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Article

Isolation and characterization of homoisoflavonoids from *Scilla persica* HAUSSKN

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Medicinal plants have many traditional claims including the treatment of ailments of infectious origin. In the evaluation of traditional claims, scientific research is extremely important. In this study, five homoisoflavonoids named 3-(4'-hydroxybenzylidene)-5,7-dihydroxy-6-methoxychroman-4-one(Autumnalin),3-(4'-hydroxybenzyl)-5,7-dihydroxy-6-methoxychroman-4-one (3,9-dihydro-autumnalin), 3-(3',4'-dihydroxybenzyl)-5,8-dihydroxy-7-methoxychroman-4-one,3-(3',4'-dihydroxybenzylidene)-5,8-dihydroxy-7-methoxychroman-4-one and 3-(3',4'-dihydroxybenzylidene)-5,7-dihydroxy-6-methoxychroman-4-one, were isolated from the bulbs of the plant *Scilla persica* HAUSSKN. Their structures were established on the basis of extensive spectroscopic analyses such as NMR, MS, IR and UV.

Uniterms: *Scilla persica* HAUSSKN/phytochemistry. Homoisoflavonoids/isolation. Homoisoflavonoids/isolation. Medicinal plants.

Plantas medicinais apresentam muitas atribuições tradicionais, incluindo o tratamento de doenças de origem infecciosa. A pesquisa científica é extremamente importante na avaliação dos usos tradicionais. Neste estudo, cinco homoisoflavonóides: 3-(4'-hidroxibenzilideno)-5,7-diidroxi-6-metoxicroman-4-ona(autumnalina), 3-(4'-hidroxibenzil)-5,7-diidroxi-6- metoxicroman-4-ona (3,9-diidro-autumnalina), 3-(3',4'-diidroxibenzil)-5,8-diidroxi-7- metoxicroman-4-ona, 3-(3',4'-diidroxibenzilideno)-5,8-diidroxi-7- metoxicroman-4-ona e 3-(3',4'-diidroxibenzilideno)-5,7-diidroxi-6- metoxicroman-4-ona foram isolados dos bulbos da planta *Scilla persica* HAUSSKN. Suas estruturas foram estabelecidas com base na extensa análise espectroscópica, como RMN, EM, IV e UV.

Unitermos: *Scilla persica* HAUSSKN/fitoquímica. Homoisoflavonóides/separação. Homoisoflavonóides/isolamento. Plantas medicinais.

INTRODUCTION

Over the years, natural medicines, especially medicinal herbs, were considered for the treatment of a variety of ailments. The ingredients contained in these plants are used in the pharmaceutical industry because of their disinfecting properties (Amin, 1991; Volak, Stodola, 1995; Yasuda *et al.*, 2013). *Scilla persica* HAUSSKN is a perennial plant that belongs to the Liliaceae family. The bulb of this plant is used as a foodstuff, a traditional medicine that increases blood circulation, as an anti-inflammatory agent as well as an analgesic (Crouch, Bangani, Mulholland, 1999). This plant has a small white bracteole that includes one or two high-centered flowers (10-13 flowers), with two to four leaf sheathes. Its petals are bluish in color, standing erect with inflorescence or a cluster of flowers. The axis of their flowers is usually single and rarely dual. In Iran, the habitat of this plant exists in the West Azerbaijan, Khorram Abad, Alvand, Hamedan, Qasr Shirin, Sanandaj and the Mountains of Rzab (Mobin, 1975; Rahimi, Aghaalinejad, Arslana, 2012).

According to phytochemical studies performed on the *Scilla* species, the chemical compounds reported contain alkaloids, cardiac glycosides, stilbenes (Bangani, Crouch, Mulholland, 1999; Crouch, Bangani, Mulholland, 1999; Kato *et al.*, 2007; Nishida *et al.*,

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2008; Ono *et al.*, 2013; Silayo, Ngadjui, Abegaz, 1999) and specially homoisoflavanones (Bangani, Crouch, Mulholland, 1999; Famuyiwa *et al.*, 2012; Ghoran *et al.*, 2014; Heller, Tamm, 1981; Kouno, Komori, Kawasaki, 1973; Shim *et al.*, 2004). Homoisoflavonoids were reported to be responsible for the biomedical activities of these plants, such as antibacterial, antifungal, anti-allergic, antioxidant, cytotoxic and anti-angiogenic activities. They also inhibit *in-vitro* the growth and sporogenesis of several microorganisms (Heller, Tamm, 1981; Kouno, Komori, Kawasaki, 1973; Mighani, Ebrahimi, 2014; Nishida *et al.*, 2013; Shim *et al.*, 2004). Stilbenes of scilla bulbs has been used in the treatment of asthma, coughs, bronchitis, heart disease (adjusted HR), rheumatism, diuretic, expectorant (Gupta, Raina, 2001), internal tumors, dilate blood vessels, anti-allergic and anti-mutagenic (Dias, Graça, Gonçalves, 2000; Yasuda *et al.*, 2013). In traditional Iranian medicine, the bulbs of scilla herb were used as the antidote, activator of blood circulation and also the treatment of eczema. It can also treat swelling and abscesses (Winnicka, Bielawski, Bielawski, 2006). Studies have shown that the bulb of *Scilla natalensis* has anti-cancer properties, antibacterial properties; it kills worms (intestinal parasite) and also prevents the formation of cancerous agents. Also sodden these plants are used for the treatment of abscesses (Chinthala, Chinde, 2014; Koorbanally *et al.*, 2007). There are reports that bulbs of *Scilla* are used for gynecological treatment of menstrual pains and to facilitate delivery in childbirth (Gerstner, 1941; Hutchings, 1989; 1996). Decoctions are also taken as enemas for female fertility and to enhance male potency and libido (Gerstner, 1941).

In a previous paper (Ghoran *et al.*, 2014), we reported the isolation and structural

elucidation of a new homoisoflavonoid from the CHCl3 extract of fresh bulbs of *S. persica* along with MTT cytotoxicity assay on AGS and WEHI-164 cancerous cell lines. As part of an ongoing study of this plant, we described the isolation and structural characterization of five other homoisoflavonoids from the CHCl3 extract from the bulbs of the plant *Scilla persica* HAUSSKN. Their structures were identified on the basis of extensive spectroscopic analyses such as NMR, MS, IR and UV.

MATERIAL AND METHODS

General experimental procedures

An Electrothermal Melting Point Apparatus was used to determine precise melting points. UV and IR spectra were recorded by a Shimadzu UV-PC 2501 and Perkin- Elmer spectrophotometer, respectively. NMR spectra were also obtained in either CD3OD or DMSO-d6 using a Bruker AV-500 instrument with TMS as the internal standard. EIMS were also recorded with a VG-Autospec-3000 spectrometer. HREIMS were recorded with a Thermo Fisher Finnegan MAT 95 XP instrument. Column chromatography (CC) was carried out using silica gel (70–230 and 230–400 mesh; E-Merck, Darmstadt, Germany). Aluminum sheets precoated with silica gel 60 F 254 (10×10 cm, 0.2 mm thick; E-Merck) were applied for TLC to check the purity of the compounds and were observed under UV light (254 and 365 nm) followed by anisaldehyde as the spray reagent and then it was heated.

Plant material

The plant used for the present study (*S. persica*) was collected and identified in March 2011 from the village of Valliv (Sardasht, West Azerbaijan Province, Iran) at an altitude of 1700-1800 m. A voucher specimen (6334) is deposited in the Herbarium of Agricultural Research Center and Natural Resources of Sari, Iran.

Phytochemical screening (qualification tests)

The tests were performed to find the presence of the bioactive chemical constituents, such as alkaloids, anthraquinones, flavonoids, glycosides, reducing sugar, saponins, tannins, terpenoids and steroids (Table I) (Iyengar, 1981; Savithramma, Rao, Suhrulatha, 2011; Siddiqui, Ali, 1997).

Extraction and isolation

The bulbs of *S. persica* (400 g) were crashed and extracted with EtOAc (3×2.5 L) at room temperature for 24 h. The combined extracts were concentrated under reduced pressure. Then the crude extract of the bulbs were subjected to column chromatography and eluted with n-hexane:EtOAc:MeOH, in the order of increasing polarity (70:30:0, 60:40:0, 50:50:0, 40:60:0, 20:80:0, 0:100:0, 0:80:20, 0:70:30 0:60:40 and 0:50:50 (v/v), respectively) to separate different fractions. Fraction **A** (190 mg) was further separated by Sephadex LH-20 column chromatography and eluted with MeOH to give **1** (7 mg) (SILAYO *et al.*, 1999) and **2** (18 mg) (Silayo, Ngadjui, Abegaz, 1999). Repeated column chromatography of fraction **B** (210 mg) using a silica gel column and isocratic elution with CHCl3: MeOH

8:2) afforded **3** (30 mg) (Adinolfi *et al.*, 1989). Moreover fraction **C** (150 mg) gave two compounds **4** (15 mg) and **5** (23 mg) (Mašterová *et al.*, 1991) upon elution with MeOH on a Sephadex LH-20 column.

RESULTS AND DISCUSSION

Table I shows the results of phytochemical screening (qualification tests) on *S. persica* HAUSSKN. These results indicated that this plant contains flavonoids, glycosides, reducing sugars, saponins and tannins especially catecholic tannins and alkaloids. The bulb of this plant is void of terpenoids and steroids.

The spectroscopic and other physical data of the isolated compounds **1-5** were indicated below and details of ^{1}H and ^{13}C-NMR spectroscopic data were also shown in Table II.

*3-(4′-Hydroxybenzylidene)-5, 7-dihydroxy-6-methoxychroman-4-one (**1**)*

Yellowish powder with $C_{17}H_{14}O_6$ molecular formula; mp 242-245 °C; IR (KBr) V_{max} (cm^{-1}) 3423 (OH), 1647 (C=O), 1510 (C=C Ar), 1458 (-CH3), 1328, 1173 (C-O); UV (MeOH) λ_{max} (log ε) 197 (4.80), 359 (4.25) nm; EIMS m/z (rel. int.) 314 $[M]^+$ (89), 299 (25), 183 (15), 167 (55), 131 (24); ^{1}H and ^{13}C-NMR spectroscopic data are shown in Table II.

*3-(4′-Hydroxybenzyl)-5, 7-dihydroxy-6-methoxychroman-4-one (**2**)*

Yellowish plates from $CHCl_3$ with $C_{17}H_{16}O_6$ molecular formula; mp 198-200 ºC; IR (KBr) V_{max} (cm^{-1}) 3356 (OH), 1638 (C=O), 1609 (C=C Ar.), 1451 (-CH3), 1265, 1153 (C-O); UV (MeOH) λ_{max} (log ε) 211 (4.30), 293 (4.421) 320 (4.98) nm; EIMS m/z (rel. int.) 316 $[M]^+$ (100), 301 (37), 283 (15), 265 (12), 210 (85), 109 (77); ^{1}H and ^{13}C-NMR spectroscopic data are shown in Table II.

*3-(3′, 4′-dihydroxybenzyl)-5, 8-dihydroxy-7-methoxychroman-4-one (**3**)*

Yellow-Brown plates from MeOH with $C_{17}H_{16}O_7$ molecular formula; mp 138-141 ºC; EIMS (70 eV) m/z (rel. int.) 332.0899 ($[M]^+$; calc for $C_{17}H_{16}O_7$ 332.0896) (95), 210 (100), 195 (15), 183 (50), 167 (35), 149 (20), 123 (95); ^{1}H and ^{13}C-NMR spectroscopic data are shown in Table II.

*3-(3′, 4′-dihydroxybenzylidene)-5, 8-dihydroxy-7-methoxychroman-4-one (**4**)*

Orange powder from MeOH with $C_{17}H_{14}O_7$ molecular formula; mp: 233-237 ºC; IR (KBr) V_{max} (cm^{-1}) 3501 (OH), 3348 (-CH Ar. and vin. Stretching), 1638 (C=O), 1568 (C=C Ar.), 1521 (C=C Ar.), 1477 (C=C Ar.), 1436 (-CH3), 1264, 1089 (-C-O); UV (MeOH) λ_{max} (log ε) 269 (4.19), 340 (shoulder) (4.30), 367 (4.29) nm; EIMS

TABLE I - Results of phytochemical screening tests on *Scilla persica* HAUSSKN

Type of Secondary Metabolites	Methods	Observations	Result
Alkaloids	Mayer's Test	A white yellowish precipitate	No Yes
	Dragendorff's Test	A turbidity or orange precipitate	
Anthraquinones	Bourne-Tragr Reaction	Formation of rose-pink color	No
Flavonoids	Shinoda Test	A red solution	Yes Yes
	Alkaline Reagent Test	A yellowish orange solution	
Glycosides	Keller-Killiani Test	A reddish brown solution	Yes
Reducing Sugars	Fehling's Test	A green solution with fehling A A brown solution with fehling B	Yes Yes
Terpenoids	Libermann-Buchard Test	A red-violet solution	No
Tannins	Ferric Chloride Test	A blue solution for gallic tannins A green-black solution for catecholic tannins	No Yes
Saponins	Froth Test	Appearance of creamy miss of small bubbles	Yes
Steroids	Libermann-Buchard Test	A green bluish solution	No

(70 eV) m/z (rel. int.) 330.0909 $[M]^+$ $C_{17}H_{14}O_7$ (100), 315 (25), 183 (38), 169 (10), 168 (12), 167 (48), 149 (18), 148 (25), 147 (12); ^{1}H and ^{13}C-NMR spectroscopic data are shown in Table II.

*3-(3′, 4′-dihydroxybenzylidene)-5, 7-dihydroxy-6-methoxychroman-4-one (**5**)*

Orange crystals from MeOH with $C_{17}H_{14}O_7$ molecular formula; mp 237-240ºC; IR (KBr) V_{max} (cm^{-1}) 3497 (OH), 3343 (-CH Ar. and vin. Stretching), 1642 (C=O), 1531 (C=C Ar.),1436 (-CH3), 1260, 1050 (-C-O); UV (MeOH) λ_{max} (log ε) 264 (4.02), 384 (4.35) nm; EIMS (70 eV) m/z (rel. int.) 330.0910 $[M]^+$ $C_{17}H_{14}O_7$ (100), 315 (25), 183 (38), 169 (10), 168 (12), 167 (48), 149 (18), 148 (25), 147 (12); ^{1}H and ^{13}C-NMR spectroscopic data are shown in Table II.

The isolated compounds **1-5** in this plant showed the presence of a 5-OH group confirmed by ^{1}H-NMR spectroscopy (δ_{OH} > 11.5) in Table II.

As it can be seen in Figures 1, 4 and 5 compounds have the same mass and due to the position of CH3 and –OH substituents on ring A are different.

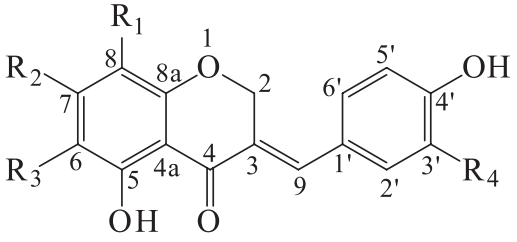
Compound 1: The spectroscopic and other physical data of compound **1** were found to be similar to those reported for autumnalin isolated from the bulbs of *Eucomis autumnalis, Colchicum doerfleri* and *Scilla nervosa* (Buckingham, 1993; Sidwell, Tamm, 1970; Silayo, Ngadjui, Abegaz, 1999).

Compound 2: Compound **2** exhibited spectral data very similar to those reported for eucomnalin (3,9-dihydro-autumnalin), a compound isolated previously from *Eucomis autumnalis* (Tamm, 1972) and it was recently reported from *S. nervosa* Silayo, Ngadjui, Abegaz, 1999). The ^{1}H-NMR of compound **2** indicated a sharp singlet at δ_H 5.93 ppm and it was assigned to H-8 in compound **2**. The ^{13}C-NMR data is in complete agreement with the given structure (Agrawal, 1989). The location of the only methoxy signal observed in **2** was deduced to be at C-6 on the basis of the downfield resonance position of the methoxy carbon at δ_C 60.47 ppm and the presence of a C-5 OH group.

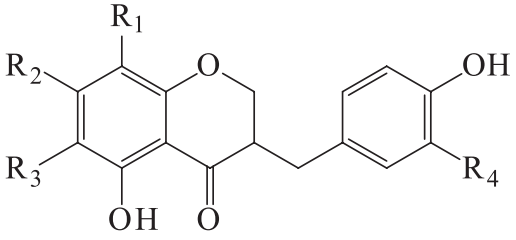
Compound 3: The B ring of 3-benzyl-4-chromanone **3** has two oxygenated functions. In fact, the base peak in the mass spectra of compound **3** was due to dihydroxytropylium fragment (m/z 123). The peak (m/z 183) in the mass spectrum of **3** also indicated that the A ring has two hydroxyl and one methoxy group. The NMR signals of the hydroxyl proton, methoxy and methine protons in the A ring at δ_H 11.83, 3.82 and 6.19 ppm, respectively, revealed that one hydroxyl group located at C-5 while the methoxy group is at C-7. The chemical shifts of the A ring carbons are very similar to those of **2**. Therefore the remaining hydroxyl group is linked at C-8.

Compound 4: The ^{1}H and ^{13}C-NMR spectral data of compound **4** are identical with those previously described (Adinolfi *et al.*, 1989; Mašterová *et al.*, 1991).

Compound 5: Substitution of a methoxy group at C-6 in compound **5** was confirmed by losing methyl group giving a characteristic $[M-15]^+$ ion like those in 6-OMe flavones (Bowie, Cameron, 1966). This cleavage is followed in ring A by a gradual elimination of CO (m/z 287) and H_2O ('ortho effect', m/z 269). The latter experiment also led to the assignment of carbon



	R_1	R_2	R_3	R_4
1	H	OH	OMe	H
4	OH	OMe	H	OH
5	H	OH	OMe	OH



	R_1	R_2	R_3	R_4
2	H	OH	OMe	H
3	OH	OMe	H	OH

FIGURE 1 - Chemical structures of the isolated homoisoflavonoids **1-5** from bulbs of the plant *Scilla persica* HAUSSKN

TABLE II - ^{1}H and ^{13}C-NMR chemical shifts (δ/ppm) of compounds 1-5 in CD_3OD[a] and DMSO-d_6[b] as the solvents (500 MHz for δ_H and 125 MHz for δ_C)

position	1[a]		2[b]		3[b]		4[b]		5[b]	
	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c
2a	5.28, d (1.5)	67.35	4.21, dd (11.5, 4.5)	69.37	4.28, dd (4.3, 11.3)	69.35	5.32, d (1.5)	67.20	5.33, d (1.6)	67.10
2b			4.03, dd (11.25, 8.5)		4.11, dd (3.9, 7.6)					
3		125.56	2.92 (m)	46.11	2.92 (m)	46.51		126.41		126.14
4		185.75		198.99		199.18		185.26		184.82
4a		102.48		101.76		102.18		102.40		101.90
5		159.23		155.80		157.40		156.73		156.06
6		126.80		128.52	6.19 (S)	93.03	6.21 (S)	92.89		129.28
7		159.40		159.88		156.44		157.24		159.47
8	5.92 (S)	94.73	5.93 (S)	95.16		126.81		125.20	5.90 (S)	94.82
8a		155.97		158.38		148.58		147.05		157.00
9a	7.73 (S)	137.21	2.99, dd (14, 5)	31.60	2.93, dd (5.7, 10.4)	31.85	7.62 (S)	137.34	7.65 (S)	137.8
9b			2.57, dd (13.8, 9.5)		2.54, dd (10.8, 13.5)					
1’		129.33		129.48		129.20		125.23		124.90
2’	7.21, d (8)	132.32	7.01, d (9.5)	130.42	6.62, d (2.1)	116.79	6.84 (S)	117.79	6.87 (S)	117.76
3’	6.88, d (9)	115.58	6.68, d (8.5)	115.71		145.62		145.45		145.20
4’		159.94		156.22		144.28		148.07		147.85
5’	6.88, d (9)	115.58	6.68, d (8.5)	115.71	6.66, d (8.1)	116.05	6.86, d (3.5)	115.92	6.89, d (8.2)	115.91
6’	7.21, d (8)	132.32	7.01, d (9.5)	130.42	6.48, dd (2.0, 8.05)	120.15	6.81, dd (2, 8.5)	123.64	6.79, dd (1.8, 8)	123.55
5-OH	12.9 (S)		12.22 (S)		11.83 (S)		12.48 (S)		12.8 (S)	
C6-OMe	3.81 (S)	59.95	3.65 (S)	60.47					3.68 (S)	59.83
C7-OMe					3.82 (S)	56.58	3.83 (S)	56.15		

resonances in this part of the molecule. These results are in good agreement with ^{13}C-NMR data of the 3, 9-dihydro derivative of **5** (Purushothaman *et al.*, 1982).

CONCLUSION

Scilla persica HAUSSKN is an edible plant that is used in traditional medicine to treat eczema and to relieve constipation, but, so far, no reports have been documented with evidence of its compounds. The results of phytochemical screening of this plant showed the presence of flavonoids, glycoside, tannin and saponin. The presence of flavonoids in this plant is probably responsible for the antibacterial effects. In this study, five homoisoflavonoids were isolated from bulbs of the plant *Scilla persica* HAUSSKN and their structures were identified using spectroscopic analyses such as NMR, MS, IR and UV. The spectroscopic and other physical data of isolated compounds were found to be similar to those reported for autumnalin isolated from the bulbs of *Eucomis autumnalis, Colchicum doerfleri* and *Scilla nervosa*.

CONFLICTS OF INTEREST

The authors report no conflicts of interest for the present study.

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