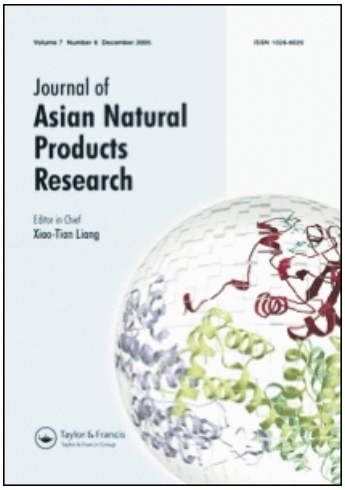

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A NEW BIOLOGICALLY ACTIVE FLAVONOL GLYCOSIDE FROM THE SEEDS OF *ABRUS PRECATORIUS* LINN

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A new flavonol glycoside m.f. $C_{29}H_{34}O_{16}$, m.p. 260–262°C, $[M]^+$ 638 (EIMS) was separated from the chloroform soluble fraction of the concentrated 80% methanolic extract of the seeds of *Abrus precatorius* (Linn). It was characterised as a new biologically active flavonol glycoside 7,3′,5′-trimethoxy-4′-hydroxy flavone-3-*O*-β-D-galactosyl-(1 → 4)-α-L-xyloside (1) by several colour reactions, spectral analysis and chemical degradations.

Keywords: *Abrus precatorius* (Linn); Leguminosae; Flavonoid

INTRODUCTION

Abrus precatorius (Linn) [1–3] belongs to the Leguminosae family and is commonly known as "Gungchi" in Hindi. It is distributed almost throughout India. Its fruit is used as tonic and useful in eye diseases. It is used in treatment of leucoderma, itching, skin diseases and wounds. Its leaves and fruits are useful in fevers and asthma. The roots of this plant are useful in curing snake-bite.

RESULTS AND DISCUSSION

A new compound **I** with m.f. $C_{29}H_{34}O_{16}$, m.p. 260–262°C, $(M)^+$ 638 (EIMS) was isolated from the chloroform soluble fraction of the 80% methanolic extract of the seeds of the plant. It gave characteristic reactions of flavonoid. Its IR spectrum showed absorption band at 3480–3520 cm^{-1} (–OH groups), 1624 (C=O), 2980 (C–H), 2865 (OMe), 1480–1010(*O*-gly) and 875 cm^{-1}. The ^{1}H-NMR spectrum of I showed three singlets at δ 3.92, 3.96, 3.96 which were assigned to three methoxy groups at C-7, C-3′, C-5′ positions and two aromatic

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TABLE I Antibacterial activity of compound (**1**)

S. No.	*Bacterial species*	*Diameters of zone of inhibition* (mm)*				
		Chloroform fraction	1:4	1:8	1:12	1:16
1.	(−) *Proteus vulgaris*	20.0	9.0	7.0	6.5	0
2.	(−) *Pseudomonas aeruginosa*	6.5	6.5	7.0	6.5	0
3.	(+) *Klebsiella pneumoniae*	13.5	12.5	10.5	10.5	7.5
4.	(+) *Escherichia Coli*	10.5	9.5	8.5	8.5	7.5
5.	(+) *Bacillus anthracis*	10.2	9.3	8.02	0	0
6.	(+) *Staphylococcus aureus*	21.0	18.5	15.5	15.5	10.5

* The zone of inhibition (mm) taken as average of four determination in four different directions and Whatman No. 1 filter paper (6 mm) were soaked with each sample tested for their activity at a concentration of 6 mg/ml of PBS (w/v).

protons as one singlet at δ 7.25 assigned to 2′ and 6′ positions. The anomeric proton signals at δ 5.53, δ 4.38 were assigned to H-1″ and H-1‴ of D-galactose, L-xylose.

The position of sugar moiety in compound **I** was established by per methylation [4] of **I** followed by acid hydrolysis. The aglycone was identified as 3,7,3′,4′,5′-penta-methoxy-flavone and methylated sugars were identified as 2,3-di-*O*-methyl xylose and 2,3,4,6-tetra-*O*-methyl galactose (Co-PC and Co-TLC) according to Petek [5], suggesting that the C-1‴ of galactose was linked with C-4″ of xylose and C-1″ of D-xylose was linked to C-3 of the aglycone. The interlinkage between sugars were further confirmed by ^{13}C-NMR spectrum. (See "Experimental section").

Acid hydrolysis of compound **I** with 9% methanolic HCl yielded aglycone 2, m.f. $C_{18}H_{16}O_7$, m.p. 172–175°C, and $(M)^+$ 344, which was identified as 7,3′,5′-tri methoxy 3,4′-dihydroxyflavonol by comparison of its spectral data with literature values [6].

The aqueous hydrolysate after the removal of the aglycone was neutralised with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography (PC) using nBAW (4:1:5) and sugars were identified as L-xylose, ($R_f = 0.29$), D-galactose ($R_f = 0.16$), Co-PC and Co-TLC). Periodate oxidation [7] of compound **I** further confirmed that both the sugars were present in pyranose form.

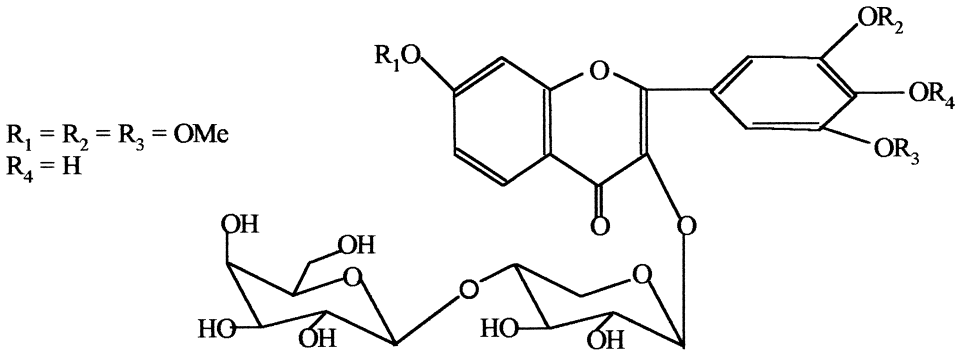
Enzymatic hydrolysis of compound **I** with almond emulsin liberated D-galactose confirming the presence of the β-linkage between galactose and xylose and on hydrolysis with Takadiastase liberated L-xylose, showing the presence of α-linkage between L-xylose and aglycone.

On the basis of above evidences, the structure of compound **I** was identified as a novel flavonol glycoside, 7,3′,5′-trimethoxy-4′-hydroxy flavone-3-*O*-β-D-galactosyl-(1 → 4)-α-L-xyloside.

TABLE II Antifungal activity of compound (**1**)

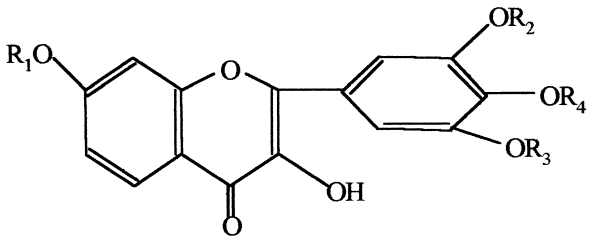
S. No.	*Fungal species*	*Diameters of zone of inhibition* (mm)*				
		Chloroform fraction	1:4	1:8	1:12	1:16
1.	*Penicillium digitatum*	6.0	5.0	3.0	0	0
2.	*Penicillium notatum*	4.0	3.5	0	0	0
3.	*Fusarium oxyporum*	15.5	12.5	10.5	10.5	9.5
4.	*A. niger*	9.5	8.5	8.5	7.5	6.5
5.	*Aspergillus fumigatus*	4.08	3.8	3.6	0	0

* The zone of inhibition (mm) taken as average of four determination in four different directions and Whatman No. 1 filter paper (6 mm) were soaked with each sample tested for their activity at a concentration of 6 mg/ml of PBS (w/v).



$R_1 = R_2 = R_3 = OMe$
$R_4 = H$

(1)



$R_1 = R_2 = R_3 = OMe$
$R_4 = H$

(2)

EXPERIMENTAL SECTION

General Experimental Procedure

Melting points are uncorrected. The IR spectra were recorded in KBr disc. ^{1}H-NMR spectra were run at 300 MHz using TMS as internal standard and $CDCl_3$ as solvent. ^{13}C-NMR spectra were run at 100 MHz using DMSO-d_6 as solvent.

Plant Material

The seeds of *A. precatorius* (Linn) were collected around Sagar region and was taxonomically authenticated by Taxonomist of Botany Department of Dr H.S. Gour University, Sagar. The voucher specimen was deposited in the Natural Products Laboratory, Department of Chemistry, Dr H.S. Gour University, Sagar (MP).

Extraction and Isolation

Air-dried and powdered seeds (3 kg) of *A. precatorius* were extracted with 90% in MeOH in a Soxhlet. The total methanolic extract was concentrated at room temperature which was successively extracted with pet-ether (60–80°C), $CHCl_3$, C_6H_6, MeOH, CH_3COCH_3, $CH_3COOC_2H_5$. The chloroform soluble fraction of the methanolic extract of the plant was

concentrated at room temperature to yield compound **I** as dark brown residue, which showed two spots on TLC examination using solvent system (*n*-BuOH–HOAC–H_2O, 4:1:5) which were separated by TLC and gave two compounds **A** and **B**. These compounds were purified by column chromatography. After purification amount of compound **B** was very less. Therefore it was not possible to analyse it further. Compound **A** yield m.p. 260–262°C, has the m.f. $C_{29}H_{34}O_{16}$, (Elemental analysis: Found C 54.53%, H 5.31% Calcd for $C_{29}H_{34}O_{16}$, C 54.54%, H 5.32%), IR (KBr) 3480–3520 cm^{-1} (–OH groups), 1624 (C=O), 2950 (C–H), 2865 (OMe), 1480–1010 (*O*-gly) and 875 cm^{-1}. ^{1}H-NMR (300 MHz-$CDCl_3$), δ 3.92 (3H, s, C-7 OMe), 3.96 (3H, s, C-3′ OMe), 3.96 (3H, s, C-5′ OMe), 7.58 (1H, d, $J = 8.0$ Hz H-5), 6.87 (1H, dd, $J = 8.0$, 2.0 Hz, H-6), 7.27 (1H, d, $J = 2.0$ Hz, H-8), 7.25 (1H, d, $J = 2.0$ Hz, H-2′), 7.25 (1H, d, $J = 2.0$ Hz, H-6′), 4.38 (1H, $J = 7.8$ Hz, H-1″), 3.28 (1H, dd, H-2″), 3.36 (1H, dd, H-3″), 3.38 (1H, H-4″), 3.16 (2H, dd, H-5″), 5.53 (1H, d, $J = 8.2$ Hz, H-1‴) 5.46 (1H, dd, $J = 8.2$, 9.8 Hz, H-2‴), 3.86 (1H, dd, $J = 3.6$, 9.8 Hz, H-3‴), 3.95 (1H, t, $J = 3.6$ Hz, H-4‴), 3.87 (1H, t, $J = 7.0$ Hz, H-5‴), 4.26 (2H, dd, $J = 5.8$, 11.3 Hz, H-6‴) ^{13}C-NMR (100 MHz, DMSO-d_6) 158.4 (C-2), 133.6 (C-3), 178.3 (C-4), 124.7 (C-5), 111.7 (C-6), 163.7 (C-7), 98.9 (C-8), 159.5 (C-9), 116.3 (C-10), 120.7 (C-1′), 105.2 (C-2′), 146.6 (C-3′), 136.2 (C-4′), 146.6 (C-5′), 105.2 (C-6′) 108.5 (C-1″), 74.8 (C-2″), 77.5 (C-3″), 70.6 (C-4″), 67.5 (C-5″), 101.4 (C-1‴), 74.6 (C-2‴), 73.5 (C-3‴), 70.7 (C-4‴), 74.8 (C-5‴), 63.8 (C-6‴).

Acid Hydrolysis of Compound I

The compound **I** (150 mg) was dissolved in MeOH (30 ml) and refluxed with 10 ml of 9% HCl on water bath for 7–8 h. The reaction mixture was allowed to cool and the residue was separated with Et_2O. The ethereal layer was washed with water and the residue was chromatographed over silica-gel G using *n*-hexane–benzene (6:4) as solvent to give compound **2**, $C_{18}H_{16}O_7$, m.p. 172–175°C, $[M]^+$ 344, (EIMS), (Elemental analysis: Found C 62.78%, H 4.64%: Calcd for $C_{18}H_{16}O_7$, C 62.79%, H 4.65%).

The aqueous hydrolysate after acid hydrolysis was neutralised with $BaCO_3$, and the $BaSO_4$ filtered off. After concentration it was subjected to PC (BAW 4:1:5) as developer and aniline hydrogen pthalate as detecting agent showed the presence of L-xylose ($R_f = 0.29$) and D-galactose ($R_f = 0.16$) (Co-PC and Co-TLC).

Permethylation Followed by Acid Hydrolysis of Compound I

Compound **I** was refluxed with MeI (5 ml) and Ag_2O (35 mg) in DMF (5 ml) for 24 h and then filtered. The filtrate was dried in vacuum and hydrolysed with 9% methanolic HCl for 7–8 h, yielded methylated aglycone identified as 3,7,3′,4′,5′-pentamethoxyflavone and methylated sugars, which were identified as 2,3-di-*O*-methylxylose and 2,3,4,6-tetra-*O*-methyl galactose according to Petek.

Periodate Oxidation of Compound I

Compound **I** was dissolved in MeOH and treated with sodium metaperiodate for one day. The liberation of formic acid, periodate were estimated by Jones method, which also showed that both the sugars were present in pyranose form.

Enzymatic Hydrolysis of the Compound I

The compound **I** (50 mg) was dissolved in MeOH (10 ml) and on hydrolysis with equal volume of almond emulsin at room temperature yielded D-galactose indicating the presence

of β-linkage between L-xylose and D-galactose and on hydrolysis with Takadiastase gave L-xylose showing the presence of α-linkage between aglycone and xylose.

Antimicrobial Study of Compound I

The antibacterial and antifungal activity of the methanol soluble of fraction of the extract of the plant were tested at its various dilutions using methylene glycol as solvent, at a concentration of 6 mg/ml of phosphate buffered saline pH = 6.8 (w/v). The various bacterial species were first incubated at 40°C for 48 h. The zone of inhibition were recorded at 34 ± 1°C after 48 h for bacteria and 33 ± 1°C after 24 h for fungi.

The antimicrobial activity was determined by Whatman No.1 filter paper discs (6 mm) method [8]. Paper discs were soaked with various samples tested and were dried at 50°C. The discs were then kept on soft nutrient Agar (2%) petri plates previously seeded with suspension of each bacterial species.

For the fungus, petri plates were placed on Sabouraud's broth [9] medium (1%). The zone of inhibition were expressed as an average of maximum diameter in four different directions. The various results are recorded in Tables I and II.

The result of Tables I and II showed that the antibacterial activity of the extract of the plant was found to fairly good against gram positive bacteria e.g. *staphylococcus aureus* and gram negative bacteria e.g. *Klebsiella pneumoniae* and *Escherichia coli* and antifungal activity of the extract was found to be more active against *A. niger* and *Fusarium oxyporum*.

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