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Antioxidant Capacity, Fatty Acid Profile and Volatile Components of the *Onopordum Anatolicum* and *Onopordum Heteracanthum* Species Seeds Grown in Van, Turkey

Ayhan BAŞTÜRK[1]*, Sümeye PEKER[1]

ABSTRACT: The crude oil contents, total phenolic contents, volatile components and antioxidant activities of *Onopordum anatolicum* and *Onopordum heteracanthum* species seeds were investigated. In addition, the fatty acid profile, tocopherol contents, peroxide values, free fatty acidity and color values of the oils obtained from species seeds were determined. The oil contents of *O. anatolicum* and *O. heteracanthum* species seeds were found to be 15.84% and 12.54%, respectively. Total phenolic contents were found as 18554 and 13015 mg-GAE kg^{-1}. DPPH (% inhibition) values in the studied seeds and BHT were determined as 84.41, 66.73, and 86.92% and ABTS values were determined as 121.18, 46.90, and 123.78 mmol Trolox eq. g^{-1}. Linoleic acid (49.38 and 38.09%) and oleic acid (30.04 and 22.07%) were the most abundant fatty acids in the oil from seeds. In addition, a significant amount of α-tocopherol (1066.99 mg kg^{-1}) was detected in *O. anatolicum* seed oil. Furthermore, 17 volatile compounds were detected in different amounts of each in the seeds of the species. In the light of all these findings, it was concluded that the seeds of *O. anatolicum* and *O. heteracanthum* could be appreciated as alternative oil raw materials, and used as natural antioxidant and polyunsaturated fatty acids sources in the formulation in functional foods.

Keywords: Antioxidant capacity, *Onopordum anatolicum*, *Onopordum heteracanthum*, volatile component, fatty acid

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This study was produced from Sümeye Peker's Master's thesis.

INTRODUCTION

Over the past few years, research fixating on the use of non-traditional oilseeds as an origin of vegetable oil has gained importance to meet the vegetable oil demand in many developing countries (Gopala Krishna and Bhatnagar, 2013). Besides their oil content, these herbs or seeds also accommodate phytochemical antioxidants such as phenolics that can be used as a radical scavenger. The antioxidant activities of these substances are the reason for their redox properties (Kumar et al., 2012). Raw plant extracts, which are rich in phenolic content, have attracted the attention of the food industry as they prevent oxidation and increase the quality and nutritional value. The antioxidants contained in plants are of increasing interest among scientists and consumers as they protect health and prevent disease (Kahkonen et al., 1999).

Onopordum L. is a genus of about 60 species of thistles associated with the family Asteraceae, native to Europe (mainly the Mediterranean region), northern Africa, the Canary Islands, the Caucasus, and southwest and central Asia (Kubitzki, 2007). This genus of 19 species, including two subspecies in Turkey, consists of a total of 20 taxa (Davis, 1970; Güner et al., 2000; Özhatay et al., 2009). This species has spiky and pale green leaves, dense spines of small purple flowers, its length varies between 30-1000 cm (Fig. 1). White feathers are found as bunches at the ends of the seeds. They spread widely in the Mediterranean geography. It is also widely grown in Turkey. *O. Davis, O. polycephalum, O. Boissier, O. carica,* and *O. anatolicum* are endemic species in Turkey (Davis, 1965). They are found in the countryside, pastures, roadsides, cultural areas, vineyards, gardens up to 1300 meters high. Among the people, they are known by local names such as akkız, deve kengeri, kengel, kıbbun, meryemana dikeni, sütlü kengel, şevkülmeryem, uslu kenger, kasna, eşek dikeni, kenger otu, köygöçüren (Taşdelen, 2013).

Some types of *Onopordum* are widely used in traditional medicine. Flowering branches of *Onopordum acanthium* are used as diuretic (diuretic) and antipyretic, and roots for diuretic, antipyretic, appetizing and abdominal pain. *Onopordum tauricum* seeds are used for the treatment of kidney disease in Turkey. Some species of *Onopordum* have been reported to exhibit antioxidant and antimicrobial activity (Csupor-Loffler et al., 2009). The worldwide interest in the antioxidant capacities and fatty acid compositions of the phenolic components of this species is still growing. Pictures of *Onopordum anatolicum* and *Onopordum heteracanthum* species are given in Figure 1.

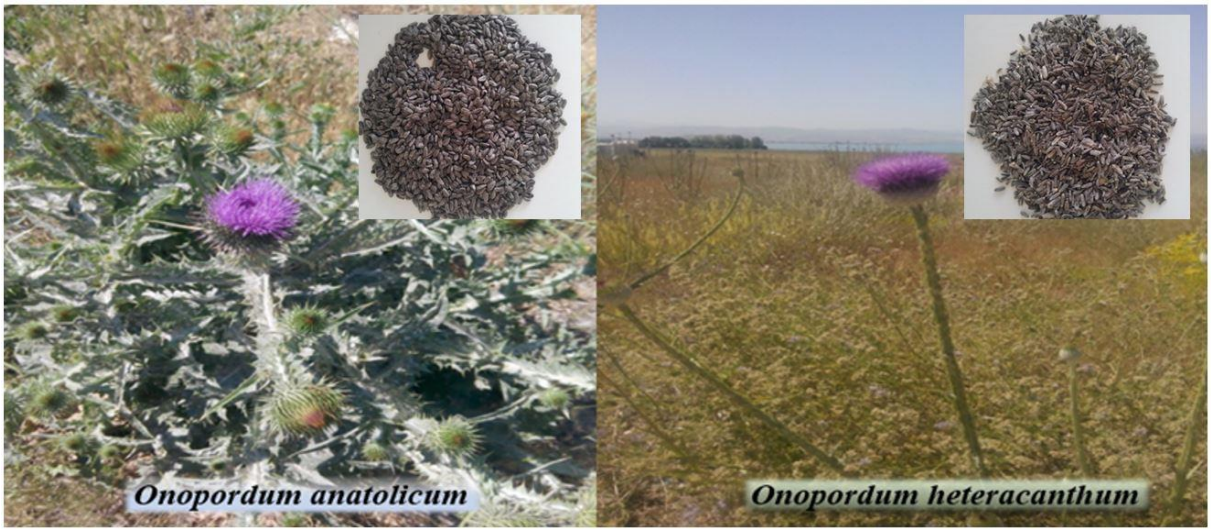

Figure 1. Pictures of the *onopordum* species and seeds

Lately, studies have been carried out on the chemistry and biological properties of the onopordum genus (Bruno et al., 2011). However, there are no reports of detailed chemical characterization of *Onopordum anatolicum* and *Onopordum heteracanthum* seeds.

Under the study, the oil content, total phenolic content (TPC), volatile components, antioxidant activities and some characteristic features of the *Onopordum anatolicum* and *Onopordum heteracanthum* species seeds belonging to the Asteraceae family, as well as the fatty acid compositions, tocopherol contents, peroxide values (PV), free acidity (FFA) and color values of the seed oils were determined. In addition, it is aimed to be used as an alternative oil source and antioxidant.

MATERIALS AND METHODS

Plant Materials and Chemicals

O. anatolicum and *O. heteracanthum* were collected from the Van region of Turkey during a harvest-maturity period in August-September 2017. The plants were identified by the biologist Professor Dr. Murat ÜNAL from the Department of Biology Education of Van Yüzüncü Yıl University. Folin-Ciocalteu, methanol, hexane, isooctane, potassium persulfate Merck (Darmstadt, Germany), 2,2-diphenyl-1picrylhydrazyl (DPPH), 2,2 + -azinobis-3-ethylbenzothiazoline-6-sulfonic acid, 5-methyl 2 hexanone was obtained from the Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical purity.

Preparation of Methanolic Extract

The obtained plants were dried in the shade in the open air, and the seeds were manually separated. It was ground in a milling machine and kept in sealed glass containers at room temperature until analysis. Accordingly, 9.5 mL methanol was added to 5 g hexane-defatted ground *O. anatolicum* and *O. heteracanthum* seeds. The contents were homogenized with a homogenizer (Heidolph, SilentCrusher M, Schwabach, Germany) at 10.000 rpm for 15 s. The homogenized sample was agitated at room temperature for 2 h at 200 rpm in a circular shaker (Heidolph, unimax 1010, Kelheim, Germany). Then, the contents were centrifuged at 8000 × g for 10 min at 4 °C. Following the centrifugation, the supernatant was separated from the residue and the residue was subjected to the same treatment twice more. The supernatants obtained at the end of the extraction were combined and completed to 25 mL with methanol.

The Analyses Performed on the Seeds

Proximate analysis of *O. anatolicum, and O. heteracanthum*

The methods recommended by the Official Society of Analytical Chemists (AOAC, 1990) were used to determine the moisture, ash, crude protein and crude oil contents. The moisture content was determined by drying the samples at 105 °C to constant weight. The ash content was determined by a laboratory furnace at 600 °C, and the temperature was increased gradually. Nitrogen content was determined by using the Kjeldhal method. Crude oil was detected by the soxhlet method. Crude oil was obtained by exhaustively extracting 10 g of each sample in soxhlet apparatus using hexane as the extractant. Each measurement was performed in triplicate and the results were averaged.

Determination of total phenolic content

The phenolic content of *O. anatolicum,* and *O. heteracanthum* seeds extracts were determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). 0.4 mL samples were placed in test tubes; 2 mL of Folin-Ciocalteu's reagent and 1.6 mL of sodium carbonate (7.5%) were added. The tubes were agitated and allowed to stand for 60 min. Absorption at 765 nm was measured in UV-spectrophotometer (Agilent 8453, Agilent technologies, CA, USA). Gallic acid was used as a standard for the calibration

curve (y = 0.0063x + 0.049). The total phenolic content was expressed as gallic acid equivalent (mg GAE kg^{-1} dry extract).

Antioxidant Activity Tests

DPPH radical scavenging assay

DPPH free radical scavenging effect of seed extracts were determined by the Blois method. (Blois, 1958). Before the procedure, the methanolic DPPH solution was prepared for the analysis. Accordingly, 0.0065 g DPPH was weighed and completed to 250 mL with methanol (0.025 g L^{-1} methanol). For the study, 0.1 mL seed extract prepared for the analysis was taken, and 3.9 mL DPPH solution was added and mixed using a vortex and kept for 60 min at room temperature in the dark. At the end of this period, the absorbance of the UV spectrophotometer was read at 515 nm. In the control sample, the spectrophotometer was reset with pure methanol using solvent instead of sample. At the end of the 60 min, the amount of DPPH inhibited in the reaction medium was determined using Eq. 1.

$$I = \frac{A_2 - A_1}{A_2} x100 \quad (1)$$

I = DPPH inhibited by the sample, %

A_1 = absorbance of the sample

A_2 = absorbance of control

ABTS assay

ABTS diammonium salt radical cation decolourisation test is also a spectrophotometric method widely used to assess antioxidant activity of various plant extracts. $ABTS^{+}$ obtained as a result of the oxidation of ABTS with potassium persulfate was presented as an excellent tool to determine the antioxidant activity of hydrogen donor antioxidants and chain-breaker antioxidants (Leong and Shui, 2002).

ABTS analysis was performed using the method proposed by Re et al. (1999). Measurements are carried out spectrophotometrically by observing the disappearance of the ABTS radical, a stable blue/green compound. The reaction between ABTS and potassium persulfate yields a blue/green $ABTS^{\cdot+}$ chromophore. Accordingly, 7 mmol of ABTS (2,2+-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and 2.45 mmol potassium persulfate were reacted at room temperature in the dark for 12-16 h to yield the stock $ABTS^{\cdot+}$ radical cation. The obtained $ABTS^{\cdot+}$ radical cation was diluted with ethanol to give 0.70 ± 0.02 absorbance at 734 nm. Then, 20 µL extract was mixed with 1980 µL $ABTS^{\cdot+}$ radical cation for 6 min at room temperature in the dark and measured in UV spectrophotometer at 734 nm. The results were calculated using the Trolox standard curve (y = 38.484x - 2.602) and Eq. 2, and were presented as mmol Trolox eq g^{-1} dry weight.

$$\text{Inhibition } \% = \frac{A_6 - A_1}{A_1} x100 \quad (2)$$

A_6: Absorbance at the 6th min

A_1: Absorbance at the 1st min

Determination of volatile compounds

Determination of volatile compounds in plant seeds were carried out by GC-MS according to Krist et al. (2006), with modifications. First, it was prepared by completing 0.1 mL of 5-methyl 2 hexanone to 10 mL with distilled water as internal standard (IS). In the 30 mL vials required for use in the analysis, 3 grams of ground seeds were placed, 10 mL pre-boiled and cooled pure water was added and homogenized using a homogenizer (Heidolph SilentCrusher M, Schwabach, Germany) at 13000 rpm. Then, it was added to 10 µL internal standard and a magnetic stirrer was added. After the lid of the vials

was sealed and conditioned for 5 min at 40 °C in the heating block, by immersing in an appropriate fiber (50/30µm-thick, DVB/CAR/PDMS as the adsorbant), left to adsorb the volatile components in the peak space for 40 minutes in a heated magnetic stirrer set to 40°C and 140 rpm. At the end of this period, the fiber was held at the injection port of the gas chromatography device for 5 min to pass the fiber-holding volatile components to the GC-MS system column. TRB-5MS (30 m length, 0.250 mm internal diameter, 0.25 µm film thickness) capillary column was used in the analyses. The operating conditions were set as follows; injection block temperature: 250 °C, detector temperature: 250 °C, carrier gas: He, flow rate: 1 mL min^{-1}, temperature of the MS source: 230 °C, MS quadrupole temperature: 150°C, injection mode: splitless, electron energy: 70 eV, mass range: 15-210 atomic mass unit, oven temperature program; hold at 40 °C for 2 min, raise from 40 to 70 °C with 5 °C increments per min, hold at 70 °C for 1 min, raise from 70 to 240 °C with 10 °C increment per min, hold at 240 °C for 30 min. Then, identifications of the components in the chromatogram were compared with the information in the Wiley and NIST libraries and the calculated retention indexes (RI). The identification of constituents was accomplished based on retention index determined with reference to a homologous series of n-alkanes (C8-C30), under the same experimental conditions. Further identification was carried out by comparing their mass spectra with those from NIST 05 and Wiley 8th version. Volatile compounds were calculated quantitatively considering the internal standard peak area-amount and given in µg kg^{-1}.

Analysis of Seed Oils

Extraction of oils

Oil samples required for planned analyses including fatty acid composition, PV, FFA, tocopherol and color parameters were obtained by cold extraction. Accordingly, 130 mL hexane was added to ground seed (35 g) and kept in the circular shaker at 180 rpm for 2 h. Extracts were filtered and hexane was evaporated at 40 ºC in a rotary evaporator. Seed oils were stored at +4 ºC in the dark until use.

Determination of FFA and PV

Methods recommended by AOCS (1989) were adopted to determine FFA content (method Ca 5a-40/93) and PV (method Cd 8-53).

Determination of fatty acid composition

For analysis, oil samples were converted into fatty acid methyl esters (FAMEs) described by Basturk et al. (2007). After the formation of methyl esters, 1 mL from the clear upper phase was injected into the injection port of the device, QP 2010 Ultra Shimadzu GC-MS with MS detector combined with FID detector. Column info and working conditions were as follows; column: DB-23 (60 m x 0.25 mm, 0.25 µm), carrier gas: He, total flow: 36.6 mL min^{-1}, column flow: 0.66 mL min^{-1}, linear speed: 21.2 cm sec^{-1}, split ratio: 50, initial temperature: 80 ºC, temperature program: 10 ºC min^{-1}, final temperature: 220 ºC, injection temperature: 250 ºC, detector temperature: 250 ºC, total analysis time: 34 min and ion source temperature: 200 ºC. Fatty acid methyl esters were identified by chromatography with authentic standards (Sigma) and from NIST 05 MS Library Database. Quantification of the fatty acids methyl ester profiles was done considering the relative areas of peaks, expressed as the relative percentage of the individual area of each one relative to total area of compounds in the chromatogram. FAMEs analyses were performed in 3 replicates.

Determination of α-tocopherol

Tocopherol content of the samples were determined on HPLC device (Shimadzu, Kyoto, Japan) according to AOCS Official Method (Ce 8-89) (AOCS, 2003). The oil samples obtained by cold extraction were diluted with n-hexane at a ratio of 1:10, then filtered through 0.45 µm (Millipare Millex-LCR Hydrophilic PTFE) filter and injected into the device. HPLC operating conditions were as follows:

column: LiChrosorb Si60 (250 × 4 mm, ID) 5 µm, flow rate: 1 mL min^{-1} (isocratic flow), mobile phase: hexane:isopropyl alcohol (99:1), wavelength: 295 nm, column temperature: 25 ℃. The compounds appearing in chromatograms were identified on retention times and spectral data by comparison with standards of α-, β-, γ- and δ-tocopherols. Results were expressed in mg kg^{-1} oil. The measurements were taken in triplicate.

Color measurement

L*, a*, b* color values of the oil samples were determined using a colorimeter (CR-400 Konica, Minolta, Tokyo, Japan). First, calibration of the device was carried out on white plate and black hole provided by the manufacturer. For absolute measurement, approximately 20 mL of oil sample was placed on the measuring head and three readings were taken in different positions. The average values of L*, a*, and b* were given based on three subsequent readings.

Statistical Analyses

Statistical analyses were performed using SPSS software (version 20.0 for Windows, SPSS Inc., Chicago, Illinois). The collected data of the different dependent variables were analyzed statistically according to the analysis of variance with three replications as a general test at each location. Differences between mean values were analyzed using Duncan's multiple range test at 0.05 level of significance.

RESULTS AND DISCUSSION

Physicochemical Properties of *O. Anatolicum, and O. Heteracanthum* Seeds

Some phytochemical properties (moisture, ash, protein and oil contents) detected in seeds are seen in Tabo 1. The protein contents of the seeds were found to be different ($p < 0.05$). Fat contents of *O. anatolicum*, and *O. heteracanthum* seeds were found as 15.84 and 12.54%, respectively. Arfaoui et al. (2014) reported the total oil content as 15.26% in *O. acanthium* L. seed. Total fat contents were determined as 15-38% in *O. acanthium* and 9.2% in *O. olgae* by Azimova et al. (2011), 12.3% in *O. nervosum* by Hachicha et al. (2007) and 21.4-23.8% in *onopordum* species by Dagne and Jonsson (1997). These variations could be due to the direct effects of genetic factors, species, specific climatic and geographical conditions as well as the ripening grade of the fruits (Arfaoui et al., 2014). The protein contents of *O. anatolicum*, and *O. heteracanthum* seeds were determined as 20.40 and 16.57%, respectively. The moisture content of the seeds was determined as 8.05 and 8.68%, respectively. Ash contents were found to be 3.34 and 4.03%. Gerçel (2011) reported the moisture and ash rate of *Onopordum acanthium* as 7.9 and 7.3%, respectively. Our results mostly overlap with these findings.

Table 1. Chemical composition of *O. anatolicum* and *O. heteracanthum* seeds (%)

Sample	Moisture	Ash	Crude Protein	Oil
O. anatolicum	8.05 ± 0.27^{a}	3.34 ± 1.14^{a}	20.40 ± 0.52^{b}	15.84 ± 0.70^{b}
O. heteracanthum	8.68 ± 0.78^{a}	4.03 ± 0.07^{a}	16.57 ± 0.68^{a}	12.54 ± 0.28^{a}

Values are mean of three replications ± standard deviation. The different small letters shown as superscripts in the same column show the difference between the mean values of the samples ($p<0.05$).

Total Phenolic Contents and Antioxidant Activities

TPC, DPPH and ABTS values determined in *O. anatolicum* and *O. heteracanthum* species seeds are given in Table 2. TPCs of seed extracts were determined by Folin-Ciocalteu reagent method. TPCs of *O. anatolicum* and *O. heteracanthum* seed extracts were determined as 18554 and 13015 mg GAE kg^{-1} extract respectively.

DPPH inhibition rates of *O. anatolicum* and *O. heteracanthum* seed extracts were found to be 84.41 and 66.73%, respectively. It seems that the scavenging ability of *O. anatolicum* is close to BHT

(86.92% inhibition) and *O. heteracanthum* is lower than BHT. Taşdelen (2013) determined DPPH inhibition values in *O. anatolicum* seed methanol extract and BHT as 69.84% and 93%, respectively. Sarikurkcu et al. (2015) reported that the DPPH radical scavenging activity of *O. anatolicum* seed extracts varied depending on the concentration. They found DPPH inhibition rates as 59.77 and 92.60% at concentrations of 1 and 2 mg ml^{-1} ethanol, respectively.

ABTS is frequently used to test the radical cleaning efficacy of antioxidant compounds of plant extracts. ABTS test results showed that similar to DPPH, *O. anatolicum* value was close to BHT and *O. heteracanthum* value was lower than BHT. Joudi and Bibalani (2010) reported that *Onopordum* extracts have antioxidant properties and have been used as an excellent protective agent in the formulation of skincare medicines.

Table 2. TPC, DPPH, ABTS values of *O. anatolicum* and *O. heteracanthum* seed extracts

Sample	TPC (mg GAE kg^{-1} extract)	DPPH (Inhibition %)	ABTS (mMol Trol. eq. g^{-1} extract)
O. anatolicum	18554 ±200^{b}	84.41 ±2.40^{c}	121.18 ±3.99^{b}
O. heteracanthum	13015 ±516^{a}	66.73 ±2.63^{b}	46.90 ±2.96^{a}
BHT		86.92 ±1.63^{a}	123.78 ±4.84^{b}

Values are mean of three replications ± standard deviation. The different small letters shown as superscripts in the same column show the difference between the mean values of the samples ($p < 0.05$).

Fatty Acid Composition of *O. Anatolicum* and *O. Heteracanthum* Seed Oils

Fatty acids identified in *O. anatolicum* and *O. heteracanthum* seed oils and individual percentages of each fatty acid are given in Table 3. In addition, chromatograms and spectra of fatty acids are shown in Figure 3-4. It was striking that in both seed oils, the major fatty acids are linoleic, oleic, palmitic and stearic acids. Linoleic acid was the most abundant fatty acid in *O. anatolicum* and *O. heteracanthum* seed oils (49.38% and 38.09%). In addition, myristic, palmitolinoleic, margaric, linolenic, arachidic and behenic acids were found in trace amounts. These results show that there is a certain resemblance between the seed oils of *O. anatolicum* and *O. heteracanthum* in view of their main component acids. It is understood that the linoleic and oleic acid ratios are higher in *O. anatolicum* seed oil compared to *O. heteracanthum* seed oil. Interestingly 16.05% Crepenynic acid was found in the composition of *O. heteracanthum* seed oil. Crepenynic acid is an 18-carbon acetylenic fatty acid with a double bond on its 9th carbon and a triple bond between its 11th and 12th carbons (Fig. 2). This type of fatty acid is very rare and has no nutritional value. Crepenynic acid is the substrate that allows many acetylenes to derive.

Matthaus et al. (2014) found that the dominant fatty acids in *O. acanthium* seed oil, a subspecies of the Asteraceae family, were linoleic acid 65.9%, oleic 18.8%, palmitic acid 5.8% and stearic acid 2.6%. In another study, the distribution of fatty acids in *O. acanthium* oil was linoleic 51.1%, oleic 34.2%, palmitic 9.9% and stearic 0.9% by Zhelev et al. (2014). The individual fatty acid ratios of *O. nervosum* were found as linoleic acid 60.34%, oleic acid 27.02%, palmitic acid 9.08 % and stearic acid 3.56% by Houachri et al. (2018). Tonguc and Erbaş (2012) determined the fatty acid profile in *O. acanthium* oil as linoleic 57.65%, oleic 28.79%, palmitic 8.81% and stearic acid 4.43%. Kurt et al. (2019) reported that linoleic acid was 70.49%, oleic 19.99%, palmitic 4.51% and 2.02% to stearic acid in *O. turcicum* seed oil. In another study, 68.26% linoleic acid, 18.40% oleic acid, 6.89% palmitic acid and 2.82% stearic acid were determined in *O. tauricum* oil (Erciyes et al., 1995).

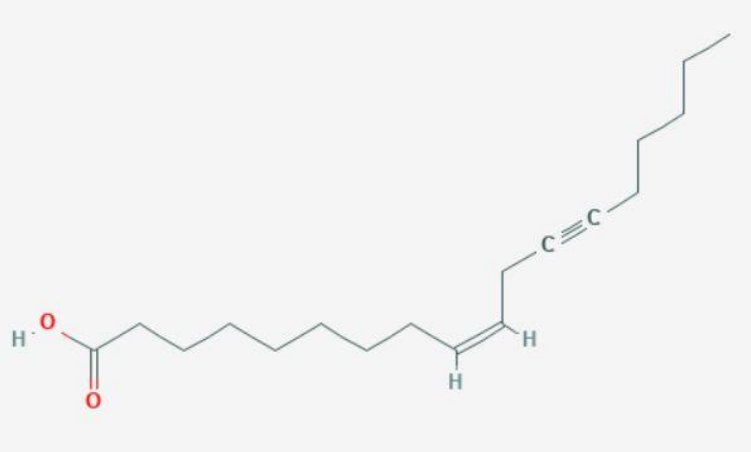

Figure 2. Crepenynic acid (9-Octadecen-12-ynoic acid)

Our study confirmed the presence of linoleic, oleic and palmitic acids as main components. Palmitic acid predominated in the fraction of saturated fatty acids. Linoleic and oleic acids were predominant among the unsaturated acids. Oleic and linoleic acid intake has been encouraged by nutritionist and the medical profession because of their ability to lower blood cholesterol levels (Goldberg, 2008). It has also been reported by Parikh et al. (2005) that oleic acid can reduce LDL and increase HDL levels. Connor (1999) reported that linolenic acid has a protective effect against heart disease and plays a role in developing the brain and retina. In *O. anatolicum* and *O. heteracanthum* seed oils, SFAs were represented as 17.22 and 20.97% in total fatty acids, while MUFAs were represented as 31.50% and 39.14% respectively. Oleic acid was the most abundant MUFA in the samples. PUFAs comprised the largest percent composition of all fatty acid groups (saturated, monounsaturated, polyunsaturated) in samples and their contents were 49.58% in *O. anatolicum* and 38.34% in *O. heteracanthum*.

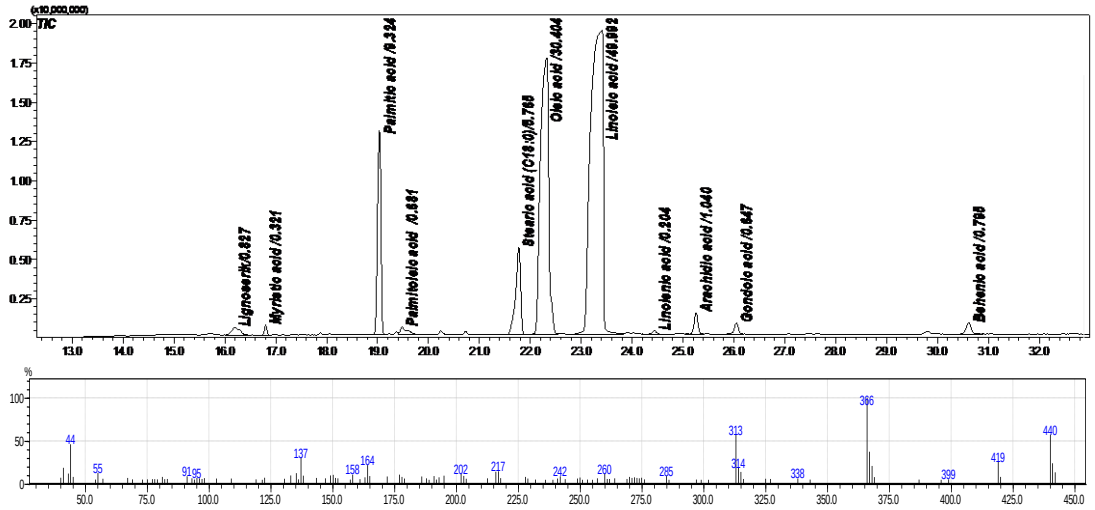

Figure 3. Fatty acid chromatogram and spectrum of *O. anatolicum* seed oil

Table 3. Percentage of fatty acids composition of *O. anatolicum* and *O. heteracanthum* seeds oils (g 100 g^{-1} FA)

Fatty acids	*O. anatolicum*	*O. heteracanthum*
Myristic acid (C14:0)	0.32 ±0.04[a]	0.51 ±0.07[b]
Palmitic acid (C16:0)	9.21 ±0.16[a]	10.40 ±1.63[a]
Palmitoleic acid (C16:1 n-7)	0.82 ±0.06[bc]	1.02 ±0.13[c]
Margaric acid (C17:0)	0.17 ±0.01[a]	1.12 ±0.13[b]
Stearic acid (C18:0)	5.70 ±0.23[a]	7.56 ±0.41[b]
Oleic acid (C18:1 n-9)	30.04 ±2.09[b]	22.07 ±1.51[a]
Linoleic acid (C18:2 n-9.12)	49.38 ±2.80[b]	38.09 ±1.92[a]
Linolenic acid (C18:3 n-9.12.15)	0.20 ±0.01[a]	0.25 ±0.01[a]
Crepenynic acid (C18: 9-en-12-ynoic)	---	16.05 ±1.47
Arachidic acid (C20:0)	1.03 ±0.14[a]	0.85 ±0.06[a]
Gondoic acid (C20:1 n-11)	0.64 ±0.07[a]	---
Behenic acid (C22:0)	0.79 ±0.06[b]	0.53 ±0.04[a]
SFA	17.22 ±1.46[a]	20.97 ±1.50[a]
MUFA	31.50 ±1.97[a]	39.14 ±4.16[a]
PUFA	49.58 ±3.93[b]	38.34 ±3.70[a]

Values are mean of three replications ± standard deviation. The means with different superscript letters within the same row vary significantly ($p< 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

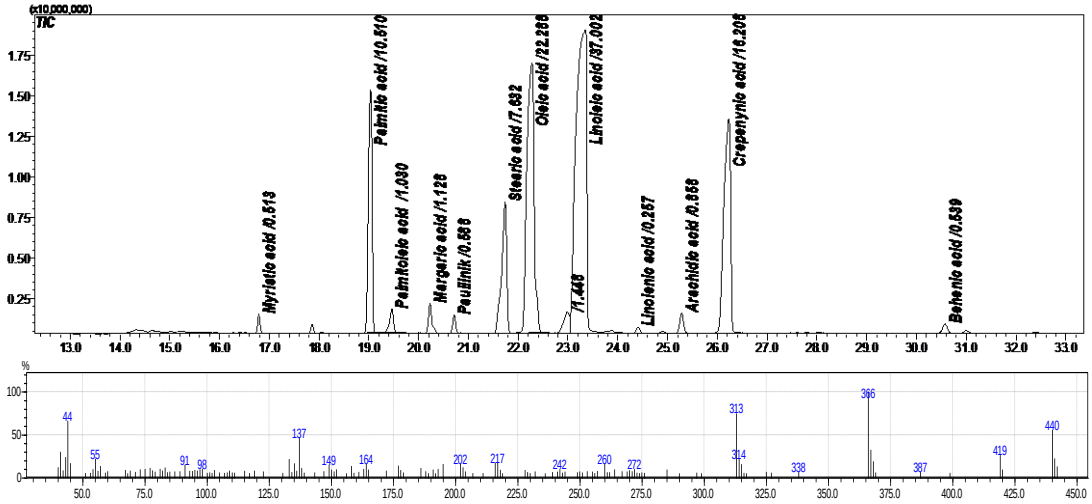

Figure 4. Fatty acid chromatogram and spectrum of *O. heteracanthum* seed oil

Volatile Compounds in *O. Anatolicum* and *O. Heteracanthum* Seeds

Volatile compounds identified in *O. anatolicum* and *O. heteracanthum* seeds are given in Table 4. It was detected 17 compounds in both species, namely *O. anatolicum* and *O. heteracanthum* seeds. The most common compounds found in *O. anatolicum* seed, respectively; hexane, hexanal, methane tetranitro, l-hexanol, benzaldehyde, butanal 3-metil, acetaldehyde, pentanal and acetic acid. The most dominant compounds in *O. heteracanthum* seed were hexanal, hexane, benzaldehyde, carbamic acid and butanal 3-metil. In terms of volatile compounds, it can be said that *O. anatolicum* seed contains more of these compounds than *O. heteracanthum.* Hexane was remarkably found about 7 times more than *O. heteracanthum* seed in *O. anatolicum* seed. Benzaldehyde was determined as 18.11 and 20.78 µg kg^{-1} in *O. anatolicum* and *O. heteracanthum* seeds, respectively. Benzaldehyde was 7.6% in *O. heteracanthum* ethanol extract by Durak and Aysu (2014). Piperazine, ethyl ether, propane, 2-nitro, acetic acid, 1-propene 3-ethoxy, silanediol dimethyl and α-thujene were found in *O. anatolicum* seed, but not detected in *O. heteracanthum* seed. Acetone, carbamic acid, butanal 2-methyl, cathine, toluene,

1-pentanol and 3-hexanone compounds were determined only in *O. heteracanthum* seed. The difference of volatile compounds among *Onopordum* species was probably related to the different subspecies, different collection times and geographic and climatic factors in Turkey.

Table 4 Volatile compounds of *O. anatolicum* and *O. heteracanthum* seeds (μg kg^{-1})

No	Compound	RI	*O. anatolicum*	*O. heteracanthum*
1	Methane, tetranitro	617	31.90 ±0.95[b]	7.51 ±0.06[a]
2	Piperazine	618	3.40 ±0.03	------
3	Acetaldehyde	622	13.15 ±1.32[b]	3.59 ±0.01[a]
4	Acetone	629	------	3.90 ±0.04
5	Ethyl ether	630	6.02 ±0.07	------
6	Carbamic acid	631	------	4.48 ±0.03
7	Propanal, 2-methyl	638	8.90 ±0.07[b]	3.07 ±0.03[a]
8	Propane, 2-nitro	640	3.51 ±0.03	------
9	Acetic acid	644	10.79 ±0.66	------
10	Hexane	647	141.88 ±8.02[b]	22.05 ±1.56[a]
11	Kloroform	654	4.74 ±0.03[b]	1.48 ±0.01[a]
12	Butanal, 3-metil	670	19.66 ±0.64[b]	4.42 ±0.03[a]
13	Butanal, 2-metil	674	------	3.90 ±0.03
14	1-Propene, 3-ethoxy	676	8.59 ±0.07	------
15	Silanediol, dimethyl	690	8.80 ±0.07	------
16	Pentanal	694	12.73 ±1.05[b]	3.98 ±0.04[a]
17	Cathine	696	------	0.47 ±0.01
18	Toluene	753	------	0.67 ±0.01
19	1-Pentanol	756	------	0.79 ±0.01
20	Hexanal	790	38.83 ±2.72[b]	46.35 ±3.58[a]
21	1-Hexanol	857	22.79 ±1.39[b]	3.56 ±0.03[a]
22	Alpha-Thujene	921	3.67 ±0.03	------
23	Benzaldehyde	948	18.11 ±1.94a	20.78 ±1.27[b]
24	3-Hexanone	1183	------	0.64 ±0.01

Values are mean of three replications ± standard deviation. The different small letters shown as superscripts in the same row show the difference between the mean values of the samples ($p<0.05$).

PV, FFA, α-Tocopherol and Hunter Color Values of *O. Anatolicum* and *O. Heteracanthum* Seed Oils

PV, FFA, α-tocopherol and hunter color values determined in oils obtained from *O. anatolicum* and *O. heteracanthum* seeds are given in Table 5. The peroxide values for *O. anatolicum* and *O. heteracanthum* seed oils determined as 3.92 and 6.21 meq O_2 kg^{-1} respectively which is well below the limit for peroxide value (<10 meqO_2 kg^{-1}) (Anonymous, 2012). Free fatty acids are produced by the hydrolysis of triglycerides. The FFA content of the tested *O. anatolicum* and *O. heteracanthum* seed oils determined 0.69 and 3.73%, as oleic acid. Only α-tocopherol homolog was detected in seed oils. α-tocopherol contents were 1066.99 and 225.26 mg kg^{-1} respectively ($p<0.05$). Compared to some other seed oils (Assumpcao et al., 2016; Gunstone et al., 2007; Sahari and Amooi, 2013; Yaqoob et al., 2016), *O. anatolicum* seed oil had a very high content of α-tocopherol. Hunter color values of *O. anatolicum* and *O. heteracanthum* seed oils L* (100 lightness / 0 darkness), a* (+ redness / - foliage) and b* (-blue / + jaundice) are given in Table 5. Seed oil color parameters of both species did not differ significantly ($p>0.05$). According to these data, it is understood that both seed oils were on the dark and yellow sides. When a * values are examined, it is seen that the scale is closer to the green color in oils.

Table 5 Peroxide value, free fatty acid, α-tocopherol and hunter color values of *O. anatolicum* and *O. heteracanthum* seed oils

		O. anatolicum	*O. heteracanthum*
PV ($meqO_2$ kg^{-1})		3.92 ±0.05[a]	6.21 ±0.59[b]
Free fatty acid (as oleic acid %)		0.69 ±0.34[a]	3.73 ±0.04[b]
α-tocopherol (mg kg^{-1})		1066.99 ±9.53[b]	225.26 ±8.23[a]
Hunter color values	L*	24.96 ±0.17[a]	25.63 ±0.99[a]
	a*	-2.47 ±0.17[a]	-1.87 ±0.15[a]
	b*	10.28 ±0.13[a]	10.00 ±0.91[a]

Values are mean of three replications ± standard deviation. The different small letters shown as superscripts in the same row show the difference between the mean values of the samples ($p<0.05$).

CONCLUSION

In this study, some characteristics of *O. anatolicum*, *O. heteracanthum* seeds and seed oils were determined. In addition, they have been investigated by comparing their usability as oil raw material and antioxidants. Oil content of *O. anatolicum* and *O. heteracanthum* seeds were 15.84 and 12.54% and crude protein contents were 20.40 and 16.57%, respectively. It was found that the antioxidant capacity of *O. anatolicum* seed extract was close to BHT and that the *O. heteracanthum* seed extract was lower. Peroxide values of the obtained oils are acceptable. Moreover, the high linoleic and oleic acids content as well as PUFA to SFAs ratio (2.87 and 1.82), which is associated with good health makes seeds nutritionally valuable. The results of the current study suggested that the seeds of *O. anatolicum* and *O. heteracanthum* can be considered as alternative oil raw materials, and that these seeds can be used in the formulation of functional foods as α-tocopherol, a natural antioxidant and polyunsaturated fatty acid sources.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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