd.Şti., Istanbul.

Candan, F., Ünlü, M., Tepe, B., Daferera, D., Polissiou, P., Sökmen, A., and Akpulat, A. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). *J. Ethnopharmacology* 87: 215-220.

Chandler, R.F., Hooper, S.N., Harvey, M.J. (1982). Ethnobotany and Phytochemistry of Yarrow, *Achillea millefolium* Compositae. *Economic Botany* 36: 203-223.

Deutsches Arzneibuch, (DAB 10) (1996). Amtliche Ausgabe, Published by Deutscher Apotheker Verlag, Stuttgart.

Duman, H., *Achillea* L., Flora of Turkey and the East Aegean Islands (Güner, A., Özhatay N., Ekim T., Başer K.H.C. eds.), vol.11, Edinburgh University Press, Edinburgh (2000) p.158-159.

Fujita, T., Sezik, E., Tabata, M., Yesilada, E., Honda, G., Takeda, Y., Tanaka, T., Takaishi, Y. (1995). Traditional medicine in Turkey VII. Folk medicine in middle and west Black Sea Regions. *Economic Botany* 49: 406-422.

Glasl, S., Presser, A., Gunbilig, D., Werner, I., Narantuya, S., Haslinger, E., Jurenitsch, J., and Kubelka W. (2001). Highly hydroxylated guaianolides of *Achillea asiatica* and Middle European *Achillea* species. *Phytochemistry*, 58: 1189-1194.

Honda G., Yeşilada E., Tabata M., et.al. (1996). Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydin provinces, *J. Ethnopharmacology* 53: 75-87.

Huber-Morath A. (1975). *Achillea* L., in Flora of Turkey and the East Aegean Islands (Davis, P.H. ed.), vol.5, Edinburgh University Press, Edinburgh.

Husain, S.R., Terao, J. And Matsushita, S. (1987). Effect of browning reaction products of phospholipids on antioxidation of methyl linoletae. *J. Am. Oil. Chem. Soc.* 72: 999-1006.

Imark, C., Kneubühl, M., Bodmer, S. (2001). Occurence and activity of natural antioxidants in herbal spirits. *Innov. Food Sci. Emer. Tech.* 1: 239-243.

Karamenderes C., Apaydin, S. (2003). Antispasmodic effect of *Achillea nobilis* L. subsp. *sipylea* (O. Schwarz) Bässler on the rat isolated duodenum. *J. Ethnopharmacology* 84: 175-179.

Kastner, U., Glasl, S., Jurenitsch, J. (1995). *Achillea millefolium* ein Gallentherapeutikum? *Zeitschrift für Phytoterapie* 16: 34-39.

Kubelka, W., Kastner, U., Glasl, S., Saukel, J., and Jurenitsch, J. (1999). Chemotaxonomic relevance of sesquiterpenes within the *Achillea millefolium* group. *Biochem. Syst. and Eco.* 27: 437-444.

Kweon, M., Hwang, H., Sung, H. (2001). Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). *J. Agric. Food Chem.* 49: 4646-4655.

Lim, K.T., Hu, C., Kitts, D.D. (2001). Antioxidant activity of a *Rhus verniciflua* Stokes ethanol extract. *Food Chem. Tox.* 39: 229-237.

Marchart, E., and Kopp, B. (2003). Capillary electrophoretic separation and quantification of flavone-O- and C-glycosides in *Achillea setacea* W. et K. *J. Chromatogr.* B 792: 25363-368

Mc Donald, S., Prenzler, P.D., Antolovich, M., and Robards, K. (2001). Phenolic content and antioxidant activity of *Olive* extract. *Food Chemistry* 73: 73-84.

Montanari, T., Ernesto de Carvalho, J., Dolder H. (1998). Antispermatogenic effect of *Achillea millefolium* L. in mice. *Contraception* 58: 309-313.

Nagai, T., Inoueb, R., Inoueb, H., Suzukia, H. (2002). Scavenging capacities of pollen extracts from *Cistus ladaniferus* on autoxidation, superoxide radicals, hydroxyl radicals, and DPPH radicals. *Nutrition Research* 22: 519–526.

Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* 269: 337-341.

Rezaeipoor, R., Saeidnia, S., Kamalinejad, M. (1999). Immunosuppressive activity of *Achillea talagonica* on humoral immune responses in experimental animals, *J. Ethnopharmacology* 65: 273-276.

Shi, X., Dalal, N.S., Jain, A.C. (1991). Antioxidant behavior of caffeine: Efficient scavenging of hydroxyl radicals. *Food Chem. Tox.* 29: 1-6.

Ünlü, M., Daferera, D., Dönmez, E., Polissiou, M., Tepe, B., Sökmen, A. (2002). Compositions and the in vitro antimicrobial activities of the essential oils of *Achillea setacea* and *Achillea teretifolia* (Compositae), *J. Ethnopharmacology*. 83: 117-121.

Wettasinghe, M., Shahidi, F. (1999). Antioxidant and free radical-scavenging properties of ethanolic extracts of defatted borage (*Borago offcinalis* L.) seeds. *Food Chemistry* 67: 399-414.

Yeşilada, E., Honda, G., Sezik, E., et.al., (1993). Traditional medicine in Turkey IV. Folk medicine in the Mediterranean subdivision. *J. Ethnopharmacology* 39: 31-38.

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