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Antioxidant and anticholinesterase constituents of *Salvia poculata*

Ufuk KOLAK[1,*], **Işıl HACIBEKİROĞLU**[1], **Mehmet ÖZTÜRK**[2]
Fevzi ÖZGÖKÇE[3], **Gülaçtı TOPÇU**[4], **Ayhan ULUBELEN**[1]
[1] *Department of General and Analytical Chemistry, Faculty of Pharmacy, İstanbul University 34116, İstanbul-TURKEY*
e-mail: ufukkolak@yahoo.com
[2] *Department of Chemistry, Faculty of Arts and Sciences, Muğla University 48121, Muğla- TURKEY*
[3] *Department of Biology, Faculty of Science and Letters, Yüzüncü Yıl University 65080, Van-TURKEY*
[4] *Department of Chemistry, Faculty of Science and Letters, İstanbul Technical University 34469 İstanbul-TURKEY*

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Two triterpenoids, namely 2α,3α-dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (**1**) and ursolic acid (**2**); 4 flavonoids, namely 5-hydroxy-7,4'-dimethoxyflavone (**3**), cirsimaritin (**4**), eupatilin (**5**), and salvigenin (**6**); a diterpenoid, namely sclareol (**7**); and a steroid, namely β-sitosterol (**8**), were isolated from the aerial parts of *Salvia poculata* Nab., a Turkish endemic *Salvia* species. Total phenolic and flavonoid contents of the crude extract were determined as pyrocatechol and quercetin equivalents, respectively. The antioxidant activity of the crude extract and the isolated compounds (**2-8**) was established using β-carotene bleaching, superoxide anion radical, and ABTS cation radical scavenging activity. In addition, the anticholinesterase activity of the crude extract and the isolated compounds (**2-8**) against the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was determined. The phytochemistry and antioxidant and anticholinesterase activities of *S. poculata* were investigated for the first time in this study. The crude extract of *S. poculata* exhibited a significant antioxidant effect as well as butyrylcholinesterase inhibitory activity. Ursolic acid (**2**) and cirsimaritin (**4**) possessed high butyrylcholinesterase inhibitory activity.

Key Words: *Salvia poculata*; Lamiaceae; antioxidant activity; anticholinesterase activity.

*Corresponding author

Introduction

Free radicals such as superoxide anion, and hydroxyl and peroxyl radicals, which are produced in biological systems and foods, are responsible for oxidation of cell lipids and DNA damage, and they may cause serious diseases (e.g. cancer, coronary arteriosclerosis, diabetes mellitus).[1] Dietary antioxidants may be effective in prevention of oxidative damage. Many scientists have focussed on medicinal and edible plants to discover natural antioxidants since some synthetic antioxidants have toxic effects. In addition, natural antioxidants may have an important role in protecting human health. Studies performed to find natural antioxidants indicated that many *Salvia* species and some of their constituents have shown significant antioxidant activity. Some *Salvia* species have been used commercially in the food industry to prevent or delay spoilage of foods.[2]

Alzheimer's disease (AD), which is the most common form of dementia, is frequently seen among elderly people all around the world. Some synthetic acetylcholinesterase inhibitors such as tacrine and donepezil have been used for the treatment of AD but they have several adverse effects.[3] As a result of the search to find natural acetylcholinesterase inhibitors from plants, more than 35 alkaloids (e.g. physostigmine and galantamine) were found to be active. In addition, some terpenoids, glycosides, and coumarins exhibited similar effects.[3] *Salvia* is one of the most investigated genera, some of its species such as *S. miltiorrhiza*, *S. officinalis* L. and *S. lavandulaefolia* Vahl. have been used traditionally for dementia therapy.[4,5] Since investigations on AD suggest that antioxidants may retard its progression,[4] both antioxidant and anticholinesterase activity tests were carried out on the crude extract and constituents of *S. poculata* in this study.

Salvia L., with 900 species, is the largest genus of the family Lamiaceae and is widespread throughout the world.[6] Its species have been widely used in traditional medicine all around the world since ancient times. They have various activities such as anti-inflammatory, analgesic, antipyretic, cardioactive, antifungal, antituberculosis, antitumor, and antioxidant.[2] In addition, they have been used for the treatment of some cases of dementia.[7] *Salvia* species have been consumed for different purposes such as tea, spices, and flavouring agents in perfumery and cosmetics; they are among the economically important species of the family Lamiaceae.[8] There are about 90 *Salvia* species growing naturally in Turkey, half of which are endemic.[9] In Turkish folk medicine, they have been used as antiseptic, antibacterial, diuretic, haemostatic, spasmolitic, and carminative agents.[10] Since 1968, the phytochemistry and biological activity of about 60 *Salvia* species have been investigated by our research group. These studies indicated that the roots of Turkish *Salvia* species are rich in diterpenoids, especially abietane-type, whereas the aerial parts contain essential oils, flavonoids, and triterpenoids. The main constituents of *Salvia* species possess various biological activities.[11]

In a continuation of our studies on Turkish *Salvia* species we report herein the constituents and the biological activity of the aerial parts of *S. poculata* Nab. for the first time. The fatty acid composition of *S. poculata* seed oil was determined.[12] In this study, 8 known compounds, namely 2α,3α-dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (**1**),[13] ursolic acid (**2**),[14] 5-hydroxy-7,4'-dimethoxyflavone (**3**),[15] cirsimaritin (**4**),[16] eupatilin (**5**),[17] salvigenin (**6**),[18] sclareol (**7**),[19] and β-sitosterol (**8**),[20] were isolated from the methanol extract of *S. poculata* (Figure 1). The antioxidant activity of the crude extract and the isolated compounds (**2-8**) was determined using 3 different methods: β-carotene bleaching, and superoxide anion radical and ABTS cation radical scavenging activity assays. In addition, the acetyl- and butyryl-cholinesterase inhibitory activity of the crude extract and the isolated compounds (**2-8**) was established using the Ellman method.

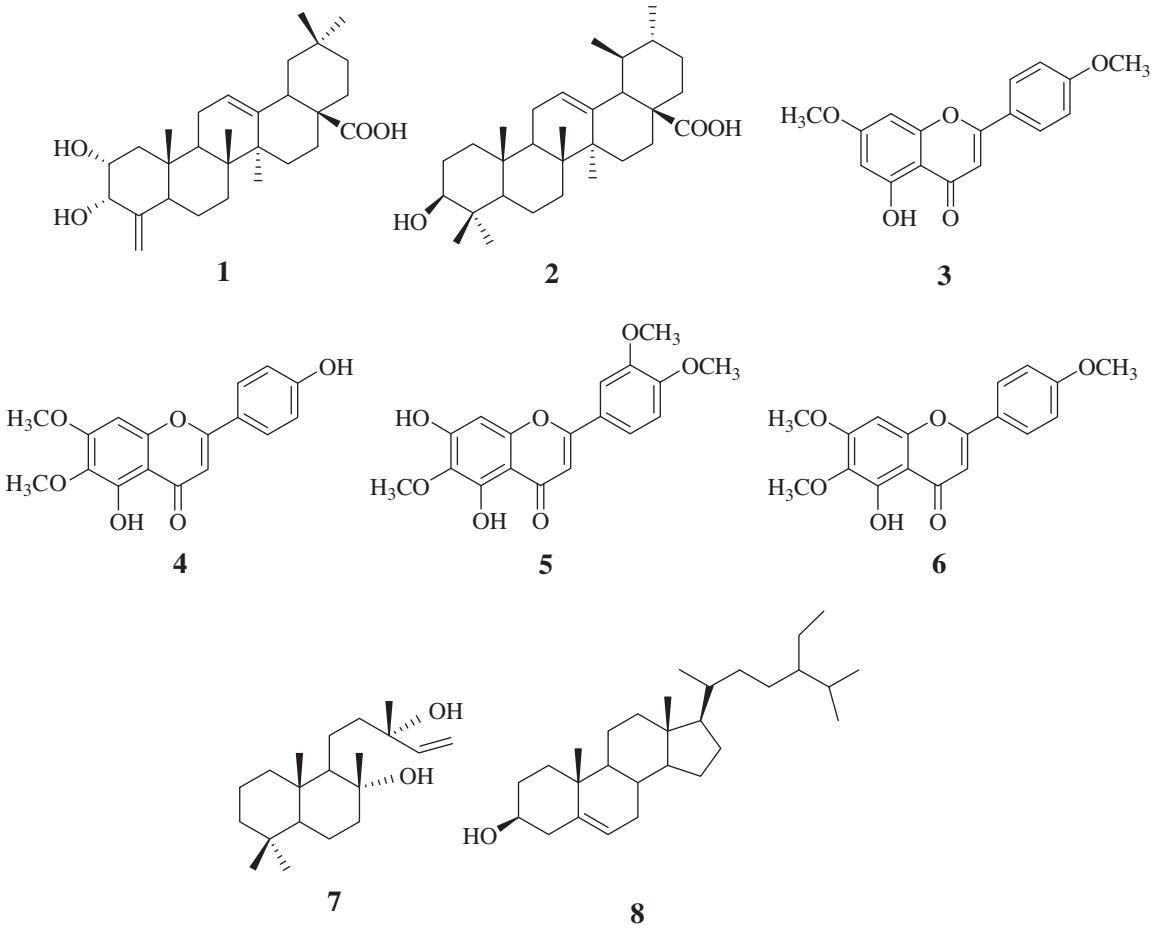

Figure 1. Chemical formulae of compounds **1-8.**

Experimental

General Experimental Procedures. The UV spectra (λ_{max}) were recorded on a Shimadzu UV-1601 in MeOH, IR spectra (ν_{max}) on a Perkin-Elmer One B in $CHCl_3$, NMR spectra on a Varian UNITY INOVA spectrometer operating at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR (TMS as an internal standard) including APT, HMQC, and HRESI-MS spectra on Bruker Microsoft Q spectrometer. Melting points were recorded on a Kofler apparatus (Reichert) and are uncorrected.

Plant Material. The aerial parts of *S. poculata* Nab. were collected from Eastern Turkey (Alacabük Dagı-Bitlis) at 2250 m altitude in July 2005, and identified by Dr. Fevzi Özgökçe. A voucher specimen was deposited in the Herbarium of Yüzüncü Yıl University (VANF 12938).

Extraction, Isolation, and Identification: The dried and powdered aerial parts of *S. poculata* (350 g) were macerated with 1.5 L of methanol at room temperature (25 °C) 3 times (24 h × 3). After filtration, the solvent was evaporated to dryness in vacuo. The crude extract (10.6 g) was fractionated on a silica gel column (2.5 × 100 cm). The column was eluted with petroleum ether (40-60°) (5 × 150 mL), and a gradient

of dichloromethane was added in 10 mL increments into 100 mL petroleum ether until reaching 100%, thus 10 × 100 mL were used, followed by methanol in 10 mL increments up to 100% (10 × 100 mL). The similar fractions were combined with TLC control and then further subjected to preparative thin layer chromatography to yield compounds **1-8** using the following solvent systems: from Frac. 13-15 β-sitosterol (22 mg) (toluene/diethylether, 7:1) and 5-hydroxy-7,4'-dimethoxyflavone (8 mg) (toluene/ diethylether, 4:1), from Frac. 18-19 salvigenin (6.5 mg) (toluene/diethylether, 7:1), from Frac. 20-25 ursolic acid (13 mg) (toluene/diethylether, 2:1), from Frac. 26-28 sclareol (12 mg) (toluene/diethylether, 2:1), and eupatilin (9 mg) (toluene/chloroform/acetone, 1:9:1), from Frac. 40-45 cirsimaritin (3 mg) (chloroform/ acetone, 9:0.5), and 2α,3α-dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (2 mg) (chloroform/acetone, 8:1).

By correlating our spectral data (UV, IR, 1D- and 2D- NMR, mass) with those of standard samples, compounds **1-8** were identified as 2α,3α-dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (**1**), ursolic acid (**2**), 5-hydroxy-7,4'-dimethoxyflavone (**3**), cirsimaritin (**4**), eupatilin (**5**), salvigenin (**6**), sclareol (**7**), and β-sitosterol (**8**).

2α,3α–Dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (1): UV (MeOH) λ_{max}: 207 nm; IR ($CHCl_3$)ν_{max}: 3437, 3080, 2950, 1695, 1650, 1462, 1382, 1053, 895, 755 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 5.26 (1H, *t*, J = 3.6 Hz, H-12), 5.00 (1H, *s*, H-23$_b$), 4.68 (1H, *d*, J = 1.45 Hz, H-23$_a$), 4.17 (1H, *d*, J = 3.66 Hz, H-3β), 3.72 (1H, *ddd*, J = 11.5; 4.76; 3.7 Hz, H-2β), 2.78 (1H, *dd*, J = 4.1; 13.2 Hz, H-18β), 2.06 (1H, *br dd*, J = 2.0; 11.9 Hz, H-5α), 1.10 (3H, *s*, Me-27), 0.87 (3H, *s*, Me-30), 0.84 (3H, *s*, Me-29), 0.78 (3H, *s*, Me-26), 0.68 (3H, *s*, Me-25); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 178.52 (*s*, C-28), 148.96 (*s*, C-4), 142.89 (*s*, C-13), 121.67 (*d*, C-12), 110.69 (*t*, C-23), 74.71 (*d*, C-3), 68.15 (*d*, C-2), 47.16 (*s*, C-17), 45.00 (*t*, C-19), 45.10 (*d*, C-9), 44.84 (*d*, C-5), 43.61 (*t*, C-1), 41.87 (*d*, C-18), 40.36 (*s*, C-14), 40.36 (*s*, C-8), 38.00 (*s*, C-10), 32.82 (*t*, C-21), 32.04 (*q*, C-29), 31.41 (*t*, C-22), 28.68 (*t*, C-7), 30.07 (*s*, C-20), 26.57 (*t*, C-15), 24.97 (*q*, C-27), 23.20 (*t*, C-11), 22.56 (*q*, C-30), 22.05 (*t*, C-16), 19.25 (*t*, C-6), 16.14 (*q*, C-26), 13.08 (*q*, C-25). HRESI-MS: m/z 456.3229 (calcd for $C_{29}H_{44}O_4$, 456.3239).

Ursolic acid (2): UV (MeOH) λ_{max}: 207 nm; IR ($CHCl_3$)ν_{max}: 3360, 2940, 2860, 1695, 1500, 1370, 1270, 1185, 1030, 995, 660 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 5.24 (1H, *t*, J = 4.0 Hz, H-12), 3.23 (1H, *dd*, J = 5.0; 10.0 Hz, H-3α), 1.24 (3H, *s*, Me-27), 1.05 (3H, *s*, Me-26), 0.97 (6H, *d*, J = 7.0 Hz, Me-29 and Me-30), 0.90 (3H, *s*, Me-25), 0.78 (3H, *s*, Me-24), 0.73 (3H, *s*, Me-23); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 180.35 (*s*, C-28), 140.36 (*s*, C-13), 123.63 (*d*, C-12), 78.24 (*d*, C-3), 55.10 (*d*, C-5), 52.62 (*d*, C-9), 47.50 (*s*, C-17), 46.50 (*d*, C-18), 42.28 (*s*, C-14), 40.11 (*s*, C-8), 40.01 (*d*, C-19), 39.01 (*s*, C-10), 38.82 (*d*, C-20), 38.42 (*t*, C-1), 38.13 (*s*, C-4), 32.89 (*t*, C-21), 31.73 (*t*, C-22), 30.35 (*t*, C-7), 28.87 (*t*, C-15), 28.12 (*q*, C-23), 25.92 (*q*, C-27), 24.83 (*t*, C-2), 23.94 (*t*, C-16), 23.40 (*t*, C-11), 21.22 (*q*, C-30), 18.15 (*t*, C-6), 17.65 (*q*, C-24), 17.23 (*q*, C-29), 15.98 (*q*, C-26), 15.20 (*q*, C-25). HRESI-MS: m/z 456.3597 (calcd for $C_{30}H_{48}O_3$, 456.3603).

5-Hydroxy-7,4'-dimethoxyflavone (3): UV (MeOH) λ_{max}: 268, 328; MeOH+ NaOMe: 288, 340; MeOH + $AlCl_3$: 277, 380; MeOH + $AlCl_3$+ HCl: 277, 380; MeOH + NaOAc: 268, 330; MeOH + NaOAc + H_3BO_3: 268, 332; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 12.80 (1H, *br s*, 5-OH), 7.85 (2H, *dd*, J= 1.95; 6.83 Hz, H-2' and H-6'), 7.02 (2H, *dd*, J = 1.95; 6.83 Hz, H-3' and H-5'), 6.58 (1H, *s*, H-3), 6.48 (1H, *d*, J = 2.44 Hz, H-8), 6.37 (1H, *d*, J = 2.44 Hz, H-6), 3.89 (3H, *s*, OMe), 3.88 (3H, *s*, OMe); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 182.41 (*s*, C-4), 164.30 (*s*, C-2), 156.41 (*s*, C-9), 154.61 (*s*, C-4'), 154.02 (*s*, C-5), 148.14 (*s*, C-7), 124.03 (*s*, C-1'), 120.32 (*d*, C-2' and C-6'), 111.54 (*d*, C-3' and C-5'), 103.42 (*s*, C-10), 103.34 (*d*, C-3), 98.06 (*d*, C-6),

93.51 (d, C-8), 61.51 (q, OMe), 56.15 (q, OMe). HRESI-MS: m/z 298.0837 (calcd for $C_{17}H_{14}O_5$, 298.0841).

Cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone) (4): UV (MeOH) λ_{max}: 274, 333; MeOH+ NaOMe: 272, 290 (sh), 383; MeOH + $AlCl_3$: 258 (sh), 296, 362; MeOH + $AlCl_3$+ HCl: 262 (sh), 299, 355; MeOH + NaOAc: 274, 339, 390; MeOH + NaOAc + H_3BO_3: 274, 335; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 12.70 (1H, *br s*, 5-OH), 7.74 (2H, d, J = 8.7 Hz, H-2' and H-6'), 6.90 (2H, d, J = 8.7 Hz, H-3' and H-5'), 6.52 (1H, s, H-3), 6.48 (1H, s, H-8), 3.89 (3H, s, OMe), 3.85 (3H, s, OMe); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 181.52 (s, C-4), 164.15 (s, C-2), 157.16 (s, C-5), 152.88 (s, C-4'), 150.22 (s, C-7), 149.97 (s, C-9), 132.15 (s, C-6), 124.38 (s, C-1'), 121.44 (d, C-2' and C-6'), 115.43 (d, C-3' and C-5'), 104.13 (s, C-10), 103.32 (d, C-3), 91.42 (d, C-8), 62.15 (q, OMe), 61.22 (q, OMe). HRESI-MS: m/z 314.0787 (calcd for $C_{17}H_{14}O_6$, 314.0790).

Eupatilin (5,7-dihydroxy-6, 3',4'-trimethoxyflavone) (5): mp = 230-232 °C; UV (MeOH) λ_{max}: 243, 275, 340; MeOH+ NaOMe: 242, 270, 377; MeOH + $AlCl_3$: 262, 291 (sh), 364; MeOH + $AlCl_3$+ HCl: 261, 295, 360; MeOH + NaOAc: 235, 275, 340; MeOH + NaOAc + H_3BO_3: 236, 275, 341; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 12.82 (1H, *br s*, 5-OH), 7.42 (1H, d, J = 2.0 Hz, H-2') 7.37 (1H, *dd*, J = 2.0; 8.79 Hz, H-6'), 6.90 (1H, d, J = 8.79 Hz, H-5'), 6.52 (1H, s, H-3), 6.48 (1H, s, H-8), 3.92 (3H, s, OMe), 3.92 (3H, s, OMe), 3.86 (3H, s, OMe); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 182.01 (s, C-4), 162.18 (s, C-2), 158.46 (s, C-5), 156.00 (s, C-4'), 152.87 (s, C-3'), 151.26 (s, C-9), 149.07 (s, C-7), 133.11 (s, C-6), 123.41 (s, C-1'), 120.13 (d, C-6'), 111.62 (d, C-5'), 108.22 (d, C-2'), 105.37 (s, C-10), 103.46 (d, C-3), 92.00 (d, C-8), 61.42 (q, OMe), 56.18 (q, OMe). 56.01 (q, OMe). HRESI-MS: m/z 344.0891 (calcd for $C_{18}H_{16}O_7$, 344.0895).

Salvigenin (6): mp = 188 °C, UV (MeOH) λ_{max}: 277, 330; MeOH+ NaOMe: 295, 332 (sh), 375; MeOH + $AlCl_3$: 235, 302 (sh), 360; MeOH + $AlCl_3$+ HCl: 235, 262, 301 (sh), 350; MeOH + NaOAc: 277, 329, 376; MeOH + NaOAc + H_3BO_3: 276, 329; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 12.76 (1H, *br s*, 5-OH), 7.86 (2H, d, J = 10.0 Hz, H-2' and H-6'), 7.02 (2H, d, J = 10.0 Hz, H-3' and H-5'), 6.59 (1H, s, H-3), 6.55 (1H, s, H-8), 3.90 (3H, s, OMe), 3.93 (3H, s, OMe), 3.97 (3H, s, OMe); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 181.52 (s, C-4), 163.12 (s, C-2), 158.13 (s, C-5), 154.20 (s, C-4'), 152.00 (s, C-7), 151.74 (s, C-9), 132.06 (s, C-6), 124.03 (s, C-1'), 120.32 (d, C-2' and C-6'), 111.54 (d, C-3' and C-5'), 104.92 (s, C-10), 102.93 (d, C-3), 91.05 (d, C-8), 62.03 (q, OMe), 61.54 (q, OMe), 56.13 (q, OMe). HRESI-MS: m/z 328.0940 (calcd for $C_{18}H_{16}O_6$, 328.0946).

Sclareol (7): UV (MeOH) λ_{max}: 217 nm; IR ($CHCl_3$)ν_{max}: 3400, 2940, 1465, 1395, 900 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 5.94 (1H, *dd*, J = 10.74; 17.08 Hz, H-14), 5.21 (1H, *dd*, J = 1.47; 17.08 Hz, H-15$_a$), 5.02 (1H, *dd*, J = 1.47; 10.74 Hz, H-15$_b$), 1.27 (3H, s, Me-16), 1.16 (3H, s, Me-17), 0.86 (3H, s, Me-20), 0.78 (6H, s, Me-18 and Me-19); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 146.23 (d, C-14), 111.43 (t, C-15), 74.98 (s, C-8), 73.84 (s, C-13), 61.89 (d, C-9), 55.51 (d, C-5), 45.24 (t, C-12), 44.64 (t, C-7), 42.24 (t, C-3), 39.96 (t, C-1), 39.50 (s, C-10), 33.61 (q, C-18), 33.46 (s, C-4), 27.52 (q, C-16), 24.48 (q, C-17), 21.71 (q, C-19), 20.75 (t, C-11), 19.33 (t, C-6), 18.66 (t, C-2), 15.57 (q, C-20). HRESI-MS: m/z 308.2710 (calcd for $C_{20}H_{36}O_2$, 308.2715).

β-Sitosterol (8): mp = 138-139 °C, UV (MeOH) λ_{max}: 205 nm; IR ($CHCl_3$)ν_{max}: 3445, 3305, 2925, 2860, 1643, 1375, 1063, 955, 835 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 5.35 (1H, m, H-6), 3.52 (1H, m, H-3α), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.4 Hz, Me-21), 0.85 (3H, t, J = 7.8 Hz, Me-29), 0.83 (3H, d, J = 6.8 Hz, Me-26), 0.81 (3H, d, J = 6.9 Hz, Me-27), 0.69 (3H, s, Me-18); ^{13}C-NMR (125 MHz, $CDCl_3$)δ: 140.71 (s, C-5), 121.63 (d, C-6), 71.73 (d, C-3), 56.75 (d, C-14), 56.02 (d, C-17), 51.13 (d, C-9), 45.82 (d, C-24), 42.37

(*s*, C-13), 42.20 (*t*, C-4), 39.79 (*t*, C-12), 37.33 (*t*, C-1), 36.43 (*s*, C-10), 36.07 (*d*, C-20), 33.95 (*t*, C-22), 31.96 (*t*, C-7), 31.81 (*d*, C-8), 31.63 (*t*, C-2), 29.15 (*d*, C-25), 28.25 (*t*, C-16), 26.10 (*t*, C-23), 24.25 (*t*, C-15), 23.13 (*t*, C-28), 21.09 (*t*, C-11), 19.77 (*q*, C-26), 19.46 (*q*, C-19), 19.21 (*q*, C-27), 18.68 (*q*, C-21), 11.84 (*q*, C-18), 11.04 (*q*, C-29). HRESI-MS: m/z 414.3857 (calcd for $C_{29}H_{50}O$, 414.3861).

Antioxidant activity

Chemicals: Methanol, chloroform, pyrocatechol, quercetin, sodium carbonate, aluminium nitrate, potassium acetate, potassium persulfate, sodium phosphate, silica gel for column chromatography (1.07734) and silica gel 60 F_{254} TLC plates (1.05554) were obtained from E. Merck (Darmstadt, Germany). Folin-Ciocalteu's reagent, β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate, butylated hydroxytoluene, butylated hydroxyanisole, trolox, (+)-catechin, nicotinamideadeninedinucleotide, acetyl-cholinesterase, butyryl-cholinesterase, 5,5′-dithiobis (2-nitrobenzoic) acid, acetylthiocholine iodide, butyrylthiocholine iodide, and galanthamine were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, Tris-HCl, nitrotetrazoliumbluechloride, and *N*-methyl-phenazoniummethylsulphate were obtained from Fluka Chemie (Fluka Chemie GmbH, Sternheim, Germany).

Determination of Total Phenolic Content: The concentrations of the phenolic content in all samples were expressed as micrograms of pyrocatechol equivalents (PEs). The phenolic content was determined as described in the literature.[21]

Determination of Total Flavonoid Content: The measurement of the flavonoid concentration was based on the method described by Moreno et al. with a slight modification and the results were expressed as quercetin equivalents.[22]

Determination of the Antioxidant Activity with the β-Carotene Bleaching Method: The antioxidant activity was established using the β-carotene–linoleic acid test system.[23]

Superoxide Anion Radical Scavenging Activity: The measurement of the superoxide anion radical scavenging activity was based on the method described by Liu et al. with slight modification.[24]

ABTS Cation Radical Decolorization Assay: The $ABTS^+$ scavenging activity was determined according to the method of Re et al. with slight modifications.[25]

Anticholinesterase Activity: Acetyl- and butyryl-cholinesterase inhibitory activities were established by slightly modifying the spectrophotometric method developed by Ellman et al.[26]

Statistical analysis: The results were mean ± SD of 3 parallel measurements. Analysis of variance was performed using ANOVA procedures. Significant differences between means were determined by Student's t-test, and p values < 0.05 were regarded as significant.

Results and discussion

Two triterpenoids, namely 2α,3α-dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (**1**) and ursolic acid (**2**); 4 flavonoids, namely 5-hydroxy-7,4'-dimethoxyflavone (**3**), cirsimaritin (**4**), eupatilin (**5**), and salvigenin (**6**); a diterpenoid, namely sclareol (**7**); and a steroid, namely β-sitosterol (**8**), were obtained from the aerial parts of

the endemic plant *S. poculata*. A rather rare nortriterpenoid 2α,3α-dihydroxy-24-nor-4(23),12-oleanadien-28-oic acid (**1**) was obtained for the first time from a Turkish *Salvia* species, previously isolated from *S. carduacea*.[13] The structure determination of the compounds (**1-8**) was established using spectral methods (UV, IR, 1D- and 2D-NMR, mass spectrometry) and their spectral data were compared to those of standard samples.

In this study, the antioxidant activity of eupatilin (**5**) and sclareol (**7**) was determined for the first time. Although some antioxidant activity tests were performed for ursolic acid (**2**),[27,28] 5-hydroxy-7,4'-dimethoxyflavone (**3**),[29] cirsimaritin (**4**),[30–32] salvigenin (**6**),[30] and β-sitosterol (**8**),[33,34] we tested their antioxidant activity using β-carotene bleaching, and superoxide anion radical and ABTS cation radical scavenging activity assays.

As shown in Table 1, the crude extract is rich in phenolic compounds. The crude extract and β-sitosterol (**8**), which is a common steroid in *Salvia* species, showed significant inhibition of lipid peroxidation by the β-carotene bleaching method (Figure 2). Sclareol (**7**), a labdane diterpenoid, exhibited the highest activity among the tested compounds as a superoxide anion radical scavenger, while the crude extract was found to be moderately active in the ABTS cation radical scavenging activity test (Table 2). Since cirsimaritin (**4**) and salvigenin (**6**) did not exhibit antioxidant activity their results are not shown in Figure 2 and Table 2.

Table 1. Total phenolic and flavonoid contents of the crude extract of *S. poculata*.[a]

Sample	Phenolic content (μg PEs/mg extract)[b]	Flavonoid content (μg QEs/mg extract)[c]
ESP	85.56 ± 1.60	35.28 ± 0.13

[a]Values expressed are means ± S.D. of 3 parallel measurements ($p < 0.05$)

[b]PEs, pyrocatechol equivalents

[c]QEs, quercetin equivalents

Table 2. $O_2^{\cdot-}$ and $ABTS^{\cdot+}$ scavenging activities of compounds **2-3**, **5-8**.[a]

Samples	O_2^- assay		$ABTS^+$ assay	
	IC_{50}(μg/mL)	r^2	IC_{50}(μg/mL)	r^2
ESP	57.99 ± 1.11	0.9804	17.99 ± 0.11	0.9944
2	94.43 ± 0.13	0.9930	> 100	0.9911
3	71.65 ± 1.95	0.9828	44.80 ± 1.56	0.9961
5	59.14 ± 0.98	0.9961	22.34 ± 0.89	0.9969
6	78.46 ± 1.55	0.9987	78.68 ± 1.32	0.9908
7	48.74 ± 0.25	0.9994	> 100	0.9970
8	> 100	0.9904	> 100	0.9818
BHA[b]	33.71 ± 0.11	0.9924	2.16 ± 0.09	0.9889
(+)-Catechin[b]	44.12 ± 0.09	0.9995	1.16 ± 0.05	0.9984

[a]Values expressed are means ± S.D. of 3 parallel measurements ($p < 0.05$)

[b]Reference compounds

Table 3. Anticholinesterase activity of the crude extract and compounds **2-8**.[a]

Samples	Inhibition % against AChE				Inhibition % against BChE			
	25 μM	50 μM	100 μM	200 μM	25 μM	50 μM	100 μM	200 μM
ESP*	1.78 ± 0.87	3.78 ± 0.58	5.81 ± 1.34	8.62 ± 0.34	32.68 ± 1.73	39.79 ± 1.09	49.09 ± 1.45	55.59 ±0 .45
2	34.60 ± 0.39	42.63 ± 0.75	50.72 ± 0.53	54.26 ± 0.21	62.84 ± 0.62	64.46 ± 0.90	66.70 ± 0.89	70.80 ± 0.17
3	na	na	na	na	na	na	na	na
4	1.78 ± 0.87	3.78 ± 0.58	7.35 ± 0.58	12.35 ± 0.99	30.59 ± 0.73	35.69 ± 1.29	42.31 ± 0.36	50.31 ± 0.94
5	na	na	0.94 ± 0.63	11.00 ± 0.72	15.22 ± 1.03	22.12 ± 1.41	32.22 ± 0.24	45.27 ± 0.33
6	na	na	na	na	4.85±0.54	5.67±0.36	7.96 ± 0.67	14.19 ± 0.51
7	na	na	na	na	0.99 ± 0.09	1.35 ± 0.07	7.99 ± 0.73	26.60 ± 0.23
8	na	na	na	na	3.83 ± 1.37	4.28 ± 0.94	6.80 ± 0.82	11.04 ± 0.88
Galantamine[b]	68.36 ± 1.10	74.38 ± 0.65	78.59 ± 0.47	81.41 ± 0.03	40.59 ± 0.88	48.73 ± 0.90	65.02 ± 0.44	75.54 ± 1.05

[a] Values expressed are means ± S.D. of t3 parallel measurements ($p < 0.05$)
[b] Standard drug
* at 200 μg/mL concentration
na: not active

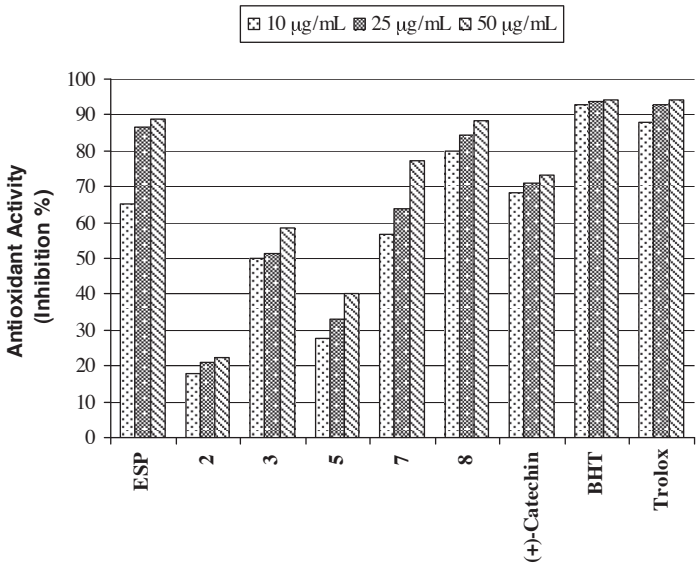

Figure 2. Inhibition (%) of lipid peroxidation of ESP, compounds (**2-3, 5, 7-8**), (+)-catechin, BHT and trolox by the β-carotene bleaching method. Values are mean ± S.D., n= 3. p < 0.05, significantly different with Student's t test. (ESP: Extract of *S. poculata*, BHT: butylatedhydroxytoluene).

The anticholinesterase activity against the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) of the crude extract and the isolated compounds (**2-8**) was investigated for the first time. While the crude extract was found to be almost inactive in the AChE inhibitory test, it exhibited high inhibition in the BChE inhibitory assay. Ursolic acid (**2**) possessed moderate AChE inhibitory activity, as seen in Table 3, which has been previously investigated by Chung et al.[35] However, ursolic acid (**2**) exhibited higher BChE inhibitory activity than the standard test compound galantamine, which is used in the treatment of mild to moderate AD. Flavonoid compounds, cirsimaritin (**4**) and eupatilin (**5**), were almost inactive against AChE, while they showed high BChE inhibition. Other isolated compounds, 5-hydroxy-7,4'-dimethoxyflavone (**3**)**,** salvigenin (**6**), sclareol (**7**) and β-sitosterol (**8**), showed no inhibition against AChE and BChE.

Conclusion

Although sclareol (**7**) and β-sitosterol (**8**) showed high total antioxidant activity in the β-carotene bleaching method, they exhibited no anticholinesterase activity. Among the isolated compounds, although triterpenoid ursolic acid (**2**) showed the highest anticholinesterase activity, especially against BChE, it exhibited neither lipid inhibition nor radical scavenging activity.

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