

Chemical constituents from *Cassia occidentalis* Linn.

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Received 2 February 2010; accepted (revised) 26 April 2011

The present paper deals with the isolation and structural elucidation of three new compounds **1**, **2** and **3** from the seeds of *Cassia occidentalis* Linn. These compounds have been characterized as 5, 7-dihydroxyflavone-5-O- β -D-xylopyranosyl-7-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)-O- α -L-arabinopyranoside (**1**), 3, 5, 7, 3', 4'-pentahydroxy flavone-3-O- α -L-rhamnopyranosyl-7-O- β -D-glucopyranosyl-(1 \rightarrow 3)-O- β -D-xylopyranoside (**2**) and 5, 7, 3', 4'-tetrahydroxy-6-methoxyflavone-5-O- α -L-arabinopyranosyl-(1 \rightarrow 4)-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)-O- β -D-galactopyranoside (**3**) respectively by various colour reactions, chemical degradations and spectral analysis. These compounds have been evaluated against various bacteria and fungi which showed good results.

Keywords: *Cassia occidentalis* Linn, leguminosae, flavone glycosides, antimicrobial activity

Cassia occidentalis^{1,2} Linn belongs to family Leguminosae which is commonly known as *Kasunda* or *Bari kasunda* in Hindi. It is distributed throughout India and in most tropical countries. Its roots are useful against ringworm infections. It is also used as a diuretic and in the treatment of snake-bite. The whole plant is useful as a purgative and used as a tonic. The seeds and leaves are used as cure for cutaneous diseases.

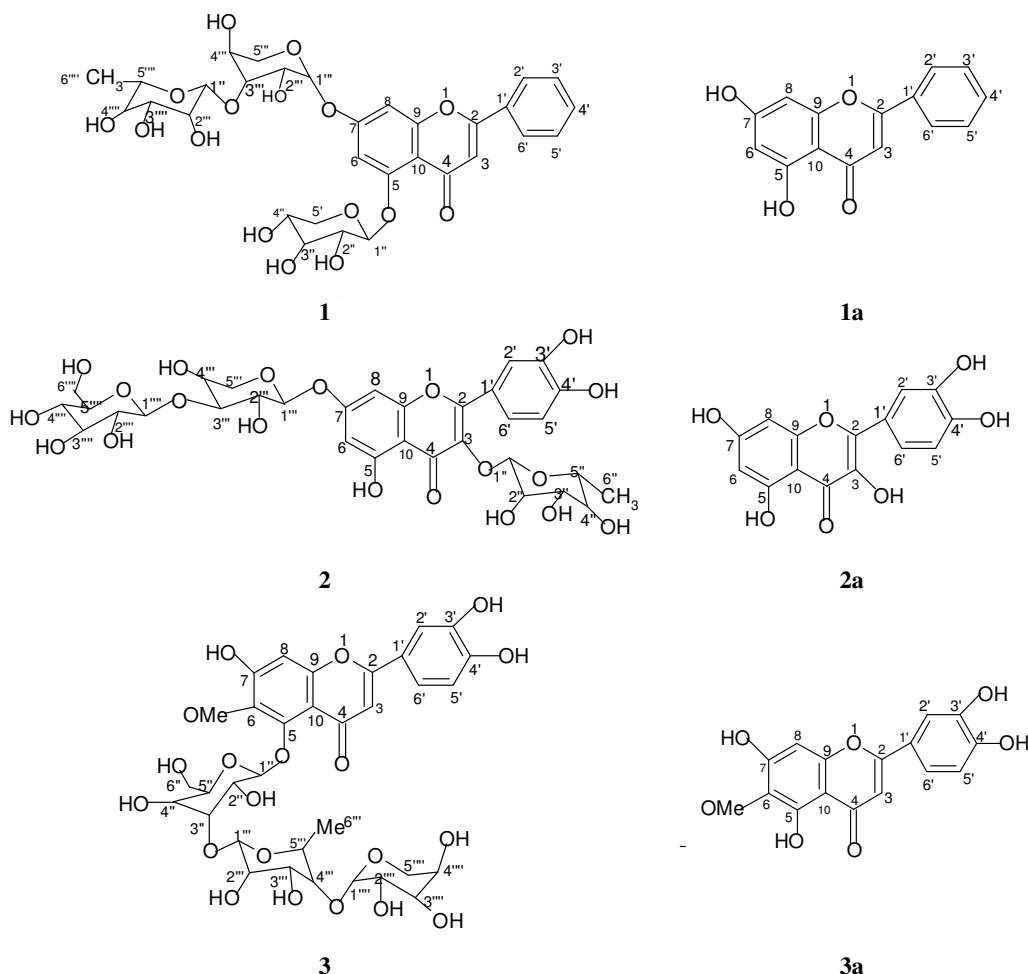
Earlier workers³⁻⁵ have reported various constituents from this plant. The present paper reports the isolation and structure elucidation of three new flavone glycosides from methanolic extract of the seeds of this plant.

Results and Discussion

Compound **1** has m.p. 210-12°C, Mol. formula C₃₁H₃₆O₁₆, [M⁺] *m/z* 664 (FABMS). It gave positive Molisch⁶ and Shinoda tests⁷ showing its flavonoidal glycosidic nature. Its UV-Vis spectrum showed absorption bands at 268 and 370 nm suggesting it to be flavone. Its IR spectrum showed absorption at 3410 (br) (-OH), 2950 (C-H aromatic), 1680 (>C=O α - β unsaturated), 1598 (aromatic ring system), 1072 (-O-glycosidic linkage), 845 and 820 cm⁻¹. In ¹H NMR spectrum of compound **1**, a singlet at δ 6.65 due to H-3 and two doublets at δ 7.95 (2H, d, *J* 6.5 Hz) and δ 6.75 (2H, d, *J* 6.2 Hz) were assigned to H-2', H-6' and H-3', H-5', respectively⁸. Two *meta* coupled

doublets (*J* 2.0 Hz) at δ 6.36 and 6.84 were assigned to H-6 and H-8 protons⁹. The anomeric proton signals at δ 4.98 (1H, d, *J* = 7.2 Hz, H-1''), δ 5.45 (1H, d, *J* = 2.5 Hz, H-1''') and δ 5.51 (1H, d, *J* = 1.8, Hz, H-1''') were assigned to H-1'', H-1''', H-1'''' of D-xylose, L-arabinose and L-rhamnose respectively. In ¹H NMR spectrum, coupling constants of *J*_{1,2} (7.3 Hz) value of anomeric proton of D-xylose, confirmed the presence of β configurations of D-xylose while coupling constants of *J*_{1,2} (2.0 Hz) and *J*_{1,2} (1.8 Hz) values of anomeric protons of L-arabinose and L-rhamnose, confirmed α configuration of L-arabinose and L-rhamnose¹⁰.

Acid hydrolysis of compound **1** with 10% ethanolic H₂SO₄ afforded aglycone **1a**, m.p. 344-45°C, Mol. formula C₁₅H₁₀O₄, [M]⁺ *m/z* 298 (FABMS), which was identified as 5,7-dihydroxy flavone by comparison of its spectral data with reported literature values¹¹⁻¹⁴. The aqueous hydrolysate was neutralized with BaCO₃ and the BaSO₄ was filtered off. The filtrate was concentrated and subjected to paper chromatography, showing the presence of D-xylose (R_f 0.26), L-rhamnose (R_f 0.35) and L-arabinose (R_f 0.21). Quantitative estimation of sugars according to the procedure of Mishra and Rao¹⁵, revealed that all the three sugars were present in equimolar ratio (1:1:1). Periodate oxidation of compound **1**, confirmed that all the sugars were present in the pyranose form¹⁶.



The position of the sugar moieties in compound **1** were determined by permethylation¹⁷ followed by acid hydrolysis, yielding methylated sugars and aglycone. The aglycone was identified as 5,7-dihydroxy flavone with reported literature values¹¹. It showed that C-5-OH and C-7-OH positions of aglycone were involved in glycosidation. The methylated sugars were identified as 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-L-rhamnose and 2,4-di-*O*-methyl-L-arabinose by paper chromatography with authentic sample.

Therefore, it was concluded that the C-1'''' -OH of L-rhamnose was linked with C-3''' of L-arabinose, C-1''' of L-rhamnose was attached with -OH group at C-7 position of aglycone and C-1'' of D-xylose was attached with -OH group at C-5 position of aglycone. The interglycosidic linkage (1→3) was found between L-rhamnose and L-arabinose¹⁸.

Enzymatic hydrolysis of compound **1** with almond emulsin enzyme liberated D-xylose, suggesting the

presence of β -linkage between D-xylose and proaglycone. Proaglycone on further hydrolysis with takadiastase enzyme, liberated L-rhamnose followed by L-arabinose, suggesting the presence of α -linkage between L-rhamnose and L-arabinose, as well as between L-arabinose and the aglycone¹⁹.

On the basis of the above evidence, the compound **1** was characterized as 5,7'-dihydroxyflavone-5-*O*- β -D-xylopyranosyl-7-*O*- α -L-rhamnopyranosyl-(1→3)-*O*- α -L-arabinopyranoside.

Compound **2** has m.p. 228-30°C, Mol. formula C₃₂H₃₈O₂₀, [M⁺] *m/z* 742 (FABMS). It gave positive Molisch and Shinoda tests showing its flavonoidal glycosidic nature. Its UV-Vis spectrum showed absorption bands at 215 and 365 nm suggesting it to be flavone. Its IR spectrum showed absorption bands at 3448 (br) (-OH), 2892 (C-H saturated), 1730 (>C=O α - β unsaturated), 1658 (aromatic ring system), 1083 (glycosidic linkage), 876 and 830 cm⁻¹. In ¹H NMR spectrum of compound **2**, a singlet at δ

6.72 due to H-8 and three doublets at δ 7.19 (1H, d, J 8.5 Hz), 7.15 (1H, d, J 8.5 Hz) and 6.80 (1H, d, J 8.8 Hz) were assigned to H-2', H-6' and H-5', respectively. Three singlets at δ 12.01, 10.34 and 10.42 were assigned to -OH groups at C-5, C-3' and C-4' position. The anomeric proton signals at δ 5.32 (1H, d, J = 1.6 Hz, H-1''), 4.95 (1H, d, J = 7.2 Hz, H-1''') and 5.22 (1H, d, J = 7.6, Hz, H-1''') were assigned to H-1'' of L-rhamnose and H-1''' of D-xylose and H-1'''' of D-glucose respectively. In ^1H NMR spectrum, coupling constant of $J_{1,2}$ (1.6 Hz) value of anomeric proton of L-rhamnose, confirmed α configuration of L-rhamnose while coupling constants of $J_{1,2}$ (7.2 Hz) and $J_{1,2}$ (7.6 Hz) values of anomeric protons of D-xylose and D-glucose confirmed β configuration of D-xylose and D-glucose.

Acid hydrolysis of compound **2** with 10% ethanolic H_2SO_4 gave aglycone **2a**, m.p. 306-308°C, Mol. formula $\text{C}_{15}\text{H}_{10}\text{O}_7$, $[\text{M}]^+$ m/z 302 (FABMS), identified as 3,5,7,3',4'-pentahydroxy flavone by comparison of its spectral data with reported literature values²⁰. 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 showing the presence of L-rhamnose (R_f 0.35), D-glucose (R_f 0.19) and D-xylose (R_f 0.26). Quantitative estimation of sugars carried out by the procedure of Mishra and Rao revealed that all the three sugars were present in equimolar ratio (1:1:1). Periodate oxidation of compound **2**, confirmed that all the sugars were present in the pyranose form.

The position of the sugar moieties in compound **2** were determined by permethylation followed by acid hydrolysis, which yielded methylated sugars and methylated aglycone. The methylated aglycone was identified as 3,7-dihydroxy 5,3',4'-trimethoxy flavone, confirming that glycosidate was involved at C-3-OH and C-7-OH position of aglycone. The methylated sugars were identified as 2,3,4-tri-*O*-methyl-L-rhamnose 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2,4-di-*O*-methyl-D-xylose (by Co-PC).

Therefore it was confirmed that the C-1'''' -OH of D-glucose was linked with C-3''' of D-xylose, C-1''' of D-xylose was attached with -OH group at C-7 position of aglycone and C-1'' of L-rhamnose was attached with -OH group at C-3 position of aglycone. Therefore it was concluded that interglycosidic linkage (1 \rightarrow 3) was found between D-glucose and D-xylose.

Enzymatic hydrolysis of compound **2** with takadiastase liberated L-rhamnose, suggesting the presence of α -linkage between L-rhamnose as well as between L-rhamnose and the proaglycone. Proaglycone on further hydrolysis with almond emulsin liberated D-glucose, followed by D-xylose indicating the presence of β -linkage between D-glucose and D-xylose as well as between D-xylose and aglycone.

Thus, the compound **2** was identified as 3,5,7,3',4'-pentahydroxyflavone-3-*O*- α -L-rhamnopyranosyl-7-*O*- β -D-glucopyranosyl-(1 \rightarrow 3)-*O*- β -D-xylopyranoside.

Compound **3** has m.p. 240-42°C, Mol. formula $\text{C}_{33}\text{H}_{40}\text{O}_{20}$, $[\text{M}^+]$ m/z 756 (FABMS). It gave positive Molisch and Shinoda test showing its flavonoidal glycosidic nature. Its UV-Vis spectrum showed absorption bands at 285 and 330 nm suggesting its flavonoidal nature. Its IR spectrum showed absorption bands at 3452 (br) (-OH), 2830 (-OMe) 2938 (C-H saturated), 1725 (>C=O α - β unsaturated), 1609 (aromatic ring system), 1084 (glycosidic linkage), 876 and 842 cm^{-1} .

In ^1H NMR spectrum of compound **3**, two singlets at δ 7.1 and 6.56 were assigned to H-3 and H-8 respectively. Three doublets at δ 7.53 and 6.95 were assigned to H-2', H-6' and H-5' respectively. Three singlets at δ 11.72, 10.78 and 10.84 were assigned to -OH groups at C-7, C-3' and C-4' positions respectively. The anomeric proton signals at δ 5.28 (1H, d, J = 7.3 Hz, H-1''), 5.48 (1H, d, J = 1.7 Hz, H-1''') and 5.19 (1H, d, J = 2.3, Hz, H-1''') were assigned to H-1'' of D-galactose, H-1''' of L-rhamnose and H-1'''' of L-arabinose respectively. In ^1H NMR spectrum coupling constant value of $J_{1,2}$ (7.3 Hz) for the anomeric proton of D-galactose confirmed β configuration of D-galactose while coupling constant values of $J_{1,2}$ (1.7 Hz) and $J_{1,2}$ (2.3 Hz) for the anomeric protons of L-rhamnose and L-arabinose confirmed the α configurations of L-rhamnose and L-arabinose.

Acid hydrolysis of compound **3** with 10% ethanolic H_2SO_4 gave aglycone **3a**, m.p. 226-28°C, Mol. formula $\text{C}_{16}\text{H}_{12}\text{O}_7$, $[\text{M}^+]$ m/z 316 (FABMS), identified as 5,7,3',4'-tetrahydroxy-6-methoxy flavone by comparison of its spectral data with reported literature values²¹. The aqueous hydrolysate was neutralized with BaCO_3 and the BaSO_4 was filtered off. The filtrate was concentrated and subjected to paper chromatography showing the presence of L-arabinose (R_f 0.21), L-rhamnose (R_f 0.35) and D-galactose (R_f

0.17). Quantitative estimation of sugars were carried out by the procedure of Mishra and Rao, which revealed that all the three sugars were present in equimolar ratio (1:1:1). Periodate oxidation of compound **3**, confirmed that all the sugars were present in the pyranose form.

The position of the sugar moieties in compound **3** were determined by permethylation followed by acid hydrolysis, which yielded methylated sugars and methylated aglycone. The methylated aglycone was identified as 5-hydroxy-6,3',4'-trimethoxy flavone which confirmed the involvement of -OH at C-5 position of the aglycone in glycosidation. The methylated sugars were identified as 2,3,4-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-L-rhamnose and 2,4,6-tri-*O*-methyl-D-galactose (by Co-PC).

Therefore it was concluded that the C-1'''' -OH of L-arabinose was linked with C-4''' of L-rhamnose, C-1''' of L-rhamnose was attached with C-3'' position of D-galactose and C-1'' of D-galactose was attached with C-5-OH position of aglycone. The interglycosidic linkages (1→4) and (1→3) were found between L-arabinose and L-rhamnose as well as between L-rhamnose and D-galactose.

Enzymatic hydrolysis of compound **3** with takadiastase liberated L-arabinose, followed by L-rhamnose suggesting the presence of α -linkage between L-arabinose and L-rhamnose as well as between L-rhamnose and D-galactose. Proaglycone was further hydrolysed with almond emulsion to liberate D-galactose suggesting the presence of β -linkage between D-galactose and aglycone.

Therefore, the compound **3** was identified as 5,7,3',4'-tetrahydroxy-6-methoxyflavone-5-*O*- α -L-arabinopyranosyl-(1→4)-*O*- α -L-rhamnopyranosyl-(1→3)-*O*- β -D-galactopyranoside.

Antimicrobial activity of the above three compounds was evaluated against various bacteria and fungi. The results reported in **Table I** show that compound **1** is more active against *Proteous vulgaris* while being less active against *Bacillus coagulans*.

Compound **2** shows good activity against *Bacillus coagulans* and is less active against *Eschecheria coli*. Compound **3** is highly active against *Bacillus coagulans* while being less active against *Staphylococcus aureus*. In case of fungicidal activity, compound **1** shows good activity against *Fusarium oxysporum* and less activity against *Penicellium digitatum*. Compound **2** is found to be highly active against *Aspergillus niger* and less active against *Fusarium oxysporum*. Compound **3** is highly active against *Fusarium oxysporum* and less active against *Aspergillus niger*.

Experimental Section

All the melting points were determined on a thermoelectrically operated melting point apparatus and are uncorrected. UV-Vis spectra were determined on Shimadzu-120 double beam spectrophotometer with MeOH as solvent. The IR spectra were recorded on Shimadzu FTIR-8400 spectrophotometer in KBr disc. ¹H NMR spectra were recorded on Varian XL 300 MHz spectrometer in CDCl₃ using TMS as internal standard. ¹³C NMR spectra were recorded on Varian XL 90 MHz spectrometer using CDCl₃. The chemical shift values are reported in δ (ppm) units and coupling constant (*J*) in Hz. The FAB mass spectra were recorded on a Jeol SX-102/DA-6000 Mass spectrometer/Data system using Argon/Xenon (6kV) as the FAB gas.

Plant Material

The seeds of *Cassia occidentalis* were procured from Sagar region in Jan-2007 and were taxonomically authenticated by the Department of Botany, Dr. H. S. Gour University, Sagar. A voucher specimen has been deposited (DK/02/15-01-2007) in the Natural Products Laboratory, Department of Chemistry, Dr. H S. Gour University, Sagar (M.P.).

Extraction and Isolation

Air dried and powdered seeds (5.0 kg) of the plant were extracted with petroleum ether (40-60°C) in

Table I — Antibacterial and antifungal activity of compounds

Compd	Antibacterial activity (Zone of inhibition in mm)				Antifungal activity (Zone of inhibition in mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. coagulans</i>	<i>P. vulgaris</i>	<i>P. digitatum</i>	<i>F. oxysporum</i>	<i>A. niger</i>	
1	12.35	11.23	9.65	15.45	9.95	15.4	11.60	
2	9.46	10.85	16.60	11.80	13.9	10.01	13.50	
3	14.4	10.51	12.05	10.70	11.85	12.10	10.04	
Streptomycin	19.58	18.80	20.00	19.10	Griseofulvin	17.2	21.0	15.5

Soxhlet extractor for 5 days. The defatted seeds were successively extracted with methanol for three days. The MeOH soluble fraction of the plant was concentrated under reduced pressure, to yield a light brown viscous mass (2.8 g) which was subjected to TLC examination over silica gel-G using *n*-BAW (4:1:5) as solvent and I₂ vapors as visualizing agent. The mixture resolved into three spots on TLC, indicating it to consist of three compounds **1**, **2** and **3**. These compounds were separated and purified by column chromatography over silica gel using CHCl₃:MeOH in various proportions (2:8, 6:4, 4:10). After removal of the solvent and crystallization from ether, the above eluates yielded compound **1** (0.76 g), compound **2** (0.58 g) and compound **3** (0.43 g) respectively.

Compound 1. m.p. 210-12°C, Mol. formula C₃₁H₃₆O₁₆, [M⁺] *m/z* 664. Anal. Found: C, 56.00; H, 5.40. Calcd for C₃₁H₃₆O₁₆: C, 56.02; H, 5.42%. UV-Vis (MeOH): λ_{max} nm 268, 370, (+AlCl₃), 278, 325, 385, (+AlCl₃ / HCl), 280, 320, 383; IR (KBr): 3410, 2950 1732, 1680, 1585, 1465, 1385, 1265, 1190, 1072, 845, 820 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.92 (2H, d, *J* = 6.2 Hz, H-2', H-6'), 7.75 (2H, d, *J* = 6.2 Hz, H-3', H-5'), 7.45 (1H, d, *J* = 6.2 Hz, H-4'), 6.65 (1H, s, H-3), 6.36 (1H, d, *J* = 2.0 Hz, H-6), 6.84 (1H, d, *J* = 2.0 Hz, H-8), 4.98 (1H, d, *J* = 7.2 Hz, H-1''), 3.42 (1H, m, H-2''), 3.37 (1H, m, H-3''), 3.46 (1H, m, H-4''), 3.41 (1H, m, H-5''), 5.45 (1H, d, *J* = 2.0 Hz, H-1'''), 4.32 (1H, d, *J* = 2.1 Hz, H-2'''), 3.45 (1H, m, H-3'''), 3.60 (1H, m, H-4'''), 3.86 (1H, m, H-5'''), 5.51 (1H, d, *J* = 1.8 Hz, H-1'''), 4.15 (1H, dd, *J* = 1.6, 3.6, Hz, H-2'''), 3.87 (1H, dd, *J* = 3.6, 9.0 Hz, H-3'''), 3.59 (1H, d, *J* = 9.2 Hz, H-4'''), 3.74 (1H, m, H-5'''), 1.58 (3H, d, *J* = 5.6 Hz, H-6'''); ¹³C NMR (90 MHz, CDCl₃): δ 165.3 (C-2), 110.5 (C-3), 184.2 (C-4), 163.1 (C-5), 99.7 (C-6), 162.8 (C-7), 96.5 (C-8), 160.1 (C-9), 106.4 (C-10), 132.6 (C-1'), 135.0 (C-2'), 129.5 (C-3'), 138.2 (C-4'), 120.2 (C-5'), 132.5 (C-6'), 105.0 (C-1''), 76.5 (C-2''), 73.5 (C-3''), 70.2 (C-4''), 67.7 (C-5''), 106.8 (C-1'''), 83.4 (C-2'''), 76.7 (C-3'''), 87.7 (C-4'''), 66.5 (C-5'''), 104.9 (C-1'''), 78.5 (C-2'''), 77.6 (C-3'''), 74.8 (C-4'''), 65.7 (C-5'''), 68.7 (C-6''').

Acid hydrolysis of Compound 1

Compound **1** (80 mg) was dissolved in ethanol (15 mL) and refluxed with 10% H₂SO₄ (10 mL) on a water bath for 7 hr. The contents were concentrated and allowed to cool and the residue was extracted

with diethyl ether. The ethereal layer was washed with water and the residue was chromatographed over silica gel using CHCl₃:MeOH as solvent to give aglycone **1a**, which was identified as 5,7-dihydroxy flavone by comparison of its spectral data. The aqueous hydrolysate was neutralized with BaCO₃ and the BaSO₄ was filtered off. The filtrate was concentrated and subjected to paper chromatography using *n*-BAW (4:1:5) solvent system and the sugars were identified as D-xylose (R_f 0.26), L-arabinose (R_f 0.21) and L-rhamnose (R_f 0.35) (by Co-PC and Co-TLC).

Permethylation of Compound 1

Compound **1** (35 mg) was dissolved in DMF (50 mL) and treated with MeI (5 mL) and Ag₂O (25 mg) in a 150 mL round bottomed flask fitted with condenser and refluxed for 2 days. The contents were filtered and washed with DMF. The filtrate was concentrated under reduced pressure and hydrolysed with 10% H₂SO₄ to give methylated aglycone, identified as 5,7-dihydroxy flavone. The aqueous hydrolysate obtained after the removal of aglycone was neutralized with BaCO₃ and the BaSO₄ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography over Whatman filter paper No.1 using *n*-BAW (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-D-xylose, 2,3,4-tri-*O*-methyl-L-rhamnose and 2,4-di-*O*-methyl-L-arabinose by m.m.p. and co-pc.

Enzymatic hydrolysis of Compound 1

Compound **1** (40 mg) was dissolved in MeOH (25 mL) and hydrolyzed with equal volume of almond emulsin. The reaction mixture was allowed to stay at RT for two days and then filtered. The hydrolysate was concentrated and subjected to paper chromatography using *n*-BAW (4:1:5) as solvent system and aniline hydrogen phthalate as a spraying reagent, to show the presence of D-xylose (R_f 0.25). The proaglycone was dissolved in MeOH (20 mL) and hydrolyzed with equal volume of takadiastase enzyme at RT as usual procedure to yield aglycone identified as 5,7-dihydroxyflavone and the sugars were identified as L-rhamnose (R_f 0.36) and L-arabinose (R_f 0.21) (Co-PC and Co-TLC).

Aglycone 1a. m.p. 273-74°C, Mol. formula C₁₅H₁₀O₄, [M⁺] *m/z* 254. Anal. Found: C, 70.83; H,

3.90. Calcd for $C_{15}H_{10}O_4$: C, 70.86; H, 3.93%. UV-Vis (MeOH): λ_{\max} nm 265, 310, (+AlCl₃), 281, 323, 388, (+AlCl₃ / HCl), 282, 325, 385; IR (KBr): 3420 1655, 1590 cm^{-1} ; ¹H NMR (300 MHz, CDCl₃): δ 12.09 (s, 5-OH), 8.05 (2H, d, $J = 6.2$ Hz, H-2', H-6'), 7.35 (2H, d, $J = 6.0$ Hz, H-3', H-5'), 7.40 (1H, d, $J = 6.5$ Hz, H-4'), 6.94 (1H, s, H-3), 6.82 (1H, d, $J = 1.3$ Hz, H-8), 6.32 (1H, d, $J = 1.0$ Hz, H-6), δ 11.49 (1H, s, 5-OH), δ 8.65 (1H, s, 7-OH); ¹³C NMR (90 MHz, CDCl₃): δ 162.6 (C-2), 104.5 (C-3), 181.2 (C-4), 162.0 (C-5), 99.5 (C-6), 162.5 (C-7), 96.5 (C-8), 158.1 (C-9), 106.5 (C-10), 132.0 (C-1'), 126.2 (C-2'), 129.5 (C-3'), 132.2 (C-4'), 120.2 (C-5'), 126.5 (C-6').

Compound 2. m.p. 228-30°C, Mol. formula $C_{32}H_{38}O_{20}$, [M⁺] m/z 742. Anal. Found: C, 51.72; H, 5.10. Calcd for $C_{32}H_{38}O_{20}$: C, 51.75; H, 5.12%. UV-Vis (MeOH): λ_{\max} nm 217, 254, 275, 285, 385; IR (KBr): 3448 2892, 1730, 1658, 1620, 1568, 1448, 1386, 1349, 1210, 1078, 1025 cm^{-1} ; ¹H NMR (300 MHz, CDCl₃): δ 12.15 (1H, s, 5-OH), 8.65 (1H, s, 3'-OH), 8.70 (1H, s, 4'-OH), 6.75 (1H, d, $J = 2.4$ Hz, H-8), 7.19 (1H, d, $J = 2.1$ Hz, H-2'), 6.80 (1H, d, $J = 8.4$ Hz, H-5'), 7.15 (1H, d, $J = 8.2, 2.2$ Hz, H-6'), 5.32 (1H, d, $J = 1.8$ Hz, H-1''), 4.11 (1H, dd, $J = 1.6, 3.6$ Hz, H-2''), 3.84 (1H, dd, $J = 3.6, 9.0$ Hz, H-3''), 3.70 (1H, d, $J = 9.0$ Hz, H-4''), 3.55 (1H, m, 5''), 1.89 (3H, d, $J = 5.8$ Hz, H-6''), 4.95 (1H, d, $J = 7.2$ Hz, H-1'''), 3.32 (1H, m, H-2'''), 3.36 (1H, m, H-3'''), 3.34 (1H, m, H-4'''), 3.21 (2H, m, H-5'''), 5.22 (1H, d, $J = 7.6$ Hz, H-1'''), 3.22-3.70 (4H, m, H-2''', 3''', 4''', 5'''), 3.78 (1H, dd, $J = 12.0, 2.2$ Hz, H-6'''); ¹³C NMR (90 MHz, CDCl₃): δ 163.0 (C-2), 104.6 (C-3), 180.8 (C-4), 161.5 (C-5), 100.5 (C-6), 163.4 (C-7), 96.2 (C-8), 158.0 (C-9), 105.5 (C-10), 130.5 (C-1'), 117.5 (C-2'), 129.0 (C-3'), 132.5 (C-4'), 116.2 (C-5'), 123.9 (C-6'), 105.0 (C-1''), 78.4 (C-3''), 77.6 (C-5''), 75.6 (C-2''), 73.1 (C-4''), 62.9 (C-6''), 103.8 (C-1'''), 73.5 (C-2'''), 75.8 (C-3'''), 72.0 (C-4'''), 78.2 (C-5'''), 104.9 (C-1'''), 78.6 (C-3'''), 76.5 (C-5'''), 76.0 (C-2'''), 74.7 (C-4'''), 68.0 (C-6''').

Aglycone 2a. m.p. 310-12°C, Mol. formula $C_{15}H_{10}O_7$, [M⁺] m/z 302. Anal. Found: C, 59.55; H, 3.30. Calcd for $C_{15}H_{10}O_7$: C, 59.60; H, 3.31%. UV-Vis (MeOH): λ_{\max} nm 256, 275, 365, 370; IR (KBr): 3408 2892, 1730, 1651, 1600, 1508, 1448, 1366, 1309, 1200, 1071, 1020 cm^{-1} ; ¹H NMR (300 MHz, CDCl₃): δ 12.29 (1H, s, 5-OH), 10.70 (1H, s, 3-OH), 9.80 (1H, s, 7-OH), 9.45 (1H, s, 3'-OH), 9.39 (1H, s, 4'-OH), 6.25 (1H, d, $J = 2.2$ Hz, H-6), 6.62 (1H, d, $J =$

2.4 Hz, H-8), 7.65 (1H, d, $J = 2.1$ Hz, H-2'), 6.85 (1H, d, $J = 8.4$ Hz, H-5'), 7.05 (1H, d, $J = 8.2, 2.2$ Hz, H-6'); ¹³C NMR (90 MHz, CDCl₃): δ 162.5 (C-2), 130.3 (C-3), 179.8 (C-4), 161.5 (C-5), 100.5 (C-6), 163.4 (C-7), 96.2 (C-8), 158.0 (C-9), 104.5 (C-10), 128.5 (C-1'), 117.5 (C-2'), 129.0 (C-3'), 135.7 (C-4'), 116.2 (C-5'), 123.9 (C-6').

Compound 3. m.p. 240-42°C, Mol. formula $C_{33}H_{40}O_{20}$ [M⁺] m/z 756. Anal. Found: C, 52.36; H, 5.25. Calcd for $C_{33}H_{40}O_{20}$: C, 52.38; H, 5.29%. UV-Vis (MeOH): λ_{\max} nm 272, 385; IR (KBr): 3452, 1680, 2915, 1725, 1609, 1522, 1476, 1270, 1084, 842 cm^{-1} ; ¹H NMR (300 MHz, CDCl₃): δ 7.1 (1H, s, H-3), 6.56 (1H, d, $J = 1.2$ Hz, H-8), 7.50 (1H, d, $J = 2.3$ Hz, H-2'), 6.95 (1H, d, $J = 8.2$ Hz, H-5'), 7.56 (1H, dd, $J = 8.2, 2.3$ Hz, H-6'), 3.74 (3H, s, 6-OMe), 12.18 (s, 5-OH), 11.08 (s, 5-OH), 11.32 (s, 7-OH), 10.15 (s, 3'-OH), 10.17 (s, 4'-OH), 5.28 (1H, d, $J = 7.6$ Hz, H-1''), 3.68 (1H, m, H-2''), 3.80 (1H, m, H-3''), 3.74 (1H, m, H-4''), 3.97 (1H, m, H-5''), 4.28 (1H, m, H-6''), 5.48 (1H, d, $J = 1.9$ Hz, H-1'''), 4.35 (1H, dd, $J = 1.6, 3.2$ Hz, H-2'''), 3.82 (1H, dd, $J = 3.6, 9.0$ Hz, H-3'''), 3.68 (1H, d, $J = 9.0$ Hz, H-4'''), 3.88 (1H, m, 5'''), 1.66 (3H, d, $J = 5.6$ Hz, H-6'''), 5.19 (1H, d, $J = 2.0$ Hz, H-1'''), 4.52 (1H, d, $J = 2.1$ Hz, H-2'''), 3.58 (1H, m, H-3'''), 3.60 (1H, m, H-4'''), 3.85 (1H, m, H-5'''); ¹³C NMR (90 MHz, CDCl₃): δ 156.4 (C-2), 132.8 (C-3), 177.6 (C-4), 104.5 (C-5), 152.4 (C-6), 131.6 (C-7), 158.2 (C-8), 94.5 (C-9), 151.8 (C-10), 121.5 (C-1'), 116.4 (C-2'), 145.0 (C-3'), 148.5 (C-4'), 115.8 (C-5'), 121.6 (C-6'), 65.05 (6-OMe), 102.0 (C-1''), 71.89 (C-2''), 74.3 (C-3''), 69.8 (C-4''), 73.2 (C-5''), 64.5 (C-6''), 104.9 (C-1'''), 78.3 (C-3'''), 77.0 (C-5'''), 76.7 (C-2'''), 72.5 (C-4'''), 65.3 (C-6'''), 106.8 (C-1'''), 83.9 (C-2'''), 78.5 (C-3'''), 91.8 (C-4'''), 68.6 (C-5''').

Acid hydrolysis, permethylation and enzymatic hydrolysis of compound **2** and **3** were carried out by similar method as described for compound **1**.

Aglycone 3a. m.p. 226-27°C Mol. formula $C_{16}H_{12}O_7$ [M⁺] m/z 316. Anal. Found: C, 60.72; H, 3.75. Calcd for $C_{16}H_{12}O_7$: C, 60.75; H, 3.79%. UV-Vis (MeOH): λ_{\max} nm 285, 330; IR (KBr): 3425, 1655, 2925, 1705, 1610, 1516, 1475, 1275, 1075, 835 cm^{-1} ; ¹H NMR (300 MHz, CDCl₃): δ 7.1 (1H, s, H-3), 6.56 (1H, d, $J = 1.2$ Hz, H-8), 7.50 (1H, d, $J = 2.3$ Hz, H-2'), 6.95 (1H, d, $J = 8.2$ Hz, H-5'), 7.56 (1H, dd, $J = 8.2, 2.3$ Hz, H-6'), 3.74 (3H, s, 6-OMe), 11.98 (s, 5-OH), 9.70 (1H, s, 7-OH), 9.46 (1H, s, 3'-OH), 9.31

(1H, s, 4'-OH); ¹³C NMR (90 MHz, CDCl₃): δ 156.4 (C-2), 132.8 (C-3), 177.6 (C-4), 104.5 (C-5), 152.4 (C-6), 131.6 (C-7), 158.2 (C-8), 94.5 (C-9), 151.8 (C-10), 121.5 (C-1'), 116.4 (C-2'), 145.0 (C-3'), 148.5 (C-4'), 115.8 (C-5'), 121.6 (C-6'), 60.15 (6-OMe).

Antimicrobial Activity of Compounds

The antimicrobial activity of compound **1**, **2** and **3** were determined by Filter Paper Disc Diffusion Method²². The various bacterial species were first incubated at 45°C for 48 hr. The sterile filter paper discs (6mm) were soaked with standard antibacterial agent and various test samples and were dried at 50°C. The discs were then placed on soft nutrient agar (2%) petri plates previously seeded with suspension of each bacterial species. The diameter of zone of inhibitions were measured at 37 ± 1°C after 24 hr.

For antifungal activity, Sabourauds broth media²³ with 4% agar was used for the preparation of plates and incubated with spores and mycelium suspension of fungi obtained from one week old culture. The diameters of zones of inhibition were measured at 28 ± 1°C after 48 hr. The results are recorded in **Table I**.

Acknowledgement

Authors are grateful to Head, RSIC, CDRI Lucknow for recording various spectral data and elemental analysis and to Head, Department of Chemistry, Dr. H.S. Gour University Sagar (M.P.) for providing necessary laboratory facilities. One of the authors Dharmendra Kumar Satnami is thankful to UGC New Delhi for award of Rajiv Gandhi National Fellowship.

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