

A NEW FLAVONE GLYCOSIDE FROM THE FRUITS OF *Pithecellobium dulce* AND ITS ANTIMICROBIAL ACTIVITY.

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ABSTRACT

The isolation and spectral data of flavone glycoside 3' prenyl, apigenine, 7-rutinoside (7-O-alpha-L-rhamno pyransoyl (1-6) O-beta-D-glucopyranoside) from the fruits extracts (in various solvents) of *Pithecellobium dulce*. The various extracts were tested for their antimicrobial activity and it has been found to possess remarkable antifungal and antibacterial activities.

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KEY WORDS : *Pithecellobium dulce*; Fruit extracts, Flavone glycoside, Antimicrobial activities.

Introduction

Pithecellobium dulce^{1,3} is commonly known as jungle jalebi or vilayti lmal. It belongs to natural order Leguminoceae. It is important indigenous plant, cultivated through-out India and a native of tropical America. The plant possesses significant medicinal value. Earlier workers reported that significant number of compounds have been isolated from the plant⁴.

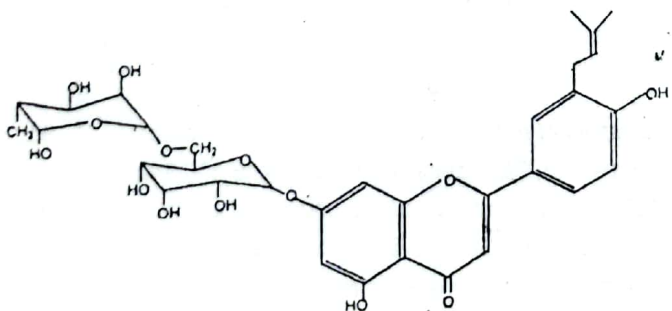
Materials and methods

The fruits of *Pithecellobium dulce* were collected locally (Bundelkhand region) and authenticated by department of botany. D.V. (P.G.) College, ORAI (U.P.). Air dried and crushed (3kg) fruits as pods of *Pithecellobium dulce*, were extracted with rectified spirit. The extract was concentrated under reduced pressure to give brown syrupy mass. Which was successively extracted with petroleum ether (60-80° C), benzene, chloroform ethylacetate, acetone and ethanol in soxhlet apparatus. After removal of solvents, syrupy mass were collected for each extract. These extracts were tested separately for antimicrobial activity. The ethyl acetate crude compound identified as flavoneglycoside⁵. This crude compound subjected to thin layer chromatography using ethylacetate: Acetone: acetic acid : water:: 5:3:1:1 (v/v). Finally the crude compound was subjected to silica-gel-G column

chromatography eluted by acetone-methanol in various proportions. Fraction acetone-methanol (2:8v/v) gave pale yellow compound. It was analysed for M.F.C₃₂H₃₈O₁₄, M.P. 128-129° C UV max: (MeOH) 268,353: (AlCl₃) 267,313,389.(AlCl₃+HCl) 270,316,400; (NaOMe)278,402; (NaOAc) 284,302, nm; IR⁵ bands (KBr): 3495 (-OH group), 1696 (α-β-unsaturated keto group), 1370, 1380 (Gemdimethyl group) cm⁻¹; ¹³C-NMR⁷ (DMSO-d₆/TMS): 164.1 (C-2), 103.1 (C-3), 181.5 (C-4), 161 (C-5), 100.1(C-6), 162.3(C-7), 95(C-8), 156.9(C-9), 105.3(C-10), 21.3(C-11), 122-2(C-12),130.3(C-13), 25.4(C-14), 17.5(C-15), 123.4(C-1'), 130.1(C-2'), 118.4(C-3') 162(C-4'), 118.7(C-5'), 130(C-6'), 90.1(C-1'') 73(C-2''), 76(C-3''), 69.3(C-4''), 75.6(C-5''), 66(C-6''), 100.1(C-1''') 70.1(C-2'''), 70(C-3'''), 71.8(C-4'''),67.8(C-5'''), 17.2(C-6'''), ¹H-NMR⁸ (400 MHz Me₂Co-d₃): δ 1.78(6H,s,2x-CH₃-13) δ 3.44 (2H,d,j 8.0Hz,-CH₂-11), δ 5.32 (1H,m,H-12), δ 5.91 (1H,d,H-1''), δ 6.23 (1H,d,j 2.0 Hz, H-6), δ 6.32(1H,d,j 8.5 Hz, H-8) 6.51(1H,s,H-3), δ 6.66 (1H,d,j 8.5 Hz H-5'), δ 7.71 (1H,d,j 2.0 Hz, H-6'), δ 7.79 (1H,d,j 2.0 Hz, H-2); MS[M⁺] 646, EIMS, M/z: 338,295,185,153.

The flavone glycoside on permethylation by Kuhn's procedure (MeI, DMF/Ag₂O) followed by acid hydrolysis yielded a glycone, tri-O-methyl-D-glucose and tri-O-methyl-L-rhamnose (by Co-Pc, Co-TLC). A glycone is soluble in ethylacetate and methanol. Its M.F. C₂₀H₁₈O₅, m.p.

Fig. : 1



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TABLE 1 : Antifungal activities of successive fruit extract of *Pithecellobium dulce*.

Fungal Species	Diameter of inhibition zone (mm)* in					
	pet.ether	Benzene	chloroform	Acetone	Ethyl Alcohol	Control**
1. <i>Bolryotrichum keratinophi</i>	-	-	8.0	8.5	9.0	19.0
2. <i>Chrysosporium tropicum</i>	8.5	10.5	18.0	12.0	11.0	16.5
3. <i>Keratinophyton terreum</i>	12.0	9.5	27.5	23.0	18.5	24.0
4. <i>Malbrachea pulchella</i>	8.0	-	8.5	12.5	10.5	14.5

TABLE-2 Antibacterial activities of successive fruit extract of *Pithecellobium dulce*.

Bacterial Species	Diameter of inhibition zone (mm)* in					
	pet.ether	Benzene	chloroform	Acetone	Ethyl Alcohol	Control**
1. <i>Bacillus anthracis</i>	18.0	15.5	18.5	22.5	24.0	36.0
2. <i>Bacillus pumilis</i>	24.5	31.5	37.5	18.5	15.5	37.0
3. <i>Staphylococcus albus</i>	19.5	15.0	16.5	14.5	22.5	22.5

*including the diameter of filter paper disc (6mm).

** Griseofulvin 1000 (ppm)

*** Acromycin and streptomycin (500ppm) against grampositive and gramnegative bacteria

103°C, UV max: (MeOH) 268,346; (AlCl₃) 272, 383, 401; (AlCl₃ + HCl) 271, 387, 403; (NaOMe) 280, 324, 398; (NaOAc) 284, 362 nm; IR bands (KBr): 3440, 1684, 1355, 1362cm⁻¹; ¹H-NMR (400 MHz, Me₂ Co-d₆) δ 1.75 (6H, s, 2 xCH₃), δ 3.4 (2H,d,J 8.0 Hz, CH₂-11), δ 5.36 (1H,m,H-12), δ 6.2(1H,d,j 2.0Hz, H-6) δ 6.36 (1H,d,j 2.0Hz, H-8) δ 6.54 (1H,s,H-3), δ 6.68 (1H,d,j 8.5Hz, H-5'), δ 7.74 (1H,d,j 2.0 8.5 Hz, H-6'), δ 7.82 (1H,d,j 2.0 Hz, H-2') MS [M⁺] EIMS, m/z : 283, 282, 185, 155, 125,110.

Aglycone on alkaline oxidation yielded, a compound of m.f. C₁₂H₁₄O₃, [M⁺] 206 EIMS m.p. 177°C and phloroglucinol. Compound C₁₂H₁₄O₃ identified as 2,2 dimethyl chroman, 6 carboxylic acid. Thus aglycone was identified as 3'-prenyl, 5,7,4'-trihydroxy flavone.

Enzymatic hydrolysis of flavone glycoside with enzyme Diastase gave one mole of L-rhamnose (Co-PC) and new glycoside identified as 3'-prenyl apigenine 7-O-β-D-glucopyranoside. This new glycoside was hydrolysed with almond emulsion, yielded aglycone and sugar as D-glucose.

The various solvents extracts of fruits were tested for antimicrobial activity. The antifungal activity was tested by filter paper disc method⁹, soft neutrient agar medium (2%) and petriplates (sterilized and previously

seeded with the test species) were used. Sabourad dextrose agar media¹⁰ was used to each antifungal activity.

Antibacterial activities against plant and human pathogenic bacteria were tested by "Oxide neutrient agar" and by Sikorowski *et al* method¹¹. The results were expressed in terms of inhibition zone.

Results and Discussion:

The flavone glycoside, isolated from an ethyl acetate extract of the fruits, was identified as 3' prenyl, apigenine, 7-rutinoside (7-O-α-L-rhamnopyranosyl(1-6) O-β-D-glucopyranoside), represented in figure. The fruit extracts of *Pithecellobium dulce* in various solvents were found to possess remarkable antifungal and antibacterial activities.

Table 1 showed that various solvent fruit extracts were found to possess promising antifungal activity. Chloroform extract showed better inhibition zones than control against chrysosporium tropicum and keratinophyton terreum. Acetone extract showed better result in comparison with control against keratinophyton terreum. From table 2 it is clear that chloroform fruit extract showed better results in comparison with control against *Bacillus pumilis*. Ethylalcohol fruit extracts showed better result in comparison with control against *staphylococcus albus*.

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