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**Original Research Article**

# Phytochemical profile and some biological activities of three Centaurea species from Turkey

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## Abstract

***Purpose:*** *To characterise the phytochemical profile of whole plants of Centaurea balsamita, C. depressa and C. lycopifolia with LC-ESI-MS/MS, and as well as their antioxidant, anticholinesterase and antimicrobial activities.*

***Methods:*** *Organic and aqueous extracts of the three Centaurea species were evaluated for DPPH free radical, ABTS cation radical scavenging and cupric reducing antioxidant capacity (CUPRAC). Acetyl- and butyryl-cholinesterase enzyme inhibition abilities of the extracts using petroleum ether, acetone, methanol and water were studied to determine anticholinesterase activity, while antimicrobial activity was determined by disc diffusion method using appropriate antimicrobial standards and organisms. The phytochemical components of the methanol extracts were assessed by LC-MS/MS.*

***Results:*** *The methanol extract of C. balsamita exhibited much higher DPPH free and ABTS cation radicals scavenging activities (with $IC_{50}$ of 62.65 ± 0.97 and 24.21 ± 0.70 mg/ml, respectively) than the other extracts. The petroleum ether extracts of the plant species exhibited moderate inhibitory activity against butyrylcholinesterase enzymes while the acetone extract of C. balsamita showed good antifungal activity against Candida albicans. Quinic acid (17513 ± 813 µg/g, 63874 ± 3066 µg/g and 108234 ± 5195 µg/g) was the major compound found in the methanol extracts of C. balsamita, C. depressa and C. Lycopifolia, respectively.*

***Conclusion:*** *These results indicate quinic acid is the major compound in the three plant species and that Centaurea balsamita has significant antioxidant, anticholinesterase and antimicrobial properties. Further studies to identify the compounds in the extracts responsible for the activities are required.*

***Keywords:*** *Centaurea, LC-ESI-MS/MS, Anticholinesterase, Antioxidant, Antimicrobial*

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## INTRODUCTION

The genus *Centaurea* L. belongs to Asteraceae family which comprises of more than 500-600 species that are widespread all over the world, particularly around the Mediterranean and western Asia [1]. In Anatolia, the genus comprises about 207 taxa including 134 endemic species classified in 4 sections with endemism 64 % [1]. *Centaurea* species are often called zerdali dikeni, timur dikeni and peygamber çiçegi in Anatolia [2]. In Turkish traditional medicine, these

species are widely used as expectorant, antidiabetic, antipyretic and antidiarrhoeal [2]. *Centaurea* species are known to have various biological activities such as antimicrobial [4], antifungal [5], antiinflammatory [6], antiulcerogenic [7], antioxidant [8], antiplasmoidal [9], antiprotozoal [4], cytotoxic [9,10] and anticancer [10].

In previous studies, sesquiterpene lactones, flavonoids and phenolic compounds have been isolated from the plants [3]. These isolates include 10 flavonoids (myricetin, fisetin, quercetin, naringenin, hesperetin, luteolin, kaempferol, apigenin, rhamnetin, chrysin), 3 flavonoid glycosides (rutin, hesperidin, hyperoside), 9 phenolic acids (gallic, chlorogenic, protocatechuic, tannic, tr-caffeic, p-coumaric, rosmarinic, 4-OH benzoic and salycylic acids), one phenolic aldehyde (vanillin), one coumarin and other 3 organic acids (quinic, malic and tr-aconitic acids). Some of the compounds obtained from other *Centaurea* species include quercetin from *C. omphalotricha* [11], luteolin and apigenin and kaempferol from *C. urvillei* subsp. *urvillei* [12], rutin and chlorogenic acid from *C. calolepis* [13], protocatechuic acid from *C. isaurica* [14], chlorogenic acid from *C. cadmea* [15], and *C. isaurica* [14], vanillin from *C. sadleriana* [16]. To the best of our knowledge, none of these earlier studies used LC-MS/MS in determination of phytochemical compositions of the plant species.

Therefore the purpose of this study was to determine the chemical profiles of *Centaurea* species using liquid chromatography coupled to tandem electrospray mass spectrometry and as well as the biological activities of the plants.

## EXPERIMENTAL

### Chemicals

Butylated hydrox-ytoluene (BHT) and 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Merck (Darmstadt, Germany). Malic acid, quercetin, protocatechuic acid, chrysin, rutin, hesperetin, naringenin, rosmarinic acid, vanillin, p-coumaric acid,caffeic acid, chlorogenic acid, hyperoside, myricetin, coumarin, kaempferol, formicacid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5,5-dithiobis-(2-nitro benzoic acid) (DTNB), copper (II) chloride dihydrate ($CuCl_2.2H_2O$), neocuproine(2,9-dimethyl-1,10-phenanthroline), EDTA (Ethylenedi-aminetetraacetic acid), acetylcholinesterase (AChE: fromelectric eel) (Type-VI-S, EC 3.1.1.7, 425.84 U/mg), and butyryl-cholinesterase (BChE: from horse serum) (EC 3.1.1.8, 11.4 U/mg) were obtained from Sigma (Steinheim, Germany). Quinic acid, tr-aconitic acid, 4-hydroxybenzoic acid, fisetin, α-tocopherol and acetylthiocholine iodide were from Aldrich (Steinheim, Germany). Gallic acid, tannic acid, salicylic acid, and galanthamine hydrobromide were purchased from Sigma–Aldrich (Steinheim, Germany). Folin Ciocalteu Phenol reagent was from Applichem (Darmstadt, Germany) while hesperidin, luteolin, apigenin, rhamnetin and butyrylthiocholineiodide were from Fluka (Steinheim, Germany). All solvents were of LC-MS and analytical grade.

### Plant material

The whole plants of *Centaurea balsamita* Lam., *Centaurea depressa* Bieb. and *Centaurea lycopifolia* Boiss et Kotschy were collected from southeastern Turkey (Diyarbakır, Malatya and Maraş, respectively) in July 2012 by Dr A Ertaş, and identified by Dr Y Yeşil. These specimens were stored at the Herbarium of Istanbul University (ISTE 97140, ISTE 97664 and ISTE 97138).

### Preparation of plant extracts for LC-ESI-MS/MS

The plants were dried, powdered and 10 g of each was extracted with MeOH for 24 h at room temperature. Resultant extracte were filtered and evaporated under vacuum. Dry filtrate was diluted to 250 mg/L and passed through the microfiber filter (0.2 µm) for LC-ESI MS/MS.

### Preparation of plant extracts for biological activities

Whole plant materials were dried, powdered and 100 g of each was sequentially macerated three times with petroleum ether, acetone, methanol and water (250 mL) for 24 h at room temperature. After filtration, the solvents were evaporated to obtain the crude extracts. The yields of the petroleum ether extracts of the three plants studied were obtained as: CBP (*Centaurea balsamita* petroleum ether extract) 0.7 %, CDP (*Centaurea depressa* petroleum ether extract) 0.6 %, CLP (*Centaurea lycopifolia* petroleum ether extract) 0.8 %; the acetone extracts as CBA (*Centaurea balsamita* acetone extract) 2.1 %, CDA (*Centaurea depressa* acetone extract) 1.3 %, CLA (*Centaurea lycopifolia* acetone extract) 2.6 %, the methanol extracts as CBM (*Centaurea balsamita* methanol extract) 7.2 %, CDM (*Centaurea depressa* methanol extract) 5.2 %, CLM (*Centaurea lycopifolia* methanol extract) 8.5 %, and the water extracts as CBW (*Centaurea balsamita* water extract) 2.3 %, CDW (*Centaurea*

*depressa* water extract) 1.8 %, CLW (*Centaurea lycopifolia* water extract) 3.1% (w/w).

**Phenolic compound identification and quantification**

LC-MS/MS analysis of the phenolic compounds were performed using a Shimadzu Nexera model UHPLC instrument coupled to a tandem MS instrument. The liquid chromatograph was equipped with LC-30AD binary pumps, DGU-20A3R degasser, CTO-10ASvp column oven and SIL-30AC autosampler. Chromatographic separation of plant extract samples was performed on a C18 reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3µm) analytical column with the column temperature fixed at 40 $^{o}$C. The elution gradient consisted of mobile phase (A) water (5mM ammonium formate and 0.1 % formic acid) and (B) methanol (5mM ammonium formate and 0.1 % formic acid). Gradient elution using the proportions of solvent B of 40 %, 90 %, 90 %, 40 % and 40 % at 0, 20, 23.99, 24 and 29 min, respectively was applied. The solvent flow rate was maintained at 0.5 mL/min and injection volume was 4 µL. MS detection was performed using Shimadzu LCMS 8040 model triple quadrupole mass spectrometer equipped with an ESI source operating in both positive and negative ionization mode (Shimadzu, Kyoto, Japan). LC-ESI-MS/MS data were collected and processed using LabSolutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analytes: the assay of phenolic compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation.

**Optimization of LC-ESI-MS/MS method**

Subsequent to several combinations of trials, a gradient of methanol (5 mM ammonium formate and 0.1 % formic acid) and water (5 mM ammonium formate and 0.1 % formic acid) system was concluded to be the best mobile phase solution. ESI source was chosen instead of APCI (Atmospheric Pressure Chemical Ionization) and APPI (Atmospheric Pressure Photoionization) sources as the phenolic compounds were small and relatively polar molecules. Tandem mass spectrometry was used for the current study since this system is commonly used for its fragmented ion stability [18]. The working conditions were determined as interface temperature of 350 $^{o}$C, DL temperature of 250 $^{o}$C, heat block temperature of 400 $^{o}$C, nebulizing gas flow using Nitrogen, 3 L/min and drying gas flow (nitrogen) of 15 L/min.

The linearity of the phenolic standards was affirmed in the range of: 0.025 to 10 mg/L (Table 1). Regression coefficient of each calibration graph was found to be higher than 0.99. Limit of detection (LOD) and limit of quantitation (LOQ) of the method reported in this study were dependent on the calibration curve established from six measurements. LOD and LOQ were determined using the equations, 3S/N and 10S/N, respectively where S/N is the signal (S) to noise (N) ratio) (Table 1). For different compounds, LOD ranged from 0.05 to 25.8 µg/L and LOQ ranged from 0.17 to 85.9 µg/L (Table 1). Furthermore, the recovery of the phenolic compounds standards ranged from 96.9 % to 106.2 %.

**Determination of total phenolic and flavonoid contents**

Total phenolic and flavonoid amounts in the crude methanol extracts were determined as previously described and expressed as pyrocatechol and quercetin equivalents using the following equations [19]:

Absorbance = 0.0251 pyrocatechol (µg) + 0.0445 ($R^2$ = 0.9945)

Absorbance = 0.0301 quercetin (µg) + 0.0553 ($R^2$ = 0.9984)

**Antioxidant activity of the extracts**

We used the DPPH free radical, ABTS cation radical scavenging activity and cupric reducing antioxidant capacity (CUPRAC) methods to determine the antioxidant activity [19].

**Anticholinesterase activity of the extracts**

A spectrophotometric method developed by Ellman *et al* [19] was used to test the acetyl- and butyryl-cholinesterase inhibitory activities.

**Antimicrobial activity of the extracts**

Five different microorganisms including Gram positive bacteria (*Streptococcus pyogenes* ATCC19615 and *Staphylococcus aureus* ATCC 25923), Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922) and yeast (*Candida albicans* ATCC10231) which were purchased from Refik Saydam Sanitation Center (Turkey) were used for detecting the antimicrobial activity of the extracts. The disc diffusion method was employed for this purpose and the minimum inhibitory concentrations (MICs) were determined

by the broth macrodilution method according to NCCLS [20]. Ampicillin and fluconazole were used as positive controls for bacteria and yeast, respectively.

### Statistical analysis

The results of the antioxidant and anticholinesterase activity assays were expressed as means ± SD. Unpaired Student's t-test and ANOVA were used for data comparison. A 2-tailed *p* values less than 0.05 was considered to be significant.

## RESULTS

### Quantitative analysis of phenolic compounds

Methanolic extracts of *C. balsamita*, *C. depressa* and *C. lycopifolia* were found to contain. 27 compounds including 10 flavonoids, 3 flavonoid glycosides, 9 phenolic acids, 1 phenolic aldehyde, 1 coumarin and 3 other organic acids (Figure 1A). These compounds were monitored by the transition from the specific deprotonated molecular ions [M-$H^+$] to the corresponding fragment ions [M-$H^+$-X]. The molecular ions and fragments observed in MS/MS, related collision energies for these fragments and the quantified results for the three *Centaurea* species are shown in Table 1.

Fisetin and kaempferol were not detected by this method from *C. balsamita*. Similarly, fisetin and hesperetin were not detected from *C. depressa*. Myricetin, fisetin, hesperetin, quercetin, naringenin and kaempferol were equally detected from the extracts of *C. lycopifolia*. However, the quamtities of quinic acid found in *C. balsamita* (17513.16 ± 813.48 µg/g), *C. depressa* (63874.33 ± 3065.97 µg/g) and *C. lycopifolia* (108233.80 ± 5195.22 µg/g) were more than the quantities of other compounds. About 10 % of the methanol extract of *C. lycopifolia* was quinic acid. *C. balsamita* and *C. lycopifolia* were found to be rich in chlorogenic acid (10770.70 ± 365.66 µg/g and 15782.64 ± 463.45 µg/g, respectıvely) while *C. depressa* was found to have high amount of malic acid (22587.95 ± 1524.52 µg/g) (Table 1, Figure 1B-D).

### Antioxidant activity assays and total phenolic and flavonoid content

The methanol extract of *C. balsamita* had the highest amount of total phenolic while the water extract of the same plant species had the highest content of total flavonoid content (Table 2).

As shown in Table 3, CBM, CDM and CLM extracts had 62.65 ± 0.97, 82.21 ± 0.92 and 81.45 ± 1.32 µg/mL $IC_{50}$ values in the DPPH free radical scavenging activity assay, respectively. The other extracts showed weak or no activity.

In the ABTS cation radical scavenging assay, CBA, CBM, CBW and CLW extracts exhibited 47.50 ± 0.63, 24.21 ± 0.70, 44.89 ± 1.09 and 35.03 ± 0.80 µg/mL $IC_{50}$ values, respectively. The CBM, and CLW extracts exhibited strong ABTS cation radical scavenging activity. CBA and CBW extracts showed moderate activity with 70.96, 65.91 and 79.49 % inhibitions, respectively (Table 3).

### Anticholinesterase and antimicrobial activities

As provided in Table 2, all the extracts had no activity against acetylcholinesterase enzyme, while CBP, CBA, CDP, CLP and CLA extracts showed moderate activity (56.69, 57.46, 48.15, 53.11 and 47.19 % inhibition, respectively) against butyrylcholinesterase enzyme at 200 µg/mL. The acetone extracts of the plants were active against all microorganisms tested with different levels of inhibition: weak (inhibition zone <12) and moderate (inhibition zone <20-12) (Table 4). Among the tested microorganisms, *C. albicans* was the most susceptible microorganism against acetone extracts. The extracts exhibited moderate antimicrobial activity against *C. albicans* and *S. pyogenes*. For a more reliable assessment of antimicrobial activity, a broth dilution assay was carried out. The susceptibility of the test microorganisms against active extracts was evaluated and results are shown as MIC (Table 4). The lowest MIC value was recorded by *C. balsamita* against *C. albicans* (45 µg mL-1) in conformity with the result of disc diffusion method. MIC values of *C. balsamita* against *E. coli* and other Gram (-) bacteria were more than 1000 µg $mL^{-1}$.

## DISCUSSION

Present study has revealed that *C. balsamita* has antioxidant, anticholinesterase and antimicrobial properties. Several studies are present in literature reporting the use of liquid chromatography electrospray ionization tandem mass spectrometry to perform quantitative analyses [18,19]. In this study, an accurate method on a mass spectrometer equipped with a

**Table 1:** Analytical parameters, identification and quantification of phenolic compounds of methanol extract of *C. balsamita*, *C. depressa* and *C. lycopifolia* by LC ESI MS/MS[a]

| Analyte no | Analytes | RT[a] | RSD%[b] | Linearity Range (µg/L) | LOD/ LOQ (µg/L)[c] | Recovery (%) | U[d] | M-H$^+$ (m/z)[e] | MS$^2$(CE)[f] | Quantification (µg analyte/g extract)[g] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | *C. balsamita* | *C. depressa* | *C. lycopifolia* |
| 1 | Quinic acid | 3.36 | 0.0388 | 250-10000 | 22.3 / 74.5 | 103.3 | 4.8 | 190.95 | 85 (22), 93 (22) | **17513.16±813.48** | **63874.33±3065.97** | **108233.80±5195.22** |
| 2 | Malic acid | 3.60 | 0.1214 | 250-10000 | 19.2 / 64.1 | 101.4 | 5.3 | 133.05 | 115 (14), 71 (17) | 3370.64±178.64 | **22587.95±1197.16** | 8534.11±452.31 |
| 3 | tr-Aconitic acid | 4.13 | 0.3908 | 250-10000 | 15.6 / 51.9 | 102.8 | 4.9 | 172.85 | 85 (12), 129 (9) | 198.99±9.75 | 551.47±27.02 | 217.05±10.64 |
| 4 | Gallic acid | 4.25 | 0.4734 | 25-1000 | 4.8 / 15.9 | 102.3 | 5.1 | 169.05 | 125 (14), 79 (25) | 16.03±3.10 | 346.09±17.65 | 550.79±28.09 |
| 5 | Chlorogenic acid | 5.29 | 0.1882 | 250-10000 | 7.3 / 24.3 | 99.7 | 4.9 | 353 | 191 (17) | **10770.07±549.27** | 1552.63±76.08 | **15782.64±773.35** |
| 6 | Protocatechuic acid | 5.51 | 0.5958 | 100-4000 | 25.8 / 85.9 | 100.2 | 5.1 | 152.95 | 109 (16), 108 (26) | 179.45±9.15 | 1872.96±95.52 | 3749.46±191.22 |
| 7 | Tannic acid | 6.30 | 0.9075 | 100-4000 | 10.2 / 34.2 | 97.8 | 5.1 | 182.95 | 124 (22), 78 (34) | 67.70±3.45 | 3413.75±174.10 | 35.96±1.83 |
| 8 | tr- caffeic acid | 7.11 | 1.0080 | 25-1000 | 4.4 / 14.7 | 98.6 | 5.2 | 178.95 | 135 (15), 134 (24), 89 (31) | 54.79±2.85 | 96.92±5.03 | 138.31±7.19 |
| 9 | Vanillin | 8.57 | 0.4094 | 250-10000 | 10.1 / 33.7 | 99.2 | 4.9 | 151.05 | 136 (17), 92 (21) | 61.69±3.02 | 55.34±2.71 | 49.58±2.43 |
| 10 | p-Coumaric acid | 9.17 | 1.1358 | 100-4000 | 15.2 / 50.8 | 98.4 | 5.1 | 162.95 | 119 (15), 93 (31) | 70.68±3.60 | 702.78±34.44 | 246.46±12.57 |
| 11 | Rosmarinic acid | 9.19 | 0.5220 | 250-10000 | 10.4 / 34.8 | 101.7 | 4.9 | 358.9 | 161 (17), 133 (42) | 110.36±5.40 | 180.53±8.85 | 160.70±8.19 |
| 12 | Rutin | 9.67 | 0.8146 | 250-10000 | 17.0 / 56.6 | 102.2 | 5.0 | 609.1 | 300 (37), 271 (51), 301 (38) | 40.87±2.04 | 133.87±6.69 | 90.17±4.51 |
| 13 | Hesperidin | 9.69 | 0.1363 | 250-10000 | 21.6 / 71.9 | 100.2 | 4.9 | 611.1 | 303, 465 | N.D. | 238.13±11.67 | 87.24±4.27 |
| 14 | Hyperoside | 9.96 | 0.2135 | 100-4000 | 12.4 / 41.4 | 98.5 | 4.9 | 463.1 | 300, 301 | 1688.96±82.76 | 1272.65±62.36 | 1998.14±97.91 |
| 15 | 4-OH Benzoic acid | 11.38 | 1.4013 | 25-1000 | 3.0 / 10.0 | 106.2 | 5.2 | 136.95 | 93, 65 | 50.24±2.61 | 290.55±15.11 | 347.92±18.09 |
| 16 | Salicylic acid | 11.39 | 0.6619 | 25-1000 | 4 / 13.3 | 106.2 | 5.0 | 136.95 | 93, 65, 75 | 52.23±2.60 | 267.83±13.39 | 308.89±15.44 |
| 17 | Myricetin | 11.42 | 2.8247 | 100-4000 | 9.9 / 32.9 | 106.0 | 5.9 | 317 | 179, 151, 137 | 189.38±11.17 | 31.32±1.85 | N.D. |
| 18 | Fisetin | 12.10 | 2.4262 | 100-4000 | 10.7 / 35.6 | 96.9 | 5.5 | 284.95 | 135, 121 | N.D. | N.D. | N.D. |
| 19 | Coumarin | 12.18 | 0.4203 | 100-4000 | 9.1 / 30.4 | 104.4 | 4.9 | 146.95 | 103, 91, 77 | D. | 26.20±1.28 | 12.75±0.62 |

**Table 1:** Analytical parameters, identification and quantification of phenolic compounds of methanol extract of *C. balsamita*, *C. depressa* and *C. lycopifolia* by LC ESI MS/MS[a]

| Analyte no | Analytes | RT[a] | RSD%[b] | Linearity Range (µg/L) | LOD/ LOQ (µg/L)[c] | Recovery (%) | U[d] | M-H$^+$ (m/z)[e] | MS$^2$(CE)[f] | Quantification (µg analyte/g extract)[g] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | *C. balsamita* | *C. depressa* | *C. lycopifolia* |
| 20 | Quercetin | 13.93 | 4.3149 | 25-1000 | 2.0 / 6.8 | 98.9 | 7.1 | 300.9 | 179, 151, 121 | 3731.86±264.96 | 67.66±4.80 | N.D. |
| 21 | Naringenin | 14.15 | 2.0200 | 25-1000 | 2.6 / 8.8 | 97.0 | 5.5 | 270.95 | 151, 119, 107 | 40.15±2.21 | 37.19±2.05 | N.D. |
| 22 | Hesperetin | 14.80 | 1.0164 | 25-1000 | 3.3/ 11.0 | 102.4 | 5.3 | 300.95 | 164, 136, 108 | 410.57±21.76 | N.D. | N.D. |
| 23 | Luteolin | 14.84 | 3.9487 | 25-1000 | 5.8 / 19.4 | 105.4 | 6.9 | 284.95 | 175, 151, 133 | 97.11±6.70 | 2019.30±139.33 | 43.53±3.00 |
| 24 | Kaempferol | 14.85 | 0.5885 | 25-1000 | 2.0 / 6.6 | 99.1 | 5.2 | 284.95 | 217, 133, 151 | N.D. | 1887.07±98.13 | N.D. |
| 25 | Apigenin | 16.73 | 0.6782 | 25-1000 | 0.1 / 0.3 | 98.9 | 5.3 | 268.95 | 151, 117 | 52.48±2.78 | 3958.61±209.80 | 2.25±0.12 |
| 26 | Rhamnetin | 18.41 | 2.5678 | 25-1000 | 0.2 / 0.7 | 100.8 | 6.1 | 314.95 | 165, 121, 300 | 56.69±3.46 | 45.69±2.79 | 12.06±0.74 |
| 27 | Chrysin | 20.60 | 1.5530 | 25-1000 | 0.05 / 0.17 | 102.2 | 5.3 | 253 | 143, 119, 107 | 7.75±0.41 | 7.88±0.42 | 5.74±0.30 |

[a]RT: Retention time, [b]RSD: Relative standard deviation, [c]LOD/LOQ(µg/L): Limit of detection/Limit of quantification, [d]U (%): Percent relative uncertainty at 95% confidence level (k=2), [e]M-H$^+$(*m/z):* Molecular ions of the standard compounds (mass to charge ratio), [f]MS$^2$(CE): MRM fragments for the related molecular ions (CE refers to related collision energies of the fragment ions), [g]Values in µg/g (w/w) of plant methanol extract, [h]N.D: not detected, [i]D: peak observed, concentration is lower than the LOQ but higher than the LOD

**Table 2:** Total phenolic and flavonoid contents and anticholinesterase activity of *Centaurea* extracts*

| Extracts | Phenolic content ($\mu$g PEs/mg extract)$^{\alpha}$ | Flavonoid content ($\mu$g QEs/mg extract)$^{\beta}$ | Inhibition % against AChE | Inhibition % against BChE |
|---|---|---|---|---|
| **CBP** | 28.88 ± 0.82[a] | 4.00 ± 0.51[a] | NA | 56.69 ± 0.92[a] |
| **CBA** | 30.88 ± 1.82[a] | 20.39 ± 1.64[b] | NA | 57.46 ± 0.79[a] |
| **CBM** | **174.30 ± 1.90[b]** | 22.60 ± 1.87[b] | NA | 30.87 ± 1.46[b] |
| **CBW** | 45.48 ± 1.05[c] | 41.43 ± 0.33[c] | NA | NA |
| **CDP** | 23.38 ± 0.92[d] | 5.39 ± 0.41[a] | NA | 48.15 ± 1.03[c] |
| **CDA** | 36.68 ± 0.83[e] | 18.04 ± 1.18[b] | NA | 34.21 ± 0.64[b] |
| **CDM** | 67.75 ± 1.24[f] | 25.82 ± 1.06[b] | NA | NA |
| **CDW** | 46.34 ± 0.74[c] | 22.71 ± 0.98[b] | NA | NA |
| **CLP** | 38.84 ± 1.45[e] | 1.89 ± 0.33[a] | NA | 53.11 ± 0.97[a] |
| **CLA** | 41.87 ± 1.41[c] | 32.57 ± 1.19[d] | NA | 47.19 ± 1.56[c] |
| **CLM** | 52.24 ± 1.15[g] | 10.97 ± 1.70[e] | NA | NA |
| **CLW** | 37.85 ± 1.14[e] | 11.75 ± 2.52[e] | NA | NA |
| **Galanthamine**[†] | - | - | 75.11 ± 0.69 | 82.49 ± 0.32 |

* Values expressed are means ± S.D. of three parallel measurements, different letters in the same column indicate a significant difference ($p< 0.05$), [†]Standard drug, NA: Not active, [α] PEs, pyrocatechol equivalents (y = 0.0251 x + 0.0445 $R^2$ = 0.9945), [β]QEs, quercetin equivalents (y = 0.0301 x – 0.0553 $R^2$ = 0.9984).

**Table 3:** Antioxidant activity of the extracts and standards[a]

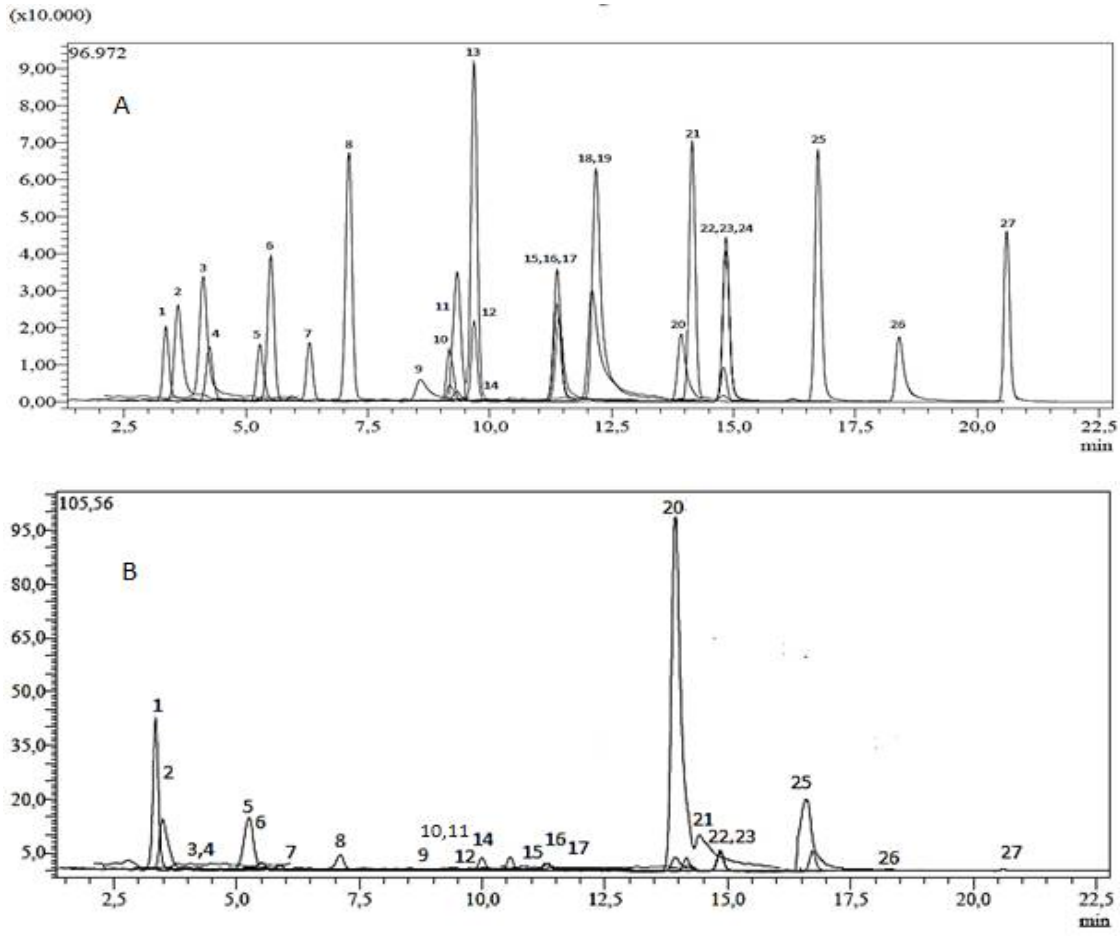
| | $IC_{50}$ values | |
|---|---|---|
| **Samples** | **DPPH Free Radical** | **ABTS Cation Radical** |
| **CBP** | >200 | >200 |
| **CBA** | 139.19±0.85 | 47.50±0.63 |
| **CBM** | 62.65±0.97 | 24.21±0.70 |
| **CBW** | 105.12±0.74 | 44.89±1.09 |
| **CDP** | >200 | >200 |
| **CDA** | >200 | 96.29±1.06 |
| **CDM** | 82.21±0.92 | 70.08±0.52 |
| **CDW** | 107.06±1.27 | 97.39±0.87 |
| **CLP** | >200 | >200 |
| **CLA** | >200 | >200 |
| **CLM** | 81.45±1.32 | 89.05±0.56 |
| **CLW** | 100.88±1.21 | 35.03±0.80 |
| **α-TOC** | 18.76±0.41 | 9.88±0.23 |
| **BHT** | 48.86±0.50 | 10.67±0.11 |

[a]Values were given as $IC_{50}$, Values are means ± S.D., n = 3

**Table 4:** Zones of growth inhibition (mm) and MIC values showing the antimicrobial activities of the acetone extracts compared to positive controls

| | | *Gram positive* | | *Gram negative* | | *Yeast* |
|---|---|---|---|---|---|---|
| | | ***S. aureus*** | ***S.pyogenes*** | ***E.coli*** | ***P. aeruginosa*** | ***C. albicans*** |
| ***Centaurea lycopifolia*** | [a]**DD** | 11±0.2 | 12±0.1 | 11±0.5 | 11±0.3 | 16±0.3 |
| | **MIC** | 250±0.4 | 100±0.5 | 180±0.5 | 300±0.2 | 50±0.3 |
| ***Centaurea balsamita*** | [a]**DD** | 11±0.2 | 12±0.3 | 10±0.3 | 8±0.6 | 17±0.2 |
| | **MIC** | 170±0.3 | 150±0.2 | >1000 | >1000 | 45±0.4 |
| ***Centaurea depressa*** | [a]**DD** | 10±0.3 | 12±0.4 | 9±0.2 | 10±0.5 | 15±0.1 |
| | **MIC** | 240±0.6 | 180±0.5 | >1000 | 500±0.1 | 50±0.2 |
| **Positive controls** | [b]**DD** | 35±0.2 | 19±0.2 | 20±0.1 | NA | 30±0.3 |
| | **MIC** | 1.95±0.3 | 7.815±0.1 | 7.815±0.4 | NA | 3.125±0.2 |

[a]DD: Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 30 mg $mL^{-1}$ of plant extracts, [b]DD: Inhibition zone in diameter (mm) of positive controls that are ampicillin for bacteria and fluconazole for yeast. Minimum inhibitory concentration (MIC) values are given as µg $mL^{-1}$, NA: Not active



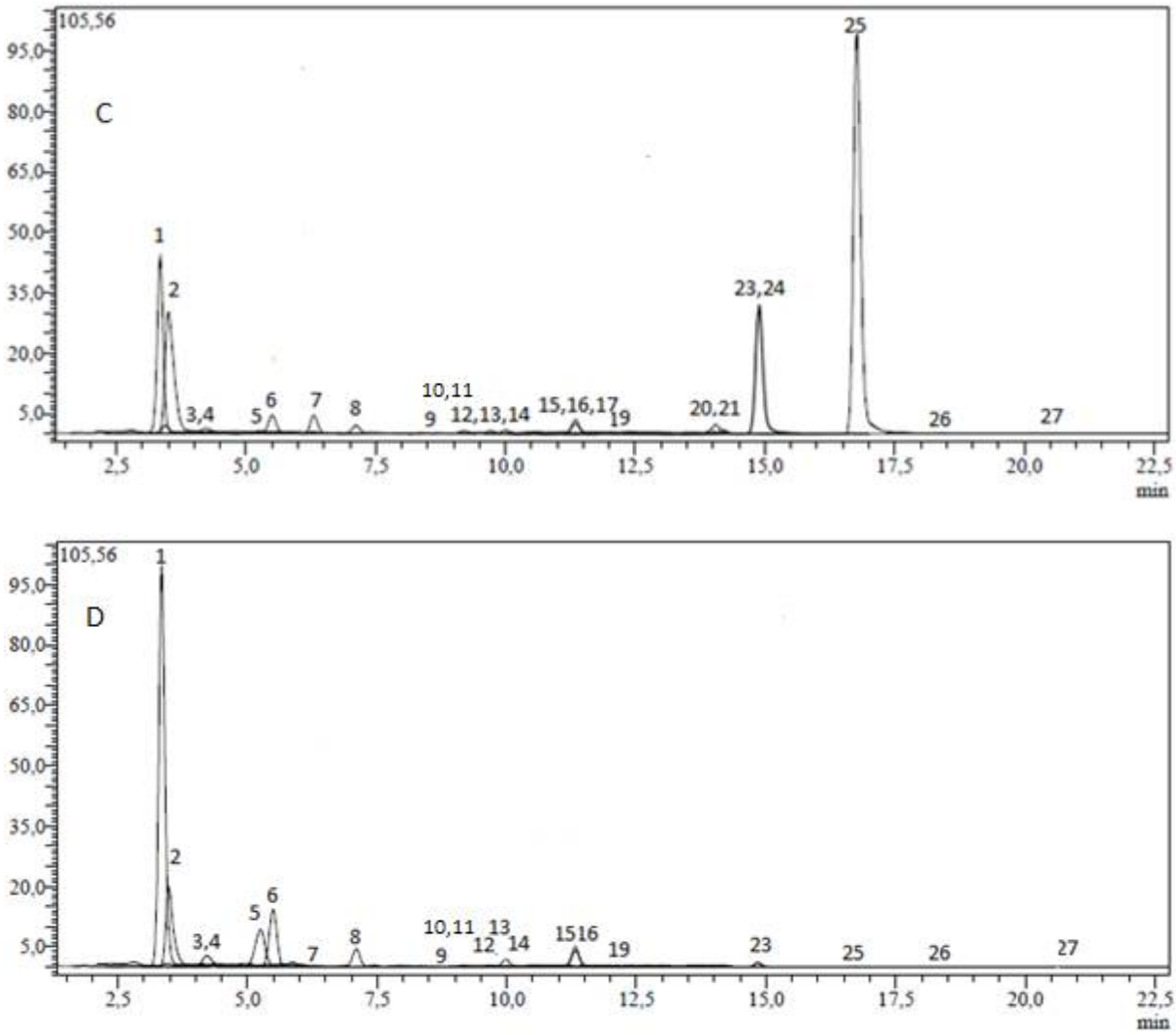



**Figure 1:** LC-MS/MS chromatograms of A: 250 ppb standard mix, B: CB methanol extract, C: CD methanol extract, D: CL methanol extract. 1: Quinic acid, 2: Malic acid, 3: tr-Aconitic acid, 4: Gallic acid, 5: Chlorogenic acid, 6: Protocatechuic acid, 7: Tannic acid, 8: tr- caffeic acid, 9: Vanillin, 10: p-Coumaric acid, 11: Rosmarinic acid, 12: Rutin, 13: Hesperidin, 14: Hyperoside, 15: 4-OH Benzoic acid, 16: Salicylic acid, 17: Myricetin, 18: Fisetin,19: Coumarin, 20: Quercetin, 21: Naringenin, 22: Hesperetin, 23: Luteolin, 24: Kaempferol, 25: Apigenin, 26: Rhamnetin, 27: Chrysin

triple quadrupole analyzer was developed for the analyses of 27 compounds in *Centaurea* species. Quinic acid was found to be the most abundant among the compounds and about 10 % of methanol extract of *C. lycopifolia* was quinic acid. For the first time, mrycetin, fisetin, naringenin, hesperetin, rhamnetin, chrysin, hesperidin, hyperoside, gallic acid, tannic acid, caffeic acid, p-coumaric acid, rosmarinic acid, 4-hydroxybenzoic acid, salicylic acid, malic acid and tr-aconitic acid were detected in the three plant species using LC-MS-MS. Also, flavonoids, flavonoid glycosides, phenolic acids and other organic acids constituents of *Centaurea* species have been detected with LC-MS-MS.

The antibacterial effect of *C. balsamita* seen in this study has been reported previously in eight *Centaurea* species [4,9]. Sımilarly, the antifungal activity of *C. balsamita* has been reported in *Candida krusei* [10]. As in an earlier report, the methanol extract of the three *Centaurea* species exhibited the highest effect in DPPH free and ABTS cation radicals scavenging activities just like *C. pulcherrima* var. *pulcherrima* [21]. The ABTS cation radical scavenging activity in our study may be caused by higher quercetin contents of *C. balsamita* than other investigated plants. In CUPRAC assay, *Centaurea* extracts showed very weak activity that verify the CUPRAC assay results of *Centaurea* species reported by Aktümsek *et al* [21].

In the report of Aktümsek *et al* [21], water extracts of *C. pyrrhoblephara* and *C. antalyense* were not active against AChE as in our results but their methanol extracts exhibited weak activity against AChE. However, all the tested extracts displayed more or less inhibition on BChE, except for methanolic extract of *C. pyrrhoblephara* [21] while our results of CBP, CBA, CDP, CLP and CLA extracts showed moderate antibutyrylcholinesterase activity. Acetone extract of *Centaurea balsamita* has good antifungal activity against *Candida albicans*. Inhibition zones and MIC values of the acetone extracts of *Centaurea* species on the yeast *Candida albicans* compared to that of positive control (fluconazole) indicated their moderate antifungal properties.

## CONCLUSION

Some compounds including mrycetin, fisetin, naringenin, hesperetin, rhamnetin, chrysin, hesperidin, hyperoside, gallic acid, tannic acid, caffeic acid, p-coumaric acid, rosmarinic acid, 4-hydroxybenzoic acid, salicylic acid, malic acid and tr-aconitic acid have been detected for the first time in *Centaurea* species using LC-MS-MS. *Centaurea balsamita* has antioxidant, anticholinesterase and antimicrobial properties. Further studies to identify the compounds in the extracts responsible for the activities are needed.

## DECLARATIONS

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

## REFERENCES


1. *Wagenitz G. Centaurea L., In Flora of Turkey and the East Aegean Islands, Vol 5. Davis PH (Ed.). Edinburgh University Press, Edinburgh, 1975; pp 465-585.*
2. *Baytop T. Therapy with Plants in Turkey (Past and Present), Publications of Istanbul University, Istanbul, 1999; p 316.*
3. *Öksüz S, Topçu G. Guaianolides from Centaurea glastifolia. Phytochem 1994; 37: 487-490.*
4. *Tekeli Y, Zengin G, Aktümsek A, Sezgin M, Torlak E. Antibacterial activities of extracts from twelve Centaurea species from Turkey. Arch Biol Sci Belgrade 2011; 63: 685-690.*
5. *Barrero AF, Oltra JE, Alvarez M, Raslan DS, Saude DA, Akssira M. New sources and antifungal activity of sesquiterpene lactones. Fitoterapia 2000; 71: 60-64.*
6. *Negrete RE, Backhouse N, Cajigal I, Delporte C, Cassels BK, Breitmaier E, Eckhardt G. Two new antiinflammatory elemanolides from Centaurea chilensis. J Ethnopharmacol 1993; 40: 149–153.*
7. *Yeşilada E, Sezik E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey IX: Folk medicine in Northwest Anatolia. J Ethnopharmacol 1999;64: 195-210.*
8. *Tepe B, Sökmen M, Akpulat HA, Yumrutas O, Sökmen A. Screening of antioxidative properties of the methanolic extracts of Pelargonium endlicherianum Fenzl., Verbascum wiedemannianum Fisch. & Mey., Sideritis libanotica Labill. subsp. linearis (Bentham) Borm., Centaurea mucronifera DC. and Hieracium cappadocicum Freyn from Turkish flora. Food Chem 2006; 98: 9-13.*
9. *Medjroubi K, Benayache F, Bermejo J. Sesquiterpene lactones from Centaurea musimomum, antiplasmodial and cytotoxic activities. Fitoterapia 2005; 76: 744–746.*
10. *Shoeb M, MacManus SM, Jaspars M, Trevidu J, Nahar L, Kong-Thoo-Lin P, Sarker SD. Montamine, a unique dimeric indole alkaloid, from the seeds of Centaurea montana (Asteraceae), and its in vitro cytotoxic activity against the CaCo2 colon cancer cells. Tetrahedron 2006; 62: 11172-11177.*
11. *Mouffok S, Haba H, Lavaud C, Long C, Benkhaled M. Chemical constituents of Centaurea omphalotricha Coss. & Durieu ex Batt. & Trab. Rec Nat Prod 2012; 6: 292-295.*
12. *Gülcemal D, Alankuş-Calışkan Ö, Karaalp C, Örs AU, Ballar P, Bedir E. Phenolic glycosides with antiproteasomal activity from Centaurea urvillei DC. subsp. urvillei. Carbohydr Res 2010; 345: 2529-2533.*
13. *Erel SB, Karaalp C, Bedir E, Kaehlig H, Glasl S, Khan S, Krenn L. Secondary metabolites of Centaurea calolepis and evaluation of cnicin for anti-inflammatory, antioxidant, and cytotoxic activities. Pharm Biol 2011; 49: 840-849.*
14. *Flamini G. Stoppelli G, Morelli I, Ertugrul K, Dural H, Tugay O, Demirelma H. Secondary metabolites from Centaurea isaurica from Turkey and their chemotaxonomical significance. Biochem Sys Ecol 2004; 32: 553-557.*
15. *Astari KA, Erel SB, Bedir E, Karaalp C. Secondary Metabolites of Centaurea cadmea Boiss. Rec Nat Prod 2013; 7: 242-244.*
16. *Csupor D, Widowitz U, Blazsó G, Laczkó-Zöld E, Tatsimo JSN, Balogh A, Boros K, Dankó B, Bauer R, Hohmann J. Anti-inflammatory activities of eleven Centaurea species occurring in the Carpathian basin. Phyther Res 2013; 27: 540-544.*

17. Demir S, Baykan Ş, Erel, Bedir E, Karaalp C. 2009. Secondary metabolites of Centaurea depressa Bieb. J. Fac. Phar. Ankara 38: 1-7.
18. Ertaş A, Boğa M, Yılmaz MA, Yeşil Y, Haşimi N, Kaya MŞ, Temel H, Kolak U. Chemical composition by using LC-MS/MS and GC-MS and viological activities of Sedum sediforme (Jacq.) Pau. J Agric Food Chem 2014; 62: 4601-4609.
19. Ertas A, Boga M, Yilmaz AM, Yesil Y, Tel G, Temel H, Hasimi N, Gazioglu I, Ozturk M, Ugurlu P. A detailed study on the chemical and biological profiles of essential oil and methanol extract of Thymus nummularius (Anzer tea): Rosmarinic acid. Ind. Crops Prod. 2015; 67, 336-345.
20. NCCLS (National Committee for Clinical Laboratory Standards). 2009. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard 8th ed. Wayne Pa. M08-A8.
21. Aktümsek A, Zengin G, Güler GO, Çakmak YS, Duran A. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic Centaurea L. species. Food Chem Toxicol 2013;55: 290-296.