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RESEARCH ARTICLE

Antioxidant and Anti-inflammatory Activity of Five *Centaurea* Species

Ali Sen[1]

[1]Marmara University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkiye

ABSTRACT

Objective: In this study, we examined the anti-inflammatory and antioxidant (ABTS radical scavenging) activity of methanol extracts of aerial parts (except capitula) and capitula of *Centaurea cuneifolia, C. iberica, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* for the first time comparatively.

Materials and Methods: The antioxidant and anti-inflammatory activity, expressed as IC_{50} values, were determined by 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic asit (ABTS) and 5-lipoxygenase methods. The total phenolic content, expressed as gallic acid equivalents, was estimated by Folin-Ciocalteu method.

Results: Methanol extract of capitula of *C. solstitialis* subsp. *solstitialis* (CSSC) with an IC_{50} value of 8.74 µg/mL showed antioxidant activity as strong as standard acarbose (4.41 µg/mL) against ABTS radicals. The IC_{50} values of ABTS radical scavenging activities of other extracts varied between 24.42 and 88.95 µg/mL. CSSC with an IC_{50} value of 122.10 µg/mL displayed moderate inhibitory activity against 5-lipoxygenase enzyme. The IC_{50} values of the antilipoxygenase activities of the other extracts were found to vary between 122.10 and 781.30 µg/mL. Also, the highest amount of total phenolic compounds was found in the CSSC (83.41 mg/g), while the lowest was found in methanol extract of aerial parts of *C. solstitialis* subsp. *solstitialis* (35.20 mg/g).

Conclusion: These results clearly indicate that CSSC has significant antioxidant and anti-inflammatory activity. As far as is known, this paper is the first comparative study on ABTS radical scavenging and lipoxygenase inhibitory activity of five different *Centaurea* species. It is also the first study on the antilipoxygenase activity of *C. iberica* and *C. solstitialis* subsp. *solstitialis*.

Keywords: *Centaurea* species, antioxidant activity, anti-inflammatory activity, total phenolic content

INTRODUCTION

Oxidative stress, an imbalance between reactive oxygen species (ROS) production, acts as significant signaling molecules in the physiological processes, and the antioxidant defenses that protect cells is associated with the development of a number of diseases, including pulmonary hypertension, asthma, cancer, heart disease, autoimmune, metabolic diseases, and others.[1] Antioxidants, known as free radical scavengers, play an important role in preventing cell damage by inhibiting the free radical chain reaction under different physiological conditions.[2] Many antioxidant compounds obtained from plants are identified as free radical inhibitors, reducing agents, or active oxygen scavengers.[3]

Recent substantial evidence suggests that oxidative stress plays a key role in various aspects of acute and chronic inflammation.[4] Inflammation is known as the body's defense mechanism against pathogens and toxic stimuli. An abnormal immune system leads to a chronic immune response, causing inflammatory diseases such as gastritis, cancer, allergies, rheumatoid arthritis, and multiple sclerosis.[5] Medicinal plants have been used in traditional folk medicine for years as anti-inflammatory and antioxidant agents against diseases caused by oxidative stress and/or inflammation.[6] It is therefore important to conduct research on medicinal plants and their secondary metabolites.

The genus *Centaurea* belonging to the family Asteraceae is represented by 159 species and 194 taxa and 110 of these are endemic.[7] These species are used in traditional medicine for the treatment of vaginal yeast infections, menstrual disorders, ulcer, liver and kidney diseases, as well as for anti-diarrhea, stomachic, tonic, appetizer, antidiabetic, antipyretic, diuretic and expectorant treatment.[8] Phytochemical studies on the *Centaurea* species reported that they are rich in sesquiterpene lactones and flavonoids. In addition, it is suggested that these species have other effects such as antimicrobial, antipyretic, antiviral, antimalarial, antiphytoviral, antiulcerogenic, anti-inflammatory, cytotoxic, hypoglycemic, neurotoxic and vasodilator effects.[9,10]

Corresponding Author: Ali Sen **E-mail:** ali.sen@marmara.edu.tr; alisenfb@yahoo.com
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This study aimed to comparatively investigate the anti-inflammatory and antioxidant activity of methanol extracts of aerial parts (except capitula) and capitula of *Centaurea cuneifolia* Sm., *C. iberica* Trev. ex Sprengel, *C. kilaea* Boiss., *C. solstitialis* L. subsp. *solstitialis* L. and *C. stenolepis* A. Kern. [syn. *C. phrygia* subsp. *stenolepis* (Kerner) Gugler] together with their total phenolic contents.

MATERIALS AND METHODS

Plant Material

Aerial parts of *Centaurea cuneifolia, C. iberica, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* were gathered from different districts of İstanbul, Turkiye in 2009 and identified by Dr. Gizem Bulut, a botanist of the Faculty of Pharmacy, University of Marmara. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE No: 11651, 11712, 11690, 11966 and 11965, respectively).

Extraction

Extracts of the *Centaurea* species were obtained from a previous study.[8] Aerial parts of five *Centaurea* species were separated from the capitula. Next, the aerial parts (except capitula) and capitula of *Centaurea* species were dried and ground, about 15 g each, and macerated with MeOH three times (24 h×180mL). All extracts were filtered and dried in a vacuum. The aerial parts (except capitula) and capitula of *Centaurea cuneifolia, C. iberica, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* were then coded as CCC, CCA, CIC, CIA, CKC, CKA, CSSC, CSSA, CSC, CSA. Extraction yields were found to be 12.693%, 10.603%, 10.750%, 12.439%, 11.331%, 12.916%, 10.901%, 8.139%, 13.224% and 13.542%, respectively. All extracts were stored at 4°C for further analysis.

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Radical Scavenging Activity

The ABTS radical scavenging capacity was tested as described by Zou et al.[11] The $ABTS^{\bullet+}$radical cation used for the determination of total antioxidant capacity was obtained by mixing 7 mM ABTS (in H_2O) with 2.45 mM potassium persulfate (in H_2O) and then kept in the dark for 12-16 h at room temperature. The $ABTS^{\bullet+}$ solution was diluted with 96% ethanol solvent of analytical purity to give an absorbance value of 0.700±0.050 at 734 nm. Ten µL of each of the solutions was prepared at five different concentrations of the extracts (250-3.91 µg/mL) were transferred to microplate wells and 190 µL of the $ABTS^{\bullet+}$ solution was added. The mixture was kept at room temperature for 30 min and the absorbance at 734 nm was read immediately afterwards. Trolox (250-0.49 µg/mL), an antioxidant molecule, was used as the standard and the results were expressed as an IC_{50} value.

In Vitro Anti-Inflammatory Activity

The anti-inflammatory activity was tested according to the reported procedure.[12,13] Ten µL of each of the samples (extract and standard) were prepared at five different concentrations (250-0.49 µg/mL) and transferred to quartz microplate wells and 20 µL ethanol, 25 µL borate buffer (0.1 M, pH 9) and 25 µL type V soybean lipoxygenase solution (pH 9, 20.000 U/mL) in a buffer were added. The mixture was incubated at 25°C for 5 min, then 100 µL of 0.6 mM linoleic acid solution was added, mixed thoroughly, and absorbance changes at 234 nm were recorded for 6 min. Indomethacin (250 - 0.49 µg/mL) was used as a reference standard and the results were expressed as an IC_{50} value.

Determination of Total Phenolic Contents (TPC)

The total phenolic content was determined according to the reported procedure.[13,14] Ten µL of the solutions prepared from each of the extracts at various concentrations (151.52 – 2.37 µg/mL) were put on microplates and 20 µL of Folin-Ciocalteau solution, 200 µL of ultrapure water and 100 µL of 15% Na_2SO_4 were added. The absorbance of the solutions after 2 h of incubation at room temperature was read at 765 nm in a spectrophotometer. For the standard curve plot, gallic acid (500-0.977 µg/mL) was used as the standard and absorbances corresponding to each concentration were measured at 765 nm. The total phenolic compound amounts of the extracts were calculated from this standard curve plot and the results expressed as mg gallic acid equivalent per gram extract (mg GAE/g plant extract).

Statistical Analysis

All analyses were conducted in triplicate, and the data presented as mean±standard deviation (SD). The data was analyzed by ANOVA followed by the Tukey's multiple comparison tests using the GraphPad Prism 5. Significance was defined at $p<0.05$.

RESULTS

The CSSC showed the highest antioxidant activity with an IC_{50} value of 8.74 µg/mL, while CSA showed the lowest antioxidant activity with an IC_{50} value of 88.95 µg/mL in the ABTS assay. CSSC demonstrated significant ABTS radical scavenging activity as compared to standard trolox having an IC_{50}=4.41 µg/mL (Table 1). From another perspective, CSSC showed a significant effect in inhibiting ABTS radical, reaching up to 78.95% at a concentration of 31.25 µg/mL. However, CIA showed the lowest inhibition against the ABTS radical with a value of 48.64% at a concentration of 31.25 µg/mL (Figure 1).

In the 5-lipoxygenase inhibitory activity experiment, CSSC exhibited the highest anti-lipoxygenase activity with an IC_{50}

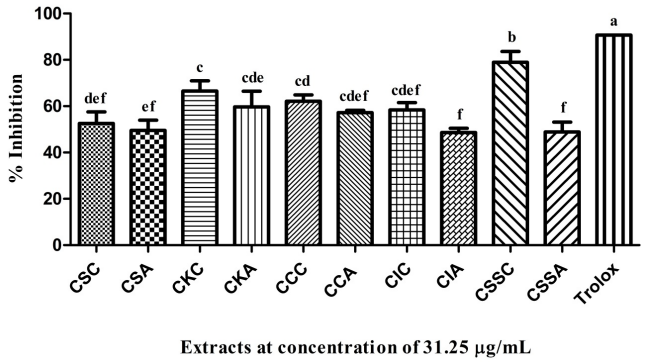

Figure 1. Percentage of Inhibition (%) of ABTS radical by various extracts. The aerial parts except capitula and capitula of *Centaurea cuneifolia, C. iberica, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* coded as CCC, CCA, CIC, CIA, CKC, CKA, CSSC, CSSA, CSC, CSA. The bars represent mean ± standard deviation of triple repetitive analysis. Different and same letters on the error bars indicate statistical significance ($p<0.05$) and non-significance ($p>0.05$), respectively.

value of 122.10 µg/mL, while CKA exhibited the lowest antioxidant activity with an IC_{50} value of 781.30 µg/mL. CSSC presented moderate anti-inflammatory activity when compared to the standard indomethacine (23.11 µg/mL) (Table 1). When CSSC was compared against the 5-lipoxygenase enzyme, it displayed a 96.47% inhibition at concentration of 250 µg/mL. However, CKA showed the lowest inhibition against the lipoxygenase enzyme with a value of 21.64% at concentrations of 250 µg/mL (Figure 2).

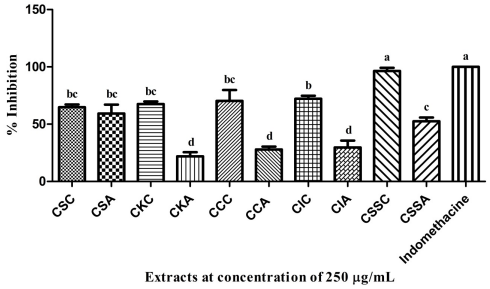

Figure 2. Percentage Inhibition (%) of lipoxygenase enzyme activities of various extracts. The aerial parts except capitula and capitula of *Centaurea cuneifolia, C. iberica, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* coded as CCC, CCA, CIC, CIA, CKC, CKA, CSSC, CSSA, CSC, CSA. The bars represent mean ± standard deviation of triple repetitive analysis. Different and same letters on the error bars indicate statistical significance ($p<0.05$) and non-significance ($p>0.05$), respectively.

The TPC values were obtained from the calibration curve y= 0.127x + 0.075 with R^2= 0.998. Among all the tested extracts, the highest amounts of total phenolic were found in the CSSC (83.41 mg/g). The total phenol contents of other extracts ranged between 35.20 and 79.23 mg GAE per gram extract (Table 1, Figure 3).

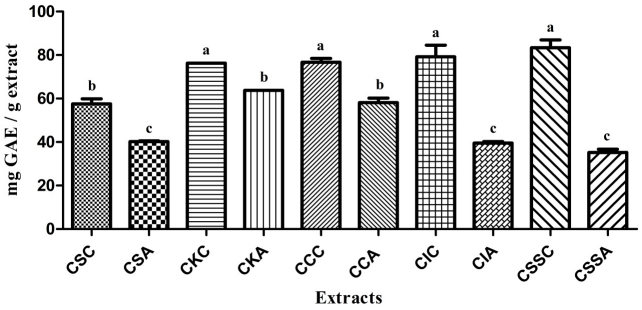

Figure 3. Total phenolic contents of various extracts. The aerial parts except capitula and capitula of *Centaurea cuneifolia, C. iberica, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* coded as CCC, CCA, CIC, CIA, CKC, CKA, CSSC, CSSA, CSC, CSA. The bars represent mean ± standard deviation of triple repetitive analysis. Different and same letters on the error bars indicate statistical significance ($p<0.05$) and non-significance ($p>0.05$), respectively.

DISCUSSION

Oxidative stress and inflammation play an important role in aging and the emergence of chronic diseases.[15] Medicinal plants with antioxidant and anti-inflammatory activity are used in traditional folk medicine for the treatment of chronic disorders caused by oxidative stress and inflammation.[16–18] The antioxidant and anti-inflammatory activity of methanol extracts of aerial parts (except capitula) and capitula of *Centaurea cuneifolia, C. iberica, C. kilaea, C. solstitialis* subsp. *solstitialis* and *C. stenolepis* along with their total phenolic amounts were investigated in the current study.

Among methanol extracts of *Centaurea* species, the CSSC (IC_{50} value: 8.74 µg/mL) had the highest antioxidant activity, followed by CCC (24.42 µg/mL), CKC (25.79 µg/mL), CKA (38.74 µg/mL), CIC (48.20 µg/mL), CCA (48.66 µg/mL), CIA (76.19 µg/mL), CSSA (85.68 µg/mL), CSC (85.74 µg/mL) and CSA (88.95 µg/mL) against the ABTS radical. Necip and colleagues reported that the methanol extract obtained from aerial parts of *C. solstitialis* inhibited the ABTS radical by 21%, 46% and 94% at concentrations of 50, 100 and 250 µg/mL.[19] In our current study, the activity of this species was much better (78.95% inhibition at a concentration of 31.25 µg/mL) than Necip et al. study.[19] This could be due to the difference in the time and place of collection and the part of the plant extracted. In another study, it was reported that C. kilaea (147.2 µg/mL), *C. cuneifolia* (119.5 µg/mL), *C. stenolepis* (129.9 and 146.4 µg/mL) aerial parts chloroform extracts have antioxidant activity against the ABTS radical.[20] Unlike this study, extracts were obtained from the *Centaurea* species using a different solvent (we used methanol) and it revealed that methanol extracts exhibited better antioxidant activity than chloroform extracts.

When comparing the antilipoxygenase activities of the *Centaurea species*, it was observed that the CSSC (IC50 value: 122.10 µg/mL) had the best lipoxygenase inhibitory activity, followed by CIC (169.20 µg/mL), CCC (180.20 µg/mL), CKC

Table 1. Anti-inflammatory/antioxidant activities and total phenolic contents of *Centaurea* extracts.

Extracts / Standards	ABTS radical scavenging activity	5-lipoxygenase inhibitory activity	TPC (mg GAE/g extract)****
	IC_{50} (µg/mL)		
CCC	24.42 ± 1.64^{c}	180.20 ± 8.63^{cd}	76.73 ± 1.78^{a}
CCA	48.66 ± 0.30^{e}	440.50 ± 2.12^{f}	58.16 ± 2.07^{b}
CKC	25.79 ± 0.84^{c}	184.80 ± 2.76^{cd}	76.31 ± 0.00^{a}
CKA	38.74 ± 0.83^{d}	781.30 ± 8.27^{h}	63.79 ± 0.00^{b}
CIC	48.20 ± 1.12^{e}	169.20 ± 3.96^{c}	79.23 ± 5.32^{a}
CIA	76.19 ± 0.62^{f}	513.80 ± 26.23^{g}	39.58 ± 0.59^{c}
CSC	85.74 ± 0.32^{g}	191.00 ± 5.23^{cd}	57.53 ± 2.36^{b}
CSA	88.95± 0.17^{g}	209.20 ± 8.49^{de}	40.21 ± 0.30^{c}
CSSC	8.74 ± 0.23^{b}	122.10 ± 1.49^{b}	83.41 ± 3.54^{a}
CSSA	85.68 ± 1.80^{g}	233.50 ± 8.13^{e}	35.20 ± 1.47^{c}
Trolox	4.41 ± 0.17^{a}		
Indomethacine		23.11 ± 1.77^{a}	

* The aerial parts except capitula and capitula of *Centaurea cuneifolia*, *C. iberica*, *C. kilaea*, *C. solstitialis* subsp. *solstitialis* and *C. stenolepis* coded as CCC, CCA, CIC, CIA, CKC, CKA, CSSC, CSSA, CSC, CSA.

** Each value in the table is the mean of three replicates and was represented as mean±SD.

*** Different and same letter superscripts in the same column show statistical significance ($p<0.05$) and non-significance ($p>0.05$), respectively..

**** GAE: Gallic acid equivalent

(184.40 µg/mL), CSC (191.00 µg/mL), CSA (209.20 µg/mL), CSSA (233.50 µg/mL), CCA (440.50 µg/mL), CIA (513.80 µg/mL) and CKA (781.30 µg/mL) against the 5-lipoxygenase enzyme. There has been no study on the antilipoxygenase activity of methanol extracts of these species until the present study. However, chloroform extracts for two species were studied. Sekerler et al. demonstrated that the *C. kilaea* (110.0 µg/mL), *C. cuneifolia* (129.4 µg/mL), *C. stenolepis* (138.5 and 154.4 µg/mL) aerial parts chloroform extracts had anti-inflammatory activities against the 5-lipoxygenase enzyme.[20] Unlike the current study, extracts were obtained using a solvent with lower polarity (chloroform) and it revealed that these extracts (except CSSC) had better anti-inflammatory activity.

The phenolic contents of crude methanol extracts as gallic acid equivalents were found to be highest in CSSC (83.41 mg/g) followed by CIC (79.23 mg/g), CCC (76.73 mg/g), CKC (76.31 mg/g), CKA (63.79 mg/g), CCA (58.16 mg/g), CSC (57.53 mg/g), CSA (40.21 mg/g), CIA (39.58 mg/g) and CSSA (35.20 mg/g). Alper et al. investigated the total phenolic content of the ethanol extract obtained from the flowers of *C. solstitialis* and found 52.31 mg/g as equivalent to gallic acid in a gram extract.[21] This value was found to be lower than the value in our current study (83.41 mg/g for CSSC). This could be due to the difference in the time and place of collection of the plant. Sekerler et al. reported that the total phenol contents of *C. kilaea, C. cuneifolia* and *C. stenolepis* aerial parts chloroform extracts were found to be 31.57, 55.40, 52.36 and 49.74 mg/g, respectively.[20] Compared to the current study, Sekerler extracts were obtained using a different solvent (chloroform) and the total phenol content of these extracts was found to be lower in general.

The highest total phenolic content with the best antioxidant and anti-inflammatory activity was observed in the methanol extract of *C. solstitialis capitula* (CSSC). Previous studies showed that *C. solstitialis* contains phenolic acids (caffeic acid, chlorogenic acid, cinnamic acid, 2,5-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, ellagic acid, ferulic acid, gallic acid, 4-hydroxybenzoic acid, p-coumaric acid, vanil-

lic acid), and flavonoids (epicatechin, naringin, quercetin, rutin) and sesquiterpene lactones (solstitialin A and acetyl solstitialin).[21,22] Phenolic acids and flavonoids were reported to have antioxidant activity.[23,24] It is suggested that sesquiterpene lactones together with these substance groups have anti-inflammatory activity.[25–27] Thus, phenolic acids, flavonoids and sesquiterpene lactones could be responsible for the antioxidant (but not sesquiterpene lactones) and anti-inflammatory activity of CSSC.

CONCLUSION

These findings indicate that the methanol extract of *C. solstitialis* capitula may be a valuable resource in the discovery of antioxidant and anti-inflammatory molecules.

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ORCID ID of the author

Ali Sen 0000-0002-2144-5741

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