SHORT REPORT

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Chemical Composition, Antioxidant and Anticholinesterase Activities of the Essential Oil of *Salvia chrysophylla* Staph

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Abstract: The essential oil from the aerial parts of *Salvia chrysophylla* Staph (Lamiaceae), endemic to Turkey, was investigated by using GC and GC-MS. Fifty-four of 55 components, represented 99.52% of the total oil, were identified. The major components of the essential oil were found to be α-terpinenyl acetate (36.31%), *β*-caryophyllene (15.29%), linalool (8.12%) and β-elemene (4.26%). The antioxidant activity of the oil was investigated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and *β*-carotene/linoleic acid tests. Anticholinesterase activity was screened against acetylcholinesterase and butyrylcholinesterase which are the chief enzymes of Alzheimer's disease. The essential oil showed weak antioxidant activity. However, at 1 mg/mL concentration, the essential oil exhibited mild acetylcholinesterase (52.5±2.0%) and moderate butyrylcholinesterase (76.5±2.7%) inhibitory activity

Keywords: *Salvia chrysophylla*; essential oil; GC and GC-MS; antioxidant activity; anticholinesterase activity

1. Plant Source

Modern scientific investigations have confirmed several biological activities such as antibacterial [1], antituberculous [2], antioxidant, anti-inflammatory, anticholinesterase [3], anticancer [4], antiviral, cytotoxic [5], cardiovascular [6] and liver protective [7] for the *Salvia* species. Some *Salvia* species in Europe, however, have been also used against memory loss [8]. Moreover, in a report, the Ottoman herbalist-physician lived between 1641 and 1693 years, had used same plant for memory enhancement [9]. Whole plant material of *Salvia chrysophylla* Staph (Lamiaceae) was collected from the Fethiye-Girdev plateau (2100 m), Turkey in August, 2009. The voucher specimen (No: TSP-1003), has been deposited in the Herbarium of Chemistry, Faculty of Arts and Science, Muğla University, Türkiye.

2. Previous Studies

Essential oil composition of *S. chrysophylla* has been reported by a recent study [10]. However, it has not been studied in detail. According to this report [10], major components of the essential oil have been determined as 3-octanol, α-phellandren-8-ol, camphor and limonene. The antioxidant activity of the only extracts such as dichloromethane, ethylacetate and methanol extracts of *S.chrysophylla* was previously studied [11-12].

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3. Present Study

In this research, the chemical composition of the essential oil and antioxidant and anticholinesterase activities of *S. chrysophylla* were studied, and compared with those of commercial and synthetic antioxidants which are commonly used in the food and pharmaceutical industries. The detail essential oil composition of *S. chrysophylla* with antioxidant and anticholinesterase activities was carried out for the first time in this study.

The essential oil from the aerial parts of the plant was obtained by hydrodistillation for 4h by using Clevenger type apparatus according to the recommendation of the *European Pharmacopoeia* [13]. The essential oil was treated with anhydrous sodium sulphate to dry and was stored under nitrogen at -20 ºC in a sealed vial until required. Qualitative and quantitative analysis of the oil were performed using GC and GC/MS.

GC and GC/MS conditions: The GC analysis of the oil was carried out on a Shimadzu GC-17 AAF, V3, 230V series gas chromatography (Japan), equipped with split injector, attached to DB-1 column (30 m x 0.25 mm, 0.25 μm film thickness) and fitted to FID. Carrier gas flow rate (He) was 1,4 mL/min, split ratio 1:50, injector temperature was 250°C, detector temperature 270°C. The initial oven temperature for both analysis were held at 60 ºC for 5 min, then increased up to 240 ºC with 4 ºC/min increments and held at this temperature for 10 min. The same analytical conditions were employed for the GC/MS analysis, where Varian Saturn 2100T (USA) system equipped with DB-1 column (30 m x 0.25 mm, 0.25 μm film thickness) was used. Transfer line temperature was heated at 290°C. Mass spectrum was acquired in EI mode (70 eV), in *m/z* range 28–650. Identification of components of the essential oil 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 [14-15] and whenever possible, by co-injection with authentic compounds.

Antioxidant and Anticholinesterase Activities: Antioxidant activity were used by β-carotene/linoleic acid bleaching assay [16], and free radical scavenging activity by 1,1-diphenyl-2-picryl-hydrazil (DPPH) assay [17]. BHA and α-tocopherol were used as antioxidant standards for comparison of the activity. Acetylcholinesterase and butyrylcholinesterase enzymes inhibitory activities were assayed by the Ellman method *in vitro* [18]. Galantamine was used as a reference compound. The data of all antioxidant activity tests were triplicated. Significant differences between means were determined by Student's-*t* test, $p<0.05$ were regarded as significant. IC_{50} values, calculated from the concentration-effect linear regression curve.

The physical properties of the essential oil were given in Table 1. Fifty-four components consisting up to 99.52% of the essential oil were identified. Their retention indices, percentage composition and identification methods were also given in Table 2. The major components were found to be α-terpinenyl acetate (36.31%), β-caryophyllene (15.29%), linalool (8.12%), β-elemene (4.26%), germacrene D (3.15%) and spathulenol (2.67%). More than half part of the essential oil was represented by monoterpenoids (51.04%), and other components were determined as sesquiterpene hydrocarbons 28.12%, sesquiterpenoids 8.24%, monoterpene hydrocarbons 5.52% and diterpenes 4.84%. According to the previous report, major components of the oil have been determined as 3-octanol, α-phellandrene-8-ol, camphore and limonene [10]. These finding were inappropriate with results obtained in this study, particularly for the main components of the oil. As known that, the collection date and the different locality including collection altitude may cause differences in the oil composition.

Table 1. The physical properties of the essential oil of *S. chrysophylla*.

Physical property	Essential oil
Density (g/mL), d_{20}	0.9814
Specific rotation, $[\alpha]_D^{20}$	+35.01
Refractive index, n_{20}^0	1.4758

Table 2. Chemical composition of the essential oil of *S. chrysophylla*.

Peak No	Compounds	RI [a]	% [b]	Idendification Methods
1	*α*-Pinene	914	0.25	Co-GC, MS, RI
2	*β*-Pinene	960	0.36	Co-GC, MS, RI
3	2,3-Dehydro-1,8-cineole	969	0.27	MS, RI
4	*β*-Myrcene	977	0.82	Co-GC, MS, RI
5	Benzene acetaldehyde	1000	0.26	MS, RI
6	*p*-Cymene	1007	*tr*	Co-GC, MS, RI
7	Eucalyptol	1015	0.91	Co-GC, MS, RI
8	Limonene	1018	1.95	Co-GC, MS, RI
9	*γ*-Terpinene	1047	2.14	Co-GC, MS, RI
10	(*E*)-3-Nonene-1-ol	1067	0.49	MS, RI
11	Linalool	1082	**8.12**	Co-GC, MS, RI
12	*trans*-*p*-Mentha-2,8-dienol	1108	0.21	MS, RI
13	*trans*-Pinocarveol	1110	0.12	MS, RI
14	Terpinene-4-ol	1142	0.80	Co-GC, MS, RI
15	*α*-Terpineol	1150	0.84	Co-GC, MS, RI
16	Carvone	1177	0.13	Co-GC, MS, RI
17	*p*-Mentha-4-en-3-one	1189	0.39	MS, RI
18	Geraniol	1224	0.45	Co-GC, MS, RI
19	Linalyl acetate	1228	1.02	Co-GC, MS, RI
20	Eugenol	1241	0.12	Co-GC, MS, RI
21	*α*-Terpinenyl acetate	1244	**36.31**	Co-GC, MS, RI
22	*γ*-4-Dimethyl-benzene butanal	1257	0.23	MS, RI
23	*α*-Copaene	1261	1.83	MS, RI
24	Geranyl acetate	1262	0.51	Co-GC, MS, RI
25	*β*-Bourbonene	1263	0.93	MS, RI
26	*β*-Elemene	1267	**4.26**	MS, RI
27	*cis*-Jasmone	1271	0.85	MS, RI
28	*β*-Caryophyllene	1277	**15.29**	Co-GC, MS, RI
29	*trans*-Geranyl acetone	1287	0.12	MS, RI
30	*α*-Humulene	1289	0.35	Co-GC, MS, RI
31	Germacrene D	1298	**3.15**	MS,RI
32	Germacrene B	1300	0.85	MS,RI
33	*γ*-Cadinene	1302	0.39	MS,RI
34	*δ*-Cadinene	1303	*tr*	MS, RI
35	*trans*-*γ*-Bisabolene	1304	1.07	MS, RI
36	Spathulenol	1326	**2.67**	Co-GC, MS,RI
37	Caryophyllene oxide	1328	0.98	Co-GC, MS,RI
38	Cubenole	1342	0.26	MS, RI
39	*τ*-Cadinol	1344	0.83	MS, RI
40	*β*-Eudesmol	1346	0.99	MS, RI
41	*α*-Cadinol	1348	0.27	MS, RI
42	Eudesm-7(11)-en-4-ol	1351	0.32	MS, RI
43	Ledene oxide-II	1354	0.49	MS, RI
44	Calarene epoxyde	1358	*tr*	MS, RI
45	*α*-Bisabolol	1361	0.98	MS, RI
46	Unidentified	1362	0.40	-
47	Benzyl benzoate	1365	0.29	MS, RI
48	Hexahydrofarnecyl acetone	1530	0.45	MS, RI
49	Sclareol oxide	1617	0.86	MS,RI

50	3,7,11,15-Tetrametil hexadeca-(E,E,E)-1,3,6,10,14-pentaen	1712	0.12	MS,RI
51	Androst-5-en-3-β-ol	1734	0.41	MS,RI
52	3,7,11,15-Tetramethyl hexadeca- (E,E,E)-1, 6,10,14-pentaen-3-ol	1790	0.52	MS,RI
53	Manoyl oxide	1807	1.08	MS,RI
54	Manool	1823	1.37	MS,RI
55	Sclareol	1841	1.12	MS, RI
	Total identified (%)		99.52	
	Monoterpene hydrocarbons		5.52	
	Monoterpenoids		51.04	
	Sesquiterpene hydrocarbons		28.12	
	Sesquiterpenoids		8.24	
	Diterpenes		4.84	
	Others		1.76	

[a]: Kovats index on DB–1 fused silica column, [b]: Percentage concentration, [Co-GC]: Co-injection with authentic compounds, [RI]: Retention Index literature comparison, [tr]: trace

Table 3 shows the β-carotene/linoleic acid assay and DPPH free radical scavenging assay of the oil. In β-carotene-linoleic acid assay, the oil exhibited 26.7±0.0% inhibition against lipid peroxidation at 800 µg/mL.

Table 3. Antioxidant activity (%) of the essential oil of *S. chrysophylla* by the β-carotene/linoleic acid and DPPH assays [a]

	β-carotene/linoleic acid assay				**DPPH assay**			
Extract	**100 µg**	**200 µg**	**400 µg**	**800 µg**	**100 µg**	**200 µg**	**400 µg**	**800 µg**
Essential oil	5.0±0.0	8.1±0.1	15.6±0.1	26.7±0.0	1.5±0.0	2.8±0.0	4.8±0.0	9.9±0.1
BHA [b]	90.7±0.0	91.9±0.2	92.9±0.1	93.7±0.0	59.0±0.0	79.3±0.5	90.8±0.2	94.1±0.1
α-TOC [b]	87.8±0.1	90.1±0.0	91.6±0.0	93.2±0.1	84.1±0.0	95.9±0.0	96.1±0.9	96.7±0.1

[a] Values expressed are means ± SD of three parallel measurements (p<0.05).
[b] Reference compounds, BHA: Butylated hydroxyanisole; α-TOC: α-tocopherol.

Table 4 shows the acetylcholinesterase and butyrylcholinesterase inhibitory activities of the essential oil at four concentrations, which is compared with that of galantamine. The essential oil showed 52.5±2.0% inhibitory activity against acetylcholinesterase and 76.5±2.7% against butyrylcholinesterase enzymes at 1 mg/mL concentration. The IC_{50} values of the oil were found to be838.8±5.11 and 96.6±1.11 µg/mL, against AChE and BChE enzymes, respectively. Galantamine, however, demonstrated 5.01±0.11 and 50.88±0.95 µM, respectively. Even if the essential oil demonstrated less AChE and BChE inhibitory activity than galantamine, it may be useful as a moderate butyrylcholinesterase inhibitory agent. But, some further studies should be done.

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Table 4. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of the essential oil of *S. chrysophylla* [a].

	AChE assay				**BChE assay**			
Extract	**125 µg**	**250 µg**	**500 µg**	**1000 µg**	**125 µg**	**250 µg**	**500 µg**	**1000 µg**
Essential oil	21.2±1.7	36.3±2.3	43.9±1.1	52.5±2.0	56.6±0.8	69.6±0.2	73.9±1.1	76.5±2.7
Galantamine [b]	75.6±1.4	79.1±1.3	82.7±0.5	95.9±0.8	53.5±0.4	72.3±1.3	95.6±2.1	98.7±1.6

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

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