Isolation and Identification of the Main Compounds of *Satureja sahendica* Bornm.

1S. Saeidnia, 2M.S. Nourbakhsh, 1A.R. Gohari, 2A. Davood

1Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, PO Box 14155-6451, Iran.
2Department of Medicinal Chemistry, Faculty of Pharmacy, Islamic Azad University, Tehran, Iran

**Abstract:** *Satureja sahendica* Bornm is generally called Marzeh in the Persian language and belongs to Lamiaceae family which comprises 13 species in Iran. In this study, the plant material (aerial parts of *S. sahendica*) was collected from North-East of Iran (Azerbayjan province). Luteolin (1), together with oleanolic acid (2), beta-sitosterol (3) and diosmetin (4) were isolated from the ethyl acetate and methanol extracts of *S. sahendica* for the first time. Different chromatographic methods were carried out on the silica gel and sephadex LH20 in order to separate of compounds. The structures of the isolated compounds were determined using the 1H, 13C-NMR and MS spectra in comparison of those reported in the literatures. Diosmetin is an important flavone which converts to luteolin in the human body and affects on the breast cancer via binding to the estrogen receptors.

**Key words:** Lamiaceae, *Satureja sahendica*, Flavonoids

**INTRODUCTION**

The genus *Satureja* (Lamiaceae) comprises around 13 species in Iran. Among them *Satureja sahendica* grows widely in north-west of Iran (Hedge 1986; Mozaffarian, 1996). *Satureja* genus consists of the fragrance shrubs which are growing on the rocky mounts and used traditionally for anti-diarrhea, antispasmodic and pesticide activities (Gohari et al., 2009). Literature reviews show that there are a few reports only on chemical compositions of the volatile oil of *S. sahendica*. Recently, composition of the essential oil of *S. sahendica* has been investigated by GLC and GC-MS. Thirty-nine components were identified in the oils. The main constituents of the essential oils were thymol (19.6–41.7%), p-cymene (32.5–54.9%) and γ-terpinene (1.0–12.8%) (Hassanpouraghdam et al., 2009, Sefidkon et al., 2004).

We have previously reported the presence of flavones (5,6,3′-trihydroxy-7,8,4′-trimethoxyflavone, 5-desmethoxynobiletin, thymonin and luteolin) from *S. atropatana* using chromatographic methods followed by 1H and 13C-NMR and MS spectra (Gohari et al., 2009).

In this paper, we aimed to report the separation and structural elucidation of the main phytochemical constituents from the aerial parts of *S. sahendica* which has not been reported in advance.

**MATERIAL AND METHODS**

**Experimental:**

Aerial parts of *S. sahendica* Bornm, at the full flowering stage, were gathered around Tabriz in East Azerbaijan Province (September, 2008). A voucher specimen of the plant deposited at the Herbarium of the Institute of Medicinal Plants, ACECR, Tehran. Plant specimen was identified by Mr. Yousef Ajani from the mentioned institute.

**General Procedure:**

The 1H and 13C-NMR spectra were measured on a Brucker Avance TM 500 DRX (500 MHz for 1H and 125 MHz for 13C) spectrometer with tetramethylsilane as an internal standard and chemical shifts are given in δ (ppm). The MS data were recorded on an Agilent Technology (HP TM) instrument with 5973 Network Mass Selective Detector (MS model). The silica gel 60F254 pre-coated plates (Merck TM) were used for TLC. The spots were detected by spraying anisaldehyde-H2SO4 reagent followed by heating (120 °C for 5 min).

**Corresponding Author:** A.R. Gohari, Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, PO Box 14155-6451, Iran.
Tel & Fax: +98-21-64122330.
E-mail: goharii_a@sina.tums.ac.ir

1450
**Isolation Process:**

The flowered aerial parts of *S. sahendica* (2150 g) was cut into small pieces and extracted with ethyl acetate and methanol, consequently, at room temperature to obtain ethyl acetate (25 g) and methanol (62 g) extracts. The ethyl acetate extract was subjected to silica gel column chromatography (CC) with hexane: AcOEt (9:1, 3:2, 0:1), AcOEt: MeOH (1:1) and MeOH as eluent to give nine fractions (A-I). The fraction D (200 mg) was submitted to sephadex LH20 CC with CHCl3; MeOH (3:7) as an eluent to obtain four fractions D1-D4. The fraction C4 (20 mg) was the pure compound 1. The fraction E (1460 g) was subjected to silica gel CC with hexane; AcOEt (8:2, 6:4, 1:1 and 0:1) to gain eight fractions (E1-E8). The fractions E1 chromatographed again on sephadex LH20 to result in compound 2 (77 mg).

The MeOH extract (30 g) was successively subjected to silica gel column chromatography and washed with CHCl3; AcOEt (1:0, 1:1, 0:1) and MeOH as eluents to result in eight fractions M1-M8. Fraction M2 (292 mg) was fractionated on silica gel CC with CHCl3: AcOEt (19:1, 6:4, 0:1) to obtain three fractions M21-M23. The fraction M22 (6 mg) was the pure compound 3. The fraction M4 (216 mg) was chromatographed on sephadex LH20 with MeOH to afford Compound 4 (3 mg).

**RESULTS AND DISCUSSION**

Isolated compounds (Fig.1) from the ethyl acetate and MeOH extracts of *S. sahendica* identified as luteolin (1), together with oleanolic acid (2), beta-sitosterol (3) and diosmetin (4) by comparison of their NMR and MS spectral data with those reported in literature (7-11). NMR data of luteolin (1) and beta-sitosterol (3) were described by Saeidnia et al. (2009) and Gohari et al. (2009; 2005).

![Fig. 1: Structures of the isolated compounds from Saturejasahendica.](image-url)
Oleanolic Acid (2):
White amorphous powder. m.p.: 271-273° C. 1H-NMR (500 MHz, CDCl3): 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 (each 3H, s, CH3 ×6), 1.13 (3H, s, H-27), 2.82 (1H, dd, J = 3.6, 13.2 Hz, H-18), 3.23 (1H, dd, J = 11.2, 4.4 Hz, H-3), 5.27 (1H, t, J=3.5 Hz, H-12). 13C-NMR (125 MHz, Pyridine-d5): δC (from C-1 to C-30) 39.0, 28.2, 78.1, 39.4, 55.8, 18.8, 33.3, 39.8, 48.2, 37.4, 23.7, 122.6, 144.8, 42.2, 28.4, 23.8, 46.7, 42.0, 46.5, 31.0, 34.3, 33.2, 28.8, 16.6, 15.6, 17.5, 26.2, 180.2, 33.3, 23.8.

Diosmetin (4):
Yellow needle crystal. 1H NMR (500 MHz, acetone-d6): δ (ppm), 12.99 (1H, s, 5-OH), 7.57 (1H, dd, J = 2.2, 8.5 Hz, H-6’), 7.50 (1H, d, J = 2.3 Hz, H-2’), 7.13 (1H, d, J = 8.5 Hz, H-5’), 6.64 (1H, s, 3-H), 6.56(1H, d, J = 2.1 Hz, H-8), 6.20 (1H, d, J = 2.1 Hz, H-6) and 3.95 (3H, s, 4 -OCH3). 13C NMR (125 MHz, acetone-d6): δ (ppm) 164.8 (C-2), 104.6 (C-3), 182.9 (C-4), 163.3 (C-5), 99.6 (C-6), 164.9 (C-7), 94.7 (C-8), 158.7 (C-9), 105.3 (C-10), 124.8 (C-1’), 113.6 (C-2’), 147.8 (C-3’), 151.6 (C-4’), 112.4 (C-5’), 119.6 (C-6’), 56.3 (OMe).

Diosmetin, the methyl ether aglycon of diosmin, is the most important flavone in S. sahendica which reported only from S. obovata among the Stureja species (Atta-ur-Rahman, 2005). Anticancer effects of diosmetin on cell cycle progression and proliferation of MDA-MB 468 breast cancer cells, has been reported (Androutsopoulos et al., 2009). The studies of flavonoids on the breast cancer showed that the flavonoid diosmetin is metabolised to the more active molecule luteolin by CYP1 family enzymes (Androutsopoulos et al., 2009). The cytoprotective effect of diosmetin, was investigated on iron-loaded hepatocyte cultures. Thus, S. sahendica which consists of both luteolin and diosmetin as the main anticancer agents, seems to be a good cytotoxic medicinal plant. The cytoprotective activity of diosmetin, which previously reported, could be ascribed to its antiradical property but also to iron-chelating effectiveness (Morel et al., 1993).

Oleanolic acid is another bioactive component of S.sahendica. As we can see in the literatures, oleanolic acid, a petacyclic triterpene, showed antibacterial, antifungal, trypanocidal and anti-inflammatory effects, antitumor and immunomodulatory activity (Gohari et al., 2009; Saeidnia et al., 2009; Gohari, et al., 2005).

Beta-sitosterol is a common sterol in the plants which decreases the symptom of benign prostatic hyperplasia and anti-inflammatory activities (Wilt et al., 1999).

Conclusion:
In conclusion, S.sahendica, the medicinal plant of Lamiaceae family, contains luteolin and diosmetin as the bioactive flavones and, oleanolic acid and beta-sitosterol as the bioactive triterpen and sterol. These components have not been reported from S.sahendica so far.

ACKNOWLEDGEMENT
This research has been supported by Tehran University of Medical Sciences and Health Services grant (No.10188).

REFERENCES


