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

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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 LH_{20} in order to separate of compounds. The structures of the isolated compounds were determined using the ^{1}H, ^{13}C-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

INTODUCTION

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 ^{1}H and ^{13}C-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 Azerbayjan 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 ^{1}H and ^{13}C-NMR spectra were measured on a Brucker Avance TM 500 DRX (500 MHz for ^{1}H and 125 MHz for ^{13}C) 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 $60F_{254}$ pre-coated plates (Merck TM) were used for TLC. The spots were detected by spraying anisaldehyde-H_2SO_4 reagent followed by heating (120 ˚C for 5 min).

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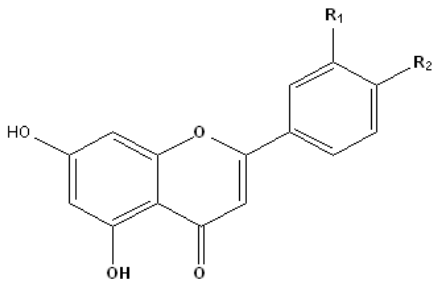
Isolation Process:

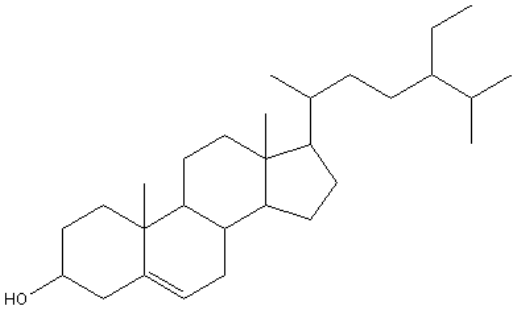
The flowered aerial parts of *S. sahendica* (2150 g) was cut into small pieces and extracted with ethyl acetate and methanol, consequently, at room temperature to obtain ethyl acetate (25 g) and methanol (62 g) extracts. The ethyl acetate extract was subjected to silica gel column chromatography (CC) 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 LH_{20} CC with $CHCl_3$: MeOH (3:7) as an eluent to obtain four fractions D_1-D_4. The fraction C_4 (20 mg) was the pure compound **1.** The fraction E (1.460 g) was subjected to silica gel CC with hexane: AcOEt (8:2, 6:4, 1:1 and 0:1) to gain eight fractions (E_1-E_8). The fractions E_5 chromatographed again on sephadex LH_{20} to result in compound **2** (77 mg).

The MeOH extract (30 g) was successively subjected to silica gel column chromatography and washed with $CHCl_3$: AcOEt (1:0, 1:1, 0:1) and MeOH as eluents to result in eight fractions M_1- M_8. Fraction M_2 (292 mg) was fractionated on silica gel CC with $CHCl_3$: AcOEt (19:1, 6:4, 0:1) to obtain three fractions M_{21} –M_{23}. The fraction M_{22} (6 mg) was the pure compound **3.** The fraction M_4 (216 mg) was chromatographed on sephadex LH_{20} with MeOH to afford Compound **4** (3 mg).

RESULTS AND DISCUSSION

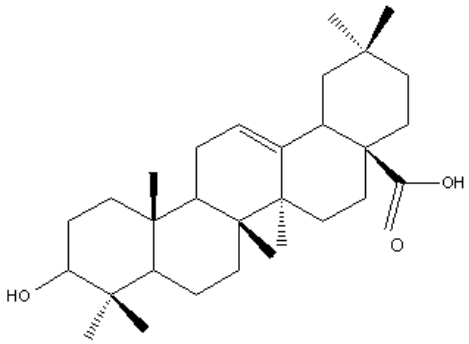
Isolated compounds (Fig.1) from the ethyl acetate and MeOH extracts of *S. sahendica* identified as luteolin (**1**), together with oleanolic acid (**2**), beta-sitosterol (**3**) and diosmetin (**4**) by comparison of their NMR and MS spectral data with those reported in literature (7-11). NMR data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





luteolin (1): R_1= OH, R_2 = OH
diosmetin (4): R_1=OH, R_2= OCH3

β – sitosterol (3)



oleanolic acid (2)

Fig. 1: Structures of the isolated compounds from *Saturejasahendica*.

Oleanolic Acid (2):

White amorphous powder. m.p.: 271-273° C. ^{1}H-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-*d*5): 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. ^{1}H NMR (500 MHz, acetone-d_6): 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 -OCH_3). ^{13}C NMR (125 MHz, acetone-d_6): 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 triterpene and sterol. These components have not been reported from *S.sahendica* so far.

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