

Phytochemical and Chemotaxonomic Investigation of *Stelleropsis iranica*.

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Abstract: *Stelleropsis* genus belongs to Thymelaeaceae and has two species in Iran of which *S. iranica* Pobed. subsp. *Iranica* Bonge (aerial parts) were collected from the North-East of Iran and extracted with AcOEt and MeOH, consequently. The main phenolic compounds were isolated by different chromatography methods and the structures identified by spectroscopic methods (¹H-NMR, ¹³C-NMR, HMBC, HMQC, H-H COSY, EI-MS) as syringin (**1**), gengkwanin 5-O-β-D-primeveroside (Yuankanin) (**2**), syringaresinol (**3**), syringinoside (**4**), β-Sitosterol (**5**) and gengkwanin (**6**).

Key words: Thymelaeaceae, *Stelleropsis iranica*, flavonoid glycoside

INTRODUCTION

Stelleropsis Pobedimova genus belongs to Thymelaeaceae, which is the moderate size family of dicotyledons consisting of 67 genera and found throughout the tropical and temperate parts of the world (Borris *et al.*, 1988). *Stelleropsis* has two species in Iran of which, *S. iranica* Pobed. Subsp. *iranica* is restrictively growing in central and North- East of Iran (Akhyani, 1994). Literature reviews show that there is no report on phytochemical identification of the chemical constituents of *Stelleropsis* genus.

Experimental:

Plant Material:

Aerial parts of *S. iranica* Pobed. subsp. *Iranica* Bonge, at flowering stage, were collected from Firuzkuh (Tehran Province, the North-East of Iran), in July 2007. A voucher specimen (6682- TEH) was deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Plant specimen was identified by Dr. Gholamreza Amin (Tehran University of Medical Sciences).

Isolation Process:

The flowered aerial parts of *S. iranica* (500 g) were cut into small pieces and extracted with AcOEt and MeOH, consequently, at room temperature. The MeOH extract (37 g) was successively subjected to silica gel column chromatography with CHCl₃: MeOH (19:1, 9:1, 8:2, 1:1) and MeOH as eluent to give seven fractions (A-G). The fraction D (1.14 g) was chromatographed on sephadex LH₂₀ with MeOH to result D1-D4 sub fractions. Compounds **1** and **2** were obtained from D2 (342 mg) and D4 (15 mg) respectively, using sephadex LH₂₀ with MeOH. Fraction E (335 mg) was subjected to silica gel CC with Hexane: Acetone (9:1, 8:2, 0:1) to yield five parts (E1-E5). Compound **3** (15 mg) resulted from fraction E4 (77 mg) via CC with CHCl₃: AcOEt (8:2). Fraction G (8.4 g) was chromatographed on reverse-phase silica gel with aqueous methanol 20%, 60% and methanol, respectively, to give G1-G5. Purification of G3 on sephadex LH₂₀ was resulted in compound **4** (87 mg).

The AcOEt extract (10 g) was subjected to silica gel CC with hexane: CHCl₃ (6:4), CHCl₃: AcOEt (8:2) and AcOEt as eluent to give six fractions (Ac1-Ac6). The fraction Ac3 (315 mg) was submitted to sephadex LH₂₀ with MeOH to result Ac31-Ac33. Ac32 (35 mg) was subjected to silica gel CC with AcOEt: MeOH (1:1) to obtain compound **5** (21 mg). The fraction Ac4 (810 mg) was submitted to silica gel CC with Hexane: Acetone (8:2) and then sephadex LH₂₀ with AcOEt: MeOH (1:1) to give compound **6** (38 mg).

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General:

¹H- and ¹³C-NMR spectra were measured on a Bruker Avance 500 DRX (500 MHz for ¹H and 125 MHz for ¹³C) spectrometer with tetramethylsilane as an internal standard and chemical shifts are given in δ (ppm). MS data were recorded on Agilent Technology (HP) instrument with 5973 Network Mass Selective Detector (MS model). Silica gel 60F₂₅₄ pre-coated plates (Merck) were used for TLC. The spots were detected by spraying anisaldehyde-H₂SO₄ reagent followed by heating.

RESULTS AND DISCUSSION

The structures of the isolated compounds (**Fig 1**), from the aerial parts of *S. iranica*, were identified by a combination of spectroscopic methods and comparisons with the literature data as syringin (**1**) (Kamel, 2003), gengkwanin 5-O-β-D-primeveroside or Yuankanin (**2**) (Granados and Buruaga, 1980), syringaresinol (**3**) (Abe and Yamauchi, 1988), syringinoside (**4**) (Nisar *et al.*, 1999), β-Sitosterol (**5**) (Goad and Akihisa, 1997) and gengkwanin (Markham *et al.*, 1978) (**6**). The results of ¹H and ¹³C-NMR spectra of syringin and gengkwanin 5-O-β-D-primeveroside summarized in **Table 1** and **2** respectively.

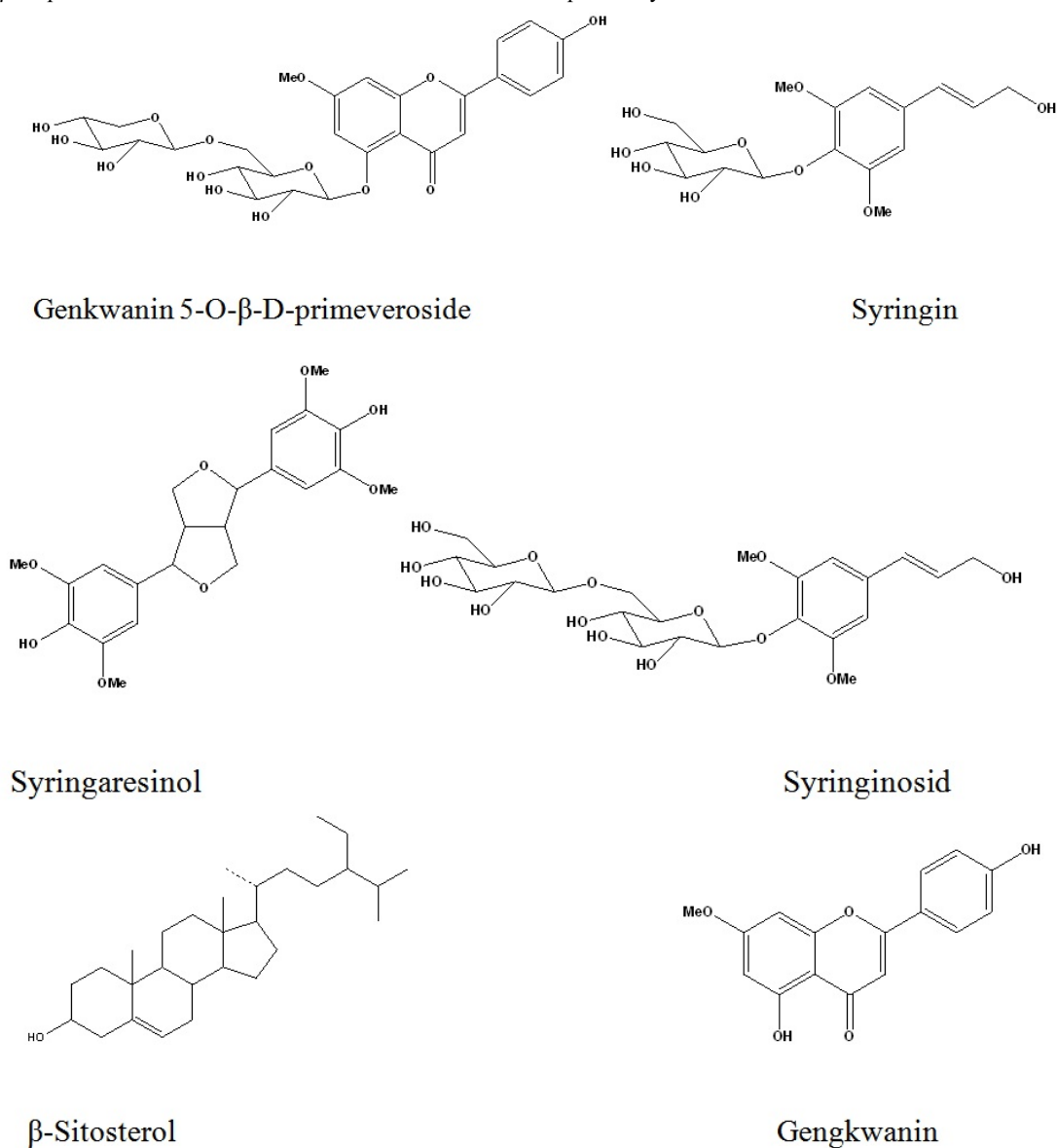


Fig. 1: Structures of the isolated compounds from *Stelleropsis iranica*.

Table 1: NMR spectra of syringin in methanol-*d*₄.

No.	¹³ C-NMR	¹ H-NMR
1	64.0	4.21 (<i>dd</i> , <i>J</i> = 5.6, 1.2 Hz, 2H)
2	130.5	6.35 (<i>dt</i> , <i>J</i> = 15.8, 5.6 Hz, 1H)
3	131.7	6.59 (<i>d</i> , <i>J</i> = 10.9 Hz, 1H)
1'	136.3	
2'	105.9	6.76 (<i>s</i> , 1H)
3'	154.7	
4'	135.7	
5'	154.7	
6'	105.9	6.76 (<i>s</i> , 1H)
OMe-3"	57.4	3.87 (<i>s</i> , 3H)
5"	57.4	3.87 (<i>s</i> , 3H)
Glc-1"	105.8	4.85 (<i>d</i> , <i>J</i> = 7.5 Hz, 1H)
2"	76.0	3.33 (<i>m</i> , 1H)
3"	78.1	3.43 (<i>m</i> , 1H)
4"	71.6	3.50 (<i>m</i> , 1H)
5"	78.6	3.23 (<i>m</i> , 1H)
6"	63.0	3.69 (<i>dd</i> , <i>J</i> = 12.0, 5.2 Hz, 1H) 3.81 (<i>dd</i> , <i>J</i> = 12.0, 2.4 Hz, 1H)

Table 2: NMR spectra of Genkwanin 5- primeveroside in DMSO-*d*₆.

No.	¹³ C-NMR	¹ H-NMR
2	161.1	
3	106.6	6.76 (<i>s</i> , 1H)
4	177.1	
5	158.1	
6	102.9	6.87 (<i>bs</i> , 1H)
7	163.8	
8	96.7	7.06 (<i>bs</i> , 1H)
9	158.6	
10	109.3	
1'	122.9	
2'	128.2	8.04 (<i>d</i> , <i>J</i> = 8.8 Hz, 1H)
3'	114.6	7.10 (<i>d</i> , <i>J</i> = 9.0 Hz, 1H)
4'	162.2	
5'	114.6	7.10 (<i>d</i> , <i>J</i> = 9.0 Hz, 1H)
6'	128.2	8.04 (<i>d</i> , <i>J</i> = 8.8 Hz, 1H)
OMe-7	56.2	3.89 (<i>s</i> , 3H)
Glc-1	103.8	4.79 (<i>d</i> , <i>J</i> = 7.5 Hz, 1H)
2	73.4	
3	76.6	
4	69.6	
5	75.7	3.66 (<i>m</i> , 1H)
6	68.7	3.97 (<i>bd</i> , <i>J</i> = 10.7 Hz, 1H)
Xyl-1	104.2	4.18 (<i>d</i> , <i>J</i> = 7.4 Hz, 1H)
2	73.4	
3	76.0	
4	69.8	
5	65.7	

The present study reports two phenyl propanoid glycoside (**1**, **4**), one flavonoid glycoside (**2**), a lignan (**3**), a sterol (**5**) and a flavone (**6**). This is the first report of those mentioned components in *S. iranica*, which is restrictively growing in Iran. Syringin (**1**), which is known to inhibit cAMP-PDE (Deliorman *et al.*, 1999) and possess immunomodulatory activity (Kim *et al.*, 2001), is mainly distributed within the Thymelaeaceae family. Syringin (**1**) and syringinoside (**4**) had been isolated from *Wikstroemia sikokiana* (Niwa *et al.*, 1988), *Daphne arisanensis* (Niwa *et al.*, 1991), *D. oleoides* (Ullah *et al.*, 1999), *D. tangutica* (Zhang *et al.*, 2007) and *Edgeworthia chrysantha* (Yan *et al.*, 2004). Syringinoside, as a disaccharide, seems to be included in the plant as stored form of coniferyl alcohol, which is susceptible to oxidation and is regarded as precursor of lignans or lignin.

Yuankanin (**2**) was isolated previously from various species of Thymelaeaceae, *D. gnidium* (Cottiglia *et al.*, 2001), *D. genkwa* (Park *et al.*, 2006), *D. giraldii* (Zhang *et al.*, 2008), *D. odora* (Zhang *et al.*, 2005), *Gnidia involucre* (Ferrari *et al.*, 2000), *Struthiola argentina* (Ayers *et al.*, 2008), *Wikstroemia indica* (Geng *et al.*, 2006) and *Ovidia pillo-pillo* (Nunez-Alarcon, 1973). More recently, it has been isolated from *Aquillaria sinensis* (Thymelaeaceae) as a laxative agent of agarwood extracts (Hara *et al.*, 2008). Syringaresinol (**3**) has also been reported to exhibit a broad range of biological activities, including antifungal, anti-inflammatory, anti-

malarial activities, inhibition of cAMP phosphodiesterase, antioxidant and cytotoxic activity (Yan *et al.*, 2004). This furofuran-type lignan was previously reported from *Dirca occidentalis* (Badawi *et al.*, 2006) and *D. genkwa* (Park *et al.*, 2008).

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