Journal of Medicinal Plants Research Vol. 5(29), pp. 6484-6490, 9 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.220

Full Length Research Paper

Antimicrobial and anticholinesterase activities of the essential oils isolated from *Salvia dicroantha* Stapf., *Salvia verticillata* L. subsp. *amasiaca* (Freyn and Bornm.) Bornm. and *Salvia wiedemannii* Boiss.

Buket Kunduhoglu[1]*, Mine Kurkcuoglu[2], Mehmet Emin Duru[3] and Kemal Husnu Can Baser[2]

[1]Department of Biology, Faculty of Science and Arts, Eskişehir Osmangazi University, 26480 Eskişehir, Turkey.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey.
[3]Department of Chemistry, Faculty of Science and Arts, Muğla University, 48121 Muğla, Turkey.

Accepted 21 October, 2011

In this study, the essential oil compositions and antimicrobial and anticholinesterase activities of three Turkish endemic *Salvia* species were investigated. The gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS) analysis of the essential oils obtained from *Salvia dicroantha, Salvia verticillata* subsp. *amasiaca,* and *Salvia wiedemannii* showed that the major constituents were caryophyllene oxide (22.4%), Germacrene D (36.6%), and α-pinene (36.2%), respectively. All essential oils exhibited antimicrobial activity against tested Gram-positive and Gram-negative bacteria, and test yeast strains. The minimum inhibitory concentrations (MICs) of oils ranged from 12.5 to 50.0 µl/ml. Additionally, essential oils were more active than standard antifungals, inhibiting test strains of yeasts such as *Candida albicans, Candida glabrata,* and *Saccharomyces cerevisiae.* At 400 µM concentration, the essential oil of *S. wiedemannii* had moderate acetylcholinesterase (55.95±2.01%), and butyrylcholinesterase (50.97±3.12%) inhibitory activity, while other two species no activity against both enzymes.

Key words: *Salvia dicroantha*, *Salvia verticillata* subsp. *amasiaca*, *Salvia wiedemannii*, essential oil, antimicrobial activity, anticholinesterase activity, minimum inhibitory concentrations (MICs).

INTRODUCTION

Salvia L. is the largest genus of the family Lamiaceae, represents nearly 1000 species around the world (Walker et al., 2004). *Salvia* has been used by people as a folk medicine since ancient times (Naghibi et al., 2005), and illnesses such as rheumatism, bronchitis, tuberculosis (Watt and Breyer-Brandwijk, 1962), psoriasis, and eczema (Janosik, 1980) have been cured using this plant. Some *Salvia* species have also been used as a remedy for memory loss (Perry et al., 1996). In Turkish folk medicine, *Salvia* species, known as "adaçayı," have been used as a tonic, stimulant, diuretic, carminative, antiseptic, to treat inflammations in the mouth, and to combat infections. They are also consumed as herbal tea and used as food flavor (Baytop, 1999). It has been determined by some studies that essential oils and various extracts of members of this genus have wide antimicrobial spectrum such as antifungal activity (Ozek et al., 2010; Bouaziz et al., 2009), antibacterial activity (Bouaziz et al., 2009; Cardile et al., 2009), antimycobacterial activity (Askun et al., 2010), and antiviral activity (Alim et al., 2009). Additionally, the essential oils of this genus exhibiting antimicrobial activity against some food borne pathogens such as *Escherichia*

*Corresponding author. E-mail: bkunduh@gmail.com. Tel: +90 222 2393750. Fax: +90 222 2393578.

coli, *Bacillus cereus, Listeria monocytogenes, Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Salmonella typhimurium* (Burt, 2004; Hayouni et al., 2008; Gutierrez et al., 2008; Kamatou et al., 2006) have shown that they have great potential as natural food preservers.

Another important finding obtained from studies carried out on the *Salvia* species is that it inhibits acetylcholinesterase (AChE) and butyrylcholinestease (BChE) enzyme activities (Kivrak et al., 2009; Loizzo et al., 2009). AChE and BChE are the chief enzymes in the pathogenesis of Alzheimer's disease (AD). Therefore, AChE and BChE inhibitors have been associated with the treatment of AD (Atta-ur-Rahman and Choudhary, 2001). Tacrine and donepezil, the synthetic drugs currently used to cure AD have side effects such as toxicity in liver, dizziness, and bradycardia. Natural drugs such as rivastigmine and galantamine are used for the therapy of mild patients of AD (Dokmeci, 2000). Therefore, utilization and development of better AChE and BChE inhibitors from natural resources are needed.

Salvia is represented by 94 taxas of 89 species in Turkey and almost 50% of these are endemic (Hedge, 1982). Although Turkey is a country rich in endemic *Salvia* species, not many studies have been conducted on the essential oil components of these endemic plants and their biological activities. *Salvia dichroantha, Salvia verticillata* subsp*. amasiaca,* and *Salvia wiedemannii* are the endemic members of this genus. To the best of our knowledge, reports on the chemical composition of the essential oils of *S. dichroantha* (Kawazoe et al., 1999) and *S. wiedemannii* (Kaya et al., 2009) are limited and there is no report on their antimicrobial and anticholinesterase (anti-ChE) potential in literature. Some studies on the chemical composition and antimicrobial profiles of *S. verticillata* subsp*. amasiaca* (Altun et al., 2007; Askun et al., 2010) have previously been published but there is no report on its anti-ChE activity. Therefore, the current research reports the chemical composition, antimicrobial and anti-ChE potential of the essential oils of three endemic *Salvia* species.

MATERIALS AND METHODS

Plant material and isolation of essential oils

The aerial parts of the flowering plants were collected during June 2008 from Bilecik and Eskişehir province, Turkey. Information about the collection dates and localities of studied plants are listed as follow:

(1) *S. dicroantha* Stapf.: Bilecik province, Bozüyük to Arapdede, 13 June 2008.
(2) *S. verticillata* L. subsp. *amasiaca* (Freyn & Bornm.) Bornm.: Bilecik province, Bozüyük to Kozpınar, 13 June 2008.
(3) *S. wiedemannii* Boiss.: Eskişehir province, Middle Sakarya to Bozan plantation area-L1, 08 June 2008.

The plant samples were identified by Assoc. Prof. Dr. Atilla Ocak and voucher specimens have been deposited in the Herbarium of the Department of Biology, Faculty of Sciences and Arts, Eskişehir Osmangazi University. Plants were dried in shade at room temperature and subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The essential oils obtained were stored in amber vial at 4℃ prior to analysis.

Gas chromatography-mass spectrometry (GC-MS) conditions

GC-MS

The GC-MS analysis was carried out using an Agilent 5975 GC-MSD system (Agilent Technologies Inc., Santa Clara, CA). Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium as a carrier gas (0.8 ml/min). The GC oven temperature was kept at 60℃ for 10 min and programmed to 220℃ at a rate of 4℃/min, and kept constant at 220℃ for 10 min and then programmed to 240℃ at a rate of 1 °C/min. The split ratio was adjusted to 40:1. The injector temperature was at 250℃. MS was taken at 70 eV. The mass range was from *m/z* 35 to 450.

Gas chromatography (GC)

The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain the same elution order with GC/MS, simultaneous injection was done using the same column and appropriate operational conditions. The flame ionization detector (FID) temperature was 300℃. The components of essent ial oils were identified by comparing their mass spectra with those of the Baser Library of essential oil constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and were confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from the FID chromatograms.

Determination of minimum inhibitory concentration (MIC)

The MIC value was determined by the broth micro-dilution susceptibility assay using 96-well microtiter plates (NCCLS, 1999). Stock solutions of the essential oils were prepared in 25% DMSO. Two-fold serial dilutions of essential oils were prepared to achieve a decreasing concentration range of 100 to 0.098 µl/ml. Each well was filled with 25 µl of serial essential oil dilution and inoculated with 25 µl of the bacterial suspension at a density of 10^6 cell/ml and of the yeast suspension at a density of 10^5 cell/ml. The microtiter plates inoculated with bacteria were incubated for 24 h at 37℃ and the fungal cultures were incubated at 30℃ for 48 h. The last row containing only serial dilutions of antimicrobial agent without microorganism was used as negative control.

Gentamicin (for bacteria), Amphotericin B, and Nystatin (for yeasts) were used as standard antimicrobial agents. MIC was defined as the lowest concentration of essential oil that prevented visible growth of test organisms. The minimum bactericidal concentrations or minimum fungicidal concentrations (MBC/MFC) of oils were determined by subculturing 10 µl from each negative well and from the positive growth control wells. The MBC/MFC was defined as the lowest concentration yielding negative subcultures. All tests were made in duplicate. Additionally, inhibitory activity of the DMSO (as 12.5% final concentration in wells) against test strains was checked.

Table 1. Essential oil constituents of *S. dichroantha*.

RRI	Main compounds*	Composition (%)
1535	β-Bourbonene	1.0
1612	β-Caryophyllene	2.1
1661	Alloaromadendrene	3.1
1704	γ-Muurolene	1.4
1726	Germacrene D	1.2
1945	1,5-Epoxy-salvial(4)14-ene	1.5
1958	(*E*)-β-Ionone	1.0
2008	Caryophyllene oxide	22.4
2037	Salvial-4(14)-en-1-one	2.9
2123	Salviadienol	2.5
2130	Spathulenol	1.1
2131	Hexahydrofarnesyl acetone	1.7
2243	Torilenol	3.2
2369	Eudesma-4(15), 7-dien-1β-ol	4.7
2392	Caryophyllenol II (=*Caryophylla-2(12),6-dien-5*β*-ol*)	5.5
2622	Phytol	5.6
2700	Heptacosane	1.0
2900	Nonacosane	1.1

* Only values of 1% and above are indicated in this table.

Anticholinesterase activity

The inhibitory activity of AChE and BChE 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′-Dithio-bis (2-nitrobenzoic) acid was used for the measurement of the cholinesterase activity. Ethanol was used as a solvent to dissolve test compounds and controls. Briefly, 150 µl of 100 mM sodium phosphate buffer (pH 8.0), 10 µl of sample solution dissolved in ethanol at different concentrations, and 20 µl AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) solution was mixed and incubated for 15 min at 25℃, and 10 µl of 0.5 mM DTNB was added. The reaction was then initiated by the addition of 10 µl of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM).

The hydrolysis of these substrates were monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, at a wavelength of 412 nm utilizing a 96-well microplate reader (SpectraMax PC340, Molecular Devices, USA). The measurements and calculations were evaluated by using Softmax PROv5.2 software. The percentage of inhibition of AChE or BChE was determined by comparing the reaction rates of the samples relative to a blank sample (ethanol in phosphate buffer pH 8) using the formula:

$$(E - S) / E \times 100$$

where E is the activity of the enzyme without the test sample and S is the activity of the enzyme with the test sample. The experiments were carried out in triplicate. Galantamine was used as a reference compound.

RESULTS

Chemical composition of essential oils

In this study, antimicrobial and anticholinesterase enzyme activities of essential oils obtained from aerial parts of three *Salvia* species endemic to Turkey were determined. The yield of essential oils obtained by hydrodistillation of *S. dicroantha, S. verticillata* subsp. *amasiaca,* and *S. wiedemannii* were determined as 0.04, 0.04, and 0.27% (w/w), respectively. The compositions of essential oils obtained from the aerial parts of three *Salvia* species are provided in Tables 1 to 3. The major constituents of *S. dicroantha* essential oil were caryophyllene oxide (22.4%), hexadecanoic acid (18.3%), phytol (5.6%), and caryophyllenol II (5.5%). *S. verticillata* subsp. *amasiaca* had high levels of Germacrene D (36.6%), β-caryophyllene (7.6%), hexadecanoic acid (6.7%), and β-copene (5.7%). The major constituents of *S. wiedemannii* were determined as α-pinene (36.2%), β-pinene (13.3%) and 1,8-cineole (14.2%).

Antimicrobial activity

The antimicrobial activity of essential oils obtained from

Table 2. Essential oil constituents of *S. verticillata* subsp. *amasiaca*.

RRI	Main compounds*	Composition (%)
1602	β-Copaene	5.7
1612	β-Caryophyllene	7.6
1687	α-Humulene	3.2
1704	γ-Muurolene	2.4
1726	Germacrene D	36.6
1755	Bicyclogermacrene	2.2
1773	δ-Cadinene	1.8
2008	Caryophyllene oxide	2.4
2123	Salviadienol	1.0
2130	Spathulenol	4.5
2243	Torilenol	1.0
2255	α-Cadinol	1.0
2931	Hexadecanoic acid	6.7

* Only the percentages over 1% are indicated in this table.

three *Salvia* species were evaluated against some pathogenic bacteria and yeasts and their potencies were quantitatively assessed by MIC values. The MIC values of essential oils are provided in Table 4. All essential oils inhibited the growth of all tested microorganisms in varying concentrations; however, this effect was not cidal but static. The minimum inhibitory concentrations (MICs) of essential oils ranged from 12.5 to 50.0 µl/ml. Additionally, essential oils were more active than standard antifungals, inhibiting test strains of yeasts such as *Candida albicans, Candida glabrata,* and *Saccharomyces cerevisiae.*

Anticholinesterase activity

The anti-ChE enzyme activity of essential oils is compared with galantamine in the same concentration in Table 5. While the essential oil of *S. wiedemannii* showed moderate AChE (55.95±2.01) and BChE (50.97±3.12) inhibitor activity, the other essential oils belonging to the other two species showed almost no inhibitor effect.

DISCUSSION

It is widely known that essential oil components of plants and their biological activities are influenced by some factors such as geographical region where the plants are collected, climate, harvest season and distillation method (Panizzi et al., 1993). In our study the major component of *S. verticillata* subsp. *amasiaca* essential oil is Germacrene D (36.6%). However, Askun et al. (2010) determined different main constituents (β-pinene: 21.4% and 1,8-cineole: 16.1%) from *S. verticillata* subsp. *amasiaca* collected from different geographical regions (Bitlis, Tatvan, Hizan- Eastern Anatolia). In this study, α-pinene (36.2%), 1,8-cineole (14.2%), β-pinene (13.3%), camphor (7.4%), and *p*-cymene (5.4%) were found to be the major components of the essential oil of *S. wiedemannii*. Similar to our results, 1,8-cineole (38.4%), β-pinene (24.5%), and α-pinene (13.6%) were found to be the major components of the essential oil of *S. wiedemannii* collected from Eskişehir region (Kaya et al., 2009).

The antimicrobial activities of the essential oils of *S. dicroantha* and *S. wiedemannii* were reported for the first time in this study. However, Askun et al. (2010) determined the MIC value of the essential oil of *S. verticillata* subsp. *amasiaca* as 196 µg/ml for *Mycobacterium tuberculosis* H37Ra (ATCC 25177).

Essential oils were antimicrobially effective in relatively lower MIC values against Gram-positive bacteria (*B. cereus*, *E. faecium*, and *S. aureus*: 12.5 to 50 µl/ml) than Gram-negative (*E. coli* and *E. aerogenes*: 25 to 50 µl/ml) ones. In antimicrobial activity studies carried out on essential oils, although it has been reported that generally Gram-positive bacteria were more sensitive compared to Gram-negative bacteria, some studies showed no significant difference between these two groups in terms of sensitivity (Burt, 2004). The MIC values of essential oils against test yeast strains have varied between 25 and 30 µl/ml. It is quite remarkable that essential oils have shown stronger anti-yeast activity than Amphotericin B and Nystatin used as positive control. The results showed that essential oils can be used as potential anticandidal agents against *C. albicans* and *C. glabrata.* Similar results have also

Table 3. Essential oil constituents of *S. wiedemanni*.

RRI	Main compounds*	Composition (%)
1032	α-Pinene	36.2
1076	Camphene	4.3
1118	β-Pinene	13.3
1132	Sabinene	1.4
1203	Limonene	1.2
1213	1,8-Cineole	14.2
1255	γ-Terpinene	1.4
1280	*p*-Cymene	5.4
1532	Camphor	7.4
1664	*trans*-Pinocarveol	1.1
1683	*trans*-Verbenol	1.0
1719	Borneol	1.9
1726	Germacrene D	1.1

Table 4. Antimicrobial activity of essential oils isolated from three *Salvia* sp.

Test strains	MIC values (µl/ml)			MIC values (µg/ml)	
	S. dicroantha	***S. verticillata* subsp. *amasiaca***	***S. wiedemannii***	**Reference antibiotics**	
				Gentamicin	
B. cereus LMG[a] 8221	25.0	50.0	50.0	0.15	
E. faecium NRRL[b] 2354	12.5	12.5	12.5	20.0	
S. aureus ATCC[c] 25923	25.0	12.5	12.5	10.0	
E. coli LMG 8223	50.0	25.0	50.0	2.5	
E. aerogenes NRRL 427	25.0	25.0	25.0	0.6	
				Amphotericin	**Nystatin**
C. albicans NRRL 12983	50.0	50.0	50.0	125	46.9
C. glabrata NRRL 1431	25.0	50.0	25.0	125	187.5
S. cerevisiae NRR 12796	12.5	25.0	12.5	62.5	46.9

[a] LMG: Belgian Co-Ordinated Collections of Micro-Organisms. BCCM/LMG, Laboratorium Voor Microbiologie, Universiteit Gent (Ugent), K.L. Ledeganckstraat 35, B- 9000 Gent, BELGIUM. b NRRL: ARS Culture Collection (Northern Regional Research Laboratory). Microbial Genomics and Bioprocessing Research, Ncaur, 1815 N. University Street Peoria, Il 61604, USA. c ATCC: The American Type Culture Collection. 10801 University Boulevard, Manassas (VA) 20110-2209, USA.

Table 5. Anti-ChE activity of essential oils isolated from three *Salvia* sp.

Essential oils	AChE assay		BChE assay	
	Inhibition (%) (400 µM)	IC_{50}[a] (µM)	Inhibition (%) (400 µM)	IC_{50} (µM)
S. dichroantha	43.25±2.06	>400	47.07±2.21	>400
S. verticillata subsp. *amasiaca*	20.36±1.01	>400	1.79±0.02	>400
S. wiedemanii	55.95±2.01	350.22±2.23	50.97±3.12	380.12±1.12
Galantamine[b]	73.97±0.8[c]	5.01±0.11	75.03±0.60[c]	50.88±0.95

[a]IC_{50} values represent the means ± standard deviation of three parallel measurements (p<0.05). [b]Standard drug. [c]in 200 µM concentration.

been obtained in studies carried out on *Salvia* species. In the study on the essential oil of *Salvia officinalis* (Pinto et al., 2007), anticandidal activity was determined with MIC values varying between 1.25 and 10 µl/ml. Kivrak et al. (2009) reported the MIC value to be 18.5 µg/ml for *C. albicans* in their study on the essential oil of

Salvia potentillifolia and they determined that the major components of *S. potentillifolia* (α-pinene and β-pinene) showed weaker anticandidal activity than the whole essential oil. It was concluded that this condition originated from a possible synergistic interaction between the major components in the composition of the essential oil and other minor components. Plant essential oils or extracts constitute the basis of several applications including raw and processed food conservation, pharmaceuticals, alternative medicine, and natural therapy (Deans and Sbodova, 1990). Essential oils, oleoresins and natural extractives of some plants, including Salvia (*Salvia lavandulaefolia* Vahl., *S. officinalis* L., *Salvia sclarea* L., and *Salvia triloba* L.), have been included in the list of "Substances Generally Recognized as Safe (GRAS)" by Food and Drug Administration (FDA, 2010). From this point of view, the essential oils we tested have antimicrobial effect on important food borne pathogens (such as *S. aureus*, *B. cereus, E. coli*) and *Candidas;* hence they have potential for use as natural antimicrobial agents. However, acute toxicity, chronic toxicity and surface contact studies should first be performed and safe doses should be determined.

The essential oil of *S. wiedemannii* showed inhibition toward AChE and BChE at 400 mM having 55.95±2.01% and 50.97±3.12% inhibition, respectively. The existence of BChE enzyme inhibition along with AChE has specific importance because during the latter phase of AD AChE level in the brain reduces up to 85% and BChE becomes predominant. Therefore, in order to slow down the progress of AD, inhibition of BChE has been targeted recently (Perry et al., 1978). In our study, the major components of the essential oil of *S. wiedemannii* are α-pinene, β-pinene and 1,8-cineole, camphor, and *p*-cymene. Similarly, Loizzo et al. (2009) determined that the major components of the essential oil of *Salvia leriifolia* were camphor (10.5%), 1,8-cineole (8.6%), camphene (6.2%), and α-pinene (4.7%). They reported that this oil showed considerable AChE and BChE inhibition with the value 32 and 0.29 µl/ml IC_{50}, respectively. Kivrak et al. (2009) determined the AChE and BChE inhibition activity with the essential oil of *S. potentillifolia* as >200 µM (21.9%) and 100 µM (66.7%), respectively. In the same study, while a remarkable AChE enzyme inhibition was observed with α-pinene which is a major component of the essential oil of *S. potentillifolia* with a value of 81.7 IC_{50} (87.50%, 200 µM), neither AChE nor BChE inhibitor activity was observed with β-pinene. While in our study α-pinene (36.2%) was the major component of the essential oil of *S. wiedemannii,* it was one of the minor components (<1%) of the other two studied *Salvia* species. From these data, it may be concluded that the α-pinene mainly contributed to the AChE inhibitor activity of *S. wiedemannii*. However, differences in the composition of essential oils belonging to various *Salvia* species which inhibit ChE enzyme raise difficulties in explaining structure-activity relation. It is thought that possible synergistic/ antagonistic interaction between major essential oil components and minor components can affect anti-ChE activity.

Conclusion

To the best of our knowledge, this is the first study in which the antimicrobial and anti-ChE activities of the essential oils of *S. dicroantha* and *S. wiedemannii* were determined. Also, the anti-ChE activity of the essential oil *S. verticillata* subsp. *amasiaca* was reported for the first time. Essential oils which were obtained from three endemic *Salvia* species which had a remarkable antimicrobial activity against food-borne pathogens and *Candidas* show that they have the potential to be used as natural antimicrobial agents. In addition, the essential oil of *S. wiedemannii* showed moderate anti-AChE and anti-BChE enzyme activity proving that it may also be used medicinally. However, additional studies as acute and chronic toxicity, and surface contact should first be performed and safe doses of essential oils should be determined.

ACKNOWLEDGEMENT

The authors wish to thank Assoc. Prof. Dr. Atilla Ocak, Department of Biology, Faculty of Science and Arts, University of Eskişehir Osmangazi, for his help in the collection and identification of the plant species studied.

REFERENCES

Alim A, Goze I, Goze HM, Tepe B (2009). *In vitro* antimicrobial and antiviral activities of the essential oil and various extracts of *Salvia cedronella* Boiss. J. Med. Plants Res., 3: 413-419.

Altun M, Unal M, Kocagoz T, Goren AC (2007). Essential oil compositions and antimicrobial activity of *Salvia* species. J. Essent. Oil Bear. Plants, 10: 251-258.

Askun T, Baser KHC, Tumen G, Kurkcuoglu M (2010). Characterization of essential oils of some *Salvia* species and their antimycobacterial activities. Turk. J. Biol., 34: 89-95.

Atta-ur-Rahman, Choudhary MI (2001). Bioactive natural products as a potential source of new pharmacophores, a theory of memory. Pure Appl. Chem., 73: 555-560.

Baytop T (1999). Therapy with medicinal plants in Turkey (past and present). Istanbul University Publications No. 3255, Istanbul: Nobel Press House, P. 480.

Bouaziz M, Yangui T, Sayadi S, Dhouib A (2009). Disinfectant properties of essential oils from *Salvia officinalis* L. cultivated in Tunisia. Food Chem. Toxicol., 47: 2755-2760.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods- a review. Int. J. Food Microbiol., 94: 223-253.

Cardile V, Russo A, Formisano C, Rigano D, Senatore F, Arnold NA, Piozzi F (2009). Essential oils of *Salvia bracteata* and *Salvia rubifolia* from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. J. Ethnopharmacol., 126: 265-272.

Deans SG, Sbodova KP (1990). The antimicrobial properties of marjoram (*Origanum majorana* L.) volatile oil. Flavour Fragr. J., 5: 187-190.

Dokmeci I (2000). Essentials of pharmacology. Nobel Press House, Istanbul, pp. 190-195.

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

FDA (2010). Food and Drug Administration, Department of Health and Human Services. Substances Generally Recognized as Safe (GRAS), 21CFR182.10, access date: 18/07/ 2011. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=182.10

Gutierrez J, Barry-Ryan C, Bourke R (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol., 124: 91-97.

Hayouni EA, Chraief I, Abedrabba M, Bouix M, Leveau JY, Mohammed H, Hamdi M (2008). Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat. Int. J. Food Microbiol., 125: 242-251.

Hedge IC (1982). Flora of Turkey and East Aegean Islands. In P. H. Davis et al., (Eds.). *Salvia* L. *()*, Edinburgh: Edinburgh University Press, 7: 400-461.

Janosik J (1980). Czechoslovakian Patent No: 185.262; Chem. Abst. (1981), 95: 68027f.

Kamatou GPP, Van Zyl RL, Van Vuuren SF, Viljeon AM, Figueiredo AC, Pedro SF, Barroso LG (2006). Biological activities and composition of *Salvia muirii* L. Bol. essential oil. J. Essent. Oil Res., 18: 48-51.

Kawazoe K, Yamamoto M, Takaishi Y, Honda G, Fujita T, Sezik E, Yesilada E (1999). Rearranged abietane-type diterpenes from *Salvia dicroantha.* Phytochemistry, 50: 493-497.

Kaya A, Baser KHC, Demirci B (2009). Composition of essential oil of endemic *Salvia wiedemannii* in Turkey. Chem. Nat. Compd., 45: 552-553.

Kivrak I, Duru, ME, Ozturk M, Mercan M, Harmandar M, Topcu G (2009). Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. Food Chem., 116: 470-479.

Loizzo MR, Menichini F, Tundis R, Bonesi M, Conforti F, Nadiafi F, Statti GA, Frega N, Menichini F (2009). *In vitro* biological activity of *Salvia leriifolia* Benth. essential oil relevant to the treatment of Alzheimer's disease. J. Oleo Sci., 58: 443-446.

Naghibi F, Mosaddegh M, Mohammadi Motamed S, Ghorbani A (2005). Labiatae family in folk medicine in Iran: from Ethnobotany to Pharmacology. Iran. J. Pharm. Res., 2: 63-79.

NCCLS (1999). National Committee for Clinical Laboratory Standards, *Performance standards for antimicrobial susceptibility testing*, 9th Informational supplement. NCCLS document M100-S9. NCCLS, Wayne, PA.

Ozek G, Demirci F, Ozek T, Tabanca N, Wedge DE, Khan SI, Baser KHC, Duran A, Hamzaoglu E (2010). Gas chromatographic-mass spectrometric analysis of volatiles obtained by four different techniques from *Salvia rosifolia* Sm., and evaluation for biological activity. J. Chromatogr. A, 1217: 741-748.

Panizzi L, Flamini G, Cioni PL, Morelli I (1993). Composition and antimicrobial activity of essential oils of four Mediterranean Lamiaceae. J. Ethnopharmacol., 39: 167-170.

Perry EK, Perry RH, Blessed G, Tomlinson BE (1978). Changes in brain cholinesterases in senile dementia of Alzheimer type. Neuropathol. Appl. Neurobiol., 4: 273-277.

Perry N, Court G, Bidet N, Court J, Perry E (1996). Cholinergic activities of European herbs and potential for dementia therapy. Int. J. Geriat. Psychiat., 11: 1063-1069.

Pinto E, Salgueiro LR, Cavaleiro C, Palmeira A, Goncalves MJ (2007). *In vitro* susceptibility of some species of yeasts and filamentous fungi to essential oils of *Salvia officinalis*. Ind. Crops Prod., 26: 135-141.

Walker JB, Sytsma KJ, Treutlein J, Wink M (2004). *Salvia* is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. Am. J. Bot., 91: 1115-1125.

Watt JM, Breyer-Brandwijk MG (1962). The medicinal and poisonous plants of Southern and Eastern Africa. Being an account of their medicinal and other uses, chemical composition, pharmacological effects and toxicology in man and animal. 2nd Ed. E. and S. Livingstone, Edinburgh, P. 525.