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Chemical Properties and Antioxidant Capacity of *Elaeagnus angustifolia* L. Fruits

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Elaeagnus angustifolia L. (oleaster) is an autumn fruit which is generally consumed dried. Although it grows almost everywhere in Turkey, use of its fruits is limited. We analyzed chemical composition and antioxidant properties of the edible parts of *Elaeagnus angustifolia* L. fruit. Total moisture, ash, protein, total soluble sugar, fat, crude fibre, total titratable acidity and major mineral content of the fruit were determined. Total polyphenols were investigated using Folin-Ciocalteau method and antioxidant capacity of the fruit was assessed by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the mesocarp and exocarp tissues. Results showed that potassium is the most abundant mineral in the fruit (8504 mg/kg) which is followed by sodium (1731 mg/kg) and phosphorus (635 mg/kg). *Elaeagnus angustifolia* L. fruit is a rich source of both several nutrients and antioxidant compounds. Average total phenolic content of aqueous, acetone and methanolic extracts of the mesocarp and exocarp in oleaster fruit were measured as 778 and 559, 390 and 361 and 414 and 524 mg gallic acid equivalents of 100 g^{-1} dried mass, respectively. In conclusion, *E. angustifolia* fruit is a rich source of chemical compounds and has a high mineral content. This fruit can be included in dietary products due to its possible health benefits.

Key Words: *Elaeagnus angustifolia* L., Fruit, Mineral content, Nutritional properties.

INTRODUCTION

Elaeagnus angustifolia (Russian olive, oleaster) belongs to *Elaeagnus* L. genus and *Elaeagnaceae* family. *Elaeagnus angustifolia* L. is a shrub or tree with a height of up to 7 m and a capacity to grow under a wide range of environmental conditions[1]. This species shows a broad geographical range, occurring widely in Asia and Europe, particularly in Turkey, Caucasia and Central Asia[2]. Although this species is used as an ornamental tree in many European cities[3], it is widely cultivated for its edible fruits in Middle and East Anatolia. The fruits are reddish-brown, elliptic, 9-12 mm long and 6-10 mm wide and they mature in September. It is consumed either freshly or in dried form. The fruits are used as diuretic, tonic, antipyretic, antidiarrheal and as a medication against kidney disorders (inflammatory or to pass kidney stone) in traditional Turkish medicine[4,5], against dysentery and diarrhea in Kingdom of Jordan[6] and for its antiinflammatory, antinociceptive and analgesic effects in Iranian folk medicine. Decoction and infusion of its fruits is considered to be a good remedy for fever, jaundice, asthma, tetanus and rheumatoid arthritis[7].

In recent years, increasing attention has been paid by consumers to the health and nutritional aspects (vitamins contents, mineral elements, antioxidants, *etc.*) of horticultural products[8]. In addition, fruits have low energy content, while the nutrient densities are very high. Daily consumption of fruits is recommended to help prevent major non-communicable diseases such as cardiovascular diseases and certain cancers. Increased consumption of fruits can help replace foods high in fats, sugar, salt and thus improve the intake of most micro-nutrients, such as elements and dietary fibre[9]. In addition, experiments in cell culture and in intact organisms reveal the importance of elements in many metabolic processes and functions throughout the life cycle. Human as well as animal studies originally showed that optimal intakes of elements such as sodium, potassium, magnesium, calcium and phosphorus could reduce individual risk factors, including those related to several diseases[10]. Therefore, chemical and mineral compositions of fruits should be determined.

There has been a growing interest in food components which may inhibit or interrupt the oxidation process and are capable of counter-balancing free radical activities that cause cell injuries leading to neoplastic lesions, inflammatory conditions or negative changes in blood vessels. Increasing numbers of research results confirm that injuries due to an excessive production of free oxygen radicals occur in many

common pathological states, such as cardiovascular diseases, some prenatal complications, neoplastic diseases, inflammatory states (*e.g.*, rheumatoid arthritis), cataract, Parkinson's disease, Alzheimer's disease or ageing of the organism[11]. Fruits have long been regarded as having considerable health benefits, due in particular to their antioxidant content, which can protect the human body against cellular oxidation reactions radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide and quenching superoxide and singlet oxygen[8,12].

To the best of our knowledge, only few studies were conducted exploring the composition of *Elaeagnus angustifolia* L. fruit. Although this species grows almost everywhere naturally in Turkey, uses of its fruits are limited in agricultural and food industry. For this reason, we aimed to investigate the nutritive compositions, five nutritional important elements (Na, Mg, K, Ca, P), total polyphenolic content and antioxidant capacity in *Elaeagnus angustifolia* L. fruits in order to establish its potential as an edible source of valuable nutrients.

EXPERIMENTAL

The fruit is not suitable for consumption when freshly harvested due to its harsh and unpleasant taste. After drying, the fruits tasted sweet[4]. For this reason, we used sun-dried fuits as sample material. Oleaster fruits were supplied from local market in December 2009 from Bursa, Turkey. The fruits had approximately same maturity (almost reddish) with uniform shape and size. The dried fruits were processed in order to separate the endocarp (seeds), mesocarp (pulp) and exocarp (skin). Only the edible portion was used for analyses. Floury mesocarps of fruits were homogenized using a Polytron homogenizer (Brikmann Instruments, Westbury, NY, USA).

All reagent used were analytical grade purity. Folin-Ciocalteau phenol reagent and DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid were purchased from Sigma-Aldrich (St. Louis, USA); Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] was purchased from Aldrich (Aldrich Chemicals Company, Steinheim, Germany). All standard solutions were prepared in methanol (Merck, Darmstadt, Germany). High quality water, obtained using a Milli-Q system (Millipore, Bedford, MA, USA), was used exclusively.

Perkin-Elmer 2100 inductively coupled plasma-optical emission spectrophotometer (ICP-OES) was used to analyze the elements in digested samples. The instrumental operating conditions for determination of the elements are summarized in Table-1. Samples were digesting by acid assisted microwave irradiation using Milostone MLS 1200. The heating programmed employed is shown in Table-2.

Physical properties: All physical properties of oleaster were determined using 10 repetitions at the dried moisture content of 26.52 %. To determine the size of fruits, ten groups of samples consisting of 100 fruits were selected randomly. Tens of fruits were taken from each group and their linear dimensions-length and diameter were measured. A micrometer measured linear dimensions with an accuracy of ± 0.01 mm.

TABLE-1
INSTRUMENT OPERATING CONDITIONS APPLIED FOR METALS DETERMINATION BY ICP-OES

Parameters	
View mode	Axial
View height	15 mm
Gas	Argon
Shear gas	Nitrogen
Gas: plasma	15 L/min
Gas flow: auxiliary	0.2 L/min
Source equilibration time	15 s
Pump flow rate	1.5 mL/min
Detector	CCD Array
RF power	1300 W
Nebulizer	0.8 L/min
Sample aspiration rate	1.5 mL/min
Read	Peak area
Number of replicates	3
Read delay	120 s
Rinse delay	20 s

TABLE-2
HEATING PROGRAM IN MICROWAVE DIGESTION SYSTEM

Step	Power	Hold
1	250	02:00
2	0	02:00
3	250	06:00
4	400	05:00
5	600	05:00

Chemical analyses: The protein, fat, moisture, ash, crude fibre and total titrable acidity values were estimated using standard methods of analysis[13]. Sugars were extracted by suspending 100 mg of mesocarp tissues in 5 mL of 80 % (v/v) ethanol in 85 °C water bath for 60, 30, 15 and 15 min, respectively. The ethanolic solutions were combined and evaporated to dryness at 55 °C. Pellets were dissolved in 1 mL distilled water. Total soluble sugar (TSS) content was determined using glucose as a standard and anthrone as reactive according to Sanchez-Castillo *et al.*[14] colorimetric method at 620 nm absorbance.

Element analyses: Approximately 0.5 g of sample was accurately weighed and transferred to a Teflon container. 6 mL of 65 % HNO_3 (Merck, Darmstadt, Germany) and 1 mL of 30 % H_2O_2 (Merck, Darmstadt, Germany) were then added. After microwave digestion cycle, digestion solutions were added with high purity deionized water to adjust the final volume to 25 mL. All samples were diluted and filtered using 0.45 μm filters (Hydropinilic PVDF Millipore Millex-HV) before analysis. Standard metal solution were prepared daily from 1000 mg/L stock (Merck, Darmstadt, Germany) in 2 % HNO_3 suprapur grade (Merck). To avoid contamination of samples, all PTFE materials (Teflon vessels, pipets, micropipette tips and auto sampler cups) were immersed in freshly prepared 15 % v/v prior to analysis HNO_3 (Merck) for 24 h, then rinsed thoroughly with doubly deionized water and dried in a dust free area before use. Each sample was decomposed into six replicates. Two water blanks were run with each batch of samples[15].

Total polyphenol content and antioxidant capacity: Extraction of phenolic compounds in food is influenced by

their chemical nature, extraction method applied, sample particle size, storage time and conditions and presence of interfering substances[16]. Therefore, we tested three different procedures for extraction of phenolics in both mesocarp and exocarp of oleaster fruits. The methods were selected on the basis of extraction tests performed using different solvents and conditions reported in the literature[17-19].

Extraction method A: Mesocarp and exocarp of oleaster fruits were ground using a domestic blender and 2 g of this material was extracted using 25 mL of deionized water. The mixture was allowed to stand at room temperature on orbital shaker (at 250 rpm) for 3 h in the dark. The aqueous extract was obtained by filtering the mixture through Whatman No. 1 filter paper and used for analysis without further treatment[17].

Extraction method B: Two g of mesocarp and exocarp of fruit were extracted with 25 mL of 80 % acetone (containing 0.2 % formic acid) using a domestic blender. The mixture was allowed to stand at room temperature on orbital shaker (at 250 rpm) for 3 h followed by centrifugation at 4000 rpm for 0.5 h. The supernatants were transferred to tubes[18].

Extraction method C: Two g mesocarp and endocarp samples were homogenized in a domestic blender. These samples were extracted overnight in 25 mL of 80 % MeOH at room temperature on orbital shaker (at 250 rpm). The obtained extracts were centrifuged at 4000 rpm for 15 min. The same procedure was repeated 3 times with 25 mL portions of 80 % MeOH on the remaining part of the fruits. All extracts were combined and diluted to 100 mL using the same solvent[19]. All obtained extracts were used for determination of total phenolics and DPPH scavenging activity.

Determination of total polyphenol: The amount of total phenols in the extracts was determined with Folin-Ciocalteu reagent using the modified method of Apak *et al.*[20]. Samples were introduced into test tubes; 1 mL of dilute plant extracts, 5 mL H_2O, 0.5 mL of Folin-Ciocalteu reagent were addded. The tubes were vortexed and the mixture was allowed to stand for 3 min. At the end of this period, 1 mL of 7.5 % Na_2CO_3 was added to mixture and shaken intermittently for 1 h at room temperaure in dark. The absorbance was measured at 750 nm against blank with ATI Unicam UV2-100 UV/vis spectrophotometer. Total phenol content was calculated from a standard curve of gallic acid and results were expressed as mg of gallic acid equivalents (GAE) per g of fresh weight (FW).

Determination of antioxidant capacity: The DPPH free radical scavenging activity of each sample was determined using the ATI Unicam UV2-100 UV/vis spectrophotometer according to the slightly modified method described by Katalinic *et al.*[21]. The initial absorbance of the DPPH in methanol was measured at 515 nm and did not change throughout the period of assay. Briefly, all diluted extracts (0.1 mL) were placed in test tubes and 3.9 mL of 6×10^{-5} M methanolic solution of DPPH radical was added and mixed. The reaction was allowed to take place in the dark for 60 min and absorbance was measured. Methanol was used as blank to determine the concentration of remaining DPPH. Standard curve was prepared using different concentration of Trolox. The results were expressed as μmol Trolox equivalent (TE) per g fresh weight.

All determinations were performed in triplicate. The per cent inhibition of the DPPH radical by the samples was calculated according to the formula of Ozturk *et al.*[22].

$$\text{DPPH Scavenging effect (\%)} = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100$$

Statistical analysis: All analyses were carried out in quintuplicate. Results were given as mean ± standard deviation of five independent determinations. All statistical analyses were performed with SPSS statistical analysis system. One-way analysis of variance (ANOVA) was used to compare the means and differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Physical properties: Table-3 shows the size distribution of the oleaster fruits. The fruit length and width ranged from 23.94 to 26.46 mm and from 14.34 to 17.17 mm, respectively. The seed average weight was 0.41 g while average fruit weight was 1.94 g. Present results of physical properties of oleaster fruits show minor differences when compared with data reported by Akbolat *et al.*[23]. However, Raj *et al.*[24] reported that average fruit length, width and weight ranged 1.43-2.33 cm, 1.08-1.45 cm and 0.43-0.99 for five fruit morphotypes of *Elaeagnus angustifolia* L., respectively. These values in *Elaeagnus angustifolia* L. were lower than these we found in present study. The variation of fruit weight in fruits could be due to different environmental conditions and the nutritional status of the soils.

TABLE-3
VARIOUS PHYSICAL PROPERTIES OF *Elaeagnus angustifolia* L. FRUIT

	Range	Mean ± SD
Fruit		
Weight (g)	1.71-2.66	1.94 ± 0.27
Length (mm)	23.94-26.46	24.81 ± 0.95
Width (mm)	14.34-17.17	16.23 ± 0.81
Stone		
Weight (g)	0.32-0.52	0.40 ± 0.07
Length (mm)	17.81-22.83	20.06 ± 1.89
Width (mm)	5.33-6.05	5.57 ± 0.29

Chemical composition: *Elaeagnus angustifolia* L. fruits tested had mealy, good and pleasant flavours, suggesting that they contained on combination of sugar, acidity, solids contents and crude fibre. The chemical composition of *Elaeagnus angustifolia* L. fruits are presented in Table-4. The moisture content change between 26.33 and 26.63 % for fruits. These results were similar to dried fruits, such as fig (30.00 %), prune (30.92 %), cranberry (16.00 %) and apricot (30.89 %)[25].

TABLE-4
CHEMICAL COMPOSITION OF *Elaeagnus angustifolia* L. FRUIT

	Range	Mean ± SD
Protein (%)	3.60-5.78	4.64 ± 0.88
Total soluble sugar (%)	66.92-75.99	70.61 ± 3.76
Fat (%)	0.4-0.6	0.47 ± 0.10
Moisture (%)	26.33-26.63	26.52 ± 0.16
Ash (%)	1.14-1.30	1.24 ± 0.09
Crude fibre (%)	3.87-4.24	4.07 ± 0.19
Total titrable acidity (%)	13.16-14.52	13.84 ± 0.68

Sugars and acidity have an important impact on the sensory quality of fruit. *Elaeagnus angustifolia* L. fruits have high sugar and acid content because of their low moisture content. The mean total soluble sugar content of the samples was 70.60 %. The total soluble sugar content of *Elaeagnus angustifolia* L. fruits is higher than that of dried apple (57.19 %), dried apricot (53.44 %) and dried cranberry (65.20 %)[25]. Ayaz and Bertoft[4], reported that the predominant sugars quantified were fructose (27.1 % dry wt.) and glucose (22.3 % dry wt.), while sucrose was not detected in this fruit. The portions of fructose, glucose and sucrose are important in perception of fruit quality since fructose is 1.8 times sweeter than sucrose, while the sweetness of glucose is only 60 % that of sucrose[18]. The mean total titrable acidy content of the samples was 13.84 %. The titrable acidic level was positively correlated with total organic acid and phenolic acid[18]. Ayaz and Bertoft[4], announced that seven phenolic acids were determined in *Elaeagnus angustifolia* L. fruits. Among these, 4-hydroxy-benzoic acid (45.8 mg/100 g dry wt) and cafeic acid (32 mg/ 100 g dry wt) were the most abundant, whereas ferulic acid (2.3 mg/100 g dry wt) and benzoic acid (11.6 mg/100 g dry wt) were least abundant. Wang and Fordham[18] indicated that malic acid, quinic acid and citric acid were found in *E. umbellata* Thunb. fruit and malic acid (range between 2.02 to 6.88 mg/100g of fresh mass) was the primary organic acid.

The mean fat content of the fruits was 0.47 %. Oleaster pulp has a low fat content, like the content of apples (0.32 %), figs (0.33 %), prune (0.38 %) and apricot (0.51 %)[25]. Although, Kusova and Luk'yanchikov[26], identified that the main esters and neutral lipids of the *Elaeagnus angustifolia* L. fruits were oleic acid, linoleic acid and linolenic acid, covering up to 92.8 % of the lipid content. Goncharova and Glushenkova[27] and Goncharova *et al.*[28] reported an abundance of palmitoleic acid (16:1) in pericarps and linoleic acid (18:0) and palmitic acid (16:0) acids in seeds.

The protein content ranged between 3.60 and 5.78 % with mean concentration of 4.64 ± 0.88 %. These values were higher than these reported for other dried fruits. Dried apricot, fig, prune, apple and cranberry have mean protein contents of 3.39, 3.30, 2.18, 0.93 and 0.07 %, respectively[25]. The ash content of *Elaeagnus angustifolia* L. fruits were 1.24 ± 0.09 %. This value is higher than the ash content of the dried cranberry (0.20 %) and dried apple (1.10 %), whereas it is lower than that of dried apricot (2.57 %) and prune (2.64 %)[25].

Epidemiological studies suggest that fibre consumption helps to reduce obesity, some kinds of cancer, cardiovascular diseases and gastrointestinal diseases. Although numerous health organizations indicate the necessity of increasing fibre consumption of up to 20-35 g per day, most people are unaware of the recommended dose[29]. The mean crude fibre content of the fruits was 4.07 %.

The major element contents of *Elaeagnus angustifolia* L. fruits are shown in Table-5. The detection and quantification limits of elements determined are shown in Table-6. The element composition of fruits depended on the species, varieties, growing conditions, such as soil and geographical conditions[30]. In this study, while the existence of five elements was determined in *Elaeagnus angustifolia* L. fruits, K was predominant, followed by Na, P, Ca and Mg. These data were in agreement with these reported obtained by Akbolat *et al.*[23]. Potassium concentration ranged from 795.83 to 909.53 mg/100 g with an average of 850.40 mg/100 g. *Elaeagnus angustifolia* L. fruits is a good source of K; this content is higher than that in dried apple (450.00 mg/100 g) and prune (731.00 mg/100 g), it is lower than dired apricot (1162.00 mg/100g). Sodium and phosphorus concentrations ranged from 151.82 to 192.17 and 60.20 to 67.31 mg/100 g. in the case of Na values, *Elaeagnus angustifolia* L. fruits has significantly higher Na than that in other dried fruits[25]. When Mg and Ca values are compared to those of other elements in *Elaeagnus angustifolia* L. fruits, the average concentration of Mg and Ca are very low. With respect to Mg content, it is higher in oleaster than that in dried apple (16.00 mg/100 g), dried cranberry (5.00 mg/100 g) and fig (2.03 mg/100 g) while lower than that in dried apricot (32.00 mg/100 g) and prune (41 mg/100 g). On the other hand, Ca content is higher than that in dried apple (14.00 mg/100 g) and dried cranberry (10.00 mg/100 g) but lower than that for dried in dried apricot (55.00 mg/100 g), dried fig (162.00 mg/ 100 g) in *Elaeagnus angustifolia* L. fruits[25]. According to present results, *Elaeagnus angustifolia* L. fruits are moderate sources of Mg and Ca. Nevertheless, present findings make a significant contrubition to the limited information available in the literature.

TABLE-5
MAJOR ELEMENT CONTENT OF *Elaeagnus angustifolia* L. FRUIT

	Range	Mean ± SD
Na (mg/ 100g)	151.81-192.17	173.10 ± 17.30
Mg (mg/ 100g)	20.32-23.81	22.10 ± 1.30
K (mg/ 100g)	795.83-909.53	850.40 ± 57.00
Ca (mg/ 100g)	36.18-42.27	40.40 ± 2.40
P (mg/ 100g)	60.20-67.31	63.80 ± 2.70

TABLE-6
PERFORMANCE CHARACTERISTICS OF THE METHOD

Metals	Detection limit (mg/kg)	Quantification limit (mg/kg)
Na	1.73	5.76
Mg	0.04	0.14
K	0.51	1.70
Ca	0.19	0.63
P	0.01	0.04

Total polyphenol content and antioxidant capacity: The exocarp or peels which are usually disposed of as waste material in many food processing industries could be used as a rich source of beneficial phytochemicals. Furthermore, this could prevent environmental pollution and economic losses[31]. For this reason, total polyphenol content and the antioxidant activity were measured both in mesocarp and exocarp of oleaster fruits using different extraction procedures and results were given in Table-7.

Average total phenolic content of aqueous, acetone and methanolic extracts of the mesocarp and exocarp in oleaster fruit are measured as 778.11 and 558.52, 390.44 and 361.24 and 413.95 and 524.40 mg gallic acid equivalents 100 g^{-1} dried mass, respectively. Water extraction is much more capable of determining antioxidant capacity and total phenolics compared to methanol and acetone extraction procedures for tested samples ($p < 0.05$).

TABLE-7
TOTAL POLYPHENOLIC CONTENT AND DPPH FREE RADICAL SCAVENGING ACTIVITY OF *Elaeagnus angustifolia* L. FRUIT EXTRACTS

Sample	Extraction procedure	Total polyphenolic content (mgGAE/ 100 g FW)	DPPH (µmol Trolox/g FW)
Exocarp	Extraction with water	558.52	27.62
	Extraction with acetone	361.24	27.95
	Extraction with methanol	524.40	27.84
Mesocarp	Extraction with water	778.11	28.03
	Extraction with acetone	390.44	27.16
	Extraction with methanol	413.95	27.82

Total phenolic contents and antioxidant capacity of the mesocarp tissues are significantly ($p < 0.05$) higher than those of the exocarp tissues in *Elaeagnus angustifolia* L. fruits. The free radical (DPPH) scavenging activity as µmol Trolox equivalents of 28.03 g^{-1} dried mass water extracts are obtained from mesocarp of *Elaeagnus angustifolia* L. fruits. We think that more than one type of antioxidant activity assay method should be performed in order to take various mechanisms of antioxidant action into account for fruits.

No data have been reported on total polyphenol content and antioxidant capacity in *Elaeagnus angustifolia* L. fruits, whereas very few studies have been performed on other *Elaeagnus* species fruits. Wang *et al.*[32] reported that autumn olive (*Elaeagnus umbellata*) berry had high scavenging free radical activities for O_2^- and $^{\bullet}OH$. Wang and Fordham[18] showed that the ED_{50} values ranged from 2.42 to 5.37 mg of fresh mass for six autumn olive genotypes.

Conclusion

Herein, we reported the physical characteristics, chemical compositions, total phenolic content and antioxidant capacities of *Elaeagnus angustifolia* L. fruits sample from a wide range of habitats in the environment. It can be concluded that *Elaeagnus angustifolia* L. fruit is a valuable horticultural product, based on their rich and beneficial nutrient composition. This study is a preliminary research for investigating the nutritional values and potential use of *Elaeagnus angustifolia* L. species and hopefully it paves the way for further research projects.

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