



Volatile Compounds, Fatty Acid Composition and Antioxidant Activity of *Centaurea albonitens* and *Centaurea balsamita* Seeds Growing in Van, Turkey

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Authors' contributions

This work was carried out in collaboration between both authors. Author AB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SP managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to determine and compare the potential of *Centaurea albonitens* and *Centaurea balsamita* seeds as alternative raw materials for edible oil production and natural antioxidant sources.

Study Design: *C. balsamita* and *C. albonitens* were harvested from the district of Van province during harvest-maturity period in August-September 2017. The plants were identified by a biologist, Prof. Dr. Murat ÜNAL. The Voucher specimens (B5495, MÜ68611) were deposited at the Virtual Herbarium of Lake Van Basin, Van Yüzüncü Yıl University, Faculty of Education.

Place and Duration of Study: The study was carried out between June 2017 - January 2019 in Yüzüncü Yıl University, Faculty of Engineering, Food Engineering Department laboratory.

Methodology: This study consists of two parts. In the first part, crude oil, moisture, ash, protein content, total phenolic content (TPC), volatile components and antioxidant activities of *Centaurea albonitens* and *Centaurea balsamita* seeds were determined. In the second part, fatty acid

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compositions, tocopherol contents, peroxide values (PV), free fatty acidity (FFA) and color values of seed oils obtained by cold extraction were determined.

Results: The antioxidant capacities were determined by the 2,2-diphenylpicrylhydrazyl (DPPH) and the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Results were 26.60 and 27.12%, and 80.61 and 95.99 mmol Trolox eq/g for *C. balsamita* and *C. albonitens* seeds, respectively. The total phenolic content of the seeds were determined to be 9019 and 11501 mg GAE/kg, respectively. The average α -tocopherol content were found to be 1186 and 1689 mg/kg oil. Oil yields of the seeds were found to be 19.36 and 17.65 %, for *C. balsamita* and *C. albonitens* seeds, respectively. In fatty acid profiles; linoleic, oleic, palmitic and stearic acids were determined as the most dominant fatty acids. 22 volatile compounds were detected in *C. balsamita* seed while this was 26 volatile compounds in *C. albonitens* seed.

Conclusion: In this study, it was concluded that *C. albonitens* and *C. balsamita* seeds may be considered as alternative raw materials for edible oil production, and these seeds can be used in the formulation of functional foods due to their high level of α -tocopherol, natural antioxidants and polyunsaturated fatty acids.

Keywords: *Centaurea albonitens*; *Centaurea balsamita*; antioxidant activity; volatile compound; fatty acid composition.

1. INTRODUCTION

In recent years, because of the gap between demand and production of vegetable oils in many developing countries, research focusing on the use of unconventional oil seeds as a source of vegetable oils has become important [1]. Further, these plants or seeds contain phytochemical antioxidants such as phenolics and flavonoids which can be used to scavenge the free radicals. The antioxidant potential of these substances is due to their redox properties [2]. Crude extracts of phenolic rich plants are raising interest in the food industry because they delay oxidative damage of lipids and improve the quality and their nutritional value. The importance of the antioxidants in plants in the maintenance of health and protection from disease is also increasingly of interest among scientists and consumers [3]. *Centaurea* is the largest genus of *Asteraceae* family and has nearly 600 species and is usually found in the Mediterranean Region and West Asia [4]. In the Anatolian peninsula, the

genus is represented by 190 species of more than 100 endemic species [5,6]. In Anatolia, different species are known as various common local names such as peygamber çiçeği, zerdali dikenli, çoban kaldıran, timur dikenli, gökbaş, sarıbaş, acımık kötürüm, kotonkiran and boğa dikenli [7-11]. Many *Centaurea* species have traditionally been used for their antidiabetic, antidiarrheal, antirheumatic, anti-inflammatory, choleric, digestive, gastric, diuretic, menstrual, astringent, hypotensive, antipyretic, cytotoxic and antibacterial properties. Many of these have been previously studied for chemical components, ecological, biological properties and fatty acid composition and antimicrobial activity [12-14]. However, there is a lack of information on the volatile compound, fatty acid composition, total phenolic content, volatile components, antioxidant activities of *Centaurea albonitens* and *Centaurea balsamita* seeds and seed oils. These parameters may be used not only for quality evaluation but also for comparison between samples. *Centaurea albonitens* and



Fig. 1. A: *Centaurea albonitens*, B: *Centaurea balsamita*

Centaurea balsamita species belonging to *Asteraceae* family shows a wide distribution for Turkey and distributed mainly in eastern and southern Anatolia [15]. *Centaurea albonitens* is a perennial plant, with erect, 35-45 cm tall stems and yellow flowers. Grows on steppe and dry stony slopes, at an altitude of 1640-2400 m (Fig. 1A) [16]. *C. balsamita* is an annual species, with yellow flowers and 30-120 cm tall stems and cultivates in the steppe and fallow fields, at an altitude of 650-1900 m (Fig. 1B) [16].

In this study, the oil contents, total phenolic content (TPC), volatile components, antioxidant activities and some characteristic properties of *Centaurea albonitens* and *Centaurea balsamita* species seeds and also fatty acid compositions, tocopherol contents, peroxide values (PV), free fatty acid (FFA) and color values of them seed oils were investigated. It was also aimed to determine their usability as alternative oil raw material and antioxidant source.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

C. balsamita, and *C. albonitens* were harvested from the district of Van province during harvest-maturity period in August-September 2017. The plants were identified by the biologist Professor Dr. Murat ÜNAL. The Voucher specimens (B5495, MÜ68611) were deposited at the Virtual Herbarium of Lake Van Basin, Van Yüzüncü Yıl University, Faculty of Education. Folin-Ciocalteu, methanol, hexane, isooctane, potassium persulfate Merck (Darmstadt, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2 + -azinobis-3-ethylbenzothiazoline-6-sulfonic acid, 5-methyl 2 hexanone was obtained from the trolex Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were of analytical purity.

2.2 Preparation of Methanolic Extract

The collected plants were dried in the shade in the open air and the seeds were manually separated and ground in wheat grinding machine and kept in closed glass jars at room temperature until the analyzes were performed. Accordingly, 9.5 mL methanol was added to 5 g hexane-defatted ground *C. balsamita* and *C. albonitens* seeds, and the contents were homogenized with a homogenizer (Heidolph, Silent Crusher 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 $8000 \times g$ 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.

2.3 The Analyses Performed on the Seeds

2.3.1 Proximate analysis of *C. balsamita* and *C. albonitens* seeds

The recommended methods of the Association of Official Analytical Chemists [17] were adopted to determine the levels of moisture, ash, crude protein and crude oil. The moisture content was determined by drying of 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.

2.3.2 Determination of total phenolic content

The phenolic content of *C. balsamita* and *C. albonitens* seeds extracts were determined using the Folin-Ciocalteu reagent [18]. 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 dry extract).

2.3.3 Antioxidant activity tests

2.3.3.1 DPPH radical scavenging assay

DPPH free radical removal activity of seed extracts were determined by the Blois method [19]. 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 methanol). For the analysis, 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 Equation 1.

$$I = \frac{A_2 - A_1}{A_2} \times 100 \quad (1)$$

I = DPPH inhibited by the sample, %

A₁ = absorbance of the sample

A₂ = absorbance of control

2.3.3.2 ABTS assay

ABTS is often used to test the anterior radical scavenging activity of antioxidant compounds or 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 [20].

ABTS analysis was performed using the method proposed by Re, et al. [21]. Measurements are carried out spectrophotometrically by observing disappearance of the ABTS radical, a stable blue/green compound. The reaction between ABTS and potassium persulfate yields a blue/green ABTS⁺ chromophore. Accordingly, 7 mmol of ABTS (2,2'-azino-bis-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⁺ radical cation. The obtained ABTS⁺ 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⁺ radical cation for 6 min at room temperature in the dark and measured in UV spectrophotometer at 734 nm. The results were calculated using Trolox standard curve ($y = 38.484x - 2.602$) and Equation 2, and were presented as mmol trolox eq/g dry weight.

$$\text{Inhibition \%} = \frac{A_6 - A_1}{A_1} \times 100 \quad (2)$$

A₆: Absorbance at the 6th min

A₁: Absorbance at the 1st min

2.3.4 Determination of volatile compounds

Determination of volatile compounds were carried out by GC-MS according to Krist, et al. [22], with modifications. Before starting the analysis, as the internal standard (IS) 0.1 mL 5-methyl 2 hexanone was completed to 10 mL with pure water and prepared for analysis. 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 Silent Crusher 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 (50/30 µm-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, 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). In addition, the mass spectra of the defined components and the mass spectra of the internal standard were used to calculate the amounts (µg/kg).

2.4 Analysis of Seed Oils

2.4.1 Extraction of oils

Oil samples of *C. balsamita* and *C. albonitens* seeds 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°C in a rotary evaporator. Seed oils were stored at +4°C in the dark until use.

2.4.2 Determination of FFA and PV

Methods recommended by AOCS [23] were adopted to determine FFA content (method Ca 5a-40/93) and PV (method Cd 8-53).

2.4.3 Fatty acid composition

First, fatty acid methyl esters (FAMES) were formed, as described by Basturk, et al. [24]. After formation of methyl esters, 1 mL from clear upper phase was injected into 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, column flow: 0.66 mL/min, linear speed: 21.2 cm/sec, split ratio: 50, initial temperature: 80°C, temperature program: 10°C min⁻¹, final temperature: 220°C, injection temperature: 250°C, detector temperature: 250°C, total analysis time: 34 min and ion source temperature: 200°C. 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 three replicates.

2.4.4 Determination of α-tocopherol

Tocopherol content of the *C. balsamita* and *C. albonitens* seed oils were determined on HPLC device (Shimadzu, Kyoto, Japan) according to AOCS Official Method (Ce 8-89) [25]. The oil samples obtained by cold extraction were diluted with n-hexane at a ratio of 1:10, then filtered through 0.45 µm (Millipore 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 (isocratic flow), mobile phase: hexane:isopropyl alcohol (99:1), wavelength: 295 nm, column temperature: 25°C.

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 oil. The measurements were taken in triplicate.

2.4.5 Color measurement

L*, a*, b* color values of the *C. balsamita* and *C. albonitens* seed oils 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.

2.5 Statistical Analyses

Statistical analyses of the data obtained from two different seeds, namely the *C. balsamita* and *C. albonitens* seeds, were performed with SPSS software (version 20.0 for Windows, SPSS Inc., Chicago, IL, USA). Experimental data were given as means±standard deviations of 3 replications and was analyzed using one-way ANOVA at a significance level of $P < 0.05$. Differences among the means were compared using Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of *C. balsamita* and *C. albonitens* Seeds

The moisture, ash, protein and fat ratios of seeds were given in Table 1. The difference between protein ratios in these species was found to be significant ($P < 0.05$). Fat content of *C. balsamita* and *C. albonitens* seeds were determined as 17.65 and 19.36%, respectively. Ayaz, et al. [26] found that the total fat content of ten *Centaurea* species obtained from different natural habitat in Turkey (5.57–19.13%) in the range. Fat contents of *C. pseudoscabiosa*, *C. pulcherrima*, *C. salicifolia* and *C. babylonica* were found in the range of 2.50-3.16% by Aktumsek, et al. [27]. These values are about 7-8 times lower than the rates of *C. balsamita* and *C. albonitens* seed oils we studied. However, it should be noted that our ratios belong to seed oils content. The protein contents of *C. balsamita* and *C. albonitens* seeds were determined as 23.83 and 19.42%,

respectively. Moisture content of the seeds was found to be 8.07 and 5.83% respectively. Ash contents were detected to be 3.04 and 4.12%.

3.2 Total Phenolic Contents and Antioxidant Activities

TPC, DPPH and ABTS values determined in *C. balsamita* and *C. albonitens* seeds were given in Table 2. TPCs of seed extracts were determined by Folin-Ciocalteu reagent method. TPCs of *C. balsamita* and *C. albonitens* extracts were determined as 9019 and 11501 mg GAE/kg extract, respectively. Zengin, et al. [28] reported that the TPCs of *Centaurea patula*, *C. pulchella* and *C. tchihatcheffii* were 25.61, 55.00 and 22.27 mg GAE/g extract respectively. TPC values in *C. calcitrapa*, *C. spicata* and *C. ptoisimopappa* methanolic extracts were found to be 17.24, 37.59 and 72.63 mg GAE/g, respectively by Erol-Dayi, et al. [8]. Our values were lower than these values. This shows that the amount of TPC in the seeds contains less phenolic than other plant organelles.

DPPH inhibition rates of *C. albonitens* and *C. balsamita* seed extracts were found to be 26.60 and 27.12%, respectively. It seems that the scavenging ability of the samples was less effective than that of BHT (50.5% inhibition). When the results were compared to BHT, the synthetic antioxidant had higher inhibition capacity than *C. albonitens* and *C. balsamita*. Zengin, et al. [29] in their study comparing the radical scavenging effect of *C. urvillei* methanolic extract and BHT IC50 values were found to be 137.06 and 3.56 µg/mL, respectively. The lower IC50 value indicates higher antioxidant activity of plant extracts. ABTS is generally used for testing the preliminary radical scavenging activity of antioxidant compounds or plant extracts. ABTS test results of *C. albonitens* and *C. balsamita* indicated that they showed lower activity compared to BHT. There was a strong positive correlation between TPC contents and ABTS values ($R=0.957$, $R^2=0.915$). As far as the relevant literature is concerned, there is no study about the ABTS scavenging ability of *C. albonitens* and *C. balsamita* species.

3.3 Fatty Acid Composition of *C. albonitens* and *C. balsamita* Seed Oils

Fatty acids identified in *C. albonitens* and *C. balsamita* seed oils and individual percentages of each fatty acid were given in Table 3. In addition, chromatograms and spectra of fatty acids were

shown in Figs. 2-3. The predominant fatty acids were linoleic, oleic, palmitic and stearic acids. Linoleic acid was determined to be the major fatty acid in *C. albonitens* and *C. balsamita* seed oils (49.94 and 47.75%). In accordance with our results, Tekeli, et al. [14] reported that linoleic acid was the most abundant fatty acid in six of the *Centaurea* species. The fatty acid composition of oil seeds grown in Turkey has been investigated previously, and the linoleic acid content was determined between 3.3% and 70.1% [30]. The fatty acid composition of 17 seed oils has shown that linoleic acid was the major fatty acid except in one species [31]. The fatty acid composition of some *Centaurea* oils, which was previously determined, indicate that linoleic acid content was 11.69 and 55.27% in *C. rigida* and *C. kotschyi* var. *kotschyi*, respectively [32,33]. Linoleic acid, one of the essential fatty acids, is very important for the nutritional value of oils [34]. The high linoleic acid content makes the oil of *Centaurea* species nutritionally valuable. Oleic acid was detected as the second most abundant fatty acid and percent of oleic acid was 30.36 and 30.07% in these samples, respectively. Tekeli, et al. [14] found that six *Centaurea* species oleic acid in the oil obtained from aerial part in the range of 8.65% to 27.22%. This shows that seed oil and plant aerial parts oil compositions differ in *Centaurea* species. In a recent study, it has been reported that oleic acid levels varied from 3.4% to 25.12% in six different *Centaurea* species' aerial parts [35]. Oleic and linoleic acid intake have been encouraged by nutritionists and the medical professions because of their ability to lower blood cholesterol levels [36]. Therefore, these seed oils can be considered as a good source of oleic and linoleic acid. In addition, palmitic acid content of *C. albonitens* and *C. balsamita* species was 8.98 and 10.64%, and stearic acid was 5.89 and 6.41%, respectively. The individual fatty acid ratios of *C. pseudoscabiosa*, *C. pulcherrima*, *C. salicifolia* and *C. babylonica*; were found as linoleic acid 20.19-29.93%, oleic acid 6.90-17.37%, palmitic acid 23.38-30.49% and stearic acid 16-8.03% by Aktumsek, et al. [27]. Among these, linoleic and oleic acid ratios were lower than our findings, palmitic acid was higher than our findings and stearic acid was close to our findings. It may be thought that these differences may originate primarily from *Centaurea* species and the latter from plant organelles (seeds, aerial parts, flowers, leaves, etc.) from which oil is obtained. In *C. albonitens* and *C. balsamita* seed oils, SFAs were represented as 17.25 and 18.81% in total fatty acids, while MUFAs were

Table 1. Chemical composition of *C. balsamita* and *C. albonitens* seeds (%)

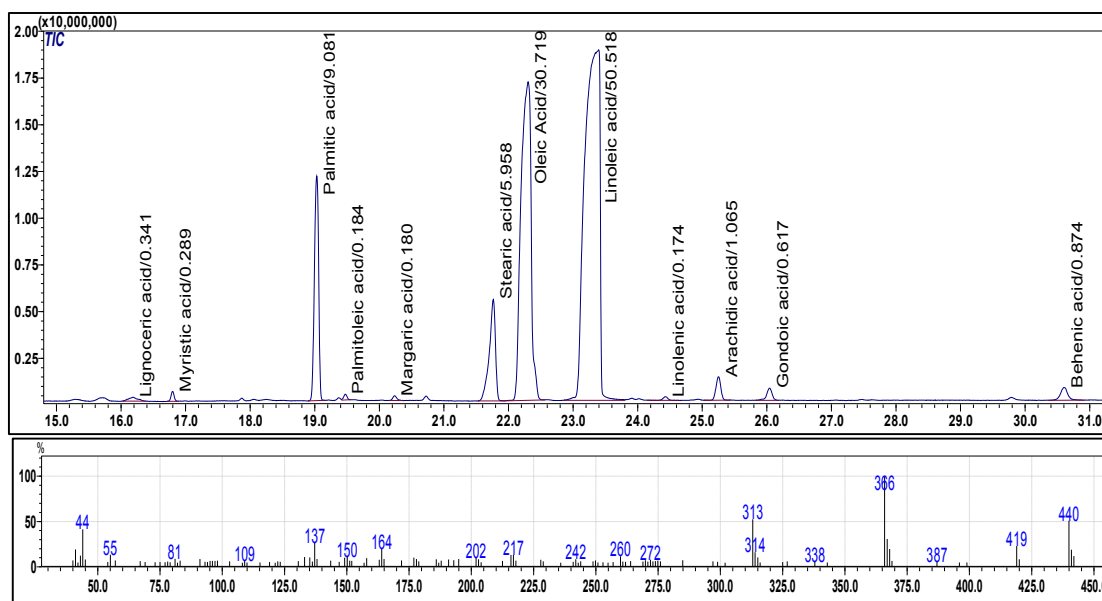
Sample	Moisture	Ash	Crude Protein	Oil
<i>C. albonitens</i>	8.07±0.31 ^a	3.04±0.11 ^a	23.83±3.55 ^b	17.65±2.53 ^a
<i>C. balsamita</i>	5.83±0.58 ^a	4.12±0.16 ^a	19.42±1.12 ^a	19.36±1.86 ^a

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 ($P < 0.05$)

Table 2. TPC, DPPH, ABTS values of *C. balsamita* and *C. albonitens* seed extracts

Sample	TPC (mg GAE/kg extract)	DPPH (Inhibition %)	ABTS (mMol Troi. eq./g extract)
<i>C. balsamita</i>	9019±336 ^a	26.60±2.84 ^a	80.61±4.42 ^a
<i>C. albonitens</i>	11501±648 ^b	27.12±2.69 ^a	95.99±6.07 ^b
BHT	--	50.5±4.47 ^b	123.78±9.12 ^c

Values were given as mean of three replications ± standard deviation. Different small letters shown as superscripts in the same column show the difference between the mean values of the samples ($P < 0.05$)

**Fig. 2. Fatty acid chromatogram and spectrum of *C. albonitens* seed oil**

represented as 31.22% in both samples. 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 50.11% in *C. albonitens* and 47.96% in *C. balsamita*. Therefore, it may be hypothesized that plants may be accumulating higher amounts of antioxidants in order to protect polyunsaturated fatty acids [27].

3.4 Volatile Compounds in *C. albonitens* and *C. balsamita* Seeds

Volatile compounds identified in *C. albonitens* and *C. balsamita* seeds were given in Table 4.

The identification of constituents was accomplished on the basis of retention index determined with reference to a homologous series of n-alkanes (C8-C30), under same experimental conditions. Further identification was carried out by comparison of their mass spectra with those from NIST 05 and Wiley 8th version. Volatile compounds were calculated quantitatively taking into account the internal standard peak area-amount and given in $\mu\text{g}/\text{kg}$. *C. balsamita* and *C. albonitens* seeds 22 and 26 compounds were detected respectively. The most common compounds found in *C. balsamita* seed were hexanal, butanal 2-methyl, butanal 3-methyl and hexane. The most dominant compounds in *C. albonitens* seed were hexane,

methane tetranitro, methane thiobis and 1-butanol 3-methyl. In terms of volatile compounds, *C. balsamita* seed seems to be richer than *C. albonitens* and contains more of these compounds. Ertas, et al. [37] identified thirty-two components that constitute 92.8% of the essential oil composition obtained by hydro-distillation from the entire *C. balsamita* plant. They identified the main components as α -selinene (8.5%), hexatriacontane (8.3%), 2,5-di-tert octyl-pbenzoquinone (7.4%) and tetracosane (6.0%). Flamini, et al. [38] identified main components as germacrene D (40.2%), bicyclogermacrene (7.1%), α -cedrene (3.5%), methyl 2,4-decadienoate (3.2%), α -cadinol (2.6%), n-pentacosane (2.5%), β -cedrene (2.3%), spathulenol (2.2%), T-murolol (2.0%), 1-heptadecene (2.0) and α -bisabolol (1.9%) in essential oil of *C. balsamita*. These volatile compounds and their proportions do not substantially coincide with our findings. The possible reason for this is that the seed of the plant and the aerial parts differ in terms of volatile components. The essential oils of *C. balsamita* collected in the same habitat from Turkey, have been investigated by Erdogan, et al. [39]. A total of 54 components were identified in *C. balsamita*. They found that the main components of essential oil were hexadecanoic acid (23.0%), spathulenol (8.9%) and germacrene D (2.1%). Nonanal were found to be 2.68 and 51.83 $\mu\text{g}/\text{kg}$, in *C. albonitens* and *C. balsamita* seeds, respectively. Benzaldehyde was found to be

11.71 $\mu\text{g}/\text{kg}$ in *C. balsamita* seed, but not in *C. albonitens* seed. Nonanal found to be 0.4% in *C. balsamita* seed essential oil by Flamini, et al. [38]. Akkurt and Celik [15] reported that the main constituents of *C. albonitens* essential oil were γ -elemene 4.45, caryophyllene 7.75, germacrene 9.23, spathulenol 7.97, caryophyllene oxide 16.45, cembrene 6.25, phytol 4.75, and β -selinene 4.57 %. It is understood that the aerial parts of *Centaurea* species are richer in terms of volatile components than their seeds. The data presented in this work differed from the Flamini, et al. [38] report about the essential oil composition of *C. balsamita* from the middle of Turkey. Germacrene D (40.2%), bicyclogermacrene (7.1%) and spathulenol (2.2%) were reported to be the main constituents of *C. balsamita* [38]. The difference of volatile compounds among *Centaurea* species were probably related to the different subspecies, different collection times and geographic and climatic factors in Turkey.

3.5 PV, FFA, α -tocopherol and Hunter Color Values of *C. albonitens* and *C. balsamita* Seed Oils

PV, FFA, α -tocopherol and hunter color values determined in oils obtained from *C. albonitens* and *C. balsamita* seeds were given in Table 5. The peroxide values for *C. albonitens* and *C. balsamita* seed oils were determined as 2.95 to 3.48 meq O_2/kg respectively that was well below

Table 3. Fatty acids composition of *C. albonitens* and *C. balsamita* seeds oils (g/100 g FA)

Fatty acids	<i>C. albonitens</i>	<i>C. balsamita</i>
Myristic acid (C14:0)	0.29±0.01 ^a	0.33±0.04 ^a
Palmitic acid (C16:0)	8.98±0.21 ^a	10.64±1.29 ^a
Palmitoleic acid (C16:1 n-7)	0.25±0.01 ^a	0.64±0.08 ^b
Margaric acid (C17:0)	0.18±0.01 ^a	0.21±0.03 ^a
Stearic acid (C18:0)	5.89±0.14 ^a	6.41±0.85 ^a
Oleic acid (C18:1 n-9)	30.36±2.26 ^a	30.07±1.40 ^a
Linoleic acid (C18:2 n-9.12)	49.94±3.42 ^a	47.75±2.12 ^a
Linolenic acid (C18:3 n-9.12.15)	0.17±0.00 ^a	0.21±0.01 ^a
Arachidic acid (C20:0)	1.05±0.07 ^a	0.79±0.10 ^a
Gondoic acid (C20:1 n-11)	0.61±0.07 ^a	0.51±0.06 ^a
Behenic acid (C22:0)	0.86±0.04 ^b	0.43±0.07 ^a
SFA	17.25±1.95 ^a	18.81±1.03 ^a
MUFA	31.22±2.81 ^a	31.22±3.95 ^a
PUFA	50.11±3.39 ^a	47.96±4.07 ^a
UFA	81.33	79.18
UFA/SFA	4.71	4.21
SFA/UFA	0.21	0.24

Values are mean of three replications \pm 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, UFA unsaturated fatty acids

Table 4. Volatile compounds of *C. albonitens* and *C. balsamita* seeds ($\mu\text{g}/\text{kg}$)

No	Compound	RI	<i>C. albonitens</i>	<i>C. balsamita</i>
1	Methane, tetranitro	617	13.11 \pm 1.58 ^a	25.96 \pm 3.04 ^b
3	Oxirane	620		15.74 \pm 1.56
4	Acetaldehyde	622	8.97 \pm 0.59	
5	Azomethane	627		20.83 \pm 2.6
6	Acetone	629		23.31 \pm 2.47
8	Carbamic acid, ethyl ester	631	11.36 \pm 1.22	
9	Methane, thiobis	633	12.32 \pm 1.74	
10	Propanal, 2-methyl	638	1.63 \pm 0.04 ^a	51.63 \pm 4.26 ^b
12	Acetic acid	644		18.06 \pm 2.35
13	Hexane	647	40.53 \pm 3.56 ^a	114.67 \pm 10.54 ^b
14	Chloroform	654	5.19 \pm 0.06	
15	Isobutane	659	1.26 \pm 0.01	
16	Butanal, 3-methyl	670	1.10 \pm 0.01 ^a	167.63 \pm 10.72 ^b
17	Butanal, 2-methyl	674	1.22 \pm 0.01 ^a	169.49 \pm 10.95 ^b
18	1-Propene, 3-ethoxy	676	1.97 \pm 0.11	
19	1-Hexen-3-ol	685	0.96 \pm 0.01	
20	Pentane, 2,2,4-trimethyl	686	1.80 \pm 0.03	
21	Silenediol, dimethyl	690	6.95 \pm 0.08 ^a	26.22 \pm 2.81 ^b
22	Pentanal	694		22.54 \pm 2.50
23	Cathine	696	2.47 \pm 0.03	
24	1-Butanol, 3-methyl	724	10.94 \pm 1.02	
25	1-Butanol, 2-methyl	727	4.43 \pm 0.04	
26	Propanenitrile, 3-hydroxy	750		41.46 \pm 2.40
27	Toluene	753	1.48 \pm 0.01	
28	1-Pentanol	756	4.02 \pm 0.07 ^a	12.17 \pm 1.91 ^b
29	Methylsilane	774	1.00 \pm 0.03	
30	Hexanal	790	10.30 \pm 1.10 ^a	192.77 \pm 12.11 ^b
31	Fural	822		44.80 \pm 3.04
32	1-Hexanol	857	35.86 \pm 2.31	
33	Heptanal	891		18.18 \pm 2.08
34	Pyrazine, 2,5-dimethyl	902		19.30 \pm 2.52
35	α -Thujene	921	2.73 \pm 0.03 ^a	17.31 \pm 1.63 ^b
36	Benzaldehyde	948		11.71 \pm 0.54
37	Isonitropropane	986	1.60 \pm 0.03	
38	Octanal	991		27.63 \pm 1.57
39	Nonanal	1090	2.68 \pm 0.03 ^a	51.83 \pm 4.23 ^b
40	3-Hexanone	1183	3.05 \pm 0.03	
41	Phthalol	1573		11.81 \pm 0.78

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

the limit for peroxide value ($<10 \text{ meqO}_2/\text{kg}$). Free fatty acids are produced by the hydrolysis of triglycerides. The FFA content of the tested *C. albonitens* and *C. balsamita* seed oils were determined to be 0.96 ve 0.98%, as oleic acid. Only α -tocopherol was detected in seed oils. α -tocopherol contents were 1689 and 1186 mg/kg respectively ($P < 0.05$). Compared to some other seed oils such as *C. sinensis* seed oil 119-210 mg/kg [40], flaxseed (*Linum usittatissimum*. L.) 18.4-38.5 mg/kg [41], organic grape seed oils 1.74-2.03 $\mu\text{g}/100 \text{ g}$ [42], Niger (*Guizotia abyssinica*) seed oils 657-853 mg/kg, canola oil

19 mg/100 g [43], *C. albonitens* and *C. balsamita* seed oils have a very high α -tocopherol content.

Hunter color values of *C. albonitens* and *C. balsamita* 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 showed statistically significant difference ($P < 0.05$). According to these data, *C. albonitens* seed oil was darker and yellower. When a * values were considered, it was seen that the scale was closer to the green color in *C. albonitens* oil.

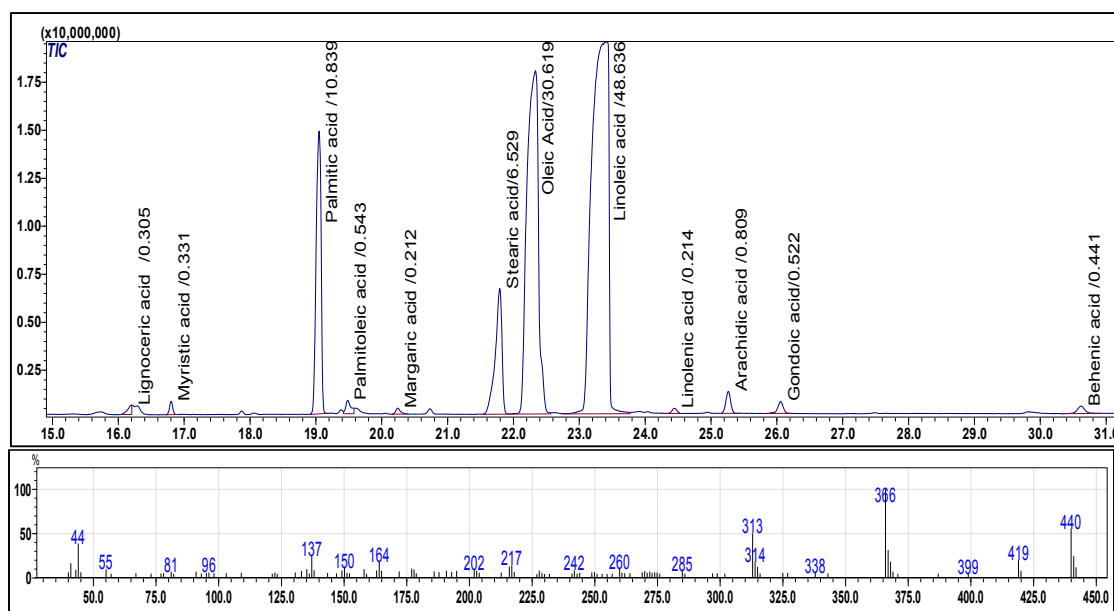


Fig. 3. Fatty acid chromatogram and spectrum of *C. balsamita* seed oil

Table 5. Peroxide value, free fatty acid, α -tocopherol and hunter color values of *C. albonitens* and *C. balsamita* seed oils

	<i>C. albonitens</i>	<i>C. balsamita</i>
PS (meqO ₂ /kg)	2.95±0.01 ^a	3.48±0.75 ^a
Free acidity (as oleic acid %)	0.96±0.07 ^a	0.98±0.21 ^a
α -tocopherol (mg/kg)	1689±35 ^b	1186±25 ^a
Hunter color values		
L*	24.71±0.21 ^b	23.00±0.08 ^a
a*	-1.81±0.29 ^b	-0.78±0.13 ^a
b*	14.21±1.51 ^b	9.15±0.04 ^a

Values are mean of three replications \pm standard deviation. The means with different superscript letters within the same row vary significantly ($P < 0.05$)

4. CONCLUSION

In this study, some characteristics of *C. balsamita*, *C. albonitens* seeds and seed oils were determined. In addition, they have been investigated by comparing their usability as raw material for edible oil and antioxidant production. Oil content of *C. albonitens* and *C. balsamita* seeds were 17.65 and 19.36% and their protein contents were 23.83 and 19.42%, respectively. The antioxidant capacity of seed extracts was found to be lower than BHT, a synthetic antioxidant. Peroxide and acidity values of the obtained oils were acceptable for human consumption. These oils were found to be rich in linoleic and oleic acids. The results of the current study suggested that the seeds of *C. albonitens* and *C. balsamita* may be considered as alternative raw materials for edible oil production, and that these seeds may be used in the

formulation of functional foods as α -tocopherol, natural antioxidant and polyunsaturated fatty acid sources.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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