Sterols and Flavonoids of *Lomatopodium staurophyllum*

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Abstract

Background: The genus *Lomatopodium* Fisch. & C.A.Mey., belongs to Apiaceae family and contains one herbaceous species growing wildly in Iran. There are only a few papers about the chemical constituents of these plants. Here, we focused on the isolation and identification of the main flavonoid glycosides and sterols from the aerial parts of *L. staurophyllum*, which has not been previously reported.

Methods: Flowered aerial parts of the plant were dried and cut into small pieces, then extracted with ethyl acetate and methanol by percolation at room temperature. The separation process was carried out using several chromatographic methods. Structural elucidation was based on NMR and MS data, in comparison with those reported in the literature.

Results: The isolated compounds from ethyl acetate and methanol extracts of *L. staurophyllum* were identified as nonanal (1), stigmasterol acetate (2), beta-sitosterol (3), quercetin 3-O-glucoside (4) and quercetin 3 – O - rhamnoglucoside (5) by comparison of their NMR spectral data with those reported in the literature.

Conclusion: Ethyl acetate and methanol extracts of *L. staurophyllum* contain various flavonol glycosides and sterols as the main components. Pelargonic aldehyde (nonanal), a growth promoting factor in plant cells, is found to be occurred in this species and might be a cause of anti - diarrhea activity.

Keywords: ***Lomatopodium staurophyllum*, Apiaceae, Nonanal, Stigmasterol acetate, Quercetin 3 - O-glucoside, Quercetin 3 – O - rhamnoglucoside**

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Introduction

Lomatopodium Fisch. & C.A.Mey. (Apiaceae), well-known as Pa-pahn in Persian language, includes one endemic species spread throughout North-East and Northern parts of Iran [1]. The mentioned species, *L. staurophyllum,* is growing on the gypsy soil [2]. This species is especially widespread in Khorasan province and Robat-Sefid aeria [1, 3]. Based on the resent researches, the important synonym of the genus *Lomatopodium* is *Seseli* L. [1].

The literature reviews show that there are only a few papers about the phytochemical investigation of this plant. It has been reported that the volatile oil of *L. staurophyllum* contained *E*-beta-ocimene (26.8%), myrcene (26.3%), *Z*-beta-ocimene (17.7%), beta-caryophyllene (4.6%) and limonene (4.6%) as the main volatile components [4]. In another study, a hydro-distilled oil of the aerial parts of *L. khorassanicum* Mozaffarian, has been analyzed by GC/MS. The major components were found to be myrcene (28.2%), E-beta-ocimene (15.4%) and limonene (10.1%) [5]. A review of the literature reveals that *L. khorassanicum* has not been mentioned in the flora of Iran and only the species *L. staurophyllum* has been found in that literature [1, 3].

Regarding to this point that there is no paper around the non-volatile constituents of this species, we aim to present the results of isolation and structural elucidation of the main compounds from the aerial parts of *L. staurophyllum* which has not been published in advance.

Materials and Methods

Aerial parts of *L. staurophyllum,* at the flowering stage, were collected from the Semnan Province (July, 2009). A voucher specimen of the plant was deposited at the Herbarium of the Institute of Medicinal Plants, ACECR, Tehran.

Extractions of the plants

Aerial parts of the plants were dried at room temperature. Dried material was reduced to small pieces. The extraction process was performed with ethyl acetate and methanol using percolation method. The solvents evaporated under reduced pressure to obtain the concentrated extracts and dried by freeze dryer to obtain powdered extracts.

Instruments

^{1}H and ^{13}C-NMR spectra were measured on a Bruker Avance TM 500 DRX (500MHz for ^{1}H and 125MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard; chemical shifts are given in δ (ppm). Pre-coated silica gel $60F_{254}$ plates (Merck TM) were used for TLC. Spots were detected by spraying anisaldehyde-H_2SO_4 reagent followed by heating.

Isolation

Dried aerial parts of *L. staurophyllum* (2 kg) were cut into small pieces and extracted with ethyl acetate and methanol, respectively, at room temperature. The ethyl acetate extract (50 g) was subjected to silica gel column chromatography (CC) with hexane: $CHCl_3$ (8: 2, 1: 1, 0: 1) and ethyl acetate to give six fractions (A–F). The fraction C (7.5 g) was submitted to silica gel CC with $CHCl_3$: AcOEt (8:2, 1:1, 0:1), yielding three fractions (C_1–C_3). Fraction C_3 (403 mg) was subjected to silica gel CC with hexane: AcOEt (7:3), yielding the pure compound 1 (11 mg). The fraction E (4.8 g) was submitted to silica gel CC with hexane: AcOEt (9:1, 7:3, 0:1), to obtain five fractions (E_1–E_5). The fraction E_2

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(132 mg) was subjected to silica gel CC with hexane: $CHCl_3$: AcOEt (3:6:1), resulting in two pure compounds **2** (7 mg) and **3** (35 mg).

The crude methanolic extract (95 g) was washed with chloroform to obtain methanol extract (75 g) which was successively subjected to silica gel column chromatography with AcOEt : MeOH (8:2, 1:1 and 0 : 1) to result in five main fractions, M_1–M_5. The fraction M_3 (1.3 g) was submitted to sephadex LH_{20} with MeOH to give nine fractions. The fractions M_{36} and M_{39} were the pure compounds **4** (7 mg) and **5** (12 mg).

Stigmasterol acetate (2): ^{13}C NMR ($CDCl_3$, 125 MHz), δ (ppm): 12.1 (C-18), 12.2 (C-29), 19.0 (C-26), 19.3 (C-19), 21.0(C-11), 21.2 (C-27), 21.4 (CH_3, acetate), 21.5 (C-21), 24.4 (C-15), 25.4 (C-28), 27.8 (C-2), 28.8 (C-16), 31.8 (C-25), 31.9 (C-7, 8), 36.6 (C-10), 37.0 (C-1), 38.1 (C-4), 39.6 (C-12), 40.4 (C-20), 42.2 (C-13), 50.1 (C-9), 51.2 (C-24), 56.0 (C-17), 56.8 (C-14), 73.9 (C-3), 122.5 (C-6), 129.3 (C-23), 138.2 (C-22), 139.6 (C-5), 170.2 (C=O, acetate).

Quercetin 3-O-glucoside (isoquercitrin) (4): ^1H-NMR (MeOH, 500 MHz), δ (ppm): 6.10 (1H, *d*, *J* = 2.0 Hz, H-6), 6.26 (1H, *d*, *J* = 2.0 Hz, H-8), 6.85 (1H, *d*, *J* = 8.0 Hz, H-5'), 7.57 (1H, *dd*, *J* = 2.0, 7.5 Hz, H-6'), 7.70 (1H, *d*, *J* = 2.0 Hz, H-2'), 5.10 (1H, *d*, *J* = 7.7 Hz, H-1''), 3.30-3.80 (6H, *m*, H-2''- H-6''); ^{13}C NMR (MeOH, 125 MHz), δ (ppm): 158.0 (C-2), 135.1 (C-3), 178.9 (C-4), 163.2 (C-5), 101.6 (C-6), 167.3 (C-7), 95.4 (C-8), 158.6 (C-9), 105.2 (C-10), 123.0 (C-1'), 116.2 (C-2'), 145.9 (C-3'), 149.5 (C-4'), 117.4 (C-5'), 122.7 (C-6'), 101.4 (Glc-1''), 74.3 (Glc-2''), 76.8 (Glc-3''), 70.3 (Glc-4''), 77.5 (Glc-5''), 61.3 (Glc-6'').

Results

The isolated compounds (Figure 1) from the ethyl acetate and methanol extracts of *L. staurophyllum* were identified as nonanal (**1**), stigmasterol acetate (**2**), beta-aitosterol (**3**), quercetin 3-O-glucoside (**4**) and quercetin 3-O-rhamnoglucoside (**5**) by comparison of their ^1H and ^{13}C -NMR and MS spectral data with those reported in the literature [6-9]. We previously reported the ^1H and ^{13}C –NMR of the compounds **3** and **5** [6, 7]. In this paper, the ^1H and ^{13}C -NMR data of the compounds **2** and **4** have been reported (see isolation section).

Discussion

This is the first report of the isolation and identification of main flavonoids and sterols from *L. staurophyllum*, which have not been isolated until now. These compounds even nonanal have been found to be effective in many pharmacological and biological tests.

The flavonol quercetin presents predominantly in herbal medicines and foods. Quercetin glycosides showed different biological activity and bioavailability because of the sugar moiety. The antiproliferative activities of quercetin 3-O-glucoside (Q3G) and quercetin 3-O-rhamnoglucoside (rutin) have been reported on six various cancer cell lines including colon, breast, hepatocellular, and lung cancer. Q3G showed the most potent growth inhibition, whereas rutin showed the least potency and could transform to Q3G by *Aspergillus niger* [10]. In addition, it is reported that the flavonol quercetin 3-O-glucoside can inhibit *in vitro* absorption of cyaniding 3-glucoside [11].

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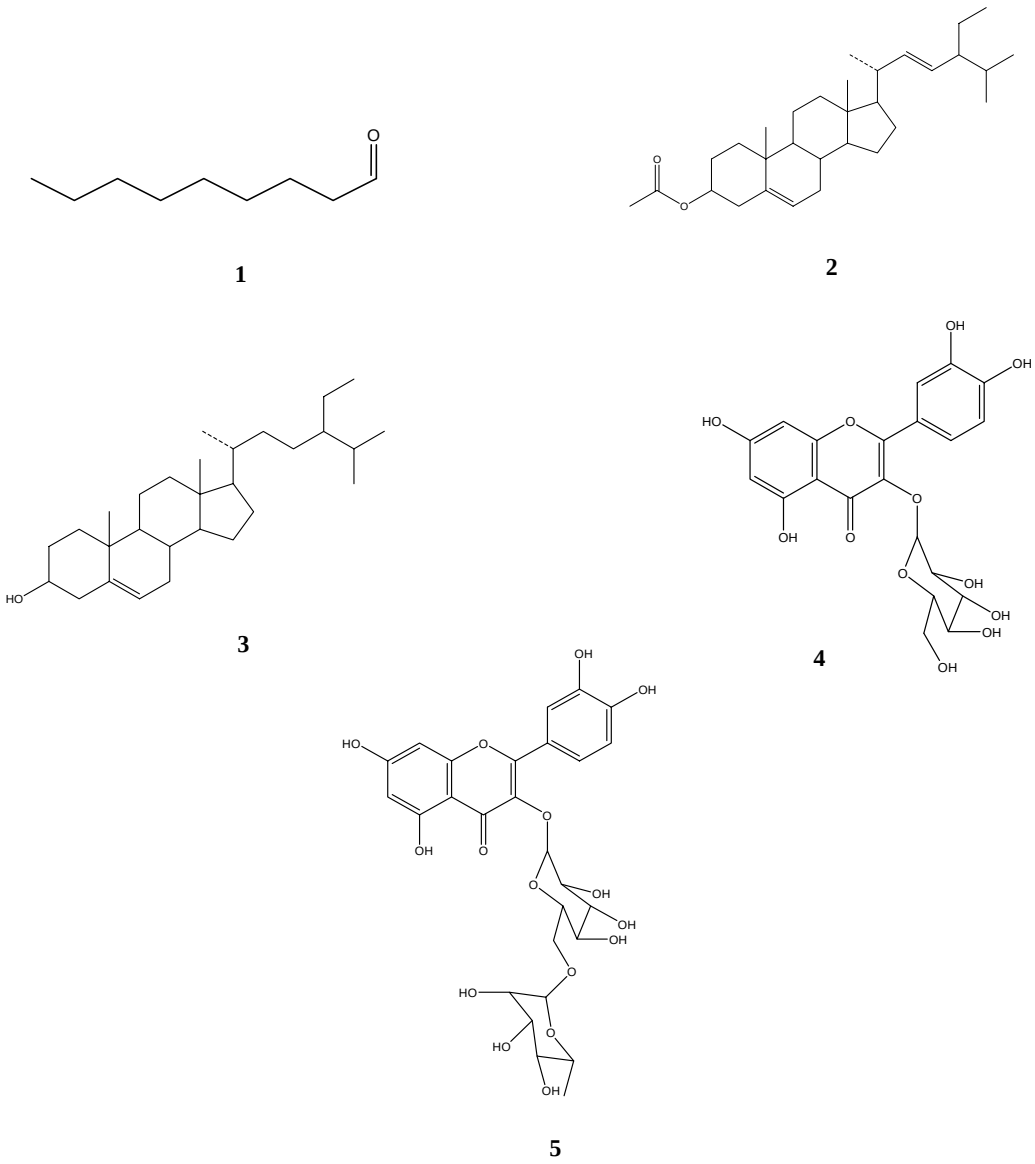


Figure 1- Structures of the isolated compounds from *L. staurophyllum*

In the literature review, it was reported that stigmasterol, stigmasterol acetate and beta-sitosterol were evaluated for their anti-nociceptive activity compare to aspirin. Their results showed that stigmasterol and stigmasterol acetate (50-200 mg/kg) could exhibit significant analgesic activity against both acetic acid- and formalin-induced nociception in mice. Also, stigmasterol and stigmasterol acetate (10-100 mg/kg) caused inhibition of both the neurogenic and inflammatory phases of formalin-induced pain [12].

The essential oil of *Artemisia ludoviciana* was found to be anti-diarrhoeal and nonanal was identified as the compound responsible for this effect. Nonanal showed a significant inhibitory effect on mice with diarrhoea induced with castor oil, $MgSO_4$ and arachidonic acid. It also showed an important

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delayed the intestinal transit activity 30min after its administration [13]. In the plants, nonanal showed the growth-promoting effect which remained unchanged when the concentration of biotin was increased. Growth-stimulating effect of nonanal is reported to be different from the effect of oleic acid [14]. Human Retinol Dehydrogenase 12 (RDH12) is an $NADP^+$-dependent oxidoreductase that acts exclusively in the reductive direction in the cells. Kinetic analysis suggests that RDH12 recognizes retinaldehyde as well as medium-chain aldehydes as substrates. Nonanal has been reported to inhibit the activity of RDH12 towards retinaldehyde, suggesting that nonanal was metabolized by RDH12 [15].

Acknowledgements

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