List of Research Papers
LIST OF RESEARCH PAPERS:


(4) Saxena, V.K. and Shukla, M., "Isolation and study of the saponin MS-I Lupeol-3-o-α-L-xylopyranosyl [1→4]-o-β-D-glucopyranoside from the stem-bark of Ficus glomerata Roxb". J. Inst. Chemists, Kolkata, (Communicated).

(5) Saxena, V.K. and Shukla, M., "Isolation and study of saponin (MS-II) β-amyrin-3-o-β-D-galactopyranosyl [1→4]-o-α-L-rhamnopyranoside from the stem-bark of Ficus glomerata (Roxb)". Indian Academy of Sciences, Bangalore, (Communicated).

(6) Saxena, V.K. and Shukla, M., "Isolation and study of the flavonoidal glycoside; Quercetol-3-o-β-D-arabinopyranoside. From the seeds of Embelia ribes Burm". J. Inst. Chemists, Kolkata, (Communicated).
(7) Saxena, V.K. and Shukla, M., “Study and isolation of steroidal saponin (MS-IV) stigmasteral-3-0-β-D-arabinopyranosyl [1→4]-0-β-D-glucopyranoside from the seeds of Embelia ribes Burm”. Indian Academy of Sciences, Bangalore, (Communicated).


PAPER PRESENTED IN SEMINARS/CONFERENCES

Carbohydrate Contents of Some Indigenous Anthelmintic Plants

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Keywords

Ficus glomerata Roxb, Embelia ribes, Burm., Anthelmintic plant, Carbohydrate contents.

Abstract

Alcoholic extract of the stem bark (250 g) of Ficus glomerata Roxb studied for their carbohydrate contents and was found to contain D-glucose, L-rhamnose, sucrose, D-ribose and seeds (250 g) of Embelia ribes, Burm. was found to contain D-glucose, D-galactose, L-rhamnose, sucrose, maltose, fructose, D-xylene, and L-ascorbic acid.

Introduction

Carbohydrates are the support of the plant tissue and are widely distributed in plant kingdom. Ficus glomerata Roxb\[1-3\] is commonly known as "Gular" in Hindi and belongs to natural order Moraceae family, and Embelia ribes Burm.[4-6] which is commonly known as "Baberang" in Hindi belongs to natural order Myrsinaceae. Both plants have been reported to possess anthelmintic property.
Result

The sugars were identified on the basis of comparison of their Rf values with those cited in the literature. The data and sugars identified are given in table 1.

**Solvent System**: n-Butanol : Acetic acid : Water (4 : 1 : 5).

**Spraying Reagent**: Aniline hydrogen phthalate

**TABLE - 1**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Rf Found</th>
<th>Rf Reported(^7)</th>
<th>Carbohydrate Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.13</td>
<td>0.14</td>
<td>Sucrose</td>
</tr>
<tr>
<td>2.</td>
<td>0.17</td>
<td>0.18</td>
<td>D-Glucose</td>
</tr>
<tr>
<td>3.</td>
<td>0.31</td>
<td>0.33</td>
<td>D-Ribose</td>
</tr>
<tr>
<td>4.</td>
<td>0.368</td>
<td>0.366</td>
<td>L-Rhamnose</td>
</tr>
</tbody>
</table>

**PLANT - EMBELIA RIBES** Burm. (Seeds)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Rf Found</th>
<th>Rf Reported(^7)</th>
<th>Carbohydrate Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.12</td>
<td>0.11</td>
<td>Maltose</td>
</tr>
<tr>
<td>2.</td>
<td>0.135</td>
<td>0.14</td>
<td>Sucrose</td>
</tr>
<tr>
<td>3.</td>
<td>0.15</td>
<td>0.16</td>
<td>D-Galactose</td>
</tr>
<tr>
<td>4.</td>
<td>0.16</td>
<td>0.18</td>
<td>D-glucose</td>
</tr>
<tr>
<td>5.</td>
<td>0.23</td>
<td>0.24</td>
<td>Fructose</td>
</tr>
<tr>
<td>6.</td>
<td>0.27</td>
<td>0.28</td>
<td>D-xylose</td>
</tr>
<tr>
<td>7.</td>
<td>0.37</td>
<td>0.366</td>
<td>L-Rhamnose</td>
</tr>
<tr>
<td>8.</td>
<td>0.39</td>
<td>0.38</td>
<td>L-Ascorbic acid</td>
</tr>
</tbody>
</table>

**Discussion**

A perusal of both the tables concluded that the plant *Ficus glomerata* Roxb. contains, D-glucose, L-rhamnose, sucrose, D-ribose, and the plant *Embelia ribes* Burm. contains, D-glucose, D-galactose, L-rhamnose, sucrose, maltose, fructose, D-xylose and L-ascorbic acid.
Experimental

The air dried and powdered stem bark (250 g) of *Ficus glomerata* Roxb., and seeds (250 g) of *Enbelia ribes* Burm., were separately extracted with ethanol (100 ml) in a 500 ml round bottom flask and the extracts were subjected to ascending paper chromatography. Whatman filter paper No. 1 was cut into strips of $5 \times 30$ cm. size and the concentrated alcoholic extract was spotted on paper with the help of a fine capillary on a line 1 cm. above the bottom. Separation was carried out using the solvent system, n-butanol : acetic acid : water (4 : 1 : 5 v/v) Aniline hydrogen phthalate was used as the spraying reagent for sugars. Sugars were visualised as brown spot.

Acknowledgements

Thanks are due to Head, Department of Chemistry, Dr. Hari Singh Gour Vishwavidyalaya, Sagar for providing necessary facilities and to Prof. J.T. Rao and Dr. R.N. Yadav.

References


"Indian Medicinal Plants - A Storage of Potential Anthelmintic Agents - I"

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Abstracts
A survey of available literature on Indian flora has revealed that a large number of herbal Indian medicinal plants which are mainly found in Indian subcontinent to possess anthelmintic activities due to the active principal present in them. An ideal anthelmintic drug should possess antibacterial, anti diarrhoeal, anti-inflammatory, antidiabetic, anti-allergic properties with minimal or no side effects. It is prudent to focus research work in this area in order to search ideal anthelmintic drugs.

The medicinal plants like:- Embelia ribes, Punica granatum, Butea frondosa, Centratherum anthemanticum, Cucurbita maxima and some others have been found to be anthelmintic and their phytochemical investigation has shown that they possess compounds like Embelin (Embelic acid; 2,5,- di hydroxy 3-undecyl-1,1,4, benzoquinone), leucoanthocyanins (Leuco cyanidine -3-0-D-glucopyranoside leucoanthocyanins, Leuco cyanidine -3-0-D-glucopyranoside, palasonin. etc.

Keywords: Indian Medicinal Plants, anthelmintic, antibacterial antidiarrhoeal, anti-inflammatory, antidiabetic, anti-allergic, review.

Introduction
Although various highly effective and selective anthelmintics are available, but such compounds must be used correctly and judiciously to obtain a favourable clinical research and also to minimize selection for anthelmintic resistance. Modern drugs have a wide range of safety and considerable activity against immature or larval stages of parasites and have a broad spectrum of activity.

Infact the ideal anthelmintic should have a broad spectrum of activity against mature and immature parasites and should be easy to administer to a large number of animals and also must have a wide margine of safety.

The anthelmintic must have selective toxic activity to parasite. This is usually achieved either by inhibiting metabolic processes vital to the parasite but which are not vital or one absent in the host or by inherent pharmacokinetic properties of the compound that cause the parasite to be exposed to higher concentration of the anthelmintic then are the host cells, where as the physiologic mode of action of anthelmintics is not fully understood, the sites of action and biochemical mechanisms of many of them are known parasitic helminths must maintain an appropriate feeding site and nematodes and trematodes must actively ingest and more food through their digestive tracts to maintains an appropriate energy states, reproductive process requires proper neuromuscular co-ordination parasites must also maintain homeostasis in the face of host immune reactions.

As such an exploration in the available literatures has revealed that some of the Indian medicinal plants have earned the reputation of possessing anthelmintic properties and the compounds already isolated are reviewed in this paper.

Natural Anthelmintic Drugs Embelia ribes
Embelia ribes Burm. is commonly known as "Babe rang" in hindi and belongs to natural order Myrsinaceae. Embelin (Embelic acid; 2,5,- di hydroxy 3-undecyl-1,1,4, benzoquinone), isolated from seeds of Embelia ribes Burm. with n-hexane. The crude embelin on crystallization from αformulæe for Embelin is C_{18}H_{13}O_{4}

Embelin contains two highly reactive hydroxyl and two ketonic groups.
Twenty four such derivative were developed and investigated for their anthelmintic activity.

**Ficus glomerata**

Ficus glomerata, Roxb. is commonly known as "Gular" in hindi and belongs to natural order Moraceae.

Two leucoanthocyanins (Leuco cyanidine -3-0-D-glucopyranoside and leucopelargonidine 3-0-a-L-rhamnopyranoside were isolated from stem bark of *Ficus glomerata* Roxb. which have been reported to posses anthelmintic activity.

This plant is used in treatment of dysentery, vulnerary, kapha, biliousness and diseaser of vagina.

**Punica granatum**: (N.O. Punicaceae)

Punica granatum, Linn, known as pomegranate, is a deciduous small tree up to 8 m in height with attractive reddish scarlet edible fruits. The species originated in Iran, Afganistan and Baluchistan is found wild in the warm valleys of the Himalayas and is cultivated through out India.

3,7,8,4'-tetrahydroxy -3'-myrtr-8-en-yl-flavone, has been isolated from flowers of Punica granatum Linn. which molecular formula is C_{25}H_{24}O_{6}.
This plant parts is used the treatment of haematuria, haemopysis, diarrhoea, dysentery, nesal hemorrhage.
The plant parts are astringent and used an anthelmintic especially against tapeworm.

**Butea frondosa**

Butea frondosa koen.ex. Roxb. which is commonly known as 'phalasa' in hindi belongs to natural order papilionaceae.

The nitrogenous acidic compound palasonin has been isolated from it's seeds.

Palasonin and its sodium and piperazine salts showed anthelmintic activity. Minimal lethal concentration being 1.0 and 0.75 mg/kg respectively against cases of lumbricoides of human origin.

![Palasonin](image)

The plant is used to the treatment of diarrhoea. It's seeds are anthelmintic, useful in flatulince and piles; while it's flowers are diuretic, depurative, astringent and aphrodisiac. It's leaves are aphrodisiac and used as febrifuge bark. The exuded gum (Butea gum) is used as an astringent and is beneficial in diarrhoea and dysentery

**Centrtherum anthelminticum (Linn)**

Centrtherum anthelminticum (Linn) kuntze which is commonly known as "Somraj" and is belongs to natural order Asteraceae. 8,14,(z),24,(28) stigmastatrient acetate was isolated from seeds of centrtherum anthelminticum (Linn) which has been reported to possess anthelmintic activity.

![8,14,(z),24,(28)- stigmastatrienol acetate](image)
The seeds have a hot sharp taste, acrid, astringent to the bowels, anthelmintic, cure ulcers, "vata" and "kapha", and used in skin disease, e.g. leucoderma, and for curing fevers (Ayurveda).

The seeds have a sharp bitter taste; anthelmintic, purgative, and is used for asthma, kidney troubles, hiccup; applied in inflammatory swellings; remove blood from liver; and is good for itching of the eyes.

The seeds are considered as powerfully anthelmintic, and are also an ingredient of powder prescribed in snake-bites. On the Malabar Coast, an infusion of the seeds is given for coughs and against flatulence. In the Punjab, it is considered a febrifuge.

The seeds are very bitter and are used instead of quinine by the Mundas of Chota Nagpur. In paralysis of the legs the powdered seeds are applied externally. When the stomach of cattle swells, e.g. after they have grazed too much on paddy or rambra plants, the powdered seeds are mixed in equal quantity with salt and soot of the fireplace. This is dissolved in water with the addition of two capsules of Spanish pepper, and given as a drink.

In Travancore, the bruised seeds, ground up in a paste with limejuice, are large employed as a means of destroying pediculi. They are also given in anasrca and used for plasters for abscesses.

The seeds are also credited with tonic, stomachic, and diuretic properties.

The juice of the leaf is given to cure phlegmatic discharges from the nostrils.

In Ceylon, the plant is used for cure fever convulsions.

The seeds of this plant are considered to possess very strong anthelmintic properties by vaidyans. They are also administered in intestinal colic and dysuria. The powdered seeds followed by castor oil were tried for removal of roundworms in ¼ to ½ drachms does and the result as far as could be ascertained showed the seeds to posses considerable anthelmintic properties. Confirmatory trials gave completely satisfactory results (Koman).

The powdered seeds, even in 4 drachms doses with the usual purges, failed to expel hookworms and roundworms (Caius and Mhaskar).

The seeds in combination with other drugs are prescribed for snake-bite (Chakara, Sushruta4) and scorpion-sting (Chakara, Sushruta, Vabhata, Varindaadvahava, Vaishyaratnavali, Chakradatta, Ashtangasangraha).

Cucurbita maxima -Duch ex. lam
Cucurbita maxima Duch ex. Lam. which is commonly known as 'Sitapal' in hindi, and is belong to natural order Cucurbitaceae.

Stigmasta -7,24, (28)-dien-3-ol and stigmasta -7,25-dienol acetate have been isolated from it's seeds. Aqueous and alcoholic extracts of it's seeds possess anthelmintic properties.
The fruit has flavour, diuretic, tonic; allays thirst; cures kapha; indigestible; increases 'vata'; causes biliousness and loss of appetite (Ayurveda). The seeds are used as a taeniacide, their oil is prescribed as a nervine.

The pulp of the fruit is often used as poultice.

In Guinea it's fruit is considered sedative, emollient, and refrigerant. The pulp is applied to burns and scalds, inflammation, abscesses, and boils; it is also prescribed in migraine and neuralgia. The seeds are used as anthelmintics, more especially as taeniacides.

References
Phytochemical Study of some Indigenous Anthelmintic Plants

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Keywords: Ficus glomerata Roxb., Embelia ribes Burm, Anthelmintic plants, Amino acids contents.

Introduction:

The present communication deals with the identification of different amino acids in the Ficus glomerata Roxb.1-3, which is commonly known as 'Gular' in hindi belongs to natural order, Moraceae, and Embelia ribes Burm.4-6, which is commonly known as "Baberang" in hindi and belongs to natural order Myrsinaceae. Both the above plants have been reported to possess anthelmintic properties. Through examination of the available literature7-13 on anthelmintic plants has revealed that a large number of compounds e.g. steroids, tetracyclic triterpenoids, leucoanthocyanins, monocarboxylic acid, higher alkanes, sugars, quinones, and alkaloids.

Experimental and discussion:

Isolation of protein:

The air dried and powdered stem bark (100 g) of Ficus glomerata Roxb. And seeds (100 g) of Embelia ribes Burm. were defatted with the petroleum ether in soxhlet apparatus separately. The defatted part is extracted from the brine extract (10% NaCl) and is acidified to get the crude protein.

The defatted part of stem bark of Ficus glomerata Roxb. and seeds of Embelia ribes Burm. were found to consist of large amount of crude protein.

Identification of Amino acid:-

The crude extract(1.0gm) was hydrolysed by refluxing with 100ml. of 6N.HCl for 24hours, at about 100°C temperature. After refluxing the solution was decolourised by animal charcoal and filtered. The filtrate was evaporated to remove acids. The residue was dissolved in water and again evaporated to dryness. This procedure was repeated three times to
remove excess of HCl. Finally, the dry residue was dissolved in 10% isopropanol and used for chromatography studies. Paper strips in ascending chromatography were developed with the upper layer on shaking a mixture of n-Butanol : Acetic acid : Water (4 : 1 : 5 and 2.67 : 3.33 : 1 v/v). After development the strip was dried and sprayed with 0.2% (w/v) ninhydrin reagent. The amino acids present in the crude protein residue were identified by comparing the Rf-values with known amino acids under the same conditions.

**Result:**

The amino acids identified on the basis of comparison of their Rf values with those cited in the literature.

**Solvent System:** n-Butanol : Acetic acid : water

Ratio (a) 4 : 1 : 5 (v/v)

(b) 2.67 : 3.33 : 1 (v/v)

**Spraying Reagent:** 0.2% ninhydrin

| PLANT : FICUS GLOMERATA Roxb. (Stem-bark) |
| Solvent ratio (a) |
|---|---|---|
| S.No. | Rf Found | Rf Reported<sup>7</sup> | Amino acid identified |
| 1. | 0.132 | 0.135 | Proline |
| 2. | 0.257 | 0.259 | r-Methyl proline |
| 3. | 0.40 | 0.39 | γ-Amino butyric acid |
| 4. | 0.50 | 0.51 | δ-Amino valeric acid |
| 5. | 0.54 | 0.53 | Valine |
| 6. | 0.66 | 0.68 | Leucine |

| PLANT - EMBELIA RIBES Burm. (SEEDS) |
| Solvent ratio (b) |
|---|---|---|
| S.No. | Rf Found | Rf Reported<sup>7</sup> | Amino acid identified |
| 1. | 0.71 | 0.70 | γ-Amino butyric acid |
| 2. | 0.73 | 0.75 | δ-Amino valeric acid |
| 3. | 0.78 | 0.77 | Valine |
| 4. | 0.80 | 0.82 | Leucine |
Acknowledgment:

Thanks are due to Head, Department of Chemistry, Dr. Hari Singh Gour University, Sagar for providing necessary facilities and to Prof. J.T. Rao and Dr. R.N. Yadav for encouragements.

Reference:


15. Asolkar, L.V., Kakkar, K.K. and Chakre, O.J. "Glossary of Indian Medicinal Plants with active principles", Parts 1, CSIR, New Delhi, p. 290-291.


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To,

The Secretary
Institute of Chemist (India)
Kolkata.

Dear Sir,

Herewith I am enclosing a research paper entitled Lupeol-3-o-α-L-xylopyranosyl [1→4]-o-β-D-glucopyranoside from the stem bark of Ficus-gglomerata (Roxb.) for the favour of publication in your esteemed journal.

Hope you will please publish in your journal and with regards.

Thanking you,

Yours faithfully

Mrityunjai Shukla
Research Scholar

Date : June 18, 2007
Place : Sagar
Lupeol-3-o-α-L-xylopyranosyl [1→4]-o-β-D-glucopyranoside from the stem-bark of *Ficus-glomerata* (Roxb.)

M. Shukla and V.K. Saxena
Department of Chemistry
Dr. H.S. Gour University, Sagar (M.P.) 470 003 India

*Ficus glomerata*1-5 Roxb which is commonly known as ‘Gular’ in hindi belongs to natural order Moraceae. It is an evergreen tree found and cultivated throughout India. Different parts of plants are used in the treatment of dysentery, vulnerary, kapha, biliousness, and diseases of vagina. The roots are useful in hydrophobia. The bark is useful in curing asthma, piles, and galactagogue. It has also been reported to posses anthelmintic properties. The present paper deals with the Isolation and study of the saponin (MS-I) Lupeol-3-O-α-L-xylopyranosyl [1→4]-O-β-D-glucopyranoside from the stem-bark of *Ficus-glomerata* (Roxb.).

**EXPERIMENTAL AND DISCUSSION**

**Extraction and Isolation** : The stem-bark of *Ficus glomerata* Roxb was procured locally around Sagar region and was identified by reputed taxonomist. The air dried and powder stem-bark of *Ficus glomerata* Roxb. was extracted with rectified sprit in a round bottomed flask on an electric water bath. The rectified sprit extract was filtered while hot. The ethanolic extract was concentrated under reduced pressure to yield brown viscous mass, which was partitioned with n-hexane, benzene, chloroform, ethyl acetate and acetone.

The methanol soluble part was concentrated under reduced pressure to get a dark brown viscous mass. It showed three spots on TLC examination using n - B:A:W (4:1:5)as solvent system and I₂ vapours as visualizing agent. Eluates for chloroform : Methanol (6:1) and (6:2) on TLC examination were found to be mixture and so were rejected. Eluates from chloroform : methanol (6:3) on TLC
examination were found to homogenous and so were mixed and concentrated, when saponin MS-I precipitated out. Crystallisation of MS-I with methanol gave light yellow crystalline compound which showed a single homogeneous spot on TLC over silica gel using (n B:A:W 4:1:5) as solvent system and I₂ vapours as visualising agent.

The saponin MS-I analysed for molecular formula C₃₈H₆₃O₁₀ (C=67.14%, H = 9.26%) m.p. 190-191°C and [M⁺] 679 (FABMS). It is responded positive to all characteristic colour reactions of the saponin. The saponin MS-I showed significant bands, in the IR spectrum at νₓ max cm⁻¹. 3906.1-3423.2 (-OH), 2817.4 (CH₃ stretching), 1596.2 (C=C stretching), 1461.2 (C–H bending vibration of methyl group), 1383.3 (CH₃ symmetric stretching), 1352.2 (CH₃ bending), 1112.7 (symmetric (C–O –C), 769.2 (C–H out of plane bending).

¹H-NMR signals were recorded at (300 MHz, DMSO): δ 0.68 (3H, s, Me-C₂₂), δ0.82 (3H, s, Me-C₂₃), δ 0.94 (3H, s, Me-C₂₄), δ0.80 (3H, s, Me-C₂₆), δ1.2-2.03 complex pattern, 28 H (polymethylene and CH proton), δ4.40 (m, C₃H), δ0.85 (2H, s, C₂₇), δ3.43 (5H, m, glucose proton), δ 2.42 (6H, m, xylose proton), δ4.32 (1H, J 7.3, d, 1'anomeric proton) and δ 4.33 (1H, J 7.1, d, 1" anomeric proton).

It's FABMS showed significant fragmentation peaks m/z = 679, 547, 385, 219, 192, 179, 177 and 166.

**Acid hydrolysis of the saponin MS-I**:

The saponin MS-I dissolved in ethanol and treated with 7% H₂SO₄ and refluxed on water bath for 10-12 hrs. The reaction mixture was concentrated and allowed to cool and the residue was extracted with ether. The aqueous layer was examined separately for identification of sugar portion and ethereal layer was graphy using Chloroform : Methanol (6:3) yielded sapogenin MS-I(A), molecular formula C₂₇H₄₅O (C=84.15%, H = 11.68%) m.p. = 185-186°C and [M⁺] = 385 (FABMS). It was identified as Lupeol by chemical degradation
and spectral analysis. The aqueous hydrolysate obtained, after acid hydrolysis of saponin MS-I, was neutralized with BaCO₃ and BaSO₄ was filtered. Filtrate was concentrated and subjected to paper chromatography. The sugar present were identified as L-xylose and D-glucose (Rf 0.26 and 0.16).

**Alkaline Hydrolysis of MS-I:**

600 mg of the saponin MS-I was treated with 0.02 N H₂SO₄ (25ml) in a 250 ml round bottomed flask and the reaction mixture kept at room temperature for 7 days and extracted with n-butanol. Thin layer chromatographic examination of the butanol extracted showed the presence of two compounds. The butanol extract was concentrated and chromatographed over a column of silica gel G. and chloroform : methanol in different proportions were used as eluant, when two compounds designated MS-I(b), m.f. C₃₃H₅₅O₆ and MS-I(c), m.f. C₃₈H₆₃O₁₀ were obtained which were crystallized from methanol.

**Permethylation of Hydrolysis of the saponin MS-I:**

50 mg of the saponin MS-I on complete permethylation followed by hydrolysis gave 2,3,4, tri-O-methyl-L-xylose and 2, 3, 6. tri-O-methyl-D-glucose. Thereby concluding that the terminal sugar L-xylose was linked to D-glucose via (1→4) Linkage and also concluded that D-glucose and L-xylose were presented in he pyranose form.

The graded hydrolysis of the saponin MS-I with (0.02 N) sulphuric acid for one day at room temperature liberated first L-xylose and finally D-glucose, thereby concluding L-xylose to be terminal sugar.

**Enzymatic Hydrolysis of the saponin MS-I**

The saponin MS-I (30 mg) was dissolved in EtOH (20 ml) and mixed with Takadiastase solution (40 ml) in a 100 ml conical flask. The contents were allowed to stand for 2 days at room temperature and filtered. The hydrolysate after concentration was examined for sugars by paper chromatography and found to contain L-xylose (Rf =0.28).
The saponin (MS-I) 30 mg was dissolved in EtOH (20 ml) and mixed with almond emulsion (40 ml) in a 100 ml conical flask fitted with a stopper. The contents were allowed to stand at room temperature for 4 days and then filtered. The concentrated hydrolysate was examined on paper chromatography for sugar moieties using Whatmann filter paper NO-I and n-Butanol : acetic acid : water (4:1:5) as solvent system. The sugar was identified as D-glucose (Rf=0.18).

Thus the structure of saponin MS-I was established as Lupeol-3-O-α-L-xylopyranosyl [1→4]-O-β-D-glucopyranoside from the stem-bark of *Ficus-gglomerata* (Roxb.).
Thanks are due to The Head, Department of Chemistry, Dr. H.S. Gour University, Sagar (M.P.) for facilities and CDRI Lucknow for spectral analysis.

**SUMMARY**

The present paper deals with isolation and structural elucidation of the saponin Lupeol-3-O-α-L-xylopyranosyl [1→4]-O-β-D-glucopyranoside from the stem-bark of *Ficus-glomerata* Roxb. (Hindi – Gular).

**REFERENCES:**


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To,

The Executive Secretary
Indian Academy of Science
Bangalore.

Dear Sir,

Herewith I am enclosing a research paper entitled $\beta$-Amyrin-3-O-$\beta$-D-galactopyranosyl [1→4]-O-{$\alpha$}-L-rhamnopyranoside from the root of Ficus-gglomerata Roxb. for the favour of publication in your esteemed journal.

Hope you will please publish in your journal and with regards.

Thanking you,

Yours faithfully

Mrityunjai Shukla
Research Scholar

Date: June 18, 2007
Place: Sagar
**β-Amyrin-3-O-β-D-galactopyranosyl [1→4]-O-α-L-rhamnopyranoside from the root of Ficus glomerata Roxb.**

M. Shukla and V.K. Saxena  
Department of Chemistry  
Dr. H.S. Gour University, Sagar (M.P.) 470 003 India  

**ABSTRACT:**

*Ficus glomerata* Roxb. which is commonly known as ‘Gular’ in hindi belongs to natural order Moraceae, it is cultivated throughout India. The roots are useful in hydrophobia. The bark is useful in asthma, piles and galactogogues. The fruit is useful in chronic bronchitis, dry cough, lose of voice, diseases of the kidney and spleen. Its root, stem-bark and leaves possesses antiinflammatory and anthelmintic properties. The present work deals with the isolation and identification of the saponin MS-II characterized as -β-Amyrin-3-O-β-D-galactopyranosyl [1→4]-O-α-L-rhamnopyranoside, which has been isolated from the root of *Ficus glomerata* Roxb.

**KEY WORDS:** *Ficus glomerata* Roxb; Moraceae; saponin; β-Amyrin-3-O-β-D-galactopyranosyl [1→4]-O-α-L-rhamnopyranoside.

**INTRODUCTION:**

*Ficus glomerata*1-4 Roxb. which is commonly known as ‘Gular’ in hindi belongs to natural order Moraceae, it is cultivated throughout India. The roots are useful in hydrophobia. The bark is useful in asthma, piles and galactogogues. The fruit is useful in chronic bronchitis, dry cough, lose of voice, diseases of the kidney and spleen. Its root, stem-bark and leaves possesses antiinflammatory and anthelmintic5 properties. The present work deals with the isolation and identification of the saponin MS-II characterized as - β-Amyrin-3-O-β-D-galactopyranosyl [1→4]-O-α-L-rhamnopyranoside, which has been isolated from the root of *Ficus glomerata* Roxb.

Earlier workers have already reported the presence of Lupeol, β-Amyrin, β-sitosterol and guananol acetate in this plant.
RESULT AND DISCUSSION:

Air dried and powdered root of *Ficus glomerata* Roxb was extracted with (95%) hot ethanol. The ethanolic extract was concentrated under reduced pressure to get brown viscous mass, which was partitioned with n-hexane, benzene, chloroform, ethyl acetate and methanol.

The methanol soluble part on concentration under reduced pressure yielded a brown viscous mass which on addition of excess of solvent ether gave precipitate which on TLC examination over silica gel 'G', using solvent system n-butanol : acetic acid : water (4:1:5) showed two spots, indicating it to be a mixture of two compounds.

The precipitate was therefore subjected to column chromatography $^6,7$ over silica gel 'G' and eluted with acetone : methanol in different proportions and studied separately.

Eluates from acetone : methanol (2:1) were of the same Rf values and so combined. On removal of the solvent it yielded an amorphous mass.

It was crystallized from methanol, molecular formula C$_{39}$H$_{66}$O$_{10}$ (m.p.) 200-202°C and analysed for $M^+$ = 694 (by mass spectroscopy). It was soluble in methanol and ethanol.

The saponin MS-II acid hydrolysis yielded a sapogenin MS-I(A) m.f. C$_{26}$H$_{46}$O, m.p. 180-182°C [$M^+$] = 374 (FABMS) sugar moieties, D-galactose R$_f$ 0.18 and L-rhamnose R$_f$ 0.36).
Permethylatation by Khun8 Procedure followed by acid hydrolysis of the saponin MS-II, yielded sapogenin MS-II(A) and methylated sugars identified as 2,3,4,6-tera-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-rhamnose (by CoPC and CoTLC), thereby indicated that C1 of the rhamnose was involved in the glycoside linkage and also suggested that L-rhamnose was present in the pyranose form.

The saponin MS-II were first hydrolysed with enzyme almond emulsion which liberated D-galactose and prosapogenin MS-II(b) indicating that the linkage between sapogenin MS-II(A) and D-galactose was β.

Thereafter another enzyme Takadiastase was added to the hydrolysed products when it was found that L-rhamnose and D-galactose were also liberated thereby confirming the presence of α-linkage between L-rhamnose and sapogenin MS-II(A).

The sugars were quantitatively estimated by procedure of Mishra and Rao, which revealed that the saponin MS-II had sapogenin MS-II (A), D-galactose and L-rhamnose in the ratio of 1:1.

From the results it was confirmed that one molecule of the saponin give one molecule of sapogenin and one molecule of D-galactose and L-rhamnose.

The bands 3904.2-3425.5 cm-1 of IR spectrum of sapogenin MS-I (A), indicated the presence of -OH group(s) in it. Acetylation of sapogenin MS-I (A), with Ac2O/NaOAc in glacial acetic acid gave mono-acetate of sapogenin. It analyzed for molecular formula C29H48O11 m.p. 185-186°C and [M+] = 572 [FABMS]. The percentage of acetyl group (7.517%) was estimated quantitatively by the process of Weisenberger9.
as described by Belcher and Godbert\textsuperscript{10}, which showed the presence of only one acetylatable hydroxyl group in the sapogenin MS-II (A).

The sapogenin MS-II (A) was oxidised with Cr\textsubscript{2}O\textsubscript{3}/pyridine when it gave a ketone m.f. C\textsubscript{23}H\textsubscript{44}O, m.p.180-182\textdegree C and [M\textsuperscript{+}]=372 [FABMS], which responded positive to Zimmermen\textsuperscript{11} test for C-3 keto group there by confirming the presence of hydroxyl group at C-3 position and further indicated its nature as see on day in sapogenin MS-II (A). The IR spectrum of sapogenin MS-II (A) displayed a band at cm\textsuperscript{-1} which indicated the presence of double bond in it.

The catalytic hydrogenation with Pd/c it gave a dihydro derivative m.f. C\textsubscript{26}H\textsubscript{48}O, m.p. 187-188\textdegree C and (M\textsuperscript{+}) = 376 (FABMS), which indicated the presence of one double bond in the sapogenin MS-II (A).

The IR spectrum of sapogenin MS-II (A) showed a band at 1350.0 cm\textsuperscript{-1} indicated the presence of methyl group(s) in the sapogenin MS-II (A). The quantitative estimation of methyl groups was done by Ziesel's methods. Methyl group was found to be 20.053\%, which indicated the presence of five methyl groups in the sapogenin MS-II (A).

The position of methyl group(s) were established by \textsuperscript{1}HNMR spectrum. The \textsuperscript{1}HNMR spectrum of MS-II (A) showed three proton intensity singlets each at δ 0.78, δ 0.85, δ 0.96, δ 0.72 and δ 0.73, assigned for the positions of methyl groups at C\textsubscript{23}, C\textsubscript{24}, C\textsubscript{25}, C\textsubscript{26} and C\textsubscript{27} respectively in sapogenin MS-II (A) was β-amyrin, which was further confirmed by superimposable, \textsuperscript{1}HNMR IR, and mass spectral studies.

On the basis of deliberations, the saponin MS-II was identified as β-Amyrin-3-O-β-D-galactopyranosyl [1→4]-O-α-L-rhamnopyranoside.
EXPERIMENTAL

PLANT MATERIAL

The root of *Ficus glomerata* Roxb. (N.O. Moraceae) was collected locally in month October-November 2004 and was identified by reputed taxonomist. A herbarium specimen No. XXXVI has been submitted in the chemistry Department, room no. 36, Dr. H.S. Gour University, Sagar (M.P.).

EXTRACTION AND ISOLATION

Air dried and powdered root of *Ficus glomerata* Roxb was extracted with (95%) hot ethanol. The ethanolic extract was concentrated under reduced pressure to get brown viscous mass, which was partitioned with n-hexane, benzene, chloroform, ethyl acetate and methanol.

The methanol soluble part on concentration under reduced pressure yielded a brown viscous mass which on addition of excess of solvent ether gave precipitate which on TLC examination over silica gel 'G', using solvent system n-butanol : acetic acid : water (4:1:5) showed two spots, indicating it to be a mixture of two compounds.

The precipitate was therefore subjected to column chromatography over silica gel 'G' and eluted with acetone : methanol in different proportions and studied separately.

Eluates from acetone : methanol (2:1) were of the same Rf values and so combined. On removal of the solvent it yielded an amorphous mass.
It was crystallized from methanol, molecular formula C_{39}H_{66}O_{10} (m.p.) 200-202°C and analysed for M⁺ = 694 (by mass spectroscopy). It was soluble in methanol and ethanol.

Saponin MS-II showed significant absorption bands in the IR spectrum at $\nu_{\text{max}}^{KBr} \text{ cm}^{-1}$. 3906.4-3427.7 (free-OH), 2926.5 (CH$_2$ asymmetric stretching), 1597.2 (ring stretching, C=C stretching), 1351.0 (C–O stretching and O–H in plane bending vibration and CH$_3$ bending), 117.0 (C–O–C stretching asymmetric), 1031.5 (ring breathing mode), 766.8 (C–H of plane bending), 671.0 (C–O out of plane bending several bands).

**Acid Hydrolysis of the Saponin MS-II**

450 mg of the saponin MS-II was taken in a 500 ml of B-14 quick fit round bottomed flask having a reflux condenser attached to it. 100 ml of 7% H$_2$SO$_4$ was added to the flask. The flask was heated on a water bath for two hour and cooled when a crystalline sapogenin MS-II A precipitated out which was separated by filtration. The aqueous part was separately extracted with solvent ether in a separating funnel. The ethereal layer was washed with distilled water and dried over anhydrous sodium sulphate. Removal of the solvent ether yielded sapogenin. MS-II(A), which was crystallized from absolute alcohol. The sapogenin MS-II(A) analysed for m.p. 180-182°C m.f. C$_{26}$H$_{46}$O and [M⁺] = 374 (FABMS). IR spectrum $\nu_{\text{max}}^{KBr} \text{ cm}^{-1}$ 3904.2 and 3425.5 (-OH group), 2924.3 (CH$_2$ asymmetric stretching), 1595.0 (ring stretching C = C stretching), 1350.0 (C–O stretching and O-
H in plane bending and CH₃ bending), 1115.0 (C-O-C stretching vibration Asymmetric), 1030.5 (Ring breathing mode), 764.4 (C-H out of plane bending), 670.0 (C-H out of plane bending several bands). The important fragmentation pattern obtained in the FABMS of MS-II(A) were as follows m/z = 532, 386, 204, 178, 179 and 166.

⁹H-NMR δ 0.79 (3H, s, Me-C₂₃), δ 0.86 (3H, s, Me-C₂₄), δ 0.97 (3H, s, Me-C₂₅), δ 0.73 (3H, s, Me-C₂₆), δ 0.74 (3H, s, Me-C₂₇), δ 1.1 – 2.0 (23H, Complex pattern, polymethylene CH₂ and CH proton), δ 4.45 (1H, m, C₃-H), δ 2.25 (1H, t, J 3.30, C₁₂-H), δ 2.56 (2H, d, J 4.21, C₁₉-H), δ 3.15 (5H, m, galactose proton), δ 3.91 (4H, m, rhamnose), δ 3.85 (3H, complex signal, Me-rhamnose proton), δ 4.47 (1H, d, J 3.2, 1'anomeric proton), δ 4.49 (1H, d, J 3.3, 1''anomeric proton), δ 2.02 (3H, s, 2'-OAc), δ 2.04 (3H, s, 3'-OAc), δ 2.06 (3H, s, 2''-OAc), δ 2.08(3H, s, 3''-OAc), δ 2.12 (3H, s, 4''-OAc) and δ 2.16 (3H, s, 6''-OAc).

Neutralized concentrated aqueous hydrolysate showed the presence of D-galactose (Rₚ 0.18) and L-rhamnose (Rₚ 0.36).
Permethylation of saponin MS-II

The acid hydrolysis of saponin\textsuperscript{17-19} MS-II with 8\% H\textsubscript{2}SO\textsubscript{4} gave sapogenin MS-II (A) which was separated and filtered and sugar moeity (ies) remained in the solution. The aqueous hydrolysate was neutralized by adding BaCO\textsubscript{3} and the white precipitate of BaSO\textsubscript{4} was filtered off and the filtrate was concentrated under reduced pressure. The sugar was examined by PC using Whatman and aniline hydrogen phthalate as spraying reagent.

The sugars were identified as 2,3,4-tri-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-galactose, obviously showing that the terminal sugar D-galactose was linked to L-rhamnose via (1\rightarrow4) linkage and also suggested that D-galactose and L-rhamnose were present in the pyranose form.

**ENZYMATIC HYDROLYSIS**

The saponin MS-II (30 mg) was dissolved in ethanol (20 ml) and mixed with almond emulsion (40 ml) in a 100 ml round bottom flask. The contents were allowed to stand at room temperature for 4
days and then filtered. The concentrated hydrolysate was examined on paper chromatography for sugar moiety(ies) using Whatmann filter paper No. 1 n B : A : W (4:1:5) as solvent system. The sugar was identified as D-galactose Rf 0.18.

The saponin MS-II (30 mg) was dissolved in ethanol (20 ml) and mixed with Takadiastase solution (40 ml) in a 100 ml conical flask. The contents were allowed to stand for 2 days at room temperature and filtered. The Hydrolysate after concentration was examined for sugar by paper chromatography and was found to contain L-rhamnose (Rf 0.36).

REFERENCES


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To,
The Secretary
Institute of Chemist (India)
Kolkata.

Dear Sir,

Herewith I am enclosing a research paper entitled Quercetol-3-O-β-D-arabinopyranoside from the seeds of Embelia ribes (Burm.) for the favour of publication in your esteemed journal.

Hope you will please publish in your journal and with regards.

Thanking you,

Yours faithfully

Mrityunjai Shukla
Research Scholar

Date : June 18, 2007
Place : Sagar
Quercetol-3-O-β-D-arabinopyranoside from the seeds of *Embelia ribes* (Burm.)

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**KEY WORDS:**

*Embelia ribes* Burm, Myrsinaceae, Anthelmintic, Seeds, Flavonoidal -glycoside.

**ABSTRACT**

Quercetol-3-O-β-D-arabinopyranoside (MS-III) has been isolated from seeds of *Embelia ribes* Burm (N.O. Myrsinaceae) MS-III analysed or m.f. C_{20}H_{18}O_{10}, m.p. 240-241°C and [M'] = 436 (FABMS) and identified by various spectral analysis and chemical degradation.

**INTRODUCTION**

*Embelia ribes* Burm, which is commonly known as Baberang in hindi belongs to natural order Myrsinaceae. It is an evergreen tree found throughout India.

Its fruit is hot, dry, with a sharp bitter taste: good appetizer, carminative, anthelmintic, laxative, alternative, aires tumours, ascites, bronchitis, mental diseases, dyspnaea, disease of heart, urinary discharge, used in snake bite, jaundice, hemiorania, and worms in wounds.
RESULTS AND DISCUSSION

The ethylacetate soluble fraction of the concentrated alcoholic extract from seeds of Embelia ribes Burm plants yielded MS-III m.f. C_{20}H_{18}O_{10}, m.p. 240-241°C and [M^+] = 436 (FABMS). It gave all the reactions of flavonoids and also responded positively to Molisch test confirming the presence of glycoside. Flavonoidal glycoside MS-III showed significant bands in IR spectrum at $V_{\text{max}}$ cm$^{-1}$. 3906.1-3426.8 (free - OH), 2936.2 (C-H stretching vibration), 1612.1 (aromatic ring system), 1383.1 (C-O-C bending vibration), 1162.4 (>C=O), 1032.5 (C-O-C stretching vibration), 830.2 (two adjacent – H atoms in ring system).

$^1$H-NMR spectrum of MS-III showed signals at δ 6.31 (1H, d, J-2.3, C$_6$-H) and δ 6.79 (1H, d, J-2.5, C$_8$-H) shows protons of ring A. The doublets at δ 7.61 (1H, d, J-2.21, C$_2'$-H), δ 5.15 (1H, d, J-2.42, C$_3'$-H), δ 7.48 (1H, d, J-2.51, C$_6'$-H) and δ 6.70 (1H, d, J-2.54, C$_5'$-H) are attributed to protons of ring B.

Mass spectrum of MS-III showed [M^+] at m/z 436 and the loss of D-arabinose moiety from molecular ion gave fragment ion peak at 286.

Acid hydrolysis of Flavonoidal glycoside MS-III with 7% H$_2$SO$_4$ yielded aglycone MS-III (A) m.f. C$_{15}$H$_9$O$_6$ m.p. 216-220°C and [M^+] 286 (FABMS) and sugar moiety(ies) which was identified as D-arabinose (R$_f$ 0.21). It responded to all characteristic colour reactions of flavonoids and identified as Quercetol by spectral analysis.
In the IR spectrum of MS-III, a bond at 3906.1 cm\(^{-1}\) showed the presence of hydroxyl groups in it. On acetylation (Ac\(_2\)O/NaOAc) MS-III furnished an acetylated product, which analysed for m.f. C\(_{32}\)H\(_{30}\)O\(_{16}\), m.p. 250-252°C and [M\(^+\)] 577 (FABMS). The quantitative estimation of acetyl group (32.089%) was done by Weisenberger\(^2\) method as described by Belcher and Godbert. The results showed the presence of five hydroxyl groups in the MS-III. IR spectrum of acetylated produced a band at 3343.1 cm\(^{-1}\) which revealed that all the hydroxyl groups were not acetylated under condition (Ac\(_2\)O/NaOAc). The C-5–OH group was not acetylated due to strong intramolecular hydrogen bonding with carbonyl group at C-4.

**POSITION OF OH GROUP IN MS-III (A)**

**Position of –OH group at C-7 and C-5**

A bathochromic shift of 43 nm in band I with AlCl\(_3\) (relative to MeOH) and 15 nm in band II with NaOAc (relative to MeOH) showed the presence of –OH group at C-5 and C-7 respectively.

**Position of –OH group at C-4’**

(i) MS-III (A) gave pink coloured solution with Mg/HCl, which became blue on addition on NaHCO\(_3\) indicating the presence of –OH group at C-4’ in it.

(ii) A bathochromic shift of 47 nm of band I in presence of NaOMe (relative to band I in MeOH) further confirmed presence of –OH group at C-4’.
Position of \( -\text{OH} \) group at C-3

Remaining fifth \( -\text{OH} \) group was shown to be present at C-3 on the basis of:

(i) Studying the degradation products of aglycone MS-III (A) upon KOH fusion.

(ii) Yellow fluorescence of the aglycone in UV light, and the spectral shift with \( \text{AlCl}_3 \) in presence of HCl relative to band (I) in MeOH suggested a free C-3-OH group in the aglycone MS-III (A).

A characteristic colour reaction with Zn/HCl and Zirconiumoxy chloride in citric acid, further suggested the presence of OH group at C-3.

Permethylolation of compound MS-III followed by acid hydrolysis yielded aglycone MS-III (A) and methylated sugar, which was identified as 2, 3, 4-tri-O-methyl-D-arabinose (CoPC and CoTLC) indicating that C\textsubscript{1} of D-arabinose was involved in the glycosilation.

The enzymatic hydrolysis of MS-III with enzyme almond emulsion yielded aglycone MS-III(A) and D-arabinose confirming C-3 OH of the aglycone was linked to C-1\textsuperscript{\prime} of D-arabinose through \( \beta \)-linkage. Thus the glycoside MS-III was assigned the following structure and was found to be 5, 7, 4\textsuperscript{\prime} tri-hydroxy flavone 3-O-\( \beta \)-D-arabino pyranoside (Quercetol-3-O-\( \beta \)-D-arabinopyranoside).
PLANT MATERIAL

The seeds of Embelia ribes Burm. (N.O. Myrsinaceae) was collected locally in month October-November, 2004 and was identified by reputed taxonomist. A herbarium specimen no XXXVII has been submitted in the Chemistry Department room no. 36 Dr. H.S. Gour University, Sagar (M.P.).
EXTRACTION AND SEPARATION

2.0 kg of air dried and powdered seeds of Embelia ribes Burm were defatted with petroleum ether in soxhlet apparatus and then extracted with hot ethanol (95%). The ethanolic extract was concentrated under reduced pressure to yield yellow viscous mass, which was successively extracted with benzene, chloroform, ethyl acetate and methanol.

The ethyl acetate soluble fraction was concentrated under reduced pressure to get light yellow viscous mass, which was subjected to TLC examination using n-Butanol : Acetic acid : Water (4:1:5) as solvent system and I₂ vapours as visualising agent. It display two spots and was therefore subjected to column chromatography over silica gel-G and eluated with acetone : benzene with ratio of 3:1, 3:2 and 1:1 respectively.

Eluates from acetone : benzene (3:2) were found to have same Rₜ value and so combined together. On removal of the solvent it gave a yellow compound which obtained and crystallized from methanol to get light yellow crystals of compound (MS-III) m.f. C₂₀H₁₈O₁₀, m.p. -240-241°C and [M⁺]= 436 (FABMS).

¹H-NMR : [DMSO 300 MHz]

δ 6.31 (1H, d, J 2.3, C₆-H), δ 6.79 (1H, d, J 2.5, C₈-H), δ 5.51 (1H, d, J 2.42, C₃'-H), δ 7.61 (1H, d, J 2.21, C₂'-H), δ 7.48 (1H, d, J 2.51, C₆'-H), δ 6.70 (1H, d, J 2.54, C₅'-H), δ 4.41 (6H, m, Arabinose proton), δ 4.48 (1H, d, J= 2.3, 1'anomeric proton), δ 2.02 (3H, s, C₂'-OAC), δ 2.04 (3H, s, C₃'-OAC), δ 2.06 (3H, s, C₄'-OAC).
IR $\nu_{max}^{KBr}$ cm$^{-1}$ 3906.1-3426.8 (free - OH), 2936.2 (C-H stretching vibration), 1612.1 (aromatic ring system), 1383.1 (C-O-C bending vibration), 1162.4 (>C=O), 1032.5 (C-O-C stretching vibration), 830.2 (two adjacent – H atoms in ring system).

**UV $\lambda_{max}$**

$\lambda_{max}^{MeOH}$ 260, 267 nm, $\lambda_{max}^{NaOMe}$ 256, 280, 320 nm, $\lambda_{max}^{NaOMe}$ 270, 272, 360 nm, $\lambda_{max}^{HCl}$ 258, 285, 418 nm, $\lambda_{max}^{HCl}$ 278, 386 nm, $\lambda_{max}^{NaOMe+HCl}$ 284, 372 nm.

**ACID HYDROLYSIS OF THE GLYCOSIDE MS-III**

200 mg of the glycoside (MS-III) was taken with 25 ml of 7% H$_2$SO$_4$ in a round bottomed flask fitted with a reflux condenser. The reaction mixture was heated for about 8 hrs on a water bath. The contents of the flask were extracted with water and aqueous layer was shaken with solvent ether in a separating funnel. The ethereal layer was separated, washed and dried over anhydrous sodium sulphate. After removal of solvent ether, yellow crystals of aglycone MS-III (A) was obtained.

The hydrolysate obtained by acid hydrolysis of glycoside MS-III was neutralized by adding BaCO$_3$ and BaSO$_4$ was filtered off. After filtration the filtrate was concentrated to a light yellow viscous mass which was subjected to paper chromatography on Whatmann filter paper No.1. Aniline hydrogen phthalate was used as visualizing agent. Spectral data of the compounds one as under.
\[1^{\text{H}}\text{-NMR : [DMSO 300 MHz]}\]

\[\delta 6.30 \ (1\text{H}, \ d, \ J \ 2.2, \ C_6\text{-H}), \ \delta 6.78 \ (1\text{H}, \ d, \ J \ 2.4, \ C_8\text{-H}), \ \delta 5.14 \ (1\text{H}, \ d, \ J \ 2.41, \ C_3'\text{-H}), \ \delta 7.60 \ (1\text{H}, \ d, \ J \ 2.20, \ C_2'\text{-H}), \ \delta 7.47 \ (1\text{H}, \ d, \ J \ 2.50, \ C_6'\text{-H}) \text{ and } \delta 6.69 \ (1\text{H}, \ d, \ J \ 2.53, \ C_5'\text{-H}).\]

IR \(V_{\text{max}}^K \text{cm}^{-1} 3900.0-3420.0 \) (free - OH), 2930.1 (C-H stretching vibration), 1620.1 (aromatic ring system), 1385.3 (C-O-C bending vibration), 1157.3 (>C=O), 1030.1 (C-O-C stretching vibration), 835.0 (two adjacent - H atoms in ring system).

\[\text{UV } \lambda_{\text{max}}\]

\(\lambda_{\text{max}}^{\text{MeOH}} 255, 353 \text{ nm}, \ \lambda_{\text{max}}^{\text{NaOAc}} 255, 396, 408 \text{ nm}, \ \lambda_{\text{max}}^{\text{NaOMe}} 255, 245, 353 \text{ nm}, \ \lambda_{\text{max}}^{\text{AlCl}_3} 252, 319, 400 \text{ nm}, \ \lambda_{\text{max}}^{\text{AlCl}_3+\text{HCl}} 270, 353 \text{ nm}, \ \lambda_{\text{max}}^{\text{NaOAc}+\text{H}_2\text{SO}_4} 272, 351 \text{nm}.

\[\text{PERMETHYLATION FOLLOWED BY HYDROLYSIS GLYCOSIDE MS-III}\]

35 mg of the glycoside MS-III was treated with methyl iodide (5ml) and Ag\(_2\text{O}\) (20 mg) in DMF (5ml) in a conical flask. The reaction mixture was left for about 48 hrs. at room temperature. The precipitate obtained was filtered and filtrate was treated with 7% H\(_2\text{SO}_4\) when aglycone and methylated sugars were obtained. The hydrolysate was neutralized by adding BaCO\(_3\) and BaSO\(_4\) was filtered off. After filtration the concentrated mass was examined by paper chromatography\(^{53}\) using n-BAW (4:1:5) as solvent system and aniline hydrogen phthalate as visualizing agent. The sugar was identified as 2, 3, 4-tri-O-methyl-D-Arabinose.
ENZYMATIC HYDROLYSIS OF THE FLAVANOIDAL GLYCOSIDE MS-III

The glycoside MS-III (25 mg) was dissolved in ethanol (10ml) and mixed with 25 mg of enzyme Takadiastase55-59 in a conical flask. The flask was allowed to stand for 8 hrs. at room temperature and then filtered. The aglycone was identified as quercetol by superimposable spectral analysis. The sugar in the hydrolysate was identified as D-arabinose by CoPC, using Whatmann filter paper no.1 and n-BAW (4:1:5) as solvent system ($R_f = 0.22$ reported, $R_f = 0.23$ found).

PERIODATE OXIDATION OF THE FLAVANOIDAL GLYCOSIDE MS-III

The glycoside MS-III (40mg) was dissolved in methanol (30 ml) in a conical flask (100ml) fitted with a glass stopper. Sodium meta periodate (15 ml) was then added and reaction mixture was left standing for 48 hrs. Simultaneously a blank experiment was run with the same procedure. The amount of sodium meta periodate consume and formic acid liberated was estimated by Jone’s method.

ACKNOWLEDGEMENTS

Thanks are due to the Head, Regional Sophisticated Instrumentation Centre (CDRI, Lucknow) for recording various spectra and Head Department of Chemistry of Dr. H.S. Gour University for providing facilities.
REFERENCE


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To,

The Executive Secretary
Indian Academy of Science
Bangalore.

Dear Sir,

Herewith I am enclosing a research paper entitled **Stigmasterol-3-0-β-D-arabinopyranosyl [1→4]-0-β-D-glucopyranoside from the seeds of Embelia ribes (Burm)** for the favour of publication in your esteemed journal.

Hope you will please publish in your journal and with regards.

Thanking you,

Yours faithfully

Mrityunjai Shukla
Research Scholar

Date : June 18, 2007
Place : Sagar
Stigmasterol-3-0-β-D-arabinopyranosyl [1→4]-0-β-D-glucopyranoside from the seeds of *Embelia ribes* (Burm)

**M. Shukla and V.K. Saxena**
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**ABSTRACT:**

*Embelia ribes* Burm, which belongs to natural order Myrsinaceae, is commonly known as 'baberang' in hindi. It is an evergreen tree found in throughout India. The fruit is hot dry, with a sharp bitter taste, good appetizer, carminative, anthelmintic, alexiteric, laxative, alternative; cures tumours, ascites, bronchitis mental diseases and seeds possesses anti-inflammatory and anthelmintic properties. The present work deals with the isolation and identification of the saponin MS-IV characterized as Stigmasterol-3-0-β-D-arabinopyranosyl [1→4]-0-β-D-glucopyranoside from the seeds of embelia ribes (Burm).

**KEY WORDS:** Embelia ribes Burm, Myrsinaceae, Saponin, Stigmasterol-3-0-β-D-arabinopyranosyl [1→4]-0-β-D-glucopyranoside, anthelmintic properties.

**INTRODUCTION**

*Embelia ribes*1-5 Burm, which belongs to natural order Myrsinaceae, is commonly known as 'baberang' in hindi. It is an evergreen tree found in throughout India.

The fruit is hot dry, with a sharp bitter taste, good appetizer, carminative, anthelmintic, alexiteric, laxative, alternative; cures tumours, ascites, bronchitis, mental diseases, dyspnoea, disease of heart, urinary
discharge and is used in snake bite, jaundice, hemicrania and worms in wounds. The seeds, leaves and stem possesses anti-inflammatory and anthelmintic properties. The present work deals with the isolation and identification of the saponin MS-IV characterized as Stigmasterol-3-0-β-D-arabinopyranosyl [1→4]-0-β-D-glucopyranoside from the seeds of embelia ribes (Burm).

Earlier workers have already reported the presence of Embelic acid, Villangin, Tannin and Quercetol in this plant.

RESULTS AND DISCUSSION

Air dried and powdered seeds of Embelia ribes Burn were defatted with petroleum ether in soxhlet apparatus and then extracted with hot ethanol (95%). The ethanolic extract was concentrated under reduced pressure to get brown viscous mass, which was partitioned with n-hexane, benzene, chloroform, methanol ethylacetate and acetone.

The benzene, chloroform and acetone soluble parts on removal of the solvent resulted in very small amount of viscous residues which were insufficient for any substantiative study.

The methanol soluble part on concentration under reduced pressure yielded a brown viscous mass which on addition of excess of solvent ether gave a precipitate which on TLC examination over silica gel G, using solvent system n-butanol : acetic acid : water (4:1:5) showed two spots indicating it to be a mixture of two compounds.
The precipitate was therefore subjected to column chromatography\(^6\) over silica gel 'G' and eluted with acetone : methanol in different fractions and studied separately.

Eluates from acetone : methanol (2:1) were of the same R\(_f\) value and so combined and subsequently removal of the solvent yielded a homogenous mass was confirmed by the TLC examination. It was therefore crystallised from pyridine and analysed for molecular formula C\(_{40}\)H\(_{71}\)O\(_{10}\), m.p. 168-169°C and [M\(^+\)] = 711 (FABMS).

The saponin MS-IV was hydrolysed with 7% H\(_2\)SO\(_4\) when the sapogenin MS-IV (A) precipitated out. It was filtered and washed with water. The sugar moiety(ies), D-arabinose (R\(_f\) 0.21) and D-glucose (R\(_f\) 0.18).

Permethylation by Khun\(^7\) Procedure followed by acid hydrolysis of the saponin MS-IV, yielded sapogenin MS-IV(A) and methylated sugars identified as 2,3,4,6-tera-O-methyl-D-glucose and 2, 3, 4 – tri-O-methyl-D-arabinose (by CoPC and Co-TLC) indicating that D-arabinose and D-glucose both are present in pyranoside form.

The steroidal saponin MS-IV was subjected to hydrolysis by enzyme almond emulsion when the sugars were liberated. The study of sugars by PC indicated the presence of D-arabinose and D-glucose thereby confirming the linkage between D-arabinose and the sapogenin as well as between D-glucose and D-arabinose was β.
From the results it was confirmed that one molecule of the saponin give one molecule of sapogenin and one molecule of D-glucose and D-arabinose in the ratio of 1:1.

Bands at $\nu_{max}^{KBr}$ 3754.3 and 3423.2 cm$^{-1}$ of the IR spectrum of the saponin MS-IV indicated the presence of $-\text{OH}$ group(s). The number of hydroxyl ($-\text{OH}$) group(s) were estimated by acetylation of saponin MS-IV with Ac$_2$O/pyridine when it yielded an acetylated product m.p. 197-198°C, molecular formula C$_{31}$H$_{53}$O and $[M^+] = 441$ (FABMS). The percentage of the acetyl group in the acetylated product was estimated by the procedure of Weisenberger$^8$ as described by Belcher and Godbert$^9$ (58.503%), which showed the presence of six-OH groups in it.

On Cr$_2$O$_3$/pyridine oxidation, sapogenin MS-IV(A) yielded a ketone, m.f. C$_{35}$H$_{63}$O$_2$, m.p. 227-229°C and $[M^+] = 739$ (FABMS), which responded to positive Zimmerman$^{10}$ test for C-3 keto group, thereby confirming the presence of hydroxyl group at C-3 and further indicated its nature as secondary in sapogenin MS-IV(A).

The characteristics band at $\nu_{max}^{KBr}$ 1594.0 cm$^{-1}$ in the IR spectrum of the sapogenin MS-IV(A) showed the presence double bond(s) in it. On catalytic hydrogenation with Pd/C sapogenin MS-IV(A) gave a tetra hydro derivative m.f. C$_{35}$H$_{67}$O m.p. 225-226°C and $[M^+] = 420$ (FABMS), which indicated the presence of two double bond in sapogenin MS-IV(A).

The IR spectrum of sapogenin MS-IV(A) showed band(s) at $\nu_{max}^{KBr}$ 1351.0 and 1381.1 cm$^{-1}$, which indicated the presence of angular
methyl group(s) in sapogenin MS-IV(A). The quantitative estimation of methyl group(s), which was done by Ziesel's methods. It found to be (21.634%), which indicated the presence of six angular methyl group(s) in sapogenin MS-IV(A).

The position of methyl group(s) was established by the study of $^1$HNMR spectrum. The $^1$HNMR spectrum of sapogenin MS-IV(A) showed three proton intensity singlet each at $\delta$ 0.71, $\delta$ 0.83 doublets each at $\delta$ 0.76 J-6.4, $\delta$ 0.70 J-6.6 and triplet $\delta$ 0.89 J-6.33, assigned for the position of methyl group at C-18, C-19, C-25, C-26, C-28 and C-29 respectively in the sapogenin MS-IV(A).

On the basis of above discussion, the saponin MS-IV was identified as Stigmasterol-3-0-\(\beta\)-D-arabinopyranosyl [1→4]-0-\(\beta\)-D-glucopyranoside.

**EXPERIMENTAL**

**Plant Material**

The seeds of *Embelia ribes* Burm. (N.O. Myrsinaceae) was collected locally in month October-November, 2004 and was identified by reputed taxonomist. A herbarium specimen no XXXVII has been submitted in the Chemistry Department room no. 36 Dr. H.S. Gour University, Sagar (M.P.).

**Extraction and Isolation**

Air dried and powdered (3 kg) seeds of *Embelia ribes* Burm. were defatted with petroleum ether in soxhlet apparatus and then extracted
with 95% hot ethanol. The ethanolic extract was concentrated under reduced pressure to get brown viscous mass, which was partitioned with n-hexane, benzene, chloroform, ethyl acetate and methanol.

**THE STUDY OF THE METHANOL SOLUBLE PART**

The methanol soluble part (180 ml) on concentration under reduced pressure yielded a brown viscous mass (15 gm) which on addition of excess of solvent ether gave a precipitate which on TLC examination over silica gel 'G' using solvent system n-butanol : acetic acid:water (4:1:5) showed two spots.

Eluates (11-15) each of 20 ml of acetone : methanol (4:3) had same \( R_f \) value and so combined and on removal of the solvent yielded a light green coloured compound (1.45 gm). The homogenous nature of the compound was confirmed by TLC examination. It analysed for m.p. 190-191°C molecular formula \( C_{40}H_{71}O_{10} \) \( M^+ = 711 \). It responded to following characteristic reaction of saponin MS-IV.

Compound MS-IV showed significant bands, in the IR spectrum at \( V_{\text{max}}^{KBr} \) cm\(^{-1}\); 3754.3-3423.2 (-OH), 2817.4 (-CH\(_3\) stretching), 1596.2 (C=C stretching), 1461.2 (C-H bending vibration of methyl group), 1383.3 (CH\(_3\) symmetric stretching), 1352.2 (CH\(_3\) bending), 769.2 (C-H out of plane bending).

**ACID HYDROLYSIS OF THE SAPONIN MS-IV**

500 mg of the saponin MS-IV was taken in a 550 ml of β-14 quick fit round bottomed flask having a reflux condenser attached to
it. 100 ml of Ti \(\text{H}_2\text{SO}_4\) was added to the flask. The flask was heated on water both for two hours and cooled when a crystalline sapogenin MS-IV (A) precipitated out which was separated by filtration. The aqueous part was separately extracted with solvent ether in a separatory funnel. The ethereal layer was washed with water and dried over anhydrous sodium sulphate removal of the solvent ether, yielded sapogenin MS-IV A which was crystallized from absolute alcohol m.p. 220-221°C.

Compound MS-IV showed significant bands, in the IR spectrum\(^{11}\) at \(\nu_{\text{max}}^{\text{KBr}}\) cm\(^{-1}\): 3753.2-3421.0 (–OH), 2815.2 (–CH\(_3\) stretching), 1594.0 (C=C stretching), 1460.1 (C–H bending vibration of methyl group), 1381.1 (CH\(_3\) symmetric stretching), 1351.0 (CH\(_3\) bending), 768.0 (C–H-out of plane bending). The significant fragmentation pattern obtained in the FAB-MS of MS-IV(A) were as follows m/z = 711, 579, 416, 382, 290, 284, 276 and 256.

\(^1\)H-NMR\(^{12-16}\) \(\delta\) 0.71 (3H, s, Me-C\(_{18}\)), \(\delta\) 0.83 (3H, s, Me-C\(_{19}\)), \(\delta\) 0.76 (3H, d, J = 6.4, Me-C\(_{23}\)), \(\delta\) 0.70 (3H, d, J = 6.6, Me-C\(_{26}\)), \(\delta\) 0.89 (3H, t, J= 53.8, Me-C\(_{28}\)), \(\delta\) 0.57 (3H, d, J = 6.33, Me-C\(_{29}\)), \(\delta\) 5.62 (1H, dd, J = 6.79, C\(_{21}\)-H), \(\delta\) 5.51 (1H, dd, J = 6.58, C\(_{22}\)-H) 1.3 – 2.01 (25H, Complex pattern, polymethylene CH\(_2\) and CH proton), \(\delta\) 4.48 (1H, m, C\(_3\)-H), \(\delta\) 4.16 (5H, m, glucose proton), \(\delta\) 4.41 (6H, m, arabinose), \(\delta\) 2.03 (3H, s, C\(_2\)-OAc), \(\delta\) 2.06 (3H, s, C\(_3\)-OAc), \(\delta\) 2.08 (3H, s, C\(_6\)-OAc), \(\delta\) 2.14 (3H, s, C\(_2\)"-OAc), \(\delta\) 2.16 (3H, s, C\(_3\)"-OAc), \(\delta\) 2.18 (3H, s, C\(_4\)"-OAc), \(\delta\) 4.56 (1H, d, J = 2.51, 1'-anomaric proton) and \(\delta\) 4.58 (1H, d, J = 2.3, 1"-anomaric proton).
Permethylolation of the saponin MS-II

The aqueous hydrolyrate obtained after separating sapogenin MS-IV(A) after hydrolysis of saponin MS-IV was neutralized with BaCO$_3$ and BaSO$_4$ was filleted off. The filtrate was concentrated to get a syrpy mass which was found to reduce Fehling's solution. It also gave colour with aniline hydrogen phthalate.

The concentrated hydrolyrate was therefore, subjected to paper chromatography with authentic sugar samples on Whatmann No.1 filter paper using aniline hydrogen phthalate as spraying reagent. The analysis revealed the presence of D-arabinose and D-glucose as sugar
moieties (confirmed) by CoPC and CoTLC with authentic sugars. The methylated sugars were identified as 2,3,4,6-tetra-O-methyl-D-glucose and 2, 3, 4 tri-O-methyl-D-arabinose (by CoPC and Co-TLC) indicating that D-arabinose and D-glucose both are present in pyranoside form.

ENZYMATIC HYDROLYSIS

The steroidal saponin MS-IV (30 mg) was suspended in an almond emulsion solution (30 ml) and kept at 40°C for 30 hours.

The hydrolysate was paper chromatographed over Whatmann No.1 filter aniline hydrogen phthalate was used as spraying reagent.

Appearance of two spots which corresponded to D-glucose and D-arabinose of authentic sample indicated that the nature of linkage was β between sapogenin MS-IV (A) and D-glucose as well as between D-glucose and D-arabinose.

ACKNOWLEDGEMENTS

Thanks are due to the Head, Regional Sophisticated Instrumentation Centre (CDRI, Lucknow) for recording various spectra and Head Department of Chemistry of Dr. H.S. Gour University for providing facilities.
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       shuklaji_ashu@yahoo.co.in
Mob: 09907048951

To,

The Editor
Research Journal of Chemistry & Environment
Indore.

Dear Sir,

Herewith I am enclosing a research paper entitled *EMBELIA RIBES BURM SEEDS: AS ANTHELMINTIC AGENTS* for the favour of publication in your esteemed journal.

Hope you will please publish in your journal and with regards.

Thanking you,

Yours faithfully

Mrityunjai Shukla
Research Scholar

Date : June 18, 2007
Place : Sagar
EMBELIA RIBES BURM SEEDS: AS ANTHELMINTIC AGENTS

M. Shukla and V.K. Saxena
Department of Chemistry
Dr. H.S. Gour University, Sagar (M.P.) 470 003 India

Abstract

The present paper deals with the study of anthelmintic activity against isolated compound of Embelia ribes Burm plants (N.O. Myrsinaceae). The earthworm (phеритима posthumа), tapeworm (Taenia solium) were used and the anthelmintic activity of isolated compound MS-III and MS-IV seeds of Embelia ribes Burm.

The compound MS-III show significant anthelmintic activity in comparison compound MS-IV and so may potentially be explored as anthelmintic agents.

Keywords: Embelia ribes Burm, anthelmintic activity, earthworm, tapeworm.

INTRODUCTION:

Embelia ribes1-5 Burm, which belongs to natural order Myrsinaceae, is commonly known as 'baberang' in hindi. It is an evergreen tree found in throughout India.

The fruit6 is hot dry, with a sharp bitter taste, good appetizer, carminative, anthelmintic, alesteric, laxative, alternative; cures tumours, ascites, bronchitis, mental diseases, dyspnoea, disease of
heart, urinary discharge and is used in snake bite, jaundice, hemicrania and worms in wounds.

**Experimental**

**Isolation of the Compounds**

Air dried, powdered and defatted seeds of *Embelia ribes* Burm were extracted with 95% ethanol. The concentrated ethanolic extract was successively extracted with various solvents. The ethyl acetate soluble fraction when worked by column chromatography yielded compounds MS-III whereas methanol soluble fraction yielded compound MS-IV.

A brief account of the isolated compound is tabulated in table.

**Anthelmintic Activity of Isolated Compound**

The anthelmintic activity\(^7\text{--}^9\) of the compounds was tested on the various species of helminth. A detailed method s described below:
TEST WORMS

Testing of anthelmintic activity the following two species of helminth were taken for this study:

Pheritima posthuma - Earthworm

Taenia solium - Tapeworm

CHEMICAL

Piperazine phosphate and aqueous tween 80 was included in the assay as standard reference drug as Anthelmintic agents.

EXPERIMENTAL

In the present study, the earthworms (Pheritima posthuma) tapeworms (Taenia solium) were used and the activity of compound MS-III and MS-IV was compared with piperazine phosphate.

Activity Against Earthworm and Tapeworms
Preparation of Standard and Sample Solution

Emulsions of compound MS-III and MS-IV were prepared in Tween 80 in the concentration of 0.5% and 1.0% w/v. Piperazine
phosphate was dissolve in normal saline solution to give 0.5% and 1.0% w/v solution.

**Procedure**

10 ml volume of emulsions were transferred to petri-dishes of 4-inches diameters. Five earthworms washed with the normal saline solution and placed in each petri-dish containing the emulsions of MS-III and MS-IV.

Same procedure was followed for controls (Tween 80 solution and piperazine phosphate solution) also.

Another control was maintained with normal saline solution. The movements of earthworms and tapeworm were stimulated and became more marked. The worms tried to get out of the petri-dish. Thereafter, they became progressively sluggish until death supervened. A duplicate was run for all these pentri-dishes.

The time taken for complete paralysis and death was observed. The mean paralysis time and mean death time taken by each drug was recorded. The time taken by the earthworms and tapeworm to become motionless was noted as paralysis time. To earthworm and tapeworm death of motionless worms, one or more worms were transferred to hot water (50°C) which stimulated and induced movement in worms if alive 216. A number of observation were made to confirm the reading and average results were recorded.

**Results**

The isolated compound MS-III, MS-IV were investigated for their anthelmintic activity against Earthworm (Phritima posthuma) and
Tapeworm (Taenia solium). The results of the present investigation are summarized in the table.

**Table 1 : Anthelmintic activity of MS-III of *Embelia ribes* Burm.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime of minute for paralysis and death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Earth worm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Flavonoidal glycoside MS-III in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>52</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Normal saline</td>
<td>0.5</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>40</td>
</tr>
</tbody>
</table>

**Table 2 : Anthelmintic activity of MS-IV of *Embelia ribes* Burm.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime of minute for paralysis and death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Earth worm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Saponin MS-IV in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>55</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Saline solution</td>
<td>0.5</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>52</td>
</tr>
</tbody>
</table>
DISCUSSION

The piperazine are present in the market in more palatable from of salts (diphenyl acetate, adipate, citrate, tartrate, dilaurate etc.) which are being used in the treatment of parasitic invasion by helminthes.

Isolated compound MS-III and MS-IV of *Embelia ribes* Burm exhibited marked anthelmintic activity.

The results revealed that the compounds MS-III show significant anthelmintic activity in comparison compound MS-IV and so may potentially be explored as anthelmintic agents.

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Mob: 09907048951

To,
Ms. Shahida Iqbal
Oriental Journal of Chemistry
Bhopal.

Dear Sir,

Herewith I am enclosing a research paper entitled **FICUS GLOMERATA ROXB ROOT AND STEM-BARK : AS ANTHELMINTIC AGENTS** for the favour of publication in your esteemed journal.

Hope you will please publish in your journal and with regards.

Thanking you,

Yours faithfully

Mrityunjai Shukla
Research Scholar

Date: June 18, 2007
Place: Sagar
FICUS GLOMERATA ROXB ROOT AND STEM-BARK : AS ANTHELMINTIC AGENTS

M. Shukla and V.K. Saxena
Department of Chemistry
Dr. H.S. Gour University, Sagar (M.P.) 470 003 India

Abstract

The present paper deals with the study of anthelmintic activity against isolated compound of Ficus glomerata Roxb (N.O. Moracea). The earthworm (phertima posthuma), tapeworm (Taenia solium) were used and the anthelmintic activity of isolated compound MS-I and MS-II of Ficus glomerata Roxb plant part stem-bark and root respectively. The compound MS-II show significant anthelmintic activity in comparison compound MS-I and so may potentially be explored as anthelmintic agents.

Keywords: Ficus glomerata Roxb, anthelmintic activity, earthworm, tapeworm.

Introduction:

Ficus glomerata\textsuperscript{1-6} Roxb. which is commonly known as ‘Gular’ in hindi belongs to natural order Moraceae, it is cultivated throughout India. The different parts of plants are used in the treatment of dysentery, vulnerary, kapha, biliousness, and diseases of vagina. The roots are useful in hydrophobia. The bark is useful in asthma, piles and galactogogues. The fruit is useful in chronic bronchitis, dry cough, lose of voice, diseases of the kidney and spleen.
Experimental

Isolation of the Compounds

Air dried and powdered stem bark of *Ficus glomerata* Roxb. was extracted with 95% ethanol and ethanolic extract was concentrated under reduced pressure. The residue obtained after concentration was partitioned into ethylacetate, chloroform, benzene, acetone, n-hexane and methanol. The methanol soluble fraction when worked up yielded compound MS-I, and MS-II.

A brief account of the isolated compound is tabulated in table.

Anthelmintic Activity of Isolated Compound

The anthelmintic activity\(^7-9\) of the compounds was tested on the various species of helminth. A detailed method s described below:
TEST WORMS

Testing of anthelmintic activity the following two species of helminth were taken for this study:

Pheritima posthuma - Earthworm

Taenia solium - Tapeworm

CHEMICAL

Piperazine phosphate and aqueous tween 80 was included in the assay as standard reference drug as Anthelmintic agents.
EXPERIMENTAL

In the present study, the earthworms (Pheritima posthuma) tapeworms (Taenia solium) were used and the activity of compound MS-I and MS-II, was compared with piperazine phosphate.

Activity Against Earthworm and Tapeworms

Preparation of Standard and Sample Solution

Emulsions of compound MS-I and MS-II were prepared in Tween 80 in the concentration of 0.5% and 1.0% w/v. Piperazine phosphate was dissolve in normal saline solution to give 0.5% and 1.0% w/v solution.

Procedure

10 ml volume of emulsions were transferred to petri-dishes of 4-inches diameters. Five earthworms washed with the normal saline solution and placed in each petri-dish containing the emulsions of MS-I and MS-II.

Same procedure was followed for controls (Tween 80 solution and piperazine phosphate solution) also.

Another control was maintained with normal saline solution. The movements of earthworms and tapeworm were stimulated and became more marked. The worms tried to get out of the petri-dish. Thereafter, they became progressively sluggish until death supervened. A duplicate was run for all these petri-dishes.
The time taken for complete paralysis and death was observed. The mean paralysis time and mean death time taken by each drug was recorded. The time taken by the earthworms and tapeworm to become motionless was noted as paralysis time. To earthworm and tapeworm death of motionless worms, one or more worms were transferred to hot water (50°C) which stimulated and induced movement in worms if alive 216. A number of observation were made to confirm the reading and average results were recorded.

Results

The isolated compound MS-I and MS-II were investigated for their anthelmintic activity against Earthworm (Phritima posthuma) and Tapeworm (Taenea solium). The results of the present investigation are summarized in the table.

Table 1: Anthelmintic activity of compound MS-I of Ficus glomerata Roxb.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime of minute for paralysis and death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Earth worm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Saponin MS-I in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Normal saline (NaCl)</td>
<td>0.5</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>38</td>
</tr>
</tbody>
</table>
Table 2: Anthelmintic activity of compound MS-II of *Ficus glomerata* Roxb.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test substances</th>
<th>Concentration % (w/v)</th>
<th>Meantime of minute for paralysis and death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Earth worm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td>1.</td>
<td>Compound Saponin MS-II in Aqueous Tween 80 (3%)</td>
<td>0.5</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>35</td>
</tr>
<tr>
<td>2.</td>
<td>Piperazine phosphate in Normal saline solution</td>
<td>0.5</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>57</td>
</tr>
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</table>

**DISCUSSION**

The piperazine are present in the market in more palatable from of salts (diphenyl acetate, adipate, citrate, tartrate, dilaurate etc.) which are being used in the treatment of parasitic invasion by helminthes.

Isolated compound MS-I and MS-II of *Ficus glomerata* Roxb exhibited marked anthelmintic activity.

The results revealed that the compounds MS-II show significant anthelmintic activity in comparison compound MS-I and so may potentially be explored as anthelmintic agents.
References:


PROCEEDINGS
OF THE
NINETY FOURTH SESSION OF THE
INDIAN SCIENCE CONGRESS

ANNAMALAINAGAR, 2007

PART II (Abstracts)

SECTION OF
CHEMICAL SCIENCES

President : Prof. Anil K. Singh

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136. Anthelmintics Agents – Indian Medicinal Plants

V. K. Saxena* and Mrityunjai Shukla
Department of Chemistry,
Dr. H. S. Gour University,
Sagar – 470003 (M.P.)

Key words: Indian medicinal plants; Anthelmintic; Antibacterial; Antidiarrhoeal; Anti-inflammatory; Antidiabetic; Anti-allergic; Review.

A survey of available literature on Indian flora has revealed that a large number of herbal Indian medicinal plants, which are mainly found in Indian subcontinent possess anthelmintic activities due to the active principal present in them. An ideal anthelmintic drug should possess antibacterial, antidiarrhoeal, anti-inflammatory, antidiabetic, anti-allergic properties with minimal or no side effects. It is prudent to focus research work in this area in order to search ideal anthelmintic drugs. The medicinal plants like: Embelia ribes, Punica granatum, Butea frondosa, Centratherum anthelminticum, Cucurbita maxima and some others have been found to be anthelmintic and their phytochemical investigation has shown that they possess compounds like Emblin (Embelic acid; 2,5-dihydroxy-3-undecyl-1, 1, 4-benzoquinone), leucoanthocyanins (Leuco cyanidine –3-O-β-D-glucopyranoside leucoanthocyanins, Leuco cyanidine-3-O- β-D-glucopyranoside, palasonin etc.

137. Microwave Assisted Synthesis and Antimicrobial Study of 1-isonicotinoyl/thiocarboxamido-3-[3-[(4-chlorophenyl)-sulphonamido] Phenyl]-5-aryl-2-pyrazolines

P. R. Bhagat* and S. P. Koshatwar
1Department of Chemistry,
J. Darda Institute of Engineering and Technology,
Lohara, Yavatmal – 445 001 (M.S.)

Key words: Microwave synthesis; Pyrazolines.

In last few decades, chemists are emphasising on the synthesis of organic compounds by the pathway of green chemistry employing ultrasound, microwaves
The Indian Science Congress Association
14, Dr. Biresh Guha Street,
Kolkata -- 700 017

Paper Presentation Certificate

This is to certify that Prof./Dr./Shri./Smt. Mrityunjai Shukla, Dept. of Chemistry of Dr. H. S. Gour University, Sagar, has presented a Paper (Oral/Poster) entitled 'Anthelmintic Medicinal Plants' abstract no. 136, in the Section of Chemical Sciences during the 94th Indian Science Congress held at Annamalai University, Annamalainagar, Chidambaram, from January 3 to 7, 2007.

His/Her Membership no is A1238-WOY

[Signature]
Prof. A.K. SINGH
Sectional President
Section
Office Seal
Signature
Year of Indian Science Congress 2007
Dear Sir / Madam,

Your paper(s) has been accepted for Oral / Poster display during the 94th Science Congress to be held in Annamalainagar from January 3 to 7, 2007. Kindly contact Local Secretaries for details about Accommodation and Registration:

Prof. T. Balasubramanian  
Director, CAS in Marine Biology  
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Prof. Venugopal P. Menon  
Dean, Faculty of Science

Annamalai University, Annamalainagar, Chidambaram-608 002, Tamil Nadu
Email: stbcas@nic.in  
biocmr@sify.com  
au-regr@yahoo.co.in

Title of Paper —

1. Physicochemical constituents...  
2. Anthelminetics agents - Indian...

Note: This paper will be presented by my research scholar Mrityunjai Shukla

Yours faithfully,

[Signature]

01.12.06

Prof. V. K. Saxena

[Signature]

Supervisor

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"Paper for 'Poster Display':

The poster display should briefly explain the importance of the work, essential background information, results and conclusions. It should be self-explanatory. A title and the author should be present for any discussion. The poster should be in large prints for easy viewing. Authors are requested to bring drawing pins and adhesive tape etc. for putting up the poster.

[Stamp]

To

V.K. Saxena

Natural Products Lab

Dept. of Chemistry

[Stamp]

Dr. H.S. Gour University, Sagar 470 003 (M.P.)
DOCTOR HARISINGH GOUR VISHVAVIDYALAYA, SAGAR (M.P.)

No. Res./Ph.D./Reg./ Chem./6237
SAGAR, DATED: 13.9.05

CERTIFICATE OF REGISTRATION AS A SCHOLAR FOR Ph.D. DEGREE

Shri/ma./sir./ Mrityunjay Shukla

Department of Chemistry

has been REGISTERED as a Research Scholar of the University

under the Supervision of Prof. V.K. Saxena

with effect from 18.10.04

Registration No. Res./Ph.D./Chem./37 - VI

Asstt./DY. REGISTRAR (ADMIN.)
Dr. H.S. Gour V.V., Sagar (M.P.)

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1. Head, Dept. of Chemistry
   Dr. H.S. Gour V.V., Sagar (M.P.)

2. The Principal/Supervisor
   Prof. V.K. Saxena, Deptt. of Chemistry,
   Dr. H.S. Gour V.V., Sagar.
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