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Research Article

Antioxidant activity of A New Flavone Glycoside from the seeds of *Albizzia Odoratissima* Benth.

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Abstract

A new compound (A) 3,5,7,3'-tetrahydroxy-4'-methoxyflavone-3-O-α-L-rhamnopyranosyl-7-O-β-D-xylopyranosyl (1→2)O-β-D-glucopyranoside alongwith with two known compounds Luteolin (B) and Acacetin (C) were isolated from methanolic extracts of the defatted seeds of Albizzia Odoratissima Benth. The structure of a new compound was elucidated on the basis of extensive spectroscopic analysis, colour reactions and chemical degradations. Compound A exhibited higher radical scavenging activity in the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) assay system.

Key Words: *Albizzia Odoratissima* Benth., Leguminosae, flavone glycoside, Antioxidant activity.

1.Introduction

Albizzia Odoratissima Benth.[1-2] belongs to family Leguminosae, commonly known as Kala Siris. The juice of the plant is applied to the eyes. The powder of the bark taken with butter is considered as tonic. It is used in leprosy and ulcer[3]. The leaves and twigs are lopped for fodder[4]. Its seeds showed marked hypoglycemic activity in normal rats but not in alloxan diabetic rats[5]. The stem having analgesic, stimulant, diuretics, anthelmintic and mostly used for diabetics[6-7]. Earlier workers have reported[8-12] various constituents from this plant. In the present study, we report the isolation and structure elucidation of a new compound **A** from methanolic extract of the seeds of this plant. The structure of a new compound has been characterized as 3, 5, 7, 3'-tetrahydroxy-4'-methoxyflavone-3-O-α-L-rhamnopyranosyl-7-O-β-D-xylopyranosyl(1→2)O-β-D-glucopyranoside.

2. Material and Methods

2.1 General experimental procedures: All the melting points were determined on a thermoelectrically melting point apparatus and are uncorrected. The IR Spectra were recorded in KBr disc. UV Spectra were determined on Shimadzu-120 double beam spectrometer in MeOH. The IR spectra were recorded on Shimadzu FT-IR 8400S in KBr disc. ^{1}H-NMR Spectra were recorded on Bruker DRX 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard. ^{13}C-NMR Spectra were recorded on Bruker DRX 75 MHz spectrometer using $CDCl_3$. The chemical shift values are reported in ppm (δ) units and coupling constant (*J*) in Hz. The FAB mass spectra were recorded on a Jeol-SX (102) Mass spectrometer.

2.2 Plant material: The seeds of *Albizzia Odoratissima* Benth. were procured from Sagar region in the month of October 2012 and were taxonomically authenticated by the Department of Botany, Dr. H.S.Gour University sagar. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr.H.S.Gour University, Sagar (M.P.) India.

2.3 Extraction and Isolation: Air dried and powdered seeds (6.5 kg) of the plant were extracted with petroleum ether (40-60°C) in Soxhlet apparatus for 6 days. The defatted seeds of the plant were successively extracted with methanol for four days. The MeOH soluble fraction of the plant was concentrated under reduced pressure, to yield a light brown viscous mass (3.90 g) which was subjected to TLC examination over silica gel-G using n BAW (4:1:5) as solvent and I_2 vapors as visualizing agent, showed three spots, indicating it to be a mixture of three compounds **A**, **B and C**. These compounds were separated and purified by column chromatography over silica gel using $CHCl_3$: MeOH in various proportions (2 : 6, 3 : 5, 4 : 4). After removal of the solvent and crystallization from ether, above eluates yielded compound **A** (1.74 g), compound **B** (0.45 g) and compound **C** (0.69 g) respectively.

3. Results and discussion

3.1 Study of Compound A: Compound A has m.p. 267-268°C, m.f. $C_{33}H_{40}O_{20}$ [M]+ 756 (FABMS). It gave Molisch and Shinoda tests[13-14] showing its flavonoidal glycosidic nature. Its UV spectrum in methanol showed absorption bands at 266, 310 and 350 nm, suggesting C-3-O-substituted flavonol skeleton. Its IR spectrum showed absorption bands at 3400 (-OH), 1651(α-β unsaturated C=O), 1600, 1560, 1506 (aromatic ring system). In ^{1}H-NMR spectrum of compound A, two singlets at δ 6.53 (1H, br, s) and 7.18 (1H, br, s) were assigned for H-6 and H-8 in ring A. Doublets at δ 7.83 (1H, br, δ, J 2.2 Hz) and δ 7.07 (1H, δ, J 8.7 Hz) were assigned for H-2' and H-5' in ring B. A double doublet at δ 7.54 (1H, dd, J 8.6, 2.1 Hz) was assigned for H-6'. In 1H-NMR a singlet at δ 11.85 was assigned for -OH groups at C-5 position. Another singlet at δ 3.85 confirmed the presence of OCH_3 group at C-4' position. The anomeric proton signals at δ 5.54 (1H, δ, J 1.2 Hz), δ 5.10 (1H, d, J 7.2 Hz) and δ 4.43 (1H, d, J 7.4 Hz) were assigned to H-1" of L-rhamnose, H-1"' of D-glucose and H-1"" of D-xylose respectively. A coupling constant at (J 1.2 Hz) of H-1" confirmed the α-configuration for the L-rhamnose. Two coupling constants at (J 7.2 Hz) and (J 7.4 Hz) confirmed the β configuration for the D-glucose and D-xylose at H-1"'and H-1"" respectively[15-16]. In the mass spectrum of the compound A, characteristic ion peaks at m/z 756 $[M]^+$, 610 [M^+-L-rhamnose], 478 [M^+-D-xylose] and 316 [M^+-D-glucose, aglycone] were found by subsequent losses from the molecular ion of each molecule of L-rhamnose, D-xylose and D-glucose revealing L-rhamnose at C-3 position and D-xylose and D-glucose were linked to aglycone at C-7 position. Acid hydrolysis of compound A with 10% ethanolic H_2SO_4 gave aglycone A-1, m.p 225-226°C m.f $C_{16}H_{12}H_7$ $[M]^+$ 316 (FABMS) which was identified as 3, 5, 7, 3'- tetrahydroxy-4'-methoxy flavone.

The aqueous hydrolysate obtained was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to Paper chromatography examination on Whatman filter Paper No. 1 and showed the presence of D-glucose (R_f 0.16), D-xylose (R_f 0.28) and L-rhamnose (R_f 0.37). Periodate oxidation of compound A confirmed that all sugars were present in the pyranose form[17]. The position of the sugar moieties in compound A were determined by permethylation followed by acid hydrolysis, yielded methylated aglycone and methylated sugars. The methylated aglycone was identified as 3, 7-dihydroxy-5, 3', 4'- trimethoxy flavone which confirmed that glycosidation was involved at C-3-OH and C-7-OH positions of aglycone. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.01), 3, 4, 6-tri-O-methyl-D-glucose (R_G 0.78) and 2, 3, 4-tri-O-methyl-D-xylose (R_G 0.94) by paper chromatography with authentic samples. Therefore it was concluded that C-1"'-OH of D-glucose was attached with OH group at C-7 position of aglycone, C-2"'-OH of D-glucose was linked with C-1""-OH of D-xylose and and C-1" of L-rhamnose was attached with OH group at C-3 position of aglycone. Thus interglycosidic linkage (1→2) was found between D-xylose and D-glucose.

Enzymatic hydrolysis[18] of compound A with takadiastase enzyme liberated L-rhamnose indicating the presence of α linkage between L-rhamnose and 3, 5, 7, 3'-tetrahydroxy-4'-methoxyflavone-7-O-β-D-xylopyranosyl-(1→2)O-β-D-glucopyranoside as proaglycone. Proaglycone on further hydrolysis with almond emulsin enzyme liberated D-xylose first followed by D-glucose and aglycone. Thus compound A was identified as 3, 5, 7, 3'-tetrahydroxy-4'-methoxyflavone-3-O-α-L-rhamnopyranosyl-7-O-β-D-xylopyranosyl(1→2)-O-β-D-glucopyranoside.

3.2 Spectral Data of compound A: It was analyzed for m.f. $C_{33}H_{40}O_{20,}$ m.p 267-268 °C, $[M]^+$ 756 found (%) C 51.90 , H 5.24, O 42.54, Calcd for m.f. $C_{33}H_{40}O_{20}$ found (%) C 52.3 , H 5.29, O 42.3, UV (MeOH) λ_{max} nm 266, 310 and 350. IR (kBr) υ_{max} (cm^{-1}); 3400, 1651, 1600, 1560, 1506. ^{1H}NMR (300 MHz, $CDCl_3$); 11.85 (1H, s, 5-OH)), 6.53 (1H, br, s, H-6), 7.18 (1H, br, s, H-8), 7.83 (1H, br, d, *J* 2.2 Hz, H-2'), 7.07 (1H, d, *J* 8.7 Hz, H-5'), 7.54 (1H, dd, *J* 8.6, 2.1 Hz, H-6'), 5.54 (1H, d, J 1.2 Hz, H-1"), 4.23 (1H, br, s, H-2"), 3.94 (1H, m, H-3"), 3.43 (1H, m, H-4"), 3.64 (1H, m, H-5"), 0.92 (3H, d, *J* 6.2 Hz, CH_3), 5.10 (1H, d, *J* 7.2 Hz, H-1"'), 4.59 (1H, dd, J 7.2, 9.9 Hz, H-2"'), 3.68 (1H, t, *J* 9.9 Hz, H-3"'), 3.15 (1H, dd, *J* 9.9, 10.2 Hz, H-4"'), 3.27 (1H, m, H-5"'), 3.48 (1H, dd, *J* 12.0, 5.30 Hz, H-6_a"'), 3.61 (1H,

dd, *J* 12.0, 2.3 Hz, H-6_b'''), 4.43 (1H, d, *J* 7.2 Hz, H- 1''''), 3.32 (1H, dd, *J* 7.2, 9.3 Hz, H-2''''), 3.37 (1H, t, *J* 9.3 Hz, H-3''''), 3.51 (1H, m, H-4''''), 3.25 (1H, dd, *J* 13.0, 10.0 Hz, H-5_a''''), 3.67 (1H, m, H-5_b''''). ^{13}C NMR (75 MHz, $CDCl_3$); d 158.60 (C-2), 142.20 (C-3), 180.10 (C-4), 160.60 (C-5), 100.30 (C-6), 162.50 (C-7), 96.90 (C-8), 156.30 (C-9), 105.40 (C-10), 122.60 (C-1'), 113.80 (C-2'), 147.0 (C-3'), 150.0 (C-4'), 112.0 (C-5'), 118.0 (C-6'), 55.40 (C-4'-OMe), 103.50 (C-1''), 71.50 (C-2''), 72.10 (C-3''),73.10 (C-4''), 71.80 (C-5''), 17.60 (OMe), 97.95 (C-1'''),82.15 (C-2'''), 76.86 (C-3'''), 69.48 (C-4'''), 76.80 (C-5'''), 60.36 (C-6'''), 104.96 (C-1''''), 74.13 (C-2''''), 75.82 (C-3''''), 69.00 (C-4''''), 65.36 (C-5'''').

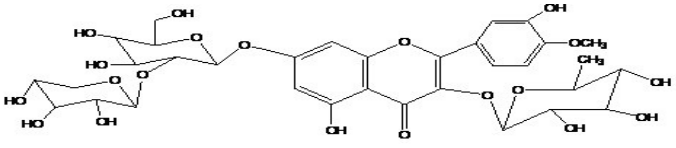
3.3 Acid hydrolysis of compound A: 70 mg of Compound A was dissolved in 15 mL of methanol and refluxed with 10% H_2SO_4 on water bath for 6 h. The contents were concentrated and allowed to cool and residue was extracted with diethyl ether. The ether layer was washed with water and the residue was chromatographed over silica gel using a mixture of $CHCl_3$ and MeOH as solvent to give aglycone **A-1**. This was identified as 3,5,7,3'-tetrahydroxy-4'-methoxyflavone.

3.4 Permethylation of compound A: Compound A (40 mg) was dissolved in DMF (35 ml) and treated with MeI (5 ml) and Ag_2O (20 mg) in a 150 ml round bottomed flask fitted with air condenser and refluxed for 2 days. The reaction mixture was filtered and washed with DMF. The filtrate was concentrated under reduced pressure and hydrolysed with 10% H_2SO_4 to give methylated aglycone 3,7-dihydroxy-5, 3',4'-trimethoxy flavone. The aqueous hydrolysate obtained after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2,3,4-tri-O-methyl-L-rhamnose (R_G 1.01), 3,4,6-tri-O-methyl-D-glucose (R_G 0.42) and 2,3,4-tri-O-methyl-D-xylose (R_G 0.94) (by m.m.p and Co-PC).

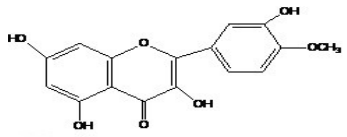
3.5 Enzymatic hydrolysis of compound A: Compound A (35 mg) was dissolved in MeOH (20 ml) and hydrolyzed with equal volume of takadiastase enzyme. The contents were allowed to stay at room temperature for 3 days and filtered. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as a spraying reagent which showed the presence of L-rhamnose (R_f 0.37) The proaglycone was dissolved in MeOH (20 ml) and further hydrolyzed with equal volume of almond emulsin enzyme at room temperature as usual procedure yielded aglycone identified as 3,5,7,3'-tetrahydroxy-4'-methoxy flavone and sugars were identified as D-glucose (R_f 0.16) and D-xylose (R_f 0.28).

3.6 Study of Compound A-1 : It was analyzed for m.f. $C_{16}H_{12}O_7$, m.p 225-226 °C, $[M]^+$ 316 found (%) C 60.74, H 3.75, O 35.47, Calcd for m.f. $C_{16}H_{12}O_7$ found (%) C 60.78, H 3.80 O 35.44, UV(MeOH) λ_{max} nm 274, 321 and 375. IR (kBr) υ_{max}(cm^{-1}); 3460, 1650, 1600, 1562, 1508. ^{1}HNMR (300 MHz, $CDCl_3$); δ 12.91 (1H, s, 5-OH)), 6.53 (1H, br, s, H-6), 7.18 (1H, br, s, H-8), 7.83 (1H, br, d, *J* 2.2 Hz, H-2'), 7.07 (1H, d, *J* 8.7 Hz, H-5'), 7.54 (1H, dd, *J* 8.6-2.1 Hz, H-6'). ^{13}C NMR (75 MHz, $CDCl_3$); δ 158.60 (C-2), 142.20 (C-3), 180.1 (C-4), 160.60 (C-5), 100.30 (C-6), 162.50 (C-7), 96.90 (C-8), 156.30 (C-9), 105.40 (C-10), 122.60 (C-1'), 113.80 (C-2'), 147.0 (C-3'), 150.0 (C-4'), 112.0 (C-5'), 118.0 (C-6'), 55.4 (C-4'-OMe).

Figure 1 Structure of Compound A and A-1



A

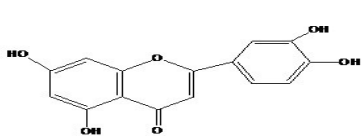


A-1

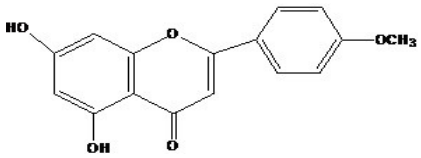
3.7 Study of known Compound B: It was analyzed for m.f. $C_{15}H_{10}O_6$, m.p 232-233 °C, $[M]^+$ 286 found (%) C 62.97, H 3.51, O 33.52, Calcd for m.f. $C_{15}H_{10}O_6$ found (%) C 62.93, H 3.49, O 33.56. UV(MeOH) λ_{max} nm 274, 321 and 375. IR (kBr) υ_{max} (cm^{-1}); 3460, 1650, 1600, 1562, 1508. ^{1}HNMR (300 MHz, $CDCl_3$); δ 6.71 (1H, s, H-3), 6.49 (1H, br, d, *J* 2.0 Hz, H-6), 7.09 (1H, br, s, H-8), 7.77 (1H, br, s, H-2'), 6.86 (1H, d, *J* 8.4 Hz, H-5'), 7.41 (1H, dd, *J* 8.4-2.3 Hz H-6'). ^{13}C NMR (75 MHz, $CDCl_3$); δ 164.40 (C-2), 102.40 (C-3), 181.80 (C-4), 160.80 (C-5), 100.10 (C-6), 162.50 (C-7), 96.50 (C-8), 159.40 (C-9), 105.50 (C-10), 121.10 (C-1'), 114.10 (C-2'), 145.90 (C-3'), 149.70 (C-4'), 115.70 (C-5'), 118.40 (C-6'). It was identified as known compound by comparison with reported literature values.

3.8 Study of known Compound C: It was analyzed for m.f. $C_{16}H_{12}O_5$, m.p 245-246 °C, $[M]^+$ 284 found (%) C 67.58, H 4.27, O 28.14, Calcd for m.f. $C_{16}H_{12}O_5$ found (%) C 67.60, H 4.22, O 28.16. UV(MeOH) λ_{max} nm 274, 321 and 375. IR (kBr) υ_{max} (cm^{-1}); 3460, 1650, 1600, 1562, 1508. ^{1}HNMR (300 MHz, $CDCl_3$); δ 6.91 (1H, s, H-3), 6.48 (1H, d, *J* 2.1 Hz, H-6), 6.81 (1H, br, s, H-8), 8.05 (1H, d, *J* 8.9 Hz, H-2'), 7.15 (1H, d, J 8.9 Hz, H-3'), 7.15 (1H, d, *J* 8.9 Hz, H-5'), 8.05 (1H, d, *J* 8.9 Hz H-6'), 3.87 (3H, s, H-4'). ^{13}C NMR (75 MHz, $CDCl_3$); δ 163.80 (C-2), 103.70 (C-3), 181.90 (C-4), 161.0 (C-5), 99.70 (C-6), 162.70 (C-7), 95.20 (C-8), 156.80 (C-9), 105.40 (C-10), 122.70 (C-1'), 128.30 (C-2'), 114.60 (C-3'), 162.30 (C-4'), 114.60 (C-5'), 128.30 (C-6'), 55.40 (C-4'-OMe). It was identified as known compound by comparison with reported literature values.

Figure 2 Structure of Compound B and C



B



C

4. Antioxidant Activity

4.1 DPPH radical scavenging assay method: DPPH quenching ability of extract was measured according to Christudas S. and Ignacimuthu S., 100 mg of dried extract were dissolved in 100 ml methanol. Various aliquotes of concentration 10-100µg/ml were prepared with methanol. 2 ml of methanolic solution of DPPH (0.1mM) was mixed with 3 ml of extracts (10–100 µg/ml) and allowed to incubate for 30 min at dark place. After 30 minute decrease in absorbance of DPPH solution was measure at 517 nm against control sample. The antiradical activity was expressed as IC_{50} (µg/ml), (the antiradical dose required to cause a 50% inhibition). Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = (A_0-A_t/ A_0) X 100

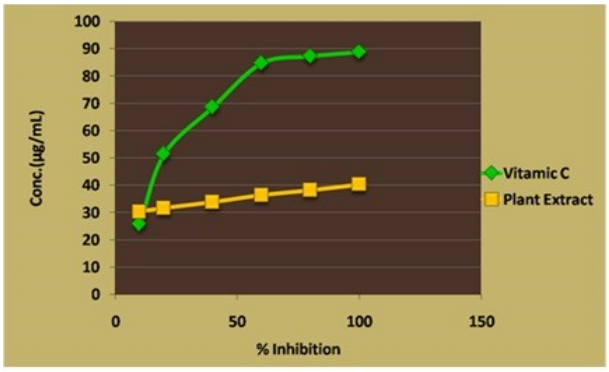
Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min.

Absorbance of 0.1mM DPPH (Ao) = 2.076

S. No	Vitamin C			Plant Extract		
	Conc.	Test	% Inhibition	Conc.	Test	% Inhibition
1	10	1.524	25.72	10	1.447	30.29
2	20	1.012	51.25	20	1.421	31.55
3	40	0.655	68.44	40	1.375	33.76
4	60	0.322	84.48	60	1.322	36.31
5	80	0.268	87.09	80	1.285	38.1
6	100	0.236	88.61	100	1.242	40.17
IC_{50} (µg/ml)			24.65	IC_{50}		189.44

From the above result we can conclude that the compound A showed good Antioxidant activity against Ascorbic acid in 1,1-diphenyl-2-pycrylhydrazyl (DPPH) system.

Figure 3 Antioxidant activity of Plant extract (DPPH Method) percentage Inhibition Vs Concentration



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