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Iridoids from Gardenia jasminoides Ellis

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Abstract: Three new iridoids, Gardenal-I (**1**), Gardenal-II (**2**) and Gardenal-III (**3**), together with nine known iridoid glycosides; geniposide **(4),** 6-*β*-hydroxy geniposide **(5),** 6-*α*-hydroxy geniposide **(6),** 6-*α*-methoxy geniposide **(7),** feretoside **(8),** genipin-1-*β*-gentiobioside **(9),** shanzhiside **(10),** lamalbidic acid **(11)** and picrocrocinic acid **(12)** were isolated from EtOH extract of *G. gasminoides*. The structures of the new and known compounds were elucidated on the basis of 1D and 2D-NMR spectroscopic techniques and available literature survey. The isolated compounds (**1-12**) were tested for antimicrobial and antiprotozoal activities.

Key words: Gardenia jasminoides**,** iridiod, iridiod glycosides, antimicrobial and antiprotozoal activities.

1. Introduction

The dried ripe fruit of *Gardenia jasminoides* Ellis (Family: Rubaceae) is a traditional Chinese medicine used as a diuretic, antipyretic, antihepatitis, anti-inflammatory agent[1]. Additionally, it is an externally used it can also act as antithrombotic[2], neuritogenic and can be used for treating ulcers of the skin and sprains[3-6]. However, there are few chemical and pharmacological studies related to *G. jasminoides* for treating sprain jaundice, acute conjunctivitis, epistaxis, hematemesis, hematuria, pyogenic infections[2] . *Gardenia fruit* extract is also used in traditional centrated pharmaceutical herbal products and the Chinese medicine to treat irritability in febrile dis- decoction herbal products have been widely adopted for clinical use in Taiwan, Japan, China, Korea and other Asian countries, and even in certain European. Several classes of compounds as e.g., iridoids, iridoid glycosides, flavonoids, monoterpenes, triterpenoids and other compounds are found in *G. jasminoides*[7]. Herein, we report the isolation of three new compounds along with nine known compounds were isolated from *G. jasminoides*. All isolated compounds were confirmed through 1D, 2D-NMR data and available literature.

2. Experimental:

2.1. General experimental procedures

^{1}H- (500 MHz), ^{13}C- (100 MHz) and 2D-NMR spectra were recorded using the residual solvent signal as internal standard on a Varian AS 500 spectrometer. IR spectra were measured on a Bruker Tensor 27 FTIR spectrometer. UV spectra were obtained on a Varian Cary 50 Bio UV-visible spectrophotometer. Optical rotations were obtained at the sodium D line at ambient temperature on a Rudolph Research Analytical Autopol IV automatic polarimeter. HRESIMS were obtained on an Agilent Series 1100 SL mass spectrometer. TLC was carried out on aluminum-backed plates precoated with silica gel F254 (20 × 20 cm, 200 µm, 60 Å, Merck). Visualization was accomplished by spraying with *p*-anisaldehyde [0.5 mL in glacial acetic acid (50 mL) and sulfuric acid (97%, 1 mL)] spray reagent followed by heating. Flash silica gel (60-120 µm, 60 Å, SiliCycle), SiliaBond C18 silica gel (40-63 µm, 60 Å, 17% carbon loading, SiliCycle) and Sephadex

LH-20 (25-100 µm, lipophilic, Sigma-Aldrich) were used for column chromatography.

2.2. Plant material

G. jasminoides fruits were obtained from the Nalgonda region, Mahatma Gandhi University, Andhra Pradesh, India.

2.3. Extraction and isolation

Powdered fruit of *G. jasminoides* (2.0 kg) were extracted through sonication in EtOH/H_2O (9:1, 3 x 3 L x 4 h) at room temperature. The combined extracts were filtered and the solvent evaporated to afford a brown powder (100 g). The powder was partitioned between H_2O and EtOAc (1:1, 3x500 ml), followed by vacuum liquid chromatography of the EtOAc fraction (35 g) over C18 silica (200 g) [H_2O (100%), H_2O/MeOH (2:8, 4:6, 6:4, 8:2), MeOH (100%)] yielding six fractions (F1-F6). Fractions F1 [H_2O (100%)] and F6 [MeOH (100%)] primarily contained sugars, aliphatic acids and esters, respectively, based on GC-MS and TLC analysis, and were not further investigated. Fractions F2 (1.8 g) and F3 (11.2 g) were combined owing to similar TLC profiles, followed by fractionation over silica (250 g) [$CHCl_3$/MeOH (8:2)] to provide six fractions [F2/3(1)-F2/3(6)]. Fractions F2/3(2) (2.50 g), F2/3(4) (1.95 g) and F2/3(6) (4.50 g) were further purified by silica chromatography [$CHCl_3$/MeOH (8:2)] to provide **1** (180.6 mg) and **2** (72.2 mg), respectively. Fraction F4 (6.0 g) was fractionated over Sephadex LH-20 (100 g) [MeOH (100%)], yielding F4(1)-F4(20). Subsequent fractionation of F4(1) (200.0 mg) by Sephadex LH-20 (100 g) [MeOH (100%)] and final purification over C18 silica (250 g) [MeCN/H_2O (4:6)] provided **3** (15.0 mg), and F4(20) (1.5 g) by C18 silica (250 g) [MeCN/H_2O (3:7)] provided **4** (36.0 mg), **5** (14.2 mg), **6** (12.8 mg) and 7 (27.8 mg). Fraction F5 (6.8 g) was fractionated by C18 silica (250 g) [MeCN/H_2O (1:1)], yielding F5(1), (1.64 g), F5(2) (0.3 g) and F5(3) (2.18 g), which gave **8** (19.0 mg) and **9** (4.0 mg), **10** (5.0 mg) and **11** (32.3 mg), and **12** (18.0 mg), respectively, after final purification over C18 silica [MeCN/H_2O (1:1)].

2.4.Gardenal-I: (**1**) White amorphous powder, $[\alpha]_D^{25}$ +3.0 (c 0.3, $CHCl_3$); UV (MeOH) max nm (log e): 239 (3.19); IR (KBr) max cm^{-1}: 3360, 2920, 1700, 1640, 1440, 1300, 1080; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 1; HRIMS (*m/z*) 210.1212 [M+H]+ (calcd. for $C_{11}H_{14}O_4$, 210.1195). C,H & N Analysis: C: 62.85; H: 6.71 & O: 30.44.

2.5.Gardenal II: (**2**) White amorphous powder, $[\alpha]_D^{25}$ -12.0 (c 0.3, $CHCl_3$); UV (MeOH) max nm (log e): 239 (3.19); IR (KBr) max cm^{-1}: 3360, 2920, 1700, 1640, 1440, 1300, 1080; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 1; HRIMS (*m/z*) 210.1712 [M+H]+ (calcd. for $C_{11}H_{14}O_4$, 210.1695). C,H & N Analysis: C: 62.85; H: 6.71 & O: 30.44.

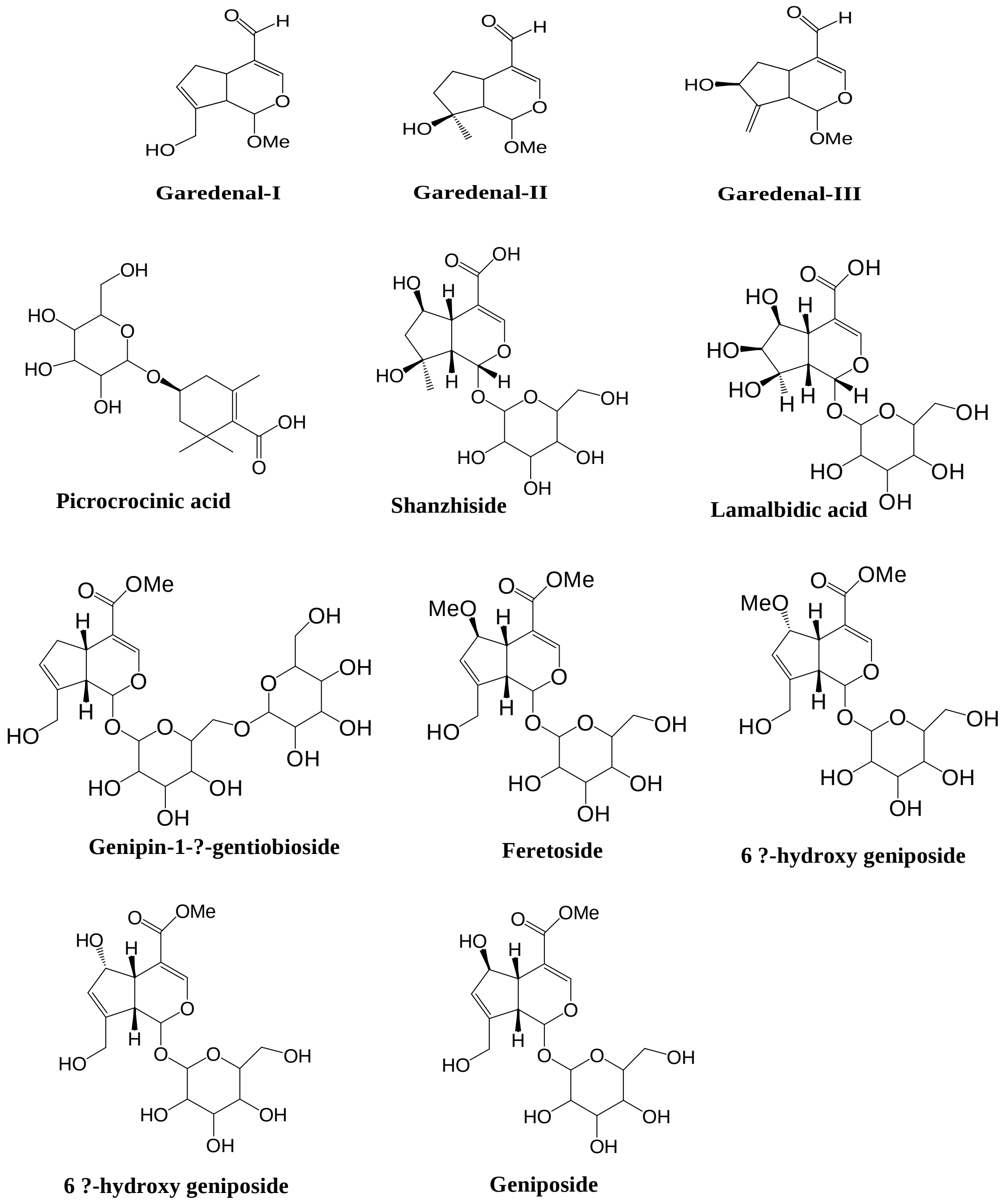
2.6.Gardenal III: (**3**) White amorphous powder, $[\alpha]_D^{25}$ +9.0 (c 0.3, $CHCl_3$); UV (MeOH) max nm (log e): 239 (3.19); IR (KBr) max cm^{-1}: 3360, 2920, 1700, 1640, 1440, 1300, 1080; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data, see Table 1; HRIMS (*m/z*) 212.1812 [M+H]+ (calcd. for $C_{11}H_{16}O_4$, 212.1795). C, H, N Analysis: C: 62.25; H: 7.60 & O: 30.15.

Table 1: ^1H- and ^{13}C-NMR data for 1-3 (δ_C and δ_H values in ppm).

Carbon	1		2		3	
	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C
1	5.38	110.5	5.45	107	5.46	110.6
2	-	-	-	-	-	-
3	7.35	159.1	7.30	160.1	7.40	159.3
4	-	126.4	-	126.0	-	126.2
5	2.57	31.2	2.18	28.5	2.33	32.3
6	2.40	29.7	1.50	30.0	1.98	30.5
7	5.59	129.4	1.70	41.5	3.90	73.6
8	-	144.2	-	61.8	-	153.4
9	2.94	47.8	2.23	50.8	2.88	49.0
10	4.20	61.4	9.88	191.6	9.75	191.2
11	9.80	191.4	3.55	56.8	4.98 & 5.21	110 .5
12	3.50	56.2	2.1	22.0	3.53	56.7

^{a}Assignments based on ^1H, ^{13}C, DEPT, HMQC, HMBC and COSY experiments.

Fig.1: Iridoids isolated from *Gardenia Jasminoides* (1-12)



Garedenal-I

Garedenal-II

Garedenal-III

Picrocrocinic acid

Shanzhiside

Lamalbidic acid

Genipin-1-?-gentiobioside

Feretoside

6 ?-hydroxy geniposide

6 ?-hydroxy geniposide

Geniposide

3. Results and Discussions:

Compound 1

The ^{1}H NMR (Table. 1) spectrum of compound (**1**) showed the presence of aldehyde moiety at δ 9.80, s, H-11) its corresponding ^{13}C NMR signal at δ 191.4 (C-11); a hydroxyl functional group is derivatized with methyl group resulting in signal at δ (3.50, s, H-1); a primary alcohol is attached to isolated double bond (H-8) position at δ (4.20, s, 2H, H-10). In addition, two degrees of unsaturation was present in the molecule at δ (7.35, s, H-3 & 5.59 H-7] and its related carbon resonance low chemical shift area at δ (159.1 & 129.4) indicating a strong unsaturation present in the molecule. Remaining feature shows that the moiety is related to iridoids. The molecular formula is assigned through HRIMS with ^{1}H and ^{13}C NMR data. The IR & ^{13}C NMR showed the presence of group aldehyde. ^{1}H, ^{13}C NMR (Table-1) and its 2D data is giving strong evidence to determine the isolated compound is related to a monoterpinoid, especially iridoids contains aldehyde group. Based on the above evidence compound (**1**) is 1, 4a, 5, 7a-tetrahydro-7-(hydroxylmethyl)-1-methoxycyclo penta pyran-4-carbaldehyde and trivially named as **Garedenol-I**.

Compound 2 was isolated as colorless oil at less polar region; its molecular formula was analyzed through [HRIMS]. The IR spectrum revealed bands of aldehyde group at 1750 cm^{-1}& 2721cm^{-1}. The ^{1}H NMR Spectrum (Table.1) showed signals of aldehyde at (9.88, s, H-10), a double bond in conjugation with aldehyde group showed at δ (7.30, s, 1H, H-12). Methyl resonance appeared at low field region at δ (2.1, s, H-11) due to presence of 3^o-hydroxyl group present at C-8 position. All other signals were similar with compound (**1**) except H-7 due to lack of double bond. The major portion of aliphatic compounds (iridoids) assembled with methylenes are methiens through the interpreted COSY, HMQC and HMBC (Fig.1) correlations based on these data and available literature. The structure of compound (**2**) was identified as 6(S)-1, 4a, 5, 6, 7, 7a-hexahydro-6-hydroxy-1-methoxy-7-methylene cyclopenta [C] pyran-4-carbaldehyde, a new iridoid and trivially named as **Gardenal-II**.

Compound **3** was amorphous white oil isolated from ethanol extract and its molecular formula was established as $C_{11}H_{16}O_4$ on the basis of HRESIMS data. The UV Spectra is similar to the compounds (**1** & **2**) except 2^o-hydroxy group and exocyclic double bond. ^{1}H NMR Spectrum (Table-1) revealed a carbonyl signal conjugated with double bond system which is attached to a highly electro negative atom which showed a signal at δ (9.82, s, H-11); a resonance at δ (7.40, s, H-3) due to conjugated double bond system near with high electronegative atom. In addition, a signal at δ (3.90, dd, H-7) presence of a hydroxyl group attached to methiene at H-7. Moreover, a exocyclic double bond is attached to H-8 at δ 4.98 and 5.21 as a singlets. All other signals were related to compound **1** & **2**. The major HMBC, COSY & HMQC correlations were showed in Fig.1. Based on the above data and available literature, the structure of compound (**3**) is a new iridoid; 7(S)-1, 4a, 5, 6, 7, 7a-hexahydro-7-hydroxy-1-methoxy-7-methyl cyclopenta pyran-4-carbaldehyde, and trivially named as **Gardenal-III**.

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