Chemical Constituents of Dichloromethane Extract of Cultivated Satureja khuzistanica

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Four compounds β-sitosterol, β-sitosterol-3-O-β-d-glucopyranoside, ursolic acid and 4’,5,6-trihydroxy-3’,7-dimethoxyflavone were characterized from the dichloromethane extract of the aerial parts of Satureja khuzistanica (Lamiaceae), a native medicinal plant growing in Iran, on the basis of spectral analysis and comparing with the data in literature. The natural occurrence of these compounds can be conclusive for the chemotaxonomic characterization of the genus Satureja.

Keywords: daucosterin – Lamiaceae – Satureja khuzistanica – sitosterol – 4’,5,6-trihydroxy-3’,7-dimethoxyflavone – ursolic acid

Introduction

Medicinal and aromatic plants have been used for many centuries and are still popular in today’s alternative therapies. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs (1). Satureja khuzistanica Jamzad (Marzeh Khuzistani in Persian, family of Lamiaceae) is an endemic plant that is widely distributed in the southern parts of Iran (2). It is a shrub, branched stem ~30 cm high, densely leafy, broadly ovaite-orbicular covered with white hairs. Base of the leaves is attenuate and petioliform.

This plant has been used as analgesic and antiseptic among the inhabitants of southern parts of Iran. Infusion of aerial parts of this plant is credited in folk medicine to relieve toothache. The composition of the essential oils of wild and cultivated S. khuzistanica has already been investigated (3). Antimicrobial activity, anti-inflammatory, antinociceptive effects and antioxidant, antidiabetic, antihyperlipidemic and reproduction stimulatory properties of this plant have been recently reported from Iran (4–6).

The chemical composition of the crude extract of this species has not yet been investigated. The current work examined the composition of dichloromethane extract of the aerial parts of cultivated S. khuzistanica.

Methods

General

Melting points were determined with a Buchi B-540 melting point apparatus. ¹H, ¹³C-NMR (DEPT) and 2D-NMR were recorded on Bruker (DRX-500 Avance) NMR spectrometer. Silica gel (kieselgel 60, 70–230 mesh, Merck) was used for column chromatography. Spots were detected on TLC under UV or by heating after spraying with 5% phosphomolybdic acid in C₂H₅OH.

Plant Material

Aerial parts of cultivated S. khuzistanica in flowering stage from open field were prepared by Khorrman Company in industrial village of Khoramabad, Lorestan Province, Iran, November 2005 (Fig. 1). The plant was authenticated by herbal museum of the Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran and a voucher specimen (No. 6650-THE) is deposited.
Extraction and Isolation

The air-dried aerial parts of cultivated *S. khuzistanica* (1 kg) was crushed and extracted with CH₂Cl₂ (3 × 3 liters) at room temperature for 6 days. The CH₂Cl₂ extracts were combined and concentrated *in vacuo* to yield a gummy extract. This residue treated with MeOH to remove waxy compounds.

The MeOH soluble portion (33.2 g) was subjected to a silica gel column chromatography (70–230 mesh, 800 g) with a gradient of hexane–EtOAc and then EtOAc–MeOH as eluent. Eight fractions were collected according to TLC analysis. The fraction of hexane–EtOAc (75:25) was subjected to another column chromatography using hexane–EtOAc (10:1) as the eluent. After recrystallization from MeOH, we obtained 75 mg of compound (1). The fraction of hexane–EtOAc (70:30) was rechromatographed over silica gel and eluted with hexane–EtOAc (9:1) to give 150 mg of compound (2). The fraction of hexane–EtOAc (60:40) was separated on another column chromatography and eluted with hexane–EtOAc (9:1) to give 270 mg of compound (3). From the fraction of EtOAc–MeOH (20:1), crude crystals were obtained, which gave after recrystallization from pyridine/MeOH 40 mg of pure compound (4).

**Results**

Isolation and Identification of Compounds

In this study, for the first time we investigated the chemical composition of cultivated *S. khuzistanica*, a plant that was used very extensively in folk medicine of southern parts of Iran.

Dichloromethane extract of aerial parts of this plant was fractionated under silica gel column chromatography and resulted in isolation of four compounds.

Compound (1) was obtained as colorless needles with a melting point of 133–135°C. According to the ¹H, ¹³C, 2D NMR (H,H-COSY, HMOC, HMBC) experiments and also MS spectra and by comparing these spectroscopic data with those reported in the literatures, this compound was assigned to be β-sitosterol (7–9).

The second compound was isolated as brown crystals with a melting point of 253–255°C. By comparing the spectral data of this compound with those reported in the literatures, it was identified as 4',5,6-trihydroxy-3',7-dimethoxyflavone (10,11).

Compound (3) was obtained as a white powder with a melting point of 267–269°C. The ¹H NMR and ¹³C NMR data
of this compound were consistent with the reported data of ursolic acid (12–14).

The structure of compound (4), isolated as colorless needles (mp: 275–277°C) was established as β-sitosterol-3-O-β-D-glucopyranoside (daucosterin) by comparison of its spectral data with literature values (15,16).

**β-Sitosterol (1)**

Colorless needles, mp 133–135°C; 1H NMR (500 MHz, CDCl3) δ 5.39 (1H, m, H-6), 3.56 (1H, m, H-3), 1.05 (3H, s, Me-19), 0.96 (3H, d, J = 6.5 Hz, Me-21), 0.89 (3H, t, J = 7.4 Hz, Me-29), 0.87 (3H, d, J = 6.7 Hz, Me-26), 0.85 (3H, d, J = 6.7 Hz, Me-27), 0.72 (3H, s, Me-18); 13C NMR (125 MHz, CDCl3, based on DEPT, HMQC, HMBC experiments) see Table 1; EIMS m/z 414 [M]+ (39), 396 (100), 381 (21), 329 (17), 303 (19), 273 (11), 255 (24), 213 (20), 161 (18), 145 (22), 107 (22), 95 (23), 55 (21).

**4,5,6-Trihydroxy-3',7-dimethoxyflavone (2)**

Brown crystals, mp 253–255°C; 1H NMR (500 MHz, DMSO-d6) δ 12.67 (1H, brs, OH-5), 7.60 (1H, dd, J = 7.6 Hz, 2.0 Hz, H-6'), 7.58 (1H, d, J = 2.0 Hz, H-2'), 6.95 (1H, d, J = 7.6 Hz, H-5'), 6.93 (1H, s, H-8), 6.91 (1H, s, H-3), 3.92 (3H, s, OMe), 3.90 (3H, s, OMe'); 13C NMR (125 MHz, DMSO-d6, based on DEPT, HMQC, HMBC experiments) see Table 1; EIMS m/z 330 [M]+ (100), 312 (29), 284 (34), 248 (7), 148 (7), 69 (8).

**Ursolic acid (3)**

White powder, mp 267–269°C; 1H NMR (500 MHz, DMSO-d6) δ 5.13 (1H, m, H-12), 4.31 (1H, brs, D2O exchangeable, OH), 3.00 (1H, m, H-3), 2.10 (1H, d, J = 11.25 Hz, H-18), 1.04 (3H, s, Me-27), 0.92 (3H, d, J = 6.5 Hz, Me-30), 0.89 (3H, s, Me-24), 0.87 (3H, s, Me-25), 0.81 (3H, d, J = 6.25 Hz, Me-29), 0.75 (3H, s, Me-26), 0.68 (3H, s, Me-23); 13C NMR (125 MHz, DMSO-d6, based on DEPT, HMQC, HMBC experiments) see Table 1; EIMS m/z 456 [M]+ (5), 248 (100), 219 (6), 207 (23), 203 (33), 190 (11), 189 (13), 133 (23), 119 (10), 85 (10), 71 (14), 69 (10), 57 (19).

**Daucosterin (4)**

White powder, mp 275–277°C; 1H NMR (500 MHz, C6D6N) δ 5.31 (1H, m, H-6), 5.02 (1H, d, J = 7.7 Hz, H-1'), 4.52 (1H, dd, J = 11.6 Hz, 2.1 Hz, H-6β'), 4.43 (1H, dd, J = 11.7 Hz, 5.2Hz, H-6α), 4.23 (2H, m, H-3', H-4'), 4.00 (1H, t, J = 7.9 Hz, H-2'), 3.92 (1H, m, H-5'), 3.87 (1H, m, H-3), 0.91 (3H, d, J = 6.4 Hz, Me-21), 0.86 (3H, s, Me-19),0.82 (3H, t, J = 7.3 Hz, Me-29), 0.80 (3H, d, J = 6.8 Hz, Me-26), 0.78 (3H, d, J = 6.9 Hz, Me-27), 0.58 (3H, s, Me-18); 13C NMR (125 MHz, DMSO-d6, based on DEPT, HMQC, HMBC experiments), see Table 1.

**Discussion**

Herbal remedies used in the traditional folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs. But it is necessary to reveal the active principles by isolation and characterization of their constituents and to validate their possible toxicity (17,18). The chemistry of the genus *Satureja* has not been thoroughly analyzed. Previous studies on the chemical composition of this genus indicate the occurrence of flavonoids in *Satureja thymbra* and *Satureja spinosa* (11). Iridoid glucosides have also been reported from *Satureja vulgaris* (19). Mono and sesquiterpenoids and also diterpenoids belonging to labdane, isopimarane and rearranged...
isopimarene groups were detected in *Satureja gilliesii* (20,21). Oleanolic acid, ursolic acid, β-sitosterol-β-d-glucoside and flavanone glycosides have been isolated from *Satureja acinos*, *Satureja montana* and *Satureja obovata* (22). Two 3,4-seco-triterpenoids along with a mixture of three sterols have been reported from *Satureja calamintha* and *Satureja graeca* (23,24).

From four compounds isolated from *S. khuzistanica*, β-sitosterol has been previously reported from *S. calamintha* and *S. graeca* (23). 4',5,6-Trihydroxy-3',7-dimethoxyflavone has been isolated from *S. thymbra* and *S. spinosa* (11). Also ursolic acid has been reported from *S. acinos* (22) and *S. graeca* (23). And β-sitosterol-β-d-glucoside has been isolated from *S. acinos*, *S. montana* and *S. obovata* (22).

The occurrence of these compounds in *S. khuzistanica* confirms the presence of flavones, triterpenoids and steroids in the genus *Satureja* (Lamiaceae). The natural occurrence of these compounds can be conclusive for the chemotaxonomic characterization of this genus.

With respect to numerous uses of this plant in folk medicine and great experiments that have been accomplished to investigate its biological properties, we decided to study the chemical composition of the crude extract. This process resulted in isolation of four known compounds. Nevertheless, we cannot attribute biological properties of *S. khuzistanica* to one of these compounds. Ursolic acid has been long recognized to have anti-inflammatory, antihyperlipidemic properties in laboratory animals. Recently, it has also been recognized to have anti-inflammatory and anti-nociceptive effect of hydroalcoholic extract of *Satureja khuzistanica* Jamzad extract. *J Pharm Pharmceut Sci* 2005:8: 102-6.

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Received January 16, 2006; accepted August 22, 2006

**Acknowledgements**

The authors would like to thank Prof. Dr P. Ruedi, University of Zurich, for the MS spectra and also Dr A.N. Salehinia, Khorraraman Co., for providing cultivated plants.

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