ORIGINAL ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF EIGHT WILD EDIBLE PLANTS IN BURSA PROVINCE OF TURKEY

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Abstract

The antioxidant properties of ethanolic extracts from eight wild edible plants, which were collected in both fresh edible as vegetable and flowering (inedible) periods were investigated by employing various established *in vitro* systems: DPPH free radical scavenging, ABTS radical cation scavenging and metal chelating activity. Results showed that ethanol extracts of *Rumex pulcher*, *A. undulata* subsp. *hybrida* and *Campanula lyrata,* which were collected in edible periods exhibited the highest DPPH free radical scavenging activity and $ABTS^{\cdot+}$ scavenging activity. Moreover, *Asparagus acutifolius*, *Galium aparine* and *Campanula lyrata,* which were collected in edible periods exhibited the highest metal chelating activity. The results obtained from this study showed that *R. pulcher, A. undulata* subsp. *hybrida, C. lyrata*, *A. acutifolius* and *G. aparine,* which were collected in edible periods can be used as sources of natural antioxidants in food industry.

Rezumat

Proprietățile antioxidante ale extractelor etanolice obținute de la opt plante comestibile, culese în perioadele de înflorire și de înmugurire, au fost cercetate folosind mai multe metode *in vitro*: capacitatea de a chelata radicali liberi (DPPH și $ABTS^{\cdot+}$) și capacitatea de chelatare a metalelor. Rezultatele experimentale au arătat că aceste extracte obținute de la *Rumex pulcher*, *A. undulata* subsp. *hybrida* și *Campanula lyrata*, plante culese în perioadele potrivite consumului, au prezentat cea mai mare capacitate de netralizare a radicalilor liberi. *Asparagus acutifolius*, *Galium aparine* și *Campanula lyrata* au prezentat capacitate de chelatare a metalelor superioară. Rezultatele obținute arată că aceste plante pot fi folosite ca surse naturale de antioxidanți pentru industria alimentară.

Keywords: $ABTS^{\cdot+}$, DPPH, metal chelating, wild edible plants

Introduction

The consumer demands for a reduced use of synthetic food preservatives have increased throughout the world. Therefore, the substitution of traditional food preservations by natural vegetables and plants materials caused great interest in research [2]. It is well known that many natural substances in plants have antioxidant activities [7]. Many natural substances (especially phenolic compounds) in plants may reduce the risk of cancer, cardiovascular disease. So, the interest in naturally occurring antioxidants has increased considerably in recent years for the use in food and pharmaceutical products [1]. The genus *Anchusa* (*Boraginaceae*) consists of about 170 taxa native worldwide [32]. Some *Anchusa* species are used as diuretic, analgesic, sedative and hypotensive agents in traditional medicine. *Anchusa* species contain some triterpene glycosides and flavonoids [38]. The radix and aerial parts of the *Anchusa undulata* L. subsp. *hybrida* (Ten.) Coutinho are consumed as vegetables in Turkey. The young flowers of this plant are eaten in soups and salads [18, 37]. *Asparagus acutifolius* L. (*Liliaceae*) is a native plant species widely distributed throughout the Mediteranean areas [31].This species is known woody with long, trailing or scrambling, striate-ridged stems, greenish, the ridges papillose at least when young, becoming purplish-brown with age [10]. The young shoots are consumed as vegetables in West Anatolia, Turkey. Moreover, the young shoots are eaten in omelette and soups, raw, boiled, sometimes with scrambled or fried eggs. This plant is used as diuretic, antirheumatic, antineuralgic in traditional medicine [5, 13, 21, 36]. *Campanula lyrata* Lam. (*Campanulaceae*) is generally herbaceous and has showy flowers. The *Campanula* species are found in the Eastern Mediterranean region, including Turkey and the Caucasus. *Campanula* species have been used to treat various diseases such as tonsillitis, laryngitis and bronchitis in traditional medicine [34]. The young leaves are consumed as vegetables in Turkey [3, 28]. *Foeniculum* Mill. (*Umbelliferae*) is a small genus of annual, biennial or perennial herbs distributed in Central Europe and Mediterranean region. *F. vulgare* is used for diabetes, bronchitis and chronic coughs and for the treatment of kidney stones in folk medicine [4]. The *Galium* L. genus (*Rubiaceae*)

is represented in Turkey by 101 species grouped into ten sections. *Galium aparine* (cleavers) is used as diuretic, choleretic and against diarrhoea in folk medicine. The aerial parts of *Galium aparine* L. are eaten as roasted [15]. *Papaver rhoeas* L. (*Papaveraceae*) is an annual herb indigenous to numerous regions in the world. The aerial parts of this plant are eaten as roasted, pie and in salads [12]. The extracts of *P. rhoeas* have been used for the treatment of inflammation, diarrhoea, sleep disorders and moreover, for cough and analgesia in folk medicine. *P. rhoeas* species shows sedative, narcotic and emollient effects [17, 29]. *Rumex pulcher* L. (*Polygonaceae*) is a polymorphic species represented in Europe by four subspecies [26]. The leaves of *R. pulcher* are used to treat furuncles. *R. pulcher* is frequently consumed as a vegetable in Turkey [37]. *Sinapis arvensis* L. (*Brassicaceae*) is an annual plant widely distributed in Europe. The young leaves are consumed as vegetable in Bursa region, Turkey [19, 37]. The whole plant is used as antirheumatic and against cough moreover, flowering branches are used as antidiabetic in folk medicine [6, 25].

These wild plants are eaten in the early spring in Westhern Turkey. They are also used for medicinal purposes in Turkey. In addition, these plants are consumed as a detox cure for one month in early spring. Therefore, the most edible plants in Bursa region (Westhern Turkey) were selected. For these reasons, the aim of the present study was to determine the antioxidant activities of eight wild edible plants, which were collected in both fresh edible as vegetable and flowering (inedible) periods by using different antioxidant tests including total phenolic compound, DPPH free radical scavenging, $ABTS^{+}$ radical cation scavenging and metal chelating activity.

Materials and Methods

Collection of plants material. All plant samples were collected by Turgut Taşkın in March and May from Bursa region (Orhangazi - Çakırlı), Turkey. The plant species were identified by Prof. PhD. Ertan Tuzlacı. Voucher specimens are deposited in the herbarium of the Faculty of Pharmacy, Marmara University, Turkey.

Table I

Names and herbarium code numbers of the wild edible plants in Bursa province of Turkey

No	Plant species	Family	Local name	Parts used	Herbarium code numbers (MARE)
1	*Anchusa undulata* subsp. *hybrida*	*Boraginaceae*	*Koyun dili*	Aerial parts	13218
2	*Asparagus acutifolius*	*Liliaceae*	*Kuşkonmaz*	Shoots	13227
3	*Campanula lyrata*	*Campanulaceae*	*Gelin mancarı*	Leaves	13219
4	*Foeniculum vulgare*	*Umbelliferae*	*Kokar ot*	Aerial parts	13224
5	*Galium aparine*	*Rubiaceae*	*Yapışkan Ot*	Aerial parts	13215
6	*Papaver rhoeas*	*Papaveraceae*	*Gelincik*	Leaves	13226
7	*Rumex pulcher*	*Polygonaceae*	*Kertilce*	Leaves	13216
8	*Sinapis arvensis*	*Brassicaceae*	*Yenen Hardal*	Leaves	13221

Preparation of ethanolic extracts. The leaves, shoots and aerial parts of plants were washed with cold water and extracted with ethanol by maceration at room temperature until it was achieved a colourless solution. The extracts were filtered and evaporated using a rotary evaporator. The obtained crude extracts were decanted into vials and deposited until use, at 4ºC.

DPPH radical scavenging method. The DPPH radical scavenging activity of ethanol extracts was measured by the DPPH method proposed by Wei *et al.* [40]. The IC_{50} value is inversely correlated to the antioxidant ability of extracts. A lower IC_{50} value reveals a higher antioxidant activity.

$ABTS^{\bullet+}$ radical cation scavenging method. The trolox equivalent antioxidant activity (TEAC) of ethanol extracts was evaluated by the $ABTS^{\bullet+}$ radical cation methods [27]. A calibration curve was prepared using Trolox in the concentration range 0.25 - 1.5 mmol/L ($R^2 = 0.9547$). The results were expressed as mM Trolox/mg extract.

Metal chelating power assay. The activity of the ethanol extracts to chelate iron (II) was evaluated according to the method of Dinis *et al.* [10]. The EDTA calibration curve was plotted as a function of the percentage of chelating activity. The results were expressed as mM EDTAE/g extract.

Determination of total phenolic compounds. The amount of total phenolic compounds in the ethanol extracts was determined according to the method of Slinkard and Singleton [33]. Results were expressed as milligrams of total phenolic per gram extract (mg GAE/g extract). The calibration equation for gallic acid is $A = 2.395x - 0.027$ ($R^2 = 0.9995$).

Statistical analysis. All data are presented as the average of analyses performed in triplicate. The data were recorded as mean ± standard deviation and analysed by the Graphpad Prism 5 Demo software. The significance of the difference between means was determined by Tukey's Multiple Comparison Test ($p < 0.05$).

Results and Discussion

Extraction yield and total phenolics contents. The percentage yields of the ethanol extract from plants different parts were shown in Table II. The extraction yield of these samples varied from 0.84% to 13.47% in the descending order: inedible leaves of *P. rhoeas* (IPr) > inedible leaves of *G. aparine* (IGa) > inedible Shoots of *A. acutifolius* (IAa) > inedible leaves of *C. lyrata* (ICl) > edible shoots of *A. acutifolius* (EAa) > edible leaves of *P. rhoeas* (EPr) > inedible aerial parts of *F. vulgare* (IFv) > edible leaves of *C. lyrata* (ECl) > inedible leaves of *S. arvensis* (ISa) > edible leaves of *R. pulcher* (ERp) > inedible leaves of *R.pulcher* (IRp) > edible leaves of *F. vulgare* (EFv) > inedible aerial parts of *A. undulata* subsp. *hybrida* (IAu) > edible aerial parts of *A. undulata* subsp. *hybrida* (EAu) > edible leaves of *S. arvensis* (ESa) > edible leaves of *G. aparine* (EGa).

Table II summarizes the total phenolic compounds in extracts expressed as gallic acid equivalents (GAE) varied between 7.00 ± 0.08 and 68.67 ± 0.12 mg/g extract. The edible leaves of *C. lyrata* exhibited the highest total phenolic content (68.67 ± 0.12 mg GAE/g extract), whereas the contents obtained with edible leaves of *S. arvensis* were the smallest one (7.00 ± 0.08 mg GAE/g extract).

Table II

Total phenols and extraction yield of ethanol extracts from eight wild edible plants different parts

Plants	Total phenolics (mg gallic acid equivalent/g extract)		Extraction yield (%)	
	Edible periods	Inedible periods	Edible periods	Inedible periods
A.undulata subsp. *hybrida*	57.67 ± 0.73^{a}	59.33 ± 0.49^{a}	4.67^{a}	5.08^{a}
A. acutifolius	14.67 ± 0.58^{b}	21.67 ± 0.5^{b}	8.96^{b}	11.85^{b}
C. lyrata	$68.67 \pm 0.12^{c,a}$	$55.67 \pm 0.99^{c,a}$	$7.86^{c,b}$	10.37^{c}
F. vulgare	25.67 ± 0.29^{d}	26.83 ± 1.3^{d}	5.74^{d}	8.50^{d}
G. aparine	31.00 ± 1.65^{e}	18.83 ± 1.12^{e}	0.84^{e}	$11.87^{e,b}$
P. rhoeas	17.50 ± 0.50^{f}	$23.50 \pm 0.5^{f,d}$	$8.95^{f,b}$	13.47^{f}
R. pulcher	40.67 ± 1.02^{g}	45.67 ± 1.04^{g}	6.96^{g}	6.51^{g}
S. arvensis	7.00 ± 0.08^{h}	$24.50 \pm 0.89^{h,f}$	$0.96^{h,e}$	7.58^{h}

These values were the mean values of three replicates ± standard deviation. Different superscript letters in each column exhibit significant differences in mean values at $p < 0.05$ according to Tukey's Multiple Comparison test.

DPPH radical scavenging activity. DPPH free radical scavenging capacity of the ethanol extracts of plants, which were collected in both edible and inedible periods were measured by DPPH assay.

Table III shows that the DPPH radical scavenging ability of extracts can be ranked as ascorbic acid > BHT > IRp > ERp > EAu > ECl > ICl = IAu > EFv > IFv > IPr > EGa > EPr > EAa > IGa > Isa > ESa > IAa.

The ethanol extracts of *R. pulcher*, which was collected in both periods, had higher DPPH free radical scavenging activity than other extracts.

ABTS radical cation scavenging activity. The $ABTS^{\cdot+}$ radical cation scavenging assay was employed for evaluation of antioxidant activity of plant extracts. The results, presented as mM Trolox/mg extract are shown in Table III.

Table III

DPPH radical, ABTS radical cation and metal chelating activity of ethanol extracts from eight wild edible plants

Extracts/ Standards	Metal chelating activity (mM EDTAE/g extract)		DPPH (IC_{50} mg/mL)		TEAC (mM Trolox/mg extract)	
	Edible periods	Inedible periods	Edible periods	Inedible periods	Edible periods	Inedible periods
A.undulata subsp. *hybrida*	2.82 ± 0.06^{a}	0.72 ± 0.13^{a}	**0.98 ± 0.02^{a}**	**1.18 ± 0.02^{a}**	**1.54 ± 0.008^{a}**	**1.47 ± 0.1^{a}**
A.acutifolius	**3.6 ± 0.19^{b}**	**2.76 ± 0.13^{b}**	5.71 ± 0.21^{b}	11.00 ± 0.25^{b}	0.73 ± 0.07^{b}	0.75 ± 0.07^{b}
C.lyrata	**$2.86 \pm 0.16^{c,a}$**	**1.08 ± 0.07^{c}**	**1.07 ± 0.01^{c}**	**$1.18 \pm 0.03^{c,a}$**	**1.83 ± 0.006^{c}**	**1.61 ± 0.08^{c}**
F.vulgare	0.49 ± 0.04^{d}	0.49 ± 0.23^{d}	2.57 ± 0.04^{d}	2.88 ± 0.04^{d}	0.36 ± 0.05^{d}	0.39 ± 0.06^{d}
G.aparine	**$2.96 \pm 0.03^{e,a}$**	0.99 ± 0.04^{e}	4.39 ± 0.05^{e}	5.93 ± 0.01^{e}	$0.7 \pm 0.05^{e,b}$	0.51 ± 0.04^{e}
P.rhoeas	1.41 ± 0.05^{f}	0.34 ± 0.03^{f}	$5.24 \pm 0.01^{f,b}$	3.67 ± 0.05^{f}	$0.36 \pm 0.03^{f,d}$	$0.75 \pm 0.06^{f,b}$
R.pulcher	2.14 ± 0.01^{g}	**1.84 ± 0.03^{g}**	**$0.86 \pm 0.01^{g,a}$**	**0.57 ± 0.01^{g}**	**$1.83 \pm 0.003^{g,c}$**	**$1.37 \pm 0.10^{g,a}$**
S.arvensis	2.59 ± 0.08^{h}	$0.69 \pm 0.06^{h,a}$	9.78 ± 0.14^{h}	7.27 ± 0.06^{h}	0.52 ± 0.10^{h}	0.69 ± 0.12^{h}
Butylated hydroxytoluene (BHT)			0.32 ± 0.03^{i}	0.32 ± 0.03^{i}		
Ascorbic acid			0.09 ± 0.006^{i}	0.09 ± 0.006^{i}		

These values were the mean values of three replicates ± standard deviation. Different superscript letters in each column exhibit significant differences in mean values at $p < 0.05$ according to Tukey's Multiple Comparison test. Bold values show significant activity.

The edible leaves of *C. lyrata* (1.83 ± 0.006 mmol/L TEAC) and *R. pulcher* (1.83 ± 0.003 mmol/L TEAC) showed the highest $ABTS^{\cdot+}$ radical cation scavenging activity, whereas the edible leaves of *P. rhoeas* (0.36 ± 0.03 mmol/L TEAC) and *F. vulgare* (0.36 ± 0.05 mmol/L TEAC) had the lowest activity among the other extracts.

Metal chelating activity. An important mechanism of antioxidant activity is the ability to chelate metals [20]. Therefore, in this study, the iron (II) chelating activity of ethanol extracts from plants was screened. As showed in Table III, the values of the iron (II) chelating activity ranged from 0.34 ± 0.03 to 3.6 ± 0.19 mMol EDTAE/g extract. The edible shoots of *A. acutifolius* showed the highest iron (II) chelating activity, whereas the inedible leaves of *P. rhoeas* had the lowest activity among the other extracts.

Phenolic compounds are secondary metabolites of plants and these compounds are generally playing an important role in chelating redox-active metal ions and scavenging free radicals [30]. Therefore, phenolic compounds (tannins, flavonoids and phenolic acids etc.) are considered to be the major contributors to the antioxidant capacity of fruits and vegetables. The biological activity of plants, such as antiinflammatory, antiatherosclerotic and anti-carcinogenic activity, may be related to their anti-oxidant activity [8]. Therefore, in this study, the total phenolic contents and antioxidant activity of ethanol extracts from eight wild edible plants in Turkey were evaluated. According to this study, usually in the flowering period, the amount of extracts and total phenolic compound contents increased. However, the plants collected during the early spring usually exhibited stronger antioxidant activity than plants collected in the flowering period. This study showed that it is meaningful use of these plants both food and for treatment. In addition, according to this study it is correct to use these food plants during early spring in the form of detox cure, because the plants collected during early spring usually showed the highest antioxidant activity. To the best of our knowledge, there are some reports on the antioxidant potential of these plants (excluding antioxidant activity of the *C. lyrata*) [9, 16, 22-24, 35, 39]. However, in our current study, the antioxidant activities of plants collected in the early spring and the flowering period were compared for the first time followed by examined total phenolic compound contents in the ethanol extracts, which were collected in both periods. As a result of this study, the ethanol extracts of plants collected in the early spring usually exhibited the higher DPPH free radical, ABTS radical cation and metal chelating antioxidant activity than the others. Therefore, after examining the toxic effects on different normal cell line of these extracts, which were collected in edible periods it is believed that these extracts might be worthy natural antioxidant sources.

Conclusions

As a conclusion, in the present study there were evaluated the antioxidant activities and total phenolic contents of eight wild edible plants in Turkey. It can be stated that the results obtained from the present study clearly showed that the highest DPPH free radical scavenging activity and $ABTS^{\cdot+}$ scavenging activity were found in *R. pulcher*, *A. undulata* subsp. *hybrida* and *C. lyrata*, which were collected in edible periods. Moreover, *A. acutifolius*, *G. aparine* and *C. lyrata,* which were collected in the edible period, exhibited the highest metal chelating activity. In addition, the edible leaves of *Campanula lyrata* showed the highest total phenolic contents. A significant linear correlation was confirmed between the values for the total phenolic content and antioxidant activity of ethanol extract from edible leaves of *Campanula lyrata.* The results obtained from this study show that *R. pulcher, A. undulata* subsp. *hybrida, C. lyrata*, *A. acutifolius* and *G. aparine*, which were collected in edible periods can be used as sources of natural antioxidants in the pharmaceutical and food industry.

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