APPENDICES
This is to certify that Prof./Dr/Shri/Smt. Shivani Jain, of H.S.G. Univ., Sagar (MP), has presented a Paper/Poster in Section of Chemistry (LP:34) during the 89th Indian Science Congress held at Lucknow on January 6-7, 2013.

Date: 06/01/2013

Sectional President
(Signature)
Thirty eighth Annual Convention of Chemists, 2001

Venue: Jai Narain Vyas University, Jodhpur, Rajasthan
December 26-29, 2001

Organised by: Indian Chemical Society
92, Acharya Prafulla Chandra Road
Kolkata-700 009

E-mail: indchemical@psnl.net
Fax-Phone: 033-3503478

CONVENTION COMMITTEE
President
Prof. S. C. Ameta

Secretary
Prof. P. L. Majumder

Treasurer
Prof. P. Bhattacharyya

Members
Editors (3rd)
Prof. D. C. Mukherjee
Prof. M. Saha

Governor
Local Committee, Annual Convention of Chemists, 2001
Dr. K. M. Gangotri

Governor
Local Committee, Annual Convention of Chemists, 2000
Prof. A. K. Indrakaran

Honorary Secretary
Institution of Chemists
(India)

Scientists-in-Charge
Prof. B. Chakravarty
Prof. R. N. Mehrotra
Prof. (Mrs.) B. Talapatra
Dr. G. L. Tembe
Prof. R. K. Verma

To Whom It May Concern

This is to certify that Dr./Mr./Ms. A. K. MAJUMDER, has attended the Thirty-eighth Annual Convention of Chemists 2001, as a delegate and presented a paper (or PPT).

No TA/DA has been paid by the Society for the Purpose.

[Signature]
Professor P.L. Majumder
Honorary Secretary

[Date] 28/11/2001
5,6,4'-Trihydroxy-7,8,3'-Trimethoxy Flavone-4'-O-β-
D-Xylopyranosyl-(1→2)-O-α-L-rhamnopyranoside: A Novel
Bioactive Flavone Glycoside from the Mucuna prurieta Hook

R.N. YADAV** and SHIVANI JAIN
Natural Products Laboratory, Department of Chemistry
Dr. H.S. Gour University, Sagar-470 003, India

A novel flavone glycoside isolated from the seeds of Mucuna prurieta
and its structure was identified as 5,6,4'-tri hydroxy-7, 8, 3'-trimethoxy
flavone-4'-O-β-D-xylopyranosyl-(1→2)-O-α-L-rhamnopyranoside by
various chemical degradations and spectral analysis. This compound
showed antimicrobial and antifungal activity against various gram +ve
gram -ve bacteria and fungi.

INTRODUCTION

Mucuna prurieta1,2 belongs to family Leguminosae which is commonly known
as ‘Kivanchhi’ in Hindi. It is found in Punjab plains, Himalaya to Ceylon and
Burma. The Ayurvedic system of medicine describes that the fruit of this plant
is used as aphrodisiac tonic, cures blood diseases, biliousness and ulcers. The seeds
are aphrodisiac and cure “Vata”. Its roots are useful in the treatment of dysentery
and in uterine troubles.

“Yunani” system of medicine also describes that the leaves of this plant are
used as tonic and anthelmintic. and it is also useful in inflammations and
headache. The seeds are alexipharmic and cure scorpion sting and are also useful
in gonorrhoea.

RESULTS AND DISCUSSION

The acetone soluble fraction of the ethanolic extract of the seeds of M. prurieta
afforded a novel compound (I), m.p. 260–262°C., m.f. C_{20}H_{14}O_{16}, and [M]+ 638.
It gave positive response to Molisch test for glycosidic nature and Shinoda test3
for its flavonoid nature. A bathochromic shift at 48 nm (MeOH + NaOMe) showed
the hydroxy group at C-4' position and bathochromic shift at 25 nm in band I
with MeOH suggested the presence of OH group at C-5 position. Its IR spectrum
showed absorption bands at 3445 (−OH), 2971 (−CH), 2873 (−OCH3), 1626
(κ=O), 1495–1020 (−O-gly) and 870 cm⁻¹.

Compound I, on acid hydrolysis with 10% HCl yielded aglycone (II), m.f.
C_{18}H_{16}O_{8}, m.p. 270–71°C and [M]+ 360 and sugars were identified as L-rhamnose (Rf 0.35) and D-xylose (Rf 0.29) (Co-PC and Co-TLC). The aglycone (II)
was identified as 5,6,4'-trihydroxy-7,8,3'-trimethoxy flavone with reported litera-
ture3.

The 1H-NMR spectrum of I showed three singlets at δ 3.85, δ 3.95, δ 3.94
which were assigned to three methoxy groups at C-7, C-8 and C-3' positions and two aromatic protons as one singlet at δ 7.21 and δ 6.78 assigned to 2', 6' and 5' positions respectively. The anomeric proton signals at δ 5.35 (1H, br, s) and 4.37 (1H, d, J = 7.6 Hz) were assigned to H-1" and H-1'" of L-rhamnose and D-xylose respectively and a doublet at δ 1.03 was due to the rhamnosyl methyl group.

The EIMS at m/z 360 corresponded for aglycone fragment. A fragment at m/z 214 in the Retro-Diels Alder fragmentation suggested the presence of two hydroxyl groups and two methoxy groups in ring A and another fragment at m/z 132 and 149 suggesting the presence of the one methoxy group and one hydroxy group in the B ring.

Permethylion of I followed by acid hydrolysis yielded 3,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-xylose, according to Petek, suggesting that the C-1" of xylose was linked with C-2" of rhamnose and C-1" of rhamnose was attached to C-4' of aglycone. The interlinkage (1→2) between both sugars was further confirmed by its 13C-NMR spectrum.

Periodate oxidation of compound I consumed 3.01 moles of periodate with the liberation of 1.18 moles of formic acid confirming the presence of both the sugars in pyranose form.

Quantitative estimation of sugars in the glycoside was done by Somogyi's procedure which showed the presence of both the sugars in equimolar ratio (1:1).

Enzymatic hydrolysis of compound I with almond emulsion liberated D-xylose first showing the presence of β-linkage between D-xylose and rhamnose, and on hydrolysis with takadiastase liberated L-rhamnose confirming the presence of the α-linkage between the aglycone and L-rhamnose.

On the basis of above evidences, the structure of compound I was assigned as 5,6,4'-trihydroxy-7,8,3'-trimethoxy flavone 4'-O-β-D-xylopyranosyl-(1→2)-O-α-L-rhamnopyranoside.
EXPERIMENTAL

The seeds of *Mucuna prurieta* were collected around sagar region and was taxonomically authenticated by the 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 (M.P.).

**Extraction and Isolation**

The air-dried and powdered seeds of *Mucuna prurieta* were extracted with 95% rectified spirit in a Soxhlet extractor. The total extract was concentrated under reduced pressure to yield light brown viscous mass, which was successively extracted with petroleum ether (60-80°C), benzene, chloroform, ethyl acetate, acetone and methanol. The concentrated acetone-soluble fraction on TLC examination using CHCl₃ : MeOH : H₂O (9 : 6 : 3) and I₂ vapour as visualizing agent gave a single spot. Therefore it was purified by column chromatography over Si-gel-G and eluted with EtOAc : acetone (3 : 1). On evaporation of the solvent it gave an amorphous compound which was found to be homogeneous on TLC examination. It was recrystallised from methanol as light brownish crystals, m.p. 260-262°C, m.f. C₁₀H₁₅O₁₀. |M|⁺ 638 (EIMS); (Found: C 54.55%; H 5.32%; Calcd. for C₁₀H₁₅O₁₀; C, 54.56%; H, 5.35%; IR (KBr) ν max: 3445, 2971, 2873, 1626, 1495-1020, 870 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃) at δ 3.86 (3H, s, C-7 OMe); 3.90 (3H, C-8, OMe); 3.87 (3H, s, C-3', OMe); δ 6.76 (1H, s, C-1'''); δ 7.21 (2H, s, H-2', H-6'); δ 6.78 (1H, s, H-5'''); 5.34 (1H, br, s, H-1'''); 4.16 (1H, br, d, J = 3.6 Hz, H-2'''); 3.81 (1H, dd, H-3'''); 3.28 (1H, dd, H-4'''); 3.66 (1H, d, H-5'''); 1.03 (3H, d, J = 6.0 Hz, Rham-Me); 4.34 (1H, J = 7.8 Hz, H-1'''); 3.24 (1H, dd, H-2'''); 3.36 (1H, dd, H-3'''); 3.38 (1H, H-4'''); 3.12 (2H, dd, H-5'''); ¹³C-NMR (90 MHz, DMSO-d₆) 161.4 (C-2); 108.5 (C-3); 177.4 (C-4); 158.1 (C-5); 103.5 (C-6); 164.8 (C-7); 96.5 (C-8); 159.1 (C-9); 107.4 (C-10); 126.4 (C-1''); 104.1 (C-2'); 153.3 (C-3'); 141.1 (C-4'); 153.5 (C-5'); 104.5 (C-6'); 56.4 (OMe-7'); 59.2 (OMe-8); 57.1 (Ome-3'); 103.6 (C-1'''); 82.9 (C-2'''); 71.8 (C-3'''); 74.5 (C-4'''); 72.0 (C-5'''); 18.8 (C-6'''); 108.1 (C-1'''); 74.8 (C-2'''); 77.1 (C-3'''); 70.6 (C-4'''); 67.1 (C-5''').

**Acid Hydrolysis of Compound-I:** The compound I was refluxed with 7% ethanolic H₂SO₄ for 8-10 h at 100°C. The contents were allowed to cool and after the removal of the solvent yielded an aglycone II, which was separated by
filtration. The aglycone (II) was recrystallised from EtOH as light brownish crystals, m.p. 270–271°C, [M]+ 360 (EIMS). UV, λ_{max} (nm); (+MeOH) 250 sh, 292, 343; (+NaOMe) 259, 392; (+AlCl_3) 241 sh, 261, 306, 380; (+AlCl_3) 241 sh, 261, 306, 380; (+AlCl_3/HCl) 240 sh, 259, 305, 369; (+NaOAc) 296, 310, 341, 392; (NaOAc/H_2BO_3) 296 sh, 333.

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 (n-BAW : 4 : 1 : 5) confirming the presence of L-rhamnose (R_f 0.35) and D-xylene (0.29) (by Co-PC and Co-TLC).

Permutation of Compound I: Compound I was treated with CH_3I and Ag_2O in DMF at room temperature for 24 h and then filtered. The filtrate was concentrated in vacuum and hydrolysed with ethanolic H_2SO_4 for 8–10 h yielding methylated aglycone identified as 5,6,7,8,3',4'-hexamethoxy flavone and methylated sugars, which were identified as 3,4-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-D-xylene according to Petek.7

Periodate Oxidation of Compound I: The compound I was dissolved in MeOH and treated with sodium metaperiodate for two days. The liberation of formic acid and consumed periodate were estimated by Jone’s method,8 which showed that both the sugars were present in pyranose form.

Microbial Activity of Compound I: The acetone soluble fraction of the ethanolic extract of the plant was tested for antibacterial and antifungal activity at its different dilutions using ethylene glycol as solvent, at a concentration of 6 mg/mL of phosphate buffered saline (w/v). The different bacterial species were first incubated at 40°C for 48 h. The zones of inhibition were recorded at 37 ± 1°C after 48 h for bacteria and at 36 ± 1°C after 24 h for fungi.

The antimicrobial activity was determined by Whatman No. 1 filter paper discs (6 mm) method10. Paper discs were soaked with various samples tested and were dried at 50°C. The discs were then placed 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 broth11 medium (1%). The zones of inhibition were expressed as an average of maximum diameter in four different directions. The various results are recorded in Tables 1 and 2.

The results recorded in Tables 1 and 2 showed that the antibacterial activity of the extract of the plant was found to be fairly good against gram +ve bacteria, e.g., Bacillus anthracis and gram –ve bacteria, e.g., Proteus vulgaris, Salmonella newport and was found to retain its activity even at dilution of 1 : 16. Antifungal activity of the plant extract was found to be more active against Aspergillus fumigatus and Penicillium notatum.

Thus the above investigations revealed that the acetone-soluble fraction of the ethanolic extract of the plant may potentially be used as therapeutic agent for diseases caused by these microorganisms.
TABLE-1
ANTIBACTERIAL ACTIVITY OF COMPOUND I

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Bacterial species</th>
<th>Diameters of zone of inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetone fraction 1:4</td>
</tr>
<tr>
<td>1.</td>
<td>(-) Klebsiella pneumoniae</td>
<td>9.5</td>
</tr>
<tr>
<td>2.</td>
<td>(+) Streptococcus agalactiae</td>
<td>21.5</td>
</tr>
<tr>
<td>3.</td>
<td>(-) Proteus vulgaris</td>
<td>18.5</td>
</tr>
<tr>
<td>4.</td>
<td>(+) Bacillus anthracis</td>
<td>8.5</td>
</tr>
<tr>
<td>5.</td>
<td>(-) Salmonella newport</td>
<td>25.0</td>
</tr>
<tr>
<td>6.</td>
<td>(-) Pseudomonas aeruginosa</td>
<td>8.5</td>
</tr>
</tbody>
</table>

TABLE-2
ANTIFUNGAL ACTIVITY OF THE COMPOUND I

<table>
<thead>
<tr>
<th>S. No</th>
<th>Fungal species</th>
<th>Diameters of zone of inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetone fraction 1:4</td>
</tr>
<tr>
<td>1.</td>
<td>Aspergillus niger</td>
<td>9.5</td>
</tr>
<tr>
<td>2.</td>
<td>A. fumigatus</td>
<td>8.0</td>
</tr>
<tr>
<td>3.</td>
<td>Microsporum gypseum</td>
<td>8.0</td>
</tr>
<tr>
<td>4.</td>
<td>Penicillium notatum</td>
<td>9.0</td>
</tr>
<tr>
<td>5.</td>
<td>Fusarium oxyporum</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*The zone of inhibition (mm) taken as average of four determinations in four different directions and Whatmann 1 (6 mm) were soaked with each sample tested for their activity at a concentration of 6 mg/mL of PBS (w/v).

ACKNOWLEDGEMENT

Thanks are due to Head, RSIC, CDRI, Lucknow for spectral analysis and Prof. S.P. Banerjee, Head, Dept. of Chemistry, Dr. H.S. Gour University, Sagar (M.P.) for providing laboratory facilities.

REFERENCES


(Received: 22 March 2001; Accepted: 4 May 2001) AJC-2340
To,

Editor-in-Chief
Professor Atta-ur-Rahaman
H.E.J. Research Institute of Chemistry
University of Karachi
Karachi - 75270
Pakistan

Respected Sir,

Herewith I am sending 3 copies of manuscript entitled "A Novel Bio-Active Flavonol Glycoside from Termanus Labialis Spreng" along with floppy for favour of publication in your esteemed journal.

Kindly do the needful
Thanking you.
With regards.

Yours sincerely,

(By R.N. Yadava)

Encl.:

(1) Three copies of the manuscript.
(2) Floppy.
A NOVEL BIO-ACTIVE FLAVONOL GLYCOSIDE FROM *TERAMNUS LABIALIS SPRENG.*

R.N. Yadava*, Shivani Jain
Natural Products Laboratory, Department of Chemistry,
Dr. H.S. Gour University, Sagar (M.P.) 470 003 INDIA

A novel biologically active flavonol glycoside m.f. C_{26}H_{28}O_{17}, m.p. 216-217° C was isolated from the chloroform soluble fraction of the rectified spirit extract of the stems of *Teramnus labialis*. Its structure was characterised as 3,5,7,3',4',5'-hexahydroxy-flavonol-3-O-β-D-glucopyranosyl(1→3)-O-α-L-arabinopyranoside by several spectral and chemical analysis. This compound (1) showed antimicrobial activity against various gram *+ve* and gram *-ve* bacteria and fungi.

*Keywords*: *Teramnus labialis* Spreng; Leguminosae; Flavonol glycoside; 3,5,7,3',4',5' - hexahydroxy-flavonol-3-O-β-D-glucopyranosyl (1→3)-O-α-L-arabinopyranoside; Antimicrobial activity.

INTRODUCTION

*Teramnus labialis* Spreng [1,2] (Leguminosae) is known as ‘Mashoni’ in Hindi and is distributed in throughout India, Ceylon and the tropies generally Natal. The fruit is used as antipyretic. It is useful in inflammation, biliousness, bronchitis, paralysis, rheumatism and affection of the nervous system.

Corresponding author: Tel. + 91-07582-26405  E-mail - rnyadava@rediffmail.com.
The present paper deals with the isolation and structure elucidation of a novel bioactive flavonol glycoside, which was characterised as 3,5,7,3',4',5'-hexahydroxy-flavonol-3-O-β-D-glucopyranosyl (1→3)-O-α-L-arabinopyranoside (1) by spectral and chemical analysis. The compound (1) showed antimicrobial activity against variously gram +ve and gram -ve bacteria and fungi.

RESULTS AND DISCUSSION

The chloroform soluble fraction of the rectified spirit extract of the stems of Teramnus labialis on column chromatography over silica gel 'G' using eluants CHCl₃ : MeOH : H₂O (15:8:3) gave a novel natural product (1), m.p. 216-217⁰ C, m.f. C₂₆H₂₄O₁₇, [M]+ 612 (EIMS). It gave positive response to Molisch test and Shinoda test [3]. The IR spectral data revealed the presence of a hydroxyl group (3401 cm⁻¹), carboxylic group (1656 cm⁻¹) and double bonds (1608 cm⁻¹).

The compound (1) on acid hydrolysis with 10% HCl gave an aglycone (2) m.f. C₁₅H₁₀O₇, m.p. 278-279⁰ C, [M]+ 318 (EIMS) and sugars which were identified as D-glucose and L-arabinose (by Co-PC and Co-TLC). The aglycone was identified as 3,5,7,3',4',5'-hexahydroxy flavonol by comparision of its spectral data with known reported literature [4].

The 'H-NMR spectrum of compound (1) showed two aromatic proton signals at δ 6.92 which were assigned to H-2', H-6', respectively and two signals at δ 6.17 and 6.36 due to H-6 and H-8 proton. The anomeric sugar
protons observed at $\delta$ 5.16 (d, $J = 7.4$ Hz) and $\delta$ 5.25 (d, $J = 7.4$ Hz) were attributed to the H-1" proton of L-arabinose and H-1" of D-glucose.

The $^{13}$C-NMR spectra of (1) showed twenty six carbon resonances. In the $^{13}$C-NMR spectrum of (1), three signals at $\delta$ 103.9, 66.3 and 62.6 were assigned to arabinopyranosyl C-1", C-4" and C-5" respectively [5]. The signals, at $\delta$ 78.7 was assigned to the arabinosyl C-3 carbon and the down field shift of arabinosyl C-3 and the concomitant upfield shift of the arabinosyl C-2 provided evidence that the glucopyranosyl anomeric carbon was attached to C-3 of the arabinopyranoside moiety. $^{13}$C-NMR spectrum of (1) further confirmed the presence of two sugar moieties by exhibiting two anomeric signals at $\delta$ 103.9 and $\delta$ 102.5 assigned to C-1" of arabinose and C-1" of glucose respectively.

Permethylated (1) followed by acid hydrolysis gave methylated aglycone identified as 5,7,3',4',5'-pentamethoxy-3-hydroxy flavonol compare by spectral data with reported literature (6) and methylated sugars characterized as 2,3,4,6-tetra-O-methyl-D-glucose(7), and 2,4-di-O-methyl-L-arabinose (Co-PC and Co-TLC). Thus confirming that C-3 of arabinose is linked to C-1 of glucose.

Periodate (8) oxidation of compound (1) consumed 3.01 moles of periodate with the liberation of 1.15 moles of formic acid suggesting that both the sugars were present in pyranose form.

Enzymatic hydrolysis of compound (1) with equal volume of almond emulsin liberated D-glucose and proaglycone showed the presence of $\beta$-linkage between D-glucose and L-arabinose and on hydrolysis with
Takadiastase liberated L-arabinose confirming the presence of α-linkage between aglycone and L-arabinose.

Thus, the structure of compound (1) was elucidated as 3,5,7,3',4',5'-hexahydroxy-flavonol-3-O-β-D-glucopyranosyl (1→3)-O-α-L-arabinopyranoside.

**MICROBIAL ACTIVITY OF COMPOUND (1)**

The chloroform soluble fraction of the compound (1) was tested for antibacterial and antifungal activity. The results given in Tables I and II showed a wide variation of antibacterial and antifungal activity. The antibacterial activity of the extract of the plant was found to be fairly good against gram +ve bacteria e.g. *Streptococcus pyogenes, Staphylococcus aureus* and was found to retain its activity even at dilution of 1:5. It is evident that the plant extract was found to be more potent against gram +ve group of bacteria in general. Antifungal activity of the extract was found to be more active against *Trichoderma viride, Helminthosporium oryzae* and *Botrytis cinerea* and was found to retain its activity even at dilution of 1:5.

The investigations thus revealed that the chloroform fractions of the compound (1) of *Teramnus labialis spreng* may potentially be used as therapeutic agent diseases caused by these microorganisms.
EXPERIMENTAL

Melting points were obtained on Reichert microscope hot-stage apparatus and are uncorrected. UV spectra were determined in MeOH and IR spectra recorded in KBr discs, $^1$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 90 MHz using DMSO-d$_6$ as of as solvent.

EXTRACTION AND ISOLATION

Plant material was collected from the United Chemicals and Allied Products, Calcutta. The air-dried powdered stems (4 Kg) of this plant were extracted with rectified spirit in a Soxhlet extractor. The rectified spirit extract was concentrated under reduced pressure to yield a light brown gummy mass which was successively extracted with pet-ether (60-80°C), CHCl$_3$, C$_6$H$_5$, CH$_3$COOCH$_3$, CH$_3$COCH$_3$, MeOH. The chloroform soluble fraction was concentrated under reduced pressure to brown viscous mass which was subjected to column chromatography over a Si-gel ‘G’ column using CHCl$_3$ : MeOH : H$_2$O (15:3:3) to give compound (1) which was crystallised from MeOH as brownish solid crystals m.p. 216-217°C, [M]$^+$ 612 (ESIMS), $\nu$ max 3401, 1656, 1608 cm$^{-1}$, UV (MeOH) $\lambda_{max}$: 258 347 (+ NaOMe); 266, 321 (sh), 398 (+ NaOAc); 270. 316.385 (+ AlCl$_3$); 273, 310 (sh), 426, (+ AlCl$_3$ + HCl) 275. 306 (sh), 363, 403 nm, $^1$H-NMR (300 MHz-CDCl$_3$), $\delta$ 6.92 (2H, s, H-2', H-6'), 6.17 (1H, d, J = 1.8 Hz, H-6), 6.36 (1H, d, J = 1.8 Hz, H-8) 5.16 (1H, d, J = 7.4, H-1") and $\delta$ 5.25 (1H, d, J = 7.4, H-1")
$^{13}$C-NMR (90 MHz, DMSO-$d_6$) δ 157.22 (C-2), 134.45 (C-3), 177.79 (C-4), 161.34 (C-5), 98.70 (C-6), 164.39 (C-7), 93.56 (C-8), 156.22 (C-9) 103.88 (C-10), 119.41 (C-1'), 107.79 (C-2') 145.84 (C-3'), 136.78 (C-4'), 145.84 (C-5'), 107.77 (C-6'), 103.9 (C-1''), 71.3 (C-2''), 78.7 (C-3''), 66.3 (C-4''), 62.6 (C-5''), 102.5 (C-1''), 74.3 (C-2'') 76.8 (C-3'') 69.6 (C-4''), 76.4 (C-5'') 60.7 (C-6'').

Acid hydrolysis of (1)

The compound (1) (15 mg) was treated with 10% of 15 ml HCl in aqueous 20 ml of MeOH for 6h and concentrated under reduced pressure. The mixture was then diluted with 20 ml of H$_2$O and extracted with chloroform. The aqueous layer was neutralized with BaCO$_3$ and BaSO$_4$ filtered off and concentrated under reduced pressure. The residue was compared with standard sugars on Si-gel plates using solvent system (n BuOH-HOAC-H$_2$O, 4:1:5). The spots were detected with aniline phthalate and sugars were identified as L-arabinose (R$_f$-0.22) and D-glucose (R$_f$ - 0.19). The aglycone (2) was crystalised as white needles, m.p. 278-279°C, m.f., C$_{15}$H$_{10}$O$_8$ (Found : C, 56.64%, H, 3.18% : C$_{15}$H$_{10}$O$_8$ requires C, 56.60%; H. 3.14%).

Permethylalation of (1) Followed by Acid Hydrolysis

Compound (1) (20 mg) was treated with MeI and Ag$_2$O in DMF at room temperature for 24 hours. The contents were concentrated and hydrolysed with 10% ethanolic H$_2$SO$_4$. After usual work-up; the methylated sugars were identified 2,3,4,6-tetra-O-methyl-D-glucose and 2,4 di-O-methyl-L-arabinose.
Enzymatic Hydrolysis of Compound (1)

Compound (1) was mixed with almond emulsin and allowed to stand at room temperature for 30 h. Water was then added to it and was then extracted with EtOAc. The aqueous layer gave D-glucose (PC).

Quantitative Estimation of Sugars

Quantitative estimation of sugars in the glycoside was done by Mishra and Rao procedure [9], which revealed that two sugars were present in equimolar ratio (1:1).

Antimicrobial Study of the Compound

The antifungal and antibacterial activity of the compound (1) of the plant were tested at its various dilutions using ethylene glycol as solvent at a concentration of 6 mg/ml of phosphate buffered saline (w/v).

Micro-organism Used

The various bacterial species were first incubated at 38°C for 48 h. The zone of inhibition were recorded at 37±1°C after 48 h for bacteria and at 28°C after 24 h for fungi.

Activity Studied

The antimicrobial activity was determined by Whatman N.1 filter paper discs (6 mm) method [10]. Paper discs were soaked with various samples tested and were dried at 50°C. The discs were then placed on soft
nutrient Agar (2%) petri plates previously seeded suspension of each of the bacterial species.

For the fungus, petri plates were placed on Sabouraud's agar [12] medium (1%). The zones of inhibition were expressed as an average of maximum diameter in three different directions. The various results are recorded in Table I and II.

Acknowledgement

Thanks are due to Head, R.S.I.C., C.D.R.I., Lucknow, for spectral analysis, and Prof. V.K. Saxena, Head Department of Chemistry, Dr. H.S. Gour University, Sagar (M.P.) for providing laboratory facilities.
Table I: Antifungal activity of compound (I)

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Incubation time</th>
<th>Inhibition (%) concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Trichoderma viridae</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Penicillium expansum</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>Helminthosporium oryzae</td>
<td>24</td>
<td>93</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>24</td>
<td>43</td>
</tr>
<tr>
<td>Rhizopus oligosporus</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Rhizobulum pneumoniae</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Rhizopus chinensis</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium Moniliforme</td>
<td>14</td>
<td>-</td>
</tr>
</tbody>
</table>

Table II: Antibacterial activity of compound (I).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Incubation time</th>
<th>I.C. (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Gram +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>12-24</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12-24</td>
<td>65-41</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>12-24</td>
<td>-</td>
</tr>
<tr>
<td>Gram -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>12-24</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>24</td>
</tr>
</tbody>
</table>
Reference:


Date: 11.12.2002

To,

Editor in Chief
Xiao Tian Liang,
Institute of Materia Medica,
Chinese Academy of Medical Sciences,
1 Xian Nong Tan Street,
Beijing 100050, China

Respected Sir,

Herewith, I am sending two copies of the manuscript entitled "A new bioactive flavone glycoside from the seeds of Melilotus indica All." for favour of publication in your esteemed journal.

Thanking you.

With regards.

Encl.:

(1) 2 copies of the manuscripts.

Yours sincerely,

(Dr. R.N. Yadava)
A new bioactive flavone glycoside from the seeds of *Melilotus indica* All.

R.N. Yadava*, S. Jain
Natural Products Laboratory,
Deptt. of Chemistry,
Dr. H.S. Gour University
Sagar, 470003 (M.P.) INDIA
ABSTRACT:

*Melilotus indica* A11. [1-3] belongs to family Leguminosae which is commonly known as ‘Banmethi’ in Hindi. It is found in North India, extending S. persia, S. Europe and Tropical zone of India. The seeds are used as anthelmintic, antipyretic, for curing heart diseases, bronchitis, leprosy, bowel complaints and infantile diarrhoea. This plant was also used as discutient, emollient, and as a fomentation. It is also useful in plaster for swelling. It is considered astringent and narcotic. Earlier workers have reported the presence of c-glycosides [4], methylene-dioxypterocarpan (MIS₆) [5], pterocarpone (MIS₂) [6] and prenylated pterocarpan [7] from this plant.

In the present work, we report the isolation of a new flavone glycoside, 5,7,4'-trihydroxy-6,3'-dimethoxy-flavone-7-O-α-L-arabinopyranosyl-(1→6)-O-β-D-galactopyranoside (I), from the seeds of this plant.

KEYWORDS:

*Melilotus indica* A11.; Leguminosae; flavonoid; A new bio-active flavone glycoside; Antimicrobial activity.

RESULTS AND DISCUSSION:

+ NaOMe) showed the hydroxy group at C-4' position and bathochromic shift at 25 nm (MeOH + AlCl₃) in band I with MeOH suggested the presence of OH group at C-5 position [9]. Its IR spectrum showed absorption bands at 3504 (-OH), 2952 (-CH), 2869 (-OCH₃), 1626 (C=O), 1616 (aromatic ring system) 1125 (-O-gly) and 826 cm⁻¹.

The compound I, on acid hydrolysis with 7% ethanolic H₂SO₄ gave an aglycone II, m.p. 194-196°C; m.f. C₁₇H₁₆O₇, [M]* 330 (EIMS) and sugars which were identified as L-arabinose (0.24) and D-galactose (0.16) (by Co-PC and Co-TLC). The aglycone was identified as 5,7,4'-trihydroxy-6,3'-dimethoxy-flavone by comparision of its spectral data with known reported literature [10].

The ¹H-NMR spectrum of compound I showed two aromatic protons as two singlets at δ 7.35 and δ 6.78 which were assigned to H-2', 6' and H-5' positions respectively and two singlets at δ 3.71 and 3.84 due to OMe-6 and OMe-3' and two singlets at δ 6.62 and 6.69 due to H-3 and H-8 proton. Signals for anomeric proton were observed at δ 5.56 (1H, d, J = 7.9, H-1"), and δ 4.94 (1H, d, J = 6.4, H-1") assigned to D-galactose and L-arabinose.

The position of sugar moiety in compound I was established by permethylation of I [11] followed by acid hydrolysis which afforded 2,3,4-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose and 5,4',3',6 tetramethoxy-7-hydroxyflavone showing that the C-1" of arabinose was linked with C-6" of galactose and the C-7 position of the aglycone (II) originally involved in glycosylation. The inter linkage
(1→6) between the sugars were further confirmed by its $^{13}$C-NMR spectrum (see Experimental).

Periodate oxidation [12] of compound I consumed 3.01 moles of periodate with the liberation of 1.16 moles of formic acid suggesting that the both of the sugars were in pyranose form.

Enzymatic hydrolysis of I with almond emulsion liberated D-galactose (Co-PC-Co-TLC) showed the presence of β-linkage with aglycone (II) and hydrolysis with Takadiastase suggested the presence of α-linkage between L-arabinose and D-galactose.

On the basis of above discussions, the compound I was identified as 5,7,4'-trihydroxy-6,3'-dimethoxy-flavone-7-O-α-L-arabinopyranosyl-(1→6)-O-β-D-galactopyranoside.

The compound I was tested for antimicrobial activity against various plant pathogenic fungi and bacteria.

**EXPERIMENTAL:**

**Plant Material:**

The seeds (3 Kg) of *M. indica* were procured from M/s. United Chemicals and Allied Products, Calcutta and authenticated by the Botany Department of this University.

**Extraction and isolation**

The air-dried and powdered seeds (2.5 Kg) at *Melilotus indica* were extracted with 95% EtOH in a Soxhlet extractor. The total extract was concentrated under reduced pressure to yield a brown viscous
mass, which was successively partitioned with petroleum ether (60-80°C), n-hexane, acetone, chloroform and ethyl acetate.

The ethyl acetate soluble fraction of the ethanolic extract was concentrated under reduced pressure to give a light yellow syrupy mass. It gave single spot on TLC examination using solvent system n-BuOII-AcOII-H2O (4:1:5) and I2 vappurs as visualising. It was therefore purified by CC over Si-gel-G and eluted with C6H6-CHCl3 in various proportions. The fractions collected from C6H6-CHCl3 (9:6) gave light yellow needles of compound-I. (95 mg), mp 210-212°C (from EtOII) m.f. C28H32O16, [M]+ m/z 624; UV_max (MeOH) 245 (sh), 274, 349, (NaOMe + MeOH) 285, 287, 397 (+AlCl3) 262, 285, 297, 374, (+ AlCl3/HCl) 254, 279, 265, 356; IR bands (KBr); 3504, 2869, 2952, 1626, 1616, 1125, 826 cm⁻¹; ¹H-NMR (300 MHz, CDCl3); δ 6.62 (1H, s, H-3), 6.69 (1H, s, H-8), 7.35 (2H, s, H-2' and H-6'), 6.78 (1H, s, H-5'), 8.94 (1H, br, s, 5-OH), 3.71 (3H, s, 6-OMe), 3.84 (3H, s, 3'-OMe), 5.56 (1H, d, J=7.9, H-1’’), 4.94 (1H, d, J = 6.4, H-1’’); ¹³C-NMR (90 MHz, DMSO-d₆); 164.8 (C-2), 105.2 (C-3), 180.9 (C-4), 155.1 (C-5), 128.9 (C-6), 166.0 (C-7), 98.0 (C-8), 160.1 (C-9), 106.2 (C-10), 122.0 (C-1’), 105.39 (C-2’), 147.10 (C-3’), 139.9 (C-4’), 147.1 (C-5’), 107.9 (C-6’), 102.1 (C-1’’), 71.5 (C-2’’), 73.4 (C-3’’), 67.9 (C-4’’), 73.8 (C-5’’), 64.9 (C-6’’), 106.1 (C-1’’), 70.9 (C-2’’), 74.8 (C-3’’), 69.1 (C-4’’), 66.3 (C-5’’).

ACID HYDROLYSIS OF COMPOUND I

On acid hydrolysis of compound (I) with 10% HCl yield aglycone (II) and sugar moiety (ies), which were separated by filtration. The
aglycone (II) was recrystallised from EtOH as yellowish needles, m.p. 194-196°C; m.f. C_{17}H_{14}O_{7}, [M]^+ m/z 330; which was identified as 4’-5-7-trihydroxy 3’-6-dimethoxy flavone by various spectral analysis with reported literature.

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 PC examination (n-BuOH-AcOH-H_2O 4:1:5) showed the presence of L-arabinose (R_f 0.24) and D-galactose (0.16). (by Co-PC and Co-TLC).

PERMETHYLATION OF I FOLLOWED BY ACID HYDROLYSIS:

Compound I was treated with MeI and Ag_2O in DMF at room temperature for 24h and then filtered. The filtrate was dried in vacuo and hydrolysed with 20% ethanolic H_2SO_4 for 7h, after the usual work up yielded aglycone (II) and methylated sugars identified as 2,3,4-tri-O-methyl-arabinose and 2,3,4-tri-O-methyl-galactose according to Petek. [13]

PERIODATE OXIDATION OF COMPOUND I:

Compound I was dissolved in MeOH and treated with sodium meta periodate for two days. The liberation of formic acid and consumed periodate were estimated by the Jone’s method [12] which revealed the presence of both sugars in pyranose form.

ENZYMATIC HYDROLYSIS OF COMPOUND I:
Compound I was treated with 5 ml of enzyme Takadiastase at 35°C for 24h to liberate L-arabinose (Rr = 0.24) (Co-PC) (BAW 4:1:5) using aniline hydrogen phthalate as detecting reagent. After complete hydrolysis with Takadiastase, the glycoside I was discussion and further MeOH treated with equal volume of almond emulsin solution and left at room temperature for 24h. The hydrolysate on examination PC (BAW 4:1:5) showed the presence of galactose (Rr 0.16).

QUANTITATIVE ESTIMATION OF SUGARS:

Quantitative estimation of sugars in the glycoside was done by Mishra and Rao procedure [14] which revealed that two sugars were present in equimolar ratio (1:1).

MICROBIAL ACTIVITY OF COMPOUND I:

The ethyl acetate soluble fraction of the ethanolic extract of the plant was tested for antibacterial and antifungal activity at its different dilutions using ethylene glycol as solvent, at a concentration of 6 mg/ml of phosphate buffer's saline (w/v). The zone of inhibition were recorded at 37°C ± 1°C after 48 hour's of incubation for bacterial species and fungi were incubated at 28°C after 2-5 days of incubation for fungal species.

The antimicrobial activity was determined by Whatman No. 1 filter paper discs (6 mm) method [15, 16]. Paper discs were soaked with various samples tested and were dried at 50°C. The discs were then placed of the centre on soft nutrient Agar petri plates previously
spreaded with suspension of each bacterial species. Then the plates were incubated at 37°C ± 1°C for 48 hour's.

For the fungal species Sabouraud's agar medium was poured and fungal species were inoculated and plates were incubated at 28°C for 2-5 days. The zone of inhibition were expressed as an average of maximum diameter in four different directions. The various results are recorded in Table I & Table II.

Table I: Antibacterial Activity of Compound (1)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bacterial Species</th>
<th>Diameters of zone of inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ethyl acetate soluble 1:4 1:8 1:12 1:16</td>
</tr>
<tr>
<td>1.</td>
<td>* Streptococcus pyogenes</td>
<td>20.9 17.9 14.5 0 0</td>
</tr>
<tr>
<td>2.</td>
<td>* Bacillus coreus</td>
<td>8.0 7.8 7.1 7.1 6.3</td>
</tr>
<tr>
<td>3.</td>
<td>* Klebsiella pneumoniae</td>
<td>9.7 7.9 6.9 0 0</td>
</tr>
<tr>
<td>4.</td>
<td>* Pseudomonas aeruginosa</td>
<td>8.7 7.4 6.7 7.7 0</td>
</tr>
<tr>
<td>5.</td>
<td>* Streptococcus agalactiae</td>
<td>20.9 17.8 15.2 0 0</td>
</tr>
<tr>
<td>6.</td>
<td>* Salmonella newport</td>
<td>25.0 19.6 18.1 18.1 12.1</td>
</tr>
<tr>
<td>7.</td>
<td>* Bacillus anthracis</td>
<td>8.6 8.0 7.1 7.1 6.4</td>
</tr>
<tr>
<td>8.</td>
<td>* Proteus vulgaris</td>
<td>18.4 12.6 11.4 10.3 10.1</td>
</tr>
<tr>
<td>9.</td>
<td>* Escherichia coli</td>
<td>8.5 7.9 7.0 7.0 6.2</td>
</tr>
</tbody>
</table>

Table II: Antifungal Activity of the Compound I

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fungal Species</th>
<th>Diameters of zone of inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ethyl acetate soluble 1:4 1:8 1:12 1:16</td>
</tr>
<tr>
<td>1.</td>
<td>Aspergillus niger</td>
<td>9.7 6.8 0 0 0</td>
</tr>
<tr>
<td>2.</td>
<td>Fusarium oxyporum</td>
<td>3.6 2.1 2.1 0 0</td>
</tr>
<tr>
<td>3.</td>
<td>Microsporum canis</td>
<td>7.9 7.6 0 0 0</td>
</tr>
<tr>
<td>4.</td>
<td>Penicillium notatum</td>
<td>8.9 8.6 7.4 7.4 6.4</td>
</tr>
<tr>
<td>5.</td>
<td>Epidermophyton floccosum</td>
<td>8.6 7.2 0 0 0</td>
</tr>
<tr>
<td>6.</td>
<td>Microsporum gypsum</td>
<td>8.2 7.7 0 0 0</td>
</tr>
<tr>
<td>7.</td>
<td>Aspergillus niger</td>
<td>9.4 6.6 0 0 0</td>
</tr>
<tr>
<td>8.</td>
<td>Penicillium purpuragenus</td>
<td>9.2 8.3 7.6 7.2 6.3</td>
</tr>
<tr>
<td>9.</td>
<td>A. fumigatus</td>
<td>8.2 7.4 7.4 7.1 7.1</td>
</tr>
</tbody>
</table>

* The zone of inhibition (mm) taken as average of four determination in four different directions and Whatman No. 1 (6mm) were soaked with each sample tested for their activity at a concentration of 6 mg/ml of PBS (w/v).
The results recorded in Table I and II showed that the antibacterial activity of the extract of the plant was found to be fairly good against gram +ve bacteria e.g. *Bacillus anthracis*, *Bacillus cereus*, *Escherichia coli*, and gram -ve bacteria e.g. *Proteus vulgaris*, *Salmonella newport* and was found to retain its activity even at dilution of 1:16. Antifungal activity of the plant extract was found to be more active against *Penicillium notatum*, *Penicillium purpurogenus* and *A. fumigatus*.

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

ACKNOWLEDGEMENT:

Thanks are due to Head, RSIC, CDRI, Lucknow for spectral analysis and Prof. V.K. Saxena, Head Deptt. of Chemistry, Dr. H.S. Gour University, Sagar (M.P.) for providing Laboratory facilities.
REFERENCES


To,

The Editor,
Institution of Chemists (India)
11/4 Dr. Biresh Guha Road,
Calcutta - 700 017.

Dear Sir,

Here with, I am sending two copies of the research paper entitled "Anti-Inflammatory activity of a new isolated flavone glycoside from the bauninia retusa root." for the favour of publication in your esteemed journal.

Kindly do the needful.

Thanking you.

With regards.

Encl.:

(1) 2 copies of the manuscript

Yours sincerely,

R.N. Yadava

( Dr. R.N. Yadava )

Date: 11.12.2002
ANTI-INFLAMMATORY ACTIVITY OF A NEW ISOLATED FLAVONE GLYCOSIDE FROM THE BAUHINIA RETUSA ROXB.

R.N. Yadava*, Shivani Jain
Natural Products Laboratory, Department of Chemistry, Dr. H.S. Gour University, Sagar 470 003 INDIA

A new flavone glycoside was isolated from the stem of Bauhinia retusa Roxb. and its structure was identified as 7,4'-dihydroxy-flavone-7-0-β-D-galactopyranosyl (1→2)-0-α-L-rhamnopyranoside by various chemical degradations and spectral analysis. The new compound (1) showed anti-inflammatory activity.

Keywords : Bauhinia retusa Roxb ; Leguminosae; Flavonoid; Anti-inflammatory activity.

Introduction

Bauhinia retusa Roxb. (Leguminosae) is known as 'Semla' in Hindi and is distributed in Sub-Himalayan tract and Outer Himalaya of the Punjab and Kumaon [1-3]. Earlier workers have reported the presence of L-arabinose, D-galactose, D-ribose, L-rhamnose and D-galacturonic acid [4] from the gum of Bauhinia retusa. In the present paper, we deals with the isolation and characterisation of a new bio-active flavone glycoside from the stems of this plant.

Results and Discussion

The acetone soluble fraction of the stems of B. retusa Roxb. afforded a new compound 1 C_{27}H_{32}O_{16}; m.p. 214-215°C; [M]+ 564 (EIMS). It gave positive response to Molisch test and Shinoda test [5]. The IR spectrum of

*Corresponding author : Tel. +91-07582-26464; E-mail : rnyadava@rediffmail.com.
1 showed absorption band at 3302(-OH), 1641 (C=O), 1602 (aromatic ring system), 1560, 1500 cm⁻¹. The UV spectrum of 1 showed a bathochromic shift of 32 nm in band I with NaOAC suggesting the presence of free hydroxyl group at C-4’ position [6].

The compound 1 on acid hydrolysis with 7% H₂SO₄ gave an aglycone 2 C₁₅H₁₂O₄; mp 280-282°C; [M]+ 256 (EIMS) and sugars which were identified as galactose and rhamnose (by Co-PC and Co-TLC). The aglycone was identified as 7,4’-dihydroxy flavone by comparision of its spectral data with known reported literature [7].

The ¹H-NMR spectrum of compound 1 showed four aromatic proton signals at δ 7.12 (2H, d, J = 8.6 Hz) and 7.46 (2H, d, J = 8.6 Hz) which were assigned to H-3’, 5’ and H-2’, 6’, respectively and doublet at δ 5.46, 7.73, 6.51, 6.35 and 2.74, 3.05 due to H-2, H-5, H-6, H-8 and H-3 proton. Signals for anomeric proton were observed at δ 5.35 (1H, br, s, H-1”) and δ 5.55 (1H, d, J=7.78, H-1’”), assigned to rhamnose and galactose, respectively and a complex signal at δ 1.02 was due to the rhamnosyl methyl group.

The position of sugar moiety in compound 1 was established by permethylation of 1 [8] followed by acid hydrolysis which afforded 3,4-di-O-methyl-L-rhamnose,2,3,4-tri-O-methyl-D-galactose and 4’-methoxy-7-hydroxy flavone showing that the C-1’” of galactose was linked with C-2” of rhamnose and the C-7 position of the aglycone 2 originally involved in
glycosylations. The inter linkage (1→2) between the sugars was further confirmed by its $^{13}$C-NMR spectrum (See Experimental).

Periodate oxidation [9] of 1 consumed 3.01 moles of periodate with the liberation of 1.15 moles of formic acid suggesting that both the sugars were in pyranose form.

Enzymatic hydrolysis of compound 1 with equal volume of almond emulsion liberated D-galactose and proaglycone confirming the presence of β-linkage between D-galactose and L-rhamnose and on hydrolysis with Takadiastase liberated L-rhamnose confirming the presence of α-linkage between aglycone and rhamnose.

On the basis of above evidences, the compound 1 was identified as 7,4'-dihydroxy-flavone-7-0-β-D-galactopyranoxy (1→2)-O-α-L-rhamnopyranoside.

The acetone soluble fraction of the ethanolic extract of the plant showed moderate anti-inflammatory activity on albino rats. The % inhibition calculated [10] for the acetone soluble was found 62% as compared to standard drug ASA.

Experimental

All the mps were determined on a thermo-electrical m.p. apparatus and are uncorrected UV spectra were determined in MeOH and IR spectra were recorded in KBr disc. $^1$H-NMR spectra were run at 400 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 stems of *B. retusa* Roxb were collected from "Pachimarhi" Hill Station in M.P. and was taxonomically authenticated by Taxonomist of Botany Department of Dr. H.S. Gour University, Sagar (M.P.) INDIA and the herbarium specimen was deposited in room no. 36 of Chemistry Department of this University.

Extraction and isolation

Air-dried and powdered stems of (3kg) *B. retusa* Roxb were extracted with 90% MeOH in a Soxhlet extractor. The methanolic extract was concentrated under reduced pressure to a viscous mass, which was then dissolved in hot H₂O and partitioned with petroleum ether, chloroform, ethyl acetate and acetone. The concentrated acetone soluble part was chromatographed on a silica-gel column using solvents with increasing polarity. The fraction collected from CHCl₃-MeOH (6:2) gave compound 1, crystallized from MeOH as light yellow crystal, which gave a single spot on TLC by using CHCl₃-MeOH-H₂O (10:6:4) as solvent system, mp 214-215⁰C and [M]+ 564 (EIMS) (Found C: 57.42%, H: 5.70% Calcd for C₂₇H₃₄O₁₃. C: 57.44%, H: 5.67%) IR (KBr): ν max 3302 1641, 1602, 1560, 1500 cm⁻¹. UV (MeOH) λ max : 311,276 ; + NaOMe 336, 254 ; + NaOAc 343, 255 ; + AlCl₃ 312.276 nm; ¹H-NMR (400 MHz - CDCl₃)

δ 7.12 (2H, d, J = 8.6 Hz , H - 3', 5'); 7.46 (2H, d, J = 8.6 Hz, H - 2', 6'); 5.46 (1H, dd, J = 12.8 and 2.8 Hz, H-2); 7.73 (1H, d, J = 8.4 Hz, H-5); 6.51 (1H, dd, J = 8.4 and 2.20 Hz, H-6); 6.35 (1H, d, J = 2.04 Hz, H-8); 2.74
(1H, \(dd, J = 17.2\) and 2.8 Hz, H-3); 3.05 (1H, \(dd, J = 17.2\) and 12.8 Hz, H-3) 5.35 (1H, \(br, s, H-1''\)), 5.55 (1H, \(d, J = 7.78, H-1''\)) 1.02 (3H, \(d, J = 6.2\) Hz, Rham - Me), 13 C-NMR (100 MHz, DMSO - d6) \(\delta 78.60\) (C-2), 43.13 (C-3); 189.69 (C-4), 128.28 (C-5), 110.5 1(C-6), 164.57 (C-7), 102.55 (C-8); 162.98 (C-9), 113.55 (C-10), 132.38 (C-1'), 127.81 (C-2'), 116.25 (C-3'), 157.43 (C-4'), 116.27 (C-5'), 127.81 (C-6'), 103.3 (C-1''); 82.5 (C-2''); 72.2 (C-3''); 73.3 (C-4''); 72.0 (C-5''); 17.6 (C-6'') 102.2 (C-1'''); 71.6 (C-2''''); 73.5 (C-3''''); 67.8 (C-4''''); 73.7 (C-5''''); 64.8 (C-6'').

**Acid hydrolysis of Compound 1**

Compound 1 was hydrolysed with 7% H₂SO₄ for 2h. The aglycone 2 which precipitated out on cooling was recrystallised from Et₂O as a yellow needles and was identified as 7,4'-dihydroxy flavone C₁₅H₁₂O₄, mp 280-282°C, [M]+ 256 (EIMS), (Anal.:C, 70.35%; H, 4.69% Calcd. for C₁₅H₁₂O₄ : C, 70.31%; H, 4.68).

The aqueous hydrolysate was neutralised with BaCO₃ and BaSO₄ was filtered off. The concentrated filtrate was developed on PC with upper phase of solvent system n-B₄OH-AcOH-H₂O (4:1:5) and using aniline hydrogen phthalate as detecting agent. The \(R_f\) value for rhamnose was 0.36 and for galactose was 0.16 (by Co-PC and Co-TLC).

**Permethylaion of 1 followed by Acid Hydrolysis.**

Compound 1 was treated with MeI and Ag₂O in DMF at room temperature for 24h and then filtered. The filtrate was dried in vacuo and hydrolysed with 20% ethanolic H₂SO₄ for 6 h. after the usual work up
yield aglycone 2 and methylated sugars identified (by Co-PC) as 3,4-di-O-methyl rhamnose and 2,3,4-tri-O-methyl-galactose according to Petek.[11]

**Periodate Oxidation of Compound 1**

Compound 1 was dissolved in MeOH and treated with sodium metaperiodate for 48 h. The liberation of formic acid and consumed periodate were estimated by the Jone's method [9] which suggests the presence of both sugars in pyranose form.

**Enzymatic Hydrolysis of Compound 1**

The compound 1 (50 mg) was dissolved in MeOH (20 ml) and on hydrolysis with equal volume of almond emulsin at room temperature yielded D-galactose confirming the presence of the β-linkage between D-galactose and L-rhamnose and on hydrolysis with takadiastase liberated L-rhamnose, showing the presence of α-linkage between L-rhamnose and aglycone.

**Quantitative Estimation of Sugars**

Quantitative estimation of sugars in the glycoside was done by Mishra and Rao Procedure [12] which revealed that both the sugars were present in equimolar ratio (1:1).

**Anti-Inflammatory activity of Compound 1**

Anti-inflammatory activity of the acetone soluble fraction of this plant was done by non-immunological carrageenin induced hind paw oedema method [13].
Adult albino rats of either male or female weighing 145-180 gm. were taken in the examination. The volume was measured by Plethysmograph. Initial volume of right hind paw of albino rats were measured by Plethysmograph with out administration of test drug. Acetyl salicylic acid was used as a standard drug. Activity was carriedout by measuring the change in the volume of inflammed foot produced by injection of 0.05 ml. of 1% freshly prepared carrageenin suspension.

Albino rats were divided into three groups, Each group consisting of four rats. First group of rats were treated intraperitonially (i.p.) with 50 mg/kg body weight of the ethanolic extract of the plant.

Second group was administered i.p. 40 mg/kg body weight of the aqueous suspension of acetyl salicylic acid and the third control group was fed with the same volume of distilled water. After one hour of the drug administration, the rats were injected 0.05 ml suspension of carrageenin in the right hind paw.

The measurement of the paw volume was carried out by mercury displacement technique with the help of Plethysmograph immediately before and after the carrageenin injection after 1, 2 and 3 hrs. The percentage inhibition (I) of inflammation after 3 hrs. was calculated by the formula given by Newbould. [14]

\[ I = 100 \left(1 - \frac{a - x}{b - y}\right)\]

Where -
x = mean foot volume of rats before the administration of carrageenin injection in the test and the standard drug.

n = mean foot volume of rats after the administration of carrageenin and the test drug injection in the test and standard drug.

y = mean foot volume of rats before the administration of carrageenin injection in the control group.

b = mean foot volume of rats after the administration of carrageenin injection in the control group.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test solutions applied</th>
<th>Does mg/kg. i.p.</th>
<th>Volume of paw after drug administration</th>
<th>Total increase in paw volume</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control Group (0.50) +</td>
<td>........</td>
<td>0.50+ 0.66+ 0.75+ 0.86+ 0.36+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Treated group</td>
<td>50</td>
<td>0.50+ 0.58+ 0.67+ 0.68+ 0.18+ 50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ASA</td>
<td>40</td>
<td>0.50+ 0.64+ 0.70+ 0.74+ 0.24+ 33%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summary:

*Bauhinia retusa* (Roxb) belongs to family Leguminosae, which is commonly known as 'Semla'. In the present paper we report the isolation and structure elucidation of a flavonol glycoside 7,4′-dihydroxy-flavone·7·O·β-D-galactopyranosyl (1→2)·O·α-L-rhamnopyranoside, from the stems of this plant and Anti-inflammatory activity of the compound.

Acknowledgement:

The authors are grateful to Head, R.S.I.C., C.D.R.I., Lucknow, for spectral analysis, anti Inflammatory studies and Prof. V.K. Saxena, Head Department of Chemistry, Dr. H.S. Gour University, Sagar (M.P.) INDIA, for providing laboratory facilities.
Reference:


