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Bioactive constituents from roots of *Salvia syriaca* L.: Acetylcholinesterase inhibitory activity and molecular docking studies

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ABSTRACT

Several *Salvia* species have been used to enhance memory in European traditional medicine. Accordingly, we aimed to evaluate the acetone extract of the roots of *Salvia syriaca* for anti-Alzheimer constituents. Structures of purified compounds were determined as ursolic acid, corosolic acid, β-sitosterol, urs-12-en-2α,3β-diol and daucosterol (1–5). β-sitosterol and daucosterol exhibited high acetylcholinesterase inhibitory activities (24.1 and 34.3 μg/mL, respectively). The experimental results were also confirmed by docking analysis.

1. Introduction

The genus *Salvia* is the largest member of the Lamiaceae family and comprises over 1000 species (Wu et al., 2012). *Salvia* species are traditionally used for medicinal purposes worldwide (Farimani et al., 2012; Bahadori and Mirzaei, 2015). Various biological activities such as antimicrobial, cytotoxicity, anti-protozoal, anti-HIV, antioxidant and anti-inflammatory activities have been reported for the genus (Topcu, 2006; Farimani et al., 2015). In addition, *Salvia* species are used for central nervous system disorders (Karimi et al., 2012). Several species from the genus are used in perfume, food and pharmaceutical industries (Bahadori et al., 2015). A broad spectrum of natural compounds such as terpenoids and phenolics are found in *Salvia* species (Lu and Foo, 2002; Bautista et al., 2013).

Salvia syriaca is used as a forage (Flamini et al., 2007). Previous phytochemical studies on *S. syriaca* reported the isolation of some flavonoids (Hatam and Yousif, 1992), one sesterterpenoid salvisyriacolide (Rustaiyan and Sadjadi, 1987), a new sesquiterpene named syriacine (Al-Jaber et al., 2012) and four novel seco-ursadiene triterpenoids (Al-Jaber et al., 2012; Al-Aboudi et al., 2015) from the aerial parts of the plant. Ulubelen et al. (2000) reported some di- and triterpenoids, sitosterol and one flavonoid from the roots of the plant with cardiovascular activities. Also, there are some studies on the essential oil of *S. syriaca* which reported germacrene B, germacrene D and bicyclogermacrene as major volatile compounds (Baser et al., 1996; Flamini et al., 2007).

Flora of Iran contains 61 *Salvia* species of which 17 are endemic (Jamzad, 2012). Our research group reported many structurally interesting and bioactive diterpenoids, sesterterpenoids, triterpenoids and isoprenoids with novel carbon skeletons from Iranian *Salvia* species in recent years (Farimani et al., 2011, 2012, 2013; Ebrahimi et al., 2014; Farimani et al., 2015).

Continuing our studies on the discovery of bioactive constituents from Iranian *Salvia* species, we studied the acetone extract of the roots of *S. syriaca* in the present work to find new cognitive enhancers. Herein, we report the isolation and identification of three triterpenoids, one steroid and one steroid-glucoside on the basis of 1D and 2D NMR data (Fig. 1). Acetylcholinesterase inhibitory activity and molecular docking studies of the isolated compounds are also reported.

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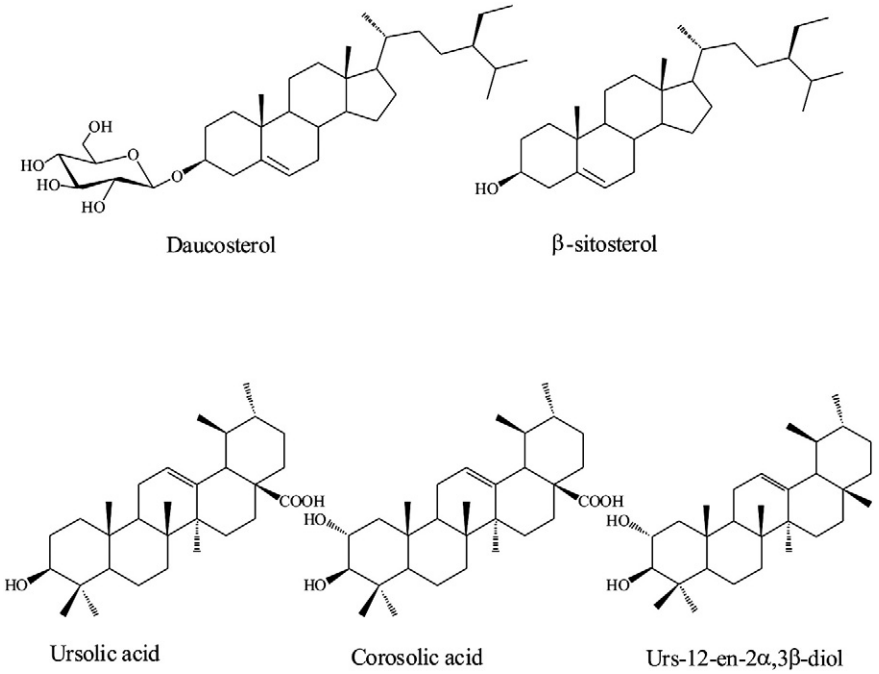

Fig. 1. Structures of isolated compounds (1–5).

2. Experimental

2.1. General experimental procedures

NMR spectra were measured on a Bruker Avance III 500 MHz spectrometer (^{1}H: 500 MHz, ^{13}C: 125 MHz). UV/Vis analysis was carried out using a Shimadzu spectrophotometer (2550 UV/Vis).

2.2. Chemicals

$CDCl_3$, methanol-d_4 and pyridine-d_5 for NMR were purchased from Armar Chemicals. Silica gel (mesh 230–400), acetylthiocholine iodide, DTNB and galantamine were obtained from Merck (Germany).

2.3. Plant material

The roots of *S. syriaca* were collected in May 2013 from Urmia, West Azarbaijan province. The plant was identified by Mr. Shahram Bahadori, a taxonomist at the Urmia School of Pharmacy Herbarium and a voucher specimen (USPH-104) has been deposited for the plant.

2.4. Extraction and isolation

The powdered roots of the plant (2.2 kg) were extracted using acetone (3 × 10 L) by maceration at room temperature. The extract was concentrated *in vacuo* to obtain 40 g reddish gummy acetone extract. Fractionation of the extract by column chromatography (5 cm × 80 cm) on silica gel (step gradient *n*-hexane-EtOAc as eluent) afforded 20 fractions. Fraction 7 (0.8 g) was separated on a silica gel column with $CHCl_3$–MeOH (95:5) to afford four fractions (7a–7d). Fraction 7c was further purified using preparative thin layer chromatography ($CHCl_3$–MeOH (90:10)) to afford compound 1 (8 mg, $R_f = 0.6$). Fraction 8 (1.1 g) was separated by column chromatography with $CHCl_3$–MeOH (95:5) as eluent to give five fractions (8a–8e). Fraction 8d was recrystallized from $CHCl_3$ to yield compound 2 (3 mg). Fraction 9 (0.4 g) was recrystallized from acetone to afford compound 3 (200 mg). Fraction 12 (0.9 g) was subjected to silica gel column chromatography with $CHCl_3$–MeOH (90:10) as eluent and afforded eight fractions (12a–12 h). Fraction 12e was further purified using preparative thin layer chromatography ($CHCl_3$–MeOH (85:15)) to afford compound 4 (10 mg, $R_f = 0.8$). Fraction 15 (1.3 g) was triturated with acetone and the residue was recrystallized from $CHCl_3$–MeOH to yield compound 5 (45 mg).

2.5. Acetylcholinesterase inhibitory activity

In vitro acetylcholinesterase inhibitory activities of the extract and purified compounds were determined using a spectrophotometric method described by Ellman et al. (1961) with some modifications using a Shimadzu spectrophotometer (2550 UV/Vis). A partially purified enzyme was prepared for the experiments according to a previously published method (Karimi et al., 2010). The stock solutions of testing compounds were prepared by dissolving them in DMSO. Briefly, 500 μL of sodium phosphate buffer (100 mM, pH = 8.0), 150 μL of DTNB (3.5 mM), 150 μL of substrate (acetylthiocholine iodide) and 150 μL inhibitor solution were added into the reaction mixture in a 1 mL cell and incubated for 15 min at 37 °C. Afterward, the reaction was started by addition of 50 μL of enzyme. The enzyme was kept on ice before the addition to the incubation mixture. After immediate mixing of the reaction mixture, the changing of the absorbance was measured at 412 nm for 10 min. The inhibition rate of the samples on acetylcholinesterase was calculated by the following formula:

$$\% \text{ Inhibition} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/\text{Absorbance}_{\text{control}}] \times 100.$$

The IC_{50} values were calculated from inhibition curves (inhibitor concentration vs. percent of inhibition). The results were expressed as mean ± SEM of three independent experiments. Galantamine was used as the standard drug.

2.6. Molecular docking studies

To study the molecular interaction and binding mode of the purified compounds, molecular docking studies were performed using AutoDock 4.2 software (Morris et al., 1998). For this purpose, the crystal structures of human acetylcholinesterase (PDB code: 4PQE) was downloaded from protein data bank (http://www.rcsb.org). Subsequently, all water molecules of acetylcholinesterase were removed from the enzyme structure