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A NEW BIOACTIVE FLAVONE GLYCOSIDE FROM THE SEEDS OF *Puraria Tuberosa* DC

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ABSTRACT

A new bioactive flavone glycoside isolated from the seeds of *Puraria tuberosa* DC was identified as 5,7,3',5'–tetrahydroxy-4'-methoxy flavone-3'-0-α-L-rhamnopyranosyl (1→3)-0-β-D-galactopyranoside by various chemical degradations and spectral analysis.

Key words : Bioactive flavone, *Puraria Tuberosa* DC.

INTRODUCTION

Puraria Tuberosa DC (Leguminosae) is known as Siali or Bedarikand in Hindi. It is found in western Himalaya to Sikkim upto a height 4000 ft. in Kumaon, also in the hills of Punjab, Mount Abu, Bengal and South-India[1,2] The flower is cooling and afrodisiac, tonic galactagogue, diuretic. Its root is used as a demulcent and refrigerant in fever. According to Ayurvedic system of medicine, its flower cures leprosy, biliousness, diseases of the blood, "vata", burning sensation and urinary discharges.

EXPERIMENTAL

Plant Material : The seeds of *Puraria tuberosa* DC were collected from M/s United Chemicals and Allied Products, Calcutta and taxonomically authenticated by the Department of Botany, Dr. H.S. Gour University, Sagar (M.P.).

Extraction and Isolation : Powdered dried seeds (2 kg) of the plant were extracted with 95% EtOH in a Soxhlet extractor. The total ethanolic extract was concentrated under reduced pressure to yield brownish viscous mass, which was successively extracted with petroleum ether (60–80°C), benzene, chloroform, ethyl acetate, acetone and methanol. The ethyl acetate fraction was concentrated and subjected to paper chromatography examination. It gave two spots, suggesting it to be mixture of two compounds **I** and **Ia**, which were separated by TLC and purified by column chromatography over silica gel G. The quantity of compound **Ia** was found in very small quantity and therefore, it was rejected. Compound **I** was further purified by column chromatography, which was found to be homogeneous on TLC examination. It

was crystallized from MeOH to give light brown needles (1.34g) m.p. 266-267°C m.f. $C_{28}H_{32}O_{16}$ and [M] 624 (EIMS) (Found:C 53.87%, H, 5.12%, calcd. for $C_{28}H_{32}O_{16}$: C, 53.85%, H, 5.13%). IR (KBr) ν_{max} 3427, 2925, 2855, 1728, 1658 and 1618 cm^{-1}. UV λ_{max} (+MeOH) 268, 335 (+NaOAc): 254, 363 (MeOH-$AlCl_3$) 276, 345, 386.

^{1}H-NMR spectrum of compound **(I)** (300 MHz, $CDCl_3$) : δ 6.68 (1, s, H-3), δ 6.44 (1, d, J = 2.1, H-6), δ 6.69 (1, d, J = 2.1, H-8), δ 6.98 (1, s, H-2'), δ 6.98 (1, s, H-6'), δ 3.77 (3, s, 4'-OCH_3), δ 5.22 (1, d, J = 7.0 Hz, H-1-), δ 5.34 (1, s, H-1'"), δ 3.37-3.75 (6, m, protons of galactose), δ 4.67-4.91 (4, m protons of rhamnose), δ 1.54-1.79 (18, m, protons of sugar acetoxyls), δ 1.05 (3, d, J = 6.1, rha Me), ^{13}C-NMR (90 MHz, DMSO-d_6): δ 164.1 (C-2), 104.5 (C-3), 182.0 (C-4), 161.2 (C-5), 99.4 (C-6), 162.5 (C-7), 94.6 (C-8), 157.0 (C-9), 105.6 (C-10), 125.6 (C-1'), 105.9 (C-2'), 151.2 (C-3'), 139.1 (C-4'), 151.2 (C-5'), 105.9 (C-6'), 59.9 (-OMe), 97.8 (C-1"), 75.6 (C-2"), 76.5 (C-3"), 70.0 (C-4"), 73.7 (C-5"), 63.3 (C-6"), 108.7 (C-l'"), 76.1 (C-2'"), 79.3 (C-3'"), 74.0 (C-4'"), 64.2 (C-5'").

Acid hydrolysis of compound (I) : The compound **(I)** was hydrolyzed with 10% H_2SO_4 for 7 h at 100°C. The reaction mixture was allowed to cool and after the removal of the solvent, it gave an aglycone, which was separated by filtration. The aglycone **(II)** was recrytallised from MeOH as yellowish needles, m.f. $C_{16}H_{12}O_7$, m.p. 232-234°C and $[M]^+$ 316 (EIMS). UV λ_{max} (+MeOH) 268, 337, (+NaOAc) 256, 365.

The aqueous hydrolysate obtained after the acid hydrolysis of compound **(I)** was neutralised with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination (nBAW 4:1:5) showed the presence of L-rhamnose (R_f 0.36) and D-galactose (R_f 0.17) (by Co-PC and Co-TLC).

Permethyllation of compound (I) : The compound **(I)** was treated with CH_3I and Ag_2O in DMF at room temperature for one day, and then filtered. The filtrate was concentrated and hydrolysed with 10% H_2SO_4 for 6-7 h. After the usual work-up, it yielded methylated aglycone and methylated sugars. The methylated sugars were identified as 2,4,6-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-rhamnose (by Co-PC and Co-TLC) according to Petek[3].

Periodate oxidation of compound (I) : The compound **(I)** was dissolved in MeOH and treated with sodium metaperiodate for two days. The quantities of sodium metaperiodate consumed and formic acid liberated were estimated by Jone's method[4].

Enzymatic hydrolysis of compound (I) : Compound **(I)** was treated with 5 mL of aqueous enzyme takadiastase at 35°C for 24 h. It liberated L-rhamnose (R_f 0.36) and proaglycone. The proaglycone **was** hydrolysed with equal volume of almond emulsin solution and left at room temperature for 24 h, which yielded D-galactose (R_f 0.17) and aglycone.

Quantitative estimation of sugars: Quantitative estimation of sugars in the glycoside was done by Mishra and Rao procedure[5], which revealed that both the sugars were present in equimolar ratio (1:1).

Antimicrobial activity

The antimicrobial activity of the ethyl acetate soluble fraction of the plant was tested at its various dilutions using ethyl acetate as solvent at various concentrations. The various bacterial species were first incubated at 40°C for 48 h. The zone of inhibition were measured at 35 ± 1°C for two days. The antimicrobial activity was carried out by Whatman No. 1 filter paper disc (6 mm) method[6]. The various results are given in Table 1.

Table 1 : Antibacterial activity of plant extract

S. No.	Bacterial species	Ethyl acetate fraction	Inhibition (%) Concentration (mm)*				
			10 : 2	8 : 4	6.6	4.8	Std**
1.	(+) Bacillus coagulas	12.4	9.3	4.1	1.5	-	21.0
2.	(+) Staphylococcus aureus	16.4	14.1	10.3	8.1	6.6	19.9
3.	(-) Escherichia coli	11.4	7.4	23.4	1.7	-	18.4
4.	(-) Psedomonas aerugenosa	14.6	13.2	11.4	10.2	9.7	22.9

* The zone of inhibition (mm) taken as average of four determinations in four different directions and Whatman No. 1 Fitter papers (6 mm) were soaked with each sample tested for their activity

**Streptomycin was used as standard antibacterial agent.

RESULTS AND DISCUSSION

The ethyl acetate soluble fraction of the ethanolic extract of the seeds of *Puraria tuberosa* DC afforded a new bioactive compound **(I)**. It was analysed for m.f. $C_{26}H_{32}O_{16}$, m.p. 266-267°C and $[M]^+$ 624. It gave positive response to Molisch test for glycosidic nature and Shinoda test[7] for its flavonoid nature. A bathochromic shift of 28 nm in band I with NaOAc (relative to MeOH) suggested the presence of free hydroxy group at C-7 position and a bathocromic shift at 46 nm in band I with $AlCl_3$-HCl (relative to MeOH) showed the presence of -OH group at C-5 position in ring A. Its IR spectrum showed absorption bands at 3437 (–OH), 2925 (-CH), 2855 (-OCH_3) and 1619 Cm^{-1} (>C = O).

The compound **(I)** on acid hydrolysis with 10% ethanolic H_2SO_4 yielded an aglycone **(II)**, m.f. $C_{16}H_{12}O_7$, m.p. 232-234°C and $[M]^+$ 316 and sugars were identified as L-rhamnose (R_f 0.36) and D-galactose (R_f 0.17) (by Co-PC, Co-TLC). The aglycone **(II)** was identified as

5,7,3',5' – tetrahydroxy-4' – methoxy flavone by its spectral analysis (with reported literature, values[8]). A fragment at m/z 274 in the retro-Diels Alder fragmentation suggested the presence of two hydroxyl groups in ring A and another fragment at m/z 244 suggesting the presence of one methoxy group in ring B.

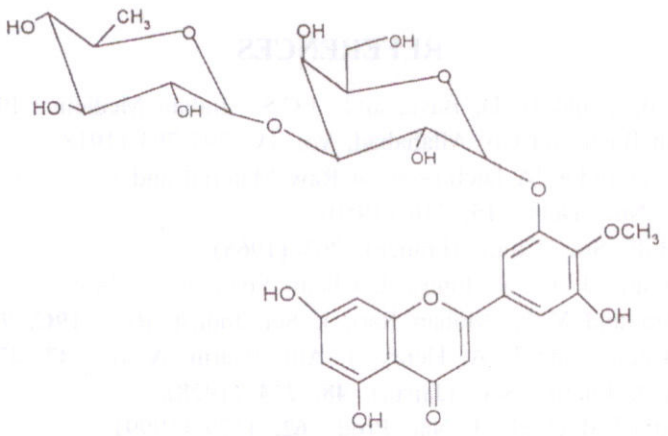
The position of the sugar residue in compound **(I)** was established by permethylation[9] of **(I)** followed by acid hydrolysis gave 3'–hydroxy-5,7,4',5'–tetramethoxy flavone and methylated sugars were identified as 2,4,6-tri-O-methyl-D–galactose and 2,3,4-tri-O-methyl-L-rhamnose according to Petek[3], which showed that C-3' position of the aglycone was involved in glycosilation.

Compound **(I)** on periodation oxidation[4] consumed 3.02 moles of periodate with the liberation of 1.20 moles of formic acid, which confirmed that the both the sugars were present in pyranose form.

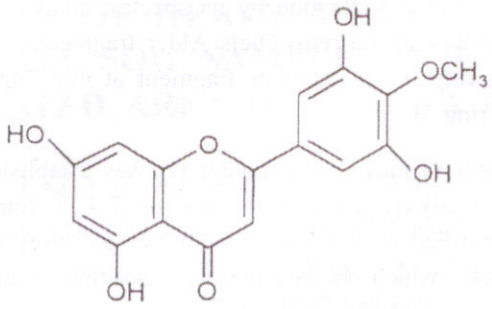
Quantitative estimation of sugars in the glycoside was done by Mishra and Rao method[8], which showed the presence of both the sugars in equimolar ratio (1 : 1).

Enzymatic hydrolysis of the compound **(I)** by takadiastase liberated L-rhamnose (R_f 0.36) and proaglycone confirming the presence of α-linkage between L-rhamnose and D-galactose and on furtfier hydrolysis by almond emulsin gave D-galactose (R_f 0.17) and aglycone suggesting the presence of β–linkage between D-galactose and aglycone.

On the basis of all the above evidences, the compound **(I)** was identified as 5,7,3',5'-tetrahydroxy-4'–methoxy-flavone-3'-O-α-L–rhamnopyranosyl (1→3)-O-β-D-galactopyranoside.



(I)



(II)

The results recorded in Table 1 showed that antibacterial activity of the plant extract was found to be more against gram +ve bacteria e.g. staphylococcus aureus and gram –ve bacteria e.g. Psedomonas aerugenosa.

Thus, above investigations revealed that the ethyl acetate soluble fraction of the ethanolic extract of the plant may be potentially used as therapeutic agent for diseases caused by these micro-organisms.

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