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New Antifungal Constituent from the *Tricholepsis glaberrima* DC.

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A new flavone glycoside **1** m.p. 242-244 °C, m.f. $C_{35}H_{44}O_{20}$, $[M]^+$ 784 (FABMS), has been isolated from the methanolic extract of the flowers of the plant *Tricholepsis glaberrima* DC. alongwith a known compound **1a**, 7,3'-dihydroxy-6,4'-dimethoxy flavone. Compound **1** was characterized as a new flavone glycoside, 5,2'-dihydroxy-3,6,7-trimethoxy flavone-5-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-O-β-D-galactopyranoside by several colour reactions, spectral analysis and chemical degradations. Compound **1** showed antifungal activity against various fungi.

Key Words: *Tricholepsis glaberrima* DC., Compositae, Flavone glycoside, Antifungal activity.

INTRODUCTION

Tricholepsis glaberrima DC.[1-3] (N.O. Compositae) is commonly known as 'Brahmadandi' in Hindi and Sanskrit. It is found in West Rajputana, Mt. abu and central part of India. Ayurvedic system of medicine describes that the plant is hot and bitter, cure 'Kapha', 'Vata', inflammation, used in leucoderma and skin diseases. It is believed to be a nervine tonic and an aphrodisiac. It is also used in seminal debility. Earlier worker's[4-7] have reported the presence of various constituents from this plant.

In present paper, the isolation and structural elucidation of a new antifungal flavone glycoside; 5,2'-dihydroxy-3,6,7-trimethoxy flavone-5-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-O-β-D-galactopyranoside (**1**) alongwith a known compound 7,3'-dihydroxy-6,4'-dimethoxy flavone **1a** from the flowers of the plant are reported.

EXPERIMENTAL

All the melting points were determined on a thermoelectrical melting point apparatus and are uncorrected. The IR spectra were recorded in FTIR, KBr discs, ^1H NMR spectra were run at 300 MHz using TMS as internal standard and $CDCl_3$ as solvent. ^{13}C NMR spectra were recorded at 90 MHz using DMSO-d_6 as solvent. UV spectra were determined in methanol and mass spectra on a Jeol SX-102 mass spectrometer.

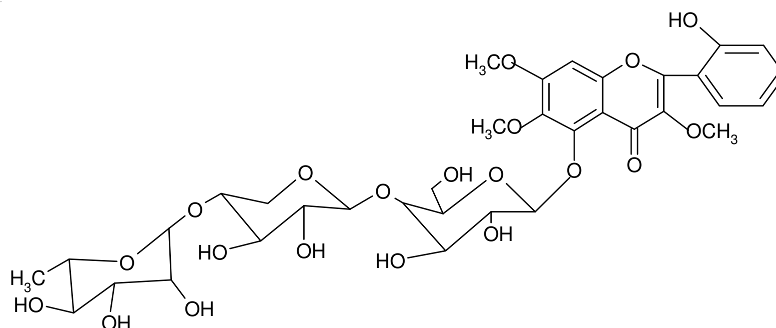
The flowers of *Tricholepsis glaberrima* DC., were collected from the Tamia region District-Chhindwara in India and were taxonomically authenticated by the Taxonomist, Department of Botany, Dr. H.S. Gour University, Sagar, India. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University Sagar, India.

Extraction and isolation: Air-dried powdered flowers (4 kg) of the plant were extracted with 95 % methanol in Soxhlet apparatus for 1 week. The total methanolic extract was successively partitioned with petroleum ether (40-60º), $CHCl_3$, CH_3COOCH_3, CH_3COCH_3 and MeOH. The acetone soluble fraction of the plant was concentrated under reduced pressure to give brown viscous mass. It gave two spots on TLC examination, indicating it to be mixture of two compounds. These were separated by column chromatography over silica gel column and purified by preparative TLC yielded compound **1** and **1a**. Compound **1** and **1a** were studied separately.

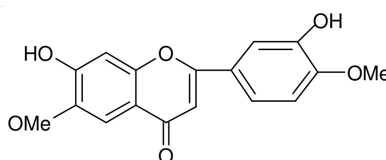
Study of compound 1: It had m.p. 242-244 ºC, m.f. $C_{35}H_{44}O_{20}$, $[M]^+$ 784 (FABMS); Found (%) C 53.54, H 5.60; Calcd. for $C_{35}H_{44}O_{20}$, C 53.57, H 5.61; IR (KBr, ν_{max}, cm^{-1}): 3245, 2910, 1655, 1632, 1598, 1210, 1145, 1068, 875, 812; UV, (MeOH λ_{max}, nm): 205, 273 355; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm); 6.54 (1H, s, H-8), 7.14 (1H, d, *J* 8.2 Hz, H-3'), 7.42 (1H, d, *J* 8.2 Hz, H-4'), 7.02 (1H, t, *J* 8.2 Hz, H-5'), 7.62 (1H, dd, *J* 8.2, 2.1 Hz, H-6'), 3.86 (3H, s, -OMe-3), 3.92 (3H, s, -OMe-6), 3.90 (3H, s, -OMe-7), 5.45 (1H, d, *J* 7.7 Hz, H-1''), 4.62 (1H, dd, *J* 3.7, 9.7 Hz, H-2''), 4.53 (1H, dd, *J* 9.7, 3.3, 1Hz, H-3''), 4.58 (1H, d, *J* 3.1 Hz, H-4''), 4.61 (1H, m, H-5''), 4.42 (2H, d, *J* 6.3 Hz, H-6'') 5.21 (1H, d, *J* 7.2 Hz, H-1'''), 4.45 (1H, dd, *J* 3.8, 10.2 Hz, H-2'''), 4.23 (1H, dd, *J* 3.8, 10.2 Hz, H-3'''), 4.21 (1H, m, H-4'''), 4.51 (2H, d, *J* 6.5 Hz, H-5'''), 5.16 (1H, d, *J* 1.8 Hz, H-1''''), 4.75 (1H, dd, *J* 3.6, 10.2 Hz, H-2''''), 4.78 (1H, dd, *J* 3.6, 10.2 Hz, H-3''''), 4.55 (1H, dd, *J* 3.8, 10.2, Hz, H-4''''), 4.25 (1H, m, H-5''''), 1.25 (3H, d, *J* 6.8, Hz, H-6''''); ^{13}C NMR (90 MHz, DMSO-d_6) δ (ppm.); 155.2 (C-2), 137.2 (C-3), 175.2 (C-4), 157.6 (C-5), 132.2 (C-6), 159.6 (C-7), 90.2 (C-8), 153.4 (C-9), 106.2 (C-10), 118.4 (C-1'), 155.8 (C-2'), 120.4 (C-3'), 133.2 (C-4'), 119.2 (C-5'), 129.1 (C-6'), 102.6 (C-1''), 71.5 (C-2''), 70.4 (C-3''), 71.1 (C-4''), 72.8 (C-5''), 62.5 (C-6''), 102.4 (C-1'''), 73.1 (C-2'''), 72.8 (C-3'''), 70.4 (C-4'''), 68.2 (C-5'''), 100.4 (C-1''''), 76.6 (C-2''''), 78.8 (C-3''''), 76.4 (C-4''''), 68.7 (C-5''''), 20.4 (C-6''''); MS (FABMS) m/z 784 $[M]^+$ 638 [M^+-rhamnose], 506 [M^+-rhamnose-xylose], 344 [M^+-rhamnose-xylose-galatose], 343, 329, 313, 301, 152, 121.

Acid hydrolysis of compound 1: 400 mg of compound **1** was dissolved in ethanol (25 mL) and refluxed with 20 mL of 10 % H_2SO_4 on water bath for 8-10 h. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether. The ethereal layer was washed with water and the residue was chromatographed over silica gel using $CHCl_3$:MeOH (6:4) to give compound **2**, which was identified as 5,2'-dihydroxy-3,6,7-trimethoxy flavone by comparison of its known spectral data. The aqueous hydrolysate was then neutralized with $BaCO_3$

and the $BaSO_4$ was 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 L-rhamnose (R_f 0.36), D-xylose (R_f 0.28) and D-galactose (R_f 0.16) (Co-PC).

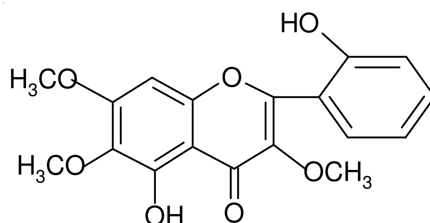


Compound 1



Compound 1a

Study of compound 2: It had m.p. 203-205 ºC, m.f. $C_{18}H_{16}O_7$, $[M]^+$ 344 (FABMS); Found (%); C 62.71, H 4.62; Calcd. for $C_{18}H_{16}O_7$, C 62.79, H 4.65. UV MeOH λ_{max}, nm; 207, 274, 358; IR (KBr, ν_{max}, cm^{-1}); 3248, 2912, 1653, 1630, 1592, 1212, 1142, 870, 815; ^1H NMR (300 MHz, $CDCl_3$) δ(ppm.); 6.50 (1H, s, H-8), 7.16 (1H, d, *J* 8.6 Hz, H-3'), 7.40 (1H, d, *J* 8.6 Hz, H-4'), 7.08 (1H, t, *J* 8.6 Hz, H-5'), 7.64 (1H, dd, *J* 8.6, 2.4 Hz, H-6'), 3.82 (3H, s, -OMe-3), 3.96 (3H, s, -OMe-6), 3.93 (3H, s, -OMe-7); ^{13}C NMR (90 MHz, DMSO-d_6) δ (ppm.); 155.8 (C-2), 137.6 (C-3), 175.8 (C-4), 152.4 (C-5), 132.6 (C-6), 159.2 (C-7), 90.6 (C-8), 153.3 (C-9), 106.7 (C-10), 118.2 (C-1'), 155.8 (C-2'), 120.5(C-3'), 133.1 (C-4'), 119.5 (C-5'), 129.4 (C-6'); MS; (FABMS) m/z, 344 $[M]^+$, 343, 329, 313, 301, 152, 121.



Compound 2

Permethylation followed by acid hydrolysis of compound 1: Compound **1** (30 mg) was refluxed with MeI (5 mL) and Ag_2O (40 mg) in DMF (60 mL) for 1 d and then filtered. The filtrate was hydrolyzed with 10 % ethanolic H_2SO_4 for 7-8 h. to give methylated aglycone, identified as 5-hydroxy-3,6,7,2',-tetra methoxy flavone and methylated sugars which were identified as 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-D-xylose and 2,3,6-tri-O-methyl-D-galactose (Co-PC and Co-TLC).

Enzymatic hydrolysis of compound 1: Compound **1** (10 mg) was dissolved in MeOH (25 mL) and hydrolyzed with equal volume of takadiastase enzyme at 25 °C in 150 mL round bottomed flask fitted with air condenser. The contents were left for 2 d and filtered. The proaglycone **3** and hydrolysate were studied separately.

The hydrolysate was concentrated and subjected to paper chromatography examination using *n*-BAW (4:1:5) solvent system which showed the presence of L-rhamnose (R_f 0.36) (Co-PC).

The proaglycone **3** (50 mg) was dissolved in MeOH (40 mL) and hydrolyzed with equal volume of almond emulsin enzyme. The reaction mixture was allowed to stay at room temperature for 2 d and filtered. The aglycone was identified as 5,2'-dihydroxy-3,6,7-trimethoxy flavone. The hydrolysate was concentrated and studied by paper chromatography examination using *n*-BAW (4:1:5) solvent system and aniline hydrogen phthalate as spraying reagent. The sugars were identified as D-xylose (R_f 0.28) and D-galactose (R_f 0.16) (Co-PC).

Study of compound 1a: It had m.p. 226-228 °C m.f. $C_{17}H_{14}O_6$ and $[M]^+$ 314 (FABMS); Found (%); C 63.78, H 4.56; calcd. for $C_{17}H_{14}O_6$, C 64.96, H 4.49. UV, MeOH λ_{max}, nm; 252, 291,336; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm.); 3.94 (12H, s, 4×-OMe), 6.64 (1H, s, H-3), 7.50 (1H, s, H-5), 6.85 (1H, s, H-8), 7.05 (1H, d, *J* 9.2 Hz, H-5'), 7.48 (2H, m, H-2', 6').

Antifungal activity of the compound 1: The antifungal activity of the acetone soluble fraction of compound **1** was tested against various fungi at different concentrations. For fungal activity, petri discs were placed on "Saboraud's broth media" with (4 %) agar was used for the preparation of plates and inoculated with spore and mycelium suspension of fungi obtained from 6 d old culture. The diameters of zone of inhibition were recorded at 27 ± 1 ºC after 48 h. The results are recorded in Table-1.

TABLE-1
ANTIFUNGAL ACTIVITY OF COMPOUND **1**

Fungal species	Diameters of zone of inhibition (mm)*					
	Compound **1** at concentration (%)					Standard**
	100	80	60	40	20	100
Trichoderma viride	16.4	12.2	9.6	7.5	5.8	23.0
Penicillium digitatum	10.6	6.5	3.6	–	–	22.0
Aspergillus niger	11.7	9.3	8.4	6.8	3.7	16.0
Penicillium notatum	8.5	5.8	2.6	–	–	13.0

*The zone of inhibition (mm) taken in different directions.
**Streptomycin used as standard antibacterial agent.
***Griseofulvin used as standard antifungal agent.

RESULTS AND DISCUSSION

The methanolic fraction of the flowers of the plant afforded a new compound **1** m.p. 242-244 ºC, m.f. $C_{35}H_{44}O_{20}$, $[M]^+$ 784 (FABMS). It gave Molisch and Sinoda test[8] showing its flavonoidal glycosidic nature. Its IR spectrum showed absorption bands at 3245, 2910, 1655, 1632, 1598, 1210, 1145, 1068, 875, 812 cm^{-1}. In UV spectrum absorption bands at 205, 273, 355 nm. suggesting a flavone skeleton with oxygen at C-3 position in compound **1**[9,10]. In ^1H NMR spectrum of compound **1**, a singlet at δ 6.54 was assigned to H-8. Two doublets at δ 7.14, δ 7.42 were assigned to H-3' and H-4'. A broad triplet at δ 7.02 was assigned to H-5'. One double doublet at δ 7.62 was assigned to H-6'. Three sharp singlets at δ 3.86, δ 3.92 and δ 3.90 confirmed the presene of three methoxy groups at C-3, C-6 and C-7 position. The anomeric proton signals at 5.78 (1H, d, *J* 8.2 Hz), 5.15 (1H, d, *J* 7.5 Hz) and δ 4.38 (1H, d, *J* 7.2 Hz) were assigned to H-1'', H-1''' and H-1'''' for D-galactose, D-xylose and L-rhamnose, respectively.

Acid hydrolysis of compound **1** with ethanolic H_2SO_4 (10 %) yielded aglycone **2**, m.p. 203-205 °C, m.f. $C_{18}H_{16}O_7$, $[M]^+$ 344 (EIMS), which was identified as 5,2'-dihydroxy-3,6,7-trimethoxy flavone by comparison of its spectral data with reported literature values[11]. The aqueous hydrolysate obtained after hydrolysis was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination showed the presence of L-rhamnose (R_f 0.36), D-xylose (R_f 0.28) and D-galactose (R_f 0.16) (Co-PC). Periodate oxidation[12] of compound **1** with sodium metaperiodate consumed 3.02 mole of periodate with the liberation of 1.04 moles of formic acid, suggesting that all the sugar were present in pyranose form.

The position of sugar moieties in compound **1** were established by its permethylation[13] followed by acid hydrolysis which yielded methylated aglycone identified as 5-hydroxy-3,6,7,2'-tetramethoxy flavone which showed that glycosylation was involved at C-5 positions in aglycone and methylated sugars were identified as 2,3,4-tri-O-methyl-L-rhamnose, 2,3-di-O-methyl-D-xylose and 2,3,6-tri-O-methyl-D-galactose (by Co-PC)[14] which showed that C-1'''' L-rhamnose was linked with C-4''' of D-xylose, C-1''' of D-xylose was linked with C-4'' of D-galactose, C-1'' of D-galactose was attached to C-5 position of aglycone. The inter linkages (1→4) between L-rhamnose and D-xylose as well as between D-xylose and D-galactose. It was further confirmed by ^{13}C NMR spectrum of the compound **1**.

Enzymatic hydrolysis of compound **1** with takadiastase enzyme liberated L-rhamnose (R_f 0.36) and proaglycone **3**, confirming the presence of α-linkage between L-rhamnose and proaglycone **3**. Proaglycone **3** on further hydrolysis with almond emulsin enzyme liberated D-xylose (R_f 0.28) first followed by D-galactose (R_f 0.16) and aglycone, suggesting the presence of β-linkage between D-xylose and D-galactose as well as between D-galactose and aglycone.

On the basis of above evidences, the structure of compound **1** was identified as 5,2'-dihydroxy-3,6,7-trimethoxy flavone-5-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-O-β-D-galactopyranoside.

Compound **1** was tested against various fungi and showed significant results. The results recorded in Table-1 showed that antifungal activity of compound **1** was found good against *Trichoderma viride* and *Aspergillus niger*.

Compound **1a** was analyzed for m.p. 226-228 °C m.f. $C_{17}H_{14}O_6$ and $[M]^+$ 314 (FABMS). It was identified 7,3'-dihydroxy-6,4'-dimethoxy flavone; by comparison of its spectral data with the reported literature values[14].

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