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Full Length Research Paper

Free radical scavenging activity and phenolic content of edible wild fruits from Kazdagi (Ida Mountains), Turkey

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The ethanolic and acetone extracts of wild fruits collected from Kazdagi (Ida Mountains) were examined for free radical scavenging activity and phenolic compounds. The extracts of the fresh fruits of *Arbutus unedo*, *Lycium europaeum*, *Prunus spinosa*, *Rosa canina*, and *Rubus sanctus* were examined for their antioxidant properties by using the free radical 1,1'-diphenyl-2-picrylhydrazyl (DPPH) scavenging method. The percentage inhibition of the extracts varied from 93.7 to 99.9%. The total phenolics, flavonoids, carotenoids, and condensed tannins of the extracts of the investigated samples ranged from 0.23 to 7.28 mg gallic acid equivalents (GAE)/g fresh fruit, 1.69 to 4.19 mg/g fresh fruit, 0.49 to 7.17 mg/g fresh fruit, and 20.18 to 315.51 µg catechin equivalent (CE)/g fresh fruit. It was determined that the fresh wild fruits collected from Kazdagi exhibited high free radical scavenging capacity. Furthermore, the fresh fruits were found to contain appreciable amounts of phenolic compounds influencing the antioxidant capacity of the samples.

Key words: Kazdagi, wild fruits, 1,1'-diphenyl-2-picrylhydrazyl (DPPH), phenolics, flavonoids, carotenoids, tannins.

INTRODUCTION

Wild fruits have been for a long time part of the human diet. In rural areas, wild fruits are consumed because of economic reasons not only as vitamin sources but also for medicinal purposes. In folk medicine, wild growing plants have been extensively used due to their curative effects (Motamed and Naghibi, 2010; Pieroni et al., 2002). Turkey's flora is very rich and people from ancient times have used different fruits for consumption as well as preparation of medicines, because of the mild climate (Guner et al., 2000; Ozgokce and Ozcelik, 2004).

Nowadays, it is becoming more important that human diet is rich in phytochemicals capable to prevent coronary diseases, cancers, neuropathological diseases, etc (Block et al., 1992; Kaur and Kappor, 2001). Our research has been focused on the wild fruits that are traditionally consumed in Turkey during autumn and these fruits have been characterized in terms of their free radical scavenging capacities and phenolic contents. According to ethnobotanical surveys, fruits of strawberry-tree (*Arbutus unedo*) (Sanjust et al., 2008), boxthorn (*Lycium europaeum*) (Pieroni et al., 2002; Said et al., 2002), blackthorn (*Prunus spinosa*) (Kultur, 2007), rose hips (*Rosa canina*) (Cakilcioglu et al., 2010; Montazeri et al., 2011; Sanjust et al., 2008), and blackberry (*Rubus sanctus*) (Cakilcioglu et al., 2010; Motamed and Naghibi, 2010; Rocabado et al., 2008) have been used for the prevention and therapy of cold and infectious diseases,

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kidney and liver disorders, diabetes, high blood pressure and also as diuretics, sedatives, etc.

The chemical composition of plants is mainly affected by the environmental conditions such as climate, altitude and soil conditions. In literature, there are several studies showing that the contents of fruits are environment dependent and therefore, samples of different areas should be investigated (Orazem et al., 2011; Ruiz-Rodriguez et al., 2011; Serce et al., 2010; Serteser et al., 2008).

The aim of this study was to comparatively investigate the radical scavenging capacities and phenolic composition of the fresh fruits of *A. unedo, L. europaeum, P. spinosa, R. canina*, and *R. sanctus*. To the best of our knowledge there is no publication dealing with the antioxidant properties of the fruits of *Lycium europaeum*, while for the other fruits this is for the first time they are investigated from Kazdagi (Ida Mountains), Canakkale, Turkey.

MATERIALS AND METHODS

Chemical reagents

All chemicals were purchased from Sigma-Aldrich (USA), SPA (Milan, Italy), Merck (Germany), and Fluka Chemie (Switzerland).

Plants

Fruits of strawberry-tree (*A. unedo*), boxthorn (*L. europaeum*), blackthorn (*P. spinosa*), dog rose (*R. canina*), and blackberry (*R. sanctus*) were collected from the Northern parts of Kazdagi, Turkey (Figure 1) and were identified according to Davis (1972) by Dr. Ahmet Gonuz, Department of Biology, Canakkale Onsekiz Mart University, Canakkale, Turkey. Specimens were deposited in the personal herbarium of Dr. Ahmet Gonuz.

Preparation of fresh fruit sample extracts

The collected and identified fruits were stored at 4°C till analysis (not more than 12 h and fresh fruits were used for extraction. Each fresh fruit sample (20 g) was extracted according to Maisuthisakul et al. (2007). Briefly, a sample (20 g) was blended with 60 ml ethanol (95%) or acetone in a blender for 1 min and was shaken for 4.5 h. The supernatant was filtered through Whatman filter paper (No. 4). All filtrates were evaporated under reduced pressure using a Rotary evaporator at 40°C, and were weighed in order to determine the yield of soluble components. Afterwards, the extracts were immediately analyzed for DPPH free radical scavenging activity, total phenolics, flavonoids, carotenoids, and condensed tannins. Unless otherwise stated, all assays were done in triplicate.

DPPH free radical-scavenging activity assay

The effect of the oxidized fruit extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated as described by Brand-Williams et al. (1995). Each sample was diluted in methanol prior to the analysis (1 mg/ml). The DPPH solution was added to the diluted sample, thoroughly mixed, then left for 30 min for the reaction to occur. After that, the absorbance of the sample was measured at 515 nm using a UV–Vis spectrophotometer (Thermo Aquamate). The absorbance of DPPH solution in methanol, without any antioxidant (control), was also measured. The percentage of DPPH radical scavenging activity was calculated by using the following equation:

$$\text{DPPH scavenging (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where A_{sample} is the absorbance of the sample after the time necessary to reach the plateau (30 min) and $A_{control}$ is the absorbance of DPPH.

Extract concentrations providing IC_{50} inhibition values (defined as the concentration of the compounds that was able to inhibit 50% of the total DPPH radicals) were calculated from graph plotting using nonlinear regression and expressed in microgram material equivalents per milliliter for sample extracts. Butylated hydroxytoluene (BHT) was used as a positive control. A lower value of IC_{50} indicates a higher antioxidant activity and vice versa.

Analysis of total phenolic content

The amount of total phenolics in the ethanol and acetone extracts of fruit samples was measured using the Folin-Ciocalteu reagent method of Djeridane et al. (2006). The ethanol/acetone solution of each extract (0.2 ml, 500 mg/ml) was taken in a test tube. 0.5 ml distilled water and 0.5 ml Folin–Ciocalteu reagent was added and the tubes were shaken thoroughly. After 1 min, 0.8 ml of sodium carbonate solution (7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. Absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Thermo Aquamate). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram fresh fruit.

Analysis of total flavonoids content

The total flavonoid content was determined according to Quettier-Deleu et al. (2000) using rutin as a standard. The total flavonoid content was expressed as rutin equivalents in milligram per gram fresh fruit. Three replicates were used for the determination of the mean total flavonoid values of the fruit samples.

Analysis of total carotenoids content

The carotenoid content present in the fresh fruit extracts was determined spectrophotometrically (UV-Vis spectrophotometer, Thermo Aquamate) and the absorbance was measured at 480 nm. For acetone, the extinction coefficient $E_{(1\%, 1\ cm)}$ was 1.900, while for ethanol $E_{(1\%, 1\ cm)}$ was 2.130. The carotenoid content was calculated using the following equation:

$$A = \alpha.c.l$$

where A is the absorbance at 480 nm, α is the specific absorbance coefficient of the solvent, c is the concentration of the carotenoids in mg/g, and l is the path length of the cuvette (1 cm) (Lichtenthaler and Buschmann, 2001).

Analysis of total condensed tannins content

Condensed tannin content was evaluated using the vanillin assay (Price et al., 1978). An aliquot of 0.5 g of the fruit extracts was placed in centrifuge tubes and 20 ml of 1% HCl in methanol was added to each sample. Then, the tubes were placed in a water bath at 30°C with constant shaking for 20 min. After incubation, the

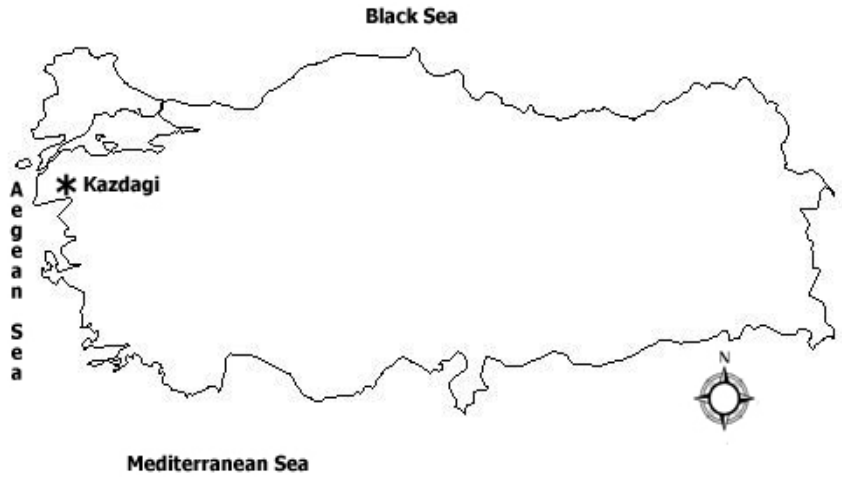

Figure 1. Kazdagi (Ida Mountains), Turkey.

Table 1. The DPPH radical scavenging activity of the fruit samples*.

Fruit sample	Extract	IC50** (µg/ml)	Inhibition** (%)
A. unedo	Ac extract	1.12 ± 0.01[a]	99.9 ± 0.1
	EtOH extract	1.24 ± 0.02[a]	95.8 ± 0.1
L. europaeum	Ac extract	4.28 ± 0.02[d]	93.7 ± 0.1
	EtOH extract	3.66 ± 0.01[c]	95.9 ± 0.2
P. spinosa	Ac extract	2.13 ± 0.01[b]	97.8 ± 0.1
	EtOH extract	2.26 ± 0.04[b]	97.2 ± 0.1
R. canina	Ac extract	3.50 ± 0.05[c]	96.4 ± 0.2
	EtOH extract	3.81 ± 0.02[c]	95.6 ± 0.2
R. sanctus	Ac extract	1.56 ± 0.01[ab]	99.5 ± 0.1
	EtOH extract	1.62 ± 0.01[ab]	99.1 ± 0.2
BHT	-	1.35 a	99.7

*The values are given as mean ± standard deviation (n = 3). **Means with different letter within a column are significantly different at $P < 0.05$.

samples were centrifuged. Aliquots of the supernatants were placed in two separate assay tubes, one for the sample determination and the other for blank determination. Samples and blanks were incubated for exactly 20 min after adding 5 ml of the vanillin reagent (0.5 g of reagent and 200 ml of 4% HCl methanol) to the samples and 4% HCl in methanol to the blanks. Afterwards, the absorbance was measured at 500 nm using a UV–Vis spectrophotometer (Thermo Aquamate). The results were expressed as microgram catechin equivalents per gram of fruits (µg CE/g).

Statistical analysis

The results were reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied to investigate the differences among means by using Statghaphics Centurion XV software. The values were considered to be significantly different at $P < 0.05$. The correlation coefficients (r) were calculated in order to determine the relationship between two variables using MS Excel software.

RESULTS AND DISCUSSION

The DPPH free radical scavenging capacities of the fruits are presented in Table 1. The lowest IC_{50} value (indicating high free radical scavenging activity) was

Table 2. Total phenolics, flavonoids, carotenoids, and tannins of five wild growing fruits from Kazdagi, Turkey*.

Fruit sample	Extract	Total phenolics** (mg GAE/g)	Total flavonoids** (mg/g)	Total carotenoids** (mg/g)	Condensed tannins** (µg CE/g)
A. unedo	Ac extract	7.28±0.04^{h}	3.05±0.04^{c}	2.13±0.01^{e}	315.51±3.12^{f}
	EtOH extract	1.67±0.02^{g}	3.15±0.03^{c}	1.57±0.01^{d}	29.25±0.60^{b}
L. europaeum	Ac extract	1.12±0.01^{f}	3.72±0.01^{d}	4.55±0.03^{h}	25.37±0.22^{a}
	EtOH extract	0.91±0.02^{e}	4.19±0.01^{e}	3.38±0.07^{g}	23.54±0.41^{a}
P. spinosa	Ac extract	0.31±0.01^{b}	1.74±0.01^{a}	2.68±0.01^{f}	95.28±1.12^{c}
	EtOH extract	0.29±0.02^{b}	1.81±0.01^{a}	1.29±0.05^{c}	105.03±1.02^{c}
R. canina	Ac extract	0.24±0.01^{a}	1.69±0.02^{a}	0.16±0.01^{a}	23.41±0.26^{a}
	EtOH extract	0.23±0.02^{a}	1.72±0.01^{a}	0.49±0.02^{b}	20.18±0.17^{a}
R. sanctus	Ac extract	0.62±0.04^{d}	1.88±0.01^{ab}	7.17±0.08^{i}	142.79±2.46^{d}
	EtOH extract	0.42±0.02^{c}	1.93±0.01^{b}	1.36±0.02^{c}	165.86±3.02^{e}

*The values are given as mean ± standard deviation (n=3) and per gram fresh fruit. **Means with different letter within a column are significantly different at $P < 0.05$. Ac: acetone, EtOH: ethanol.

determined for the ethanolic and acetone extracts of *A. unedo*, while the lowest free radical scavenging activity was observed for the ethanolic extract *L. europaeum*. Similar IC_{50} values and DPPH percentage inhibition for *R. sanctus* were found in literature (Motamed and Naghibi, 2010). For all fruit extracts, no matter what the extraction medium was, percentage inhibition level of DPPH was as high as that of BHT.

The total phenolics, flavonoids, carotenoids, and tannins of the wild fruits are shown in Table 2. The amount of total phenolics was found to range from 0.23 to 7.28 mg GAE/g of fresh fruits. The total phenolic compounds were highest in *A. unedo* and lowest in *R. canina*. The acetone extracts of *A. unedo*, *L. europaeum*, and *R. sanctus* were higher in total phenolics than that of the ethanolic extracts. This is mainly due to the polarity of the solvent used for extraction. The fruits were found to be rich in total flavonoids (Table 2). The highest total flavonoid content was detected in the ethanolic extract of *L. europaeum*, while the lowest was found in the ethanolic extract of *R. canina*. The extraction medium did not affect the flavonoid content for *A. unedo*, *P. spinosa*, and *R. sanctus*, while for the other fruits, ethanol was found to be a better solvent for the extraction of flavonoids. The highest total carotenoid content was found in *L. europaeum* and lowest in *R. canina* both for the acetone and ethanolic extracts. Except for *R. canina*, in the samples of *A. unedo*, *L. europaeum*, *P. spinosa*, and *R. sanctus*, the acetone extracts were significantly higher in total carotenoids.

The condensed tannin content varied in different fruit samples and was found to range between 20.18 and 315.51 µg CE/g of fresh fruit. The highest tannin content was detected in the acetone extract of *A. unedo*. There was no significant difference between the tannin content of the ethanolic and acetone extracts of *L. europaeum*, *P. spinosa*, and *R. canina*.

Egea et al. (2010) investigated the antioxidant activity and the phenolic composition of several wild fruits including *R. canina* and *P. spinosa*. Based on the factthat the plants were collected from different areas of the Mediterranean region, the results obtained in our study showed differences in total phenolics and carotenoids per gram fresh fruit. Barros et al. (2010) investigated the dried fruits of strawberry-tree, blackthorn and dog rose and found that the ethanolic extracts of the fruits contain considerable amounts of phenolic compounds, affecting the antioxidant capacity of the fruits. Because in the Kazdagi region, the local people consume the investigated fruits in their fresh form, in our study, we examined the free radical scavenging capacity as well as the bioactive compounds content of the fresh fruits.

The correlation between percentage inhibition levels of DPPH and the total phenolics, total flavonoids, total carotenoids, and condensed tannins are as shown in Figure 2. There was a high correlation only between percentage inhibition level of DPPH and the condensed tannins ($r = 0.85$). In previous studies, the correlation between total phenolics and antioxidant activity of various fruits were well recorded (Egea et al., 2010; Giorgi et al., 2005), while Motamed et al. (2010) found medium correlation between DPPH radical scavenging activity and total flavonoids and phenolic compounds of the analyzed fruits.

Nevertheless, there are also reports about no correlation between total phenolic contents and radical scavenging capacity (Yu et al., 2002). The high free radical scavenging capacity of the wild plants might be attributed not only to the phenolic composition, but also to the presence of other bioactive compounds, such as vitamins (ascorbic acid, tocopherols) and pigments (anthocyanins) as well as the structural interaction among these compounds (Barros et al., 2010; Djeridane et al., 2006; Serteser et al., 2008).

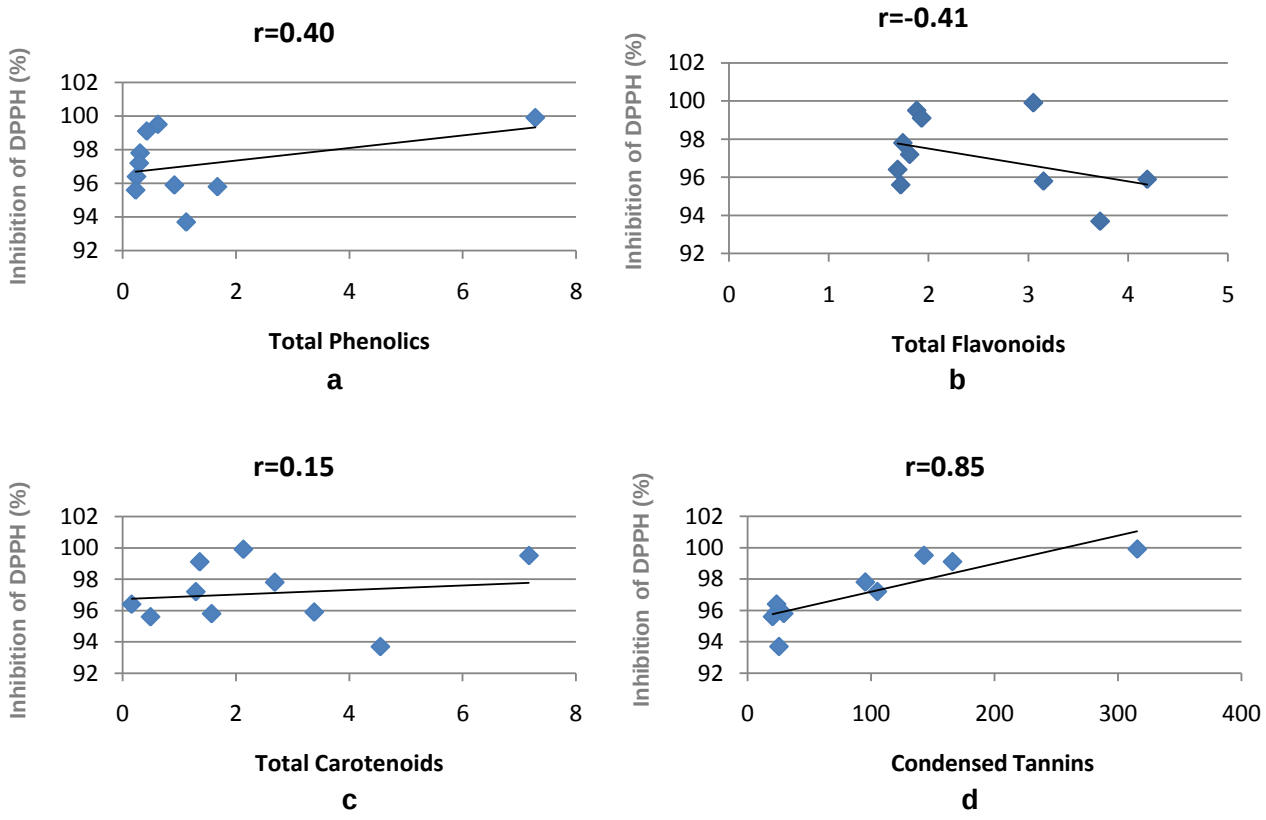

Figure 2. Correlation between percentage inhibition of DPPH and total phenolics (mg GAE/g), total flavonoids (mg/g), total carotenoids (mg/g), and condensed tannins (µg CE/g).

Conclusion

The wild fruits collected from Kazdagi (Ida Mountains) during autumn were found to contain substantial amounts of phenolic compounds, which affect the free radical scavenging capacity. Nevertheless, a comprehensive investigation of the bioactive compounds affecting the antioxidant capacity of the wild fruits is needed to explain the high free radical scavenging activity of these fruits.

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REFERENCES

Barros L, Carvalho AM, Morais JS, Ferreira ICFR (2010). Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties. Food Chem. 120:247-254.

Block G, Patterson B, Subar A (1992). Fruits vegetables and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer 18:1-29.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28:25-30.

Cakilcioglu U, Sengun MT, Turkoglu I (2010). An ethnobotanical survey of medicinal plants of Yazikonak and Yurtbasi districts of Elazig province, Turkey. J. Med. Plants Res. 4:567-572.

Davis PH (1972). Flora of Turkey and the East Aegean Islands. Edinburgh Press University, Edinburgh, UK 6:444; 9:124; 11:31-446.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocher, Vidal N (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97:654–660.

Egea I, Sanchez-Bel P, Romorjaro F, Pretel MT (2010). Six edible wild fruits as potential antioxidant additives or nutritional supplements. Plant Food. Hum. Nutr. 65:121-129.

Giorgi M, Capocasa F, Scalzo J, Murri G, Battino M, Mezzetti B (2005). The rootstock effects on plant adaptability, production, fruit quality, and nutrition in the peach (cv. 'Suncrest'). Sci. Hort. 107:36-42.

Guner A, Ozhatay N, Ekim T, Baser KHC (2000). Flora of Turkey and the East Aegean Islands. In Davis PH (ed) Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh UK. 11(Suppl. 2):654.

Kaur C, Kapoor HC (2001). Antioxidants in fruits and vegetables – the millenium's health. Int. J. Food Sci. Technol. 36:703-725.

Kultur S (2007). Medicinal plants used in Kirklareli Province (Turkey). J. Ethnopharmacol. 111:341-364.

Lichtenthaler HK, Buschmann C (2001). Current Protocols in Food Analytical Chemistry. John Wiley and Sons, Inc., New York F4.3.1-F.4.3.8.

Maisuthisakul P, Suttajit M, Pongsawatmanit R (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem. 100:1409-1418.

Montazeri N, Baher E, Mirzajani F, Barami Zi Yousefian S (2011). Phytochemical contents and biological activities of *Rosa canina* fruit

from Iran. J. Med. Plants Res. 5(18):4584-4589.
Motamed SM, Naghibi F (2010). Antioxidant activity of some edible plants of the Turkmen Sahra region in Northern Iran. Food Chem. 119:1637-1642.
Orazem P, Stampar F, Hudina M (2011). Quality analysis of 'Redhaven' peach fruit grafted on 11 rootstocks of different genetic origin in a replant soil. Food Chem. 124:1691-1698.
Ozgokce F, Ozcelik H (2004). Ethnobotanical aspects of some taxa in East Anatolia, Turkey. Econ. Bot. 58:697-704.
Pieroni A, Nebel S, Quave C, Münz H, Heinrich M (2002). Ethnopharmacology of *liakra*: traditional weedy vegetables of the Arbereshe of the Vulture area in southern Italy. J. Ethnopharmacol. 81:165-185.
Price ML, Vanscoyoc S, Butler LG (1978). Critical evaluation of Vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26:1214-1218.
Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet J, Luyck M, Cazin M, Cazin JC, Bailleul F, Trotin F (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacol. 72:35-40.
Rocabado GO, Bedoya LM, Abad MJ, Bermejo P (2008). Rubus – a review of its phytochemical and pharmacological profile. Nat. Prod. Commun. 3:423-436.
Ruiz-Rodriguez BM, Morales P, Fernandez-Ruiz V, Sanchez-Mata MC, Camara M, Diez-Marques C, Pardo-de Santayana M, Molina M, Tardio J (2011). Valorization of wild strawberry-tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. Food Res. Int. 44:1244-1253.
Said O, Khalil K, Fulder S, Azaizeh H (2002). Ethnopharmacological survey of medicinal herbs in Israel, the Gollan Heights and the West Bank region. J. Ethnopharmacol. 83:251-265.
Sanjust E, Mocci G, Zucca P, Rescigno A (2008). Mediterranean shrubs as potential antioxidant sources. Nat. Prod. Res. 22:689-708.
Serce S, Ozgen M, Torun AA, Ercisli S (2010). Chemical composition, antioxidative activities and total phenolic content of *Arbutus andrachne* L. (Fam. Ericaceae) (the Greek strawberry tree) fruits from Turkey. J. Food Compos. Anal. 23:619-623.
Serteser A, Kargioglu M, Gok V, Bagci Y, Ozcan MM, Arslan D (2008). Determination of antioxidant effects of some plants species wild growing in Turkey. Int. J. Food Sci. Nutr. 59:643-651.
Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002). Free radical scavenging properties of wheat extracts. J. Agr. Food Chem. 50:1619-1624.