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Original Article

Investigation of antioxidant properties, essential oil, and fatty acid composition of *Onobrychis armena* Boiss. & Huet

Sukru Karatas[1], Abdurrahman Aktumsek[2], Sengul Uysal[3]

[1]Mugla Sitki Kocman University, Koycegiz Vocational School, Mugla, Turkiye
[2]Selcuk University, Science Faculty, Department of Biology, Konya, Turkiye
[3]Erciyes University, Halil Bayraktar S.H.M.Y.O., Kayseri, Turkiye

ORCID IDs of the authors: S.K. 0000-0003-0348-1076; A.A. 0000-0002-5151-2650; Ş.U. 0000-0003-4562-1719

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ABSTRACT

Background and Aims: In this study, *Onobrcyhis armena* Boiss. & Huet was screened for its antioxidant potential, fatty acids and volatile compounds.
Methods: Antioxidant activities of different extracts (ethyl acetate, methanol and water) were measured using the phosphomolybdenum assay, free radical scavenging assay, β-carotene/linoleic acid method, and ferric and cupric reducing power assay. Total phenolic and flavonoid contents were also calculated spectrophotometrically.
Results: GC analysis revealed that the oil was dominated by palmitic (22.67%) and linoleic (15.09%) acids. Unsaturated acids levels were higher than saturated fatty acids. The essential oil was analyzed by GC-MS system and twenty-two volatile compounds were identified. The identified major components were n-hexadecanoic acid, 9-12 octadecanoic acid, tetradecanoic acid and hexahydro farnesyl acetone.
Conclusion: The results of this study show that *O. armena* can be used as an easily accessible source of natural antioxidants and unsaturated fatty acids in food and pharmaceutical industries.

Keywords: *Onobrychis*, Fatty acids, Essential oil, Phenolic compounds, GC-MS

INTRODUCTION

Free radicals are atoms or groups of atoms having an unpaired electron in their last orbital. Generally, they are known as reactive oxygen species consisting of hydroxyl radical, hydrogen peroxide, peroxynitrite, nitric oxide, peroxyl radical, singlet oxygen and superoxide anion. Oxidative stress is caused by the discrepancy between the production of free radicals in the body and the attempt to render their bad effects harmless with antioxidants. These render free radicals harmless by complementing their unpaired end electrons with extra electrons or by breaking them down (Halliwell & Gutteridge, 1984).

Antioxidants play a significant role in the prevention of diseases with their ability to repair or cleanse the damage caused by free radicals (Alonso, Guillen, Barroso, Puertas, & Garcia, 2002). Epidemiological studies have revealed an inverse relationship between dietary antioxidant-rich foods, degenerative processes, and death (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Pietta, 2000). Natural chemicals obtained from plants, especially phenolic compounds with high antioxidant activity, have enabled them

Address for Correspondence:
Şükrü KARATAŞ, e-mail: sukrukaratas@mu.edu.tr

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to be used in the pharmaceutical and food industry. In addition to their antioxidative properties, herbal ingredients are valuable for their antiviral, antimicrobial and anti-inflammatory activities (Rice-Evans, Miller, & Paganga, 1997).

Essential oils and chemical components obtained from medicinal plants are used as deodorizing and flavoring additives in the cosmetics and food industry. Also, essential oils play an important role in the protection of plants against insects in nature (Bakkali, Averbeck, Averbeck, & Waomar, 2008).

Plant-based products used against oxidative damage and diseases, such as various foods, dietary supplements, and pharmaceuticals, have attracted worldwide attention in recent years (Huang, Ou, & Prior, 2005).

In recent years, besides natural antioxidants or plant-based foods containing antioxidants, synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), PG (propyl gallate) and TBHQ (tertiary butyl hydroquinone) have been used to prevent oxidative damage in the body, as well as to prevent lipid oxidation in foods. However, some studies have shown that synthetic antioxidants can harm human health (Valentao et al., 2002).

Fabaceae comprise the second largest plant family after the Asteraceae family with more than 18,000 species common worldwide (Zengin et al., 2015). The Fabaceae plant family is medicinally, economically, and culturally important (Erbil, Duzguner, Durmuskahya, & Alan, 2015).

Onobrychis is a member of the Fabaceae family of which 162 species have been identified in the world. In Turkiye there are a total of 52 Onobrychis genera,27 of which are endemic. These have a rich phenolic content (such as *p*-hydroxybenzoic acid, ferulic acid, rutin, benzoic acid, caffeic acid, *p*-coumaric acid, quercetin) (Karakoca, Asan-Ozusaglam, Cakmak, & Teksen, 2015), and therefore these have a significant antioxidant (Karamian & Asadbegy, 2016) and antimicrobial activity (Usta, Yildirim, & Turker, 2014).

In this study, antioxidant properties, fatty acids and essential oil composition of the aerial parts of *Onobrychis armena* Boiss. & Huet plant collected from Konya (Turkiye) were studied. For this purpose, different extracts (ethyl acetate, methanol and water) of *Onobrychis armena* were prepared and its antioxidant activities were measured using the phosphomolybdenum experiment, free radical scavenging test, *β*-carotene/linoleic acid method, and ferric and copper reducing power test. Also, the total phenolic and flavonoid contents were determined to understand the usefulness of this plant as a foodstuff as well as in medicine.

MATERIAL AND METHODS

Plant materials

Onobrychis armena was collected during the flowering period from the area between Yükselen and Kestel villages in Konya province (Turkiye). The plants were identified by Prof. Dr. Murad Aydin Sanda from the Division of Botany within the Department of Biology, Science Faculty, Selcuk University (Konya). The voucher specimens were deposited in the KNYA herbarium at the Department of Biology, Selcuk University.

Chemicals

Potassium ferricyanide, Folin–Ciocalteu's reagent, BHT, BHA and methanol were purchased from Merck (Germany); 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene/linoleic acid and Tween 40 were purchased from Sigma–Aldrich GmbH (Germany). All other chemicals and solvents were analytical grade.

Preparation of the extracts

The collected plant samples were dried in the shade, then they were ground thoroughly in the mill. 15 g of powdered samples were weighed and extracted separately with ethyl acetate and methanol for 6-8 hours in the soxhlet apparatus. After this time, the mixture obtained was filtered with Whatman paper. Then the solvents were completely evaporated at 40 ^{0}C in a rotary evaporator. The obtained dry extracts were stored at +4 ^{0}C until analysis. 15 g of dry herbal material was boiled in 250 mL of water for 30 minutes to obtain a water extract. The water extract was frozen after filtering and lyophilized to completely remove the water. The lyophilized dry herbal drug was stored at +4 ^{0}C until analysis. The extraction yields were 2.4%, 10.8% and 14.2% for ethyl acetate, methanol, and water, respectively.

Total phenolic (folin-ciocalteu method) substance determination

A concentration of plant extracts was prepared consisting of 2 mg/mL, and 200 µl of each concentration was taken into separate test tubes. Then 1.5 mL of water and 100 µL of Folin-Ciocalteu reagent were added to each tube. After this, 500 µL of 2% Na_2CO_3 solution was added to each tube. After the mixtures were left in the dark for 2 hours at room temperature, their absorbance was measured at 765 nm. Spectrophotometric measurements in all antioxidant capacity determination tests were carried out using the Shimadzu UV-1800 spectrophotometer. The same procedures were repeated for the standard gallic acid. The phenolic content of the plants was given as gallic acid equivalent (mg GAE/g) (Slinkard & Singleton, 1977).

Total flavonoid substance determination

The total flavonoid content in plant extracts was determined spectrophotometrically. Accordingly, 1 mL of the methanolic solution of 2% $AlCl_3$ was taken and mixed with the same volume and 2 mg/mL concentration of plant extract. After waiting for 10 minutes, the absorbance of the mixture against blank was determined at 415 nm. The same procedures were done for the standard flavonoid routine, and the calibration curve for the routine was drawn. As a result, the total flavonoid substance contents of the extracts were given as routine equivalent (mg RE/g) (Arvouet-Grand et al., 1994).

Determination of total antioxidant capacity

The basis of the method is the reduction of Mo (VI) to Mo (V) and the formation of green colored phosphate/Mo (V) complex in acidic medium. Following this method, the first step was to prepare solutions of plant extracts with a concentration of 2 mg/mL.

The reagent solution was prepared by mixing the prepared solutions in a cylinder. 0.3 mL of herbal solutions at a concentration of

1 mg/mL was taken into a tube and 3 mL of the reagent solution was added. Tubes were mixed vigorously and stored at 95 ^{0}C.

The incubation lasted for 90 minutes,at the end of which the absorbance of the solutions was read at 695 nm. The same procedures were followed for ascorbic acid, which is used as a standard antioxidant. Antioxidant activity was calculated as ascorbic acid equivalent (mg AAE/g) (Prieto, Pineda, & Aguilar, 1999).

β-carotene/Linoleic acid emulsion system

In this method, the emulsion solution was first prepared. For this, 1 mg of β-carotene was dissolved in 2 mL of chloroform. 50 µL linoleic acid and 200 mg Tween 40 were added to this mixture. The mixture was thoroughly mixed. Chloroform was thoroughly evaporated at 40 ^{0}C in a rotary evaporator. 200 mL of pure water was added on the remaining part. Thus, the emulsion solution was prepared.

350 µl of herbal drugs and standard substances with a concentration of 2 mg/mL were taken and 2.5 mL of emulsion solution was added to them. As soon as the emulsion solution was added, the absorbances were read at 490 nm. The tubes were then incubated at 50 ^{0}C for 120 minutes. In addition, the control solution was prepared by adding 350 µL of methanol instead of the plant material and then adding 2.5 mL of emulsion solution. The absorbance of the control solution was also read as soon as the emulsion solution was added and was likewise incubated at 50 ^{0}C for 120 minutes (Sokmen et al., 2004). The reduction percentage was given as shown below.

$$I\ (\%)=(A_0-A_1)/A_0x100$$

In this formula, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

DPPH free-radical scavenging assay

Free radical removal activities of plant samples were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). 0.4 mM DPPH solution was subjected to dilution with ethanol by controlling the absorbance at 517 nm. Subsequently, 40 µL of different concentrations of plant samples were placed in microplates. Then, 120 µL of ethanol and 40 µL of DPPH solution were added to be incubated for 30 minutes in a dark condition. The absorbances were read at 517 nm. The absorbance results of the herbal extracts were examined against the control. Free radical removal activity was used as shown below and the percentage of the inhibition values was calculated from these absorbance values (Sarikurkcu et al., 2009).

$$I\ (\%)=(A_0-A_1)/A_0x100$$

In this formula, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

Reducing power activity [Iron (III) to iron (II) reduction]

In this method, concentrations of 0.2 to 2 mg/mL of herbal extracts were used. 2.5 mL of herbal solutions of different concentrations were taken. After that 0.2M pH:6.6 2.5 mL phosphate buffer and 2.5 mL 1% potassium ferricyanide were added. The tubes were left to incubate at 50 °C for 20 minutes. 10% Trichloroaceticacid (TCA) was added to the tubes after incubation. After mixing the tubes, 2.5 mL were transferred from their upper part to another tube. 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ solution were added into this tube. Absorbances of the solutions were read at 700 nm (Oyaizu, 1986).

CUPRAC assay

The cupric ion reducing activity (CUPRAC) was determined according to the method of Apak et al. (2006). Sample solution (0.5 mL) was added to premixed reaction mixture containing $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and NH_4Ac buffer (1 mL, 1M, pH:7.0). Similarly, a blank was prepared by adding a sample solution (0.5 ml) to the premixed reaction mixture (3 mL) without $CuCl_2$. Then, the sample and blank absorbances were read at 450 nm after a 30 min incubation at room temperature. The absorbance of the blank was subtracted from that of the sample. CUPRAC activity was expressed as Trolox equivalent (TE/g extract).

Determination of fatty acid composition of plants
oil extraction from plants

The ground and powdered 10 g of plant material was first extracted with petroleum ether for 6-8 hours in the soxhlet apparatus. At the end of the extraction, the residue after the solvent was evaporated in the evaporator was used in fatty acid analysis.

Preparation of methyl esters of fatty acids

0.1-0.2 g of the oil samples were transferred to the flasks. 4 mL of 2% NaOH solution was added to the oil samples and boiled for 10 minutes for saponification to occur. After saponification was completed, 5 mL of 14% BF_3-methanol complex was added and boiled for 5 minutes. Then, 2 mL of n-heptane was added to the mixture and left to boil for one minute. After boiling was complete, 4 mL of saturated NaCl solution was added. After mixing thoroughly, the balloons were transferred to separation funnels for phase separation and left for 5-10 minutes. At the end of this period, the lower aqueous part was discarded, and the upper yellow phase was transferred to vials and stored at -20 ^{0}C until analyzed (IUPAC, 1979).

Gas chromatography-mass spectrometry

The samples to be analyzed for essential oil were ground and subjected to water distillation with the Clevenger apparatus. The extracts obtained were made in HP Agilent 7890A Gas Chromatography using Agilent 5975C MS detector and HP Innowax column. Wiley and Nist libraries were used for the identification of essential oil components.

Helium was used as carrier gas in the analyzes and the flow rate was set at 1.2 mL/min. The initial temperature of the column was determined to be 60 ^{0}C and held at this temperature for 10 minutes. Later, by increasing 4 ^{0}C per minute, 220 ^{0}C was reached. After being kept at this temperature for 10 minutes, it reached 240 ^{0}C by increasing 1 ^{0}C per minute. Finally, it was held at this temperature for 30 minutes. Thus, the total analysis time was determined as 110 minutes. The temperature of the injector block was set at 240 ^{0}C. Mass spectrums were recorded at 70 eV.

RESULTS AND DISCUSSION

Total phenolic and flavonoid content

The total phenolic and flavonoid contents of the ethyl acetate, methanol and water extracts of *Onobrychis armena* are given in Table 1. Among the extracts, the phenolic content was mostly observed in the methanol extract. 62.199±0.001 mg GAE/g phenolic substance was found in the methanol extract. Methanol extract is followed by water (34.689±1.136 mg GAE/g) and ethyl acetate (18.323±0.852 mg GAE/g) in terms of content, respectively. Godevac et al. (2008), in their study investigating the antioxidant properties of nine Fabaceae members, determined the phenolic content in *O. scardica* methanol extract to be higher (115.23 mg GAE/g) than *O. armena*. However, *Astragalus glycyphyllos* L. (44.6 mg GAE/g) and *Coronilla emerus* L. (38 mg GAE/g) extracts had lower phenolic content than *O. armena*. The total phenolic contents determined in our study agree with the values given for some species of the Fabaceae family (Orhan et al., 2009; Orhan et al., 2011).

Flavonoids constitute the largest and most important group of phenolic compounds. Flavonoid contents of *O. armena* were calculated as routine equivalents. The richest extract in terms of flavonoid content is methanol extract with 32.589±0.465 mg RE/g content. Methanol extract is followed by water (20.046±0.141 mg RE/g) and ethyl acetate (16.846±0.061 mg RE/g) in terms of content, respectively. Hayet et al. (2008) determined the flavonoid contents of ethyl acetate and methanol extracts as 53.81 and 41.58 mg CAE/g, respectively, in their study on the biological activities of *Retama raetam* (Forssk.) Webb, a member of Fabaceae.

Total antioxidant capacity (Phosphomolybdate test)

The phosphomolybdate test is based on the reduction of the antioxidant compounds Mo (VI) to Mo (V) in an acidic environment and spectrophotometric measurement of the phosphate/Mo (V) complex formed.

The results of the method are given using a standard antioxidant and usually ascorbic acid. According to the results of this test, methanol extract has the highest efficiency (103.118±0.795 mg AAE/g). This extract is followed by ethyl acetate (38.933±0.596 mg AAE/g) and water (35.562±1.390 mg AAE/g) extracts, respectively (Table 1).

DPPH free radical removal method

The change in free radical scavenging activity of *O. armena* extracts depending on the concentration is presented in Table 2. These results show the methanolic extract to be the highest radical scavenging efficiency among the extracts. Although the water and methanol extracts show relatively similar activity, the activity of the ethyl acetate extract is very low. On the other hand, the free radical scavenging efficiency of BHA and BHT, which are synthetic antioxidants, is considerably higher than the extracts despite their low concentrations.

DPPH scavenging activity of *Vicia sativa* ssp. *nigra* was determined as 8.7% and 15.4% at a concentration of 0.5 mg/ml and 1 mg/mL, respectively (Orhan et al., 2009). The activity of the methanolic extract at a concentration of 1 mg/mL of *O. armena* used in our study is higher compared with the water extract. Of the other concentrations studied, the activities of water and methanol extracts were relatively close to each other.

Ferric reducing power

Reducing power or potential is one of the most important indicators of antioxidant capacity. This potential antioxidant activity indicates the electron donating ability of the compounds or extracts investigated. Therefore, high reducing power means high antioxidant activity. For this purpose, the ferric and copper reduction powers of *O. armena* extracts were investigated. The ferric reducing power is based on the investigation of the ability of antioxidant molecules to convert Fe^{+3}-Fe^{+2} and the resulting Prussian Blue color spectrophotometric determination. The higher the absorbance, the higher the reducing power. The ferric reducing power results are shown in Table 3, depending on the concentrations of the studied *O. armena* extracts (Table 3). Among the extracts, the best reducing power belongs to

Table 1. Total phenolic, flavonoid contents and total antioxidant capacity of *O. armena* extracts.

	Ethyl acetate	Methanol	Water
Total Phenolic (mg GAEs/g*)	18.323±0.852	62.199±0.001	34.689±1.136
Total Flavonoid (mg REs/g*)	16.846±0.061	32.589±0.465	20.046±0.141
Total Antioxidant (mg AAEs/g*)	38.933±0.596	103.118±0.795	35.562±1.390

*GAEs-gallic acid equivalents, *REs/g-rutin equivalents, *AAEs-Ascorbic acid equivalents

Table 2. DPPH free radical % scavenging activities of *O. armena* extracts and synthetic antioxidant.

	0.125 mg/mL	0.25 mg/mL	0.5 mg/mL	1 mg/mL
Ethyl acetate	6.540±0.001	11.958±1.368	16.796±0.274	26.529±2.764
Methanol	10.700±0.958	23.084±0.629	44.021±0.520	80.611±0.930
Water	14.222±1.450	23.491±1.806	44.621±2.080	76.645±0.137
BHA	95.627±0.328	-	-	-
BHT	62.481±0.301	-	-	-

the methanolic extract and the ferric reduction power at a concentration of 1 mg/mL is 1.457±0.047. However, although synthetic antioxidants are at 10 times more dilute concentration, they have a stronger reducing potential than all the extracts. The concentration of 1 mg/ml of *Vicia sativa* ssp. nigra, a member of Fabaceae, was determined as 0.230±0.1 at 700 nm (Orhan et al., 2009). This value is quite low compared to *O. armena*. Likewise, in a study investigating the antioxidant and DNA protective effects of *Bauhinia variegata* L., a Fabaceae member, the highest reducing power was reported in the methanol extract (Sharma, Bhardwaj, Kumar, & Kaur, 2001). Similarly, methanol extract of *O. armena* also exhibited stronger activity compared to water. Another study on the nutritional characteristics of Fabaceae members shows that the ferric reduction power of seeds and fruit extracts is quite weak compared to *O. armena* (Chanda, Dudhatra, & Kaneria 2010).

CUPRAC test

As with ferric reducing power, the copper reducing power of extracts increases with concentration. The concentration-dependent copper reduction capabilities of *O. armena* extracts are shown in Table 4. Similar to the ferric reduction power, the highest copper reduction capacity was found in the methanol extract of *O. armena*. Similarly, many studies have reported a strong correlation between ferric and copper reduction powers. The extracts studied and synthetic antioxidants can be listed in terms of copper-reducing efficiency as follows: BHA> BHT>Methanol> Water> Ethyl acetate. Various other studies also state that methanol extracts have stronger copper reducing power compared to water extracts (Zahin, Aqil, & Ahmad, 2010).

β-carotene/Linoleic acid test system

The percentages of the inhibition values of *O. armena* extracts and synthetic antioxidants in β-carotene/linoleic acid test system are given in Table 5. Unlike other methods, the highest inhibition rate (84.437%) was observed in ethyl acetate extract in this method. Methanol extract showed 58.557% inhibition, while the water extract showed 33.649% inhibition. The results observed in this test system may be due to the higher presence of other antioxidant compounds with antioxidant activity other than phenolic compounds that prevent linoleic acid oxidation in the ethyl acetate extract. Not only do phenolic compounds play an active role in the method, but also other molecules with antioxidant properties. Similar situations have been reported for this method in various studies (Mishra, Yosouf, & Singh, 2009; Baghiani et al., 2012). Methanolic extract of *Sesbania sesban*, a member of the Fabaceae family, like *O. armena*, inhibited linoleic acid oxidation by 49.80%. Although this value seems low compared to *O. armena*, considering that the *S. sesban* extract has a concentration of 1 mg/mL, it is not correct to make a direct comparison. In a study on 15 *Lathryus* species, inhibition values in the β-carotene/linoleic acid test vary between 28.47% and 57.83% (Pastor-Cavada, Juan, Pastor, Alaiz, & Vioque, 2009).

Results of fatty acid analysis

As a result of the gas chromatographic analysis of the oil of *Onobrychis armena* Boiss. & Huet, it was determined that the fatty acid composition was formed by 25 different fatty acids. Carbon numbers of these fatty acids vary between 8 and 22.

Table 3. Ferric reducing power of *O. armena* extracts and synthetic antioxidants.

	0.2 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL
Ethyl acetate	-	0.062±0.008	0.209±0.037	0.441±0.023
Methanol	0.072±0.025	0.307±0.017	0.710±0.023	1.457±0.047
Water	0.036±0.001	0.229±0.032	0.458±0.010	0.843±0.001
BHA	2.685±0.028	-	-	-
BHT	1.810±0.028	-	-	-

Table 4. Copper reducing power of *O. armena* extracts and synthetic antioxidants.

	0.2 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL
Ethyl acetate	0.115±0.021	0.328±0.01	0.629±0.022	1.374±0.004
Methanol	0.395±0.094	0.820±0.069	1.557±0.020	3.045±0.007
Water	0.170±0.008	0.453±0.025	0.942±0.005	1.731±0.020
BHA	3.678±0.081	-	-	-
BHT	3.435±0.046	-	-	-

Table 5. Percentage (%) of *O. armena* extracts and synthetic antioxidants to inhibit Linoleic acid oxidation.

	Inhibition (%)
Ethyl acetate	84.437±4.657
Methanol	58.557±0.540
Water	33.649±0.618
BHT	94.395±0.052
BHA	95.735±0.052

When the fatty acid composition was examined, it was found that the major fatty acid was C 16:0, palmitic acid (Table 6). Palmitic acid is followed by C 18:2 ω6 with 15.09%, linoleic acid and C 20:3 ω6 with 12.03% (cis-8-11-14 eicosatrienoic acid). Similarly, palmitic (34.92%) and linoleic acid (25.91%) were determined as the fatty acids with the highest percentage in the fatty acid composition of *Teramnus labialis* belonging to the Fabaceae family such as *Onobrychis* (Wiswanathan, Thangadurai, Vendan, & Ramesh, 1999). Thangadurai et al. (2001), in their study investigating the nutritional properties of *Galactia longifolia* (Jacq.) Benth., determined that the fatty acid composition consists of five fatty acids and that palmitic acid has the highest percentage of these fatty acids. In their study on the fatty acid compositions of some Fabaceae members,Bagci et al. (2004) determined the fatty acid compositions of four species belonging to the genus Onobrcyhis showing them to be linoleic, oleic and palmitic acids, respectively. In the same study, the percentage of linoleic acid in *Onobrychis major* (Boiss.) Hand.-Mazz. increased up to 51.8%.

It was observed that the monounsaturated fatty acids (MUFA) content of *O. armena* was mostly composed of C 18:1 C9 oleic acid (4.47%) in the fatty acid composition. Oleic acid accounts for about 50% of the total monounsaturated fatty acids content. This fatty acid is followed by C 16:1 ω7 palmitoleic acid (1.51%), C 15:1 ω5 pentadecanoic acid (1.37%) and C 14:1 ω5, myristoleic acid (1.22%), respectively. Others of the monounsaturated fatty acids are at very low levels. Similarly, in many studies on fatty acid compositions of Fabaceae members, oleic acid has been reported to be the fatty acid that contributes the greatest to MUFA content (Mao et al., 2012; Uzun, Arslan, Karhan, & Toker, 2007).

When looking at the saturated (SFA), mono (MUFA) and polyunsaturated (PUFA) fatty acid contents of the studied *O. armena* oil, the polyunsaturated fatty acids are higher than saturated and monounsaturated fatty acids. In *O. armena*, PUFA, SFA, and MUFA contents were determined as 56.62%, 33.84% and 9.54%, respectively (Table 7). This situation has been similarly reported in *Onobrychis* and many Fabaceae members. The polyunsaturated fatty acids (PUFA) content of *O. armena* is mostly C 18:2 ω6 and C 20:3 ω6. Linoleic acids cannot be synthesized by humans and are considered "essential fatty acids" since they must be taken from the diet. The percentage of essential fatty acids in *O. armena* is 26.96%. This high level of essential fatty acids suggests that *O. armena* oil may be considered as an important source of essential fatty acids. In addition, the positive effects of polyunsaturated fatty acids on health and the high levels of polyunsaturated fatty acids in *O. armena* oil increase the importance of this oil on health.

Various values such as ω3/ω6, atherogenic index (AI) and thrombogenic index (TI) have recently been used more frequently in evaluating the nutritional quality of fat. A high ratio of ω3/ω6 and low AI and TI values is desirable in the nutritional quality of the oil. The 3/ω6 ratio, AI and TI values of *O. armena* oil used in the study were determined as follows: 0.65, 0.47 and 0.42. It has been reported that the AI value of various vegetable oils, for example cocoa butter, is between 13-20, and this value is around 7 for palm oil. From this , it might be concluded that the indicated indexes increase due to the increase in saturated fatty acid content, but on the contrary, the increase in unsaturated fatty acids contributes to the decrease of these values. Therefore, the high unsaturated fatty acid content of *O. armena* oil is the most important indicator that this oil might also be nutritionally valuable.

Results of essential oil composition

During the flowering period of *Onobrcyis armena*, the essential oils of the above-ground parts of this plant were obtained by water distillation method, and the compositions of these essential oils were investigated by GC-MS. Analysis results of these plants are given in Table 8. A total of 22 different essential oil components were determined in the examined *O. armena* essential oil. The components of the essential oil were identified at a rate of 96.957%. N-hexadecanoic acid was determined as the major component of essential oil. This component makes up 56.609% of the total essential oil content. In the same way, n-hexadecanoic acid was determined as the major

Table 6. Fatty acids of *O. armena*.

Carbon Number	Common and Systematic Name
C8:0	Caprylic acid (Octanoic acid)
C10:0	Capric acid (Decanoic acid)
C11:0	Andesilic acid (Andecanoic acid)
C12:0	Lauric acid (Dodecanoic acid)
C13:0	Tridesilic acid (Tridecanoic acid)
C14:0	Myristic acid (Tetradecanoic acid)
C14:1ω5	Myristoleic acid (cis-9-Tetradecanoic acid)
C15:0	Pentadecylic acid (Pentadecanoic acid)
C15:1ω5	Pentadecanoic acid (cis-10-Pentadecanoic acid)
C16:0	Palmitic acid (Hexadecanoic acid)
C16:1ω7	Palmitoleic acid (cis-9-Hexadecanoic acid)
C17:0	Margaric acid (Heptadecanoic acid)
C17:1ω8	Margaroleic acid (cis 10-Heptadecanoic acid)
C18:0	Stearic acid (Octadecanoic acid)
C18:1ω9	Oleic acid (cis-9-Octadecanoic acid)
C18:1ω7	cis-vaccenic acid (cis-11-Octadecanoic acid)
C18:2ω6	Linoleic acid (cis-9-12-Octadecadienoic acid)
C18:3ω6	γ-Linolenic acid (cis-6-9-12-Octadecatrienoic acid)
C18:3ω3	Linolenic acid (α-linoleic acid.ALA) (cis-9-12-15-Octadecatrienoic acid)
C20:0	Arachidic acid (Eicosanoic acid)
C20:1ω9	Gadoleic acid (cis -11 Eicosenoic acid)
C20:3ω3	cis-11,14,17- Eicosatrienoic acid
C20:3ω6	cis-8-11-14 Eicosatrienoic acid
C22:1ω9	Erucic acid (cis-13 Docosanoic acid)
C22:6ω3	Docosahexaenoic acid (DHA) (cis-4,7,10,13,16,19-Docosahexaenoic)

Table 7. *O. armena* fatty acid composition (%).

Carbon Number	*O.armena*	Carbon Number	*O.armena*
C8:0	0.05±0.01*	C18:2ω6	15.09±0.01
C10:0	0.10±0.01	C18:3ω6	11.81±0.07
C11:0	0.04±0.01	C18:3ω3	11.87±0.01
C12:0	0.45±0.01	C20:3ω6	12.03±0.03
C13:0	0.84±0.01	C20:3ω3	0.11±0.01
C14:0	1.97±0.01	C22:6ω3	5.71±0.35
C15:0	1.09±0.01	**ΣPUFA****	**56.62±0.26**
C16:0	22.67±0.07	**ΣUFA****	**66.16±0.01**
C17:0	0.64±0.03	**ΣEFA****	**26.96±0.01**
C18:0	5.87±0.01	**PUFA/SFA**	**1.67±0.01**
C20:0	0.12±0.02	**ω3**	**17.69±0.35**
ΣSFA*	**33.84±0.01**	**ω6**	**27.17±0.02**
C14:1ω5	1.22±0.01	**ω3/ω6**	**0.65±0.01**
C15:1ω5	1.37±0.20	**ω6/ω3**	**1.54±0.03**
C16:1ω7	1.51±0.10	**AI****	**0.47±0.01**
C17:1ω8	0.33±0.01	**TI****	**0.42±0.01**
C18:1ω9	4.47±0.01		
C18:1ω11	0.46±0.01		
C20:1ω9	0.04±0.01		
C22:1ω9	0.14±0.05		
ΣMUFA*	**9.54±0.27**		

*Arithmetic mean ± standard deviation
**SFA:Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, UFA: Unsaturated fatty acids, EFA: Essential fatty acids, AI: Atherogenic index, TI: Thrombogenic index.

Table 8. *O. armena* essential oil components (%).

	RTa	Component Name	%
1	27.500	Tetradecane	0.517
2	28.910	Farnesan	0.127
3	44.371	β-Ionone	0.262
4	45.363	2-propenoicacid, pentadecy-lester	0.097
5	47.718	Heneicosane	0.484
6	48.575	Hexahydrofarnesylacetone	5.222
7	48.903	Spathulenol	0.923
8	50.939	Spiro[4,5]dec-6-en-8-one,1,7-dimethyl-4-1(1-methylethyl)	0.156
9	52.468	Docosane	0.955
10	57.097	9-octadecanoicacid	0.289
11	58.400	Dodecanoicacid	3.153
12	58.600	Tetratetracontane	0.880
13	58.891	9-15octadecanoicacid	0.569
14	63.481	Phytol	1.538
15	67.274	Heneicosane,11-(1-ethylpro-pyl)	0.951
16	67.541	Tetradecanoicacid	6.783
17	75.026	2-ısobutyl-1,3,2-oxazaborolane	0.549
18	77.780	Nonocosane	2.888
19	78.717	n-Hexadecanoicacid	56.609
20	80.652	15-Tetracosenoicacid	3.567
21	90.053	Hexadecane	1.453
22	99.995	9-12-Octadecanoicacid	8.985
		Total defined component	**96.957**

RT: RetentionTime

component in *Samanea saman* (Jacq.) Merr. essential oil (Oguwande, Walker, Setzer, & Essien, 2006). Studies have reported that the essential oil composition varies depending on certain factors such as climatic conditions, season, and altitude (Daferera, Ziogas, & Polissiou, 2000; Grosso et al., 2007).

Major components after n-hexadecanoic acid in the essential oil composition of the studied *O. armena* can be listed as follows: 9-12 Octadecanoic acid (8.985%), tetradecanoic acid (6.783%), hexahydro farnesyl acetone (5.222%), 15-tetracosenoic acid (3.567%) and dodecanoic acid (3.153%). In *Retama raetam* essential oil, a member of the Fabaceae family, 12 components were determined quite differently from *O. armena* essential oil and-linalool (50.9%) was reported as the major component. Differently, 1,8-cineol (39.8%) was determined as the major component in the composition of the essential oil of *Cassia alata*, which belongs to the same family (Bhaksu & Raju, 2009). Kicel et al. (2010) identified hexahydro farnesyl acetone in the essential oil composition of *Trifolium repens* at a rate of 6.2%, close to our study. Phytol, which is relatively low as 1.538% in *O. armena*, is the major component of the essential oil of *Prosopis farcta* leaves, a member of the same family.

CONCLUSION

In this study, the *in vitro* antioxidant properties of ethyl acetate, methanol and water extracts of *Onobrychis armena* Boiss. & Huet were investigated using five different chemical test systems including total antioxidant, free radical capture, β-carotene/linoleic acid, copper and ferric reduction powers. In addition, total phenolic and flavonoid content was also determined. The antioxidant activity of methanol extract of *O. armena* was found to be stronger than other extracts in all test systems (except the β-carotene/linoleic acid test). This shows that, as in many other studies, the antioxidant activity changes depending on the solvent used. When the results of these test systems and the total phenolic and flavonoid content are evaluated, the antioxidant activity of *O. armena* extracts can be shown as follows: Methanol>Water>Ethyl acetate.

The fatty acid composition of *O. armena* was investigated using GC and it was found that the main part of the fatty acid composition was composed of unsaturated fatty acids (66.16%), which are of great importance for health. The major fatty acids

in the oil composition are palmitic acid (22.67%) and linoleic acid (15.09%). The composition of the essential oil obtained by hydrodistilation from *O. armena* was investigated by GC-MS and a total of 22 components were identified, the major component being n-hexadecanoic acid (56.609%).

In recent years, the search for new natural raw material resources has been increasing day by day, especially in the field of food and pharmacology. In this study, antioxidant properties and polyunsaturated fatty acids of *Onobrychis armena* were investigated. According to the determined results, it can be concluded that *Onobrychis armena* can be used as a natural raw material source in the field of food and pharmacology.

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