SHORT REPORT

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Determination of Antioxidant, Anticholinesterase, Tyrosinase Inhibitory Activities and Fatty Acid Profiles of 10 Anatolian *Klasea* Cass. Species

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Abstract: In search of new natural fatty acid sources, extract of 10 different Turkish *Klasea* species were studies. Fatty acids of *Klasea* species were studied by GC and GC-MSD. Oleic acid (4.8-45.8%), palmitic acid (15.6-51.8%), linoleic acid (0.3-45.5%), palmitoleic acid (0.8-28.4%) and linolenic acid (15.6-34.6%) were the main fatty acids elucidated. All extracts were also subjected to acetylcholinesterase, butyrylcholinesterase, tyrosinase, β-carotene-linoleic acid, DPPH$^{\bullet}$ scavenging, CUPRAC and ferrous ion-chelating ability activities. Total flavonoid and phenolic contents were determined as quercetin and pyrocatechol equivalents. All extracts showed significant antioxidant activity in all tests, except hexane extracts of *K. serratuloides* and *K. cerinthifolia* that showed weak inhibition against BChE and AChE. The hexane extract of *K. coriaceae* and methanol extract of *K. serratuloides* exhibited notable tyrosinase inhibitory activity.

Keywords: *Klasea* species; tyrosinase inhibitory activity; anticholinesterase activity; antioxidant activity; phenolic and flavonoid contents; fatty acids.

1. Plant Source

Ten Anatolian *Klasea* Cass. (Asteraceae) species were collected and identified by Professor Ahmet Duran and Dr. Bekir Doğan. Voucher specimens were deposited at the Herbarium of Department of Biology, Selçuk University, Konya, Turkey. The collection dates, collection locations as well as voucher numbers of these species were given in Table 1.

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2. Previous Study

New classification of genus *Serratula* counts species understudy in genus *Klasea* (formerly a section of *Serratula* genus) [1] that represents 16 species; 5 of which are endemic to Turkey [2]. Due to their ecdysteroid contents, *Serratula* species are considered as food supplement and source of herbal remedies [3] that plays pharmacological effects in mammals and physiological functions in arthropods [4]. Rhizomes of *S. strangulate and S. chinensis* (as whole) have been used to treat toxicosis, chickenpox, high cholesterol since long in China [5,6].

Table 1. Endemism, voucher numbers, collection dates and localities of the studied *Klasea* species.

***Klasea* Species**	**Endemism**	**Herbarium numbers**	**Collection localities and dates**
K. bornmuelleri (Azn.) Greuter & Wagenitz	E	B. Doğan 2110 & A. Duran (KNYA)	Malatya, Darende near Akçatoprak, 1010 m 17st of July 2009
K. cerinthifolia (Sm.) Greuter & Wagenitz	NE	B. Doğan 2107 & A. Duran (KNYA)	Kahramanmaraş province, Ahir mountain on 990 m altitude on 16th July 2009
K. coriaceae (Fisch. & C. A. Mey. ex DC.) Holub	NE	B. Doğan 2122 & A. Duran (KNYA)	Kars province, Tuzluca to Kağızman at 1055 m altitude on 20st July of 2009
K. grandifolia (P. H. Davis*)* Greuter & Wagenitz	NE	B. Doğan 2130 & A. Duran (KNYA)	Antalya province, Akseki, Süleymanlı village at 1425 m altitude on 31st July of 2009
K. haussknechtii (Boiss.) Holub	NE	B. Doğan 2113 (KNYA)	Muş province, Malazgirt, Karıncalı village at 1840 m altitude on 18st July of 2009
K. kotschyi (Boiss.) Greuter & Wagenitz	NE	B. Doğan 2131 & A. Duran (KNYA)	Bitlis province, Tatvan, Sapur village at 1965 m altitude on 06st August of 2009
K. kurdica (Post) Greuter & Wagenitz	NE	B. Doğan 2106 & A. Duran (KNYA)	Osmaniye province, Yarpuz at 1465 m altitude on 15st July of 2009
K. oligocephala (DC.) Greuter & Wagenitz	NE	B. Doğan 2108 & A. Duran (KNYA)	Kahramanmaraş province, Ahir mountain on 990 m altitude on 16th June 2009
K. quinquefolia (M. Bieb. ex Willd.) Greuter & Wagenitz.	NE	B. Doğan 2139 & A. Duran (KNYA)	Artvin, Ardanuç, Boyalı village at 1210 m on 11st of August 2009
K. serratuloides (DC.) Greuter & Wagenitz	NE	B. Doğan 2117 & A. Duran (KNYA)	Van province, Van to Gurpinar at 2125 m altitude on 15st of July 2009

E: endemic; NE: not endemic

Alzheimer’s disease is a progressive neurological disorder that causes behavioral abnormalities and cognitive deficit [7], and the using of antioxidants can prevent Alzheimer’s progress and neuronal degeneration [8]. The widely accepted hypothesis for the primary cause of Alzheimer's disease is the lack of acetylcholine; and thus the disease has been treated by using acetylcholinesterase inhibitory drugs [9]. Nevertheless, some of these drugs have side effects. Tyrosinase inhibitors are widely used to treat some melanin hyper-pigmentation related dermatological disorders. They are also used in cosmetics for whitening and de-pigmentation sunburns [10,11].

Linoleic acid and linolenic acid are essential for our basal metabolism including other health benefits [12]. Lack of essential fatty acids in diet may cause cardiovascular diseases [13]; that awake a need to find out new natural sources of fatty acids. So far, there is no antioxidant, tyrosinase, anticholinesterase, and fatty acid composition studies of *Klasea* species available in the literature.

3. Present study

Twenty extracts were prepared from the aerial parts of ten *Klasea* species. These extracts were subjected to anticholinesterase, antioxidant, and tyrosinase inhibitory activities. In addition, total flavonoids and phenolics were also determined as quercetin and pyrocatechol equivalents, respectively. The fatty acid profiles were studied by GC and GC-MSD. Biological activities of the extracts of *Klasea* species were compared with those of standards used in pharmaceutical and food industries.

Among the hexane extracts *K. oligocephala* (78.7±0.1 µg PEs/mg extract) showed the highest phenolic content followed by *K. serratuloides* (54.6±0.1 µg PEs/mg extract) and *K. coriaceae* (50.7±0.1 µg PEs/mg extract) (Figure 1). *K. bornmuelleri* (261.2±0.1 µg PEs/mg extract) was the richest in phenolics followed by *K. kotschyi* (256.9±0.1 µg PEs/mg extract). *K. haussknechtii* (32.6±0.1 µg PEs/mg extract) showed the least content.

The highest flavonoid amount was observed in methanol extract of K. cerinthifolia (58.4±0.1 µg QEs/mg extract) followed by K. oligocephala (56.4±0.1 µg QEs/mg extract) and K. grandifolia (39.4±0.1 µg QEs/mg extract). The hexane extract of K. oligocephala (3.4±0.0 µg QEs/mg extract) exhibited the least flavonoid content among the all.

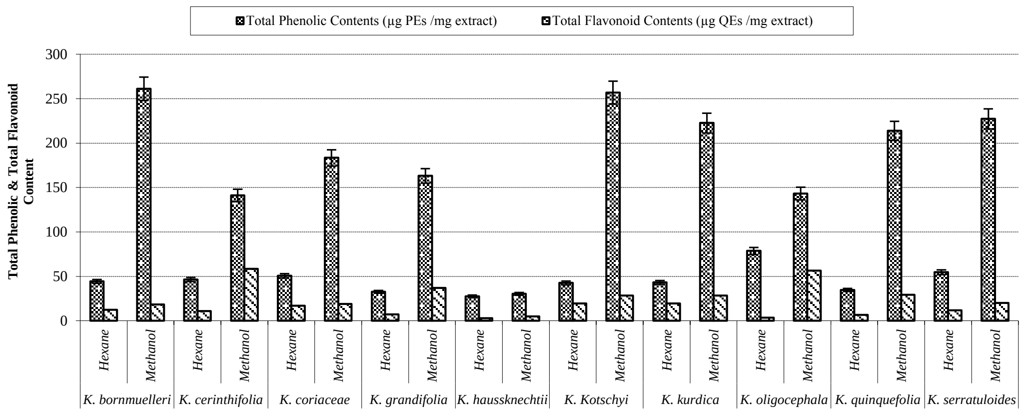

Figure 1. Total phenolic and total flavonoid contents of the *Klasea* species PEs: pyrocatechol equivalents. QEs: quercetin equivalents. Values are mean ± S.E.M, $n = 3$. $p < 0.05$, significantly different with student's *t*-test when compared with the control.

All hexane and methanol extracts were screened for their antioxidant activity using β-carotene-linoleic acid, DPPH, CUPRAC and metal chelating assays, and compared with those of α-tocopherol, BHA and EDTA (Table S1 and S2).

Klasea species demonstrated good lipid peroxidation inhibition activity performed by β-carotene-linoleic acid assay (Table S1). Hexane extracts *K. quinquefolia* and *K. haussknectii* showed higher activity, followed by *K. kurdica* and *K. kotschyi*. Methanol extracts showed better activities than hexanes due to the presence of flavonoids and phenolics [14]. At 100 µg/mL concentrations, methanol extracts of *K. cerinthifolia* (82.9±1.1%), *K. haussknechtii* (77.9±0.6%) and *K. grandifolia* (77.6±2.1%), *K. oligocephala* (73.2±1.6%), *K. coriaceae* (66.5±1.6%), and *K. kurdica* (51.9±4.1%) showed higher lipid peroxidation inhibition activity.

DPPH radical is very stable molecule and used to determine free radical scavenging activity of natural compounds [15]. As shown in Table S1, the activity increased with increasing the amount of the extracts (Table S1). The difference between the control and *Klasea* extracts was statistically significant ($p < 0.05$). The hexane extracts demonstrated less activity in this assay. However, methanol extracts of *K. kurdica*, *K. coriaceae* and *K. cerinthifolia* showed higher DPPH$^{\bullet}$ scavenging activity; higher than that of BHA and close to that of α-tocopherol at all studied concentrations.

The cupric reducing antioxidant capacity (CUPRAC) of ten *Klasea* species are given in Table S2. Activity was increased with increase of extracts amount. Hexane extracts of *K. haussknectii* and *K. kotschyi* exhibited better activity, while *K. kurdica*, *K. bornmuelleri*, *K. kotschyi*, *K. quinquefolia*, *K. coriaceae* and *K. serratuloides* extracts of methanol were higher reductants, respectively.

Table 2. The fatty acids compositions (%) of 10 *Klasea* species.

Fatty acids	***K. kotschyi***	***K. oligocephala***	***K. kurdica***	***K. grandifolia***	***K. haussknechtii***	***K. serratuloides***	***K. cerinthifolia***	***K. bornmuelleri***	***K. quinquefolia***	***K. coriaceae***
Nonanoic acid ($C_{9:0}$)	-	-	-	1.9	-	-	-	-	-	-
Decanoic acid ($C_{10:0}$)	-	-	-	-	-	-	0.8	-	-	-
Dodecanoic acid ($C_{12:0}$)	1.2	0.4	1.3	-	-	4.5	1.4	1.8	7.5	-
Nonanedioic acid	-	-	-	-	5.2	-	0.3	-	-	-
(*Z*)-11-Tetradecenoic acid ($C_{14:1}$)	-	-	-	1.4	-	-	-	-	-	-
Myristic acid ($C_{14:0}$)	2.2	0.9	1.2	4.3	1.2	4.0	3.1	4.0	3.2	4.6
Pentadecanoic acid ($C_{15:0}$)	0.4	0.3	-	0.2	-	-	0.6	-	-	1.1
Palmitoleic acid ($C_{16:1}$)	0.8	2.6	**10.5**	**28.4**	**11.2**	6.6	4.5	-	-	-
Palmitic acid ($C_{16:0}$)	**24.4**	**15.6**	**20.2**	**20.9**	**17.4**	**45.3**	**32.5**	**29.2**	**35.5**	**51.8**
Margaric acid ($C_{17:0}$)	0.8	0.3	-	0.3	-	-	0.7	-	-	1.2
Linoleic acid ($C_{18:2}$)	**17.2**	**45.5**	**18.3**	**10.1**	**16.4**	**17.7**	**21.0**	11.9	0.3	0.3
Linolenic acid ($C_{18:3}$)	**25.5**	-	-	-	-	**15.6**	-	**16.4**	**34.6**	**25.9**
Oleic acid ($C_{18:1}$)	7.5	**26.9**	**43.9**	**24.6**	**45.8**	6.4	**18.1**	**14.1**	4.8	**9.5**
Stearic acid ($C_{18:0}$)	5.9	-	4.7	4.3	1.7	-	5.6	5.7	**10.2**	5.7
10-Methyl octadecanoic acid	-	**4.2**	-	-	-	-	-	-	-	-
Arachidonic acid ($C_{20:4}$)	0.4	-	-	-	-	-	-	-	-	-
Gondoic acid [(*Z*)-11-eicosenoic acid] ($C_{20:1}$)	-	-	-	0.3	-	-	-	-	-	-
Arachidic acid ($C_{20:0}$)	5.9	1.6	-	2.2	1.0	-	**9.0**	2.0	3.4	-
Heneicosanoic acid ($C_{21:0}$)	0.5	0.3	-	-	-	-	-	-	-	-
Behenic acid ($C_{22:0}$)	5.0	1.2	-	1.2	-	-	2.3	**15.1**	0.7	-
Tricosanoic acid ($C_{23:0}$)	0.3	-	-	-	-	-	-	-	-	-
Tetracosanoic acid ($C_{24:0}$)	2.1	0.4	-	-	-	-	0.2	-	-	-
Total saturation	48.7	20.9	27.3	35.2	21.3	53.7	56.1	57.7	60.4	64.4
Total unsaturation	51.3	74.9	72.7	64.8	73.4	46.3	43.6	42.3	39.6	35.6
Others	0	4.2	0	0	5.2	0	0.3	0	0	0
Saturation/Unsaturation ratio	0.9	0.3	0.4	0.5	0.3	1.2	1.3	1.4	1.5	1.8
Linoleic acid/Oleic acid ratio	2.3	1.7	0.4	0.4	0.4	2.8	1.2	0.8	0.1	0.03

Metal chelating activity of the extracts was performed on ferrous ions and compared with that of EDTA used as a standard (Table S2). Hexane extract of *K. oligocephala* (74.9±0.7%) showed the highest activity at 0.8 mg/mL followed by hexane extract of *K. grandifolia* (69.7±0.7%) at same concentration. Methanol extracts showed less activity than those of hexane extracts in this assay.

The butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) inhibitory activities of the extracts and galantamine are given in Table S3. Hexane extracts of *K. serratuloides* (IC_{50}: 134.7±2.1 µg/mL) showed significant activity against AChE.

Extracts of *Klasea* species were also investigated for tyrosinase inhibitory activity by L-DOPA [16] and compared with that of kojic acid (Table S3). Hexane extract of *K. coriaceae* showed the highest activity (48.2±4.1%). The methanol extracts of *K. coriaceae*, *K. bornmuelleri*, *K. serratuloides*, *K. kurdica* and *K. quinquefolia* reflected moderate inhibitory activity on tyrosinase enzyme.

22 compounds were detected —where 20 were fatty acids —using GC-FID and GC-MSD from all studied *Klasea* species (Table 2). Major fatty acids were palmitic acid (15.6-51.8%), oleic acid (4.8-45.8%) and linoleic acid (10.1-45.5%). Total unsaturated fatty acid percentages were in the range of 35.6–74.9%. Stearic (1.7-10.2%) and palmitoleic (0.8-28.4%) acids were also observed in hexane extracts. Interestingly, arachidic ($C_{20:0}$), dodecanoic ($C_{12:0}$) and behenic ($C_{22:0}$) acids that are unusual in plant kingdom were also detected. The concentration of dodecanoic acid ($C_{12:0}$) for *K. quinquefolia*, and behenic acid for *K. bornmuelleri* were high. Similarly the arachidonic acid concentration of *K. cerinthifolia* was also higher, even more than 5% of the crude oil extract. Fatty acids i.e. $C_{9:0}$, $C_{10:0}$, $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{21:0}$, $C_{23:0}$ and $C_{24:0}$ were also detected in low quantities (less than 5.0% in concentration) [14]. Linoleic, linolenic, oleic, palmitic and palmitoleic acid were found as the main fatty acids in the most of *Klasea* species (Table 2). Five species of *Klasea* were found with higher concentrations of unsaturated fatty acids; more than 51 % of the total fatty acids. During the analysis, total saturated fatty acid composition was found between 20.9 and 64.4%, while unsaturated fatty acid composition was as 35.6–74.9%.

Few studies considered linolenic:linoleic acid ratio as an important chemo-taxonomic criterion among species of the same genus [17]. However, linolenic acid was undetected in 5 species out of 10. Therefore, linoleic:oleic acid ratio is considered important criterion from chemo-taxonomic point of view (Table 2).

Long story short, 10 species of *Klasea* were found as new natural sources of fatty acids. These species are also found as good sources of antioxidants. The oleic acid rich extracts demonstrate good cholinesterase inhibitory activity [18]; however, the origin of the activity is not be only related with the presence of this compound. Further phytochemical studies are needed to reach the molecular level to understand the origin of these activities. Particularly, major constituents should be tested for their antioxidant, anticholinesterase and tyrosinase inhibitory activities.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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