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Isolation and characterization of novel flavan glycosides from *Viscum angulatum* B. Heyne ex DC. and screening for hypoglycemic activity

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Abstract

Methanolic extract of *Viscum angulatum* B. Heyne ex DC., whole plant was subjected to flash chromatography, using different ratio of chloroform and methanol. Total of three new metabolites were isolated and characterized by UV, IR, NMR and Mass spectroscopy and these compounds were further confirmed by characterization of its acetylated derivatives. Isolated metabolites are flavan glycosides, 5, 4'-dihydroxy flavan-7-0-β-D- glucopyranoside, 5, 4'-dihydroxy flavan-7-O-β-D-arabinopyranoside and 5, 4'-dihydroxy flavan-7-O-β-D-[erythro-apiofuranosyl (1-2)-β-D glicopyranoside] were isolated from *V. angulatum*. All these isolated compounds were tested for their antidiabetic activity in cell based metabolic assays which showed promising antidiabetic potential.

Key words: *Viscum angulatum* B. Heyne ex DC., isolation, characterization, flavan glycoside hypoglycemic activity

1. Introduction

Viscum angulatum B. Heyne ex DC., is a perennial, semi parasitic plant belonging to the Loranthaceae family and was found, distributed in the central parts of India. Of the twelve species of Viscum found in India, *Viscum album* also known as European mistletoe, was used for the treatment of hypertension, arteriosclerosis, tumors, and arthritis. While *Viscum tubeculatum* was used for the treatment of disorders associated with liver whereas *V. angulatum* was being used for the treatment of arthritis, hypertension and diabetes (Chiu and Chang, 1985). The chemical constituents of Viscum generally belong to the class of flavonoids and may vary with the host species and ecological factors.

Previous phytochemical studies have resulted in the isolation of long chain alkanes, alcohols and fatty acids along with β-amyrin fatty acids esters, β-amyrin acetate, betulic acid, oleanolic acid, pinocembrin 7-O-apiosyl (1-5) apiosyl (1-2)-β-D-glucopyranoside, 2', 3', 4', 3"- tetramethoxy-1, 3-diphenylpropane 5', 4"-di-O-β-D-gluco-pyranoside, viscumneoside V, naringenin and homoeriodictyol (Lin *et al*., 2002).

In our effort towards identifying the novel lead compounds that possessed antidiabetic activity, chemical investigation of *V. angulatum* was undertaken on the basis of its earlier reported use by folklores in diabetes management. In our endeavors to identify novel compounds that possessed antidiabetic activity, we have isolated and identified three flavans from *V. angulatum*, which were unknown. Only a few flavans have been reported so far to occur naturally. These flavans were not readily visible on chromatograms and have simple spectroscopic features compared to other flavonoids (Bohm, 1998).

2. Materials and Methods

2.1 Extraction and isolation

The shade-dried whole plant (1 kg) was extracted successively with hexane, acetone, methanol and water. The methanolic extract was evaporated under reduced pressure to give a dark brown powder (110 g). Methanolic extract (38 g) was subjected to flash chromatography over silica gel (100-200 mesh), using a gradient of $CHCl_3$:CH_3OH (10-30%) to give 62 fractions of 200 ml each. These were pooled based on TLC profile into nine fractions (A-I).

Fraction F (5 g) was subjected to flash chromatography, using a gradient of $CHCl_3$:CH_3OH (85:15). 35 fractions of 200 ml each were collected and pooled into five fractions (1-5). Fraction 3 (1.1 g), obtained from column chromatography of fraction F, was subjected to repeated chromatography in $CHCl_3$:CH_3OH: H_2O: AcOH (77.5:29:2.5:0.2, v/v/v/v), followed by preparative TLC to obtain compound 1 (147 mg).

Fraction H (1 g) was subjected to flash chromatography, using a gradient of $CHCl_3$:EtOAc: CH_3OH (45:40:15, v/v/v). Total of 183 fractions of 7.5 ml each was collected. Out of these, fractions 86-

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117 were pooled together, dried and subjected to preparative TLC, using solvent system EtOAc: CH_3OH: H_2O: AcOH (8.5:1:0.5: 0.05, v/v/v/v) to yield compound 5 (109 mg).

2.2 Characterization of isolated compounds

Melting points were determined on a Buchi B-545 melting point apparatus. IR spectra were recorded on a Perkin-Elmer FTIR RXI spectrophotometer. Ultraviolet spectra were recorded on a Shimadzu UV 2450 spectrophotometer in chloroform or in methanol. ^{1}H NMR spectra were run on a 200 MHz and 400 MHz, while ^{13}C NMR spectra were run on 50 MHz and 100 MHz. 1D and 2D NMR were recorded on 400 MHz in $CDCl_3$, MeOD, and DMSO with D_2O exchange. Mass spectra were recorded on a Perkin-Elmer SCIEX. API 3000 LC/MS/MS. Column chromatography was performed with silica gel (100-200 mesh). HPLC was carried out on a Shimadzu LC2010C. TLC has carried out on glass precoated silica gel 60 F_{254} plates. Spots were visualized under UV light and by spraying with vanillin-H_2SO_4, followed by heating at 100ºC. EtOAc:CH_3OH:H_2O: AcOH (85:10:5:0.25, v/v/v/v), Hexane: EtOAc (50:50 and 30:70) and benzene-acetone (80:20) were used as developing solvents.

2.3 Acid hydrolysis

25 mg of each compound was refluxed with 2N CF_3COOH for 2 h. After extraction with $CHCl_3$, the aqueous layer was co-distilled with methanol until neutral. Three sugars obtained were identified as glucose, arabinose and apiose by comparison with authentic samples on TLC in $CHCl_3$:CH_3OH:H_2O (8:5:1, v/v/v). The optical rotation was measured for each of the sugar obtained after the preparative TLC.

2.4 Acetylation

Compounds **1**, **3**, and **5** (10 mg) were acetylated in pyridine (0.5 ml) and acetone (0.5 ml) to yield the corresponding colorless amorphous solids **2**, **4**, and **6**, respectively.

2.5 *In vitro* cell based assays

2.5.1 *Adipocyte differentiation assay*

Adipogenesis assay was performed according to established procedures (Mukharjee *et al*., 2000). 3T3L1 preadipocytes were seeded at $1X10^4$ cells per wall in a 24 well plate. The cells were grown in DMEM +10% FBS for 48 h. After reaching confluence, the cells were induced for 48 h with a mixture of 1 µM dexamethasone, 5 µg/ml insulin, and 0.6 µM isobutylmethylxanthine (IBMX) in addition to test compounds. Cells were re-fed with the same medium without insulin, dexamethasone, and IBMX with only the test compounds for an additional 72 h. at the end of the incubation, cells were washed with PBS and lysed in 200 µl of 1% Triton in PBS. Triglyceride and protein measurement was done in the cell lysates by enzymatic method and the triglycerides were normalized with protein and expressed as mg/dl. Rosiglitazone (1µM) was used as reference control and triglyceride accumulation was considered as 100%.

2.5.2 *Glucose uptake in* 3T3L1 *cells*

Glucose uptake in differentiated 3T3L1 ($1X10^4$) cells was done as per the method described by Mukharjee *et al*. (2000). 3T3L1 cells were grown in 24 well plates for 48 h as for adipocyte differentiation and differentiated in presence of 5 µg/ml insulin, 0.25 M dexamethasone and 0.5 Mm IBMX. Cells were re-fed with the same medium with insulin and test compounds for 72 h. cells were allowed to completely differentiate for an additional 48 h. The cells were placed in serum-free DMEM medium for 3 h. and rinsed with freshly prepared KRPH buffer (5 Mm phosphate buffer, pH 7.4, 20 mM HEPES, 1mM $MgSO_4$, 136 mMNaCl, 4.7 mM KCl). Cells were incubated with or without 100 nM insulin in KRPH buffer at 37ºC for 30 min. The buffer was replaced with 1µCi/well of [^{14}C]-2-deoxy-D-glucose (Amersham Biosciences) supplemented with 100 µM 2-deoxy-D-glucose, test compounds and incubated at 37ºC for 10mins. The plate was rinsed with cold PBS and cells lysed in 200 µl of 1% Triton in PBS. 100 µl volume of the cell lysate was counted in a Top Count (Perkin Elmer) and the values expressed as CPM/mg protein. Rosiglitazone was used as a reference compound and the value was defined 100%. Percent change was calculated with respect to rosiglitazone (1µM).

3. Results and Discussion

The whole plant of *V. angulatum* was extracted with methanol. The methanolic extract was subjected to column chromatography followed by preparative TLC. This resulted in the isolation of three novel compounds **1**, **3**, and **5**. Acetyl derivatives **2**, **4**, and **6** of these compounds were also prepared to enable the structure elucidation. Compounds **1**, **3**, and **5** showed a positive ferric chloride test confirming the presence of hydroxyl functionality and similarity in spectral data. These compounds showed ^{1}H and ^{13}C NMR signal (Tables 1 and 2) characteristic of flavan moiety. Out of the three compounds isolated, **1** and **3** were found to have a monosaccharide attached to flavan ring. In compound **5**, the glycon was identified to be a disaccharide. Sugar moieties were identified as glucose, arabinose and apiose, based on the NMR data and were further confirmed by TLC of the acid hydrolysed compounds with authentic sugars. The glycoside linkage was established to be at C-7 by NOESY and HMBC experiments.

3.1 Compound 1

Compound **1** was isolated as an amorphous hygroscopic powder and mp 238-240ºC. The UV spectrum (λ_{max} 330 nm) revealed the presence of an aromatic nucleus. IR (KBr) ν_{max} 3432, 2927, 1623, 1438, 1077, 834 cm^{-1} showing that it has both hydroxyl and aromatic absorption bands. The compound showed positive ferric chloride test suggesting the presence of phenolic hydroxyl group, MS (ESI Source, Positive mode): m/z 443 $[M + Na]^+$. Together with ^{1}H and ^{13}C NMR spectral data (Tables 2 and 3), suggested the molecular formula 1 as $C_{21}H_{24}O_9$.

^{1}H NMR (DMSO-d6 + D_2O, 400 MHz) and ^{13}C NMR (DMSO-d6 + D_20, 100 MHz); see Tables 2 and 3. ^{1}H NMR showed signals corresponding to the presence of four aromatic protons at δ 7.18 (2H, d, *J* = 8 Hz) and 6.47 (2H, d, *J* = 8.4 Hz) assignable to C-2', 6' and C-3', 5' of the B ring. A pair of broad singlet at δ 5.94 and 6.08 was attributed to H-6 and H-8 of ring A, the signals due to one methine proton of benzyl ether system at δ 4.83 (1H, d, *J* = 9.6 Hz) and two methylene multiplets centered at δ 1.88-2.01 (2H, m) and δ 2.52 (2H, m) confirmed the presence of a flavan moiety in the molecule. The glycon moiety was identified to be β-D-glucose by characteristic NMR signal of the anomeric proton appearing at δ 4.68 (1H, d, *J*=7.6 Hz) and also by the anomeric carbon signal at δ101.0. the linkage of the glucose moiety C-7 of the flavan ring was confirmed by the NOESY correlation of anomeric proton at δ 4.68 with that of the proton at 5.94 (1H, brs) and 6.08 (1H, brs)corresponding to

H-6 and H-8, respectively. The ^{1}H and ^{13}C NMR data of 1 were in close accordance with those reported for the flavans (Bohm, 1998) acid hydrolysis of compound 1 afforded glucose, which was identified in the filtrate by TLC after evaporation. These data supported the initial identification of compound 1 as a flavan glycoside.

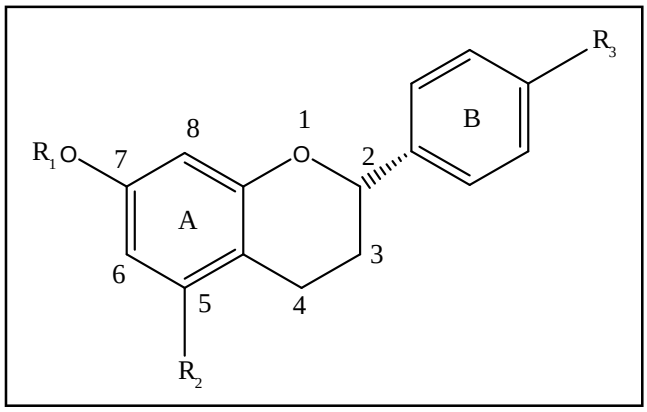

Figure 1: Compounds (1-6) isolaed from *V. angulatum*

Comp.	R_1	R_2	R_3
1	-Glc	OH	OH
2	-Glc$(OCOCH_3)_4$	$OCOCH_3$	$OCOCH_3$
3	-Ara	OH	OH
4	-Ara$(OCOCH_3)_4$,	$OCOCH_3$	$OCOCH_3$
5	-β-D-erythro-apiofuranosyl (1 → 2)-β-D-glucopyranoside	OH	OH
6	-β-D-erythro -apiofuranosyl (1 → 2) -β-D-glucopyranoside acetate	$OCOCH_3$	$OCOCH_3$

3.2 Compound 2

Compound **2** is white amorphous powder; mp 126-127ºC; Mol. Formula $C_{33}H_{36}O_{15}$, UV ($CHCl_3$) λ_{max} 232 nm; IR (DCM) ν_{max} 3435, 2928, 1619, 1561, 1419, 1046, 834 cm^{-1}. ^{1}H NMR (DMSO-d6 + D_2O, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz); see Tables 1 and 2, LCMS (ESI source, positive mode): m/z 690 $[M + NH_4]^+$ and 695 $[M + Na]^+$.

Compound **1** on acetylation afforded a hexa-acetate 2 is known as compound **2**, which had four acetate groups derived from the hydroxyl groups of the glucose and phenolic acetates from the flavan moiety. The ^{1}H NMR signal at δ 5.05 (1H, d, *J* = 7.6 Hz) was assigned to the anomeric proton of the β-D glucose. NOESY experiments revealed the correlation of H-1" with aromatic protons at δ 6.37 (H-6) and 6.51 (H-8). This establishes the presence of a 7-O-glucoside. The attachment of glucose was further confirmed by HMBC correlation of anomeric proton at δ 7.42 (2H, d, *J* = 8.4 Hz) and 7.13 (2H, d, *J* = 8.4 Hz) were assigned to H-2', 6' and H-3', 5'. Similarly, protons appearing as a multiplet at δ 2.08-2.19 (2 H,m) and 2.65 (2 H, m) were assigned to the H-3 and H-4 protons of the flavans nucleus. All the assignments were made on the basis of critical comparison of ^{1}H NMR, ^{13}C NMR, COSY, HSQC and NOESY data. Thus, compound 1 was assigned as 5,4- dihydroxy flavan-7-O-β-D-glucopyranoside (Figure 1).

3.3 Compound 3

Compound **3** was isolated as an hygroscopic amorphous powder; mp 243; Mol. Formula $C_{20}H_{22}O_8$, UV (MeOH) λ_{max} 211nm; IR (KBr) ν_{max} 3435, 2928, 1619, 1561, 1419, 1046, 834 cm^{-1}. ^{1}H NMR (DMSO-d6 + D_2O, 400 MHz) and ^{13}C NMR (DMSO-d6 + D_2O, 100 MHz), see Tables 1 and 2, LC-MS (ESI source, positive mode): m/z 413 $[M + Na]^+$.

^{1}H NMR spectrum of compound **3** was similar to that of compound 1 and displayed signals assignable to 2', 6' and 3', 5' protons at δ 7.17 (2H, d, *J* = 8.4 Hz) and 6.74 (2 H, d, *J* = 8.4 Hz) of flavan moiety. The spectrum further showed the presence of signals at δ 5.90 and δ 6.06 assignable to H-6 and H-8 protons. The NOESY spectrum showed aclear correlation of the anomeric proton H-1" (δ 4.66 d, *J* = 7.6 Hz) with H-6 and H-8 indicating the attachment of the glycan moiety at C=7 position. The methane proton was observed at δ 4.81 (H-2, 1H, d, *J* = 10 Hz). Two sets of methylene multiplets were present at δ 1.81-2.03 and 2.50 (m, 2H), which were assignable to H-3 and H-4 protons. ^{13}C NMR showed the presence of five carbons at δ 101.60, 76.94, 69.85, 73.48 and 66.12, revealing the presence of pentose sugar, which was further characterized by comparing the TLC of the residue obtained after the acid hydrolysis of compound **3** with authentic arabinose.

3.4 Compound 4

Compound **4** is white amorphous powder; mp 235-238ºC, Mol. formula $C_{30}H_{32}O_{13}$, UV (DCM) λ_{max} 240 and 279 nm; IR (DCM) ν_{max} 3437, 1626, 1371, 1220, 1074, 910 cm^{-1}. ^{1}H NMR ($CDCl_3$,400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2, LCMS (ESI source, positive mode): m/z 623 $[M + Na]^+$ and 618 $[M + NH_4]$.

Acetylation of compound **3** afforded a penta acetate 4, which had three acetate groups derive from the hydroxyl groups of arabinose moiety and phenolic acetates from the flavan moiety. ^{1}H NMR, ^{13}C NMR, HMBC, HMQC, and NOESY data supported the assignments and lead to the surface of compounds **3** as **5**, 4'- dihydroxy flavan-7-O-β-D-arabinopyranoside (Figure 1).

3.5 Compound 5

Compound **5** is hygroscopic amorphous powder; mp 230-238ºC, Mol. Formula $C_{26}H_{32}O_{13}$, UV (MeOH) λ_{max} 208 nm; IR (KBr) ν_{max} 3400, 2927, 1623, 1438, 1077, 832 cm^{-1}. ^{1}H NMR (DMSO-d6 + D_2O, 400 MHz) and ^{13}C NMR (DMSO-d6 + D_2O, 100 MHz), see table 1 and 2, LCMS (ESI source, positive mode): m/z 575 $[M + Na]^+$.

Compound **5** was isolated as a white amorphous hygroscopic powder, m.p. 181-182ºC. The LCMS data, where the quasi-molecular ion peak was observed at m/z 574 $[M+ Na]^+$, together with ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2), suggested the molecular formula of 1 as $C_{26}H_{32}O_{13}$. The UV spectrum revealed the presence of an aromatic nucleus. IR spectrum showed hydroxyl and aromatic absorption bands. The compound showed positive ferric chloride test suggesting the presence of phenolic hydroxyl group.

Table 1: ^{1}H NMR Data of Compounds **1 - 6** (DMSO- d_6 + D_2O and $CDCl_3$, δ ppm)

Position	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6
2	4.83 (1H, d, J= 9.6 Hz)	5.01 (1H, d, J= 10.0 Hz)	4.81 (1H, d, J= 10 Hz)	5.00 (1 H, d, J = 8.8 Hz)	4.82 (1H, d, J= 10 Hz)	5.00 (1H, d, J= 9.2 Hz)
3	1.88, 2.01 (2H, m)	2.19, 2.08 (2H, m)	1.81-2.03 (2H, m)	2.19 (1 H, m), 2.00 (1 H, m)	2.01 (1H, m), 1.85 (1H, m)	Merged in acetate signal
4	2.52 (2H, m)	2.65 (2H, m)	2.50 (m, 2H, m)	2.62 (2H, m)	2.50 (2H, m)	2.64 (2H, m)
6	5.94 (1H, brs)	6.37 (1H, d, J= 2.4 Hz)	5.90 (1H, brs)	6.35 (1H, brs)	5.90 (1H, brs)	6.41 (1H, d, J= 2.0 Hz)
8	6.08 (1H, brs)	6.51. (1H, d, J= 2.4 Hz)	6.06 (1H, brs)	6.48 (1H, brs)	6.04 (1H, brs)	6.54. (1H, d, J= 2.4 Hz)
2' & 6'	7.18 (2H, d, J= 8.0 Hz)	7.42 (2H, d, J= 8.4 Hz)	7.17 (d, J= 8.4 Hz)	7.40 (2H, d, J= 8.0 Hz)	7.17 (2H, d, J= 8.0 Hz)	7.41 (2H, d, J= 8.8 Hz)
3' & 5'	6.47 (2H, d, J= 8.4 Hz)	7.13 (2H, d, J= 8.4 Hz)	6.74. (d, J= 8.4 Hz)	7.10 (2H, d, J= 8.0 Hz)	6.74 (2H, d, J= 8.4 Hz)	7.12 (2H, d, J= 8.4 Hz)
1"	4.68 (1H, d, J= 7.6 Hz)	5.05 (1H, d, J= 7.61 Hz)	4.66 (d, J=7.2 Hz)	5.12 (1H, m)	4.74 (1H, d, J= 7.2 Hz)	4.95 (1H, d, J= 7.6 Hz)
2"	3.17 (1H, m)	5.25 (1H, m)	3.32 (1H, m)	5.20 (1H, m)	3.27- 3.31 (1H, m)	3.93 (1H, dd, J= 7.6, 9.2 Hz)
3"	3.25 (1H, m)	5.25 (1H, m)	3.21 (1H, m)	5.15 (1H, m)	3.49-3.51 (1H, m)	5.28 (1H, m)
4"	3.27 (1H, m)	5.14 (1H, m)	3.16 (1H, m)	4.98 (1H, m)	3.27- 3.31 (1H, m)	5.02 (1H, t)
5"	3.12 (1H, m)	3.86 (1H, m)	3.19 (1H, m), 3.70 (1 H, m)	3.50 (1H, dd), 4.18 (1 H,dd)	3.70- 3.75 (1H, m)	3.82 (1H, m)
6"	3.64 (1H, br, d) 3.47 (1H, m)	4.18, 4.26 (2H, dd,)	_	_	3.39-3.62 (2H, m)	4.13 (1H), 4.21 (1H)
1"'	-	-	-	-	5.24 (1H, brs)	5.18 (1H, d, J= 3.6 Hz)
2"'	-	-	-	-	3.80- 3.84 (1H, m)	5.18 (1H, d, J= 3.6 Hz)
4"'	-	-	-	-	3.65- 3.85 (2H, m)	4.37, 4.15 (2H, d, J= 10 Hz)
5"'	-	-	-	-	3.30 (2H, m)	4.58 (2H, dd, J= 12.4, 2.4 Hz)
OCO CH_3	_	2.04, 2.05, 2.05, 2.06, 2.31, 2.33	_	2.06, 2.06, 2.06, 2.29, 2.30	_	1.99, 2.01, 2.02, 2.04, 2.09, 2.11, 2.31, 2.32

^{1}H NMR spectrum of compound **3** was similar to that of compound **1** and displayed signals assignable to 2', 6' and 3', 5' protons at δ 7.17 (2H, d, *J* = 8.4Hz) and 6.74 (2H, d, *J* = 8.4 Hz) of flavan moiety the spectrum further showed the presence of signals at δ 5.90 and δ 6.06 assignable to H-6 and H-8 protons. The NOESY spectrum showed a clear correlation of the anomeric proton H-1" (δ 4.66 d, *J* = 7.6 Hz) with H-6 and H-8, indicating the attachment of the glycan moiety at the C-7 position. The methine proton was observed at δ4.81 (H-2, 1H, d, *J* = 10 Hz). Two sets of methylene multiplets were present at δ 1.81-2.03 and 2.50 (m, 2H), which were assignable to H-3 and H-4 protons. ^{13}C NMR showed the presence of five carbons at δ 101.60, 76.94, 69.85, 73.48, and 66.12, revealing the presence of pentose sugar, which was further characterized by comparing the TLC of the residue obtained by the acid hydrolysis of compound **3** with authentic arabinose.

^{1}H NMR and ^{13}C NMR of compound **5** showed a similar pattern as that of compound **1** for the aglycone moiety. However, the filtrate obtained after the acid hydrolysis of compound **5** had afforded two monosaccharides, which were further confirmed as glucose and apiose after acid hydrolysis of 5 and comparing with authentic standards. NOESY experiments confirmed compound **5** to be a 7-O glycoside. HMBC experiments showed the correlation between ^{1}H-1" of glucose and C-7 of flavan. HMBC correlation between H-2" of glucose and C-1" of apiose has suggested that C-2" of glucose is linked to C-1" of apiose. The correlation observed between H-2" of glucose and H-1" of apiose in the NOESY experiments, were in close agreement with the above findings.

Table 2: ^{13}C NMR Data of Compounds **1 - 6** (DMSO- d_6 + D_2O and $CDCl_3$, ä ppm)

Position	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6
2	77.37	77.51	77.26	77.44	77.40	77.47
3	29.17	29.05	29.14	29.10	29.19	29.09
4	19.63	19.69	19.70	19.68	19.66	19.71
5	156.59	149.73	156.58	149.72	156.63	149.76
6	95.64	103.91	95.41	103.65	96.38	103.75
7	157.65	156.47	156.59	156.44	157.56	156.43
8	96.31	102.93	96.61	102.94	95.00	102.97
9	156.73	150.41	156.89	150.36	156.63	150.39
10	95.34	110.15	103.68	109.95	109.19	110.01
1'	132.11	138.47	131.97	138.56	132.20	138.53
2' & 6'	127.99	127.18	127.98	127.18	128.02	127.21
3' & 5'	115.51	121.71	115.53	121.74	115.49	121.69
4'	157.13	155.94	157.80	155.71	156.55	155.81
1"	101.0	98.96	101.60	98.43	99.14	99.40
2"	73.27	72.77	76.94	70.65	76.19	76.22
3"	76.35	71.07	73.48	60.97	76.41	74.04
4"	76.79	68.34	69.85	68.44	70.24	68.59
5"	69.78	72.09	66.12	61.88	70.38	71.91
6"	60.09	61.99	_	_	60.96	62.06
1"'					109.18	106.61
2"'					77.16	76.50
3"'					77.24	83.74
4"'					74.49	73.05
5"'					64.82	63.11
$OCO\underline{C}H_3$	_	20.58, 20.63, 20.63, 20.63, 20.74, 21.13	_	20.72, 20.72, 20.77, 20.80, 21.16	_	21.12, 21.06, 20.76, 20.64, 20.60, 20.58, 20.54, 20.52
$O\underline{C}OCH_3$	_	168.74, 169.28, 169.41, 169.41, 170.19, 170.63	_	168.87, 169.38, 169.50, 169.87, 169.96	_	170.61, 170.33, 170.00, 169.69, 169.69, 169.40, 169.18, 168.55

Table 3: Effect of compounds **1** - **6** isolated from *V. angulatum* on adipogenesis and glucose uptake

Compound	Percent increase	
	In adipogenesis	In glucose uptake
1	0	89
2	0	82
3	17	0
4	52	48
5	0	80
6	9	53

3.6 Compound 6

Compound **6** was white amorphous powder; mp 181-182°C; Mol. Formula $C_{42}H_{48}O_{21}$, UV ($CHCl_3$) λ_{max} 206 nm; IR (KBr) ν_{max} 3449, 2931, 1752, 1624, 1372, 1229, 1034 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Tables 1 and 2, LCMS (ESI source, positive mode): m/z 906 $[M + NH_4]^+$.

Compound **5** was acetylated to give compound **6**, an octa acetate confirming the presence of eight hydroxyl groups, six were derived from hydroxyl groups of glucose and apiose, while remaining two are phenolic in nature.

The coupling constant of H-$J_{1,2}$apiose was 3.6 suggesting a β-D-erythrofuranoside from [5,8], which was the most stable conformation of apiose. Hence, the structure of compound **5** was elucidated as 5, 4'-dihydroxy flavan-7-O-β-D-[erythro-apiofuranosyl (1-2)-β-D- glucopyranoside] (Figure 1).

Flavans were generally derived from flavanones and are found to co-occur with the flavanone of identical substitution pattern. All natural flavans have the 2 S absolute configuration, as would be expected from the flavanone origin (Porter and Harborne, 1988).

3.7 Effect on adipogenesis: differentiation of 3T3L1 preadipocytes

Activation of PPAR-gamma receptor induces differentiation of preadipocytes to adipocytes. The isolated compounds were subjected to the test and moderate adipogenesis was observed for compound **4**. However, no adipogenesiswas observed for compounds **1**, **2** and **5** (Table 1).

3.8 Effect on glucose uptake in 3T3L1 cells

PPAR-gamma agonists act as insulin sensitizers and are known to improve insulin sensitivity in peripheral tissue and decrease hyperglycemia and hyperinsulinemia. Insulin-stimulated glucose uptake in 3T3L1 cells is a well-established assay for peripheral insulin-mediated glucose utilization. Compounds **1**, **2**, and **5** (1μM, 0.1% DMSO) showed a moderate increase in glucose uptake compared to rosiglitazone (1μM) at 100% (Table 1).

4. Conclusion

Three new flavan glycosides (1, 3, and 5) were isolated from plant *V. angulatum*. Acetyl derivatives (2, 4, and 6) of all three compounds were also prepared and characterized.

All compounds were tested for their antidiabetic activity in cell based metabolic assays. Compounds **1**, **2** and **5** might be acting as selective modulator/antagonists of PPAR gamma receptor, thereby, not causing any adipogenesis but increase the glucose uptake. Compound **4** might be a PPAR-gamma activator, causing adipogenesis in fat cells as well as increasing glucose uptake. Both classes of the molecule have potential as an antihyperglycemic and antihyperinsulinemic agent. These could be capable enough to improve the insulin sensitivity in peripheral tissues, particularly in diabetic conditions. Owing to the non-adipogenic potential, compounds **1** and **2** might have an added advantage over the existing PPAR-gamma activating drugs like rosiglitazone, which were known to increase the body weight. However, our data is very preliminary and additional work has to be carried out in genetic animal models of diabetes and obesity.

Conflict of interest

We declare that we have no conflict of interest.

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