GC-MS analysis of *Ornithogalum procerum*

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ABSTRACT

Background and objectives:Ornithogalum procerum Stapf. (Family: Liliaceae) is an Iranian medicinal plant found mainly in the east Azarbaiijan province of Iran. As part of our on-going phytochemical and bioactivity studies on medicinal plants from Iranian flora, various extracts and essential oils of this plant were analysed by GC-MS.

Methods: The hydrodistillation of the ground aerial parts of *O. procerum*, Soxhlet-extraction of the dried and ground bulbs, and the hydrolysis of the methanol extract of the bulbs yielded, the essential oils, *n*-hexane extrcat and the hydrolysed methanolic extract respectively, which were analysed by the GC-MS.

*Results:*A total of 23 compounds were identified from the essential oils of the the aerial parts. The identified compounds represented 70.27% of the total essential oils. The main components of the aerial parts were phenylacetaldehyde (7.57%), hexahydrofarnesyl acetone (8.13%), docosan (5.52%) and 5-methyl octadecane (4.63%). From the *n*-hexan extract of the bulbs, seven hydrocarbons representing 99.39% of the total extract, were identified. Finally, from the hydrolyzed methanolic extract of the bulbs, four polysterol-type compounds accounting for 59.81% of the extract, were detected.

Conclusion: The GC-MS analyses reavealed that the essential oils are mainly composed of oxygenated hydrocarbons, the *n*-hexane extract contains predominatly hydrocarbons, and the hydrolyzed methanolic extract comprises polysterol-type compounds.

Keywords: *Ornithogalum procerum*, Liliaceae, Essential oils, Terpenoids, Phytosterols

INTRODUCTION

The genus *Ornithogalum* (family: Liliaceae) comprises ca. 150 species, distributed in the temperate Europe, Asia, and Africa. Some *Ornithogalum* plants are known to be poisonous, of which several cardenolide glycosides have been isolated and identified (1). Several species of *Ornithogalum* have long been implicated to live-stock poisoning (2). In addition, it has been reported that the raw extract of the bulbs inhibited the growth of some Gram-positive bacteria (3). Phytochemical studies revealed that the bulbs of some species contain a variety of steroidal compounds, steroidal glycosides, such as cholestane glycosides, acylated cholestane bisdesmosides, saponins and spirostanol glycosides, some of which exhibited significant cytotoxic activities against cultured tumor cells and have anticancer potential (4-8).

Information gathered from the Iranian ethnopharmacologists, local herbal drug sellers and traditional medicine practioners indicate that in the East Azarbaiijan province of Iran, the aerial parts of *Ornithogalum procerum* Stapf. (common Persian name: 'Shir-morghe dayhimi') (1) are used as food additives and also in the traditional medicine as an anti-irritant and relaxant by soothing the throat and brochial tubes during dry coughs. Because of medicinal importance, the interest in chemical constituents of various species of the genus *Ornithogalum* has increased significantly in recent years (9-12). However, no phytochemical investigation has yet been carried out on *O. procerum*, a native perennial to Iran, Iraq and Turkey. The main reason for the paucity of information is its restricted geographical distribution. As part of our on-going phytochemical studies on Iranian medicinal plants (13-17), we now report on the composition of the essential oils of the aerial parts, n-hexane extract of the bulbs and the hydrolysed methanolic extract of the bulbs of *Ornithogalum procerum* using GS-MS.

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MATERIALS AND METHODS

Plant material

Aerial parts and bulbs of *Ornithogalum procerum* Stapf. were collected during April-May 2006, from around of Maraghe in the northwest of Iran. A voucher specimen (TUM-ADE 0284) has been retained in the herbarium of the Faculty of Pharmacy, Tabriz University of Medical science, Iran.

Extraction of essential oil

Plant materials were dried at room temperature. Crushed aerial parts (30 g) were subjected to hydrodistillation for 3 hrs using a Clevenger-type apparatus. The essential oils (0.4% v/w) were dried over anhydrous sodium sulfate and stored at 4-5 °C.

Soxhlet extraction

The dried and ground bulbs (100 g) were Soxhlet-extracted, successively, with *n*-hexane, and methanol (1.1 L each). Both extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45 ^{o}C to yield 616 mg and 6.63 g of dried *n*-hexane and methanolic extracts, respectively.

Hydrolysis of MeOH extract

The MeOH extract of bulb (300 mg) in 0.5M HCl (dioxane:H_2O, 1:1, 100 mL) was heated at 95 ◦C for 1h under an N_2 atmosphere. After cooling, the reaction mixture extracted by $CHCl_3$, and was concentrated under reduced pressure.

GC-MS analyses

GC-MS analyses were carried out in a Shimadzu GCMS- QP 5050A gas chromatograph fitted with a DB5 (methyl phenyl sylonane, 60 m × 0.25 mm i.d.) capillary column. Operating conditions for analysis of the essential oil, *n*-hexane extract and hydrolyzed methanolic extract are summarized below.

The essential oil

Carrier gas, helium with a flow rate of 0.7 mL/min ; column temperature, 5 min at 60°C, 60-290°C at 3°C/min and finally 5 min at 290°C; injector temperature, 250 °C; detector temperature, 290°C , Volume injected, 1 µL of the oil in chloroform (0.5%); Split ratio, 1:53. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 290°C; quadrupole 100°C, Solvent delay 4.0 min , scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

n-Hexane extract

Carrier gas, helium with a flow rate of 0.6 mL/min ; column temperature, 3 min at 190°C, 190-310°C at 3°C /min and finally 2 min at 310°C ; injector temperature, 280°C detector temperature, 310°C , Volume injected, 1 µL of the n-hexane extract in chloroform (1%); Split ratio, 1:76. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 290°C; quadrupole 100°C , Solvent delay 2.0 min , scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

Hydrolyzed methanol extract

Carrier gas, helium with a flow rate of 0.6 mL/min; column temperature, 5 min at 60°C, 60-290°C at 3°C/min and finally 2 min at 180°C; injector temperature, 280°C detector temperature, 310°C, Volume injected, 1 µL of the hydrolyzed methanolic extract in chloroform (2%); Split ratio, 1:12. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 290°C; quadrupole 100°C, Solvent delay 4.0 min , scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

Identification of components

Identification of components of the essential oil, the *n*-hexane extract and the hydrolyzed methanolic extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and 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 (18-19).

RESULTS AND DISCUSSION

The results of the GC-MS analyses on the essential oils of the aerial parts, *n*-hexane extract of the bulbs and the hydrolyzed methanolic extract of the bulbs of *O. procerum* are presented in Table 1.

A total of 20 compounds were identified from the essential oils of the aerial parts of *O. procerum.* The identified compounds represented 70.27% of the total essential oils. Cyclohexane, phenylacetaldehyde, hexahydrofarnesyl acetone, 5-methyl octadecane and docosane were the major components of the essential oils, and Hexahydrofarnesyl acetone was the most abundant one (8.13%). Sixteen of these compounds had at least one oxygen in the molecule, and the number of oxygen atoms present in these molecules varied from one to two. While cyclohexane was first which came out from the column (t_R = 7.364 min), 2,4-dimethyl docosane was retained in the column for the longest of all (t_R = 80.702 min).

From the *n*-hexane extract of the bulbs, seven hydrocarbons, representing 99.39% of the total

Table 1. Composition of the essential oils of the aerial parts, *n*-hexane extract of the bulbs and the hydrolysed methanolic extract of the bulbs of *Ornithogalum procerum*

Essential oils of the aerial parts				
Compound	Retention time (min)	Amount (%)	Molecular mass	Chemical formula
Cyclohexane	7.364	4.05	84	C_6H_{12}
3-Hexanol	18.001	3.31	102	$C_6H_{14}O$
2-Hexanol	18.502	3.63	102	$C_6H_{14}O$
Phenylacetaldehyde	23.632	7.57	120	C_8H_8O
L-Linalool	26.686	2.34	154	$C_{10}H_{18}O$
Nonanal	26.892	1.66	142	$C_9H_{18}O$
Menthol	30.906	1.56	156	$C_{10}H_{20}O$
Carvacrol	36.720	2.44	150	$C_{10}H_{14}O$
p-Vinylguaiacol	38.045	2.32	150	$C_9H_{10}O_2$
(+)-Spathulenol	41.758	3.58	220	$C_{15}H_{24}O$
β-Ionone	46.312	1.82	192	$C_{13}H_{20}O$
Megastigmatrienone	50.401	3.66	190	$C_{13}H_{18}O$
Myristaldehyde	51.288	2.70	212	$C_{14}H_{28}O$
(*E*)-Phytol	60.059	2.43	296	$C_{20}H_{40}O$
Hexahydrofarnesyl acetone	60.281	8.13	268	$C_{18}H_{36}O$
Palmitic acid methyl ester	63.056	3.09	270	$C_{17}H_{34}O_2$
5-Methyl octadecane	68.891	4.63	268	$C_{19}H_{40}$
Linolenic acid methyl ester	69.100	2.93	292	$C_{19}H_{32}O_2$
Docosane	75.051	5.52	310	$C_{22}H_{46}$
2,4-Dimethyl docosane	80.702	2.90	338	$C_{24}H_{50}$
n-Hexane extract of the bulbs				
(*E*)-11(12-Cyclopropyl)dodecen-1-ol	27.460	3.71	224	$C_{15}H_{28}O$
2,6,10,15–Tetramethyl heptadecane,	32.721	3.43	296	$C_{21}H_{44}$
Docosane	40.500	9.43	310	$C_{22}H_{46}$
Heptacosane	48.001	11.71	380	$C_{27}H_{56}$
Tetratriacontane	55.130	18.26	478	$C_{34}H_{70}$
Hexatriacontane	61.841	26.57	506	$C_{36}H_{74}$
Dioctadecyloxypropane	68.770	26.28	580	$C_{39}H_{80}O_2$
Hydrolysed methanolic extract of the bulbs				
5β, 6β-Epoxycholest-7-en-3β-ol	87.983	20.00	400	$C_{27}H_{44}O_2$
24-Methyl-ergosta-5,22-dien-3-ol	91.581	13.25	412	$C_{29}H_{48}O$
Campestrol	97.961	15.21	400	$C_{28}H_{48}O$
Ergosta-5-en-3-ol	97.892	11.35	400	$C_{28}H_{48}O$

extract, were identified. Hexatriacontane (26.57%) and dioctadecyloxypropane (26.28%) were the two most abundant components in the extract. Five of these components lacked in any oxygen in the molecule.

The GC-MS analysis of the hydrolyzed methanolic extract of the bulbs of *O. procerum* revealed the presence of four polysterol-type compounds which accounted for 59.81% of the total weight of the extract. It is interesting to note that like many other species of the genus *Ornithogalum*, the hydrocarbons and plant sterols were accumulated mainly in the bulbs of *O. procerum.*

CONCLUSION

The GC-MS analyses revealed that the essential oils are mainly composed of oxygenated hydrocarbons, the *n*-hexane extract contains predominantly hydrocarbons, and the hydrolyzed methanolic extract comprises polysterol-type compounds.

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