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Original Article

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Major phytosteroid from *Polygonatum orientale* Desf. Rhizome

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Abstract

Polygonatum orientale Desf. (Asparagaceae) is herbaceous and perennial plant grown in northern parts of Iran. The rhizomes of *Polygonatum*, known as Iranian Shaghagho, has been used as Iranian traditional medicine as a tonic, aphrodisiac, wound healing, lightning, kidney stone remedy, gynecological and internal wounds healer, anti-gout and rheumatism, and anti-diabetic. The aim of this study was to evaluate 2,2 Diphenyl -1- Picryl Hydrazyl (DPPH) free radical scavenging activity, isolation, purification and identification of secondary metabolites from *Polygonatum orientale* Desf. rhizome collected from north of Iran. The 80% methanol extract from the rhizomes of P. orientale was partitioned between 80% methanol and hexane, chloroform, ethyl acetate, and n-butanol respectively. The inhibitory concentrations 50% (IC50) for ethyl acetate fraction and BHT were measured from the concentration-inhibition curve and were 315.02 μg/ml and 42.3 μg/ml, respectively. Chloroform fraction was loaded on a silica gel chromatography column and eluted with hexane: ethylacetate (90:10 to 100% ethyl acetate).Two major compounds, 12-Hydroxystigmast 4-en 3-one, a phytosteroid, and methyl oleate, a fatty acid ester, were isolated using TLC plates and identified by spectroscopic methods; H and 13C NMR. These major compounds have been reported from *Polygonatum* species, for the first time. A further phytochemical study is recommended to find more compounds from this medicinal plant.

Keywords: *Polygonatum orientale*, DPPH, 12-Hydroxystigmast-4-en 3-one, Methyl oleate

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INTRODUCTION

Iranian "Shaghaghol" is a perennial herbaceous plant with the scientific name of *Polygonatum orientale* Desf. belongs to the family of asparagus (Asparagaceae). The common name of this plant is Solomon seal which grows in the north of Iran and the rhizome of this plant is used as a jam. Its rhizome has been used traditionally as wound healing, lightening, kidney stone treatment, gynecological and internal wounds, anti-gout and rheumatism, aphrodisiac and anti-diabetic. (Zargari, 1990; Khorasani, 2009)

Defferent species of *Polygonatum* are used in traditional medicines around the world. *Polygonatum* odoratum rhizomes have been used in traditional Chinese medicines for the treatment of diabetes, hypoimmunity, rheumatic heart disease and also used to treat different diseases due to its procoagulant property, hypoglycemic effect, glucose tolerance improvement and anti herpes simplex virus II and apoptosis inducing activities. Several studies have been reported that *P. odoratum* increases the level of antibody production, and exerts anti inflammatory, antiviral and tumor inhibitory effects. (Tai et al., 2016) Gu et al (2013) reported that *P. odoratum* root extract improves metabolic disorders in obese animals. (Gu et al., 2013) Furthermore, tracheorelaxant, anti-inflammatory, antipyretic, antioxidant, and antibacterial activities of rhizomes of *Polygonatum* verticillatum have been showed.(Khan et al., 2013)) *P. alte-lobatum* extracts have been proved to be a possible agent with an antioxidant and anti-fatigue properties. Two new homologous series of 1, 4-benzoquinones, an alkaloid, polygonapholine, were isolated from rhizomes of *P. alte-lobatum.* (Horng et al., 2014)

Anti-inflammatory effects and decrease in amyloid-β-induced neurotoxicity by *Polygonatum* sibiricum have been reported. Additionally, the Plant-derived polysaccharides had significant panti-ovariectomy (OVX)-induced osteoporosis effects by stimulating osteoblast formation and blocking osteoclastogenesis. (Du et al., 2016)

A variety of phytochemical constituents have been isolated from different species of the genus *Polygonatum*; alkaloid, homoisolflavanone, triterpenoid, steroidal saponin, and polysaccharides with different pharmacological effects. (Bai et al., 2014; Han et al., 2016; Yang et al., 2015; Gvazava and Skhirtladze, 2016; Gvazava and Kikoladze, 2014) lectins, 5- hydroxymethyl-2-furaldehyde and diosgenin have been reported from the rhizomes of *P. verticillatum.* (Khan et al., 2013) Several compounds such as stilbenes, coumarins like umbelliferone (7-hydroxycoumarin), and scopoletin (6-methoxy-7-hydroxycoumarin), and steroidal sapogenins from *Polygonatum* polyanthemum, flavonoids, also have been identified.(Gvazava and Kikoladze, 2011a; Gvazava and Kikoladze, 2011b; Gvazava and Kikoladze, 2012)

This study was aimed to evaluate DPPH free radical scavenging activity, isolate, purify and identify the secondary metabolites from *Polygonatum orientale* Desf. rhizome collected from north of Iran.

MATERIALS AND METHOD

Experimental

The 1H and 13C NMR spectra were measured in CDCL3 on a Bruker Avance spectrometer (500 MHz, tetramethylsilane (TMS) as an internal reference. Silica gel (35-70 mesh ASTM, 0.2-0.5 mm) (Merck, Germany) was used for column chromatography. All solvents were prepared from Merck, Germany. TLC has performed on Silica gel 60G F_{254} plates (Merck).

Plant material

The rhizomes of *P.orientale* were collected from the local area of (Mazandaran province), Iran in July 2015 and identified by Majid Eskandari (MSc.) (Department of Botany, Iranian Research Institute of Plant Protection (IRIPP).A voucher specimen (70101/1) is deposited in the Herbarium Ministerii Iranici Agriculture, Tehran, Iran.

Extract preparation

The air-dried rhizomes (400 gram) were powdered with a grinder and extracted with 80% methanol (Merck, Germany) (3×1 L) at room temperature. The excessive solvents were evaporated with a rotary vacuum evaporator (60 rpm at 40 °C). The concentrated extract suspended in water and then partitioned between water and hexane, chloroform, ethyl acetate, and n-butanol, respectively. The chloroform extract was selected for phytochemical studies.

Table 1: ^{1}H NMR and ^{13}C NMR data of compound 1 (500 MHz in CDCL3)

Number	^{1}H-NMR	^{13}C-NMR
	(δ=ppm)	(δ=ppm)
1		35.74
2		33.95
3		199.67
4	5.72 (1H,s)	123.92
5		171.38
6		32.96
7		32.09
8		35.74
9	0.98 (m)	53.87
10		38.63
11		21.07
12	3.66 (1H,t,J=5Hz)	79.17
13		42.43
14		55.93
15		24.20
16		28.19
17		56.08
18	0.71 (3H,s)	13.99
19	1.18 (3H,s)	17.41
20		36.13
21	0.92 (3H,d,J=5Hz)	18.72
22		33.95
23		26.19
24	0.97 (m)	46.00
25		29.24
26	0.83 (3H,d,J=5Hz)	19.80
27	0.81 (3H,d,J=5Hz)	19.05
28		23.12
29	0.86 (3H,t,J=5Hz)	11.96

Extraction and isolation

The chloroform extract (1.56 g) was subjected to silica gel column chromatography and eluted with a gradient of hexane: ethyl acetate (90:10 to 100% ethyl acetate) to afford 6 fractions. Fraction 2 was loaded on TLC plates and two major compounds of this fraction were isolated and purified.

Spectroscopic data

12a-Hydroxystigmast-4-en-3-one (1): yellow gum; molecular formula: $C_{29}H_{48}O_2$, ^1H, ^{13}C NMR (500 MHz, in $CDCL_3$); see Table 1.

Methyl oleate (Oleic acid methyl ester) (2): yellow oily liquid; molecular formula: $C_{19}H_{36}O_2$, ^1H NMR (500 MHz, CDCl3): 0.87 (3H, d, J = 5 Hz, H-18), 1.25 (2H,m,H- (4-7,12-15)), 1.61 (2H,s, H-3), 2.03 (2H, d, J = 6 Hz , H-8,11), 2.26 (2H, t, J = 6 Hz, H-2), 3.63 (3H, s, H (CH3O)), 5.33 (1H, bs, H-9,10); 13C NMR (500 MHz, $CDCl_3$): 129.76 (C-9), 129.63 (C-10), 173.85 (CO), 51.09 (CH3O), 34.15 (C-2), 31.25 (C-16), 28-29 (C-4-7, 12-15), 27.03 (C-8,11), 24.79 (C-3), 22.42 (C-17).

DPPH assay

The samples were assessed for their free radical scavenging activity using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method according to Brand Williams et al. (1995).(Brand-Williams et al., 1995) Different concentrations (100, 250, 500, and 100 μg/ml) of sample solutions (1 mL) in methanol and butylated hydroxytoluene (BHT) solutions and 1 ml of methanol were added to DPPH methanol solution (2 ml, 400 μg/mL). After 30 min, the absorbance was measured at 517 nm. Percentage of radical scavenging activity of samples was calculated according to the following equation: Inhibition% = [(A0–AS)/A0] × 100 where A0 is the absorbance of the control and AS is the absorbance of the sample. Half maximal inhibitory concentrations (IC50) were calculated from the plotted graph of scavenging activity versus the concentration of the extract, using linear regression analysis.(Kahkeshani et al., 2013)

RESULTS AND DISCUSSION

The 80% methanol extract from the rhizomes of *P. orientale* was partitioned between 80% methanol and hexane, chloroform, ethyl acetate, and n-butanol respectively.

DPPH free radical scavenging activity

Antioxidant activity of some *Polygonatum* species has been reported previously. Through this approach, we decided to evaluate the antioxidant property of *Polygonatum orientale*, grown in Iran. According to our results, comparison of the antioxidant activity of total methanol extract and four fractions showed low free radical scavenging activity of all samples except for ethyl acetate fraction. The inhibitory concentrations 50% (IC50) of ethyl acetate fraction and BHT were measured using the concentration-inhibition curve which were 315.02 μg/ml and 42.3 μg/ml, respectively.

Polygonatum odoratum is widely used as Chinese traditional food supplement exhibited strong antioxidant activity. Homoisoflavonoids isolated from the ethanol extract of *P. odoratum* showed potent antioxidant activities, among them, compounds with dehydroxylated B-rings exhibited stronger antioxidant activities (IC50 values at 3.8 ± 0.5, 4.9 ± 0.3 and 3.9 ± 0.4 μg/mL) than ascorbic acid (IC50 value at 5.3 ± 0.6 μg/mL).(Zhou et al., 2015) In a study by Wang et al. (2013) two C-methylated homoisoflavanones, 3-(4'-hydroxy-benzyl)-5,7-dihydroxy-6-methyl-8-methoxy-chroman-4-one (1) and 3-(4'-hydroxyl-benzyl)-5,7-dihydroxy-6-methyl-chroman-4-one (2), isolated from *P. odoratum* flavonoid extract. Based on that result, compound 1 showed significant antioxidant activity on DPPH radical with IC50 5.90 ± 0.150 μg/mL, almost twofold stronger than compound 2, IC50 11.64 ± 0.296 μg/mL.(Wang et al., 2013). Also, it was reported that polysaccharides from *P. odoratum* exhibited strong antioxidant activities, using DPPH radical scavenging activity assay. (Liu et al., 2015)

Horng et al (2014) evaluated the antioxidant activity of 20% ethanolic extract of *P. alte-lobatum* with a DPPH free-radical scavenging assay. The activity of DPPH inhibition was dose-dependent with an IC50 value of 9μg/mL.(Horng et al., 2014) In addition, The antioxidant effect of two compounds, diosgenin and santonin, isolated from *Polygonatum* verticillatum rhizomes showed strong free radical scavenging effect. IC50 values for both diosgenin and santonin were 65.80 and 50.03 μg/ml, respectively. (Khan et al., 2016)

Purification and isolation of compounds

The chloroform fraction was separated by silica gel column chromatography, as well as preparative TLC, to afford two major compounds. 12-Hydroxystigmast 4-en 3-one (1) (Figure 1) and methyl oleate (2) (Figure 2) were identified by 1H and 13CNMR analysis and comparison with literature data. (Chowdhury et al., 2003)

Phytosteroids are comprised of cyclopentane per hydro phenanthrene ring system, similar to cholesterol, distributed in plants and varies only in

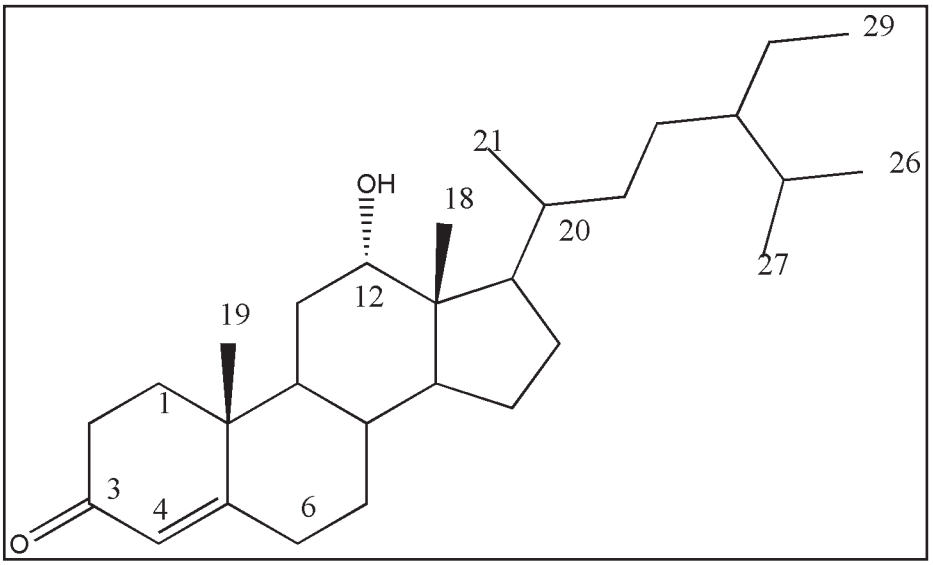

Figure1: Chemical structure of 12α-Hydroxystigmast-4-en-3-one

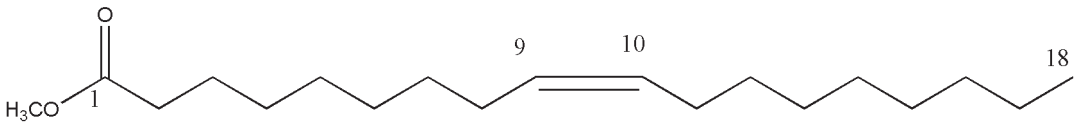

Figure2: Chemical structure of methyl oleate

carbon side chains and/or presence or absence of a double bond. Sterols, steroidal saponins, cardioactive glycosides, bile acids, corticosteroids, and mammalian sex hormones are important natural products with steroidal structure. (Singh et al., 2013)

12-hydroxystigmast-4-en-3-one with a hydroxyl steroidal ketone structure first was reported from Toona ciliate (Meliaceae). This compound exhibited a cytotoxic effect in a brine shrimp lethality bioassay with LC50 of 9.9mg/ml and it also showed important antitumor activity with Ti50valueof 14.1mg/ml in a potato disc bioassay. (Chowdhury et al., 2003)

Several steroidal saponins were reported from different species of *Polygonatum*, for example, seven steroidal saponins including two furostanol glycosides from the rhizomes of *P. prattii* (Zhang

et al., 2016), steroidal sapogenins from the rhizomes of *P. polyanthemum* and *P. glaberrimum.* (Gvazava and Kikoladze, 2014; Gvazava and Kikoladze, 2012) Six new spirostane glycosides, one furostanol glycoside, one cholestane glycoside, and one steroidal sapogenin, were isolated from a 90% MeOH extract of the fibrous roots of *P. odoratum*. (Zhang et al., 2014) Furthermore, phytochemical investigation of the rhizomes of *P. odoratum* resulted in the isolation of three cholestane-type steroidal glycosides as well as two spirostane-type steroidal saponins and three steroidal glycosides.(Bai et al., 2014) Gvazava and Skhirtladze (2016) reported a 25S-spirostane spiroketal steroidal glycoside from the rhizome of *P. verticillatum*. (Gvazava and Skhirtladze, 2016)

Previously, two steroidal compounds sceptrumgenin 3-O-β-d-lycotetraoside, akyrogenin 3-O-β-d-glucopyranosyl-(1→3)-[β-d-glucopyranosyl-(1→2)]-β-d-glucopyranosyl-(1→4)-β-d-galactopyranoside (spiroakyroside) were isolated from the fresh rhizomes of *P. orientale*. (YeşİIada and Houghton, 1991)

CONCLUSION

The total methanol extract and some fractions of the rhizomes of *Polygonatum orientale* Desf., collected from the north of Iran, were investigated to find possible antioxidant effects using DPPH assay. According to the results, all extracts had not shown a significant radical scavenging effect in comparison to BHT. Phytochemical studies of this species have been conducted in Iran for the first time. A steroidal compound, 12-Hydroxystigmast- 4-en 3-one, and a fatty acid ester, methyl oleate were isolated from chloroform fraction of the methanol extract. These major compounds have been reported for the first time from *Polygonatum* species. In order to find more compounds from this medicinal plant, further phytochemical studies are recommended.

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CONFLICT OF INTEREST (COI) DISCLOSURE:

The Authors declare that there is not any conflict of interest.

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