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# Antioxidant and anticholinesterase evaluation of selected Turkish *Salvia* species

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**Abstract**

Since *Salvia* species (Lamiaceae) have been recorded to be used against memory loss in European folk medicine, we herein examined in vitro anticholinesterase and antioxidant activities of 56 extracts prepared with petroleum ether, chloroform, ethyl acetate and methanol obtained from 14 *Salvia* species (*Salvia albimaculata* Hedge and Hub, *Salvia aucheri* Bentham var. *canescens* Boiss and Heldr, *Salvia candidissima* Vahl. ssp. *occidentalis*, *Salvia ceratophylla* L., *Salvia cryptantha* Montbret and Bentham, *Salvia cyanescens* Boiss and Bal., *Salvia frigida* Boiss, *Salvia forskahlei* L., *Salvia halophila*Hedge, *Salvia migrostegia* Boiss and Bal., *Salvia multicaulis* Vahl., *Salvia sclarea* L., *Salvia syriaca* L., *Salvia verticillata* L. ssp. *amasiaca*) growing in Turkey. The antioxidant activities were assessed by both chemical and enzymatic methods against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and xanthine/xanthine oxidase (XO) system generated superoxide anion radical inhibition. Anticholinesterase effect of the extracts was tested against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) at concentrations of 0.2 and 1 mg/ml using a microplate-reader assay based on the Ellman method. Most of the extracts did not show any activity against AChE at 0.2 mg/ml, while the chloroform extracts had noticeable inhibition against BChE between 47.7% and 74.7%. The most active extracts at 1 mg/ml for AChE inhibition were observed to be petroleum ether extract of *Salvia albimaculata* (89.4%) and chloroform extract of *Salvia cyanescens* (80.2%), whereas ethyl acetate extracts of *Salvia frigida* and *Salvia migrostegia*, chloroform extracts of *Salvia candidissima* ssp. *occidentalis* and *Salvia ceratophylla*, as well as petroleum ether extract of *Salvia cyanescens* were found to inhibit potently BChE (92.2%, 89.6%, 91.1%, 91.3%, and 91.8%, respectively). Particularly, the ethyl acetate and methanol extracts were observed to be highly active against both DPPH and XO. Our data indicates that nonpolar extracts of *Salvia* species for anticholinesterase activity and the polar extracts for antioxidant activity are worth further phytochemical evaluation for identifying their active components.
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*Keywords:* *Salvia*; Lamiaceae; Acetylcholinesterase; Butyrylcholinesterase; Alzheimer's disease; Antioxidant; DPPH; Free radical; Xanthine oxidase

## 1. Introduction

Alzheimer's disease (AD) is a degenerative neurological disorder characterized by senile plaques containing amyloid β protein and loss of cholinergic neuromediators in the brain (Lawrence & Shakian, 1998; Whitehouse et al., 1982). The most remarkable biochemical change in AD patients is a reduction of acetylcholine (ACh) levels in the hippocampus and cortex of the brain (Jaen, Gregor, Lee, Davis, & Emmerling, 1996). Therefore, inhibition of acetylcholinesterase (AChE), the enzyme responsible for hydrolysis of ACh at the cholinergic synapse, is currently

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the most established approach to treating AD (Schneider, 1996; Tariot et al. galanthamine USA-10 study group, 2000). While AChE is found in all excitable tissue, whether nerve or muscle, in most erythrocytes and in placental tissue, BChE is present more commonly in the body including within the central and peripheral nervous system, liver and plasma (Massoulie, Pezzementi, Bon, Krejci, & Vallette, 1993). On the other hand, oxidative stress, caused by reactive oxygen species (ROS), is known to cause the oxidation of biomolecules leading to cellular damage. It is also speculated to be pathologically important in various neurodegenerative processes including cognitive deficits that occur during normal cerebral aging, Alzheimer's (AD), and Parkinson's diseases (Bastianetto & Quirion, 2002; Behl & Moosman, 2002; Butterfield et al., 1999; Gray et al., 2003; Jenner, 1996; Smith et al., 1996). Nowadays, the most accepted theory about the disturbing effect of free radicals in the process of aging was reported by Harman (1956). Later on, it was also reported that oxidative stress is associated with the pathogenesis of AD and cellular characteristics of this disease are either causes or effects of oxidative stress (Smith, Harris, Sayre, & Perry, 1997; Smith et al., 1996; Vina, Lloret, Orti, & Alonso, 2004). These evidences clearly show that oxidative stress, an early event in AD, may play a key pathogenic role in the disease (Zhu et al., 2004). Interestingly, intake of polyphenols through diets rich in fruits, vegetables and beverages such as red wine was stated to reduce incidence of certain age-related neurological disorders including macular degeneration and dementia (Bastianetto & Quirion, 2002; Commenges et al., 2000). Therefore, these data suggest that high dietary or supplemental consumption of antioxidants in people may reduce the risk of AD.

On the other hand, *Salvia* species (sage) were reported to be used for memory-enhancing purposes in European folk medicine (Perry, Bollen, Perry, & Ballard, 2003). For this purpose, we aimed to examine in vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of 14 *Salvia* species (*Salvia albimaculata* Hedge and Hub, *Salvia aucheri* Bentham var. *canescens* Boiss and Heldr, *Salvia candidissima* Vahl. ssp. *occidentalis*, *Salvia ceratophylla* L., *Salvia cryptantha* Montbret and Bentham, *Salvia cyanescens* Boiss and Bal., *Salvia frigida* Boiss, *Salvia forskahlei* L., *Salvia halophila*Hedge, *Salvia migrostegia* Boiss and Bal., *Salvia multicaulis* Vahl., *Salvia sclarea* L., *Salvia syriaca* L., *Salvia verticillata* L. ssp. *amasiaca*) growing in Turkey by the Ellman method. Besides, this study was also designed to evaluate the antioxidant capacity of the aforementioned *Salvia* species by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method and xanthine/xanthine oxidase (XO) system generated superoxide anion radical inhibition assay.

## 2. Materials and methods

### 2.1. Plant materials

Fourteen Salvia species (*S. albimaculata* Hedge and Hub, *S. aucheri* Bentham var. *canescens* Boiss and Heldr, *S. candidissima* Vahl. ssp. *occidentalis*, *S. ceratophylla* L., *S. cryptantha* Montbret and Bentham, *S. cyanescens* Boiss and Bal., *S. frigida Boiss*, *S. forskahlei* L., *S. halophila* Hedge, *S. migrostegia* Boiss and Bal., *S. multicaulis* Vahl., *S. sclarea* L., *S. syriaca* L., *S. verticillata* L. ssp. *amasiaca*) were collected throughout Turkey. Collection sites, dates, and herbarium numbers (AEF) are listed in Table 1. The plants were identified by Prof. Dr. H. Duman from the Department of Biology, Faculty of Art and Science, Gazi University, Ankara, Turkey and Dr. G. Yilmaz from the Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, Ankara, Turkey. The voucher specimens were preserved at the Herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey. Only, *S. forskahlei* was identified by Dr. S. Terzioglu of Department of Forest Botany, Faculty of Forestry, Karadeniz Technical University, Trabzon, Turkey. The voucher specimen of *S. forskahlei* is preserved at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey (Table 1).

Table 1
Collection sites, dates and herbarium numbers of Turkish *Salvia* species

| *Salvia* species examined | Collection site | Collection date | Herbarium number |
|---|---|---|---|
| *S. albimaculata* Hedge and Hub | Balkusan-Ermenek, Konya | June, 2005 | AEF 23520 |
| *S. aucheri* Bentham var. *canescens* Boiss and Heldr | Ermenek-Gülnar, Konya | June, 2005 | AEF 23525 |
| *S. candidissima* Vahl. ssp. *occidentalis* | Ermenek, Konya | June, 2005 | AEF 23522 |
| *S. ceratophylla* L. | Ergani-Maden, Diyarbakir | June, 2005 | AEF 23559 |
| *S. cryptantha* Montbret and Bentham | Beynam, Ankara | June, 2005 | AEF 23614 |
| *S. cyanescens* Boiss and Bal. | Beynam, Ankara | June, 2005 | AEF 23620 |
| *S. frigida* Boiss | Ermenek, Konya | June, 2005 | AEF 23528 |
| *S. forskahlei* L. | Sümela Monestry, Trabzon | August, 2001 | GUE 1987 |
| *S. halophila* Hedge | Karakulluk, Konya | June, 2005 | AEF 23649 |
| *S. migrostegia* Boiss and Bal. | Ermenek-Tekecati, Konya | June, 2005 | AEF 23523 |
| *S. multicaulis* Vahl. | Ergani-Maden, Diyarbakir | June, 2005 | AEF23561 |
| *S. sclarea* L. | Bozkir, Konya | June, 2005 | AEF 23521 |
| *S. syriaca* L. | Hadim-Bozkir, Konya | June, 2005 | AEF 23530 |
| *S. verticillata* L. ssp. *amasiaca* | Cubuk-Karagöl National Park, Ankara | July, 2005 | AEF 23552 |

### 2.2. Preparation of crude extracts

Each plant material was dried in shade at room temperature and then ground to a fine powder in a mechanic grinder and approximately 6.0 g weighed accurately on a digital balance (Mettler Toledo AG245). Then, each plant was successively extracted with petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and then methanol (MeOH). After filtration of each solvent, the organic phases were independently concentrated under vacuum by evaporating to dryness. Yields of the crude extracts obtained a given in Table 2.

### 2.3. Determination of AChE and BChE inhibitory activities

AChE and BChE inhibitory activities were measured by slightly modifying the spectrophotometric method developed by Ellman, Courtney, Andres, and Featherstone (1961). Electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma) and horse serum BChE (EC 3.1.1.8, Sigma) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5,5′-Dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. All the other reagents and conditions were same as described in previous publications (Atta-ur-Rahman, Parveen, Khalid, Farooq, & Choudhary, 2000; Orhan, Şener, Choudhary, & Khalid, 2004). Briefly, in this method, 140 μl of 0.1 mM sodium phosphate buffer (pH 8.0), 20 μl of DTNB, 20 μl of test solution and 20 μl of AChE/BChE solution were added by multichannel automatic pipette (Gilson pipetman, France) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of 10 μl of acetylthiocholine iodide/butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate-reader (Spectramax Plus-384, Molecular Devices, CA, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH=8) using the formula $(E-S)/E \times 100$, where $E$ is the activity of enzyme without test sample and $S$ is the activity of enzyme with test sample. The experiments were done in triplicate. Galanthamine, the anticholinesterase alkaloid-type of drug isolated from the bulbs of snowdrop (*Galanthus* sp.), was purchased from Sigma (St. Louis, MO, USA) and was used as reference.

Table 2
Percentage yields (w/w) of PE, $CHCl_3$, EtOAc, and MeOH extracts of Turkish *Salvia* species

| *Salvia* species examined | PE | $CHCl_3$ | EtOAc | MeOH |
|---|---|---|---|---|
| *S. albimaculata* | 1.13 | 2.75 | 1.13 | 4.38 |
| *S. aucheri* var. *canescens* | 1.63 | 2.77 | 1.14 | 2.77 |
| *S. candidissima* ssp. *occidentalis* | 2.59 | 1.13 | 1.46 | 10.84 |
| *S. cetrophylla* | 7.63 | 1.30 | 0.49 | 2.92 |
| *S. cryptantha* | 3.02 | 2.86 | 0.79 | 9.05 |
| *S. cyanescens* | 2.04 | 2.04 | 0.63 | 7.23 |
| *S. frigida* | 7.65 | 1.83 | 0.67 | 2.99 |
| *S. forskahlei* | 0.63 | 1.10 | 0.31 | 5.98 |
| *S. halophila* | 0.47 | 0.31 | 0.31 | 20.41 |
| *S. migrostegia* | 4.45 | 1.15 | 0.66 | 6.42 |
| *S. multicaulis* | 2.07 | 6.21 | 0.64 | 6.05 |
| *S. sclarea* | 3.15 | 3.15 | 1.57 | 6.61 |
| *S. syriaca* | 1.15 | 2.30 | 0.66 | 5.75 |
| *S. verticillata* ssp. *amasiaca* | 0.48 | 1.75 | 0.63 | 11.75 |

### 2.4. Antioxidant activity

#### 2.4.1. DPPH free radical-scavenging assay

The antiradical activity of the plant extracts and the reference were assessed on the basis of the radical-scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) free radical (Lee et al., 1998). The concentration of DPPH was kept as 300 μM. The extracts and reference were dissolved in dimethylsulfoxide (DMSO), an effective solvent that is miscible in all proportions with water, while the DPPH solution was prepared in ethanol. Ten microlitres of each extract and reference was allowed to react with 200 μl of stable free radical DPPH at 37 °C for 30 min in a 96-well microtiter plate. After incubation, decrease in absorption for each solution was measured at 515 nm using an ELISA microplate-reader (Spectra MAX-340 Molecular Devices, USA). The corresponding blank readings were also taken and the remaining DPPH was calculated. Percent radical-scavenging activity by samples was determined in comparison with a DMSO treated control group. Butylated hydroxyanisol (BHA), a widely used antioxidant for long preservation of food products, was used as reference. Inhibition of free radical DPPH in percent ($I\%$) was calculated in following way:

$I\% = (1 - A_{sample}/A_{blank}) \times 100$, where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the test sample), and $A_{sample}$ is the absorbance of the extracts/reference.

#### 2.4.2. Xanthine oxidase (XO) inhibition assay

The xanthine–xanthine oxidase (X–XO) reaction is a suitable system to generate superoxide anion ($O_2^-$). Compounds/extracts which interact with XO can affect the kinetics of reaction of oxidation of xanthine to uric acid (Fridovich, 1970). The xanthine oxidase (XO) inhibition assay was assessed in phosphate buffer (0.1 M, pH = 7.5). Twenty microliters XO (0.003 unit/well) and various concentrations of test samples in 10 μl of DMSO were mixed in a 96-well microplate and pre-incubated for 10 min at room temperature. The reaction was initiated by adding 20 μl of 0.1 mM xanthine and uric acid formation was measured spectrophotometrically at 295 nm by using

Molecular Devices, Spectramax 384 (Lee et al., 1998). Allopurinol, a xanthine oxidase inhibitor also known as 1,5-dihydro-4H-pyrazolo[3,4-*d*]pyrimidin-4-one, was used as the reference compound.

*2.4.3. Statistical analysis of data*

Data obtained from in vitro experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were evaluated by ANOVA test. $P < 0.05$ was considered to be significant [$^{*}P < 0.05$; $^{**}P < 0.01$;$^{***}P < 0.001$].

## 3. Results

*3.1. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity*

At 0.2 mg/ml; PE, EtOAc and MeOH extracts did not exhibit any activity against AChE, while the most active extract was found to be the chloroform extract of *S. cryptantha* (74.7%), followed by *S. aucheri* var. *canescens* (47.72%) (Table 3). Against BChE at the same concentration, none of the methanolic extracts were active, while PE extracts of *S. cyanescens* and *S. cryptantha* were observed to be the most effective (67.1% and 82.9%, respectively). The EtOAc extracts had inhibition under 50%, except *S. cyanescens* (63.3%). However, among the $CHCl_3$ extracts, *S. sclarea* and *S. syriaca* did not show any inhibition at all, while the rest had inhibition over 50%, excepting *S. halophila* and *S. migrostegia* (Table 3). Only two PE extracts belonging to *S. albimaculata* and *S. cryptantha* displayed remarkable inhibition over 50% (89.4% and 71.8%, respectively) at 1 mg/ml against AChE (Table 4). The PE extract of *S. albimaculata* (89.4%) and the $CHCl_3$ extract of *S. cyanescens* (80.2%) were revealed to be the most active extracts for AChE inhibition, whereas the EtOAc extracts of *S. frigida* and *S. migrostegia*, the $CHCl_3$ extracts of *S. candidissima* ssp. *occidentalis* and *S. ceratophylla*, as well as PE extract of *S. cryptantha* appeared to inhibit potently BChE (92.2%, 89.6%, 91.1%, 91.3%, and 92.0%, respectively). However, the MeOH extracts were found to be completely inactive against AChE at 1 mg/ml. The PE extracts were effective against BChE, excluding *S. ceratophylla* and *S. migrostegia*. With the exception of *S. multicaulis* and *S. cryptantha*, the rest of the $CHCl_3$ extracts had significant BChE inhibitory activity. The EtOAc extracts, excluding *S. ceratophylla* and *S. halophila*, were also observed to be highly effective, while all of the MeOH extracts, apart from *S. frigida*, were shown to be totally ineffective against BChE.

*3.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and xanthine oxidase (XO) inhibitory activity*

The PE, $CHCl_3$, EtOAc, and MeOH extracts of the above mentioned *Salvia* species were also screened for their antioxidant capacity by both chemical and enzymatic methods. Butylated hydroxyanisole (BHA) for DPPH

Table 3
Percentage of inhibitions ±SEM[a] of Turkish *Salvia* species against AChE and BChE at 0.2 mg/ml

| *Salvia* species used | Inhibition % against AChE | | | | Inhibition % against BChE | | | |
|---|---|---|---|---|---|---|---|---|
| | PE | $CHCl_3$ | EtOAc | MeOH | PE | $CHCl_3$ | EtOAc | MeOH |
| *S. albimaculata* | 17.2 ± 1.11 | 8.4 ± 0.86 | NI | NI | 31.4 ± 1.10[*] | 83.8 ± 1.61[*] | 30.8 ± 0.62 | 25.3 ± 0.67 |
| *S. aucheri* var. *canescens* | NI[b] | 47.7 ± 3.15[*] | NI | NI | 13.4 ± 0.46 | 87.3 ± 2.48 | 10.7 ± 1.06[*] | 20.9 ± 1.82[*] |
| *S. candidissima* ssp. *occidentalis* | NI | 26.5 ± 0.71 | NI | NI | 43.4 ± 2.73[**] | 74.8 ± 2.09 | 37.9 ± 4.23[**] | NI |
| *S. ceratophylla* | NI | 7.4 ± 0.42 | NI | NI | NI | 57.4 ± 2.58 | 30.7 ± 1.13[*] | 27.0 ± 1.76[*] |
| *S. cyanescens* | NI | 41.3 ± 2.02 | NI | NI | 67.1 ± 0.52[***] | 81.3 ± 1.83[*] | 63.3 ± 4.05[**] | NI |
| *S. cryptantha* | 20.9 ± 1.12 | 74.7 ± 0.36 | NI | NI | 82.9 ± 1.06[*] | 66.3 ± 1.33[*] | 31.9 ± 1.55[*] | NI |
| *S. frigida* | NI | NI | NI | 46.1 ± 3.54[*] | 11.7 ± 0.38 | 67.8 ± 5.23[**] | 48.8 ± 4.49[**] | NI |
| *S. forskahlei* | NI | NI | NI | NI | 36.9 ± 1.02 | 46.0 ± 8.97[***] | 8.1 ± 1.66[*] | 17.6 ± 1.46[*] |
| *S. halophila* | NI | NI | NI | NI | 13.9 ± 0.71 | 39.8 ± 2.39[*] | 15.5 ± 0.66 | NI |
| *S .migrostegia* | NI | NI | NI | NI | NI | 26.0 ± 2.32[*] | 38.2 ± 1.78[*] | NI |
| *S. multicaulis* | NI | NI | NI | NI | 23.9 ± 2.06[*] | 78.2 ± 5.11[**] | 42.4 ± 1.89[*] | NI |
| *S. sclarea* | NI | 16.9 ± 8.52[**] | NI | NI | 33.1 ± 0.73 | 59.9 ± 2.62[**] | 45.8 ± 1.53[*] | 34.8 ± 2.87[**] |
| *S. syriaca* | NI | NI | NI | NI | 36.4 ± 0.94 | NI | 32.9 ± 0.94 | NI |
| *S. verticillata* ssp. *amasiaca* | 10.5 ± 0.61 | NI | NI | NI | 45.2 ± 5.13[**] | NI | NI | NI |
| Galanthamine | 100.05 ± 0.64 | | | | 98.60 ± 0.59 | | | |

$P > 0.05$.
[a] Values were expressed as mean ±SEM ($n = 3$).
[b] NI = No inhibition.
[*] $P < 0.05$.
[**] $P < 0.01$.
[***] $P < 0.001$.

Table 4
Percentage of inhibitions ±SEM[a] of Turkish *Salvia* species against AChE and BChE at 1 mg/ml

| *Salvia* species used | Inhibition % against AChE | | | | Inhibition % against BChE | | | |
|---|---|---|---|---|---|---|---|---|
| | PE | $CHCl_3$ | EtOAc | MeOH | PE | $CHCl_3$ | EtOAc | MeOH |
| *S. albimaculata* | $89.4 \pm 2.07^{*}$ | NI[b] | $51.7 \pm 3.22^{***}$ | $38.9 \pm 3.22^{***}$ | $73.9 \pm 0.76^{***}$ | $87.9 \pm 0.22$ | $69.8 \pm 1.99^{***}$ | $27.4 \pm 1.32^{***}$ |
| *S. aucheri var. canescens* | $27.3 \pm 0.98^{***}$ | $64.5 \pm 1.03^{***}$ | $53.4 \pm 1.59^{***}$ | $39.9 \pm 1.17^{***}$ | $59.9 \pm 378^{***}$ | $77.6 \pm 3.78^{***}$ | $69.6 \pm 2.15^{***}$ | $12.6 \pm 1.05^{***}$ |
| *S. candidissima ssp. occidentalis* | $39.4 \pm 4.31$ | $48.6 \pm 5.13^{***}$ | $46.1 \pm 1.28^{***}$ | NI | $55.6 \pm 0.28$ | $91.1 \pm 1.98$ | $77.8 \pm 0.93^{***}$ | NI |
| *S. ceratophylla* | NI | $30.8 \pm 5.25^{***}$ | $19.3 \pm 1.57^{***}$ | $27.8 \pm 2.82^{***}$ | $38.8 \pm 4.94^{***}$ | $91.3 \pm 1.63$ | $29.2 \pm 0.77^{***}$ | $34.9 \pm 6.50^{***}$ |
| *S. cyanescens* | $37.7 \pm 5.35$ | $80.2 \pm 4.35^{***}$ | $51.2 \pm 3.78^{***}$ | $9.0 \pm 0.88^{***}$ | $67.4 \pm 3.59^{***}$ | $91.8 \pm 0.54$ | $56.9 \pm 1.03^{***}$ | $13.1 \pm 0.70^{***}$ |
| *S. cryptanhta* | $71.8 \pm 2.62^{***}$ | $24.9 \pm 1.65$ | $73.3 \pm 2.55^{***}$ | $47.2 \pm 5.18^{***}$ | $92.0 \pm 0.41$ | NI | $53.6 \pm 0.67^{***}$ | $36.3 \pm 2.79^{***}$ |
| *S. frigida* | $6.2 \pm 0.24^{***}$ | $53.7 \pm 2.25^{***}$ | $59.5 \pm 0.45^{***}$ | $32.6 \pm 0.01^{***}$ | $54.9 \pm 1.95^{***}$ | $77.8 \pm 0.21^{***}$ | $92.2 \pm 0.29$ | $59.9 \pm 2.30^{***}$ |
| *S. forskahlei* | $25.2 \pm 4.46^{***}$ | $41.3 \pm 2.91$ | $47.0 \pm 2.31^{***}$ | $35.8 \pm 2.46^{***}$ | $69.3 \pm 1.65^{***}$ | $60.2 \pm 4.42^{***}$ | $62.9 \pm 0.67^{***}$ | $46.7 \pm 3.69^{***}$ |
| *S. halophila* | $18.9 \pm 1.21^{***}$ | NI | $36.1 \pm 1.21^{***}$ | NI | $50.9 \pm 4.20^{***}$ | $53.9 \pm 2.16^{***}$ | $37.2 \pm 3.88^{***}$ | NI |
| *S. migrostegia* | NI | $36.4 \pm 5.45^{***}$ | $37.1 \pm 3.15^{***}$ | $23.6 \pm 0.61^{***}$ | $22.1 \pm 2.70^{***}$ | $62.5 \pm 1.31^{***}$ | $89.6 \pm 0.67^{*}$ | $32.6 \pm 3.40^{***}$ |
| *S. multicaulis* | $21.4 \pm 3.91^{***}$ | NI | NI | $47.7 \pm 3.58^{***}$ | $68.8 \pm 3.80^{***}$ | NI | $64.3 \pm 1.02^{***}$ | $36.2 \pm 0.93^{***}$ |
| *S. sclarea* | $25.8 \pm 4.51^{***}$ | $55.3 \pm 0.98^{***}$ | $33.5 \pm 4.94^{***}$ | $25.3 \pm 1.86^{***}$ | $52.6 \pm 2.92^{***}$ | $59.9 \pm 0.50^{***}$ | $75.7 \pm 1.83^{***}$ | $15.1 \pm 1.76^{***}$ |
| *S. syriaca* | $33.4 \pm 2.98$ | $66.9 \pm 2.49^{***}$ | $49.8 \pm 2.41^{***}$ | $12.1 \pm 1.22^{***}$ | $63.5 \pm 2.12^{***}$ | $87.3 \pm 1.99$ | $70.9 \pm 2.69^{***}$ | $12.3 \pm 1.10^{***}$ |
| *S. verticillata* ssp. *amasiaca* | $45.6 \pm 4.17$ | NI | NI | $39.1 \pm 3.10^{***}$ | $85.0 \pm 53.10^{*}$ | $55.7 \pm 0.55^{***}$ | $53.3 \pm 5.50^{***}$ | $72.0 \pm 2.99^{***}$ |
| Galanthamine | $99.87 \pm 0.31$ | | | | $80.31 \pm 1.14$ | | | |

$P > 0.05$.
[a] Values were expressed as mean ± SEM ($n = 3$).
[b] NI = No inhibition.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

scavenger activity and allopurinol for XO inhibition were used as reference drugs. While all of the extracts were tested against DPPH, only the EtOAc and MeOH extracts were screened against XO. As listed in Table 5, the results showed that the EtOAc extracts had high antioxidant activity against XO, ranging between 66.1% and 162.4%, whereas the MeOH extracts belonging to *S. albimaculata*, *S. aucheri* var. *canescens*, *S. candidissima* ssp. *occidentalis*, *S. ceratophylla*, *S. cyanescens*, *S. cryptantha*, *S. frigida*, *S. migrostegia*, *S. multicaulis*, *S. sclarea*, and *S. syriaca* possessed remarkable inhibition over 50% against XO. The PE extracts of selected *Salvia* species did not seem to have DPPH scavenger activity, excepting *S. cryptantha* (74.6%). Only half of the $CHCl_3$ extracts displayed scavenging effect on DPPH over 50%. The most noticeable $CHCl_3$ extracts in the DPPH assay belonged to *S. syriaca* (92.2%), *S. frigida* (82.7%), and *S. cryptantha* (71.1%), which had inhibition rates close to that of BHA. The EtOAc extracts were highly active against DPPH, excluding *S. syriaca*, while all of the MeOH extracts exhibited quite noticeable DPH radical-scavenging activity, which were identical to that of BHA.

## 4. Discussion

Up to date, a number of studies on AChE inhibitory activity of several *Salvia* species have been reported. Among these, the essential oil and ethanolic extract of *S. officinalis* along with the essential oil of *S. lavandulaefolia* have been shown to possess anticholinesterase activity (Perry, Court, Bidet, Court, & Perry, 1996). Moreover, the essential oil as well as its major components, α-pinene, 1,8-cineole, and camphor were determined to have uncompetitive and reversible acetylcholinesterase inhibitory activity (Perry, Houghton, Theobold, Jenner, & Perry, 2000). The activity of the essential oil was concluded mainly to be due to its monoterpenoids. In another study, the acetone extract of the dried root of *S. miltiorrhiza* was subjected to activity-guided isolation for its acetylcholinesterase inhibitory activity by the Ellman method and afforded four diterpenes; dihydrotanshinone, cryptotanshinone, tanshinone I, and tanshinone IIA (Ren, Houghton, Hider, & Howes, 2004). On the other hand, the essential oils of *S. fruticosa*, *S. lavandulaefolia*, *S. officinalis* and *S. officinalis* var. *purpurea* were screened for their anti-BChE activity by the Ellman method and a time-dependent increase was shown in the inhibition of BChE by the oils of *S. fruticosa* and *S. officinalis* var. *purpurea*. In this study, it was concluded that synergy was the most possible interaction for anti-BChE activity of the oils and their constituents (Savelev, Okello, & Perry, 2004).

All these data indicate that the terpenoids, monoterpenes in particular, may have anticholinesterase activity which prompted us to investigate the above-mentioned *Salvia* species for their terpenoid-derivative compounds. For this purpose; we analyzed the extracts by thin layer

Table 5
Percentage of inhibitions ±SEM[a] of Turkish *Salvia* species against DPPH and xanthine oxidase (XO)

| *Salvia* species screened | Inhibition % against XO | | Inhibition % against DPPH | | | |
|---|---|---|---|---|---|---|
| | EtOAc | MeOH | PE | $CHCl_3$ | EtOAc | MeOH |
| *S. albimaculata* | 81.9 ± 1.12 | 95.8 ± 1.08 | 7.7 ± 0.98*** | 15.1 ± 1.34*** | 83.1 ± 2.53* | 89.4 ± 0.92 |
| *S. aucheri* var. *canescens* | 81.4 ± 1.38 | 94.4 ± 0.92 | 34.3 ± 4.94*** | 8.4 ± 1.17*** | 90.9 ± 0.54 | 92.0 ± 0.63 |
| *S. candidissima* ssp. *occidentalis* | 90.5 ± 1.97 | 69.4 ± 1.22 | 21.2 ± 1.89*** | 59.9 ± 4.29*** | 92.4 ± 0.67 | 87.7 ± 1.07 |
| *S. ceratophylla* | 90.1 ± 2.49 | 81.7 ± 2.16 | 15.9 ± 2.03*** | 53.3 ± 1.78*** | 88.9 ± 0.84 | 84.8 ± 1.11 |
| *S. cyanescens* | 77.8 ± 0.97 | 72.6 ± 5.73 | 30.6 ± 3.06*** | 55.3 ± 3.30*** | 51.8 ± 1.51*** | 87.2 ± 2.58 |
| *S. cryptanhta* | 66.3 ± 1.18 | 74.4 ± 7.12 | 74.6 ± 2.13*** | 71.1 ± 0.91*** | 82.5 ± 0.75* | 91.5 ± 1.07 |
| *S. frigida* | 93.2 ± 3.47 | 80.8 ± 0.69 | 18.9 ± 3.93*** | 82.7 ± 1.71 | 80.1 ± 2.80** | 91.5 ± 0.09 |
| *S. forskahlei* | 82.6 ± 2.68 | 46.8 ± 1.19*** | 4.8 ± 0.51 | 28.3 ± 0.83*** | 82.2 ± 0.38* | 93.5 ± 1.18 |
| *S. halophila* | 75.4 ± 1.74 | 18.4 ± 2.61*** | NI | 34.6 ± 0.42*** | 90.49 ± 0.97 | 83.9 ± 1.32 |
| *S. migrostegia* | 91.5 ± 0.91 | 74.3 ± 1.81 | 17.8 ± 1.36*** | 22.2 ± 0.73*** | 86.4 ± 0.79 | 89.7 ± 1.27 |
| *S. multicaulis* | 100.4 ± 8.96 | 73.3 ± 3.45 | 4.9 ± 0.96*** | 26.8 ± 2.31*** | 85.3 ± 3.32 | 92.6 ± 0.38 |
| *S. sclarea* | 90.5 ± 1.58 | 87.2 ± 1.78 | 22.6 ± 0.73*** | 54.2 ± 3.93*** | 72.8 ± 2.66*** | 87.8 ± 1.13 |
| *S. syriaca* | 83.2 ± 0.99 | 70.8 ± 2.32 | 13.0 ± 0.43*** | 92.2 ± 0.28 | 36.7 ± 4.91*** | 90.7 ± 1.00 |
| *S. verticillata* ssp. *amasiaca* | 66.1 ± 0.98 | 45.4 ± 2.27*** | 18.0 ± 0.42*** | 30.5 ± 1.37*** | 72.8 ± 0.42*** | 93.6 ± 0.67 |
| Reference drugs | | | | | | |
| BHA (for DPPH) | – | – | 92.75 ± 0.21 | | | |
| Allopurinol (for XO) | 80.52 ± 0.85 | – | – | – | – | |

$P > 0.05$.
[a] Values were expressed as mean ±SEM ($n = 3$).
[b] NI = No inhibition.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

chromatography (TLC) on silica gel, whose spots were revealed by vanillin–sulphuric acid reagent and the violet colored-spots occurred after application of this reagent that pointed toward the presence of terpenoids in the $CHCl_3$ and EtOAc extracts of the *Salvia* species. At 1 mg/ml concentration (Table 4), it was observed that, as the polarity increased, anticholinesterase effects of the PE extracts belonging to *S. albimaculata* and *S. crypthantha* gradually decreased. This may be most likely due to anticholinesterase activity of nonpolar compounds found in high amounts within these extracts, which is in accordance with our supposition that the MeOH extracts, containing the polar compounds, exerted the least inhibitory activity at both concentrations, with the exception of *S. verticillata* ssp. *amasiaca*.

On the other hand, the role of oxidative stress in the pathogenesis of diseases such as macular degeneration, certain types of cancer, and Alzheimer's disease (AD) has received substantial attention. For that reason, we also aimed to look into antioxidant capacities of selected *Salvia* species (sage) as well as their anticholinesterase activity. Our literature survey highlighted that there have been a number of studies on the antioxidant potential of various *Salvia* species. In earlier studies, sage was shown to contain phenolic compounds for the most part and the antioxidant activity of the plant was mainly attributed to carnosic and rosmarinic acids (Cuvelier, Richard, & Berset, 1996). In Lu and Foo's study (2001), the sage polyphenols, including flavone glycosides and some rosmarinic acid derivatives, from *S. officinalis* were found to display potent antioxidant activity against DPPH and superoxide anion radicals.

Polyphenolic compounds from various *Salvia* species have been reported to have potent antioxidative effect (Lu & Foo, 2001; Madsen & Bertelsen, 1995; Whu, Lee, Ho, & Chang, 1982). For instance; salvianolic acid, a rosmarinic acid dimer isolated from *S. officinalis*, had a very strong free radical-scavenging activity for DPPH and superoxide anion radicals (Lu & Foo, 2001). The same researchers investigated the antioxidant capacity of rosmarinic acid, salvianolic acids K and I, sagecoumarin and sagerinic acid isolated from the same plant as well as a number of flavon glycosides such as luteolin 7-glucoside, 7-glucuronide, 3′-glucuronide, 6-hydroxyluetolin 7-glucoside, and apigenin 6,8-di-*C*-glucoside (Lu & Foo, 2001). Consequently, the flavonoid glycosides were found to possess weaker DPPH-scavenging activity. Interestingly, β-sitosterol isolated from *S. plebeia* was also found to be a strong antioxidant by the oxidative stability instrument (OSI) (Weng & Wang, 2000). Therefore, it made us think that the high antioxidative property of PE extract of *S. cryptantha* against DPPH in our study could be related to its sterol content. Moreover, beyond that, we have just started to analyze our 14 *Salvia* species by reversed-phase HPLC for their caffeic, rosmarinic, chlorogenic, and gallic acids by a new method to be validated, which, later on, may allow us to be able to make a correlation between their antioxidant capacity and quantity of these phenolic acids, known as the strong antioxidant components.

## 5. Conclusion

According to data we obtained, out of 56 extracts from 14 *Salvia* species, only 2 extracts against AChE and 13 extracts against BChE had noticeable activity at 0.2 mg/ml, whereas 13 extracts against AChE and 37 extracts against BChE showed remarkable activity at concentration of 1 mg/ml. From these results, we can point out that the nonpolar *Salvia* extracts, including PE and $CHCl_3$, seemed to be much more effective then EtOAc and MeOH extracts at both concentrations. Besides, the *Salvia* extracts studied were more active against BChE than AChE (Tables 3 and 4). To the best of our knowledge, this is the first evaluation of the AChE and BChE inhibitory activities of Turkish *Salvia* species.

The difference observed between the AChE and BChE inhibitory activities of Turkish *Salvia* species seems to be due to their different phytochemical contents. Since it is evident that preventive and symptomatic treatment of AD needs a multitarget drug strategy, it is quite rational to examine the phytochemistry of the *Salvia* species having both anticholinesterase and antioxidant activities. Consequently, ingredients of the active extracts, which are primarily expected to be terpenoid-type compounds, should be identified and activity of the single compounds should also be compared with that of the crude extract to reveal possible synergistic interaction. Thus, the further work on identification of the active component(s) of the *Salvia* species effective in both assays is in progress in our laboratory.

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