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Chemical composition of the essential oil and hexane extract of *Salvia chionantha* and their antioxidant and anticholinesterase activities

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ABSTRACT

The essential oil and methyl ester of hexane extract of *Salvia chionantha* Boiss. were analysed by GC and GC–MS. Totally, 54 components were detected in the essential oil and all of them were fully determined. Germacrene D (25.03%), *β*-caryophyllene (8.71%), spathulenol (5.86%) and *α*-humulene (4.82%) were identified as the major compounds. In the methylated hexane extract, 3-hydroxy hexadecanoic acid (39.39%), 3-hydroxy tetradecanoic acid (12.66%) and palmitic acid (12.02%) were the major fatty acids elucidated. The antioxidant activity of the essential oil and the hexane extract was determined by using four complementary test systems; namely, β-carotene-linoleic acid, DPPH$^{\cdot}$ scavenging, ABTS$^{\cdot+}$ scavenging, and CUPRAC assays. In β-carotene-linoleic acid assay, the extract showed 81.2 ± 0.1% lipid peroxidation inhibition at 0.8 mg/mL concentration, while in ABTS$^{\cdot+}$ assay the essential oil exhibited 77.4 ± 0.5% inhibition at same concentration. Since, acetylcholinesterase and butyrylcholinesterase enzymes are taking place in pathogenesis of Alzheimer's disease, *in vitro* anticholinesterase activity of the essential oil and the extract was also studied spectrophotometrically. At 0.5 mg/mL concentration, the essential oil showed moderate acetylcholinesterase (56.7 ± 1.9%) and butyrylcholinesterase (41.7 ± 2.9%) inhibitory activity, while the extract was only exhibited activity (63.1 ± 0.8%) against butyrylcholinesterase enzyme. Hence, the essential oil may be useful as a moderate anticholinesterase agent, particularly against acetylcholinesterase.

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1. Introduction

Salvia species have been widely used as folk medicines. Literature survey shows that *Salvia* species are antibacterial (Ulubelen et al., 2001), antituberculous (Ulubelen et al., 1997), antiviral, cytotoxic (Topçu et al., 2003; Topçu and Ulubelen, 1999) cardiovascular (Ulubelen, 2003; Ulubelen et al., 2002), and liver protective (Zhou et al., 2005). Due to its antioxidant properties, the plant *Salvia officinalis* is also used to preserve foods (Daniela, 1993).

In Turkey there are about 89 species and 97 taxa, 45 of them being endemic (Güner et al.,2000; Hedge, 1982). The leaves of *Salvia triloba* L. (Syn = *Salvia fruticosa* Mill.) are consumed as apple tea, and the infusion of this plant is used for simple disorders, especially in rural areas (Baytop, 1999). Moreover, in a report, the Ottoman herbalist-physician lived between 1641 and 1693 years, had used same plant for memory enhancement (Tuncer, 1978). Some *Salvia* species have been also used against memory loss in Europe (Perry et al., 1996; Şenol et al., 2009).

Synthetic compounds such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), propylgallate and *tert*-butylhydroquinone are used in food industry because of their high antioxidant capacity. But, these antioxidants are responsible for liver damage and carcinogenesis in F344 rats (Ito et al., 1983). Due to these reasons, use of synthetic antioxidants is limited in some countries (Branien, 1975). So, the interest to the naturally occurring antioxidants has been increasing in the view of their protection of human from oxidative stress damage (Scalbert et al., 2005). On the other hand, excess amount of free radical species causing oxidative stress is also associated with pathology of some diseases including Alzheimer disease which is a progressive neurological disorder characterized by cognitive deficit and behavioral abnormalities in the patient (Soholm, 1998). It is also reported that reactive oxygen species contribute to cellular ageing and neuronal damage (Sastre et al., 2000). Therefore, the use of antioxidants may reduce the progression of Alzheimer's disease and minimize neuronal degeneration (Atta-ur-Rahman and Choudhary, 2001). Thus, the development and utilization of more effective antioxidants of natural origin as well as anticholinesterase compounds are desired.

So far, a few studies on *Salvia chionantha* have been carried out. However, no anticholinesterase, and/or antioxidant activities of the

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essential oil and hexane extract of *S. chionantha* have been previously published. We aimed to investigate the essential oil and hexane extract constituents of the *S. chionantha* with anticholinesterase and antioxidant activities. The objective of this study is to compare antioxidant and anticholinesterase activities with those of commercial antioxidants and galanthamine which are commonly used in the food and pharmaceutical industries.

2. Materials and methods

2.1. Plant materials

Whole plant material of *S. chionantha* Boiss. (Lamiaceae) was collected from the Söğüt-Altınyayla region (Burdur), Turkey in July, 2007 by the authors, and plant sample was identified by Dr. Ersin Karabacak, and Dr. Tuncay Dirmenci. The voucher specimen (No: TSP-1002), has been deposited in the Herbarium of Chemistry, Faculty of Arts and Science, Mugla University, Turkey.

2.2. Spectral measurements and chemicals used

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

Ethanol, *n*-hexane, ammonium acetate, copper (II) chloride, potassium persulfate and boron trifluoride-methanol complex (BF_3:MeOH) were obtained from E. Merck (Darmstadt, Germany). Terpenoids used for GC and GC–MS analysis (authentic compounds used for co-injection), β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), neocuproine, α-tocopherol, butylatedhydroxyl anisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), electric eel acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg, Sigma), horse serum butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg, Sigma), 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB), acetylthiocholine iodide and butyrylthiocholine chloride, galanthamine were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were in analytical grade.

2.3. Isolation of the essential oil

The essential oil of dried aerial parts of *S. chionantha* was obtained via the hydro-distillation by using a Clevenger type apparatus for 4 h. The oil was dried over anhydrous sodium sulphate and stored under nitrogen at −20 °C until required.

2.4. Preparation of the hexane extract and derivation of hexane extract

The dried and powdered aerial parts of *S. chionantha* (100 g) were extracted with 200 mL of hexane (4 × 24h) at room temperature. After filtration, the solvent was evaporated to dryness in vacuum to give 2.80 g of hexane extract. The hexane extract was used for antioxidant and anticholinesterase activities as well as to derivate its methyl ester. The derivative was analysed by GC and GC–MS.

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 °C), 2 mL BF_3:MeOH was added. The mixture was boiled for 2 min, and then left alone until it cooled down, and then flask was filled to 25 mL with saturated NaCl solution. The 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 over anhydrous Na_2SO_4 and filtered. The organic solvent was removed under reduced pressure on a rotary evaporator to give methyl esters.

2.5. Analysis of the essential oil

2.5.1. Gas chromatography (GC)

A Flame Ionization Detector (FID) and a DB-1 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. Sample size was 1.0 μL with a split ratio of 50:1. For the essential oil, 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. For derivative of hexane extract analysis, the initial oven temperature was held at 100 °C for 5 min, then increased up to 240 °C with 4 °C/min increments and held at this temperature for 5 min. The percentage compositions of the essential oil and derivative of hexane extract were determined with GC solution computer program.

2.5.2. Gas chromatography–mass spectrometry (GC–MS)

An Ion trap MS spectrometer and a DB-1 MS fused silica non-polar capillary column (30 m × 0.25 mm ID, film thickness 0.25 μm) were used for the GC–MS analyses. Carrier gas was helium at a flow rate of 1.4 mL/min. The oven temperature for the essential oil 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. For derivative of hexane extract, the oven temperature was held at 100 °C for 5 min, then increased up to 240 °C with 4 °C/min increments and held at this temperature for 5 min. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. Ion source temperature was 200 °C. The injection volume was 0.2 μL with a split ratio of 1:30. EI–MS 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 interscan 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 (Adams, 1989; Swigar and Silverstein, 1981) and when ever possible, by co-injection with authentic compounds.

2.6. Determination of antioxidant activity

2.6.1. β-carotene/linoleic acid bleaching assay

The total antioxidant activity was evaluated by using β-carotene-linoleic acid test system developed by Marco (1968). α-Tocopherol and BHA were used as antioxidant standards for comparison of the activity.

2.6.2. DPPH free radical scavenging assay

The free radical-scavenging activity was determined spectrophotometrically by the DPPH assay as described by Blois (1958). α-Tocopherol and BHA were used as antioxidant standards for comparison of the activity.

2.6.3. ABTS cation radical decolorization assay

The spectrophotometric analysis of $ABTS^{+\cdot}$ scavenging activity was determined according to the method of Re et al. (1999). α-Tocopherol and BHA were used as antioxidant standards for comparison of the activity.

2.6.4. Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined according to the method of Apak et al. (2004) Results were given as absorbance comparing that those of standards, α-tocopherol and BHA.

2.7. Determination of anticholinesterase activity

Acetylcholinesterase and butyrylcholinesterase inhibitory activity was measured, by slightly modifying the spectrophotometric method developed by Ellman et al. (1961). 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 (5,5′-dithiobis(2-nitrobenzoic)acid was used for the measurement of the anticholinesterase activity. Galanthamine was used as reference compound.

2.8. Statistical analysis

All data on both antioxidant and anticholinesterase activity tests were the average of triplicate analyses. The data were recorded as mean ± standard deviation. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by student's-*t* test, *p* values <0.05 were regarded as significant.

3. Results and discussions

The essential oil, which was a greenish yellow color, was obtained by hydro-distillation (0.15%, v/w) of the dried aerial parts of the plant. The chemical composition of the essential oil was given in Table 1, as well as the physical properties of the essential oil were in Table 2. The essential oil was analysed by GC and GC–MS, and resulted in the identification of 54 components representing 99.43% of the total oil. The major components of the oil were germacrene D (25.03%), *β*-caryophyllene (8.71%), spathulenol (5.86%), α-humulene (4.82%), α-elemene (4.18%) and bornyl acetate (4.15%). Caryophyllene oxide was also detected as a constituent of the oil, with a yield of 3.89% (Table 1). Sesquiterpene hydrocarbons represented 56.21% of the essential oil, monoterpene hydrocarbons 6.24%, monoterpenoids 10.02%, sesquiterpenoids 17.25%, diterpenes 1.82% and the remaining percentage (7.89%) consists of aliphatic alcohols, aldehydes, esters, hydrocarbons and ketones.

Table 1
Chemical composition of the essential oil of *S. chionantha*.

Peak no.	Compounds	RI [a]	%[b]	Idendification methods
1	α-Pinene	914	0.95	Co-GC, MS, RI
2	Camphene	925	tr	Co-GC, MS, RI
3	β-Pinene	960	0.42	Co-GC, MS, RI
4	2,3-Dehydro-1,8-cineole	969	0.34	MS, RI
5	α-Phellandrene	989	0.26	MS, RI
6	Limonene	1018	1.82	Co-GC, MS, RI
7	*cis*-Ocimene	1026	0.25	MS, RI
8	*trans*-Ocimene	1037	0.41	MS, RI
9	γ-Terpinene	1047	1.97	Co-GC, MS, RI
10	*cis-p*-Menth-2-en-1-ol	1049	0.11	MS, RI
11	Terpinolene	1074	0.16	Co-GC, MS, RI
12	(*E*)-2-Nonene-1-ol	1075	0.1	MS, RI
13	Linalool	1082	0.42	Co-GC, MS, RI
14	Fenchyl acetate	1099	0.38	MS, RI
15	Isoamyl isovalerate	1102	tr	MS, RI
16	Camphor	1106	1.46	Co-GC, MS, RI
17	*cis*-Verbenol	1112	0.86	Co-GC, MS, RI
18	Borneol	1132	1.97	Co-GC, MS, RI
19	Terpinene-4-ol	1142	1.46	Co-GC, MS, RI
20	α-Terpineol	1150	0.72	Co-GC, MS, RI
21	*trans* -Carveol	1168	0.29	MS, RI
22	Carvone	1177	0.47	Co-GC, MS, RI
23	*p*-Mentha-4-en-3-one	1189	0.39	MS, RI
24	β-Methyl cinnamaldehyde	1197	tr	MS, RI
25	Bornyl acetate	1211	4.15	Co-GC, MS, RI
26	Carvacrol	1216	0.65	Co-GC, MS, RI
27	Thymol	1218	0.88	Co-GC, MS, RI
28	δ-Elemene	1242	2.96	MS, RI
29	α-Copaene	1261	2.91	Co-GC, MS, RI
30	α-Bourbonene	1264	1.75	MS, RI
31	α-Elemene	1267	4.18	MS, RI
32	β-Bourbonene	1269	tr	MS, RI
33	*cis*-Jasmone	1271	0.18	MS, RI
34	β-Caryophyllene	1277	8.71	Co-GC, MS, RI
35	β-Gurjunene	1282	1.02	MS, RI
36	Aromadendrene	1283	1.68	MS, RI
37	α-Guaiene	1285	1.32	MS,RI
38	α-Humulene	1289	4.82	Co-GC, MS, RI
39	γ-Gurjunene	1291	0.81	MS, RI
40	Germacrene D	1298	25.03	MS,RI
41	Viridiflorene	1299	0.13	MS,RI
42	γ- Elemene	1305	0.89	MS,RI
43	Spathulenol	1326	5.86	MS, RI
44	Caryophyllene oxide	1328	3.89	Co-GC, MS,RI
45	Aristolene epoxide	1330	1.24	MS, RI
46	γ-Gurjunene epoxide-I	1335	1.52	MS, RI
47	*tau*-Cadinol	1344	0.94	MS, RI
48	7,8-Dehydro-8α-hydroxy isolongifolene	1347	1.02	MS, RI
49	Ledene oxide-II	1354	0.92	MS, RI
50	4-Methyl hexadecane	1359	0.45	MS, RI
51	(*Z*)-α-*trans*-Bergamatol	1421	1.86	MS, RI
52	Hexahydrofarnesyl acetone	1530	1.68	MS, RI
53	Eicosanol	1770	0.79	MS,RI
54	Phytol	1795	1.03	Co-GC, MS, RI
	Total identified (%)		99.43	
	Monoterpene hydrocarbons		6.24	
	Monoterpenoids		10.02	
	Sesquiterpene hydrocarbons		56.21	
	Sesquiterpenoids		17.25	
	Diterpenes		1.82	
	Others		7.89	

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

In previous studies, germacrene D was found to be the major compound in the essential oil of *Salvia longipedicellata* (13.4%) (Özer et al., 2007), *Salvia ceratophylla* (27.4%) (Bağcı and Koçak, 2007), *Salvia aethiopis* (29.0%) (Güllüce et al., 2006), *Salvia tomentosa* (7.5%) and *Salvia palaestina* (16.5%) (Bağcı and Koçak, 2008).

Table 2
The physical properties of the essential oil of *S. chionantha*.

Physical property	Essential oil
d_{20}	0.9792
$[\alpha]_D^{20}$	+33.80
n_{20}^{0}	1.4127

d_{20}: Density (g/mL) 20 °C, n_{20}^{0}: refractive index at 20 °C, $[\alpha]_D^{20}$: specific rotation at 20 °C.

Germacrene D is important compound since it demonstrated larvicidal activity against *Aedes aegypti* and *Anopheles stephensi* (Kiran et al., 2006) as well as an effective inhibition of aphid alarm pheromone activity (Bruce et al., 2005).

Individual fatty acids of the *S. chionantha* given as percentage concentrations were summarized in Table 3. The major fatty acids were elucidated as 3-hydroxy hexadecanoic acid (39.39%), 3-hydroxy tetradecanoic acid (12.66%) and palmitic acid (12.02%) in the oil. Oleic acid was also found in the extract as a percentage of 3.0%. Unsaturation was calculated as 9.07% while saturated fatty acids was 88.66% in the methylated hexane extract.

The essential oil and the hexane extract of *S. chionantha* were screened for their possible antioxidant activity using four complementary test systems, namely β-carotene-linoleic acid, DPPH, ABTS and CUPRAC assays. The total antioxidant activity of the essential oil and the hexane extract, compared with α-tocopherol and BHA were shown in Table 4. Total antioxidant activity increased with increasing amounts of the extracts. The results were found to be statistically significant ($p < 0.05$) when compared with the control. The hexane extract exhibited better lipid peroxidation activity than the essential oil; however, none of them showed greater activity than those of antioxidant standards.

DPPH free radical and ABTS cation radical-scavenging activities of the essential oil and the extract were given in Table 4. Because of the steric effect of DPPH molecule, ABTS assay has a superiority to the DPPH assay. The antioxidant may not approach to free radical and seems not to be an antioxidant. Therefore, both the assays were carried out. The difference between the extracts and control was statistically significant ($p < 0.05$) for both antiradical assays when compared with the control. In both assays, radical-scavenging activity increased with increasing amount of the extracts. Essential oil was found to be more active than the hexane extract; demonstrating 77.4 ± 0.5% inhibition in ABTS assay and 11.6 ± 0.1% inhibition in DPPH assay at 800 μg/mL concentration, while the

Table 3
The composition (%) of methyl ester of the hexane extract of *S. chionantha*.

Peak no.	Compound	Concentration (%)
1	Myristoleic acid ($C_{14:1}$)	3.70
2	3-Hydroxy tetradecanoic acid ($C_{14:0}$)	12.66
3	14-Methyl pentadecanoic acid ($C_{16:0}$)	7.59
4	Palmitic acid ($C_{16:0}$)	12.02
5	3-Hydroxy hexadecanoic acid ($C_{16:0}$)	39.39
6	Stearic acid ($C_{18:0}$)	1.89
7	Oleic acid ($C_{18:1}$)	3.00
8	Unidentified	1.85
9	10-Oxo-octadecanoic acid ($C_{18:0}$)	5.88
10	18-Methoxy-octadecanoic acid ($C_{19:0}$)	5.51
11	Arachidic acid ($C_{20:0}$)	3.70
12	Unidentified	0.42
13	Eruric acid ($C_{22:1}$)	2.37
	Total saturation	88.66
	Total unsaturation	9.07
	Unidentified	2.27

Table 4
Antioxidant activity (%) of the essential oil and the hexane extract of *S. chionantha* by the β-carotene-linoleic acid, DPPH and ABTS assays.[a]

Extracts	β-Carotene–linoleic acid assay				DPPH assay				ABTS assay			
	100 μg	200 μg	400 μg	800 μg	100 μg	200 μg	400 μg	800 μg	100 μg	200 μg	400 μg	800 μg
Essential oil	12.8 ± 0.0	20.1 ± 0.0	31.4 ± 0.1	50.6 ± 0.0	2.2 ± 0.0	3.7 ± 0.1	6.9 ± 0.0	11.6 ± 0.1	10.8 ± 0.9	22.4 ± 0.0	39.4 ± 0.2	77.4 ± 0.5
Hexane extract	27.7 ± 0.1	40.7 ± 0.1	55.6 ± 0.0	81.2 ± 0.1	0.4 ± 0.0	0.7 ± 0.0	2.2 ± 0.0	3.9 ± 0.0	4.5 ± 0.1	9.94 ± 0.8	18.4 ± 0.2	36.4 ± 0.1
BHA[b]	90.7 ± 0.0	91.9 ± 0.1	92.9 ± 0.1	93.7 ± 0.0	59.0 ± 0.0	79.3 ± 0.5	90.8 ± 0.2	94.1 ± 0.1	94.1 ± 0.0	97.9 ± 0.0	98.1 ± 0.9	99.7 ± 0.1
α-Tocopherol[b]	87.8 ± 0.1	90.1 ± 0.0	91.6 ± 0.0	93.1 ± 0.1	84.1 ± 0.0	95.9 ± 0.0	96.1 ± 0.9	96.7 ± 0.1	95.1 ± 0.0	97.9 ± 0.0	98.1 ± 0.9	99.7 ± 0.1

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

Table 5
Cupric reducing antioxidant capacity (CUPRAC) of the essential oil and the hexane extract of *S. chionantha* using spectrophotometric detection of the Cu^{+2}–Cu^{+1} transformation.[a]

Extracts	CUPRAC assay				
	0.00 μg	100 μg	200 μg	400 μg	800 μg
Essential oil	0.06 ± 0.00	0.17 ± 0.01	0.27 ± 0.02	0.48 ± 0.01	0.84 ± 0.06
Hexane extract	0.06 ± 0.00	0.21 ± 0.00	0.36 ± 0.02	0.57 ± 0.00	0.91 ± 0.03
BHA[b]	0.06 ± 0.00	3.51 ± 0.01	3.73 ± 0.01	3.81 ± 0.01	3.99 ± 0.01
α-Tocopherol[b]	0.06 ± 0.00	1.85 ± 0.01	2.22 ± 0.01	2.85 ± 0.01	3.21 ± 0.01

[a] Values, absorbance at 450 nm, expressed are means ± SD of three parallel measurements ($p < 0.01$).
[b] Reference compounds.

Table 6
Acetylcholinesterase and butyrylcholinesterase inhibitory activities of the essential oil and the hexane extract of *S. chionantha*.[a]

Extracts	AChE assay				BChE assay			
	62.5 μg	125 μg	250 μg	500 μg	62.5 μg	125 μg	250 μg	500 μg
Essential oil	35.4 ± 1.9	36.8 ± 0.2	49.6 ± 1.0	56.7 ± 1.9	–[b]	8.9 ± 1.3	25.31 ± 1.3	41.7 ± 2.9
Hexane extract	–	–	3.0 ± 0.1	7.0 ± 0.1	27.5 ± 0.0	32.6 ± 0.6	47.76 ± 0.3	63.1 ± 0.8
Galanthamine[c]	75.6 ± 1.4	79.1 ± 1.3	82.7 ± 0.5	95.9 ± 0.8	53.5 ± 0.4	72.3 ± 1.3	95.63 ± 2.1	98.7 ± 1.6

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).
[b] Exhibited no activity.
[c] Reference compound.

hexane extract exhibited 36.4 ± 0.1% and 3.9 ± 0.0% inhibitions at some concentration.

Table 5 shows the cupric reducing antioxidant capacity (CUPRAC). 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. The essential oil and the hexane extract were demonstrated close activity to each other. The essential oil and the hexane extract at all concentrations exhibited higher activities than the control given in first column; the differences were found to be statistically significant ($p < 0.01$).

Antioxidant activity by DPPH and ferric reducing power assays of the dichloromethane, ethyl acetate and methanol extracts of *S. chionantha* was reported, and among them only the methanol extract was exhibited highest activity (Şenol et al., 2009).

Table 6 shows the anticholinesterase activity of the essential oil and the hexane extract, compared with galanthamine at the same concentration. The essential oil showed moderate acetylcholinesterase (56.7 ± 1.9%) and butyrylcholinesterase (41.7 ± 2.9%) inhibitory activity. In contrast, the extract was only exhibited activity (63.1 ± 0.8%) against butyrylcholinesterase enzyme, at 500 μg/mL concentration. Previously, only the acetylcholinesterase inhibitory activity of the dichloromethane and ethyl acetate extracts of *S. chionantha* were studied, and no activity was observed in both extract (Şenol et al., 2009). Other *Salvia* species studied for their antioxidant and anticholinesterase activities in the literature (Şenol et al., 2009; Kıvrak et al., 2009; Kolak et al., 2009) point out that polar extracts of the *Salvia* species include antioxidant compounds. However, polar extracts are unable to inhibit acetylcholinesterase enzyme, in general. In addition, the essential oil and the non-polar extracts of the *Salvia* species studied indicate that non-polar extracts can be considered as acetyl-, and butyrylcholinesterase enzyme inhibitors.

4. Conclusions

The essential oil constituents and the lipid profile of the of *S. chionantha* were studied for the first time. In addition, the results presented in this study are the first information on the antioxidant and anticholinesterase activities of the essential oil and hexane extract of the plant. Among the four used antioxidant methods, the highest activity was observed for inhibition of lipid peroxidation in the β-carotene-linoleic acid system by the hexane extract (81.2%). The essential oil showed moderate activity (50.6%) at 0.8 mg/mL concentration. The essential oil also demonstrated mild acetylcholinesterase inhibitory activity as well as butyrylcholinesterase inhibitory activity. Hence, the essential oil may be useful as a moderate anticholinesterase agent, particularly against AChE.

However, further studies are needed to understand the origin of the activity. Particularly, major constituents of the essential oil need to be tested for their anticholinesterase and antioxidant activities.

Conflict of the Interest

The authors declare that there are no conflicts of interest.

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