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NEW ANTIMICROBIAL ACTIVE BIS-ISOFLAVONOID GLYCOSIDE FROM THE ROOTS OF *CHLOROPHYTUM TUBER SUM* ROXB.

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ABSTRACT

Phytochemical investigation of the roots of *Chlorophytum tuberosumRoxb* resulted in the isolation of a new Bis-isoflavonoid glycoside characterized as Bis(8-methyl-4'-methoxy-7-O-α-L-rhamnopyranoside)I-5,II-5 Bisisoflavone. Its structure has been determined by various chemical reactions as well as by using spectroscopic techniques like UV, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and FABMS. The new isolated bisisoflavanoid was found to possess significant antimicrobial and anti fungal activity.

Keywords: Antimicrobial Activity, *Chlorophytum tuberosum* Roxb., Isoflavoniod Glycoside, Rhamnopyranoside

INTRODUCTION

The indigenous people of India use C*hlorophytum tuberosum* to cure various ailments along with its use in Aurveda, sidha and unani system of medicines **[1]**. Earlier workers have reported the isolation of polyphenolics, proteins, steroid from C*hlorophytum tuberosum* **[2]**. As a part of our investigation on the chemical constituents of medicinal plants here in we report the isolation and structure elucidation of a new antimicrobial active isoflavone glycoside from the ethanolic extract of the roots of *Chlorophytum tuberosum Roxb.*

MATERIALS AND MEHODS

General

Melting point was determined in sulphuric acid-bath and uncorrected. the UV spectra were recorded on a unichem UV-2 spectrometer, the IR spectra were recorded on a perkin- elmer 842 IR

spectrophotometer in KBr pellets, the ^{1}H and ^{13}C NMR spectra were determined on brucker 300 spectrometer at 300 MHz respectively (chemical shift in δ,ppm), instrument using DMSO-d_6 as solvent with TMS as an internal standard and HMBC experiments were carried out using conventional pulse sequence. The protonated FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6KV, 10MA) as the FAB gas. Si gel GF_{254} was used in TLC and spots were visualized by exposure over ammonia vapour and UV light as per requirement.

Plant Material

The roots of *Chlorophytum tuberosum* Roxb. were collected at Babai farm of Bundelkhand region of the district Jalaun. The specimen of the plants were deposited in herbarium of Bipin Bihari college and identified by staff of Botany department, Bipin Bihari College, Jhansi.

Extraction and Isolation

The roots of *Chlorophytum tuberosum* (Fam. Liliaceae) were dried and pulverized and (2.5kg) were extracted successively under reflux with ethanol at room temperature and dried to a thick syrupy dark brown colour mass (80gm). The ethanolic extract was washed subsequently with benzene, ether and ethyl acetate. The ethyl acetate soluble part after concentration under vacuum chromatographed over silica gel (60-120 mesh). It was charged on sephadex LH-20 column (30 x 2.5 cm.). The column was eluted with ethyl acetate and methanol in different ratio. Obtained methanol extract (90:10) exhibited one spot by paper chromatography was subjected on silica gel (60-120 mesh) column chromatography. The gradient elution of column with chloroform and methanol solvent system in different ratios, results yielded compound-**1** (75mg) from $CHCl_3$:MeOH (30:70) and monitored by TLC.

Acid Hydrolysis of Compound-1

20mg compound in 10 ml ethanol and 35 ml of 7% alc. H_2SO_4 was taken in 100 ml round bottom flask fitted with water condenser & refluxed on a steam bath for 7 – 8 hours. After usual work up, the contents were concentrated and allowed to cool and the resultant mixture was extracted with Et_2O and concentrated to obtain aglycone part.

The aqueous hydrolysate after neutralization with $BaCO_3$ and

precipitate of $BaSO_4$ was filtered off. The filtrate was decolourised and lypholized to concentrate . It was then examined over paper chromatography using n-BAW (4:1:5) as solvent system and aniline hydrogen phthalate as detecting agent, confirmed the presence of L-rhamnose (R_f=0.38) (Co-PC).

Antimicrobial Activity of Compound -1

The isolated bioflavonoid compound was screened for their antimicrobial activity by filter paper disc method **[15]**, which were expressed in terms of diameter of inhibition zone.

The Potato Dextrose Agar method **[15]** were used to check antibacterial and antifungal activities respectively. Standard antibacterial and antifungal were also screened under similar conditions for comparison.

RESULTS AND DISCUSSION

The compound was isolated as a brown amorphous powder, m.p.218±2 ^{0}C, responded all the characteristic test of isoflavonoids and sugar, gave pink colour with Na-Hg/HCl, shinoda test **[3, 4]** indicating glycosidic isoflavonoid nature which was further confirmed by its UV and IR spectral data **[5, 6]**. Its molecular formula $C_{46}H_{46}O_{16}$, was deduced from molecular ion peak at $[M+H]^+$ m/z 855, by protonated FABMS. Its high molecular weight and the magnitude of the molar extinction coefficient value of compound 6.56, 6.6 led to conclude dimeric nature of biisoflavinoid **[7]**.

Its IR spectrum showed a strong absorption bands at 2975 (C-H stretching), 2860(O-CH_3), 1625(C=O, non chelated), 1640(C=C, aromatic ring), 1392(C-Me bending), 1245(C-O-C stretching), 1183-1130 cm^{-1}(O-glycosidic linkage) **[8]**.

The ^{1}H NMR spectrum (DMSO-d_6 at 300MHz) demonstrated the presence of characteristic sharp singlet in the higher downfield region of C-2 proton at δ_H 7.90 (2H,S) was indicative of two isoflavonoids units in compound **[9]** .The appearance of a singlet of two protons at δ_H 6.63(2H,s) indicated free protons at C-6 of A-ring of 1&2 unit. Further the availability of resonance for two ortho coupled protons forming two sets of AA' BB' spin system at δ_H 7.57(4H,s) and 7.28(4H,s) for H-2', 6',&H-3', 5' positions respectively. In the higher upfield region a singlet of 6 protons at δ_H 2.09(2x3H,s) was ascribable for two methyl groups in compound which were affixed at C-8 of A-ring with the availability of ^{2}J

correlation **[10]**. A singlet at δ_H 3.90(2 x 3H,s) equivalent to 6 protons corroborated the presence of 2x OCH_3 groups at C-4' on B-ring of I&II unit **[11]**. The absence of chelated hydroxyl as well as hydrogen bonded proton in ^1H NMR spectrum of compound led to deduce interflavonyl linkage through C-5 (I,II) **[12]**.

The anomeric proton signal at δ_H 5.33(2H, br, s) (I&II) was assigned to H-1" of L-rhamnose, the remaining sugar protons resonated between δ_H 3.16-4.89, a triplet at δ_H 1.23(2x3H, t) in the upfield region of compound due to the two rhamnosyl methyl protons **[13]**.

In ^{13}C NMR spectrum , the chemical shift appeared in the upfield region at δC- 60.2 and18.4 each of 6 protons intensity confirmed the presence of two methoxyl and methyl groups , assigning two positions at C-4' and C-8 respectively of both the unit, with complementary data of HMBC. The inter-isoflavonoid linkage between I&II isoflavonoid unit at C-5 was determined by the presence of downfield shifting of quaternary carbon-carbon linkage at δC-161.3 ppm(I&II) for C-5 **[14]**. the anomeric carbon signal at δC- 98.7 (I, II) was assigned to C-1" of L-rhamnose sugar attached to C-7 of I&II unit of compound.Further in upfield region the presence of singlet at δC-17.5(I,II) was confirmatory of rhamnosyl methyl groups of sugar. Thus the C-7 site of the glycoside exhibited a shielding effect which confirmed that the attachment of sugar was at C-7 of the glycoside via a C-O-C linkage **[14]**.

Complete ^1H and ^{13}C NMR spectral assignments of compound was made with the aid of HMBC, HMQC experiments. The placements of CH_3 was established by the evidence of ^{2}] coupling correlation with quaternary carbon C-8 (δC- 104.1).On the basis of above discussion the structure of the compound was assigned as Bis (8-methyl-4'-metoxy-7-0-α-L-rhamnopyranoside) I-5, II-5 bisisoflavone **(Figure 1)**.

Table 1: ^{1}H and ^{13}C NMR Spectral Data of Compound -1

Position	Carbon(δ_H)	Proton (δ_H)	HMBC(C-H)
2 (I,II)	156.5	7.90(2H,S)	
3(I,II)	121.1	_	C-3,4
4(I,II)	177.8	_	
5 (I,II)	161.3	_	
6 (I,II)	98.7	6.63(2H,S)	
7 (I,II)	164.2	_	C-5,8
8 (I,II)	104.1	_	
9 (I,II)	157.3	_	
10 (I,II)	101.8	_	
1' (I,II)	121.1	_	
2' (I,II)	120.1	7.57 (2H,s)	
3' (I,II)	115.7	7.28 (2H,s)	C-3'
4' (I,II)	148.5	_	C-5'
5' (I,II)	115.7	7.28 (2H,s)	
6' (I,II)	120.1	7.57 (2H,s)	C-1',6'
CH_3 at C-8 (I,II)	18.4	2.09 (2x3H,s)	C-1',4'
OCH_3 at C-4' (I,II)	60.2	3.90 (2x3H,s)	C-8 C-4'
Rhamnose	98.7	5.33 (2H,br,s)	
1" (I,II)	70.6	4.89 (2H,d,J=6.0 Hz)	C-7
2" (I,II)	70.4	4.61 (2H,s)	
3" (I,II)	71.2	3.39 (2H,s)	
4" (I,II)	70.1	3.16 (2H,s)	
5" (I,II)			
(CH_3) 6" (I,II)	17.5	1.23 (2x3H, t)	

Table 2: Antibacterial Activity

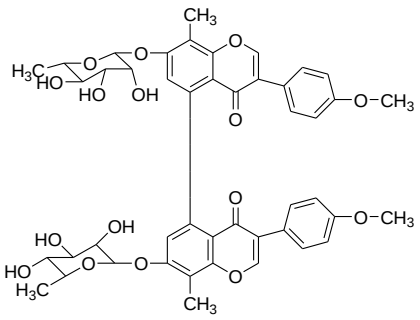
S. NO.	Test Bacteria	Diameter of inhibition	Control*
1	*E. Coli* at 250 µg/disc	12	18
2	*B.Subtilis* at 250 µg/disc	14	24

***Streptomysin Against gm^+ and gm^- Bacteria**

Table 3: Antifugal Activity

S. NO.	Test Fungi	Diameter of inhibition	Control*
1	*Rhizopus stolonifer*	8	17
2	*Aspergillus niger*	7	15
3	*Penicillium expansum*	5	11

***β naphthol(20.0ppm)**



4'Methoxy 8 Methyl 7-0-α L Rhahmnopyranoside bis Isoflavone

Figure 1

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