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Essential Oil Composition, Antioxidant, Anticholinesterase and Anti-tyrosinase Activities of Two Turkish Plant Species: *Ferula elaeochytris* and *Sideritis stricta*

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The aim of the present study is to characterize chemical compositions and antioxidant, anticholinesterase and anti-tyrosinase activities of *Ferula elaeochytris* and *Sideritis stricta* essential oils. The hydrodistilled essential oils were analyzed by GC/FID and GC/MS. A total of thirty-three and twenty-seven compounds representing 99.6 % and 99.4 % were identified in *F. elaeochytris* and *S. stricta*, respectively. The main compounds of essential oil of *F. elaeochytris* were *β*-cubebene (21.3 %), caryophyllene oxide (17.5 %) and *β*-caryophyllene (14.9 %), while the major compounds of *S. stricta* essential oil were *δ*-cadinene (18.3 %), cubenol (17.6 %) and *β*-caryophyllene (14.4 %). The antioxidant activity was tested by *β*-carotene-linoleic acid, DPPH free radical scavenging, ABTS cation radical scavenging, CUPRAC and metal chelating assays. The essential oil of *F. elaeochytris* showed the highest antioxidant activity in all assays. Also, the anticholinesterase and anti-tyrosinase activities of essential oils were performed against acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase enzymes. *F. elaeochytris* essential oil indicated the highest anticholinesterase and anti-tyrosinase activities as well. This is the first report describing antioxidant, anticholinesterase and anti-tyrosinase activities of essential oils of *F. elaeochytris* and *S. stricta.*

Keywords: *Ferula elaeochytris*, *Sideritis stricta*, Essential oil, Antioxidant activity, Anticholinesterase activity, Anti-tyrosinase activity.

Essential oils (EOs) are aromatic and volatile liquids generally extracted by steam or hydro-distillation from flowers, seeds, leaves, stems, bark and roots of plants. The chemical composition of essential oils is generally composed of terpenes and a few classes of phenolic compounds [1]. EOs are considered as secondary metabolites due to their antimicrobial, antifungal, anti-ulcer, anthelminthic, antioxidant, anti-inflammatory, repellent, insecticidal, antifeedant, cytotoxic, antiviral, ovicidal, anesthetic, molluscicidal, immunomodulatory, antinociceptive and larvicidal properties [2-16]. Therefore, EOs have been used in the pharmaceutical, cosmetic and agricultural industries since ancient times. Also, EOs have been served as food additives in the food industry as spices and herbs [17].

The *Ferula* genus, belonging to Apiaceae family, composed of 170 species and widely distributed in Central Asia and Mediterranean [18]. *Ferula* species are named as 'Çakşır', 'Çakşır otu' or 'Çaşır' in Turkey [19]. In traditional medicine, the roots and leaves of *Ferula* species are used as antispasmodic, diuretic, anticonvulsant, carminative, aphrodisiac, anthelminthic, anti-hysteric, tonic, laxative and decongestant to treat various diseases [18]. The chemical composition and biological activities of this species were studied by many research groups and sesquiterpenes and sulphur compounds showing anti-inflammatory, anti-tumor, anti-angiogenic and cancer chemopreventive properties have been isolated [20-22].

The *Sideritis* genus, a member of the Lamiaceae family, consists of more than 150 species in the world. This species is mainly grown in temperate and tropical regions of the Northern Hemisphere and Turkey and Spain which have the highest number of different species [23]. *Sideritis* species are known as 'dağ çayı' and 'yayla çayı' and the aerial parts of these species are consumed as the herbal and traditional tea in Turkey [24]. To date, many extracts, essential oils, and compounds such as terpenes, flavonoids, coumarins, sterols, iridoids and lignans with therapeutic properties such as anti-inflammatory, analgesic, antioxidant, antimicrobial, antiviral and anti-ulcer effects have been reported from *Sideritis* species [25].

Nowadays, interest in exploring of alternative natural food additives sources such as plant extracts and essential oils has become popular. In this study, chemical compositions of the essential oils of *F. elaeochytris* and *S. stricta* were identified by GC/FID and GC/MS. Antioxidant, cholinesterase and tyrosinase enzyme inhibitory activities of essential oils were investigated. This is the first study on the bioactivities of the essential oils in details. GC/FID and GC/MS techniques were used to investigate chemical compositions of the essential oils of *F. elaeochytris* and *S. stricta*. The chemical compositions of the essential oils, relative percentages (%) and Kovats index of compounds are presented in Table 1.

A total of thirty-three compounds were found in the essential oil of *F. elaeochytris*, representing about 99.6 % of total oil. *β*-cubebene (21.3 %), caryophyllene oxide (17.5 %), *β*-caryophyllene (14.9 %) and *δ*-cadinene (13.0 %) were major compounds in the essential oil. The highest quantitative classified components were found as sesquiterpene hydrocarbons (62.5 %) and sesquiterpenoids (33.7 %) in the essential oil. In a previous investigation, the essential oil composition of *F. elaeochytris* collected from Konya was studied and nonane (27.1 %), *α*-pinene (12.7 %) and germacrene-B (10.3 %) were recorded as main compounds [26].

Twenty-seven components were identified in the essential oil of *S. stricta*, representing 99.4 % of the total oil with *δ*-cadinene (18.3 %), cubenol (17.6 %), *β*-caryophyllene (14.4 %) and caryophyllene oxide (10.5 %) as major components. The most abundant compounds in the essential oil were sesquiterpene hydrocarbons (55.9 %) and oxygenated sesquiterpenes (37.9 %), respectively. The

Table 1: Chemical composition of the essential oils of *F. elaeochytris* and *S. stricta*

No	Compounds	RI[a]	LRI[b]	*F. elaeochytris* (%[c])	*S. stricta* (%[c])	Identification Methods
1	*α*-Pinene	930	939	0.1	-	Co-GC, MS, RI
2	*β*-Pinene	972	979	0.1	-	Co-GC, MS, RI
3	*β*-Phellandrene	1025	1025	*tr*	-	MS, RI
4	τ-Terpinene	1046	1048	-	0.1	MS, RI
5	Terpinolene	1084	1086	*tr*	-	Co-GC, MS, RI
6	*cis*-Verbenol	1140	1089	0.8	-	Co-GC, MS, RI
7	*α*-Terpineol	1178	1176	-	0.1	Co-GC, MS, RI
8	*p*-Cymene-8-ol	1179	1179	0.1	-	MS, RI
9	Borneol	1189	1169	0.1	-	Co-GC, MS, RI
10	Verbenone	1205	1210	0.3	-	MS, RI
11	Myrtenal	1216	1194	0.1	-	Co-GC, MS, RI
12	Bornyl acetate	1270	1289	0.6	2.8	Co-GC, MS, RI
13	Eugenol	1338	1356	-	0.1	MS, RI
14	*α*-Cubebene	1347	1355	4.6	0.6	MS, RI
15	*α*-Copaene	1371	1379	0.1	0.6	Co-GC, MS, RI
16	*β*-Bourbonene	1381	1386	0.1	0.1	Co-GC, MS, RI
17	*β*-Elemene	1390	1389	*tr*	-	MS, RI
18	*β*-Cubebene	1394	1390	21.3	1.9	MS, RI
19	*α*-Gurjunene	1408	1413	-	0.7	MS, RI
20	*β*-Caryophyllene	1424	1421	14.9	14.4	Co-GC, MS, RI
21	Aromadendrene	1441	1439	0.1	-	MS, RI
22	τ-Elemene	1442	1445	-	0.1	MS, RI
23	*β*-Farnesene	1450	1440	-	3.9	MS, RI
24	*α*-Humulene	1458	1452	3.6	*tr*	Co-GC, MS, RI
25	Alloaramadendrene	1460	1465	2.0	*tr*	Co-GC, MS, RI
26	Germacrene D	1474	1479	*tr*	6.1	MS, RI
27	β-Ionone	1479	1484	0.1	-	MS, RI
28	*β*-Guaiene	1482	1486	2.2	2.3	MS, RI
29	τ-Gurjunene	1487	1492	0.1	4.6	MS, RI
30	*α*-Muurolene	1495	1496	0.5	2.3	MS, RI
31	*α*-Selinene	1498	1498	*tr*	-	MS, RI
32	*δ*-Cadinene	1512	1522	13.0	18.3	Co-GC, MS, RI
33	Spathulenol	1576	1572	3.4	2.2	Co-GC, MS, RI
34	Caryophyllene oxide	1580	1578	17.5	10.5	Co-GC, MS, RI
35	Ledol	1590	1592	-	0.4	MS, RI
36	Viridiflorol	1596	1602	-	*tr*	MS, RI
37	Aromadendrene oxide	1598	1604	2.8	0.1	MS, RI
38	*δ*-Cadinol	1608	1608	1.5	7.1	MS, RI
39	T-Cadinol	1620	1625	1.9	-	MS, RI
40	Cubenol	1621	1605	-	17.6	MS, RI
41	Hexahydrofarnesyl acetone	1833	1843	-	2.5	MS, RI
42	Ledene oxide	1865	1871	6.6	-	MS, RI
43	Phytol	2100	1942	1.1	-	MS, RI
	Monoterpene hydrocarbons			0.2	0.1	
	Monoterpenoids			2.0	3.0	
	Sesquiterpene hydrocarbons			62.5	55.9	
	Sesquiterpenoids			33.7	37.9	
	Others			1.2	2.5	
	Total identified (%)			99.6	99.4	

[a]: Retention indices on DB–5 fused silica column, , [b]: Retention indices of literature on DB-5 column [30],[c]: Percentage concentration, [Co-GC]: Co-injection with authentic compounds, [RI]: Retention Index literature comparison, [tr]: trace.

chemical composition of the essential oil of *S. stricta* collected from Antalya, Turkey was studied by Kirimer *et al.* [27]. *β*-pinene (30.0 %), *α*-pinene (12.9 %), *β*-caryophyllene (9.6 %) and *epi*-cubebol (9.6 %) were found as major compounds and sesquiterpenoids (26.6 %) were also reported as the most abundant compounds. In a different study, *β*-pinene (21-48 %) and *α*-pinene (7-24 %) were identified as the major compounds in the essential oil of *S. stricta* [28]. Location of collection area, climatic conditions, and genetic factors cause these differences in the essential oil composition of *F. elaeochytris* and *S. stricta* [29].

The antioxidant activities related to the contents of essential oils of *F. elaeochytris* and *S. stricta* were evaluated by *β*-carotene-linoleic acid, DPPH free radical scavenging, ABTS cation radical scavenging, CUPRAC and metal chelating assays. The results are summarized in Table 2. The lipid peroxidation inhibition abilities of the essential oils were determined by *β*-carotene-linoleic acid assay. In the assay, *F. elaeochytris* essential oil (40.6±0.4 %) showed higher lipid peroxidation inhibition activity than *S. stricta* essential oil (28.6±0.1 %).

The essential oil of *F. elaeochytris* exhibited a higher radical scavenging activity (14.3±0.4 % and 23.9±0.7 %) than *S. stricta* essential oil in $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, respectively.

According to obtained results in CUPRAC assay, the essential oil of *F. elaeochytris* (0.3±0.02 Absorbance) was found to be higher reductant than the essential oil of *S. stricta* (0.1±0.00 Absorbance).

Both studied essential oils showed low ability to chelate ferrous ions (9.2±0.2 % for *F. elaeochytris* and 6.8±0.3 % for *S. stricta*).

Although *Ferula* and *Sideritis* genus have more than 170 and 150 species in worldwide, respectively, studies on chemical composition and biological properties of their essential oils are limited. Amanzadeh *et al*. [31] studied the essential composition and antioxidant activity of *Lallemanita iberica*, *β*-cubebene and *β*-caryophyllene were reported as major compounds. Major compounds (*β*-cubebene, caryophyllene oxide, *β*-caryophyllene) and antioxidant properties of *F. elaeochytris* essential oil are similiar to *L. iberica* essential oil. Antioxidant activities and major compounds of *S. stricta* (*δ*-cadinene, cubenol and *β*-caryophyllene) and *Annona muricata* (*δ*-cadinene, *β*-caryophyllene) resemble. The results are consistent with the literature. Low or moderate antioxidant activity can be explained by the fact that the essential oils of both species are rich in sesquiterpenes [32]. To the best of our knowledge, no work has been carried out on the antioxidant activities of the essential oils of studied species.

As it seen from Table 3, the essential oil of *F. elaeochytris* (20.8±0.2 %) and *S. stricta* essential oil (17.7±0.2 %) showed low inhibitory activity against AChE. Also, *F. elaeochytris* essential oil exhibited moderate inhibitory activity against BChE (54.9±0.4 % at 200 µg/mL).

Anticholinesterase activities of the essential oils of *S. galatica, F. lutea*, and *F. communis* have been previously studied. AChE (IC_{50}: 0.618 mg/mL) and BChE (IC_{50}: 0.632 mg/mL) inhibition of the essential oil of *S. galatica* were found to be weak [33]. *F. communis* essential oil was reported to show potent inhibitory activity against BChE (64.623 % at 10 mg/mL) [34]. In the study of Znati *et al.* [35], *F. lutea* exhibited high inhibitory activity against AChE (IC_{50}: 70.25±5.41 µg/mL). Both essential oils rich in sesquiterpene compounds may cause the moderate anticholinesterase activity. The results are similar to those of our study.

F. elaeochytris essential oil (29.1±0.4 %) indicated mild tyrosinase enzyme inhibition and *S. stricta* essential oil showed no activity

Table 2: Antioxidant activities of the essential oils of *F. elaeochytris* and *S. stricta* by β-Carotene-linoleic acid, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, CUPRAC and metal chelating assays (Inhibition %)[a].

		Antioxidant Activity				
		β-Carotene-linoleic acid assay	**$DPPH^{\bullet}$ assay**	**$ABTS^{\bullet+}$ assay**	**CUPRAC assay[b]**	**Metal chelating assay**
Species	*F. elaeochytris*	40.6±0.4	14.3±0.4	23.9±0.7	0.3±0.02	9.2±0.2
	S. stricta	28.6±0.1	3.5±0.04	10.9±0.2	0.1±0.00	6.8±0.3
Standards	α-Tocopherol[c]	91.6±0.1	96.1±0.1	98.2±0.5	2.2±0.10	NT[d]
	BHA[c]	92.8±0.04	90.8±0.1	98.1±0.1	3.7±0.20	NT[d]
	EDTA[c]	NT[d]	NT[d]	NT[d]	NT[d]	94.7±0.6

[a]: % inhibition of 200 µg/mL concentration of essential oils represent the means ± SEM of three parallel sample measurements ($p < 0.05$). [b]: Absorbance values of 200 µg/mL concentration of essential oils [c]: Reference compounds [d]: NT: not tested.

Table 3: Cholinesterase and tyrosinase inhibitory activities of the essential oils of *F. elaeochytris* and *S. stricta* (Inhibition %)[a].

		Cholinesterase Inhibitory Activity		Tyrosinase Inhibitory Activity
		AChE assay	BChE assay	
Species	*F. elaeochytris*	20.8±0.2	54.9±0.4	29.1±0.4
	S. stricta	17.7±0.2	48.9±0.2	NA[d]
Standards	Galantamine[b]	80.4±0.2	82.2±0.7	NT[c]
	Kojic acid[b]	NT[c]	NT[c]	83.6±0.2

[a]: % inhibition of 200 µg/mL concentration of essential oils represent the means ± SEM of three parallel measurements (p<0.05).[b]: Reference compounds [c]: NT: not tested [d]: NA: not active.

(Table 3). There is no study about the anti-tyrosinase activity of the essential oils of *Ferula* and *Sideritis* species in the literature.

This study can be considered as the first comprehensive investigation about antioxidant, anticholinesterase and anti-tyrosinase activities of the essential oils of *F. elaeochytris* and *S. stricta.* Recent studies have revealed the adverse effects on human health (oxidative stress related disease) of synthetic chemical additives used in the food industry. Therefore, the use of essential oils is a natural alternative to the synthetic for preservation of foods. It has been found that both essential oils can be used as a promising resource of natural agents for food additives and drugs for the treatment of AD and diseases associated with oxidative stress.

Experimental

Plant materials: The aerial parts of *S. stricta* were collected from Muğla, *F. elaeochytris* were collected from Bayburt, Turkey in 2016. The voucher specimen has been deposited at the herbarium of Natural Products Laboratory of Muğla Sıtkı Koçman University with voucher no MUMED1051 (for *F. elaeochytris*) and MUMED1121 (for *S. stricta*).

Isolation of the essential oil: The essential oils of dried aerial parts of *F. elaeochytris* and *S. stricta* were hydrodistillated in a Clevenger-type apparatus for 4 h. The oils were dried over anhydrous sodium sulfate and stored under +4°C until analyzed. The essential oil yields were 0.24 % for *F. elaeochytris* and 0.31 % for *S. stricta*.

Gas chromatography-flame ionization detector (GC/FID): A Flame Ionization Detector (FID) and a DB-5 fused silica capillary non-polar column (30 m×0.25 id., film thickness 0.25 µm) were used for GC analyses. The injector temperature and detector temperature were adjusted 250 and 270°C, respectively. Carrier gas was He at a flow rate of 1.4 mL/min. The sample size was 1.0 µL with a split ratio of 20:1. The initial oven temperature was held at 60°C for 5 min, then increased up to 240°C with 4°C/min increments and held at this temperature for 10 min. The percentage composition of the essential oil was determined by the ClassGC10 GC computer program.

Gas chromatography–mass spectrometry (GC/MS): An Ion trap MS spectrometer and a DB-5ms fused silica non-polar capillary column (30 m×0.25 mm ID, film thickness 0.25 µm) were used for the GC/MS analyses. The carrier gas was helium at a flow rate of 1.4 mL/min. The oven temperature was held at 60°C for 5 min, then increased up to 240°C with 4°C/min increments and held at this temperature for 10 min. Injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. The ion source temperature was 200°C. The injection volume was 0.2 µL with a split ratio of 1:20. EI–MS measurements were taken at 70 eV ionization energy. Mass range was from m/z 28 to 650 amu. Scan time 0.5 s with 0.1 inter scan delays. Identification of components of the essential oils was based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature and whenever possible, by co-injection with authentic compounds [30].

Antioxidant activity

β-carotene/linoleic acid assay: The total antioxidant activity was determined by *β*-carotene-linoleic acid method based on the measurement of the inhibition of conjugated diene hydroperoxides resulting from linoleic acid oxidation with slight modifications [36].

DPPH free radical scavenging assay: The free radical scavenging activity was determined spectrophotometrically by the DPPH assay described by Blois with slight modification [36].

ABTS cation radical scavenging assay: The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re *et al*. with slight modifications [37].

Cupric reducing antioxidant capacity (CUPRAC) assay: The cupric reducing antioxidant capacity was determined according to the method of Apak *et al*. with slight modifications [36].

Metal chelating assay: The chelating activity of the extracts on Fe^{2+} was measured as reported by Decker and Welch with slight modifications [37].

Enzyme inhibitory activity

Cholinesterase inhibition: Acetylcholinesterase and butyrylcholinesterase inhibitory activities were measured by the spectrophotometric method developed by Ellman as previously reported [36] with slight modification.

Tyrosinase inhibition: Tyrosinase enzyme inhibitory activity was measured by the spectrophotometric method as described by Masuda *et al*. [38] with slight modification.

Statistical analysis: All data on antioxidant and enzyme inhibitory activity tests were the average of three parallel sample measurements. Data were recorded as mean ±S.E.M. Significant differences between means were determined by student's-*t* test, p values <0.05 were regarded as significant.

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