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A New Antifungal Flavone Glycoside from *Acacia arabica* Willd

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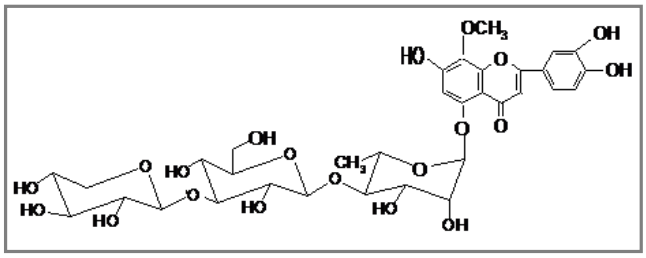
ABSTRACT

Acacia arabica willd. (Leguminosae) is commonly known as 'Babul' or 'Kikar' in Hindi. It is a moderate-sized, spiny evergreen tree found throughout the greater part of India. Its bark, gum and leaves are astringent to bowels. Its bark cures cough, bronchitis, diarrhoea, dysentery, biliousness, piles, leucoderma and urinary discharges. A new flavone glycoside 1, m.p.238-240°C, molecular formula $C_{33}H_{40}O_{20}$, $[M]^+$ 756 (FABMS) has been isolated from methanolic extract of the roots of Acacia arabica willd along with a known compound ***2****, kaempferol. Compound 1, characterized as 5,7,3'4'-tetrahydroxy-8-methoxyflavone-5-O-β-D-xylopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside by various color reactions, chemical degradations and spectral analysis. It was observed that it showed good activity against several fungi.*

Graphical Abstract



Structure of compound 1

Keywords: *Acacia arabica* willd, Leguminosae, Flavone glycoside, Antifungal.

INTRODUCTION

Acacia arabica (Lam.) Willd. (syn. *Acacia nilotica L.)* belongs to family Leguminosae/Fabaceae, commonly known as 'Babul' or 'Kikar' in Hindi. It is a moderate- sized, spiny evergreen tree, found throughout the dried parts of India [**1-4**]. Its bark, gum, pods, roots, and leaves are used in medication [**1, 5**]. Its bark is a good astringent [**6-8**] and used in cures cough, bronchitis, diarrhoea, dysentery, biliousness, piles, leucoderma, and urinary discharges [**8-10**]. Its roots are used for burning sensation and wound healing [**3**].

Earlier workers [**11-29**] have reported various constituents from this plant. The present paper deals with the isolation and structural elucidation of a new bioactive flavone glycoside, as 5,7,3'4'-tetrahydroxy-8-methoxyflavone-5-O-β-D-xylopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside **(1)** and a known compound kaempferol **(2)**, from a methanolic extract of the roots of *Acacia arabica*. Flavonoids are polyphenolic compounds, which represent one of the most interesting groups of biologically active compounds isolated from wide varieties of medicinal plants [**30-33**]. Several flavonoids have been considered valuable phytomedicines for different body system like urinary, digestive, cardiovascular, nervous and skin [**34**]. Flavonoids have been used extensively for the treatment of various diseases caused by microbes and proposed for the use against fungal pathogens [**35-36**].

MATERIALS AND METHODS

General: All the melting points were determined by thermoelectrical apparatus and are uncorrected. The IR spectra were recorded on Shimadzu 8201 PC spectrophotometer in KBr pellets; NMR spectra were obtained with a Bruker DRX-300 spectrometer operating at 300 MHz for ^{1}H using solvent $CDCl_3$ and ^{13}C using solvent DMSO-d_6; UV spectra were recorded on UV/VIS Perkin Elmer Lambda 15 spectrometer and Mass spectra on Jeol SX-102 (FAB) Mass spectrometer.

Plant Material: The roots of *Acacia arabica* were collected around the Sagar region and taxonomically authenticated by the Department of Botany, Dr. H.S. Gour University, Sagar (M.P.) India. A voucher Specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar (M.P.) India.

Extraction and Isolation: Dried powdered roots (3 kg) of the plant were extracted with ethanol in a Soxhlet apparatus for 8 hours. The ethanolic extract was concentrated under reduced pressure and was successively extracted with pet-ether (40-60^{0}C), $CHCl_3$, EtOAc, acetone, and MeOH. The methanol-soluble fraction of the ethanolic extract of the plant was concentrated at room temperature to yield (2.27 gm) and gave two spots on TLC examination, indicating it to be a mixture of compound 1 and 2. These compounds were separated by column chromatography over silica gel and purified by preparative TLC and studied separately. Compound 1 was recrystallized by methanol as light brown crystals (1.85gm).

Study of compound 1: MF $C_{33}H_{40}O_{20,}$ m.p. 238-240°C, $[M]^+$ 756 (FABMS); found (%); C 52.30, H 5.27, calcd. (%) for $C_{33}H_{40}O_{20}$; C 52.38, H 5.29; UV (MeOH) λ_{max}(nm) 253, 272 sh, 335; (+$AlCl_3$) 254, 272 sh, 335; (+$AlCl_3$-HCl) 257, 274sh, 335; (+NaOMe) 254, 272sh, 350; (+NaOAc) 273,272sh, 367 (+NaOAc/H_3BO_3) 252, 274sh, 360; IR ν_{max}^{KBr} (cm^{-1}); 3465 (-OH), 3010 (-C-H aromatic), 2940 (-C-H-saturated), 2875 (OMe), 1669 (>C=O α-β unsaturated), 1620 (aromatic ring system), 870, 830; 3484(-OH), 3015 (C-H aromatic), 2920 (C-H saturated), 2878 (-OMe), 1640 (>C=O α-β unsaturated), 1625 (aromatic ring system), 1250, 1130, 1090, 875, 830; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm); 3.80 (3H, s, OMe-8), : : 6.85 (1H, s, H-3), 6.80 (1 H, d, *J* 2.2 Hz, H-6), 7.75 (1 H, d, *J* 1.8 Hz, H-2'), 7.30(1 H, d, *J* 8.5Hz, H-5'), 7.70 (1H, dd, *J* 1.8, 8.5Hz,H-6'), 5.50 (1H, s, H-1"), 4.58 (1H, dd, *J* 3.8, 10.2 Hz, H-2"), 4.13 (1H, dd, *J* 3.8, 10.1 Hz, H-3"), 4.19 (1H, m, H-4"), 4.38 (2H,d, *J* 6.8 Hz, H-5"), 1.25 (3H, d, *J* 6.4 Hz, H-6"), 5.30 (1H, d, *J* 7.4 Hz, H-1'"), 3.82 (1H, dd, *J* 3.8,10.2 Hz, H-2'"), 3.70 (1 H, dd, *J* 3.8, 10.2 Hz, H-3'"), 3.51 (1H, dd, *J* 3.7,10.2 Hz, H-4'"), 3.67 (1H, m, H-5'"), 3.20 (2H, d, *J* 6.4 Hz, H-6'"), 4.90 (1 H, d, *J* 7.4 H-1'""), 3.52 (1H, dd, *J* 3.6, 10.1 Hz, H-2'""), 3.30 (1 H, d, *J* 6.4 Hz, H-3'""), 3.10 (1H, m, H-4'""), 3.40 (2H, m, H-5'""); ^{13}C-NMR (300 MHz, DMSO-d_6) δ (ppm); 158.9 (C-2), 105.3 (C-3), 178.5 (C-4), 162.2 (C-5), 99.4 (C-6), 166.2 (C-7), 96.1 (C-8), 158.0 (C-9), 105.5 (C-10), 122.2 (C-1'), 113.0 (C-2'), 148.3 (C-3'), 149.8 (C-4'), 115.5 (C-5'), 123.0 (C-6'), 102.4 (C-1"), 80.2 (C-2"), 71.1 (C-3"), 75.6 (C-4"), 68.2 (C-5"), 18.5 (C-6"), 101.9 (C-1'"), 78.5 (C-2'"), 70.3 (C-3'"), 71.5 (C-4'"), 77.5 (C-5'"), 69.5 (C-6'"), 100.7 (C-1'""), 75.1 (C-2'""), 76.6 (C-3'""), 72.1 (C-4'""), 68.2 (C-5'"") 56.9 (OMe-8) ; MS: (FABMS) *m/z*, 756 $[M]^+$, 624 [M^+-xylose moiety], 462 [M^+- xylose

moiety-glucose moiety], 316 [aglycone]$^+$, 315 [M$^+$-H], 301[M$^+$-CH_3], 298 [M$^+$-H_2O], 287 [M$^+$-H-CO], 285 [M$^+$-OCH_3] ,184,183,167,166, 155, 137, 134,109.

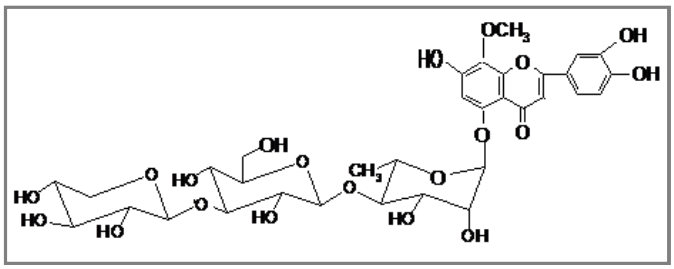


Figure 1. Compound 1.

Study of compound 2: It was analyzed for MF $C_{15}H_{10}O_6$ [M]$^+$ 286 found (%) C 63.10, H 3.17, Calcd (%) MF $C_{15}H_{10}O_6$ C 63.15, H 3.15 :UV(MeOH) λ_{max}(nm) 266, 294 and 360. IR (kBr) ν max (cm^{-1}); 3400,1650, 1608, 1545, 1508. ^{1}H NMR (300 MHz, $CDCl_3$); 6.08 (1H, d, J 2.0 Hz, H-6), 8.00 (2H, d, J 9.0 Hz, H-2' and H-6'), 7.90 (2H, d, J 9.0 Hz, H- 3' and 5'). ^{13}C NMR (500MHz, DMSO-d6); δ: 158.48 (C-2), 136.48 (C-3), 166.58 (C-4), , 160.97 (C-5), 97.85 (C-6), 163.67 (C-7), 95.88 (C-8), 57.10 (C-9),106.01 (C-10), 123.197 (C-1'), 127.28 (C-2' and C-6'), 116.84 (C-3' and C-5').

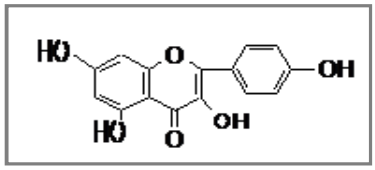


Figure 2. Compound 2.

Study of compound 3 (aglycone): MF $C_{16}H_{12}O_7$, m.p. 215-217^{o}C, 316 [M]$^+$ (FABMS). Found (%):C 60.77, H 3.73 calcd.(%) for $C_{16}H_{12}O_7$: C 60.78, H 3.80; UV (MeOH) λ_{max}(nm): 255, 270sh, 330; (+$AlCl_3$) 252, 270sh, 377; (+$AlCl_3$-HCl) 254, 271sh, 377; (+NaOMe) 254,271sh, 345 (+NaOAc) 275, 270sh, 330; (+NaOAc/H_3BO_3) 253, 270sh, 354; IR ν_{max}^{KBr} (cm^{-1}): 3465 (-OH), 3012 (-C-H aromatic), 2930 (-C-H-saturated), 2870 (OMe), 1660 (>C=O α-β unsaturated), 1620 (aromatic ring system), 870, 830; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm)), 3.80 (3H, s, OMe-8), : 6.85 (1H, s, H-3), 6.80 (1 H, d, *J* 2.2 Hz, H-6), 7.70 (1 H, d, *J* 1.8 Hz, H-2'), 7.30(1 H, d, *J* 8.5Hz, H-5'), 7.70 (1H, dd, *J* 1.8, 8.5Hz,H-6'), 7.64 (1H, dd, *J* 1.8, 8.5 Hz, H-6'); ^{13}C-NMR (300 MHz, DMSO-d_6) δ (ppm): 158.1 (C-2), 105.3 (C-3), 178.2 (C-4), 164 (C-5), 98.6 (C-6), 166.0 (C-7), 94.6 (C-8), 158.0 (C-9), 104.5 (C-10), 122.2 (C-1'), 113.0 (C-2'), 148.3 (C-3'), 149.8 (C-4'), 115.5 (C-5'), 123.0 (C-6'), 56.3 (OMe-8); MS: (FABMS) *m/z*: 316 [M]$^+$, 315 [M$^+$-H], 301[M$^+$-CH_3], 298 [M$^+$-H_2O], 287 [M$^+$-H-CO], 285 [M$^+$-OCH_3], 184,183,167,166, 155, 137, 134,109.

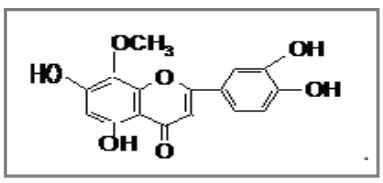


Figure 3. Compound 3.

Acid hydrolysis of compound 1: Compound 1 (50 mg) was dissolved in MeOH (50 mL) and refluxed with 10% ethanolic H_2SO_4 for 6 h. The reaction mixture was concentrated and allowed to cool and the residue was shaken with diethyl ether. The ethereal layer was washed with water and the residue was chromatographed over silica gel using MeOH:$CHCl_3$ (6:10) to afford compound 3, which was identified as 5,7,3'4'-tetrahydroxy-8-methoxyflavone by comparison with its known spectral

data. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination using *n*-BAW [4:1:5] as solvent and aniline hydrogen phthalate as spraying agent which showed the presence of D-glucose (R_f 0.18), D-xylose (R_f 0.25), and L-rhamnose (R_f 0.35).

Permethylation of compound 1: Compound 1 (100 mg) was refluxed with CH_3I (5 mL) and Ag_2O (30 mg) in DMF (50 mL) for two days and then filtered. The filtrate was hydrolyzed with 10% ethanolic H_2SO_4 for 6-7 h, to give the methylated aglycone, identified as 5-hydroxy-7,8,3',4'-tetramethoxy flavone and the methylated sugars which were identified as 2,3-di-*O*-methyl-L-rhamnose, 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-xylose (by Co-PC).

Enzymatic hydrolysis of compound 1: Compound **1** (25 mg) was dissolved in MeOH (25 mL) and hydrolyzed with almond emulsion at room temperature. The mixture was left for 48 hours and filtered. The proaglycone and hydrolysate were studied separately.

The hydrolysate was concentrated and subjected to paper chromatography using *n*-BAW (4:1:5) as solvent system, which showed the presence of D-xylose (R_f 0.25) and D-glucose (R_f 0.18), thereby confirming the presence of an β-linkage between D-xylose and D-glucose , as well as between D-glucose and proaglycone.

Enzymatic hydrolysis of **1** with almond emulsion liberated D-xylose first, followed by D-glucose and 5,7,3'4'-tetrahydroxy-8-methoxyflavone-5-O--α-L-rhamnopyranoside as a proaglycone, and confirming the presence of an β-linkage between D-xylose and D-glucose , as well as between D-glucose and L-rhamnose. The proaglycone was further hydrolyzed with takadiastase liberated L-rhamnose and aglycone, revealing the presence of a α -linkage between L-rhamnose and the aglycone

The proaglycone, on further hydrolysis with an equal volume of takadiastase by the same process as described above, liberated L-rhamnose(R_f 0.35)) and the aglycone, which confirmed the presence of an α -linkage between L-rhamnose and the aglycone.

Antifungal activity of the compound 1: For the antifungal activity of compound 1, Saboraud's [37] broth media with 4% agar was used for the preparation of plates and inoculated with the spore and mycilium suspension of fungi, obtained from 7 days old culture. The plates after inoculation were incubated at room temperature for 48 h and the zone of inhibition is reported in table 1.

Table 1. Antifungal activity of the compound 1

S. No.	Fungal species	Diameters of the zone of Inhibition (mm)*				
		Concentration of compound 1 (%)				Std**
		100	80	60	40	
1.	*Aspergillus niger*	8.4	6.2	2.0	-	17.8
2.	*Tricoderma viride*	18.2	16.0	14.2	9.5	23.5
3.	*Fusarium oxysporium*	12.8	8.9	5.0	2.2	22.1
4.	*Penicillium digitatum*	16.5	14.0	12.4	7.5	23.8

* The zone of inhibition (mm) taken as average of four determination direction.
** Griseofulvin (1000 ppm) used as standard antifungal agent.

RESULTS AND DISCUSSION

Compound 1 had a MF $C_{33}H_{40}O_{20}$, m.p. 238-240°C, $[M]^+$ 756 (FABMS), and as isolated from the methanolic extract of the roots. It gave Molisch and Shinoda tests [38] showing its flavonoidal glycosidic nature. Its IR spectrum showed absorption bands at 3465 (-OH), 3010 (-C-H aromatic), 2940 (-C-H-saturated), 2875 (OMe), 1669 (>C=O α-β unsaturated), 1620 (aromatic ring system), 870, and 830 cm^{-1}.

The bathochromic shifts of 15 nm with NaOMe in band **I** and 25 nm with $NaOAc/H_3BO_3$ revealed the presence of–OH groups at C-3' and C-4' position in compound **1**[39]. The bathochromic shift of 20 nm with NaoAc in band **II** confirmed the presence of –OH group at C-7 position [40]. In the ^{1}H-NMR spectrum of compound **1,** singlet at δ 3.80 was assigned to OMe-groups at the C-8 position. The signals at δ 7.75 (1H, d, *J* 1.6 Hz), δ 7.30 (1H, d, *J* 8.5 Hz) and δ 7.70 (1H, dd, *J* 1.6, 8.5 Hz) were assigned to H-2', H-5' and H-6', respectively [41]. Two singlets at δ 6.85 and 6.80 were assigned to H-3 and H-6 protons [40]. The anomeric proton signals at δ 5.50 (1H, s), δ 5.30 (1H, d, *J* 7.2Hz) and δ 4.90(1H, d, *J* 7.4 Hz) were assigned to H-1'', H-1''', H-1'''' of L- rhamnose, D-glucose, D-xylose, respectively. In ^{1}H-NMR spectrum coupling constants of $J_{1,2}$ 7.2 Hz and 7.4 Hz for the anomeric protons of glucose and xylose, respectively, confirmed the β configurations of D-glucose and D-xylose, while a broad singlet of anomeric proton of L-rhamnose, confirmed α configuration of L-rhamnose [42].

Acid hydrolysis of compound **1** with 10% ethanolic H_2SO_4 afforded aglycone **3**, molecular formula $C_{16}H_{12}O_7$, m.p. 215-217^{0}C, $[M]^+$ 316(FABMS), identified as 5,7,3'4'-tetrahydroxy-8-methoxyflavone by comparison of its spectral data (UV, IR, ^{1}H-NMR and ^{13}C-NMR) with literature values [43-45].

The aqueous hydrolysate, obtained after acid hydrolysis of compound **1**, was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination, using *n*-butanol, acetic acid and water (4:1:5) as solvent and aniline hydrogen phthalate as detecting agent. Sugars were identified as L-rhamnose (R_f 0.35), D- glucose(R_f 0.18) D-xylose (R_f 0.25) [46].

Quantitative estimation of sugars according to the procedure of Mishra and Rao [47], 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 form [48].

The relative locations of the sugar moieties in compound **1** were determined by Permethylation [49] followed by acid hydrolysis, yielded methylated sugars and methylated aglycone. The methylated aglycone was identified as 5-hydroxy-7,8,3',4'-tetramethoxy flavones which confirmed that glycosidation was involved at OH of C-5 position of aglycone [50]. The methylated sugars were identified as 2,3-di-*O*-methyl-L-rhamnose, 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-xylose (by Co-PC) (see experimental section). Therefore it was concluded that the C-1'''' of D-xylose was linked with C-3''' of D-glucose, C-1''' of D-glucose was linked C-4'' of L-rhamnose and C-1'' of L-rhamnose was attached with C-5 of aglycone. Glycosylation at C-5 position was further confirmed by comparing the ^{13}C-NMR spectral data of compound **1** and aglycone **3**, in which an up field shift of 1.8 ppm for C-5 signal and downfield shifts of 0.8 ppm, 1.0 ppm and 1.5 ppm were attributed for ortho related C-6 and C-10 and para related C-8 signals, respectively [51].

Enzymatic hydrolysis of **1** with almond emulsin liberated D-xylose first, followed by D-glucose and 5,7,3'4'-tetrahydroxy-8-methoxyflavone-5-O--α-L-rhamnopyranoside as a proaglycone, and confirming the presence of an β-linkage between D-xylose and D-glucose , as well as between D-glucose and L-rhamnose. The proaglycone was further hydrolysed with takadiastase liberated L-rhamnose and aglycone, revealing the presence of an α-linkage between L-rhamnose and the aglycone.

On the basis of the above evidence, the structure of compound **1** was characterized as 5,7,3'4'-tetrahydroxy-8-methoxyflavone-5-O-β-D-xylopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside.

Compound **2** was analyzed for molecular formula $C_{20}H_{18}O_5$, m.p. 238-240°C and $[M]^+$ 338 (FABMS). It was identified as kaempferol by comparison of spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with the reported literature values [52-54].

Compound **1** was tested for antifungal activity against various fungi. The results given in table 1 revealed that **compound 1** showed high antifungal activity against *Tricoderma viride* and *Penicillium digitatum* even on very dilute concentration. Therefore, It was cleared that compound **1** may potentially be used as therapeutic agent against diseases caused by these fungi.

APPLICATION

The new **compound 1:** 5,7,3'4'-tetrahydroxy-8-methoxyflavone-5-O-β-D-xylopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside, extracted from the roots of *Acacia Arabica* Willd. has significant antifungal activity and can be used as antifungal agent for diseases caused by fungi.

CONCLUSION

This research work conclude that the new **compound 1** extracted from the roots of *Acacia Arabica* has antifungal activity on the tested strains and can be used as a therapeutic agent for the diseases caused by tested fungi. Its spectral and analytical data would be of interest to researchers for characterization of chemical constituents and drug formulation. This paper provides a scientific basis for the concordance between popular practices and results.

In overview, this research will be helpful in the utilization of the full potential of Acacia Arabica. Now there is a need for deep biochemical and microbiological research to better understand its traditional therapeutic properties and development of an integrated approach to get full benefit from these potential resources in India.

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