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Authors: Gokhan Zengin, Dimitrina Zheleva-Dimitrova, Reneta Gevrenova, Paraskev Nedialkov, Andrei Mocan, Ana Ćirić, Jasmina Glamočlija, Marina Soković, Abdurrahman Aktumsek, M.Fawzi Mahomoodally

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Identification of phenolic components *via* LC-MS analysis and biological activities of two *Centaurea* species: *C. drabifolia* subsp. *drabifolia* and *C. lycopifolia*

Gokhan Zengin[1*], Dimitrina Zheleva-Dimitrova[2], Reneta Gevrenova[2], Paraskev Nedialkov[2], Andrei Mocan[3], Ana Ćirić[4], Jasmina Glamočlija[4], Marina Soković[4], Abdurrahman Aktumsek[1], M. Fawzi Mahomoodally[5]

[1] *Selcuk University, Science Faculty, Department of Biology, Campus, 42250, Konya, Turkey*

[2]*Department of Pharmacognosy, Faculty of Pharmacy, Medical University-Sofia, 2, Dunav str., 1000 Sofia, Bulgaria*

[3]*Department of Pharmaceutical Botany, "Iuliu Hatieganu" University of Medicine and Pharmacy, 8, V. Babes Street, Cluj-Napoca, Romania*

[4]*Institute for Biological Research "Siniša Stanković" University of Belgrade, Belgrade, Serbia*

[5] *Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit, Mauritius*

[*] Corresponding author: (Dr. Gokhan ZENGIN Tel.: +90 332 223 27 81; Fax: +90 332 2410106, E-mail address: gokhanzengin@selcuk.edu.tr)

Highlights

- Biological and chemical fingerprints of two *Centaurea* species were studied.
- Antioxidant, enzyme inhibition, and antimicrobial activity were assessed.
- Chemical composition was examined by liquid chromatography-mass spectrometry detection.
- The extracts exhibited remarkable biological abilities.
- These plants may be considered as potential sources of natural bioactive agents.

Abstract

The *Centaurea* genus has great potential in traditional systems and has attracted much interest in the design of novel drug formulations. The present study was focused on the chemical fingerprints and biological properties of *Centaurea drabifolia* subsp. *drabifolia* and *Centaurea lycopifolia* extracts. Spectrophotometric and LC-MS techniques were used to establish the chemical profiles of the studied extracts. Enzyme inhibitory potential was assessed against key enzymes linked to global health problems, namely neurodegenerative diseases (acetylcholinesterase), pigmentation (tyrosinase), and diabetes (α-amylase and α-glucosidase). The antimicrobial propensities of the extract were evaluated against 16 bacterial

and fungal strains using the microdilution method. The antioxidant abilities were assessed using DPPH and ABTS radical scavenging, ferric, and cupric reducing powers, phosphomolybdenum, and ferrous metal chelation. The total phenolic compounds varied from 18.33 to 32.84 mgGAE/g extract. Total flavonoid content of the extracts were in the range of 2.88-22.39 mgRE/g extract. Methanol and water extracts showed stronger antioxidant abilities compared to the ethyl acetate extracts. However, the latter extracts were most efficient towards the target enzymes (except for tyrosinase). The water extracts also exerted considerable antimicrobial effects. Findings from the present work tend to support the idea that *C. drabifolia* subsp. *drabifolia* and *C. lycopifolia* may be utilized as effective bio-resources for designing novel health-promoting products or ingredients. It is anticipated that results amassed from this still will open new avenues for research and contribute towards establishing primary data on these species for designing novel phytopharmaceuticals.

Keywords: *Centaurea*; phenolics; biological effects; natural bioactive agents; functional products.

1. Introduction

Since time immemorial, plants or plant products have been widely used by humans for several purposes including foods, drugs, or shelter. This fact has been growing day by day and the last century has witnessed a growing number of herbal consumers and could be considered as “herbal products era” against synthetics. From this point, global awareness on natural products have resulted in a plethora of investigations focused towards the discovery of novel and effective compounds from plants [1, 2]. One famous example is the discovery of the antimalarial drugs, Artemisinin isolated from the traditionally used medicinal plant, *Artemisia annua*, which was awarded the 2015 Nobel Prize in Physiology or Medicine [3]. Moreover,

several plant species have been suggested as reservoir of potential sources for designing health-promoting food products as witnessed by the growing number of publications in this area [4]. Within the framework of these information, new studies on plants, especially wild food plants are becoming one of the most attractive topics in the scientific area of natural products.

Turkey is famous for its floral diversity, which contains about 12000 plant species. It has been suggested that this biodiversity offers an excellent repertoire of potential new bioactive candidates that remains largely unexplored [5]. The *Centaurea* genus is one of the largest genera in Turkish flora and it is represented by more than 200 species, with an endemism ratio >60%. The members of this genus are known as "Timur dikeni", "peygamber çiceği" or "pıtrak" and they are traditionally used for different purposes, including food, against hemorrhoids, cold, wound healing or gastrointestinal disturbances [6, 7]. In this context, there has been an increasing amount of literature concerning chemical characterization and biological effects of different plants from *Centaurea* genus [8]. However, there is still a dearth of knowledge and a pressing need for comprehensive studies focused towards the chemical characterization and biological properties of *C. drabifolia* subp. *drabifolia* and *C. lycopifolia*. Hence, the main objective of this study is to establish the chemical profiles and biological effects of *C. drabifolia* subp. *drabifolia* and *C. lycopifolia* extracts. It is anticipated that results amassed from this study will open new avenues for research and contribute towards establishing primary data on these species for designing novel phytopharmaceutics.

2. Materials and Methods

2.1. Plant materials and extraction procedure

Aerial parts of two *Centaurea* species were collected in June 2015 and the locations are given below. Identification plants were performed by botanist Dr. Murad Aydin Sanda. Voucher specimens of the studied *Centaurea* species were stored at Department of Biology of Selcuk University, Turkey.

1. *Centaurea drabifolia* Sm. subsp. *drabifolia* Sm.: Between Avanos and Kayseri, 38°42'37"N, 34°53'52"E, 992 m.

2. *Centaurea lycopifolia* Boiss. et Kotschy: Kahramanmaraş, between Andırın and Kahramanmaraş, 37°33'59"N, 36°33'59"E, 1273 m.

The aerial parts of the selected species were analyzed. First, they were dried in the dark at room temperature. They were then ground to a fine powder using a laboratory mill. Samples (10 g) were stirred with ethyl acetate or methanol at room temperature for 24 h. Then, the extracts were concentrated to dryness using a rotary evaporator. As for the water extracts, 5 g of plant material was boiled in 100 mL water for 30 min. Then, the extracts were lyophilized. The extracts were then stored in dark glass at 4°C until further analyses.

2.2. Quantification of total phenolics and flavonoids

The total bioactive compounds (phenolics and flavonoids content) of the studied *Centaurea* extracts were obtained using Folin-Ciocalteu and $AlCl_3$ methods, respectively [9]. The contents were expressed as gallic (mg GAEs/g extract) acid and rutin equivalents (mg REs/g extract), respectively.

2.3. Screening for biological activities

Antioxidant potentials, enzyme inhibitory effects, and antimicrobial properties were evaluated for the biological activities of the studied *Centaurea* species. Antioxidant methods used were: free radical scavenging (ABTS and DPPH), ferric (FRAP), and cupric reducing power (CUPRAC); phosphomolybdenum, and metal chelating assays. The antioxidative potentials were expressed as trolox equivalents (EDTA was only used for metal chelating assays). Anti-cholinesterase, anti-tyrosinase, anti-amylase, and anti-glucosidase assays were tested for detecting enzyme inhibitory effects. The enzyme inhibitory effects were evaluated as standard

compound equivalents. Briefly, galantamine was used for AChE and BChE, kojic acid was used for tyrosinase, while acarbose was used for α-amylase and α-glucosidase inhibition assay). Antimicrobial evaluation was also performed with some bacteria and fungi strains and all experimental details are as per previous publication [10].

2.4. LC-MS analysis

Protocatechuic (**1**), neochlorogenic = 3-CQA (**2**), chlorogenic = 5-CQA (**3**), caffeic (**5**) acids, orientin (**7**), vitexin (**8**), and apigenin (**12**) were obtained from Extrasynthese (Genay, France). LC-MS analysis was performed using a Thermo Scientific Dionex Ultimate 3000 RSLC (Germering, Germany) consisting of 6-channel degasser SRD-3600, high pressure gradient pump HPG-3400RS, autosampler WPS-3000TRS and column compartment TCC-3000RS coupled to Thermo Scientific Q Exactive Plus (Bremen, Germany). LC-MS conditions were reported in our previous paper [10].

2.5. Statistical analysis

Statistical evaluation was performed using SPPS v. 14.0 program (one-way ANOVA with Tukey's assay) and $p < 0.05$ was considered as significant.

3. Results and Discussion

3.1. LC-MS phenolic profiles

LC-MS profile of *Centaurea* extracts revealed the presence of protocatechuic, caffeic, monocaffeoylquinic acids (CQA), monoferuylquinic acid (FQA), and flavonoids (Table 1). Protocatechuic (**1**), 3-CQA (**2**), 5-CQA (**3**), caffeic acids (**5**), orientin (**7**), vitexin (**8**), and apigenin (**12**) were identified by comparison of their retention time (t_R), and LC-MS-MS-fragmentation with standard compounds. All other derivatives were readily distinguished by their MS^2 fragmentation pattern and chromatographic behavior on reverse phase column.

Acylquinic acids were identified according to the guide for identification of phenolic acids [11, 12] supported by UV λmax and retention time relative to commercially available 3-caffeoylquinic acid (neochlorogenic acid) and 5-caffeoylquinic acid (chlorogenic acid). Patuletin-O-hexoside and hispidulin were identified by comparison of their LC-MS/MS-fragmentation with literature data [13, 14]. Both *Centaurea* extracts showed similar phenolic LC-MS profile. However, 1,3-diCQA was found only in *C. drabifolia*, while 1-CQA and vitexin were presented only in *C. lycopifolia* (Figure 1).

3.2. Total bioactive components and antioxidant capacity

Total phenolic content in the studied *Centaurea* extracts varied from 18.83 to 32.84 mgGAE/g extract (Table 2). The water and methanol extracts contained higher concentration of phenolics compared to ethyl acetate. The highest concentration was detected in CL-Water (32.84 mgGAE/g), followed by CL-MeOH (28.82 mgGAE/g), and CD-MeOH (24.70 mgGAE/g). The methanol extracts contained highest total flavonoids with the following order; CD-MeOH (22.39 mgRE/g)>CL-MeOH (14.10 mgRE/g)>CL-EA (11.90 mgRE/g)>CD-EA (10.38 mgRE/g). Apparently, flavonoids accounted for more than 60% of total phenolics in the studied methanol extracts. Our findings are in agreement with earlier studies, which also reported higher concentration of total bioactive components in the methanol extracts [15]. In addition, methanol extracts from several *Centaurea* species showed higher concentration of these bioactive components as reported previously [16].

The use of multiple assays has been argued to be useful to highlight the antioxidant propensities of herbal extracts. In this context, the antioxidant profiles of the studied *Centaurea* extracts were tested by different methods, namely free radical scavenging (DPPH and ABTS), reducing power (CUPRAC and FRAP), phosphomolybdenum, and metal chelation (Table 3). DPPH and ABTS assays are the most common standard assays for evaluating radical scavenging

abilities of different biological samples. In these two assays, the best activities were obtained in CL-MeOH (52.49 mgTE/g in DPPH and 92.62 mgTE/g in ABTS) CL-Water (49.80 mgTE/g in DPPH and 97.97 mgTE/g in ABTS), and CD- MeOH (42.71 mgTE/g in DPPH and 91.51 mgTE/g in ABTS), which contained the highest concentration of total phenolic compounds. Our findings appear to be well supported by several previous studies, which reported linear correlation between total phenolic and free radical scavenging abilities of plant extracts [17]. Several studies have been published on the radical scavenging abilities of *Centaurea* species in order to evaluate the antioxidant potential [18]. As observed in the present study, several variations have been reported in the literature (from low to strong activity). At this point, the *Centaurea* genus could be considered as a powerful scavenger of free radicals.

Reducing ability of biological samples is reflected by the electron-donation ability, which is considered as an important strategy to behave as an antioxidant. In this respect, CUPRAC and FRAP assays were performed. As shown in Table 3, the ferric and cupric reducing ability were highest for CL-Water extracts (154.20 mgTE/g in CUPRAC and 81.46 mgTE/g in FRAP). The lowest reducing ability was also detected in CL-EA (59.54 mgTE/g in CUPRAC and 23.79 mgTE/g in FRAP). Generally, the reducing ability of the studied extracts followed the same ranking order with the values of total phenolics. At this point, phenolics in the extracts are main reductants and this behavior was also confirmed by several authors [19]. Similar to CUPRAC and FRAP, phosphomolybdenum assay is based on the reduction of Mo (VI) to Mo (V) by antioxidants (phenolic, non-phenolics, and carotenoids etc.) under acidic condition. In contrast to other antioxidant assays, the greatest ability was observed in CD-EA, followed by CD-MeOH and CL-MeOH. The noteworthy differences may be linked to the presence of compounds other than the phenolics, such as the presence of ascorbic acid and tocopherol. In this direction, phosphomolybdenum assay can reflect total antioxidant capacity assay. Indeed,

no correlation between total phenolics and phosphomolybdenum ability has been reported previously [20].

Transition metals are involved (by Fenton and Haber-Weiss reactions) in the production of hydroxyl radical, which is known to be the most damaging of all free radicals. From this perspective, the chelation of these metals is regarded as a valuable approach to study the antioxidant mechanisms. For this purpose, ferrozine assay was used to evaluate ferrous ion chelation ability of the studied *Centaurea* extracts. From Table 3, the water extracts (19.19 mgEDTAE/g for CD-Water and 16.57 mgEDTAE/g for CL-Water) exerted the best chelating ability compared to methanol and ethyl acetate extracts. These findings tend to corroborate with earlier studies on *Centaurea*, which reported the best chelation ability of the water extracts. Contrary to our expectations, the CD-Water was ranked fourth in terms of total phenolic content. In addition, chelating ability was not detected in CD-EA. In the literature, different approaches have been observed regarding metal chelating capacities of plant extracts. Some researchers have argued that a linear relationship exists between the total phenolics and chelation ability [21]. However, some studies have also highlighted that the metal chelating ability of phenolics can be considered as only minor effect [22].

3.3. Enzyme inhibitory effects

In the 20th century, synthetic chemicals have been introduced for treating and/or managing major health problems including type-II diabetes mellitus, Alzheimer's disease, and cancer. However, such practices bear serious concerns such as toxicity and carcinogenic effects. With this in mind, natural products have proved to be important pharmacophores and are gaining much momentum in terms of managing the aforementioned diseases [23]. As an example of these solutions, natural enzyme inhibitors are considered as one of the most accepted strategies for alleviating the symptoms of these diseases (cholinesterase inhibitors

for Alzheimer disease; α-amylase, and α-glucosidase inhibitors for diabetes; and tyrosinase inhibitors for hyperpigmentation etc.) [24]. From this point, many scientists have focused on novel enzyme inhibitors from natural products such as wild plant species, which may be considered as potential sources of bioactive compounds.

For the above mentioned reasons, the studied *Centaurea* species were tested against several key enzymes including acetylcholinesterase, tyrosinase, α-amylase, and α-glucosidase. The results are listed in Table 4. The acetylcholinesterase inhibitory effects varied significantly among the studied extracts and the greatest activities were recorded for the ethyl acetate, followed by methanol and water extracts. The strong ability of the ethyl acetate extracts might be related to their non-phenolic compounds, such as alkaloids. This argument has been reported by several researchers, who found no correlation between phenolics and anti-cholinesterase activity [25]. In relation to the tyrosinase inhibitory effects, the water extracts were more potent than methanol extracts. In addition, the ethyl acetate extracts were not active against tyrosinase. This observation can be attributed to the higher levels of phenolics in the extracts, which also corroborate with those reported in earlier studies [26]. In contrast to anti-tyrosinase effects, the higher α-amylase and α-glucosidase effects of the ethyl acetate extracts are worth noting. The methanol extracts were more effective against both α-amylase and α-glucosidase than the water extracts. More recently, literature has emerged that offers contradictory findings about the relationship between phenolics and anti-diabetic effect (α-amylase and α-glucosidase inhibitory effects). Some authors have reported a linear correlation among these parameters [27], together with some contradictory findings [28]. The differences may be justified based on the complex nature of phytochemicals and their possible interactions (antagonism or synergism). As expected, knowledge of enzyme inhibitory effects for the genus *Centaurea* is very limited [29, 30]. The present study can be considered the first of its kind geared towards the enzyme inhibitory activities of the studied *Centaurea* species. In this

respect, it is anticipated that the present study could open new avenues for research and focused towards the isolation of bioactive compounds with potent enzyme inhibitory activities.

3.3. Antimicrobial activity

The antibacterial activity of the water, ethyl acetate, and methanol extracts of *C. lycopifolia* and *C. drabifolia* are presented in Table 5. *C. lycopifolia* extracts were observed to possess higher antibacterial potential than *C. drabifolia.* CL-Water exhibited the best antibacterial capacity with inhibition effect at 0.01-0.02 mg/ml and bactericidal activity in range of 0.02-0.04 mg/ml. Ethyl acetate and methanol extracts of both plants showed almost the same potential, with exception of CD-EA which showed higher effect towards *B. cereus* and *L. monocytogenes.* Streptomycin showed inhibitory activity (MICs: 0.05-0.25 mg/ml; MBCs: 0.10-0.50 mg/ml). MICs and MBCs for ampicillin were also detected as 0.10-0.30 and 0.20-1.00 mg/ml, respectively. CL-Water extract possessed much better antibacterial potential than both antibiotics. The other extracts showed better antibacterial activity than ampicillin, and most of the extracts were more active than streptomycin or showed slightly lower effect (Table 5).

Antifungal potential of *C. lycopifolia* and *C. drabifolia* extracts are presented in Table 6. Antifungal potential could be summarised with the following order: CL-Water > CL-MeOH = CD-MeOH > CD-Water > CL- EA> CD-EA. The best antifungal activity was recorded for CL-Water (MIC: 0.008-0.03 mg/ml, MBC: 0.011-0.40 mg/ml). In general water and methanol extracts of both plants showed higher antifungal potential, while ethyl acetate the lowest antifungal capacity. Interestingly, commercial antifungal agents exhibited lower activity compared with investigated extracts (Table 6). Water and methanol extracts of both species were more effective then bifonazole and ketoconazole, while the ethyl acetate showed the same or lower fungicidal activity then bifonazole, but higher than ketoconazole (Table 6). In general the fungal strains were the most sensitive towards the *Centaurea* extracts.

Conclusion

In summary, this study endeavours to study the chemical characterization and biological activities of *C. drabifolia* subsp. *drabifolia* and *C. lycopifolia*. The extracts were observed to possess considerable amounts of bioactive compounds. Individual phenolics were also detected by LC-MS. Biological effects varied significantly among the studied extracts. Methanol and water extracts showed the least antioxidant and antimicrobial effects, while the ethyl acetate extracts displayed the best inhibitory effects against key enzymes (except for tyrosinase). Taken together, our findings highlight the importance of two *Centaurea* species for designing novel phytopharmaceuticals. However, further experimental studies (*in vivo* animal studies, and isolation of active compounds) could be planned in light of the present findings.

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Table 1. Peak assessment of phenolic compounds in methanol extracts of *C. drabifolia* and *C. lycopofolia*

Peak №	$[M-H]^-$ m/z Molecular formula	MS/MS data m/z	t_R min	Theoret. mass	Delta ppm	Proposed compound
1	153.0187 $C_7H_5O_4$	109.03 (100), 153.02 (12.23)	2.99	153.0182	2.907	Protocatechuic acid[1]
2	353.0881 $C_{16}H_{17}O_9$	191.06 (100), 179.03 (62.89), 135.04 (45.87), 353.08 (41.29)	3.20	353.0867	4.026 3.431	3-CQA[1,2]
3	353.0880 $C_{16}H_{17}O_9$	191.05 (100), 179.03 (1.53), 135. 04 (1.57), 353.08 (4.07)	4.10	353.0867	3.601 4.224	5-CQA[1,2]
4	353.0881 $C_{16}H_{17}O_9$	191.05 (100), 179.03 (0.55), 173.04 (0.84), 135. 04 (0.60), 353.08 (5.17)	4.62	353.0867	3.941	1-CQA[2]
5	179.0339 $C_9H_7O_4$	135.04 (100), 179.03 (17.78)	4.83	179.0339	0.027 2.429	Caffeic acid[1,2]
6	367.1035 $C_{17}H_{19}O_9$	191.05 (100), 173.04 (11.08), 367.10 (16.5)	5.40	367.1036	1.588 2.973	5-FQA[1,2]
7	447.0927 $C_{21}H_{19}O_{11}$	447.09 (76.17), 357.06 (24.87), 327.05 (100), 297.04 (10.55)	5.52	447.0932	1.034 2.465	Orientin[1,2]
8	431.0984 $C_{21}H_{19}O_{10}$	431.09 (96.66), 341.06 (10.68), 312.05 (11.96), 311.05 (100), 283.06 (39.66)	5.96	431.0984	2.591	Vitexin[2]
9	493.0989 $C_{22}H_{21}O_{13}$	493.09 (26.55), 331.04 (100), 316.02 (2.32), 287.01 (2.32), 165.98 (10.06)	6.60	494.1060	2.582 3.129	Patuletin-O-hexoside[1,2]
10	515.1203 $C_{25}H_{23}O_{12}$	191.05 (100), 353.08 (36.04), 179.03 (5.44), 135.04 (7.58), 335.08 (2.49)	6.76	515.1184	3.684 2.034	1,5-diCQA[1,2]
11	515.1195 $C_{25}H_{23}O_{12}$	191.05 (100), 353.09 (63.64), 179.03 (36.35), 173.05 (52.54), 335.07 (3.21)	7.13	515.1184	2.150	1,3-diCQA[1]
12	269.0457 $C_{15}H_9O_5$	269.04 (100), 228.98 (13.28), 151.00 (6.50), 117.03 (21.33)	10.07	270.0528	4.461 4.126	Apigenin[1,2]
13	299.0559	299.05 (62.98), 284.03 (100), 256.03 (2.07), 297.03 (3.46), 183.04 (2.43)	10.34	299.0560	3.276 3.596	Hispidulin[1,2]

[1] *C. drabifolia* subsp. *drabifolia*; [2]*C. lycopofolia*

Table 2. Total phenolic and flavonoid contents of the studied extracts.

Plants	Extracts	Total phenolic (mgGAE/g extract)	Total flavonoid (mgRE/g extract)	DPPH radical scavenging (mg TE/g extract)	ABTS radical scavenging (mgTE/g extract)	Abbreviations
C. drabifolia subsp. *drabifolia*	Ethyl acetate	18.98±0.48*	10.38±0.18	30.18±0.18	67.09±1.06	CD-EA
	Methanol	24.70±0.62	22.39±0.16	42.71±0.18	91.51±1.31	CD-MeOH
	Water	22.03±0.44	2.88±0.42	24.81±0.88	44.04±2.02	CD-Water
C. lycopifolia	Ethyl acetate	18.83±0.57	11.90±0.10	13.93±0.72	52.59±1.59	CL-EA
	Methanol	28.82±0.59	14.10±0.27	52.49±0.44	92.62±1.05	CL-MeOH
	Water	32.84±0.16	6.45±0.16	49.80±2.41	97.97±5.17	CL-Water

*Values expressed are means ±S.D. of three parallel measurements; GAE: Gallic acid equivalent; RE: Rutin equivalent; TE: Trolox equivalent.

Table 3. Antioxidant properties of the studied extracts.

Plants	Extracts	CUPRAC (mgTE/g extract)	FRAP (mgTE/g extract)	Phosphomolybdenum (mmolTE/g extract)	Metal chelating activity (mgEDTAE/g extract)
C. drabifolia subsp. *drabifolia*	Ethyl acetate	83.59±0.42*	43.03±0.28	1.87±0.16	na
	Methanol	102.35±1.81	65.79±0.53	1.61±0.01	5.05±0.32
	Water	79.32±2.87	51.03±0.64	1.12±0.04	19.19±0.06

C. lycopifolia	Ethyl acetate	59.54±2.13	23.76±0.50	1.36±0.07	4.12±0.27
	Methanol	129.98±2.85	64.44±3.08	1.45±0.12	8.63±0.37
	Water	154.20±1.62	81.46±0.71	1.33±0.04	16.57±0.07

*Values expressed are means ±S.D. of three parallel measurements; TE: Trolox equivalent; EDTAE: EDTA equivalent; na: not active.

Table 4. Enzyme inhibitory properties of the studied extracts.

Plants	**Extracts**	**AChE Inhibition**	**Tyrosinase Inhibition**	**α-amylase Inhibition**	**α-glucosidase Inhibiton**

		(mgGALAE/g extract)	(mgKAE/g extract)	(mmolACAE/ g extract)	(mmolACAE/g extract)
C. drabifolia subsp. *drabifolia*	Ethyl acetate	2.58±0.03*	na	0.48±0.02	4.68±0.23
	Methanol	1.98±0.02	6.34±0.01	0.41±0.01	4.15±1.01
	Water	0.60±0.08	7.65±0.72	0.07±0.01	2.29±0.11
C. lycopifolia	Ethyl acetate	2.25±0.03	na	0.53±0.02	15.06±0.23
	Methanol	1.94±0.03	2.26±0.01	0.40±0.01	5.98±1.21
	Water	0.34±0.04	7.68±1.10	0.07±0.01	5.60±0.25

*Values expressed are means ±S.D. of three parallel measurements. GALAE: Galatamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active.

Table 5. Antibacterial activity of the studied *Centaurea* extracts (MIC and MBC in mg/ml)*.

Bacteria/ plants		*S. typhimurium*	*E. coli*	*E. cloacae*	*M. flavus*	*S.aureus*	*B. cereus*	*L. monocytogenes*	*P.aeruginosa*
CL- Water	**MIC**	0.02±0.000^{a}	0.015±0.003^{a}	0.01±0.003^{a}	0.02±0.003^{a}	0.02±0.004^{a}	0.015±0.003^{a}	0.02±0.003^{a}	0.02±0.003^{a}
	MBC	0.04±0.005^{a}	0.020±0.001^{a}	0.02±0.002^{a}	0.04±0.002^{a}	0.04±0.002^{a}	0.02±0.002^{a}	0.04±0.002^{a}	0.04±0.001^{a}
CL-EA	**MIC**	0.15±0.06^{c}	0.08±0.006^{b}	0.037±0.001^{b}	0.05±0.003^{b}	0.11±0.01^{b}	0.025±0.003^{b}	0.11±0.02^{c}	0.15±0.03^{bc}
	MBC	0.22±0.05^{c}	0.11±0.02^{b}	0.05±0.003^{b}	0.11±0.02^{b}	0.22±0.03^{b}	0.05±0.002^{b}	0.22±0.03^{c}	0.22±0.04^{b}
CL-MeOH	**MIC**	0.15±0.02^{c}	0.08±0.002^{b}	0.037±0.005^{b}	0.08±0.003^{bc}	0.15±0.02^{bc}	0.05±0.003^{c}	0.11±0.01^{c}	0.15±0.06^{bc}
	MBC	0.22±0.03^{c}	0.11±0.02^{b}	0.05±0.002^{b}	0.11±0.05^{b}	0.22±0.03^{b}	0.11±0.02^{c}	0.22±0.02^{c}	0.22±0.03^{b}
CD-Water	**MIC**	0.30±0.03^{d}	0.15±0.03^{c}	0.10±0.02^{c}	0.20±0.02^{d}	0.30±0.02^{d}	0.15±0.03^{e}	0.20±0.03^{c}	0.20±0.02^{c}
	MBC	0.40±0.01^{d}	0.20±0.01^{c}	0.20±0.03^{d}	0.40±0.04^{d}	0.40±0.02^{c}	0.20±0.04^{d}	0.40±0.02^{a}	0.40±0.04^{c}
CD-EA	**MIC**	0.11±0.04^{c}	0.08±0.005^{b}	0.037±0.000^{b}	0.05±0.002^{b}	0.11±0.01^{b}	0.025±0.004^{b}	0.08±0.003^{b}	0.11±0.02^{b}
	MBC	0.22±0.03^{c}	0.11±0.02^{b}	0.05±0.002^{b}	0.11±0.04^{b}	0.22±0.03^{b}	0.05±0.06^{b}	0.11±0.02^{b}	0.22±0.02^{b}
CD-MeOH	**MIC**	0.15±0.02^{c}	0.08±0.004^{b}	0.037±0.003^{b}	0.08±0.000^{bc}	0.15±0.04^{bc}	0.05±0.02^{c}	0.15±0.03^{c}	0.15±0.03^{bc}
	MBC	0.22±0.03^{c}	0.11±0.02^{b}	0.05±0.003^{b}	0.11±0.02^{b}	0.22±0.02^{b}	0.11±0.02^{c}	0.22±0.04^{c}	0.22±0.04^{b}

Streptomycin	**MIC**	0.05±0.003[b]	0.10±0.009[b]	0.05±0.006[b]	0.125±0.03[c]	0.25±0.06[c]	0.05±0.003[c]	0.15±0.030[c]	0.10±0.010[b]
	MBC	0.10±0.030[b]	0.50±0.060[c]	0.10±0.020[c]	0.25±0.030[c]	0.50±0.060[d]	0.10±0.010[c]	0.30±0.030[d]	0.50±0.030[d]
Ampicillin	**MIC**	0.15±0.030[c]	0.30±0.000[c]	0.10±0.030[c]	0.30±0.060[e]	0.30±0.060[d]	0.10±0.030[d]	0.15±0.030[c]	0.20±0.060[c]
	MBC	0.20±0.030[c]	1.00±0.100[d]	0.20±0.010[d]	0.50±0.030[e]	0.50±0.030[d]	0.30±0.060[e]	0.50±0.060[e]	1.00±0.060[e]

*Data marked with different letters within the same row indicate statistically significant differences ($p < 0.05$)

Table 6. Antifungal activity of the studied *Centaurea* extracts (MIC and MFC in mg/ml)*.

Microfungi/ plant		*A.niger*	*A.versicolor*	*A.fumigatus*	*A.ochraceus*	*P.funiculosum*	*P.ochrochloron*	*P. veruccosum*	*T.viride*
CL-Water	**MIC**	0.03±0.003[b]	0.01±0.002[a]	0.02±0.003[a]	0.02±0.003[a]	0.02±0.002[a]	0.01±0.003[a]	0.03±0.003[b]	0.008±0.000[a]
	MFC	0.04±0.03[c]	0.02±0.003[b]	0.04±0.004[b]	0.04±0.002[a]	0.04±0.000[a]	0.02±0.002[a]	0.04±0.002[a]	0.011±0.003[a]
CL-EA	**MIC**	0.22±0.02[g]	0.15±0.01[c]	0.15±0.02[c]	0.15±0.06[c]	0.30±0.03[d]	0.22±0.02[c]	0.22±0.02[c]	0.15±0.008[de]
	MFC	0.45±0.06[g]	0.45±0.01[d]	0.45±0.03[d]	0.45±0.03[d]	0.45±0.02[c]	0.45±0.02[d]	0.45±0.02[c]	0.22±0.009[d]
CL-MeOH	**MIC**	0.0125±0.002[a]	0.09±0.003[b]	0.09±0.003[b]	0.025±0.003[a]	0.025±0.006[a]	0.0125±0.003[a]	0.025±0.003[b]	0.0125±0.003[b]
	MFC	0.025±0.06[b]	0.0125±0.002[a]	0.0125±0.002[a]	0.05±0.002[a]	0.05±0.003[a]	0.025±0.002[a]	0.05±0.003[a]	0.025±0.003[b]
CD-Water	**MIC**	0.10±0.02[e]	0.15±0.03[c]	0.20±0.03[d]	0.07±0.004[b]	0.15±0.02[b]	0.10±0.03[b]	0.20±0.02[c]	0.07±0.003[c]
	MFC	0.20±0.03[e]	0.20±0.02[c]	0.40±0.02[d]	0.10±0.02[b]	0.20±0.03[b]	0.20±0.02[c]	0.40±0.03[bc]	0.10±0.04[c]
CD-EA	**MIC**	0.08±0.00[d]	0.22±0.01[d]	0.22±0.01[d]	0.22±0.03[d]	0.22±0.04[c]	0.22±0.03[c]	0.22±0.02[c]	0.11±0.02[d]
	MFC	0.11±0.03[d]	0.45±0.06[d]	0.45±0.03[d]	0.45±0.01[d]	0.45±0.03[c]	0.45±0.02[d]	0.45±0.06[c]	0.22±0.03[d]
CD-MeOH	**MIC**	0.05±0.003[c]	0.0125±0.000[a]	0.025±0.000[a]	0.025±0.000[a]	0.025±0.000[a]	0.0125±0.000[a]	0.0125±0.000[a]	0.006±0.002[a]
	MFC	0.0125±0.03[a]	0.025±0.000[b]	0.05±0.000[b]	0.05±0.001[a]	0.05±0.003[a]	0.05±0.003[b]	0.05±0.000[a]	0.0125±0.003[a]
Bifonazole	**MIC**	0.20±0.060[f]	0.15±0.030[c]	0.15±0.060[c]	0.15±0.030[c]	0.20±0.020[c]	0.20±0.000[c]	0.20±0.060[c]	0.10±0.010[d]

	MFC	0.30±0.020^{f}	0.20±0.060^{c}	0.20±0.030^{c}	0.20±0.060^{c}	0.25±0.030^{b}	0.25±0.010^{c}	0.30±0.030^{b}	0.20±0.030^{d}
	MIC	0.20±0.010^{f}	0.20±0.000^{d}	0.20±0.060^{d}	0.15±0.010^{c}	2.50±0.060^{e}	0.20±0.030^{c}	1.00±0.030^{d}	0.20±0.020^{e}
Ketoconazole	**MFC**	0.50±0.060^{h}	0.50±0.030^{d}	0.50±0.000^{d}	0.20±0.000^{c}	3.50±0.300^{d}	0.50±0.060^{d}	1.00±0.100^{d}	0.30±0.060^{e}

*Data marked with different letters within the same row indicate statistically significant differences ($p < 0.05$)

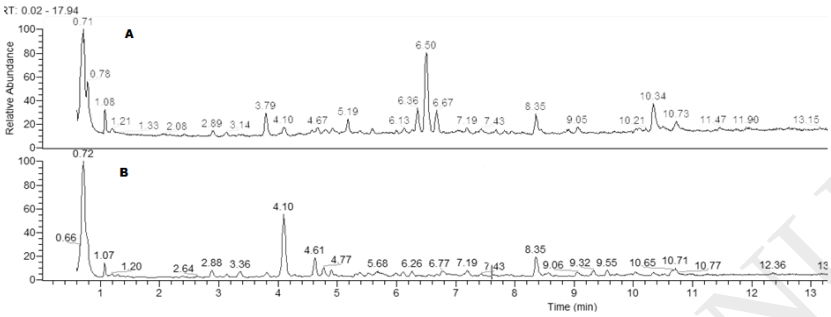

Figure 1. Total Ion Chromatogram (TIC) of methanol extracts from the studied *Centaurea* species (A-*C. drabifolia* subsp. *drabifolia*; B- *C. lycopofolia*).