Pak. J. Bot., 55(4): 1419-1427, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-4(28)

DETERMINATION OF SOME PHENOLICS AND FATTY ACID COMPOUNDS FROM AN ENDEMIC *CEPHALARIA* SPECIES IN ANATOLIA ALONG WITH ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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Abstract

In this study, HPLC analyses of total extract prepared with aqueous methanol, butanol fraction, and water fraction of *Cephalaria duzceënsis* N.Aksoy & Göktürk (Caprifoliaceae), which is a local endemic species in Turkey, were conducted and contents of chlorogenic acid were calculated. The total antioxidant capacity, ABTS and DPPH free radical scavenging activity of the total extract were determined to evaluate its antioxidant activity. Assessment of anti-inflammatory activity was done using human red blood cell membrane stabilization and protein denaturation inhibition activity tests *In vitro*, whereas carrageenan-induced hind paw edema test *In vivo*. Moreover, the fatty acid composition inside the fixed oil extracted from *C. duzceënsis* seeds was determined by GC-MS. As the result of GC-MS analysis, linoleic and oleic acids were found to be the predominant fatty acids in the fixed oil. The total phenolic contents of the total extract and butanol fraction were determined as 63.4029 and 95.0131 mg GAE/g dry extract, respectively. The chlorogenic acid amounts of the total extract, butanol, and water fractions were calculated as 3.7494%, 3.5335%, and 1.2354% by HPLC analysis. IC_{50} values of the total extract were calculated as 45.1385 µg/ml against $ABTS^{\bullet+}$ and 28.6407 µg/ml against $DPPH^{\bullet}$ radicals and the total antioxidant capacity was 45.84 mg ascorbic acid equivalent/g dry extract. IC_{50} values of the total extract were calculated as 1.4084 mg/ml for human red blood cell membrane stabilization and 1.8601 mg/ml for protein denaturation inhibition method showing its moderate activity. *In vivo* tests revealed that total extract caused almost as much inhibition on edema as 10 mg/kg diclofenac sodium.

Key words: Anti-inflammatory, Antioxidant, *Cephalaria*, Fatty acid, GC-MS, HPLC

Introduction

The genus *Cephalaria* Schrad. ex Roem. et Schult. has long been regarded as belonging to the Dipsacaceae, whereas according to APG III it is included within the larger family Caprifoliaceae (Dipsacales) and consist of 100 species (Reveal & Chase, 2011). The genus Cephalaria which has 43 taxa in Turkey, which of 24 are an endemic (Göktürk & Sümbül, 2014; Göktürk & Sümbül, 2016; Ranjbar & Ranjbar, 2018; Sarikahya *et al.*, 2019). The members of this genus widely grow especially in the Mediterranean region and the Middle East (Godjevac *et al.*, 2004). *Cephalaria* species contain several bioactive compounds including phenolic acids, flavonoids, iridoids, saponins, and terpenic compounds (Sarikahya *et al.*, 2019; 2018; 2011). The members of this genus widely grow especially in the Mediterranean region and the Middle East (Godjevac *et al.*, 2004). *Cephalaria* species contain several bioactive compounds including phenolic acids, flavonoids, iridoids, saponins, and terpenic compounds (Sarikahya *et al.*, 2019; 2018; 2011). The members of this genus are medicinal plants exhibiting antioxidant, antibacterial, antifungal, and cytotoxic activities (Kirmizigül *et al.*, 1996; Godjevac *et al.*, 2004; Tabatadze *et al.*, 2007). *Cephalaria* species are used for therapeutic purposes, such as hypothermal, alleviative, sedative, and anti-inflammatory in Europe, western and central Asia and Africa. *Cephalaria microcephala* Boiss., *Cephalaria anatolica* Shkhiyan, *Cephalaria tchihatchewii* Boiss. are used in folk medicine to treat wounds and scar in Turkey (Godjevac *et al.*, 2004; Tabatadze *et al.*, 2007; Mbhele *et al.*, 2015; Mükemre *et al.*, 2015). Furthermore, the fixed oil extracted from the seeds has antioxidant properties (Kavak & Baştürk, 2020).

Cephalaria duzceënsis N.Aksoy & Göktürk is a local endemic species that grows naturally in a single geographic area of Duzce in the western part of the Black Sea Region of Turkey. *C. duzceënsis* is distributed singly or in small colonies under *Pinus sylvestris* L. forests and on stony slopes. *C. duzceënsis* plants are perennial, herbaceous, slender and rhizomatous (Aksoy *et al.*, 2007).

The study aimed to evaluate phytochemical analysis as well as the antioxidant and anti-inflammatory potential of *C. duzceënsis* using *In vitro* and *In vivo* approaches. For this purpose, HPLC analyses were conducted and the chlorogenic acid contents of the extracts were detected. The fatty acid composition of the fixed oil obtained from the seeds was also determined by GC-MS. Total antioxidant capacity and free radical scavenging activity were determined to evaluate antioxidant activity. The anti-inflammatory potential was evaluated using two different *In vitro* methods and carrageenan-induced paw edema model *In vivo*. To the best of our knowledge, this is the first study on the phytochemical analysis and the antioxidant and anti-inflammatory effects of *C. duzceënsis* which is growing in Turkey.

Material and Methods

Chemicals: 2,2'-azino bis-(3-ethyl benzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picryl hydrazyl (DPPH), ammonium molybdate, ascorbic acid, butyl hydroxytoluene (BHT), carrageenan, carboxymethyl cellulose (CMC), Folin Ciocalteu reagent, chlorogenic acid, gallic acid and Trolox were obtained from From Sigma-Aldrich (St. Louis, MO, USA). Diclofenac sodium, dimethyl sulfoxide (DMSO), disodium hydrogen phosphate, phosphate buffered saline tablets, sodium carbonate, sulphuric acid, potassium persulphate, sodiumchloride, sodium hydroxide, sodium phosphate, acetonitrile, ethanol, methanol, phosphoric acid, and other solvents were bought from Merck (Darmstadt, Germany).

Plant material: *C. duzceënsis* samples were collected from Düzce during its flowering time middle July to middle August. Aerial parts were dried at 25˚C, avoiding exposure to sunlight. The voucher sample was stored in the Herbarium of Ankara University Faculty of Pharmacy (AEF) (Herbarium no: AEF28917!). The seeds of the plant were also collected along with the aerial parts and dried at 25˚C.

Extraction procedure: The aerial parts of the plant material was dried and powdered. The samples (307.93 g) were extracted using 80% (*v/v*) aqueous methanol in an ultrasonic bath for 1 hour. Then, the material was left for maceration for 24 hours and filtered. The filtrate was concentrated at 40°C under decreased pressure. *C. duzceënsis* total extract (CTE) was obtained. The extraction yield was calculated as 17.84%. The total extract was subjected to liquid-liquid partitioning in a separating funnel with butanol (saturated with water) and water in equal volumes, respectively. This procedure was repeated five times with each solvent. The fractions were concentrated until dryness.

Extraction of fixed oil: Fixed oil was obtained by using *n*-hexane as a solvent by Soxhlet extraction. 10 g of powdered seeds were extracted for 6 hours and *n*-hexane was evaporated at 40°C. The yield of fixed oil was calculated as 9.9%.

Total phenolic content: The extent of entire phenolic compounds in *C. duzceënsis* extracts was evaluated by the Folin-Ciocalteu method based on the procedure of Singleton *et al.*, 1999. The same procedure was conducted for extracts and gallic acid, which was used as a standard, in triplicate. Results were expressed in terms of mg gallic acid equivalent/gram dry extract (GAE/g dry extract).

Chromatographic analyses

GC–MS analysis: A GC-MC Agilent 7890B GC 5977B Mass Selective Detector System and an HP Innowax column (60 m x 0.25 mm x 0.25 μm) were employed for GC-MS analysis. A 70eV electron ionization system and an ion source at 230°C in the scanning range of 35-450 m/z were used to identify the components. Separated components were defined according to Wiley 9-Nist 11 Mass Spectral Database.

The flow rate (helium) of the mobile phase was 0.7 mL/min. The sample was derivatized according to Boron Trifluoride (BF_3) transmethylation method (Metcalfe *et al.*, 1966). Fatty acids converted to methyl esters were injected with hexane (10%, v/v) in 1 μL of the sample by split injection (40:1). The column temperature was initially at 60°C for 10 minutes, then the temperature was increased 4°C per minute up to 220°C. Finally, it was increased 1°C each minute until reached up to 240°C. Temperatures at the injection port and detector were maintained at 250°C.

HPLC analysis: The phytochemical content of the extract was evaluated by HPLC technique (LC 1200, Agilent Technologies, California, USA). The components of the extract were separated by using gradient elution composed of acetonitrile (A) and phosphoric acid solution in water (0.2%) (B) on ACE 5 C18 column (250 mm× 4.6 mm; 5 μm). While the rate of flow kept at 1 mL/min, the column kept at 25˚C during the analysis process. The samples were injected at the volume of 10μL and the chromatograms were evaluated at the wavelength of 330 nm. The composition ratio of the mobile phase was A:B 10:90 (v/v) at the beginning of the analysis, this ratio was linearly changed to 14.5:85.5 in 9 min; and to 16.5:83.5 in the next 8 min. Between 17-40 min, the solvent ratio was changed from the ratio of A:B 16.5:83.5 to 100:0 and the solvent ratio was kept at this ratio for the last 5 min of the analysis.

Determination of chlorogenic acid content: Solutions of chlorogenic acid at five concentrations (at the concentrations of 0.05; 0.1; 0.2; 0.6; 0.8 mg/mL) were prepared for the quantification using HPLC. The equation obtained for calibration was determined with the aid of areas of the peaks obtained for each concentration. Methanol was used to dissolve the extracts at the concentration of 10 mg/mL. Three copies of analyses were conducted for each level of concentration and the sample.

Limit of quantification (LOQ) and limit of detection (LOD) values: Nine times repetitive injections were performed for the verification of LOD and LOQ values.

In vitro biological activity analysis

In vitro antioxidant and anti-inflammatory capacities of the samples were investigated.

Antioxidant activity: $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radical scavenging activity and total antioxidant capacity of the samples were investigated to determine their antioxidant potentials.

$ABTS^{\bullet+}$ free radical scavenging activity: ABTS free radical scavenging activity of the samples was determined using the method of Re *et al.*, 1999 with minor modifications. Trolox was used as a reference compound and the analyses were performed in triplicates. The inhibition percentage was calculated for each concentration and sample, and then the half-maximal inhibitory concentration (IC_{50}) was computed.

DPPH$^{\bullet}$ free radical scavenging activity: DPPH free radical scavenging activity of the samples was evaluated according to the method of Blois**,** 1958. BHT was used as a reference compound and the experiments were performed in triplicates. The inhibition percentage was calculated for each concentration and sample, and then IC_{50} was computed.

Total antioxidant capacity: Phosphomolybdenum method was used in order to examine the total antioxidant capacity of the samples (Prieto *et al*., 1999). Ascorbic acid was used as a reference compound and the experiments were carried out in triplicates. The results were given as mg of ascorbic acid equivalent (AAE) per gram dry extract.

Anti-inflammatory activity: Human red blood cell membrane stabilization and protein denaturation inhibitory activity of the samples were evaluated to determine their anti-inflammatory activity.

Human red blood cell membrane stabilization activity: The human red blood cell membrane stabilization capacity of the samples was examined with the methods of Shinde *et al*., 1999 and Gunathilake *et al*., 2018. The ethical committee approval was obtained from the Human Research Ethics Committee of the Faculty of Medicine of Ankara University (14.05.2020 / I5-273-20). Diclofenac sodium was used as a reference compound and the experiments were conducted in triplicates. The protection percentage of the cell membrane was calculated for each concentration and sample, and then IC_{50} was computed.

Protein denaturation inhibition activity: The inhibitory potential of samples on protein denaturation was examined using the methods of Ruiz-Ruiz *et al*., 2017 and Janarny *et al*., 2021. Diclofenac sodium was used as a reference compound and the experiments were conducted in triplicate. The inhibition percentage of protein denaturation was calculated for each concentration and sample, and then IC_{50} was computed.

In vivo biological activity analysis

The research was conducted in accordance with internationally accepted principles for laboratory animal use and care contained in the European Community directives (EEC Directive 1986; 86/609/EEC).

Animals: Female Wistar albino rats which were used in the study (150-200 g) were obtained from a private company, Kobay DHL A.Ş. (Ankara, Turkey). The diet of the animals was standard pellet and water according to pleasure and kept in animal room conditions for 3 days. Each group included 6 animals. The animals were preserved according to the directions of the Guide for the Care and Use of Laboratory Animals, and the experiment was approved by the Experimental Animal Ethics Committee of Kobay DHL A.Ş. (25.08.2020/501).

Carrageenan-induced hind paw edema: The method of Kasahara *et al.*, 1985 was slightly modified for the evaluation of the effects of the samples on carrageenan-induced hind paw edema (Öz *et al.*, 2017). The volumes of each paw were measured every 60 min for 6 hours and at the 24th hour following the induction of inflammation. A water plethysmometer was used for the measurements in accordance with the instructions of the manufacturer (Ugo Basil, Italy). The experiment was performed in 4 groups of animals namely; group 1 (control): 0.5% CMC group; group 2 (positive control): 10 mg/kg body weight (bw) diclofenac sodium; group 3 (low-dose): 100 mg/kg bw CTE; group 4 (high-dose): 200 mg/kg bw CTE.

Statistical analysis

Statistical analysis was done with SPSS v25.0 statistical software. The experiments were performed at least three times and the data were given as the mean ± standard error (SE). One-way analysis of variance (ANOVA) followed by a post-hoc least significant difference (LSD) test was used to compare the data in each group. A p-value less than 0.05 is typically considered to be statistically significant.

Results

Phytochemical analysis

Total phenolic content, GC-MS and HPLC analysis were performed as phytochemical analysis.

Total phenolic content: The highest total phenolic content was observed in butanol fraction followed by CTE. The results are given in Table 1 [y = 1.4605x + 0.0658 (r2 = 0.9992)].

GC-MS analysis: Relative contents of the components found in the fixed oil of *C. duzceënsis* was evaluated (Table 2).

HPLC analysis: HPLC analysis revealed that CTE contains three major components, one of which is determined to be chlorogenic acid. The chromatograms of CTE, water, and butanol fractions are given along with the chromatogram and UV spectrum of chlorogenic acid (Fig. 1). Likewise chlorogenic acid, the other two major compounds detected in the chromatogram, were caffeoylquinic acid derivatives with regard to their UV spectrums. The chlorogenic acid contents of the CTE, water, and butanol fraction were determined by the external standard method (Table 3). LOD and LOQ values are determined as 1.778×10^{-3} mg/mL and 5.9250×10^{-3} mg/mL, respectively.

In vitro analysis

Antioxidant activity: The scavenging effects of the samples on ABTS and DPPH free radicals and total antioxidant capacity were evaluated to determine their antioxidant activity. Trolox and BHT were used as reference compounds in ABTS and DPPH assays, respectively (Table 4). Both CTE and Trolox possessed a significant inhibition on ABTS compared to the solvent control ($p<0.05$). Trolox displayed better ABTS free radical scavenging activity than CTE ($p<0.05$), therefore CTE is considered a moderate inhibitor of ABTS compared to

Trolox. The inhibition profiles of both samples were concentration-dependent. The relative antioxidant ability of CTE to quench DPPH free radical was provided and compared with BHT. Both CTE and BHT exhibited significant inhibition on DPPH than the solvent control ($p<0.05$). These inhibition profiles were in a dose-depended manner. Since BHT noted a stronger scavenging effect than CTE ($p<0.05$), the extract can be considered as a moderate inhibitor of DPPH free radical. The total antioxidant capacity of the extract was evaluated and calculated as mg AAE per g dry extract. The regression equation obtained for ascorbic acid was $y=33.066x-0.0502$ ($r^2=0.9998$). The total antioxidant capacity of CTE was 45.8437±0.0012 mg AAE/g dry extract.

Table 1. Total phenolic content of *C. duzceënsis*.

Plant material	mg GAE /g extract
CTE	63.4029 ± 1.0270
Butanol fraction	95.0131 ± 1.7231
Water fraction	-

The results were expressed as mean± SE of three independent experiments

Table 2. Relative contents of the components found in the fixed oil of *C. duzceënsis* by GC-MS analysis

Fatty acid composition	Relative content (%)
Lauric acid (C12:0)	0.6
Myristic acid (C14:0)	14.8
Palmitic acid (C16:0)	8.3
Stearic acid (C18:0)	2
Oleic acid (C18:1); ω-9	24.8
Elaidic acid (C18:1); ω-9	0.6
Linoleic acid (C18:2); ω-6	45.6
13-Hydroxy-12-methoxy-9-octadeconeic acid; ω-9	1.1
12-Hydroxy-12-methoxy-9-octadeconeic acid; ω-9	2.1
Total	**99.9**

Table 3. The chlorogenic acid contents of the extracts.

Plant material	Chlorogenic acid content %
CTE	3.7494 ± 0.0265
Butanol fraction	3.5335 ± 0.0067
Water fraction	1.2354 ± 1.6978

The results were expressed as mean ± SE

Anti-inflammatory activity: *In vitro* anti-inflammatory activity of the samples was assessed by using human red blood cell stabilization and protein denaturation inhibition activity methods. Table 5 displays the anti-inflammatory effects of the samples. Diclofenac sodium and CTE exhibited stronger human red blood cell membrane protection effects than the negative control ($p<0.05$). Dose-dependent stabilization of human red blood cell membrane was observed in both groups. CTE possessed moderate membrane stabilization activity compared to the reference compound. Both diclofenac sodium and CTE exhibited better protein denaturation inhibition activity than the solvent control ($p<0.05$). Diclofenac sodium, which served as a positive control, displayed stronger inhibition on protein denaturation than CTE ($p<0.05$). Therefore, CTE is considered to denote a moderate inhibitor of protein denaturation.

In vivo analysis

In vivo anti-inflammatory activity of the samples was evaluated using a carrageenan-induced rat paw edema assay. The inhibition percentage values in each hour from 0 to 24 are presented in Table 6. The inhibition rate in all groups from the 2nd to 6th hour after the induction of inflammation was statistically significant compared to the control, ($p<0.05$). The inhibition was decreased in all groups and none of them was statistically significant at the 24th hour. The high-dose CTE group (200 mg/kg bw) produced a higher reduction in the volume of the paw compared to the low-dose CTE group (100 mg/kg bw). The highest inhibition rate was observed at 3rd hour in the high-dose CTE group (200 mg/kg bw). The low-dose CTE (100 mg/kg bw) decreased paw edema with the values of 11.30, 19.83 and 18.02%, whereas diclofenac sodium (10 mg/kg) reduced by 12.96, 20.15 and 19.26% at the 4th, 5th and 6th hours, respectively. The administration of 100 mg/kg bw CTE appears to cause almost as much inhibition on edema as 10 mg/kg bw diclofenac sodium.

Table 4. Antioxidant activity of the samples.

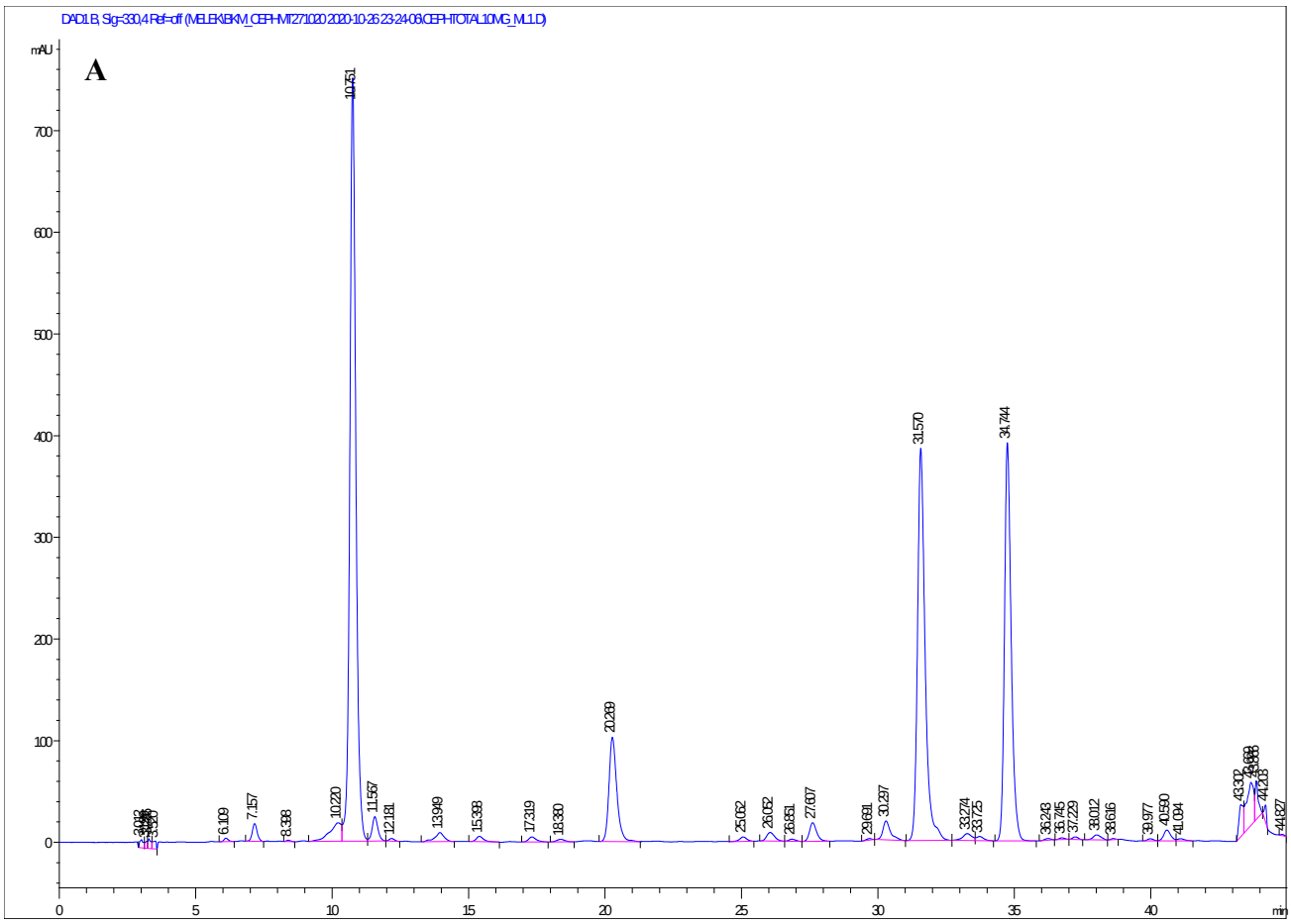
Sample	IC_{50} (µg/ml)		mg AAE/g dry extract
	ABTS assay	DPPH assay	Total antioxidant capacity
CTE	45.1385 ± 1.6708*	28.6407 ± 0.0807*	45.8437 ± 0.0012*
Trolox	8.6610 ± 0.1999*	-	-
BHT	-	23.1740 ± 0.3433*	-
Control	NE	NE	NE

(*) $p<0.05$; compared to the control (One way-ANOVA, post-hoc LSD test). The results were expressed as mean±SE of three independent experiments. AAE: ascorbic acid equivalent, NE: no effect

Table 5. *In vitro* anti-inflammatory activity of the samples.

Sample	IC_{50} (mg/ml)	
	Human red blood cell membrane stabilization activity	Protein denaturation inhibition activity
CTE	1.4084 ± 0.0415*	1.8601 ± 0.0637*
Diclofenac sodium	1.1325 ± 0.0001*	0.2822 ± 0.0059*
Control	NE	NE

(*) $p<0.05$; compared to the control (One way-ANOVA, post-hoc LSD test). The results was expressed as mean ± SE of three independent experiments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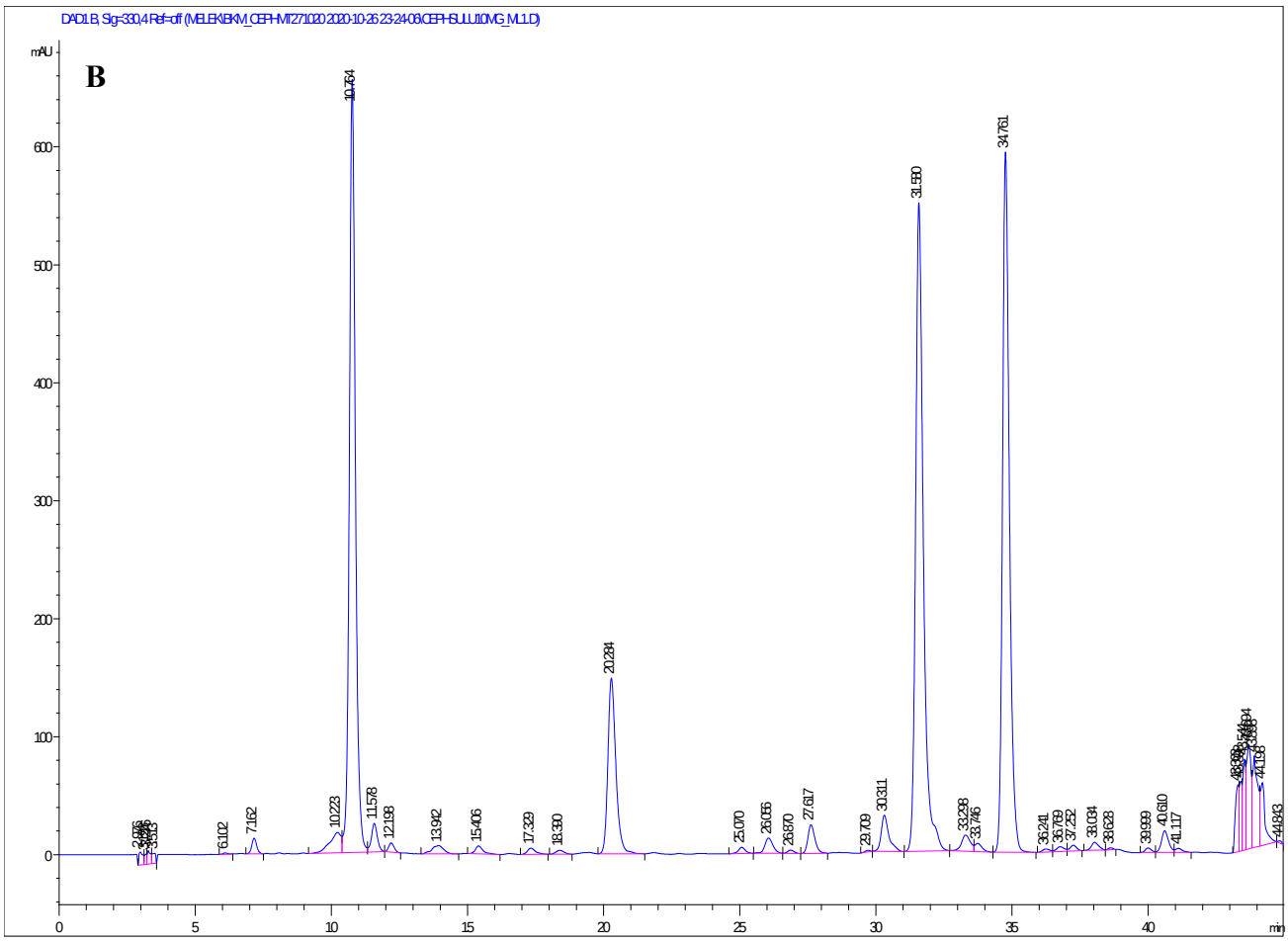
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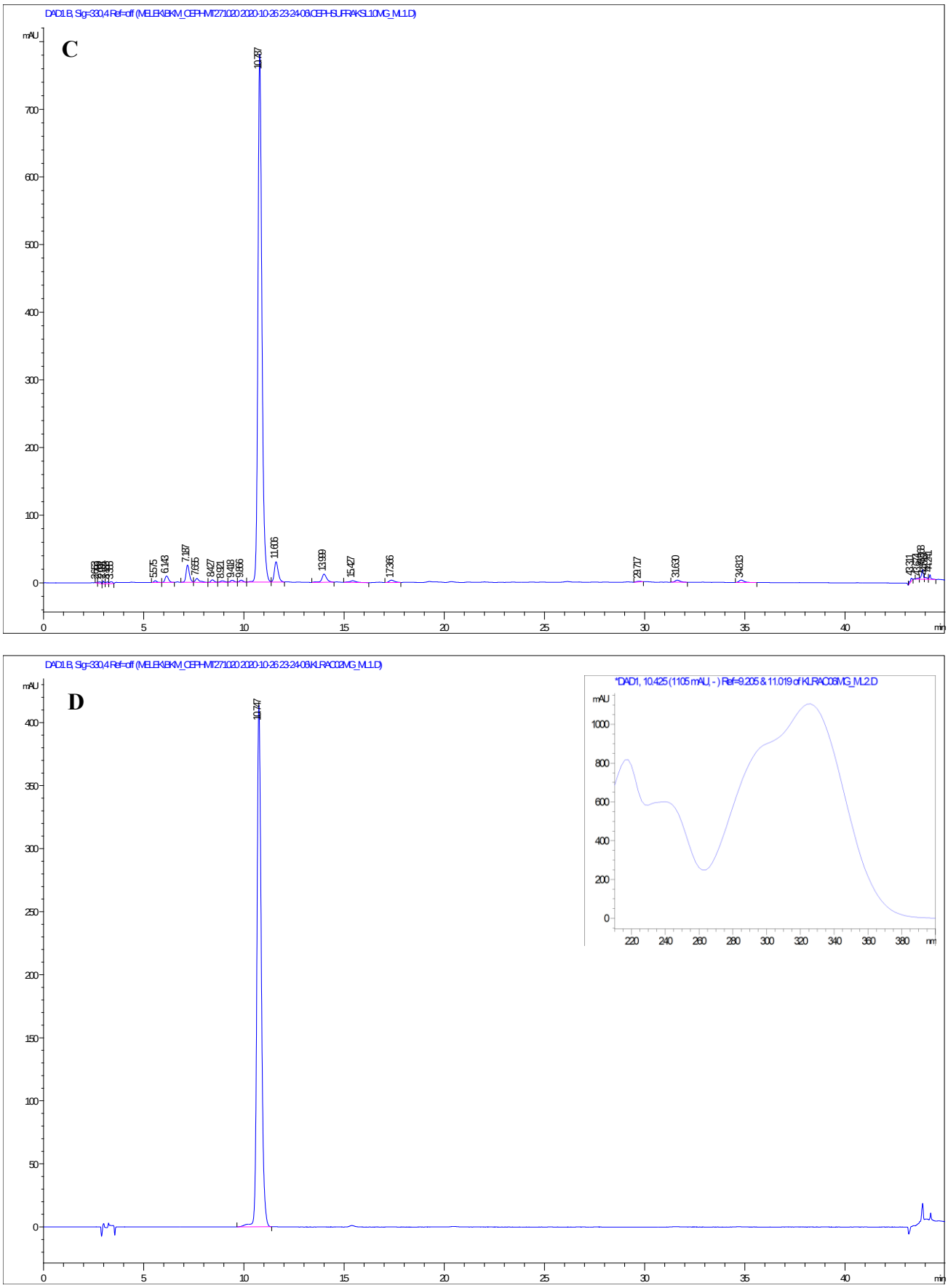

Fig. 1. The chromatograms of *C. duzceënsis* extracts and chlorogenic acid (A: Total extract, B: Butanol Fraction C: Water Fraction, D: Chromatogram and UV spectrum of chlorogenic acid).

Table 6. Anti-inflammatory effect of the samples against carragenan-induced paw edema in rats.

Group	Percentage inhibition							
	V0	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V24**
CTE (100 mg/kg)	NE	10.35±2.74*	3.16±5.49	12.42±4.42*	11.30±3.35*	19.83±2.45*	18.02±2.91*	6.92±4.67
CTE (200 mg/kg)	7.03±4.30	13.51±3.78*	12.53±4.01*	24.65±3.13*	17.17±1.53*	21.03±1.34*	20.39±1.58*	7.20±2.03
Diclofenac sodium (10 mg/kg)	NE	6.32±3.45	10.02±3.53*	16.57±1.43*	12.96±2.28*	20.15±1.82*	19.26±1.30*	3.25±2.32
Control	NE	NE	NE	NE	NE	NE	NE	NE

*p<0.05, Statistically significant compared to control (One way-ANOVA, post-hoc LSD test). The results were expressed as mean±SE. NE: No effect

Discussion

Humans are exposed to free radicals either by exogenous factors or by endogenous reactions which produced these radicals as byproducts. These reactive oxygen species can impair biological systems leading to aging, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, diabetes, and even cancer. Natural and synthetic compounds were required to counteract detrimental effects of free radicals and oxidative stress. Several natural products with antioxidant properties reduce inflammation and exhibit anti-inflammatory activities (Arulselvan *et al.*, 2016). Phenolic compounds, which were prevalent structures found in natural products, had a wide range of bioactivities including antioxidant, anti-inflammatory, immunomodulatory, anticancer, antimicrobial, cardioprotective, and neuroprotective effects. The antioxidant properties of phenolic compounds arise from their capacity to reduce free radicals and inhibit enzymes involved in the generation of reactive oxygen species. Besides, various studies had informed that the anti-inflammatory activity of these compounds correlated with their antioxidant activity (Arulselvan *et al.*, 2016; Ferreira *et al.*, 2017). Chlorogenic acid, an active dietary polyphenol, had been linked to many therapeutic activities with its immunomodulatory, antioxidant, and anti-inflammatory potential (Wang *et al.*, 2022; Sarıaltın *et al*., 2023).

Cephalaria species exhibited several therapeutic effects due to their rich phytochemical content (Mbhele *et al*., 2015; Mükemre *et al.*, 2015). Considering these characteristics, we studied the phytochemical composition along with the antioxidant and anti-inflammatory potential of *C. duzceënsis*, an endemic plant in Turkey, with *In vitro* and *In vivo* methods. As the result of the current study, the total extract, butanol, and water fractions of *C. duzceënsis* were shown to contain significant amounts of phenolic compounds. HPLC analyses confirmed these results and the chlorogenic acid levels of the total extract, butanol, and water fractions were determined as almost 3.75%, 3.53%, and 1.24%, respectively. GC-MS analysis was also performed on fixed oil obtained from the seeds of *C. duzceënsis*. The results indicated that the predominant fatty acids were oleic and linoleic acids with relative content values of 24.8% and 45.6%, respectively. The total amount of unsaturated fatty acids was observed to be 74.2%; 45.6% of these were omega-6 and 28.6% were omega-9.

Kırmızıgül *et al.*, 2012 examined the fixed oil contents of eight *Cephalaria* species growing in the southwest of Turkey using GC-FID and GC-MS techniques. The findings revealed that the amounts of unsaturated fatty acids were higher than the saturated ones and the main constituents were α-linoleic, linoleic, and oleic acids in these samples. Myristic and palmitic acids were detected as major ones among saturated fatty acids (Kırmızıgül *et al.*, 2012). The fatty acid compositions of the hexane extracts of 10 *Cephalaria* species were investigated and the predominant fatty acids were found to be oleic, linoleic, and palmitic acids with the values of 10.28-31.65%, 17.81–37.67%, and 10.54–23.81%, respectively. Total phenolic contents of the hexane extracts were also determined in the same study as well as *In vitro* antioxidant capacity and a correlation between phenolic content and antioxidant activity was observed (Sarikahya *et al.*, 2015). Another study of Sarikahya *et al.*, 2019 reported that salicylic acid and cafeic acid were major phenolic acids in 19 *Cephalaria* species growing in Turkey.

The DPPH and ABTS free radical scavenging assays were great tools to examine the antioxidant activity of hydrogen donating and chain-breaking antioxidants. Therefore, the ability of *C. duzceënsis* to scavenge these free radicals was evaluated within the scope of our study. Our results revealed that *C. duzceënsis* is a remarkable free radical scavenger and also had considerable total antioxidant capacity. Other members of the *Cephalaria* genus had also been reported to display antioxidant effects. Godjevac *et al.*, 2004 issued that *C. pastricensis*, possessed antiradical effects on DPPH and the isolated flavonoid compounds exhibited significant antioxidant potential. The total phenolic contents of *C. syriaca* were calculated between 4339 -11907 mg GAE/kg dry seeds, while the results of the ABTS and DDPH assays ranged from 0.0-41.8 mmol Trolox/g dry weight (dw) and 18.8-67.3% inhibition, respectively (Kavak & Baştürk, 2020). Wojdyło *et al.*, 2007 evaluated the relationships between phenolic content and antioxidant capacity of 32 *Cephalaria* species. The antioxidant capacity was measured by ABTS, ferric-reducing antioxidant power (FRAP), and DPPH assays, revealing the antioxidant capacity ranges of 1.76-346, 7.34-2021, and 13.8-2133 μmol Trolox/100 g dw, respectively. Cai *et al.*, 2004 screened several plants (n=112), which were employed in traditional Chinese medicine, regarding their antioxidant capacity and total phenolic contents. The antioxidant capacity, as determined by the ABTS method, ranged from 43.9 to 17.674 mol Trolox/100 g dw, and the total phenol levels were calculated to be between 0.19 and 50.20 g GAE/100 g dw. The findings of this study suggested that the plants with higher phenolic content had better antioxidant activity.

Nonsteroidal anti-inflammatory medicines were used to shield cell membranes from the damaging effects of inflammation since increased inflammation could cause severe damage to these membranes (Anosike *et al*., 2012).

Additionally, protein denaturation could cause inflammation, which can lead to a number of illnesses including cancer, cardiovascular disease, and rheumatoid arthritis (Sangeetha & Vidhya, 2016; Ruiz-Ruiz *et al*., 2017). Increased inflammation can cause severe damage to cell membranes, thus nonsteroidal anti-inflammatory drugs were used to shield these membranes against these harmful effects (Anosike *et al.*, 2012). Moreover, protein denaturation can provoke inflammation resulting in several diseases such as rheumatoid arthritis, cardiovascular diseases, and cancer (Sangeetha & Vidhya, 2016; Ruiz-Ruiz *et al*., 2017). Therefore, human red blood cell membrane stabilization and protein denaturation inhibition capacity of the total extract was determined to evaluate the anti-inflammatory potential *In vitro*. The total extract effectively diminished the inflammatory process compared to the negative control in both methods. However, the efficacy was lower than diclofenac sodium, which was used as a reference compound, at the same concentration. Furthermore, carrageenan-induced paw edema test, which was a well-known acute inflammation model, was conducted for the determination of anti-inflammatory activity *In vivo*. Inflammation generated by carrageenan is a biphasic process. Inflammation arises from the agents such as histamine and serotonin in the first phase (90-180 min), and then from arachidonic acid metabolites in the second phase (270-360 min) (Deliorman *et al.*, 2007). The highest inhibition of the edema was observed in the treatment with 200 mg/kg b.w. at all time intervals. The total extract possessed the maximum activity at 180th min at 200 mg/kg b.w. In general, 100 mg/kg b.w. extract treatment was as effective as 10 mg/kg b.w. diclofenac sodium. Our results stated that the total extract possessed moderate anti-inflammatory potential in comparison with the reference drugs in both *In vivo* and *In vitro* anti-inflammatory activity assays.

In conclusion, the outcomes of this study suggested that *C. duzceënsis* could be a promising source with its high antioxidant and moderate anti-inflammatory potential. This bioactive potential is assumed to be significantly influenced by the high phenolic content. Moreover, quite high levels of unsaturated fatty acids in the fixed oil obtained from the seed indicate that the antioxidant capacity of not only the total extract but also the oil will be high. Further studies are needed to explore the other phytochemical components and isolate drug-candidate molecules.

Acknowledgements

We wish to thank Anadolu University, Medicinal Plants, Drugs and Scientific Research Center (AUBIBAM) for technical support in analyzing fixed oil of *C. duzceënsis* with GC-MS.

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(Received for publication 17 April 2022)