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Original article

Isolation and characterization of phytochemicals of *Johrenia paucijuga* (DC.) Bornm.

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Abstract

Background and objectives: The genus *Johrenia* belongs to Umbelliferae family and contains five species that are endemic to Iran. *Johrenia paucijuga* grows widely in the north-west, west and center of Iran. So far, there has been no research about phytochemistry of *J. paucijuga*. In the present study, phytochemicals of the plants have been isolated and their structures have been elucidated. **Methods**: The aerial parts were dried and cut into small pieces, then extracted with ethyl acetate and methanol using perculator apparatus at room temperature. The methanol extract was extracted again with, petroleum ether and butanol. The separation and isolation process was carried out using column (silica gel and Sephadex LH-20) and thin layer chromatographic (TLC) methods. Structure elucidation of the purified compounds were based on ^{1}H and ^{13}C-NMR data, in comparison with those reported in the previous literatures. **Results**: The isolated compounds from the ethyl acetate and butanol extracts of *J. paucijuga* were identified as β-sitosterol, β- stigmasterol and quercetin. Quercetin is a bioactive flavonoid widely used as a health supplement. β- sitosterol and β-stigmasterol are phytosteroles (plants sterols) with chemical structure similar to that of cholesterol and are sometimes used in treating hypercholesterolemia. **Conclusion:** Regarding the valuable biological properties of the isolated compounds, different biological effects could be expected from the plant.

Keywords*:* β-sitosterol, β-stigmasterol, *Johrenia paucijuga*, phytochemistry, quercetin

Introduction

Medicinal plants are important in maintaining the health of people. Plants life is more ancient than human and plants are sources of food, air and medicines for human health. Nature of medicinal plants makes them more compatible with the human body and eliminates some side effect developed by synthetic chemicals. About fifty percent of the drugs in the world have natural sources and about twenty-five percent of the drugs have plant origin. Anticancer drugs such as vincristine and vinblastine or cardiac glycosides that are derived from plants secondary metabolites are just examples of these compounds [1]. The genus *Johrenia* belongs to the Umbelliferae family with about 300 genera and 3000 species, which is cosmopolitan in distribution, chiefly in north temperate regions. Plants of this family are grassy, permanent,

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biennial or annual and mostly grow in the north hemisphere [2]. The genus *Johrenia* has several species including *J. paucijuga, J. golestanica, J. aromatica, J. ramosissima* and *J. platycarpa*, all of which are endemic to Iran. *Johrenia paucijuga* grows in north-west, west and center of Iran and its flowering season is the late of the spring [3]. So far, there has been no research about phytochemicals or other biological properties of this species. The present study deals with the isolation and identification of three compounds of *J. paucijuga*.

Experimental

General experimental procedures

^{1}H- and ^{13}C-NMR spectra were measured on a Brucker Avance 500 DRX (500 MHz for ^{1}H and 125 MHZ for ^{13}C) spectrometer (Germany) with tetramethylsilane as the internal standard. Chemical shifts were given in δ (ppm). Column chromatography was carried out using silica gel (35-70 mesh) obtained from Merck (Darmastadt, Germany) and sephadex LH-20 procured from Fluka (Switzerland). Pre-coated silica gel F_{254} plates and silica gel RP-18 F_{254} S plates (Merck, Drmastadt, Germany) were used for TLC. Spots were observed under UV at 254 and 366 nm and sprayed with anisaldehyde-H_2SO_4 reagent (Sigma-Alderich Chemie, Germany) and heated at 120 °C for 5 min.

Plant material

Johrenia paucijuga (DC.) Bornm was collected from road sides of Khoy, West Azerbaijan province, Iran, in June 2011. The herbarium sample was identified by Y. Ajani and A.R. Gohari and deposited in the Herbarium of Faculty of Sciences, University of Tehran, Iran (No. 1625).

Extraction and isolation

The aerial parts of *J. paucijuga* (800 g) were cut into small pieces and air dried. They were extracted by methanol and ethyl acetate using percolation method three times, each time at least for 48 h. The ethyl acetate extract was transferred to a silica gel column, eluting with hexan–chloroform (3:7) and gradually increasing polarity. This process was continued by chloroform, chloroform-ethyl acetate, ethyl acetate–methanol and methanol resulting in 11 fractions (A-K). Fraction C (240 mg) was selected and loaded to a smaller silicagel column. Eluting with chloroform which was continued with chloroform-ethyl acetate (98:2) yielded 8 fractions (C_1-C_8). Fraction C_6 (14 mg) consisted of compounds **1** and **2**. About 250 mL distilled water (DW) was added to the methanol extract (80 g) and subsequently it was fractioned with petroleum ether. Afterwards butanol was added to the aqueous phase to obtain the butanol fraction. These three fractions were concentrated using a rotary evaporator (figure1). Butanol fraction (6 g) was dissolved in methanol- water (7:3) and was loaded on a sephadex LH-20 column, eluted with methanol to obtain 20 fractions (B_1-B_{20}) among which fraction 19 was identified as a pure compound **3** (6 mg) [4].

Results and Discussion

Two steroids (compounds **1** and **2**) and a flavonoid (compound **3**) (figure 2) were isolated and purified from the ethyl acetate and methanol extracts of *J. paucijuga*. Their structures were determined by comparison of their NMR spectral data with those reported in literature [4-6].

The isolated compounds were identified as β-sitosterol, β-stigmasterol and quercetin. The two former are phytosterols (plant sterols) with chemical structure similar to cholesterol. They reduce blood levels of cholesterol, and are sometimes used in treating hypercholesterolemia [7]. β-stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast and colon cancers [8]. Quercetin is a flavonoid, a plant pigment, found in fruits, vegetables, leaves and grains. It can be used as an ingredient in supplements, beverages, or foods. Quercetin works as an anti-inflammatory, antioxidant, anticancer and antiviral agents [9,10]. The NMR data of β-sitosterol (**1**) and β-stigmasterol (**2**) are presented in table 1 [11,12].

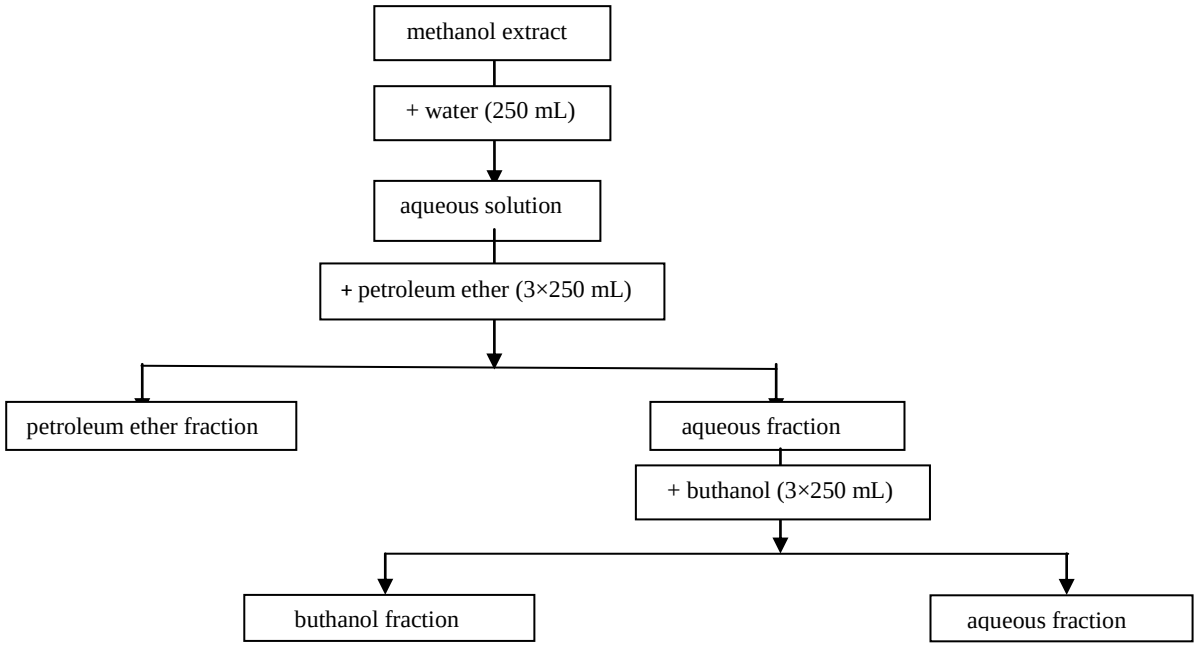

Figure 1. Schematic representation of fractioning the methanol extract

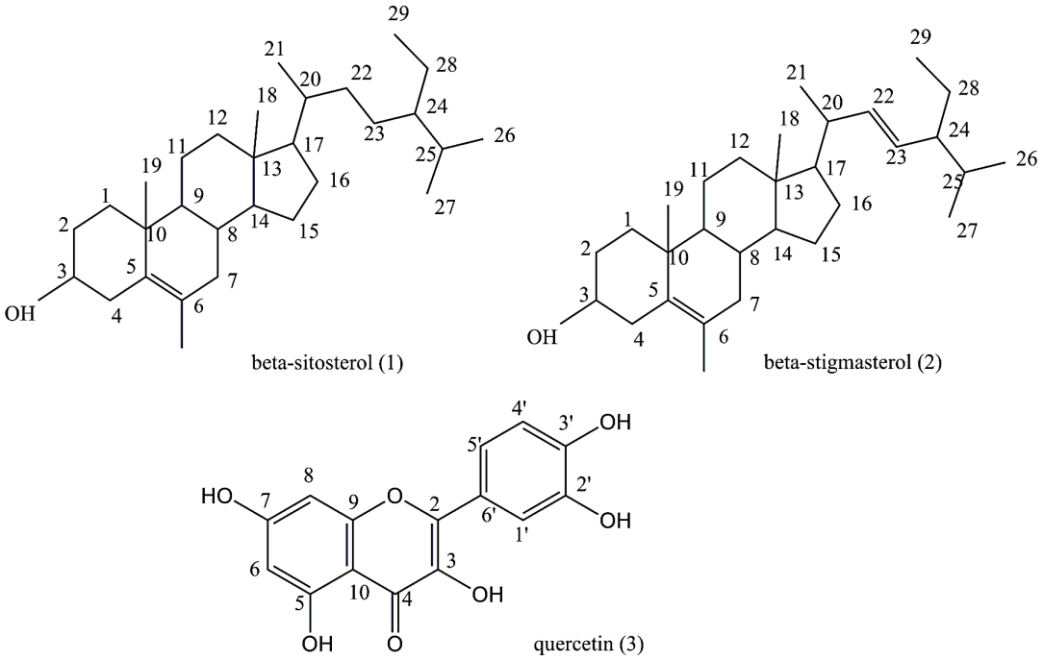

Figure 2. The structures of the purified compounds of *Johrenia paucijug*

Spectral data of compound 3 are presented. Quercetin (**3**). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 6.18 (1H, *d*, *J*=2 Hz, H-6), 6.14 (1H, *d*, *J*=2 Hz, H-8), 7.53 (1H, *d*, *J*=8.4 Hz, H-1'), 6.89 (1H, *d*, *J*=8.8 Hz, H-4'), 7.55 (1H, *dd*, *J*=2.4 and 8.5 Hz, H-5'). ^{13}C-NMR (125 MHz, DMSO-d_6): δ 146.71 (C-2), 135.68 (C-3), 175.77 (C-4), 160.66 (C-5), 98.14 (C-6), 163.9 (C-7), 93.3 (C-8), 156.88 (C-9), 102.91 (C-10), 121.89 (C-1'), 114.98 (C-2'), 145.01 (C-3'), 147.65 (C-4'), 115.54 (C-5'), 119.91 (C-6') [13].

Table 1. ^{1}HNMR and ^{13}CNMR chemical shift values for β-sitosterol (**1**) and β-stigmasterol (**2**) recorded in $CDCl_3$.

position	1		2	
	^{1}HNMR	**^{13}CNMR**	**^{1}HNMR**	**^{13}CNMR**
1		37.23		37.23
2		31.6		31.6
3	3.52 (*m* , 1H)	71.83	3.51 (*tdd*, 1H, *J*=4.5,4.2 and 3.8 Hz)	71.83
4		42.26		42.26
5		140.72	5.31 (*t*, 1H, *J*=6.1 Hz)	140.72
6	5.34 (*s*, 1H)	121.74		121.74
7		31.8		31.8
8		31.8		31.8
9		50.11		50.11
10		36.15		36.15
11		21.07		21.07
12		39.26		39.66
13		42.26		42.26
14		56.75		56.85
15		24.30		24.36
16		28.25		28.24
17		56.03		55.93
18	0.68 (*s*, 3H)	11.86	0.71 (*s*, 3H)	12.04
19	1.01 (*s*, 3H)	19.40	1.03 (*s*, 3H)	19.40
20	0.92 (*d* , *J*=6.5 HZ, 3H)	35.88		40.52
21		18.25	0.91 (*d*, 3H, *J*=6.2 Hz)	21.21
22		33.80	4.98 (*m*, 1H)	138.33
23		26.02	5.14 (*m*, 1H)	129.25
24		45.81		51.24
25		29.11		31.8
26	0.83 (*m*, 3H)	19.83	0.82 (*d*, 3H,*J*=6.6 Hz)	19.07
27	0.84 (*m*, 3H)	19.02	0.80 (*d*, 3H, *J*=6.6 Hz)	18.77
28	0.84 (*m*, 2H)	23.04		25.42
29	0.84 (*m*, 3H)	11.98	0.83 (*t*, 3H, *J*=7.1 Hz)	12.26

Although the chemical constituents of the essential oil of *J. paucijuga* has been investigated before [14], to the best of our knowledge, this is the first report about the secondary metabolites of *J. paucijuga* resulting in the isolation and identification of three compounds, β-sitosterol (1) and β-stigmasterol (2) and quercetin (3) using different column chromatographic methods. Further investigation is recommended for isolation of other phytochemicals and also investigation of pharmacological and biological properties.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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