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GC-MS Analysis of the Dichloromethane Extract of the Bulbs of *Ornithogalum cuspidatum* Bert. (Family: Liliaceae) from Iran

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Abstract: The gas chromatography-mass spectrometry (GC-MS) analysis of the vacuum liquid chromatographic (VLC) fractions of the dichloromethane (DCM) extract of the bulbs of *Ornithogalum cuspidatum*, a native perennial of Iran, Iraq and Turkey, led to the identification of a number of steroidal compounds. The free radical scavenging activity of the DCM extract was assessed by the 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay, and found to be much weaker (RC_{50} = 0.5197 mg/mL) than that of the positive control Trolox® (RC_{50} = 3.07 x 10^{-3} mg/mL).

Keywords: *Ornithogalum cuspidatum*; Liliaceae; steroids; GC-MS; Antioxidant capacity; DPPH.

1. Introduction

Ornithogalum cuspidatum Bert. is an Iranian species of the genus *Ornithogalum* L. (family: Liliaceae) that encompasses well over 150 perennial bulbous species, mostly distributed in the temperate regions of Europe, Asia, and Africa [1-3]. No phytochemical investigation has yet been carried out on steroids of *O. cuspidutum*. However, previous phytochemical studies on some other *Ornithogalum* species revealed the presence of steroidal glycosides [4-8], monoterpene lactone [9] and homoisoflavanone [10] some of which were found to possess antimicrobial, cytotoxic, cytostatic,

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anticancer, mould-inhibiting and insect deterrant properties [8]. In continuation of our phytochemical and bioactivity studies on the plants from the Iranian flora [11-21], we report on the GC-MS analysis VLC fractions of the DCM extract of the bulbs of *O. cuspidatum.*

2. Materials and Methods

2.1. Plant Material

Bulbs of *Ornithogalum cuspidatum* Bert. were collected from Maraghe in the northwest of Iran during April-May 2006. A voucher specimen (TUM-ADE 0284) representing this collection has been retained in the herbarium of the Faculty of Pharmacy, Tabriz University of Medical science, Iran.

2.2. Extraction

The dried and ground bulbs (100 g) were Soxhlet-extracted, successively, with *n*-hexane, and DCM (1.1 L each). The DCM extract was concentrated using a rotary evaporator at a maximum temperature of 45°C to yield 619 mg of dried extract.

2.3. Vacuum liquid chromatography

A portion (550 mg) of the DCM extract was subject to vacuum liquid chromatography (VLC) on silica gel 60H using a step gradient of *n*-hexane:ethylacetate to obtain six VLC fractions (fractions 1-5, *n*-hexane:EtOAc = 90:10, 80:20, 60:40, 40:60 and 20:80, respectively), which were concentrated using a rotary evaporator at a maximum temperature of 45°C.

2.4. Preparation of trimethylsilyl (TMS) ether derivatives:

The steroidal compounds present in the VLC fractions of the DCM extract were converted to their trimethylsilyl derivatives. Each fraction was mixed with Tri-Sil reagent (0.1 mL) in glass sealed tubes using an ultrasonic bath for 2 min and then vortexing briefly. The tubes were then incubated at 60°C for 45 min. Thereafter, the solvent was evaporated under a stream of nitrogen and the TMS ether derivatives were dissolved in 0.2 mL of *n*-hexane, the tubes were sonicated in an ultrasonic bath for 2 min, vortexed and centrifuged for 3 min. The *n*-hexane layer was transferred to another tube, avoiding any solid particles, and analyzed by the GC–MS. After derivatization, the tubes were stored at -20°C for subsequent analyses within 3 days.

2.5. Gas Chromatography-Mass Spectrometry

The GC-MS analyses were carried out in a Shimadzu GC-MS-QP 5050A gas chromatograph fitted with a DB1 (methylphenylsiloxane, 60 m × 0.25 mm i.d.) capillary column. Carrier gas, helium with a flow rate of 0.7 mL/min; column temperature, 5 min in 180°C, 180-260°C at 3°C/min, 5 min in 260°C, 260-280°C at 0.2°C/min, and finally 5 min in 280°C; injector temperature, 280°C detector temperature, 290°C, Volume injected, 1 µL of TMS ether derivatives in *n*-hexane (2%); Split ratio, 1:8. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 290°C; quadrupole 100°C, Solvent delay 6.0 min , scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

2.6. Identification of the compounds

The identification of components present in the VLC fractions of the of DCM extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [22-24].

2.7. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Fluka Chemie AG, Bucks. Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma-Aldrich, UK. The method used by Takao et al. [25] was adopted with suitable modifications [26]. DPPH (8 mg) was dissolved in MeOH (100 mL) to obtain a concentration of 80 μg/mL.

2.7.1 Qualitative analysis

Test samples were applied on a TLC plate and sprayed with DPPH solution using an atomiser. It was allowed to develop for 30 min. The colour changes (purple on white) were noted.

2.7.2. Quantitative analysis

Serial dilutions were carried out with the stock solutions (10 mg/mL) of the DCM extract to obtain concentrations of $5x10^{-1}$, $5x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$, $5x10^{-5}$, $5x10^{-6}$ $5x10^{-7}$, $5x10^{-8}$, $5x10^{-9}$, $5x10^{-10}$ mg/mL. Diluted solutions (2 mL each) were mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control Trolox®. The RC_{50} value, which is the concentration of the test material that reduces 50% of the free radical concentration, was calculated as mg/mL.

3. Results and Discussion

The DCM extract of *O. cuspidatum* displayed weak free radical scavenging activity in the DPPH assay . The RC_{50} values of the extract was 0.5197 mg/mL, and that of the positive control, Trolox®, was 3.07×10^{-3} mg/mL.

The results of the GC-MS analyses leading to the identification of a number of steroidal compounds from the VLC fractions of the DCM extract of *O. cuspidatum* are summarised in Table 1.

A total of 25 steroids were found, and 24 were identified from the VLC fractions for the DCM extract of *O. cuspidatum*. Fungisterol was found to be the most abundant steroid in the DCM extract, and it was detected in three VLC fractions 2-4. The first two VLC fractions (1 and 2) yielded the highest number of steroids; eight steroid in each fraction. In fraction 1, cholesta-3,6-dione and spinasterone were the major steroids. However, an unidentified steroid was also present in significant amounts (1.14%) in this fraction. In fraction 2, sitosterol, fungisterol and stigmasterol were the most significant steroids in terms of abundance. Sitosterol and stigmasterol are the two most common steroids generally found in various plant species. In addition to fungisterol, fraction 3 produced two other major steroids, e.g. 3-(*t*-butyldimethylsilyoxy)-9,19-cyclolanostane and stigmast-5-en-3-oleate. In fraction 4, except

fungisterol, there were four minor steroids present (0.11-0.17%). Three steroidal compounds were detected in fraction 5, and they were of low to medium abundance (Table 1).

Table 1. Steroidal compounds present in the VLC fractions of the DCM extract of *O. cuspidatum*

Compounds	Retention time (min)	Amount (%)	Molecular mass	Molecular formula
Fraction 1 (10% EtOAc in n-hexane)				
14-Methyl-cholest-8-en-3-one	91.563	0.14	398	$C_{28}H_{46}O$
4-Methyl-cholestan-3-one	99.842	0.16	400	$C_{28}H_{48}O$
17-(1,5-Dimethylhexyl)-10,13-dimethyl-1,7,8,9,10,11,12,13,14,15,16,17-dodecahydrocyclopenta[a] phenanthren-4-one	103.343	0.12	382	$C_{27}H_{42}O$
4,4-Dimethyl-cholestan-3-one	106.400	0.13	414	$C_{29}H_{50}O$
Cholest-4-ene-3,6-dione	110.056	0.73	398	$C_{27}H_{42}O_2$
Spinasterone	114.779	0.38	410	$C_{29}H_{46}O$
Sitosterol acetate	115.858	0.16	456	$C_{31}H_{52}O_2$
Unidentified steroid	123.569	1.14	412	$C_{27}H_{40}O_3$
Fraction 2 (20% EtOAc in n-hexane)				
4,4-Dimethyl-cholesta-6,22,24-trien	60.808	0.37	394	$C_{29}H_{46}$
Sitosterol	71.739	1.53	414	$C_{29}H_{50}O$
Cholesterol	90.557	0.43	386	$C_{27}H_{46}O$
Fungisterol	103.501	2.94	400	$C_{28}H_{48}O$
Stigmasterol acetate	108.038	2.1	454	$C_{31}H_{50}O_2$
Progesterone	110.304	0.87	314	$C_{21}H_{30}O_2$
Stigmasterol	116.27	2.48	412	$C_{29}H_{48}O$
Stigmast-4-en-3-one	123.884	0.41	412	$C_{29}H_{48}O$
Fraction 3 (40% EtOAc in n-hexane)				
4,4-Dimethyl-cholest-7-en-3-one	54.583	0.15	412	$C_{29}H_{48}O$
Fungisterol	103.501	1.14	400	$C_{28}H_{48}O$
3-(*t*-butyldimethylsilyoxy)-9,19-cyclolanostane	107.805	1.20	470	$C_{33}H_{58}O$
Stigmast-5-en-3-oleate	115.980	1.55	678	$C_{47}H_{82}O_2$
Fraction 4 (60% EtOAc in n-hexane)				
Fungisterol	103.501	1.14	400	$C_{28}H_{48}O$
Stigmastan-3,5-diene	115.665	0.11	396	$C_{29}H_{48}$
Cholestan-7-one, cyclic 1,2-ethanediyl acetal	119.248	0.19	430	$C_{29}H_{50}O_2$
Cholestane-3,5-diol, diacetate	126.818	0.13	488	$C_{31}H_{52}O_4$
3-Acetyloxy-ergosta-5,24-diene	132.165	0.17	440	$C_{30}H_{48}O_2$
Fraction 5 (80% EtOAc in n-hexane)				
Cholest-5-en-3-ol, tetradecanoate	126.993	0.26	596	$C_{41}H_{72}O_2$
3-Acetyloxy-20-hydroxy-25-methoxy-16-oxo-lanost-9(11)-en-18-oic acid, γ.-lactone	132.971	0.11	542	$C_{33}H_{50}O_6$
17-(1,5-Dimethylhexyl)-10,13-dimethyl-1,7,8,9,10,11,12,13,14,15,16,17-dodecahydrocyclopenta [a]phenanthren-4-one	141.852	0.25	382	$C_{27}H_{42}O$

Steroids are biologically active molecules. Steroidal glycosides are considered to be a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants' [27, 28]. Thus, the identification of a number of steroidal compounds from the DCM extract of *O. cuspidatum* might have some ecological significance. As steroidal compounds have previously been reported from a number of other *Ornithogalum* species, the occurrence of steroids in *O. cuspidatum* might also contribute to the chemical taxonomy of this genus.

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