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Fatty Acid Composition, Antioxidant, Anticholinesterase and Tyrosinase Inhibitory Activities of Four *Serratula* Species from Anatolia

Gülsen Tel[1*], Mehmet Öztürk[1], Mehmet Emin Duru[1], Bekir Doğan[2] and Mansur Harmandar[1]

[1]*Faculty of Sciences, Department of Chemistry, Muğla Sıtkı Koçman University, 48121 Kötekli, Muğla Türkiye*

[2]*Faculty of Ahmet Keleşoğlu Education, Division of Science Education, Necmettin Erbakan University, Meram, Konya, Türkiye*

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Abstract: *Serratula* L. (Astareceae) rich in ecdysteroid, phytoecdysteroids and flavonoids some have various biological activities including antibacterial and antitumor. The fatty acid profiles of four *Serratula* species were investigated by using GC and GC–MS techniques. Palmitic, oleic, linoleic and linolenic acids were found to be the main fatty acids. The unsaturation percentage was between 27.24-50.47%. The antioxidant activity of the extracts was determined by using four complementary tests; namely, β-carotene-linoleic acid, DPPH• scavenging, CUPRAC and ferrous-ions chelating assays. The methanol extract of *S. lasiocephala* showed the highest activity in β-carotene-linoleic acid, DPPH• scavenging and CUPRAC assays, while the hexane extract of *S. radiata* exhibited the best metal chelating activity. In addition, total phenolic and total flavonoid contents in the extracts were determined as pyrocatechol and quercetin equivalents, respectively. The *in vitro* anticholinesterase activity of extracts were tested against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which are the key enzymes taking place in pathogenesis of Alzheimer's disease. Besides, the extracts were tested *in vitro* against tyrosinase enzyme which is associated with melanin hyperpigmentation. Except the hexane extracts of *S. radiata* and *S. lasiocephala*, the extracts showed moderate inhibition against AChE and BChE, while the only hexane extract of *S. erucifolia* and methanol extract of *S. radiata* exhibited tyrosinase inhibitory activity.

Keywords: *Serratula* species; Fatty acid; Antioxidant activity; Anticholinesterase activity; Tyrosinase inhibitory activity; Total phenolic and flavonoid.

1. Introduction

The genus *Serratula* L. is one of the most important genera within Asteraceae, and it comprises about 50-70 species in total [1]. Native distribution of *Serratula* is specifically involving Central Asia, Iran, Turkey and the Mediterranean region. *Serratula* is represented with 16 species within Mediterranean and Irano-Turanian phytogeographic regions of Turkey. Five of these species are endemic to Turkey resulting in an endemism ratio of 31.25 % [2,3]. The *Serratula* species are sources of herbal remedies or food supplements due to their ecdysteroid contents [4] as well as

* Corresponding author: E-Mail: gulsentel@mu.edu.tr; Phone: +90-252-211-1514; Fax: +90-252-211-1472.

accumulate ecdysteroids which have attracted attention because of their physiological function in arthropods and their pharmacological effects in mammals [5].

To the best of our knowledge, the roots of *S. chinensis* and the rhizomes of *S. strangulate* have been used as a folk medicine in China for treatment of chickenpox, toxicosis and high cholesterol since ancient times [6,7]. Therefore, the chemical studies were started to investigate the *Serratula* species. Antioxidant activity studies were carried out on *S. coronota*, and *S. cichoracea* [8,4]. From the *S. strangulate* seven glyceroglycolipids were isolated and these compounds were found to be posses significant antibacterial and antitumor activities [9]. *S. coronota* afforded three novel phytoecdysteroid showing antimicrobial activity [10]. Agonist activity against *Drosophilla melanogaster* of phytoecdysteroids from *S. coronota* [11] and oral aphid tests against *Acyrthosiphon pisum* of ecdysteroids from *S. wolfii* were also reported [12].

Reactive oxygen species can cause oxidative damage to proteins, lipids, enzymes and DNA and they have also been linked to pathogenesis of oxidative diseases [13]. In addition, excess amount of reactive oxygen species causing oxidative stress is also associated with pathology of many diseases including Alzheimer's disease which is a progressive neurological disorder characterized by cognitive deficit and behavioral abnormalities in the patient [14]. Using of antioxidants may reduce the progression of Alzheimer's disease and minimize neuronal degeneration [15]. In fact, the known valid hypothesis being accepted is the lack of in amount of acetylcholine which is a neuromediator [16]. Thus, the acetylcholinesterase inhibitor drugs were used for the treatment of Alzheimer's disease. However, most of these drugs have side effects. Tyrosinase, also known as polyphenol oxidase (PPO), is a multifunctional copper-containing enzyme widely distributed in plants and animals. Tyrosinase inhibitors may therefore be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation [17] and also important in cosmetics for whitening and depigmentation after sunburn [18].

Polyunsaturated fatty acids such as linoleic acid and linolenic acid terming essential fatty acids are essential for human's basal metabolism and have many beneficial effects on human health [19]. Lack of dietary essential fatty acids or their inefficient metabolism has been implicated in aetiology of disease including cardiovascular disease and its progression [20]. Therefore, investigation of the fatty acid content in natural origin has become a topic of great interest.

We aimed to investigate the fatty acid profile of *Serratula erucifolia*, *S. hakkiarica*, *S. lasiocephala* and *S. radiata* subsp. *biebersteiniana* with antioxidant, anticholinesterase and tyrosinase inhibitory activities. The fatty acid compositions, the antioxidant, the anticholinesterase and the tyrosinase inhibitory activities of these four *Serratula* species were investigated for the first time in this study. The objective of this study is to compare the bioactivities above mentioned with those of commercial antioxidants, galantamine and kojik acid, which are commonly used in the food and/or pharmaceutical industries.

2. Materials and Methods

2.1. Chemicals and spectral measurements

Bioactivity measurements were carried out on a 96-well microplate reader, SpectraMax 340PC^{384}, Molecular Devices (USA), at Department of Chemistry, Muğla Sıtkı Koçman University. The measurements and calculations of the activity results were evaluated by using Softmax PRO v5.2 software. GC analyses were performed on a Shimadzu GC-17 AAF, V3, 230 V series gas chromatography (Japan), GC–MS analyses were carried out on Varian Saturn 2100T (USA).

Ethanol, *n*-hexane, methanol, ammonium acetate, copper (II) chloride, ferrous chloride, pyrocatechol, quercetin, ethylenediaminetetraacetic acid (EDTA) and boron trifluoride-methanol complex (BF_3:MeOH) were obtained from E. Merck (Darmstadt, Germany). β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), neocuproine, α-tocopherol, butylatedhydroxyl anisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent (FCR), 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5′,5″-disulfonic acid disodium salt (Ferene), acetyl-cholinesterase from electric eel (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg, Sigma), butyrylcholinesterase from horse serum (BChE, EC 3.1.1.8, 11.4 U/mg, Sigma), 5,5′-dithiobis (2-

nitrobenzoic) acid (DTNB), acetylthiocholine iodide and butyrylthiocholine chloride, galantamine, L-DOPA (3,4-Dihydroxy-D-phenylalanine), kojic acid, tyrosinase from mushroom (EC 232-653-4, 250 KU, ≥1000 U/mg solid, Sigma) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were in analytical grade.

2.2. Plant materials

The species names, endemism information, herbarium numbers, collection localities and dates of four *Serratula* species are listed in Table 1. All species were identified by Dr. Bekir Doğan and Professor Ahmet Duran. Voucher specimens were deposited in the Herbarium of Department of Biology, University of Selçuk, Konya, Turkey.

Table 1. Endemism information, collection dates and localities, and herbarium numbers of studied *Serratula* species.

No	*Serratula* Species	Endemism	Herbarium numbers	Collection localities and dates
1	*S. erucifolia (Linnaeus)* Boriss.	NE	B. Doğan 2137 & A. Duran (KNYA)	Erzurum province, Köprüköy, Eğirmez village at 1635 m altitude on 09st August of 2009
2	*S. hakkiarica* P. H. Davis	E	B. Doğan 2132 & A. Duran (KNYA)	Hakkari province, Cilo mountain, Kırıkdağ, near dez stream at 2210 m altitude on 07st August of 2009
3	*S. lasiocephala* Bornm.	E	B. Doğan 2105 & A. Duran (KNYA)	Antalya province, Gazipasa, Çayıryaka mountain pasture at 1730 m altitude on 30st of June 2009
4	*S. radiata* (Waldst. et Kit.) Bieb. subsp. *biebersteiniana* Iljin ex Grossh.	NE	B.Doğan 2124 & A.Duran (KNYA)	Kars province, Kağızman, Akçay to Cumaçay at 1830 m altitude on 20st of June 2009

2.3. Extraction

Each *Serratula* species were extracted separately with 2.5 L hexane for four times (24 h x 4) at room temperature (25 ^{0}C), filtered and evaporated to dryness *in vacuum*. Then the residue plant materials were similarly extracted, filtered and evaporated by using aqueous methanol solvent, successively.

2.4. Derivation of the hexane extract

The hexane extracts were used for antioxidant, anticholinesterase and tyrosinase inhibitory activities as well as to derivate to methyl ester. The derivatives were analysed by GC and GC–MS.
Briefly the hexane extract (100 mg) was dissolved in 0.5 M NaOH (2 mL) in a 25 mL flask. After the flask was heated by using a water bath (50 ^{o}C), 2 mL BF_3:MeOH was added. The mixture was boiled for 2 minutes, and then left until it cooled down, and then the volume was completed to 25 mL with saturated NaCl solution. Esters were extracted with *n*-hexane; thus, the organic layer was separated. The hexane layer was washed with a potassium bicarbonate solution (4 mL, 2 %) and dried with anhydrous Na_2SO_4 and filtered. The organic solvent was removed under reduced pressure by a rotary evaporator to give methyl esters [21].

2.5. Analysis of the fatty acid

2.5.1. Gas chromatography (GC)

A Flame Ionization Detector (FID) and a DB-1 fused silica capillary non-polar column (30m x 0.25 id., film thickness 0.25 µm) were used for GC analyses of the methyl derivatives of fatty acids. Injector and detector temperatures were 250 and 270 ºC, respectively, Carrier gas was He at a flow

rate of 1.3 mL/min; sample size, 1.0 µL; split ratio, 50:1. The initial oven temperature was held at 100 ºC for 5 min, then increased up to 240 ºC with 3 ºC/min increments and held at this temperature for 10 min. The percentage compositions of the fatty acid methyl derivatives were determined with GC Solution computer program.

2.5.2. Gas chromatography-Mass spectrometry (GC-MS)

An Ion trap mass spectrometer (MS) and a DB-1 MS fused silica non-polar capillary column (30 m x 0.25 mm ID, film thickness 0.25 µm) were used for the GC-MS analyses of the methyl derivatives of fatty acids. For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium (15 psi) at a flow rate of 1.3 mL/min. Injector and MS transfer line temperatures were set at 220 ^{o}C and 290 ^{o}C, respectively. The oven temperature was held at 100^{o}C for 5 min, then increased up to 240 ^{o}C with 3 ^{o}C/min increments and held at this temperature for 10 min. Diluted samples (1/25, w/v, in hexane) of 0.2 µL were injected manually in the split mode. Split ratio was 50:1. EI-MS were taken at 70 eV ionization energy. Mass range was from *m/z* 28 to 650 amu. Scan time 0.5 sec with 0.1 inters scan delays. The library search was carried out using NIST and Wiley 2005 (Gas Chromatography-Mass Spectrometry) GC-MS libraries. Supelco™ 37 components of (Fatty acid Methyl ester) FAME mixture (Catalog no: 47885-U) was used for the comparison of the GC chromatograms.

2.6. Determination of total phenolic content and total flavonoid content

Total phenolic content in all extracts were determined as microgram of pyrocatechol equivalents (PEs), by FCR according to the method of Slinkard and Singleton [22]. Total phenolic contents of the extracts were calculated according to the following equation obtained from standard pyrocatechol graph:

A = 0.0036 [pirokatekol (µg)] + 0.0196 (r^2: 0.9977)

Total flavonoid content of the extract was based on the aluminum chloride method [23] with a slight modification and results were expressed as quercetin equivalents. Total flavonoid content of the extracts was calculated according to following equation obtained from the standard quercetin graph:

A = 0.0068 [quercetine (µg)] + 0.0102 (r^2: 0.9997)

2.7. Determination of antioxidant activity

The antioxidant activity of the extracts was evaluated by four complimentary tests; namely, β-carotene-linoleic acid assay [24], free-radical scavenging activity by DPPH assay [25], Cupric reducing antioxidant capacity assay by neocuproine-Cu^{+} complexation [26], as well as metal chelating activity by the ferrene-Fe^{2+} complexation assay [27].

2.8. Determination of acetylcholinesterase- (AChE) and butyrylcholinesterase- (BChE) inhibitory activity

AChE and BChE inhibitory activities were measured by the spectrophotometric method developed by Ellman et al. [28]. AChE from electric eel and BChE from horse serum were used, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. DTNB (5,50-dithiobis(2-nitrobenzoic)acid was used for the measurement of the anticholinesterase activity.

2.9. Determination of tyrosinase inhibition activity

Tyrosinase enzyme inhibitory activity was measured by the spectrophotometric method as described by Masuda et al. [29]. Mushroom tyrosinase was used, while L-DOPA was employed as substrates of the reaction.

2.10. Statistical analysis

All data on all activity tests were the average of triplicate analyses. The data were recorded as mean ± standard deviation. Significant differences between means were determined by Student's-*t* test, p values <0.05 were regarded as significant.

3. Results and Discussion

3.1. Fatty acid composition

The fatty acid compositions of the four *Serratula* species were given in Table 2. Totally twenty fatty acids were detected by using GC and GC–MS in all species tested herein. The dominants were found to be palmitic acid (23.75–42.88%) and linoleic acid (14.73–22.08%). The oleic acid ratio was found to be between 8.67–19.64%. Linolenic acid was detected only in *S. hakkiarica* (25.14%) and *S. radiata* (12.15%) as well as palmitoleic was only in S. erucifolia (12.57%) and S. radiata (0.89%). The total unsaturated fatty acid percentages were found to be between 27.24% and 50.47%. Stearic acids (4.44–7.68%) was also found in the *Serratula* species. Other fatty acids such as $C_{9:0}$, $C_{10:0}$, $C_{12:0}$, $C_{17:0}$, $C_{20:4}$, $C_{20:1}$, $C_{21:0}$, $C_{22:0}$, $C_{23:0}$ and $C_{24:0}$ were also found in the all species tested, but all of them were in small quantity (less than 4.0% in concentration). The linoleic:oleic acid ratio was between 0.75-2.55 that could provide an important criterion from a chemotaxonomic viewpoint and could be useful for the taxonomical differentiation between species of the same genus.

3.2. Total phenolic and total flavonoid content

Since the phenolic compounds are very important constituents of plants and known as powerful chain-breaking antioxidants [30], total phenolic content of the extracts was investigated and expressed as micrograms of pyrocatechol equivalents (PEs)per milligram of extract, as shown in Table 3. Methanol extracts of *S. lasiocephala* (260.1±0.0 µg PEs per mg extract) and *S. hakkiarica* (184.6±0.0 µg PEs per mg extract) had higher phenolic contents than the other extracts. In general, methanol extracts were more effective than the hexane extracts. Flavonoids are natural phenolic compounds and well known antioxidants and their concentration in the extracts were expressed as micrograms of quercetin equivalents (QEs) per milligram of extract, as shown in Table 3.

The flavonoid contents of the methanol extracts were found to be higher than the hexane extracts, as well. Most flavonoid-rich extracts were found to be the methanol extract of *S. lasiocephala* (60.3±0.0 µg QEs per mg extract).

3.3. Antioxidant activity

Table 4 shows the antioxidant activity of the extracts of four *Serratula* species, compared with BHA and α-tocopherol, by the β-carotene-linoleic acid assay. In this assay the lipid peroxidation inhibition was screened via observation of the β-carotene colour at 470 nm. The methanol extract of *S. lasiocephala* showed the highest lipid peroxidation inhibition activity indicating 89.1±0.7% inhibition at 800 µg/ml concentration followed by, hexane extract of *S. hakkiarica*, and methanol extracts of *S. erucifolia* and *S. hakkiarica*. In general methanol extract of *S. lasiocephala* showed the best activity in all concentrations tested.

Table 2. The fatty acid composition (%) of *Serratula* species.

Fatty acid	*S. hakkiarica*	*S. radiata*	*S. lasiocephala*	*S. erucifolia*
Nonanoic acid ($C_{9:0}$)	-	-	-	0.27
Decanoic acid ($C_{10:0}$)	-	-	0.53	0.62
Dodecanoic acid ($C_{12:0}$)	2.21	3.98	1.82	3.47
Nonanedioic acid	-	-	-	0.54
(*Z*)-11-Tetradecenoic acid ($C_{14:1}$)	-	-	-	-
Myristic acid ($C_{14:0}$)	2.15	0.99	3.5	2.76
Pentadecanoic acid ($C_{15:0}$)	0.48	0.68	1.09	0.74
Palmitoleic acid ($C_{16:1}$)	-	0.89	-	12.57
Palmitic acid ($C_{16:0}$)	**26.48**	**23.75**	**42.88**	**37.25**
Margaric acid ($C_{17:0}$)	-	0.78	1.13	0.53
Linoleic acid ($C_{18:2}$)	**14.97**	**22.08**	**15.35**	**14.73**
Linolenic acid ($C_{18:3}$)	**25.14**	**12.15**	-	-
Oleic acid ($C_{18:1}$)	**9.19**	**8.67**	**11.89**	**19.64**
Stearic acid ($C_{18:0}$)	6.14	6.78	7.68	4.44
10-Methyl octadecanoic acid	0.78	-	-	-
Arachidonic acid ($C_{20:4}$)	0.23	-	-	-
Gondoic acid [(*Z*)-11-eicosenoic acid] ($C_{20:1}$)	0.94	-	-	-
Arachidic acid ($C_{20:0}$)	7.45	11.3	6.53	2.44
Heneicosanoic acid ($C_{21:0}$)	0.93	-	0.43	-
Behenic acid ($C_{22:0}$)	2.68	6.45	3.23	-
Tricosanoic acid ($C_{23:0}$)	-	-	0.51	-
Tetracosanoic acid ($C_{24:0}$)	0.23	1.5	3.43	-
Total saturation	48.75	56.21	72.76	52.52
Total unsaturation	50.47	43.79	27.24	46.94
Others	0.78	0	0	0.54
Saturation/Unsaturation ratio	0.97	1.28	2.67	1.12
Linoleic acid/Oleic acid ratio	1.83	2.55	1.29	0.75

Table 3. Total phenolic and total flavonoid contents of the *Serratula* species [a].

Serratula species	Extracts	Phenolic contents µg PEs/mg extract [b]	Flavonoid contents µg QEs/mg extract [c]
S. erucifolia	Hexane	34.9±0.0	2.4±0.0
	Methanol	85.8±0.0	25.6±0.0
S. hakkiarica	Hexane	40.5±0.0	8.3±0.1
	Methanol	184.6±0.0	24.1±0.0
S. lasiocephala	Hexane	49.2±0.0	4.1±0.0
	Methanol	260.1±0.0	60.3±0.0
S. radiata	Hexane	34.4±0.0	4.9±0.0
	Methanol	119.4±0.0	28.9±0.0

[a] Values expressed are means ± S.D. of three parallel measurements. (p<0.05)
[b] PEs, pyrocatechol equivalents. [c] QEs, quercetin equivalents.

Table 4 also shows the free radical scavenging activity of extracts of four *Serratula* species. Radical scavenging activity increases with increasing the amount of the extracts. In DPPH$^{\bullet}$ assay, the methanol extract of *S. lasiocephala* was found to be the highest activity (84.5±0.1%) followed by methanol extract of *S. hakkiarica* (81.4±0.6%) and the methanol extract of *S. erucifolia* (72.7±1.5%). In general, both assays were supported each other (Table 4).

Table 4. The inhibition (%) of linoleic acid oxidation in β-carotene/linoleic acid assay and the (%) free radical scavenging activity by DPPH assay of extracts of *Serratula* species [a].

Serratula species	Extracts	% Inhibition							
		β-carotene-linoleic acid assay				DPPH Assay			
		100 µg	200 µg	400 µg	800 µg	100 µg	200 µg	400 µg	800 µg
S.erucifolia	Hexane	8.1±1.3	22.4±2.2	39.9±1.4	47.3±0.6	15.2±0.6	16.8±0.7	25.5±0.1	33.1±0.8
	Methanol	55.9±2.1	70.3±1.3	77.1±5.7	84.6±4.1	15.6±0.2	33.8±0.9	56.8±1.2	72.7±1.5
S.hakkiarica	Hexane	26.6±3.3	65.1±1.4	71.6±6.6	87.7±3.4	16.2±1.4	17.5±1.3	23.9±1.3	28.9±2.7
	Methanol	75.1±1.6	78.7±0.2	80.4±2.6	82.6±3.0	26.7±0.9	50.9±1.1	74.2±0.5	81.4±0.6
S. lasiocephala	Hexane	15.1±0.9	30.3±0.5	33.0±1.8	53.2±1.2	14.3±0.1	18.1±1.1	23.9±1.2	28.5±1.9
	Methanol	79.6±0.6	83.8±0.0	86.2±0.4	89.1±0.7	32.8±1.0	66.9±1.6	83.8±0.9	84.5±0.1
S.radiata	Hexane	39.5±0.2	49.4±2.4	57.5±0.6	81.2±1.0	-	-	-	-
	Methanol	42.3±1.7	53.9±0.4	63.3±0.2	68.8±2.5	23.8±1.0	48.9±0.4	64.3±3.0	72.5±0.6
BHA [b]		90.6±0.1	91.8±0.2	92.8±0.0	93.7±0.0	59.0±0.0	79.3±0.5	90.8±0.2	94.1±0.1
α-Tocopherol [b]		87.8±0.4	90.1±0.1	91.6±0.3	93.1±0.5	84.1±0.0	95.9±0.0	96.1±0.9	96.7±0.1

[a] Values expressed are means±S.D. of three parallel measurements. (p<0.05).
[b] Reference compounds.
BHA: Butylatedhydroxyanisole

Table 5 shows the cupric reducing antioxidant capacity of the four *Serratula* species. The results were given as absorbances. Higher absorbance exhibited higher activity. This method is based on the measurement of absorbance at 450 nm by the formation of a stable complex between neocuproine and copper (I), the latter is formed by the reduction of copper (II) in the presence of neocuproine. Increasing activity was seen with increasing amount of the extracts. Among the hexane extracts *S. radiata* exhibited the highest activity. Among the methanol extract, however, *S. lasiocephala* and *S. radiata* were found the higher reductants, respectively.

Table 5 also shows the chelating effects of the extracts of *Serratula* species compared with EDTA as standard on ferrous ions. The difference between the extracts and control was found to be statistically significant (p< 0.05). Metal chelating activity increased with increasing concentration of the extracts. The hexane extract of *S. radiata* (72.0±0.5%) at 0.8 mg/mL showed the highest metal chelating activity among the extracts studied. However, none of the extracts have comparable results with that of EDTA.

Table 5. The cupric reducing antioxidant capacity (CUPRAC) by Cu^{2+} Cu^{+} transformation and metal chelating activity (Inhibition %) by Ferrene–Fe^{2+} assays of the extracts of *Serratula* species [a].

Serratula species	Extracts	Absorbance				Inhibition %			
		CUPRAC Assay				Metal Chelating Activity			
		100 µg	200 µg	400 µg	800 µg	100 µg	200 µg	400 µg	800 µg
S. erucifolia	Hexane	0.2±0.0	0.4±0.0	0.6±0.0	1.0±0.0	8.7±6.6	53.8±0.9	63.2±0.4	63.9±1.1
	Methanol	0.3±0.0	0.5±0.0	0.9±0.1	1.5±0.1	34.2±0.9	40.6±0.2	45.8±0.6	51.8±1.8
S. hakkiarica	Hexane	0.3±0.0	0.4±0.0	0.7±0.0	1.0±0.0	-	20.2±5.7	50.1±2.6	54.1±0.8
	Methanol	0.6±0.1	0.9±0.1	1.5±0.0	2.2±0.1	32.1±0.6	35.8±0.2	38.4±1.9	41.9±0.9
S. lasiocephala	Hexane	0.2±0.0	0.3±0.0	0.5±0.0	0.7±0.0	16.0±1.4	43.3±0.0	60.8±0.4	65.0±0.1
	Methanol	0.9±0.0	1.4±0.0	2.2±0.1	3.1±0.0	34.0±1.3	36.2±2.9	39.0±0.4	47.4±6.7
S. radiata	Hexane	0.4±0.0	0.7±0.1	1.4±0.2	2.3±0.1	43.1±0.2	57.7±1.6	63.3±0.2	72.0±0.5
	Methanol	0.5±0.0	0.9±0.1	1.3±0.2	2.5±0.1	-	2.1±0.5	4.8±0.6	20.4±1.3
BHA [b]		0.1±0.0	3.5±0.0	3.7±0.0	3.8±0.0	NT	NT	NT	NT
α-Tocopherol [b]		0.1±0.0	1.9±0.1	2.2±0.1	2.9±0.0	NT	NT	NT	NT
EDTA [b]		NT	NT	NT	NT	92.5±1.4	94.7±0.6	95.2±0.1	96.3±0.1

[a] Values expressed are means±S.D. of three parallel measurements. (p<0.05).
[b] Reference compounds.
BHA: Butylatedhydroxyanisole, EDTA: Ethylenediamine tetraacetic acid, NT: not tested.

3.4. Anticholinesterase activity

Table 6 shows the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the extracts, compared with that of galantamine used as a standard drug for the treatment of mild Alzheimer's disease. The tests were carried out spectrophotometrically in a 96 well plate microplate reader at 25, 50, 100 and 200 µg/mL concentrations. Among them against AChE enzyme, the hexane extracts of *S. radiata* (IC_{50}: 52.9±1.1 µg/mL) was found to be significant. Against BChE enzyme, the most active extract was also found to be the hexane extract of *S. radiata* (IC_{50}: 147.5±1.8 µg/mL), as well.

The hexane and methanol extracts of *S. lasiocephala* also demonstrated inhibitory activity. Against BChE enzyme, the IC_{50} values of last two extracts were found be 148.4±1.3 and 195.4±2.3 µg/mL, respectively. In general, the extracts exhibited better activity against BChE enzyme. Hence, these species, particularly *S. radiata* due to the demonstrating inhibitory activity against both AChE and BChE, may be useful as moderate anticholinesterase agents.

Table 6. Acetyl-, and butyryl-cholinesterase inhibitory activities of extracts of *Serratula* species [a].

No	*Serratula* species	Extract	Anticholinesterase assay							
			AChE Assay				BChE Assay			
			25µg	50 µg	100 µg	200 µg	25µg	50 µg	100 µg	200 µg
1	*S. erucifolia*	Hexane	-	-	5.9±0.1	25.4±0.8	14.1±0.8	16.0±2.0	28.8±2.3	36.6±1.5
		Methanol	19.2±0.3	28.1±0.4	32.1±2.2	33.5±4.6	5.1±1.3	19.4±0.2	26.7±0.6	38.4±0.8
2	*S. hakkiarica*	Hexane	-	-	1.4±0.5	6.4±1.8	13.7±0.9	17.1±0.6	18.2±0.1	19.1±0.0
		Methanol	20.1±0.3	21.7±0.9	25.3±0.1	31.3±0.7	9.8±0.7	12.7±1.7	25.5±3.5	32.4±1.1
3	*S. lasiocephala*	Hexane	-	-	5.1±0.9	7.3±1.4	21.4±2.2	28.7±1.4	40.8±3.6	60.7±1.9
		Methanol	19.9±0.2	24.5±0.6	25.1±0.9	32.7±0.3	18.2±1.6	24.8±2.2	35.9±0.6	49.6±0.4
4	*S. radiata*	Hexane	42.9±1.2	49.7±0.9	61.5±0.1	81.9±3.6	8.6±2.7	16.5±1.9	35.9±3.7	66.9±0.4
		Methanol	-	-	-	-	2.5±0.7	8.1±0.7	24.4±0.8	27.2±1.5
	Galantamine [b]		68.4±1.1	74.4±0.7	78.6±0.5	81.4±1.0	40.6±2.9	48.7±0.9	65.0±0.4	75.5±1.1

[a] Values expressed are means±S.D. of three parallel measurements. (p<0.05)
[b] Reference compounds.

3.5. Tyrosinase inhibitory activity

Tyrosinase inhibitory activity of *Serratula* species were investigated *in vitro* spectrophotometrically by using L-DOPA as a substrate. Table 7 shows the tyrosinase inhibitory activity of the extracts of *Serratula* species, comparing with that of kojic acid. According to Table 7 the only hexane extract of *S. erucifolia* and methanol extract of *S. radiata* exhibited tyrosinase inhibition activity.

Table 7. Tyrosinase inhibitory activity of extracts of *Serratula* species [a].

Serratula species	Tyrosinase inhibitory activity							
	Hexane Extracts				Methanol Extracts			
	25µg	50 µg	100 µg	200 µg	25µg	50 µg	100 µg	200 µg
S.erucifolia	-	-	2.90±2.5	2.99±3.2	-	-	-	-
S.hakkiarica	-	-	-	-	-	-	-	-
S.lasiocephala	-	-	-	-	-	-	-	-
S.radiata	-	-	-	-	-	1.9±5.6	21.7±4.7	26.2±6.6
Kojic acid [b]	36.9±2.6	57.1±3.2	79.8±0.6	83.6±0.2				

[a] Values expressed are means±S.D. of three parallel measurements. (p<0.05)
[b] Reference compounds.

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