Research Article

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Fatty acids and antioxidant capacities of three *Centaurea* L. species

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ABSTRACT: The aim of this study is to determine the fatty acid compositions and antioxidant activities of three Centaurea species as *Centaurea iberica, C. urvillei* ssp. *hayekiana*, and *C. urvillei* ssp. *nimrodis* and compare them with each other. As a result of fatty acid determination by GC MS method, oleic acid and linoleic acid were found as the main fatty acids in these Centaurea species. Polyunsaturated fatty acids (PUFAs) were determined at 58.66%, 46.98%, 62.22% in *C. iberica, C. urvillei* ssp. *hayekiana* and *C. urvillei* ssp. *nimrodis*, respectively. Antioxidant activities of methanolic extracts of three *Centaurea* species were evaluated by DPPH, ABTS and CUPRAC assays. The methanol extract of *C. urvillei* ssp *hayekiana* showed the highest antioxidant activity in direct proportion to the total amount of phenolic and flavonoid substances. The fact that the antioxidant activities of these three species and fatty acid analyzes have not been compared in previous studies is important in that this study is the first.

KEYWORDS: Fatty acid; *Centaurea iberica*; *C. urvillei* ssp. *hayekiana; C. urvillei* ssp. *nimrodis;* antioxidant activity.

1. INTRODUCTION

Turkey has a wide flora with approximately 9000 plants. Asteraceae is one of the most widespread families in Turkey. The genus *Centaurea* contains 180 taxa, 120 of which are endemic in Turkey. *C. urvillei* DC. ssp. *hayekiana* Wagenitz and *C. urvillei* DC. ssp. *nimrodis* Boiss. & Hausskn. are endemic species which are spread in the east Anatolia [1-3]. In ethnobotanical use, these species are used as antirheumatic, expectorant, antidiarrhoeal, diuretic, choleretic, stomachic, astringent, cytotoxic, antibacterial, antipyretic and tonic [4-6]. Based on the literature information, *Centaurea* species have antioxidant, antimicrobial, antirheumatic and anti inflammatory properties [7-10]. These effects are known to be due to the secondary metabolites as flavonoids, steroids, volatile constituents, sesquiterpene lactones and fatty acids which is common in *Centaurea* species [11-16].

There are various studies on the fatty acid contents and antioxidant activities of *Centaurea* species. [12, 17-22]. Bouafia and friends made comparison of fatty acid content and antioxidant activities of various *Centaurea* species according to months and different drogs of plants. According to the results, the most important difference was that the roots had a high amount of UFA C18:2 and C18:3, while the roots' bark and the leaves were mainly high in C20:1. However, in general, all results show that the fatty acid content of the plant is abundant and the plant has antioxidant activity. In a different research, total phenolic content (mg GAE/g), DPPH (IC_{50}) and reducing power (IC_{50}) of *C. kroumirensis* (Coss.) and *C. sicula* L. subsp. *sicula* were investigated. In the comparison made using different extraction techniques, the total phenolic content ranged from 61.11 to 4.79 mg GAE/g and from 28.25 to 8.32 mg GAE/g for *C. kroumirensis* and *C. sicula*, respectively [23]. In the study examining the fatty acid profile of *C. stenolepis*, it was found that it is rich in sesquiterpene hydrocarbons, hexadecanoic acid and stem oil is rich in higher alkanes, fatty acids [24].

The fatty acid composition of *C. iberica* Trev. were previously investigated [12]. There were some antioxidant activity studies on different *C. urvillei* subspecies. *C. urvillei* ssp. *armata, C. urvillei* ssp. *hayekiana* and *C. urvillei* ssp. urvillei were analyzed in some previous studies [25,26]. Even if it is the same species, it is very difficult to obtain the same results with the effect of many factors such as the place where the plants were collected, the time, the parts used and the solvent used in the preparation of the extracts. The fatty acid

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composition of *C. urvillei* ssp. *nimrodis* has not been previously studied. The aim of our study is to determine the fatty acid composition of plants by GC-FID analysis and antioxidant activities by various methods.

2. RESULTS AND DISCUSSION

The fatty acid compositions of *C. iberica, C. urvillei* ssp. *hayekiana*, and *C. urvillei* ssp. *nimrodis* are given in Table 1.

Table 1. Fatty acid compositions (%, mean±SD, n=3) of investigated *Centaurea* species.

Fatty acids	***C. iberica***	***C. urvillei* ssp.*hayekiana***	***C. urvillei* ssp. *nimrodis***
C 16:0 (palmitic acid)	6.09±0.07[a]	12.09±0.02	2.82±0.03
C 17:0 (heptadecanoic acid)	0.59±0.01	0.09±0.02	2.37±0.05
C 18:0 (stearic acid)	5.87±0.02	4.25±0.01	5.24±0.01
C 20:0 (arachidic acid)	1.70±0.16	2.44±0.21	0.93±0.09
C 22:0 (behenic acid)	0.32±0.02	2.62±0.11	2.05±0.01
∑**SFA**[b]	14.57±0.28	21.49±0.37	13.41±0.19
C 18:1ω9 (oleic acid)	18.65±0.03	28.09±0.08	23.04±0.06
C 20:1ω9 (gondoic acid)	5.91±0.02	0.14±0.01	3.00±0.01
C 24:1ω9 (nervonic acid)	-	0.23±0.01	0.34±0.03
∑**MUFA**[b]	24.56±0.05	28.46±0.10	26.38±0.10
C 18:2ω6 (linoleic acid)	29.85±0.07	23.09±0.02	31.08±0.02
C 18:3ω3 (α-linolenic acid)	14.75±0.48	8.27±0.42	10.96±0.51
C 18:3ω6 (γ-linolenic acid)	6.09±0.01	4.75±0.01	3.06±0.06
C 20:3ω3 (eicosotrienoic acid)	3.08±0.02	1.29±0.03	4.04±0.01
C 20:4ω6 (arachidonic acid)	2.89±0.06	4.58±0.07	6.08±0.04
∑**PUFA**[b]	56.66±0.49	41.98±0.06	55.22±0.09
∑**major fatty acids**	95.79	91.93	95.71
∑**other fatty acids**	4.21	8.07	4.99

[a]Values reported are means ±S.D., [b]SFA:Saturated fatty acids, MUFA:Monounsaturated fatty acids, PUFA:Polyunsaturated fatty acids

Twelve fatty acids were identified in *C. iberica* oil. The major fatty acids were linoleic acid (29.85%), oleic acid (18.65%) and linolenic acid (14.75%). MUFAs were present with 24.56% and PUFAs were 56.66%. Totaly, unsaturated fatty acids were present with 81.22% of total fatty acids. For *C. urvillei* ssp. *nimrodis* major fatty acids were determined as oleic acid (23.04%) and linoleic acid (31.08%). We also identified 13 fatty acids in oil of *C. urvillei* ssp. *hayekiana* and the major fatty acid was oleic acid (28.05%). Other majör fatty acids were linoleic acid (23.09%) and palmitic acid (12.09%). MUFAs were present with 28.46% and PUFAs were 41.98%. Totaly, unsaturated fatty acids were present with 70.44% of total fatty acids. For *C. urvillei* ssp. *nimrodis* major fatty acids oleic acid (23.04%), linoleic acid (31.08%) and linolenic acid (10.96%). And also MUFAs were present with 26.38% and PUFAs were 55.22%. Totaly, unsaturated fatty acids were present with 81.6% of total fatty acids for *C. urvillei* ssp. *nimrodis.* The antioxidant activities of our extracts of *Centaurea* species reported in Table 2.

C. urvillei ssp. *hayekiana* was determined as the most active plant of the three plants with 17.18±0.86 µg/ml IC_{50}, 98.43±1.34 % (ABTS) and 1230±0.26 µg/ml (CUPRAC) values. A positive correlation between total flavonoid and phenolic contents and antioxidant activity of the extracts were observed. In addition, the yields of the extracts prepared from the plants were obtained in harmony with each other.

3. CONCLUSION

The fatty acid composition of *C. urvillei* ssp. *nimrodis* was investigated for the first time. In our study, oleic acid was detected as the major fatty acid in *C. urvillei* ssp. *hayekiana* (28.09%) and *C. urvillei* ssp. *nimrodis* (23.04%). However, linoleic acid was obtained as the major fatty acid in *C. iberica* oil. Oleic acid, linoleic acid, linolenic acid and palmitic acid were the most common major substances in different *Centaurea* species [12, 17-22]. There is a report about the fatty acid composition of *C. urvillei* ssp. *hayekiana*, however there is no report including fatty acid compositions of *C. urvillei* ssp. *nimrodis*. Similar to our results Zengin et al. reported linoleic acid (35.92%) as the major unsaturated fatty acid and palmitic acid (16.24%) as the major saturated fatty acid in *C. urvillei* ssp. *hayekiana* from Konya, Turkey [22].

Table 2. Results of antioxidant activity, total phenolic and flavonoid contents of investigated *Centaurea* species.

Species	Yield of methanol extracts (%)	DPPH IC_{50} (μg/ml)	ABTS (%)	CUPRAC (μg/ml)	TPC (mg/g)[a]	TFC (mg/g)[b]
C. iberica	10.06	55.82 ± 0.61	50.13±0.88	190±0.49	182.46 ± 1.19	79.08 ± 5.11
C. urvillei ssp. *hayekiana*	12.41	17.18 ± 0.86[c]	98.43±1.34	1230±0.26	402.08 ± 5.74	186.08± 2.05
C. urvillei ssp. n*imrodis*	16.18	36.71 ± 1.02	75.04±1.07	310±0.05	243.91 ± 3.72	84.07 ± 1.46
Ascorbic acid	-	4.23 ± 0.32	-	-	-	-
α-tocopherol	-	-	123.65±0.6	-	-	-
Trolox	-	-	-	2520±0.92	-	-

[a] TPC: Total phenolic content (mg/g dry mass) was determimed by using y = 0,0044x - 0,0556 [Eq. 2]
[b] TFC: Total flavonoid content (mg/g dry mass) was determimed by using y = 7,453x + 0,0628 [Eq. 3]
[c] Results are mean ± SD of three replicate analysis.

Oleic acid (C 18:1 ω9) and linoleic acid (C 18:2 ω6) were determined to be major fatty acids in the oil of our samples. Oleic acid is the monounsaturated fatty acid which is important in human nutrition. Monounsaturated fat intake leads to a decrease in low-density lipoprotein (LDL) cholesterol and increased high-density lipoprotein (HDL) cholesterol. It is important to have a sufficient amount of linoleic acid in our diets because of its necessity. Lack of essential fatty acids such as linoleic acid in the body, mostly causes cardiovascular (and many other) diseases and its progression [27].

According to previous studies, the antioxidant capacity of extracts and their flavonoid content are related [28,29]. The TPC of the extracts of *C. iberica, C. urvillei* ssp. *hayekiana*, and *C. urvillei* ssp. *nimrodis* were determined as 182.46, 402.08, and 243.91 gallic acid equivalents (mg/g) dry matter. TFC of *C. iberica, C. urvillei* ssp. *hayekiana*, and *C. urvillei* ssp. *nimrodis* extracts 79.08%, 186.08%, and 84.07%, respectively. Similarly results in antioxidant capacity values, TPC and TFC concentrations *Centaurea* species were reported in previous studies [19,21,22]. Epidemiologic studies have shown an inverse relationship between foods with high antioxidant content and the rate of death from degenerative diseases such as cancer and heart disease [28]. Therefore the high antioxidant capacity of *C. urvillei* ssp. *hayekiana* is a very important result. Our results suggested that *Centaurea* oil and polyphenolics can be used as a potential source of natural antioxidants and for nutritional and pharmacological applications.

The present study was a preliminary attempt to characterize the nutritional value of *C. iberica, C. urvillei* ssp. *hayekiana*, and *C. urvillei* ssp. *nimrodis* as a potential natural source of antioxidants, proteins and oils. *C. urvillei* ssp. *hayekiana* showed relatively high unsaturated fatty acid content. Indeed, *C. urvillei* ssp. *hayekiana* methanol extract in particular exhibited significant total phenolic, flavonoid and high antioxidant potency.

Genetic and environmental factors regulate the phytochemical response of wild plants. Further studies that take into account environmental factors and genetic diversity are definitely needed. , *C. urvillei* ssp. *hayekiana* should attract attention as a natural source of protein, unsaturated fatty acids and natural antioxidants that can be used for nutritional, industrial and pharmaceutical purposes.

4. MATERIALS AND METHODS

4.1. Plant materials

The aerial parts of *C. urvillei* ssp. *hayekiana, C. iberica*, and *C. urvillei* ssp. *nimrodis* were collected during flowering period from rocky areas of Buzluk Cave, Harput, Elazıg, Turkey in June 2018. The plants were identified by Ugur Cakilcioglu from Tunceli University. Voucher specimens (no. 1460, 1461, and, 1459,

respectively) were deposited in the Herbarium of Faculty of Pharmacy, Department of Pharmacognosy, Ege University, Izmir, Turkey.

4.2. Chemicals

All standarts and chemical compounds were purchased from Sigma Chemical Co. (Sigma-Aldrich Gmbh, Germany).

4.3. Fatty acid analysis

40 grams of ground sample were extracted for oil, using petroleum ether at 60 °C for 6 h in a Soxhlet system. The solvent was evaporated by rotary evaporator. The obtained oil was esterified to determine fatty acid composition [27].

The method described by Ichihara et al. was followed to prepare fatty acid methyl esters (FAME) using transmethylation [28]. FAMEs were analyzed on a HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), Agilent 5975C mass selective detector (MSD) and fitted to a HP-88 capillary column (100 m length, 0.25 mm i.d. and 0.2 µm thickness). Injector and detector temperatures were set at 240 and 250 ºC, respectively. The oven was set at 160 ºC for 2 min. The temperature was increased up to 140 ºC at rate of 4 ºC/min then increased at up to 200 ºC at rate of 2 ºC/min and held at 200 ºC for 46.75 min. Total run time was arrenged as 70 min and as carrier gas helium was used (1 mL/min). Identification of fatty acids was done by comparision of mass spectra and retention times with standarts (Supelco 37 Component FAME Mix and with the NIST 11 and Wiley 7 commercial mass spectral libraries). Quantification methyl esters of fatty acids was done using the internal standard mass and relative percentage of GC in relation to each component. Each reported result is given in the average value of three GC analyses. The results were determined as means±S.D.

4.4. Extraction

The aerial plant materials were dried at optimum conditions in the room temperature. Methanol extracts were prepared from 40 g batches of the air-dried and powdered plant materials by extracting with 400 mL methanol in a Soxhlet apparatus for 6-8 h. The solvents were evaporated to dryness in vacuo (40 °C). Extracts were stored at +4 °C in dark until use.

4.5. Antioxidant activity assays

4.5.1. DPPH

DPPH free radical scavenging activity measurement was performed with some modifications on the Fukumoto method [29]. The measurement was made with a 96-well microplate. Microdilution sample batches (1 mg/mL, dissolved in HPLC grade MeOH) were prepared starting with 150 µL. 50 µL of DPPH reagent (100 µM, prepared with HPLC grade MeOH) was added to each well to give a total volume of 200 µL. The microplate was stored at room temperature in dark conditions and then absorbance was measured at 550 nm after 30 minutes with the aid of a BMG Labtech FluoStar Optima plate reader. For the blank control, HPLC grade MeOH was used instead of the sample. Ascorbic acid (0.01 mg/mL in HPLC grade MeOH) was used as standard. Evaluation of IC_{50} values was also done using the GraphPad Prism 6.05 program.

4.5.2. ABTS

ABTS [2,2′-azinobis (3-ethylbenzotiazolyn-6-sulfonic acid) diamonium salt] solution was prepared and diluted with ethanol until giving 0.750 absorbance in 734 nm by ABTS assay [30]. 0.1 ml of extracts and 10 µl α-tocopherol were added to 1 ml $ABTS^+$ solution and absorbance changing was observed in 734 nm during 6 minutes. For standard solution, α-tocopherol was used. % ABTS rate was calculated by this equation:

$$\% \text{ ABTS rate} = [Abs_1 - Abs_2] / Abs_1 \times 100. \quad [\text{Eq. 1}]$$

Abs_1: First measurement

Abs_2: Second measurement after 6 minutes

4.5.3. CUPRAC

In accordance with some modifications of the Apak method, the reducing power of Cu(II) was analyzed [31]. After the mixture of Neocouprin and Cu(II) solutions (pH 7) was prepared, sample solut ons at various concentrations were added and after waiting for 30 minutes at room temperature, measurements were made at 450 nm absorbance. Trolox solution prepared at different concentrations was used for the standard.

4.5.4. Determination of total phenolic and flavonoid contents

Total phenolic content (TPC) was quantified using the Folin-Ciocalteu phenol reagent, while total flavonoid content (TFC) was measured using aluminium nitrate nonahydrate according to a slightly modified experimental procedure. The results were expressed as mg GAE/g and mg QE/g of dry extract, respectively by using Equation 2 and 3 [32-34].

4.5.5. Statistical analysis

All analysis of each sample were carried out three times and obtained results were showed as means ± SD. One-way-analysis of variance (ANOVA) ($p<0.05$) was applied and Person's correlation was used to determine the correlation coefficient of total phenolic content and antioxidant activity (Microsoft excel 2013).

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