Chemical characterization and anticholinesterase effects of essential oils derived from *Salvia* species

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ABSTRACT

Inhibitory effect of *Salvia* species herbal preparations on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity may contribute to regulation of cognitive performance and impaired cholinergic functions in patients with Alzheimer’s disease. This functional role of *Salvia* species and their components makes the investigations on *Salvia* valuable in medicine-related plant research. Within this work it was aimed to investigate the *in vitro* anti-cholinesterase effect of essential oils derived from ten *Salvia* species, which grow in Turkey. The chemical composition of essential oils were characterized by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), respectively. Results showed that all of the essential oils exhibited AChE inhibitory activity. *S. pseudeuphratica*, *S. hydrangea* and *S. divaricata* essential oils demonstrated the most potent AChE inhibitory effect \[50\% inhibition concentration (IC\(\text{_{50}}\) = 26.00 ± 2.00 μg/mL, 40.0 ± 4.00, 64.68 ± 4.16, respectively\]. The essential oil of *S. pseudeuphratica* demonstrated the highest inhibitory activity against AChE and BuChE among the tested *Salvia* essential oils. Evidences from the our study augment the importance of essential oils obtained from *Salvia* species and may support utilization of *Salvia* species for symptomatic treatment of Alzheimer disease.

KEYWORDS

acetylcholinesterase; butyrylcholinesterase; *Salvia* species; essential oil; phytochemistry

ARTICLE HISTORY

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Introduction

Enzymes are important biochemical targets for treatment of several diseases and nearly 30% of all drugs in clinical use show their therapeutic effect by inhibition of enzymes (1). Beside synthetic drugs, plants and natural product-based therapeutics are an important potential source for treatment of a wide spectrum of pathologies. Ethnobotanical use of various plants may be either important in demonstrating the efficacy of natural products in treatment of diseases or a pathfinder in studies of drug-effect mechanism. Nowadays, as an enzyme inhibitor, natural compounds have a wide range of usage on many pathological conditions from analgesia to the symptomatic therapy of Alzheimer’s Disease (AD). Main treatment strategy for AD is restoration of decreased brain neuromediator acetylcholine levels with inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes. For treatment of AD, researchers have focused on screening the phytochemical contents and inhibitory effects of chemical contents of different plant fractions on enzyme activities. Because of their reputed inhibitory effect on cholinesterases *Salvia* L. species are an attractive research topic (2, 3).

The number of species known is now 100, demonstrating that Turkey is a major center of diversity for the genus in Asia (4). *S. ballisiana*, *S. cyanescens*, *S. divaricata*, *S. kronenburgii*, *S. nydegeri* and *S. pseudeuphratica* are species endemic to Turkey (5). Both *S. ballisiana* and *S. pseudeuphratica* are also local species. The main chemical constituents like flavonoids, polyphenols, monoterpenes, diterpenes and triterpenes of *Salvia* species have been subjected to phytochemical studies published in several reviews (6–10).

*Salvia* species are used in traditional medicine all around the world and their essential oils possess antimi- crobial, antioxidant, antidiabetic, antimutagenic, antitumor
The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 2.

Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (15, 16) and in-house ‘Başer Library of Essential Oil Constituents’ built up by genuine compounds and components of known oils, as well as MS literature data (17, 18), was used for the identification.

Determination of AChE activity and BuChE activity

Different concentrations of the test samples were initially prepared in methanol. Essential oils tested at final concentration range 5–80 μg/mL and six different concentrations. Twenty micro litre of AChE enzyme (1 U/mL), 10 μL sample added to 2.4 mL buffer, the mixture was
Table 2. The chemical composition of the essential oils of *Salvia* species.

| Compound                        | KI  | RRI  | Sb% | Sc% | Sd% | Shy% | Sk% | Sm% | Sn% | Sp% | Spc% | Sr% | Identification method |
|---------------------------------|-----|------|-----|-----|-----|------|-----|-----|-----|-----|------|-----|----------------------|
| Tricyclene                      | 1012 | 1016 | Tr  | 0.3 | 0.2 | 0.3  | 0.2 | 0.3 | 0.2 | 0.3 | 0.2  | 0.3 | tr                   |
| α-Pinene                        | 1025 | 1032 | 7.5 | 6.4 | 17.1 | 3.7  | 0.5 | 1.2 | 15.8 | 12.2 | 0.6  | 2.8 | RRI, MS              |
| α-Thujene                       | 1026 | 1027 | 1.4 | 0.6 | -    | -    | -   | -   | -   | -   | -    | -   | tr                   |
| Camphene                        | 1077 | 1076 | 1.2 | 2.3 | 7.7  | 9.4  | 0.2 | 1.6 | 0.4 | 0.5 | 2.3  | 2.3 | RRI, MS              |
| β-Pinene                        | 1117 | 1118 | 1.6 | 6.2 | 1.5  | 1.6  | -   | 5.1 | 9.2 | 24.0 | -    | 20.4 | RRI, MS              |
| Sabine                          | 1122 | 1132 | 2.8 | 0.5 | -    | 0.3  | -   | 0.9 | 1.3 | 1.1  | -    | 0.5 | RRI, MS              |
| Thuja-2,4(10)-diene             | 1122 | 1135 | 0.7 | -   | -    | -    | -   | -   | -   | -    | -    | -   | tr                   |
| Myrcene                         | 1160 | 1174 | 1.3 | -   | 1.0  | 0.1  | 0.3 | 0.3 | 2.3 | 0.5  | -    | 0.8 | RRI, MS              |
| α-Terpine                       | 1177 | 1188 | 0.9 | 0.2 | -    | -    | -   | -   | -   | -    | -    | -   | tr                   |
| Limonene                        | 1212 | 1203 | 3.2 | 0.4 | 2.3  | 3.9  | 6.2 | 3.7 | 2.6 | 4.3  | -    | 2.1 | RRI, MS              |
| 1,8-Cineole                     | 1213 | 1213 | 2.9 | 9.1 | 30.9 | 7.4  | 21.5 | 27.4 | 4.8 | 6.5  | 18.2 | 9.3 | RRI, MS              |
| β-Pheillardrene                 | 1209 | 1218 | 0.2 | -   | -    | -    | -   | -   | -   | -    | -    | -   | tr                   |
| (Z)-β-Ocimene                   | 1271 | 1234 | 0.2 | 0.1 | 0.3  | 0.6  | -   | -   | -   | 0.2  | -    | -   | tr                   |
| α-Thujene hydrate               | 1530 | 1540 | 0.6 | -   | -    | -    | -   | -   | -   | 0.6  | -    | -   | -                   |
| trans-Linalool oxide            | 1540 | 1466 | 1.6 | 0.7 | -    | -    | -   | -   | 0.4 | 1.2  | -    | 0.5 | RRI, MS              |
| 1-Octen-3-ol (Furanoid)         | 1444 | 1452 | 0.4 | -   | -    | -    | -   | -   | -   | 0.2  | -    | -   | tr                   |
| Octenyl acetate                 | 1386 | 1386 | -   | 0.1 | -    | -    | -   | -   | -   | -    | -    | -   | tr                   |
| trans-Linalool oxide (Furanoid) | 1450 | 1450 | -   | 0.6 | -    | -    | -   | -   | -   | -    | -    | -   | tr                   |
| α-Cubebene                      | 1480 | 1466 | 0.3 | -   | -    | -    | -   | -   | 0.9 | 0.4  | -    | 1.1 | MS                   |
| α-Copaene                       | 1488 | 1497 | 0.6 | 0.2 | 0.3  | 0.3  | -   | 1.3 | -   | -    | -    | -   | MS                   |
| α-Copaene aldehyde              | 1499 | 1499 | 0.9 | -   | -    | -    | -   | -   | -   | -    | -    | -   | tr                   |
| α-Bourbonene                    | 1528 | 1538 | 1.1 | 0.3 | 10.1 | 46.9 | 2.5 | 0.7 | 0.8 | 0.4  | 53.6 | -   | RRI, MS              |
| Camphor                         | 1513 | 1532 | 0.3 | -   | -    | -    | 10.1 | 46.9 | 2.5 | 0.7 | 0.8  | 53.6 | -   | RRI, MS              |
| β-Bourbonone                    | 1523 | 1535 | 1.4 | -   | -    | -    | 0.5 | 1.4 | 0.1 | 2.8  | -    | 2.8 | MS                   |
| α-Gurjunene                     | 1529 | 1544 | 0.2 | -   | -    | -    | -   | -   | -   | 4.8  | -    | 4.8 | MS                   |
| Linool                          | 1543 | 1553 | 0.7 | 0.1 | 1.6  | 3.9  | -   | -   | -   | -    | -    | -   | RRI, MS              |
| α-Sabinene hydrate              | 1556 | 1556 | 0.2 | -   | -    | -    | -   | -   | -   | 0.6  | -    | -   | MS                   |
| trans-Sabinene hydrate          | 1568 | 1568 | -   | 1.8 | 6.7  | 0.2  | -   | -   | -   | -    | -    | -   | RRI, MS              |
| Pinocarvone                     | 1575 | 1586 | 1.7 | -   | 0.5  | -    | -   | -   | 0.4 | 0.3  | -    | 1.3 | RRI, MS              |
| β-Ylangene                      | 1576 | 1589 | -   | -   | -    | 4.3  | -   | -   | -   | -    | -    | -   | MS                   |
| Bornyl acetate                  | 1579 | 1591 | -   | 0.9 | 1.1  | 0.3 | 0.3 | 3.8 | 0.7 | 16.4 | -    | 0.4 | RRI, MS              |
| β-Copaene                       | 1579 | 1597 | -   | -   | -    | -    | -   | -   | -   | -    | -    | -   | 1.5 | MS                   |
| β-Elemene                       | 1590 | 1600 | -   | -   | -    | -    | -   | -   | -   | -    | -    | -   | MS                   |
| Calane 1,4-diyl-β-gurjunene     | 1596 | 1610 | 1.7 | -   | -    | -    | -   | -   | -   | 0.4  | -    | -   | MS                   |
| Terpinen-4-ol                   | 1601 | 1611 | 0.2 | 0.4 | 0.6  | -    | -   | -   | 1.5 | -    | -    | -   | RRI, MS              |
| β-Caryophyllene                 | 1608 | 1612 | 8.2 | 0.5 | 0.5  | -    | -   | 26.4 | 3.3 | 2.8  | -    | 1.7 | RRI, MS              |
| Hotrienol                       | 1602 | 1616 | -   | -   | -    | 0.6  | -   | -   | -   | -    | -    | -   | MS                   |
| trans-Dihydrocarvone            | 1623 | 1624 | -   | -   | -    | 0.4  | -   | -   | -   | -    | -    | -   | MS                   |
| Aromadendrene                   | 1650 | 1658 | 0.2 | -   | -    | -    | -   | -   | 0.2 | -    | -    | -   | MS                   |

(Continued)
| Compound                                      | KI<sup>a</sup> | RRI<sup>b</sup> | Sb% | Sc% | Sd% | Shy% | Sk% | Sm% | Sn% | Sp% | Sps% | Sr% | Identification method |
|----------------------------------------------|----------------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|------------------------|
| *cis*-p-Mentha-2-en-1-ol                     | 1614<sup>c</sup>| 1638           | tr  |    |    |     |     | 0.1 |     |     |     | 0.4 | MS                      |
| *trans*-p-Mentha-2-8-dien-1-ol               | 1639<sup>e</sup>| 1639           |     |    |    |     |     |     |     |     |     | 0.4 | MS                      |
| *cis*-β-Terpineol                           | 1639<sup>e</sup>| 1641           |     |    |    |     |     | 0.4 |     |     |     | 0.4 | 1.6 MS                  |
| Myrenal                                      | 1631<sup>e</sup>| 1648           | 2.4 | 0.5 | 0.4 |     | 0.4 | 0.4 | 0.4 |     |     | 1.6 MS                  |
| γ-Elemene                                    | 1642<sup>e</sup>| 1650           |     |    |    |     |     |     |     | 0.2 |     |     | MS                      |
| Sabinaketone                                 | 1651<sup>e</sup>| 1651           |     |    |    |     |     |     |     | 0.8 |     |     | MS                      |
| Alloaromadendrene                            | 1649<sup>e</sup>| 1661           |     |    |    |     | 1.2 |     | 0.5 |     |     | 0.5 | MS                      |
| *cis*-Verbenol                               | 1659<sup>e</sup>| 1663           |     |    |    |     | 0.3 |     |     |     |     |     | MS                      |
| Nonanol                                      | 1655<sup>e</sup>| 1664           |     |    |    |     |     |     |     |     | 0.7 |     | tr, MS                  |
| *(2R,3R)-Farnesene                          | 1651<sup>e</sup>| 1668           | 0.5 |    |    |     |     |     |     |     |     |     | RRI, MS                |
| *trans*-Pino carveol                         | 1661<sup>e</sup>| 1669           | 2.5 | 0.3 | 1.0 | 0.7 |     | 0.6 | 0.4 |     |     | 0.9 | RRI, MS                |
| epi-Zonarane                                 | 1677           | 1677           | 0.1 |    |    |     |     |     |     |     |     |     | MS                      |
| *cis*-p-Mentha-2,8-dien-1-ol                 | 1652<sup>e</sup>| 1678           |     |    |    |     |     | 0.1 |     |     |     |     | MS                      |
| 6-Terpineol                                  | 1679<sup>e</sup>| 1682           |     |    |    |     |     | 0.1 |     |     |     |     | MS                      |
| *trans*-Verbenol                             | 1680<sup>e</sup>| 1683           | 1.9 | 1.5 | 0.9 |     | 0.7 | 0.2 |     |     |     |     | RRI, MS                |
| α-Humulene                                   | 1663<sup>e</sup>| 1687           | 1.4 |    |    | 0.8 |     | 2.0 | 2.8 | 2.1 |     | 0.5 | RRI, MS                |
| Cryptone                                     | 1674<sup>e</sup>| 1690           |     |    |    |     |     |     |     |     |     |     | 5.2 MS                  |
| p-Mentha-1,8-dien-4-ol                       | 1700           | 1700           |     |    |    |     |     | 0.3 |     |     |     |     | MS                      |
| Myrtenyl acetate                             | 1691<sup>e</sup>| 1704           |     |    |    |     |     |     |     |     |     |     | MS                      |
| y-Muurole                                    | 1689<sup>e</sup>| 1704           |     |    |    |     | 1.1 |     |     |     |     |     | 3.0 MS                  |
| α-Terpinol                                   | 1694<sup>e</sup>| 1706           | 0.3 |    |    |     | 1.0 |     | 1.0 |     |     |     | RRI, MS                |
| Ledene                                       | 1708           | 1708           |     |    |    |     |     |     |     | 0.1 |     |     | MS                      |
| α-Terpinyl acetate                           | 1694<sup>e</sup>| 1709           | 2.8 |    |    |     |     | 1.3 |     |     |     |     | RRI, MS                |
| Borneol                                      | 1699<sup>e</sup>| 1719           | 0.6 | 1.8 |     | 0.4 | 0.7 | 0.7 | 0.2 | 0.6 |     |     | RRI, MS                |
| Bicyclosesquiphellandrene                    | 1722           | 1722           | 0.2 |    |    |     |     |     |     |     |     |     | MS                      |
| Verbenone                                    | 1720<sup>e</sup>| 1725           | 0.1 |    | 0.4 | 0.4 |     |     |     |     | 0.3 |     | MS                      |
| Germacrene D                                 | 1708<sup>e</sup>| 1726           | 0.5 |    |     | 0.6 | 4.3 | 2.3 |     |     |     | 0.8 | MS                      |
| Neryl acetate                                | 1718<sup>e</sup>| 1733           |     | 0.5 | 0.9 |     |     |     |     |     |     |     | RRI, MS                |
| p-Mentha-1,5-dien-8-ol                       | 1674<sup>e</sup>| 1738           | 0.3 |    | 0.2 |     |     |     |     |     |     |     | MS                      |
| α-Muurole                                    | 1723<sup>e</sup>| 1740           | 0.2 | 0.4 |     |     |     |     |     |     |     | 0.6 | MS                      |
| Phellandral                                  | 1723<sup>e</sup>| 1744           |     |    |    |     |     |     |     |     |     |     | RRI, MS                |
| *trans*-Carvyl acetate                       | 1723<sup>e</sup>| 1747           |     |    |    |     | 0.8 |     |     |     |     |     | RRI, MS                |
| Carvone                                      | 1733<sup>e</sup>| 1751           | 1.3 | 11.9|     |     |     |     |     |     |     | 0.4 | RRI, MS                |
| Bicyclogermacrene                            | 1734<sup>e</sup>| 1755           | 2.8 |    |     |     |     |     |     |     |     | 0.9 | MS                      |
| Naphthalene                                  | 1735<sup>e</sup>| 1763           | 1.4 | 1.5 | 2.1 | 0.5 |     |     |     |     |     | 2.1 | RRI, MS                |
| Geranyl acetate                              | 1751<sup>e</sup>| 1765           | 1.2 | 1.5 |     |     |     |     |     |     |     |     | RRI, MS                |
| δ-Cadinene                                   | 1755<sup>e</sup>| 1773           | 1.3 | tr  | 0.6 |     | 2.4 | 0.9 |     |     |     | 2.4 | MS                      |
| γ-Cadinene                                   | 1763<sup>e</sup>| 1776           | 0.2 | tr  |     | 0.4 | 0.4 |     |     |     |     | 1.0 | MS                      |
| *ar*-Curcumene                               | 1781<sup>e</sup>| 1786           |     | 0.4 |     |     |     |     |     |     |     |     | MS                      |
| p-Methyl aceto phenone                       | 1773<sup>e</sup>| 1797           |     | 0.4 |     |     |     |     |     |     |     |     | MS                      |
| Cadin-1,4-diene (=Cubenene)                  | 1773<sup>e</sup>| 1799           |     | 0.4 |     |     |     |     |     |     | 0.3 |     | MS                      |
| Cumin aldehyde                               | 1784<sup>e</sup>| 1802           |     | tr  |     |     |     |     |     |     |     | 2.4 | RRI, MS                |
| Myrenol                                      | 1790<sup>e</sup>| 1804           | 1.7 | 0.5 |     |     |     |     |     |     |     | 0.4 | 0.4 | 0.6 MS                  |
| Perilla aldehyde                             | 1793<sup>e</sup>| 1807           | 0.7 |    |     |     |     |     |     |     |     |     | MS                      |
| *trans*-p-Mentha-1(7),8-dien-2-ol             | 1803<sup>e</sup>| 1811           |     |    |    |     |     |     |     |     |     |     | MS                      |
| p-Mentha-1,3-dien-7-al                       | 1811           | 1811           |     |    |    |     |     |     |     |     |     |     | MS                      |
| *trans*-Carveol                              | 1836<sup>e</sup>| 1845           |     | 0.7 | 3.4 |     |     |     |     |     | 0.3 |     | RRI, MS                |
| Calamene                                     | 1927<sup>e</sup>| 1849           | 0.4 | 0.5 |     |     | 0.7 | 2.1 |     | 0.5 |     |     | MS                      |
| Compound                                    | KI | RRI | Sb% | Sc% | Sd% | Shy% | Sk% | Sm% | Sn% | Sp% | Sps% | Sr% | Identification method |
|--------------------------------------------|----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----------------------|
| Geraniol                                   | 1839c | 1857 | -   | -   | 0.2 | -    | 1.6 | -   | -   | -   | -    | -   | RRI, MS               |
| p-Cymen-8-ol                               | 1848c | 1864 | -   | -   | -   | -    | 0.1 | -   | -   | -   | -    | 0.5 | RRI, MS               |
| Epi-Cubebol                                | 1900c | 1900 | 0.5 | -   | -   | -    | -   | 1.4 | 1.0 | -   | 0.6  | -   | MS                    |
| a-Calacorene                               | 1921c | 1941 | -   | -   | -   | -    | -   | -   | -   | -   | 0.5  | -   | MS                    |
| 1,5-Epoxy-salvial-4(14)-ene                | 1945 | -    | 1.8 | -   | -   | -    | -   | -   | -   | -   | -    | -   | MS                    |
| Piperitene                                 | 1909c | 1949 | -   | -   | -   | -    | -   | -   | -   | 0.2 | -    | -   | RRI, MS               |
| Cubebol                                    | 1918c | 1957 | 0.8 | -   | -   | -    | -   | 6.2 | 1.7 | -   | 0.6  | -   | RRI, MS               |
| Furapelargone A                            | 1984 | -    | -   | -   | -   | -    | -   | -   | -   | 0.6  | -   | -   | MS                    |
| γ-Calacorene                               | 1984 | -    | -   | -   | -   | -    | -   | -   | -   | -   | 0.3  | -   | MS                    |
| trans-Sequinsabinene hydrate               | 2092c | 2000 | -   | -   | -   | -    | -   | -   | -   | -   | -    | -   | MS                    |
| Iso-caryophyllene oxide                    | 2001 | 1.1  | -   | -   | -   | -    | -   | 1.6 | -   | 0.2  | -    | -   | MS                    |
| Caryophyllene oxide                        | 1962c | 2008 | 34.1 | 4.0 | -   | -    | 1.4 | -   | 22.2 | 5.6  | 4.5  | -   | 1.6 RRI, MS          |
| Perilla alcohol                            | 2006c | 2029 | tr  | -   | -   | -    | -   | -   | -   | -   | -    | -   | MS                    |
| Salvia-4(14)-en-1-one                      | 2016c | 2037 | -   | 1.1 | -   | -    | -   | -   | -   | -   | -    | 0.5 | MS                    |
| Humulene epoxide-I                         | 2015c | 2045 | 0.4 | -   | -   | -    | -   | 1.4 | 1.0 | -   | 1.0  | -   | MS                    |
| (E)-Nerolidol                              | 2036c | 2050 | -   | 3.3 | -   | -    | -   | -   | -   | -   | -    | -   | MS                    |
| Humulene epoxide-II                        | 2047c | 2071 | 3.7 | 0.1 | 0.7 | 2.1  | -   | 1.6 | 2.5 | 2.0  | -    | 0.8 | MS                    |
| Cubenol                                    | 2047c | 2080 | 0.5 | -   | -   | -    | -   | 1.4 | -   | 0.5 | 0.5  | -   | MS                    |
| Humulene epoxide-III                       | 2081c | 2081 | 0.3 | -   | -   | -    | -   | -   | -   | -   | 0.3  | -   | MS                    |
| 1-epi-Cubebol                              | 2088c | 2088 | 0.6 | -   | -   | 0.7  | -   | 0.9 | 0.3 | -   | -    | MS                    |
| cis-Sequinsabinene hydrate                 | 2096 | -    | -   | -   | -    | -   | 0.4 | -   | -   | -    | -   | MS                    |
| Viridiflorol                               | 2089c | 2104 | -   | -   | -   | -    | -   | 7.7 | -   | -    | -   | MS                    |
| Furapelargone B                            | 2105 | -    | -   | -   | -    | -    | -   | -   | -   | 3.2  | -   | MS                    |
| Cumin alcohol                              | 2058c | 2113 | -   | -   | -   | -    | -   | -   | -   | -    | 1.4  | -   | RRI, MS               |
| Hexahydrofarnesyl acetone                  | 2124c | 2131 | -   | -   | -   | -    | -   | -   | -   | -    | 0.4  | -   | MS                    |
| Valeranone                                 | 2145c | 2144 | -   | -   | 2.6 | 23.2 | 1.1 | 1.1 | 2.8 | 1.7  | -    | 10.4 | MS                    |
| Spathulenol                                | 2126c | 2144 | -   | -   | 2.6 | 23.2 | 1.1 | 1.1 | 2.8 | 1.7  | -    | -   | MS                    |
| T-Cadinol                                  | 2165c | 2187 | 0.5 | -   | 0.8 | -    | -   | -   | -   | 0.2  | -    | tr  | MS                    |
| Nonanoic acid                              | 2159c | 2192 | -   | -   | -   | -    | -   | -   | -   | tr   | -    | -   | RRI, MS               |
| α-Turmerol                                 | 2214 | -    | -   | -   | -    | -   | -   | -   | -   | -    | -   | -   | MS                    |
| α-Bisabolol                                | 2235c | 2232 | -   | -   | -   | -    | -   | -   | -   | -    | -    | -   | MS                    |
| 4-Isopropyl phenol                         | 2232 | -    | -   | -   | -    | -   | -   | -   | -   | -    | 1.9  | -   | MS                    |
| trans-α-Bergamotol                         | 2247 | tr   | 0.1 | -   | -    | -    | -   | -   | -   | 0.6  | -    | -   | MS                    |
| α-Eudesmol                                 | 2222c | 2250 | -   | -   | -    | -    | -   | -   | -   | -    | -    | -   | MS                    |
| α-Cadinol                                  | 2227c | 2255 | -   | -   | -    | -    | -   | -   | -   | -    | 0.4  | 0.5 | MS                    |
| Cadalene                                   | 2233c | 2256 | -   | -   | 2.3 | -    | -   | -   | -   | -    | 0.3  | -   | MS                    |
| β-Eudesmol                                 | 2238c | 2257 | 1.0 | -   | 1.8 | 0.7  | 1.7 | -   | -   | -    | 1.5  | -   | MS                    |
| Alismol                                    | 2264 | -    | -   | -   | -    | 0.6 | -   | 3.9 | -   | -    | -    | -   | MS                    |
| Guai-6,10(14)-dien-4β-ol                   | 2269 | -    | -   | -   | -    | -   | -   | -   | 0.7  | -    | -   | MS                    |
| Torilenol                                  | 2278 | 1.4  | -   | -   | -    | -   | 0.7 | -   | -   | -    | -    | -   | MS                    |
| Decanoic acid                              | 2273c | 2298 | -   | -   | -    | -   | -   | -   | -   | -    | -    | -   | RRI, MS               |
| Caryophylla-2(12)-6(13)-di-en-5β-ol (=Caryophylladienol I) | 2316 | -    | -   | -   | -    | -   | -   | -   | -    | -    | -   | MS                    |

(Continued)
incubated at 37°C for 15 minutes. After the 15 minutes incubation, 50 μL of 0.01 M DTNB and 20 μL of 75 mM acetylthiocholine iodide (ATCI) were added, and the final mixture was incubated at room temperature for 30 minutes. BuChE enzyme activity was determined using 20 μL of 25 mM butyrylthiocholiniodide (BTCI) as substrate and 20 μL of BuChE enzyme (1 U/mL). A control mixture and blank was prepared by using 10 μL of methanol instead of the oil sample, with all other procedures similar to those used in the case of the sample mixture. Absorbances were measured at 412 nm and 37°C using polystyrol cuvets using a spectrophotometer (Shimadzu, UV-1700) as previously reported (19, 20). Experiments were repeated in triplicates. Experimental data were calculated using the Microsoft Excel. All data are presented as mean ± standard deviation (SD) in Table 3 and Table 4.

The percent inhibition of enzymes activities (I%) was calculated by using the equation:

$$I\% = \left( \frac{OD_{sample}}{OD_{control}} \right) \times 100$$

Results and discussion

Chemical characterization of essential oils

Essential oils were obtained by hydrodistillation from the air-dried aerial parts of *Salvia ballsiana*, *S. cyanescens*, *S. divaricata*, *S. hydrangea*, *S. kronenburgii*, *S. macrochlamys*, *S. nydeggeri*, *S. pachystachys*, and *S. russelli*, respectively. The oils were subsequently analyzed by GC and GC-MS, and the characterized

| Table 3. Inhibition percentage (%) values of the in vitro tested *Salvia* essential oils. |
|---------------------------------|-----------------|-----------------|
| Essential Oil (80 μg/mL) | AChE % inhibition | BuChE % inhibition |
| S. ballsiana | 43.23 ± 0.40 | Nd |
| S. cyanescens | 15.45 ± 3.8 | 41.87 ± 4.14 |
| S. divaricata | 56.16 ± 3.28 | - |
| S. hydrangea | 53.34 ± 2.35 | 7.95 ± 0.66 |
| S. kronenburgii | 41.49 ± 3.60 | 12.81 ± 1.12 |
| S. macrochlamys | 42.69 ± 3.11 | Nd |
| S. nydeggeri | 49.35 ± 2.56 | 6.55 ± 2.01 |
| S. pachystachys | 35.43 ± 0.90 | Nd |
| S. pseudoeuphratica | 80.97 ± 2.74 | 49.18 ± 1.15 |
| S. russelli | 43.93 ± 2.75 | 24.66 ± 0.93 |

| Table 4. IC50 values of the in vitro tested *Salvia* essential oils and standard compound. |
|---------------------------------|-----------------|-----------------|
| Essential Oil (μg/mL) | AChE (IC50) | BuChE (IC50) |
| S. pseudoeuphratica | 26.00 ± 2.00 | >80 |
| S. hydrangea | 40.00 ± 4.00 | >80 |
| S. divaricata | 64.68 ± 4.16 | - |
| Galanthamine | 0.55 ± 0.10 | 6.1 ± 0.27 |

Note: No inhibition; nd: Not determined with interference; SD: Standard Deviation.
individual components are given in Table 2 with their relative percentages.

Fifty-six components which were representing 99.8% of the total essential oil of S. ballsiana were identified; caryophyllene oxide (34.1%), β-caryophyllene (8.2%) and α-pinene (7.5%) were main constituents.

Main constituents of S. cyanescens essential oil were spathulenol (23.2%), p-cymene (10.3%), 1,8-cineole (9.1%), α-pinene (6.4%) and β-pinene (6.2%) among the other fifty-one components comprising 93.1% of the total components.

Thirty-two components were identified representing 97.9% of the total essential oil of S. divaricata with 1,8-cineole (30.9%), α-pinene (17.1%), camphor (10.1%) and camphene (7.7%) as main constituents. In our previous work on S. divaricata essential oil, 1,8-cineole (40.0%), α-pinene (16.6%) and camphor (5.0%) were found as major components (12).

Main constituents of S. hydrangea were camphor (46.9%), camphene (9.4%) and 1,8-cineole (7.4%) among the other thirty-six components comprising 97.0% of the total components. Camphor (54.2%), humulene (4.0%), cis-sesquisabinene hydrate (2.8%), myrtenol (2.6%), beta-bisabolol (2.2%) and 1,8-cineole (2.1%) were found as predominant components of S. hydrangea essential oil by Kotan et al. (21). In the oil of S. hydrangea from Iran; β-caryophyllene(33.4%) and caryophyllene oxide (25.4%) were reported as major constituents (22).

GC-MS analysis of S. kronenburgii essential oil has shown that geranyl acetate (16.0%), 1,8-cineole (12.5%), carvone (12.0%) and limonene (6.2%) were the main constituents. Forty-four compounds were characterized representing 94.1% of the total oil. The essential oil composition was previously investigated by Altun et al. either (23).

Twenty-eight compounds were identified representing 97.2% of the total essential oil of S. macrochlamys with β-caryophyllene (26.4%) and caryophyllene oxide (22.2%) as main constituents. In our previous work, the oil of S. macrochlamys was characterized with 1,8-cineole (27%), borneol (13%), and camphor (11%) as main constituents (24).

This is the first report on the chemistry of S. nydeggeri. Forty-nine components were identified representing 92.0% of the total oil. α-Pinene (15.8%), β-pinene (9.2%), cubebol (6.2%) and caryophyllene oxide (5.6%) determined as major components.

GC-MS analysis of the oil of S. pachystachys has shown that β-pinene (24.0%), α-pinene (12.2%), spathulenol (10.4%), viridiflorol (7.7%) and 1,8-cineole (6.5%) were the main constituents. Sixty-two compounds were characterized representing 97.7% of the total oil.

A total of twenty-six compounds were characterized at S. pseudoeuphratica essential oil which were representing 96.0% of the total oil. This oil was characterized with a relatively high content of camphor (53.6%). The other main component was found as 1,8-cineole (17.4%).

Forty-eight compounds were identified representing 92.8% of the total essential oil of S. russellii with β-pinene (20.4%), 1,8-cineole (9.5%), α-copaene (8.7%), valeranone (8.7%) and α-gurjunene (4.8%) as main constituents.

In our study, it has been demonstrated that there is a qualitative and quantitative difference between investigated Salvia species among constituents of essential oils. The differences about chemical compositions of Salvia species evaluated in our study and the Salvia species investigated at either in our work or previous studies in the literature may be related with source of the plant, individual genetic variability, collection time of the plant, the proportions of distilled parts, variations in biosynthetic pathways and metabolism (25).

**Inhibition effect of essential oils on cholinesterases**

The AChE and BuChE inhibitory activity of essential oils derived from ten Salvia species has not been reported to date. This is the first study performed to demonstrate this activity. AChE and BuChE inhibitory activities of the essential oils are reported in Table 3 and Table 4. It is also known that Salvia species are used traditionally at various nervous system disorders (26). Previous studies demonstrated that Salvia essentials oils have potential therapeutic effects on mood and cognitive functions through cholinesterase inhibition which could be attributed to terpenes (11, 27).

The main finding of this study was that all investigated Salvia essential oils inhibited AChE enzyme activity. However, Essential oils were less active than galantamin; the standart inhibitor of AChE enzyme. While essential oil of S. pseudoeuphratica showed the highest inhibitory effect on AChE (IC_{50} = 26 ± 2 μg/mL), S. cyanescens and S. pachystachys showed the lowest inhibitory effects on AChE at the same concentration (80 μg/mL) among the species investigated. Our results also confirmed previous findings about AChE inhibitory activity of Salvia species (26).

Main component of S. pseudoeuphratica essential oil, generally attributed to the biological activity, is a naturally occurring monoterpenoid, camphor with a relative biological activity of 53.6%. Whereas, S. cyanescens (0.3%) and S. pachystachys (0.4%) were the Salvia species with the lowest camphor content. Svalve et al. (28) have demonstrated that AChE enzyme inhibitory effect of essential oil derived from poor camphor containing Salvia officinalis.
was lower than essential oils rich in camphor content. These findings suggested that camphor may be responsible from inhibitory effect on AChE enzyme.

Perry et al. (2) demonstrated that camphor is an uncompetitive reversible inhibitor of human erythrocyte AChE. Camphor, 1,8-cineole, and α-pinene inhibited the enzyme in a dose-dependent manner. When compared with standard drug physostigmine (IC50 = 4.5 × 10−8 M), the most active monoterpenes were 1,8-cineole (IC50 = 0.67 mM), α-pinene (IC50 = 0.63 mM) and camphor (4.7 mM), respectively. Inhibitory effect of camphor enantiomers on AChE activity was reported as (+)-camphor: 26.4%, (-)-camphor: 21.2% has been reported by Miyazawa et al. (29).

Other effective essential oils for AChE enzyme inhibition in our study were the essential oils obtained from S. hydrangea (40 ± 4%) and S. divaricata (64.68 ± 4.16%), respectively. While main component of Salvia hydrangea was camphor (46.9%), main component of S. divaricata was 1,8-cineol (30.9%). 1,8-cineole and α-pinene are two common monoterpenes in Salvia. AChE inhibitory activity of these Salvia species may be contributed with the presence of 1,8-cineole. Common property of forthcoming three Salvia with highest inhibitory effect on AChE enzyme was markedly rich camphor content among Salvia species investigated in our study. Combination of 1,8-cineole and camphor may result with either synergy or antagonism (30). Although Svalv et al. reported that Salvia species rich in 1,8-cineole, but not camphor, may provide oils with more potent cholinergic activities data obtained our study have showed that essential oils rich in camphor content also have inhibitory effect on cholinesterase activity (28). While camphor is known for therapeutic effects it should not be ignored that toxic effects of Salvia essential oils might be related with it (11, 31).

In enzyme inhibition assays, the percentage inhibition effect on AChE activity of essential oils obtained from S. nydegeri, S. russelli, S. ballisana, S. macrochlamys, S. kronenburgii was higher than 40% at 80 μg/mL concentration.

BuChE activity, which is also responsible for the hydrolysis of ACh; may increase for the compensation of deteriorated AChE activity in AD. In addition to esterase function, BuChE have peptidase effect. Peptidase activity ameliorates formation of β-amyloid by degredation of amyloid precursor protein (APP) which can be determined in large amounts at brain tissue of Alzheimer Disease patients (32). Therefore, inhibition BuChE (in addition to AChE) seems to have a therapeutic impact in AD.

Another finding of this study was inhibition of BuChE by essential oils obtained from some investigated Salvia species inhibitory activity of the oils on BuChE varied among the studied species. Although essential oils of S. pseudeuphratica and S. cyanesens showed the highest inhibitory effect on BuChE activity (49.18 ± 1.15% and 41.87 ± 4.14%, respectively) at the same test concentrations (80 μg/mL), they did not show 50% inhibition even at their highest concentration. Common property of S. cyanesens (30.9%) and S. pseudeuphratica (18.2%) essential oils were possessing the highest 1,8-cineole content.

Loizzo and co-workers reported that although 1,8-cineole has an inhibitory on BuChE activity (IC50 = 0.93 mM), camphor did not show inhibitory effect even at the highest tested concentration (10 mM). 1,8-cineole may have a role for the inhibitory effect of S. pseudeuphratica, S. cyanesens, S. russelli on BuChE enzyme (33).

Despite no camphor content, S. russelli have exhibited a high level of AChE inhibition and although main component of S. divaricata was 1,8 cineole, it did not demonstrate any BuChE inhibition. In spite the major factor that takes part in cholinesterase enzyme inhibition seems to be the main component of the essential oils, synergistic or antagonistic chemical interactions of essential oil components may play an important role.

Şenol and co-workers showed neither dichloromethane nor ethyl acetate extracts of the S. pseudeuphratica has inhibitory effect on AChE at a concentration of 100 μg/mL. AChE inhibitory effect of dichloromethane extract obtained from S.russelli (100 μg/mL) was 1.70 ± 0.85% and ethyl acetate extract of S. russelli (100 μg/mL) was 11.54 ± 0.34%. While dichloromethane extracts of S. pachystachys showed an inhibitory effect on AChE (2.34 ± 0.34%–100 μg/mL), ethyl acetate extract of S. pachystachys has no inhibitory effect on AChE activity at same concentration. However, relevant components of extracts were not reported in study of Senol et al. (34). Hence no inhibitory effect of S. pseudeuphratica ethyl acetate extracts on AChE activity was determined in the previous study, dual inhibitory effect of S. pseudeuphratica volatile fraction obtained in our work has showed the importance of investigation different fractions from the plants.

Evidences from our study augment the importance of essential oils obtained from Salvia species and support the utilization of Salvia species, especially the essential oil of S. pseudeuphratica, which has demonstrated the highest inhibitory activity against both enzymes, for symptomatic treatment of Alzheimer disease. Main constituents, camphor and 1,8-cineole are relevant compounds associated with cholinesterase inhibition. Contribution ratio of the active components effects cholinesterase activity in a important manner. Due to these dual efficacy on both cholinesterase enzymes, investigations on cholinesterase inhibitory effect of S. pseudeuphratica volatile fractions at in vivo studies can be worthwhile.
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No potential conflict of interest was reported by the authors.

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