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Original Article

Determining the fatty acid composition and antioxidant activities of *Centaurea virgata* Lam.

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ABSTRACT

Background and Aims: *Centaurea* is a significant widespread genus in the *Asteraceae* family. Türkiye has 194 species, of which 118 are endemic. Free radicals can alter lipids, proteins, and DNA and trigger many diseases. However, a person with an insufficient immune system needs outside assistance to scavenge free radicals. Antioxidants protect the body from oxidative stress by scavenging free radicals. Fats are also very important because they contain fat-soluble vitamins, form lipoproteins with proteins, and affect health. Fatty acids serve as part of molecules in cells or alone as building blocks of cell membranes. They also promote energy and signaling molecules. Polyunsaturated fatty acids (PUFAs) have significant antioxidant activity. Studies are found showing PUFAs to be able to be used as a preservative in foods. These effects are due to phenolic hydroxyl groups. The aim of the current study is to evaluate the fatty acid profile of *C. virgata* to determine the fatty acid content and examine the antioxidant activities related to major fatty acids.

Methods: Gas chromatography Mass Spectrometry (GC/MS) analyses were carried out to determine the fatty acid content. Furthermore, the *in vitro* antioxidant activities of methanol extract were determined using DPPH (2, 2'-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging and the reducing power assay methods. The program IBM SPSS Statistics (ver. 25) was used to evaluate antioxidant activities.

Results: *C. virgata*'s major components were found to be palmitic acid (30.13%), oleic acid (9.17%), and linoleic acid (11.64%). The DPPH results indicated the IC_{50} value of the methanol extract to be 378.86 ± 0.60 µg/mL, while ABTS was found to be 19.38 ± 0.49 µg/mL. The results from the reducing power assay found the absorbance of ascorbic acid to be 0.55 at a 100 µg/mL concentration and it measure 0.12 in *C. virgata*.

Conclusion: According to the evaluation results, harmony is seen to exist between the groups regarding the activities.

Keywords: ABTS, *Centaurea*, DPPH, Fatty acid, GC-MS

INTRODUCTION

The genus *Centaurea* is one of the most significant extensive genii of the *Asteraceae* family. *Centaurea* is predominantly distributed in Western Asia and the Mediterranean region and is common in the Southwest, Central Anatolian, and Eastern Anatolian regions of Türkiye. *Centaurea* is also known as the largest genus after *Astragalus* and *Verbascum* in Türkiye and has more than 700 species worldwide (Zengin et al., 2019). Türkiye has 194 species, of which118 are endemic (Albayrak, Atasagun & Aksoy, 2017).

Centaurea is a genus known to have significant potential in both conventional systems and drug development (Zengin et al., 2018). It has traditionally been used for asthma, abdominal pain, headache, hemorrhoids, diarrhea, rheumatoid arthritis, and stomach, as well as for its antipyretic, astringent, and antibacterial effects (Albayrak et al., 2017; Baytop, 1999; Köse, İşçan et al., 2016; Zengin et al., 2019b).

The genus contains intensely phenolic structures and flavonoids, in addition to sesquiterpene lactones, lignans, and alkaloids that are occasionally seen. The genus shows a wide variety of biological activities with its rich chemical content, with studies having observed antioxidant, antimicrobial, antiproliferative, hepatoprotective, anti-inflammatory, antiulcerogenic, antiplasmodial, and enzyme inhibitor activities in *Centaurea* species (Boğa et al., 2016; Köse et al., 2016).

Free radicals are formed by the body as a result of metabolic reactions. Free radicals can alter lipids, proteins, and DNA and trigger many diseases. The control of this production is provided by the endogenous and exogenous systems. However,

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when produced in excess, the immune system may be insufficient to scavenge these free radicals. In such cases, external assistance is needed to scavenge them. The oxidative stress caused by overproduction can cause neurodegenerative disorders, atherosclerosis, cancer, hypertension, aging, and similar diseases. Antioxidants protect the body from oxidative stress by scavenging free radicals. Plants also contain phytochemical antioxidants such as phenolic substances that act as powerful natural antioxidants (Köse et al., 2016; Zengin et al., 2011).

An important relationship is known to exist between a person's nutritional habits and disease. Carbohydrates, proteins, and fats are essential for the survival of all living organisms. Fats are made up of glycerol and fatty acids and are an important essential ingredient for their high energy and for being highly suitable for storage. While glycerol is the same in every plant, fatty acids occur differently in each plant. In addition to being a source of energy, fats are very important because they contain fat-soluble vitamins, form lipoproteins with proteins, and affect health. Saturated fatty acids increase the rate of fat in the blood and low density lipoprotein (LDL) levels and are also effective in the formation of insulin resistance. Unsaturated fatty acids are known as important sources of prostaglandins that increase the amount of high density lipoproteins (HDLs), which have protective properties against cardiovascular diseases, inflammatory diseases, and cancer (NRC, 1989; Ros& Mataix, 2006).

This study aims to determine the fatty acid content of *Centaurea virgata* Lam. and to examine its *in-vitro* antioxidant activities related to major fatty acids.

MATERIALS AND METHODS

Plant Collection

The aerial parts of *C. virgata* Lam. were collected from the Harput district in Elazığ in 2014. These were registered under No. 1461 in the herbarium of Ege University Faculty of Pharmacy, Department of Pharmacognosy.

Plant Extraction

The aerial parts of *C. virgata* Lam were dried in the shade at room temperature and then ground into fine powder with the help of a mill. Methanol extract was prepared by continuous shaking and maceration 5 g of the fine powder in 100 mL of methanol. The study then investigated the antioxidant activities of the prepared extract after filtration and evaporation of the solvents.

Fatty Acid Analysis

40 g of the powdered plant was extracted with petroleum ether in a Soxhlet extractor at 60ºC for 6 h. It was first saponified with 0.5 N NaOH. It was then treated with 14% boron trifluoride-methanol complex and n-heptane mixed with a saturated solution of NaCl to achieve phase separation. The oily part that was obtained forms the part to be analyzed in gas chromatography (GC; UPAC, 1979; Yıldırım et al., 2009). The GC operating conditions are given in Table 3. GC analysis was performed in three parallels.

GC/MS Analysis

Hewlett-Packard (HP) Agilent 6890 N Gas Chromatograph was used and equipped with a flame ionization detector (FID). It was fitted to the Supelco SP-2380 Phased Silica Capillary Column (60 m*0.25 mm, 0.2 µm). Injector temperature was 250°C and detector temperature was 260°C. After waiting 5 min at 140°C, the temperature was then raised to 240°C in 3°C increments. Helium was used as the carrier gas at a flow rate of 41.33 cm/sec. Total run time was set for 70 min. Fatty acids were identified by comparing the mass spectra and retention time to standard values (Supelco Component FAME mix and NIST 11 and WILEY 7 commercial mass spectral libraries). Quantification was performed by internal standard mass and relative percentage of GC. The results were determined as Mean ± SD.

Antioxidant Activities

2, 2'-Diphenyl-1-picrylhydrazyl assay (DPPH)

1 mL from each of the extracts prepared at concentrations between 100-1000 µg/mL was taken and mixed with 4 mL of DPPH solution. After incubating for 30 min at room temperature, the absorbance at 517 nm was measured. Higher absorbance indicates less activity. Ascorbic acid was used as the standard (Esmaeili & Sonboli, 2010). Experiments were performed in three parallels.

$$I(\%) = 100\, x\, (A_0 - A_S)/(A_0) \qquad (1)$$

A_0 (absorbance of control)
A_S (absorbance of sample)
I (inhibition)

Reducing Power Assay

According to Oyaizu (1986), 1.25 mL of the 0.2 M sodium phosphate buffer (pH 6.6) and 1.25 mL of a 1% potassium ferricyanide mixture were added to 50, 100, and 200 µg/mL of each extract. The mixture was incubated using the LAB-LINE MaxQ6000 instrument at 50ºC for 20 min. After incubation, 1.25 mL of 10% trichloroacetic acid and 0.5 mL of 1% $FeCl_3$ were added. All the mixtures' absorbance values were measured at 700 nm (Oyaizu, 1986). Ascorbic acid was used as the control [23]. Experiments were performed in three parallels.

2,2'-Azino-Bis-(3-Ethylbenzothiazoline-6-Sulfonic) Acid (ABTS) Radical Scavenging Assay

1 mL from each of the methanol extracts prepared at concentrations between 100-1000 µg/mL was taken, and 3 ml of ethanol and 1 mL of ABTS radical solution were added to it. After 6 min, the absorbance at 734 nm was measured against the blank (ABTS solution and ethanol mixture without the extract). α-tocopherol was used as the standard (Çelik, 2011). The experiments were performed in three parallels. Calculated according to Eq 1.

Statistical Analysis

All results are expressed as Mean ± SD. Analysis of variance was also performed, and significant differences between means were determined using Duncan's multiple range tests at a significance level of $p < 0.05$ in IBM SPSS (ver. 25).

RESULTS AND DISCUSSION

The fatty acid content of *C. virgata* Lam. was determined, with Table 1 showing the results. Accordingly, the major fatty acids of *C. virgata* Lam. have been determined as palmitic acid (30.13%), oleic acid (9.17%) and linoleic acid (11.64%). 43.50% of these are saturated fatty acids. Palmitic acid (hexadecanoic acid) constitutes the largest part of the saturated fatty acids at 30.13%, followed by heneicosanoic acid and myristic acid. Palmitic and myristic acid have an important role in the acylation of membrane proteins, which are important in fixing proteins to the plasma membrane. A positive relationship is known to exist for myristic and palmitic acid concentrations with type 2 diabetes and to be inversely proportional to that of pentadecanoic and heptadecanoic acids, which are odd-numbered saturated fatty acids. Still, myristic and palmitic acid are present in small amounts in the plant being researched here. Saturated fatty acids are known to stimulate inflammation (i.e., NF-kB). Because NF-kB has an immunomodulatory role, it being stimulated has great significance; NF-kB is important for designing treatments that can prevent or minimize acute inflammatory damage associated with critical illness. Monounsaturated fatty acids (MUFA) comprise 15.98% of *C. virgata* Lam., with oleic acid being the most prevalent here. Oleic acid is generally known for its LDL cholesterol lowering effect. It has no effect on HDL, has a slight anti-inflammatory effect, and also regulates insulin. Polyunsaturated fatty acids (PUFA) comprise 19.96% of *C. virgata* Lam. with linoleic acid being the most prevalent here. Linoleic acid is one of the basic building blocks of membrane phospholipids and is also an important component of ceramides, especially those in the skin. It provides skin integrity and prevents water loss and is also an important cholesterol lowering agent. It has little effect on inflammation. PUFAs have significant antioxidant activity. Studies are found to have shown it can be used as a preservative in foods. These effects are due to the phenolic hydroxyl groups (Cakmakci & Kahyaoglu, 2012; Neitzel, 2010).

Various studies have occurred on the fatty acid content and antioxidant activities of the extracts in the genus *Centaurea*. Tekeli et al.'s (2011) study identified the fatty acid compositions of *Centaurea balsamita, C. calolepis, C. carduiformis, C. cariensis* subsp. *maculiceps, C. cariensis* subsp. *microlepis*, and *C. iberica*, with C 18:2 6 linoleic acid, C 16:0 palmitic acid, C 18:3 3 linolenic acid, and C 18:1 oleic acid generally being found as major components in all these species. They also found polyunsaturated fatty acids to occur more than saturated and monosaturated fatty acids. For *Centaurea balsamita, C. calolepis, C. carduiformis, C. cariensis* subsp. *maculiceps, C. cariensis* subsp. *microlepis*, and *C. iberica* PUFA values are given as 55.10%, 50.25%, 51.41%, 41.02%, 46.18% and 58.80% respectively. Erdoğan et al.'s (2014) study investigated the fatty acid profiles for *C. behen, C. saligna, C. depressa, C. urvillei* subsp. *urvillei, C. urvillei* subsp. *hayekiana*, and *C. aggregata* subsp. *aggregata* plants collected from Elazig. The found the ratio of saturated fatty acids to vary between 24.61%-50.92%, of monosaturated fatty acids to vary between 3.40%-37.96%, and of polyunsaturated fatty acids to vary between 2.21%-20.57%. They also found the most common fatty acids in all species to be palmitic acid C 16:0, oleic acid C 18:1 and linoleic acid C 18:1. Zengin et al. (2010) investigated the fatty acid profiles of *C. pulchella, C. patula*, and *C. tchihatcheffii* and identified 33 fatty acids in the plants. They found linoleic acid to be the most common fatty acid in *C. pulchella* and *C. tchihatcheffii* and alpha-linolenic acid to be the most common fatty acid in *C. patula*. Aktümsek et al. (2013) examined the fatty acid profiles of *C. pseudoscabiosa* subsp. *aratica, C. pulcherrima* var. *pulcherrima, C. salicifolia* subsp. *abbreviata*, and *C. babylonica* and detected the most common fatty acids as palmitic acid (23.38–30.49%) and linoleic acid (20.19–29.93%). Aktümsek et al. also examined the fatty acid contents of *C. kurdica, C. rigida, C. amanicola, C. cheirolopha*, and *C. ptosimopappoides* and determined palmitic, linoleic, oleic, and linolenic acids as the major components. Zengin et al. (2011) found *C. urvillei* subsp. *hayekiana* to contain high amounts of C 18:2 omega 6 (linoleic acid) and C 18:1 omega 9 (oleic acid). In the study of Ayaz et al.'s (2017) study examined the fatty acid profiles of 10 *Centaurea* species and found their most common fatty acids to be palmitic acid C16:0 (5.22-12.06%), oleic acid C18:1n9 (8.57-30.29%), and linoleic acid C18:2n6 (49.15-79.15%). (Janackovic et al. (2017) determined the fatty acid profiles of the cypselae part of *C. galicica* and *C. tomorosii*, identified 11 fatty acids, and found palmitic acid to have the highest concentration. Zengin et al.'s (2011) study determined the fatty acid profile and antioxidant properties of *C. kotschii* and identified the most prevalent fatty acid as α-linolenic acid.

Author Senatore et al.'s (2008) study examined the volatile components of *Centaurea* species and identified high amounts

Table 1. Fatty acid content of *C. virgata* Lam.(%)

Fatty Acids	*C. virgata*
C 4:0 (butyric acid)	-
C 6:0 (caproic acid)	0.13
C 8:0 (caprylic acid)	0.57
C 12:0 (lauric acid)	1.02
C 13:0 (tridecyclic acid)	0.28
C 14:0 (myristic acid)	3.75
C 15:0 (pentadecanoic acid)	0.18
C 16:0 (palmitic acid)	30.13
C 17:0 (heptadecanoic acid)	0.45
C 18:0 (stearic acid)	1.87
C 21:0 (heneicosanoic acid)	4.09
C 22:0 (behenic acid)	0.64
C 23:0 (trichosanoic acid)	0.39
C 24:0 (lignoceric acid)	-
∑SFA[b]	**43.50**
C 18:1 ω9 (Oleic acid)	9.17
C 20:1 ω9 (Gondoic acid)	6.81
C 24:1 ω9 (Nervonic acid)	-
∑MUFA[b]	**15.98**
C 18:2 ω6 (Linoleic acid)	11.64
C 18:3 ω6 (γ-linolenic acid)	1.78
C 20:3 ω3 (Eicosatrienoic acid)	4.98
C 20:5 ω3 (Eicosopenteanoic acid)	1.56
∑PUFA[b]	**19.96**

*C:D ratio (the total number of *carbon* atoms to the number of *carbon-carbon* double bonds)

of fatty acid content, with *C. nicaeensis* oil containing 66.4% fatty acids and hexadecanoic acid and 9-12-octadecadienoic acid being the most common. They found the *C. parlatoris* plant oil to contain 22% fatty acids, with the highest amount being 18.1% hexadecanoic acid. Finally, . solstitialis subsp *schouwii* oil was found to contain 43.6% fatty acids and their esters, with hexadecanoic acid (29.4%) being the main component. Formisano et al.'s (2008) study examined endemic *Centaurea* plants and observed *C. amanicola* to be characterized by high amounts of caryophyllene oxide (12%) and caryophyllene (5.4%) and *C. consanguinea* to have germakren B (8.3%) and caryophyllene oxide (7.3%). Finally, they found a high amount os sesquiterpene inthe *C. ptosimopappa* plant, the concentrations were quite low, with only β-ödesmol is high. Lazari et al. (2000)examined the volatile components of *C. pelia, C. thessola*, and *C. zuccariana* and observed the plants to contain 31.9%, 13%, and 17.3% fatty acids, respectively, with hexadecanoic acid being the most common in all three plants. It was also observeda composition of 32.5% fatty acids in the volatile components of *C. sipthorpii*, with hexadecanoic acid again being determined as the most common, and additionally investigated the fatty acid contents and antioxidant activities of *C. iberica, C. urvillei* ssp. *hayekiana*, and *C. urvillei* ssp. *nimrodis* (Taştan et al. 2017). Oleic acid was major for three of the speciesIn the end, when evaluating all the results, the components that were found to be most common are compatible with the literature, with the most common saturated fatty acid being palmitic acid. Various fatty acid and antioxidant activity studies on *C. virgata* collected from different places have occurred. The current research has evaluated the similarity *C. virgata* collected from Elazig has with C. virgata collected from elsewhere. A study (Tekeli et al., 2013) on C. virgata collected from Konya showed the most common fatty acids that were found to be linoleic (29.15%), palmitic (28.41%), oleic (9.04%), and α-linoleic (21.33%) acids. These results also differ from the current study regarding the amounts and most common components, this difference is considered to be due to the growing conditions. The most common fatty acids in the *Centaurea virgata* Ayaz et al. (2017) collected from Gümüşhane were found as palmitic (5.75%), oleic (18.40%), and linoleic (62.99%) acids. Meanwhile the current study found the same substances to be the most common in the species collected from Elazig, though the amounts were found to be quite different. The reason for the change in the amount of content is thought to be region-based (Çelik, 2011).

Table 2. Antioxidant activity results for *C. virgata* Lam.

	DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)
CVM	378.86±0.60	19.38±0.49
Ascorbic acid	4.68±0.21	-
Trolox	-	18.22±1.15

CVM = MeOH extract of *C. virgata* Lam.

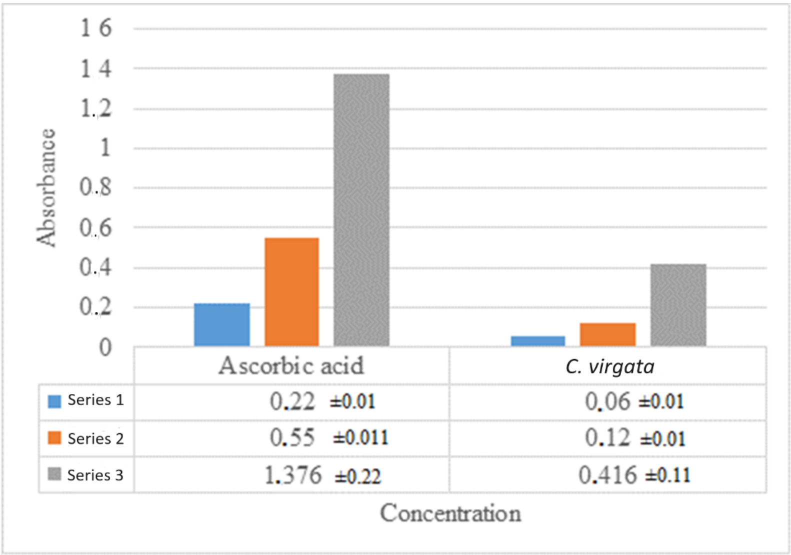

Figure 1. Reducing Power Results of the *C. virgata* Lam.

Table 2 and Figure show the results regarding the antioxidant activities, with the results appearing statistically significant at $p \leq 0.05$. The study evaluated DPPH, FRAP, and CUPRAC antioxidant activities and obtained respective values of 135.56 µmol TE/ 100 g dw, 57.63 µmol TE/ 100 g, and 68.19 µmol TE/ 100 g. The data indicate the *C. virgata* to have antioxidant effects similar to the *C. virgata* collected from Gümüşhane (see Ayaz et al., 2017), but at different levels. *C. kotschii* was investigated for its antioxidant potential. The current study has observed the antioxidant properties to be at a significant level. The IC_{50} value of the extract in the DPPH assay was determined as 37.09 g/mL. The extract exhibits a 65.22% linoleic oxidation in the beta-carotene/linoleic acid system. The amount of total phenolic content and total antioxidant capacity were detected as 36.52 mg gallic acid equivalent (GAE) per gram and 74.93 mg ascorbic acid equivalent (AE) per gram, respectively (Zengin et al., 2011). Zengin et al. (2010) had also investigated the antioxidant activities of the methanol extracts from *C. pulchella, C. patula*, and *C. tchihatcheffii*, and their results determined all of these to show antioxidant activity, with *C. pulchella* being the most active. Aktümsek et al. (2013) had investigated the antioxidant activities of *C. pseudoscabiosa* subsp. *aratica, C. pulcherrima* var. *pulcherrima, C. salicifolia* subsp. *abbreviata*, and *C. babylonica* and found *C. pulcherrima* var. *pulcherrima* to be the most active extract. Aktümsek et al. (2011) also identified the antioxidant activities of *C. kurdica, C. rigida, C. amanicola, C. cheirolopha*, and *C. ptosimopappoides* and found them to all have antioxidant activity.

CONCLUSION

In conclusion, the plants of the genus *Centaurea* and *C. virgata* Lam. have been the subject of much research because of their fatty acid content and antioxidant activities, which are known to be significant. However, no previous research could be found on the fatty acid and antioxidant activities of *C. virgata* collected from the Elazığ region. After evaluating the results, differences were determined to be present in the amounts and types of fatty acids, as well as in antioxidant activities, all due to effects such as growing and climatic conditions as well as soil richness.

According to this research, *C. virgata* can be considered an important source of antioxidants and fatty acids.

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