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Antioxidants in different parts of oleaster as a function of genotype

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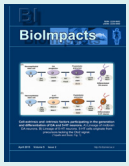

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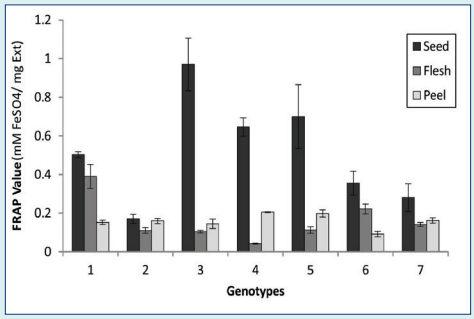
Abstract

Introduction: Fruits of oleaster (*Elaeagnus angustifolia* L.) were used in traditional medicine to treat various diseases. The aim of this study was to evaluate and compare the phenol and flavonoid contents and antioxidant activity of methanol extracts from the fruit peel, flesh and seed of seven genotypes of oleaster.



Methods: The phenol and flavonoid contents were determined using spectrophotometric methods. Antioxidant and antiradical activities were determined using reducing power, ferric-reducing antioxidant potential (FRAP) and ability to scavenge DPPH radical assays.

Results: Significant differences ($P<0.05$) were found in phenol and flavonoid contents and antioxidant activity among components of fruit and within various genotypes.

Conclusion: Results indicated that *oleaster* has good fruit quality varying among different genotypes. Seeds of fruits have excellent antioxidant activity and phenolic contents in comparison to flesh and peel.

Introduction

Beneficial effects of plant products are related to the presence of phytochemical compounds with antioxidant properties such as phenols. Natural phytochemical compounds are able to control the oxidative stress derived from excessive production of free radicals. Oxidative stress can cause damage to biological macromolecules. This may trigger many human diseases including cancer, cardiovascular, diabetes and neurodegenerative diseases like Alzheimer's and Parkinson's.[1,2] Because of the effect of genotypes on the phytochemicals and so biological activity of plants, screening and selection of potential genotypes with high antioxidant content is important to breed genotypes with better functional properties for human consumption.[3,4]

Elaeagnus angustifolia L. is a member of the *Elaeagnaceae* family and commonly called Russian olive or oleaster.[5] *E. angustifolia* has many uses in folk medicine. Fruits and flowers are used as a tonic, nutritious, anti-ulcerogenic and antipyretic agent. It is also used for treating urinary diseases, diarrhea, nausea, vomiting, gastric disorders, jaundice, asthma and flatulence.[6,7] Recent pharmacological studies have represented muscle relaxant activity, antibacterial, anti-inflammatory and antinociceptive effects.[8,9] Phytochemical studies have shown that the fruit of *E. angustifolia* is rich in compounds such as flavonoids compounds, sitosterol, terpenoid, coumarins, carotenoids, vitamins, and tannins which can have many benefits in the food and pharmaceutical industries.[9-11]

E. angustifolia grown in Iran is genetically diverse with a wide distribution. Despite widespread use of fruits of *E. angustifolia* under traditional medicine in Iran, little attention has been paid to the phenol content and antioxidant activity of fruit components and the effect of genotype. The aim of this study was to determine and compare phenol and flavonoid contents in different genotypes of *E. angustifolia* seed, flesh, and peel growing in East Azerbaijan province, Iran, and evaluate their

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potential antioxidant and antiradical activities in order to establish its potential as an edible source of valuable nutrients.

Materials and methods

Materials

DPPH (1, 1-diphenyl-2-picrylhydrazyl), TPTZ (2, 4, 6-tripyridyl-S-triazine), and quercetin were purchased from Sigma chemical company (Steinheim, Germany). Folin–Ciocalteu's reagent was obtained from Sigma–Aldrich Chemical Co (St. Louis, MO USA). DMSO (Dimethyl sulfoxide) was obtained from Scharlau Chemie (Barcelona, Spain). All other reagents were of analytical reagent grade.

Plant material and preparation of extract

E. angustifolia from the selected genotypes IEa-1 to IEa-7 (Iranian *Eleaeagnus angustifolia*-1) were collected from Esfahlan, Tabriz, East Azerbaijan province, Iran, during autumn 2011. Different parts of the fruits (peel, flesh and seed) were separated, dried, and powdered. Methanol (MeOH, 90%) extracts of the powders were prepared by maceration method. The extraction was repeated three-times and the solvent was evaporated in a vacuum, and dried extracts were stored at 4°C until use. All extracts obtained were re-dissolved in DMSO and were used in antioxidant measurement and determination of phytochemical content.[12]

Determination of total phenol

Total phenolic content (TPC) of extracts of each genotype was determined with the Folin-Ciocalteau's reagent according to the method of Singleton and Rossi[13] with some modifications. Briefly, 0.1 mL aliquot of the sample was mixed with 2.0 mL of 2% Na_2CO_3 and was allowed to remain at room temperature for 2 min. 0.1 mL of 50% Folin-Ciocalteau's phenol reagent was added, and the reaction mixture was mixed thoroughly and allowed to remain for 30 min in the dark. After incubation, absorbance of all the sample solutions was measured at 720 nm using a spectrophotometer. Different volumes of quercetin (mM) were used as a standard for the calibration curve. TPC was expressed as mM quercetin equivalents (QE) per mg of extract (mM/mg).

Determination of total flavonoid

Total flavonoid content of oleaster peel, flesh and the kernel extract from seven studied genotypes was measured by a colorimetric assay.[14] To 0.25 mL of samples, 75 µL $NaNO_2$ solution (5%), 0.15 mL $AlCl_3$ solution (10%), and 0.5 mL NaOH solution (1M) were added. Final volume of solution was increased to 2.5 mL by adding deionized water. Prepared solution was allowed to stand for 5 min. Then its absorbance was measured at 507 nm against the same mixture, without the sample as a blank. Quercetin was used as a standard for the calibration curve. Total flavonoid content of extracts was expressed as mM quercetin equivalents (QE) per mg of sample (mM/mg).

Reducing power assay

Reducing power of oleaster peel, flesh and the kernel extract from different genotypes were determined according to the method of Oyaizu.[15] One milliliter of 0.2 M phosphate buffer (pH 6.6) and 1 mL of $K_3Fe(CN)_6$ (1% w/v) were added to 1.0 mL of oleaster seed, flesh and peel methanol extract. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 1 mL of Trichloro acetic acid (10% w/v). The mixture was centrifuged at 13400 rpm for 5 min to collect the upper layer of the solution (1 mL) and mixed with 1 mL of distilled water and 0.1 mL of $FeCl_3$ (0.1%, w/v). The absorbance was then measured at 700 nm against blank sample. The increased absorbance of the reaction mixture indicated the increased reducing power.

FRAP assay

Total antioxidant capacity of oleaster peel, flesh and the kernel extract from different genotypes was measured as ferric-reducing antioxidant potential (FRAP). This assay was performed according to the method of Benzie and Strain[16] with some modifications. The FRAP assay seems to be a good tool to indicate the differences in total antioxidant power between cultivars and genotypes.[17] The freshly-prepared FRAP solution contained 25 mL of 300 mM acetate buffer (pH 3.6) and 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM ferric chloride ($FeCl_3.6H_2O$). This solution was used as blank. This method evaluates the ability of the antioxidants in a sample to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) that absorbs light at 593 nm. The sample was incubated at 37°C throughout the monitoring period. FRAP values for all samples were calculated by standard calibration curve obtained by using different concentrations of $FeSO_4.7H_2O$.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to the method described by Wettasinghe and Shahidi.[18] Different concentrations (30, 50, 70 and 100 µg/mL) of extracts were added to 2 mL of DPPH solution (0.1 mM in methanol) and reduction of DPPH absorbance was followed by monitoring at 517 nm (A_S). As a control, the absorbance of the blank solution of DPPH (2 mL) was also determined at 517 nm (A_C). The percentage of radical scavenging activity (RSA %) was calculated according to the following equation:

$$RSA\ \% = \frac{100\ (A_C - A_S)}{A_C}$$

Data analysis

The results are expressed as mean values and standard deviation (SD) of the mean. The differences within the oleaster genotypes and among components of fruit were analyzed using one-way analysis of variance (ANOVA) followed by Tukey multiple range test at $P < 0.05$. These analyses were carried out using SPSS version 11.5.

Results

Determination of total phenols and flavonoids contents

One of the most significant current discussions around the world is to identify genotypes with high amounts of polyphenols for targeting enhanced performance properties in edible plants including fruits and vegetables. In this study the content of total phenolic in each genotype of oleaster peel, flesh and seed was determined spectrometrically according to the Folin-Ciocalteu method and calculated as quercetin equivalents (QE). Fig. 1 shows the total phenolic content of the seven oleaster seed, flesh and peel. Differences in phenolic content among different genotypes were statistically significant ($P < 0.05$). As shown in Fig. 1, *E. angustifolia* seed contains high amounts of phenol content as compared to the other parts of fruit in each genotype. The results imply that the methanol extract of seeds could be effective in the antioxidant and free radical scavenging activity. Total phenolic content in seed ranged from 2.14 ± 0.12 (IEa-6) to 6.26 ± 0.04 (IEa-3) mM Q/mg Ext. For flesh, it ranged from 0.14 ± 0.07 (IEa-4) to 1.54 ± 0.04 (IEa-1) mM Q/mg Ext and in peels ranged from 0.12 ± 0.05 (IEa-1) to 2.59 ± 0.15 (IEa-5) mM Q/mg Ext (Fig. 1 and Table 1).

Total flavonoid content was expressed as quercetin equivalents (QE) in mM per milligram of extract. A variation in terms of total flavonoid content was observed among genotypes and different parts of fruits and the differences were statistically significant ($P < 0.05$). Results showed that the seed had the highest flavonoid content compared to the other components. The flavonoid content in the seed ranged from 4.7 ± 0.11 (IEa-1) to 17.6 ± 0.15 (IEa-3) mM Q/mg Ext. In flesh, it fluctuated from 0.62 ± 0.11 (IEa-7) to 1.90 ± 0.04 (IEa-1) mM Q/mg Ext and in peel, it ranged from 0.64 ± 0.02 (IEa-3) to 1.13 ± 0.05 (IEa-5) mM Q/mg Ext (Fig. 2).

Determination of reducing power, FRAP and Radical-scavenging activity

In the present study, we used some methods such as reducing power, FRAP and DPPH assays to determine the antioxidant potential of different parts of *E. angustifolia* fruits. For the determination of the reductive ability, Fe^{3+} to Fe^{2+} transformation in the presence of methanol extract was investigated. All samples revealed a high reducing power that varied among the genotypes (Fig. 3). The absorbance values of the seed extract in different genotypes were found to be more than that of peel and flesh extract. The average of reducing power value in seven genotypes of oleaster seed extract was 0.79 at 700 nm. Maximum reducing power was 1.1 ± 0.04 for IEa-3 and minimum reducing power was 0.47 ± 0.09 for IEa-2. The average of reducing power value in seven genotypes of oleaster flesh extract was 0.548 at 700 nm. Maximum reducing power was 0.61 ± 0.02 for IEa-2 and minimum reducing power was 0.45 ± 0.01 for IEa-6. The average of reducing power value in seven studied genotypes of oleaster peel extract was 0.246 at 700 nm. Maximum reducing power was 0.44 ± 0.08 for IEa-5 and minimum reducing power was 0.13 ± 0.01 for IEa-1.

The FRAP assay showed greater variability among various extracts. These differences in antioxidant activities among oleaster peel, flesh and seed are shown in Fig. 4. Methanol extract of seed was significantly more active than all other components ($P < 0.05$). These activities also were different among the oleaster genotypes. The highest FRAP value in the oleaster seeds was 0.80 ± 0.14 mM Fe^{2+}/mg Ext for IEa-3 and the lowest FRAP value, 0.17 ± 0.02 mM Fe^{2+}/mg Ext, was obtained for IEa-2. The maximum FRAP

Table 1. The comparison of DPPH IC_{50} (mg/ml) in each genotype of *E. angustifolia* L. seed, flesh and peel

	Seed	Flesh	Peel
IEa-1	30.1	24.2	90.2
IEa-2	75.16	83.7	80.9
IEa-3	24.5	116.6	105.4
IEa-4	25.02	165.7	50
IEa-5	22.06	75.9	69.35
IEa-6	37.6	77.7	123.14
IEa-7	36.4	98.7	89.7

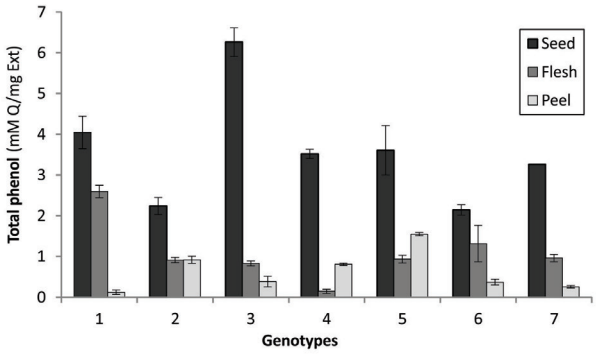

Fig. 1. Total phenolic content (TPC) of seeds, fleshes and peels from *E. angustifolia* fruit. 1: IEa-1, 2: IEa-2, 3: IEa-3, 4: IEa-4, 5: IEa-5, 6: IEa-6, 7: IEa-7. Means of triplicate measurements ± SD, $P < 0.05$.

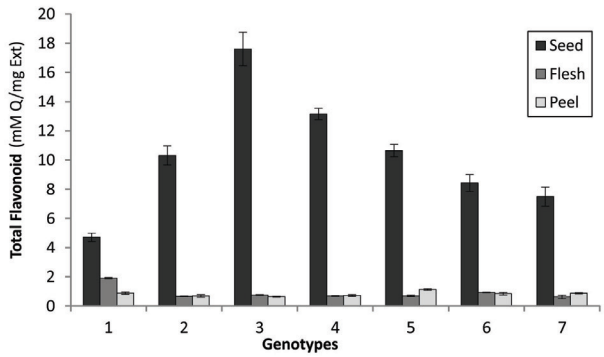

Fig. 2. Total flavonoid content (TFC) of seeds, fleshes and peels from *E. angustifolia* fruit. 1: IEa-1, 2: IEa-2, 3: IEa-3, 4: IEa-4, 5: IEa-5, 6: IEa-6, 7: IEa-7. (Mean ± SD, n = 3), $P < 0.05$.

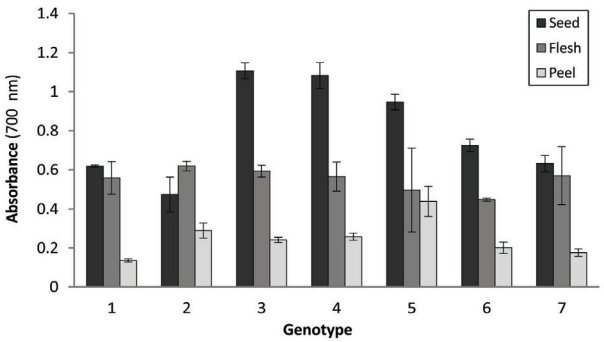

Fig. 3. Reducing power of seeds, fleshes and peels from *E. angustifolia* fruit. 1: IEa-1, 2: IEa-2, 3: IEa-3, 4: IEa-4, 5: IEa-5, 6: IEa-6, 7: IEa-7. (Mean ± SD, n = 3), $P < 0.05$.

value in flesh extracts was 0.39 ± 0.062 mM Fe^{2+}/mg Ext for Ea-1 and the lowest, 0.04 ± 0.003 mM Fe^{2+}/mg Ext, was obtained for IEa-4. The maximum FRAP value in peel extracts was 0.2 ± 0.019 mM Fe^{2+}/mg Ext for IEa-5 and the lowest of it,0.09 ± 0.014 mM Fe^{2+}/mg Ext was obtained for IEa-6.

Radical scavenging activity of the extracts was determined through the reduction in the optical absorbance at 517 nm due to scavenging of the stable DPPH free radical. A positive DPPH test suggests that the samples are free radical scavengers. The radical scavenging activities of samples were calculated against the blank solution and samples showed various results that are presented in Fig. 5. The inhibition percentage of all extracts on the DPPH radical was found to be dependent on the concentration. It was shown that seed was stronger in the scavenging of DPPH radical as compared to other components. In order to quantify the antioxidant activity, IC_{50} was further calculated (Table 1). The IC_{50} is the concentration of sample required to decrease the absorbance at 517 nm by 50%. A lower value of IC_{50} would reflect greater antioxidant activity of the sample. In the current study, the highest radical scavenging activity was observed in the seed extract of IEa-3 (IC_{50} = 24.5 mg/mL), whereas the

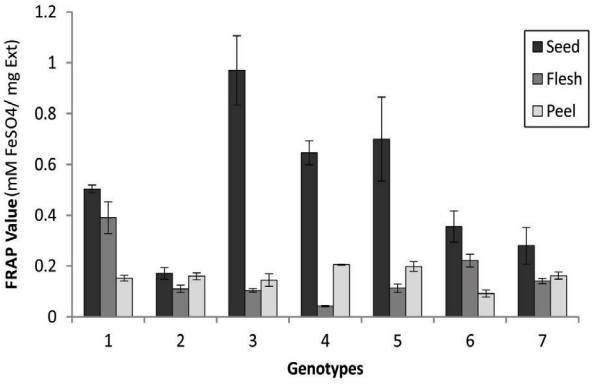

Fig. 4. FRAP value of seeds, fleshes and peels from *E. angustifolia* fruit. 1: IEa-1, 2: IEa-2, 3: IEa-3, 4: IEa-4, 5: IEa-5, 6: IEa-6, 7: IEa-7. (Mean ± SD, n = 3), $P < 0.05$.

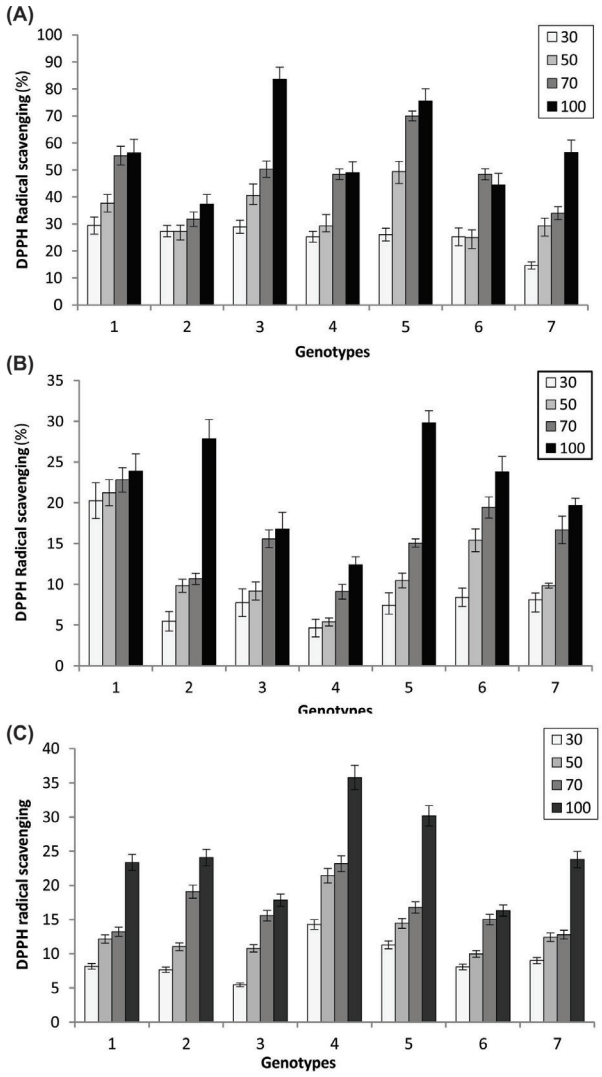

Fig. 5. DPPH radical scavenging activity percentage of various concentrations of seeds (A), fleshes (B) and peels (C) from *E. angustifolia* fruit. 1: IEa-1, 2: IEa-2, 3: IEa-3, 4: IEa-4, 5: IEa-5, 6: IEa-6, 7: IEa-7. The kinetics of scavenging effects was determined in 10 minutes.

methanol extract of peel of IEa-6 showed lower activity (IC_{50} = 123.14 mg/mL).

Correlation between antioxidant components and antioxidant properties

The results of correlation analyses among total phenolic content, flavonoid content, reducing power, FRAP values and DPPH radical scavenging activity are shown in Table 2. In seed, significant correlations ($P < 0.05$) were observed between FRAP values with TPC (total phenolic content) (r = 0.87) and DPPH (r = 0.87) scavenging activities and TPC of the seed extracts. Correlation between reducing power and TPC was positive, but not significant (r = 0.65). The correlation between flavonoid content and reducing power also was significant at the $P < 0.5$ level (r = 0.76); however, between flavonoid and FRAP value (r = 0.69), DPPH scavenging (r = 0.59) was not significant (Table 2). In flesh of investigated fruit genotypes, correlation

Table 2. Correlation coefficients (r) between antioxidant components and antioxidant properties on methanol extracts from fruit components of seven *E. angustifolia* genotypes

	FRAP value (mM $FeSO_4$/mg Ext)	Reducing power (700 nm)	DPPH radical scavenging (%)
		Seeds	
Phenolic content[a]	0.87[c]	0.64[b]	0.87[c]
Flavonoid content[a]	0.69[b]	0.76[c]	0.59[b]
		Fleshes	
Phenolic content[a]	0.98[d]	-0.16[b]	0.95[d]
Flavonoid content[a]	0.93[d]	-0.09[b]	0.97[d]
		Peels	
Phenolic content[a]	0.60[b]	0.97[d]	0.50[b]
Flavonoid content[a]	0.22[b]	0.43[b]	0.33[b]

[a] Expressed as milligram quercetin equivalents per milligram extract.

[b] No significant, [c] $P < 0.05$, [d] $P < 0.01$.

between FRAP value and TPC (r = 0.98), total flavonoid content (r = 0.93) was very high and significant at the level of 0.01, but the same correlation between reducing power and TPC and TFC was negative. The correlation between antiradical activity and TPC was positive. Strong correlation coefficients in DPPH, TPC and TFC were found (r = 0.95 and 0.97) (Table 2). In the peel, correlation between TPC and reducing power was significant at the 0.01 level (r = 0.98). But there was moderate correlation between the FRAP value (r = 0.61), DPPH radical scavenging activities (r = 0.49) and TPC. Correlation between flavonoid content and FRAP value (r = 0.22), reducing power (r = 0.43), DPPH (r = 0.33) were positive but not significant.

Discussion

A large and growing body of literature has investigated the variation in phenolic content among genotypes.[3,4,17,19-21] 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 and Fordham reported significant differences in levels of phenolic contents of the fruits of six genotypes of autumn olive (*Elaeagnus umbellate*).[19] Important and variable genotypic effects on the physicochemical parameter of the berries of sea buckthorn (*Hippophae rhamnoides* L.) has been revealed from the *Elaeagnaceae* family, grown in Turkey.[21] The chemical composition, total phenolic content and antioxidant capacities of *E. angustifolia* fruit samples from a wide range of habitats in the environment was also reported. It can be concluded that *E. angustifolia* fruit is a valuable horticultural product, due to its rich and beneficial nutrient composition.[22]

Seeds showed a much higher antioxidant capability and phenolic content than the edible portions in the fruits, as supported by Soong and Barlow.[23] Most fruits commonly consumed in China such as pomegranate, grape and hawthorn peel and seed fractions were stronger than the pulp fractions in antioxidant activity based on their FRAP values.[24] It has been shown that genetic background (species and cultivars) play an important role in determining the antioxidant potential of fruits.[19]

Our results are in accordance with Dehghan et al,[12] in which it was reported that scavenging effects of *Ferula szovitsiana* extracts on the DPPH radical had a concentration-dependent manner. The radical scavenging percentages were varied among different components of fruits (seeds, fleshes and peels) and among different genotypes in all components of fruits. A statistically significant difference was found among the genotypes of cornelian cherry in Iran, in the antioxidant activity results using the DPPH method.[25] Wang and Fordham[20] showed different genotypes of autumn olive (*E. umbellate*) had high scavenging radical activities that varied among genotypes.

The significant differences among *E. angustifolia* genotypes in terms of total phenolic, flavonoid content and also antioxidant activity is supposed to be largely because of the genotype; since all plants were grown in the same ecological condition. However, there have been no controlled studies that have compared differences in phytochemical compounds and antioxidant activity in different genotypes of *E. angustifolia*.

In general, the correlation between antioxidant capacity and the content of bioactive compounds varied remarkably among genotypes. These results are supported by Chirinos et al.[26] A correlation has been reported between TPC and the radical scavenging activity/FRAP values, for carica, raisins, blueberries, spinach and broccoli.[23,27] Strong correlations were also observed between the content of TP and TF and antioxidant capacity of fruit from *Elaeagnus angustifolia*.[28]

Moyer et al[29] observed that the correlation coefficient of total phenolics and FRAP vary among genotypes of Vaccinium, Rubus and Ribes. The antioxidant activity of some vegetables and free phenolic compounds showed a positive, tough not strong, correlation (r = 0.57).[30] In the case of leguminous seed extracts, a statistically significant ($P < 0.01$) correlation was observed for total phenolics

versus total antioxidant activity.[31] The strong correlation between FRAP values with TPC (r = 0.92) and DPPH numbers with TPC (r = 0.96) has been found in the organic extracts of *Ferula szovitsiana*, as reported by Dehghan et al.[12] A strong correlation was also found between the content of total phenolics/flavonoid and reducing power/DPPH radical scavenging in the extracts of hulls and shells of four wild almonds (*P. amygdalus* L.) as reported by Jahanban Isfahlan et al.[32] Further, correlation between the phenolic content and antioxidant activity using the ferric reducing antioxidant power assay for methanol extracts of 30 Chinese medicinal plants was significant (r = 0.758).[33] The antioxidant capacity might not always correlate with the amount of phenolics.[34] The comparative study of the antioxidant activity of flowers and young branches soft extracts of *E. angustifolia* and their polyphenols content represents rather a linear interrelationship between them. The correlation between the antioxidant activity and their flavonoids content does not show a linear dependence upon those parameters.[35] Termentzi, Kefalas and Kokkalou[36] reported a low correlation between DPPH and phenolic content (r = 0.25). Of course all data are experimental and rather controversial, so there is no general agreement about correlation between antioxidant activities and phenolic contents.

Conclusion

In this study, results were obtained for the first time regarding the total phenol and flavonoid content, reducing power, FRAP value, and DPPH radical scavenging activities of seven genotypes of oleaster (*Elaeagnus angustifolia* L.) peel, flesh, and seed, growing in Iran. In conclusion, *E. angustifolia* fruit showed high phenolic content, antioxidant and antiradical activities. The present study revealed a significant difference ($P < 0.05$) among the investigated genotypes and also among different parts of fruit. Results showed that *E. angustifolia* can be used easily as an accessible source of natural antioxidants and as a potential food supplement or even in the pharmaceutical industry. In particular, seed of fruit was found to be most potent, although seed, flesh and peel extract contained a certain amount of phenol and flavonoids. Further research should be carried out to identify the predominant compounds responsible for the antioxidant activity.

Ethical issues

There is none to be declared.

Competing interest

Authors declare that there is no conflict of interest.

Research Highlights

What is current knowledge?

√ Fruits of oleaster have been used to treat various diseases in traditional medicine.

What is new here?

√ Antioxidant content and activity of different parts of seven genotypes of oleaster fruit were investigated.

√ Significant differences were found in antioxidant content and its antioxidant activity among different parts of fruit and genotypes.

√ Results indicated that oleaster has good fruit quality that is variable among different genotypes.

√ Seeds of fruits have excellent antioxidant activity and phenolic content in comparison to fleshes and peels.

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