Pharmacognostical Investigation and Preliminary Phytochemical Screening of Leaves of *Myxopyrum Smilacifolium B.*

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**ABSTRACT**

**Objective:** The current study deals with detailed pharmacognostical study and preliminary phytochemical screening of leaf of *Myxopyrum smilacifolium* Blume. *Myxopyrum smilacifolium* Blume is a twining shrub belongs to the family Oleaceae. It is used traditionally in the treatment of cough, rheumatism, cephalalgia, notalgia and otopathy. Scrutinization of literature revealed that there is a lack of pharmacognostical and Phytochemical investigations of *Myxopyrum smilacifolium* Blume. **Methods:** The macroscopic and microscopic features were evaluated. The leaves of *Myxopyrum smilacifolium* Blume was subjected for successive solvent extraction and further preliminary phytochemical screening was carried out and also the behaviour of powder with different reagents were evaluated by fluorescence analysis. **Results:** The detailed study of pharmacognostical evaluation showed the presence of thick walled epidermal cells covered with thick cuticle, xylem and phloem elements, Glandular trichome and slightly concave collateral vascular bundles. Preliminary Phytochemical examination revealed the presence of various phytoconstituents viz., alkaloids, glycosides, tannins, sapopinoids, terpenoids, carbohydrates and fixed oils. The fluorescence analysis manifested the behavioral variation of the powdered drug. **Conclusion:** The findings of the present study will be a referential information for identification and also useful for standardization of the plant material.

**Key words:** Oleaceae, Microscopy, Phytochemical screening, Standardization, Myxopyrum.

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**INTRODUCTION**

*Myxopyrum smilacifolium* Blume¹ is a large woody twining shrub commonly called as chaturdhuratala in telugu. The leaf of this plant is used traditionally for the treatment of rheumatism, cough, cephalalgia, notalgia and otopathy. Vegetatively, the genus Myxopyrum is very distinct plant belonging to the family Oleaceae. The anatomical and chemical investigations on Myxopyrum genus are very limited. *Myxopyrum smilacifolium* Blume grows at an altitude of below 700–1000 m. Relating to *Myxopyrum* species 6 taxas are recognized.² Among them one of the species is *Myxopyrum smilacifolium* Blume. It is distributed at tropical and subtropical regions of Eastern Asia. Earlier investigation was made on a single leaf of *Myxopyrum smilacifolium* and reported that it contains irridoid glucosides and triterpenoids.³ In Ayurveda the herb is used in vitiated conditions of Kapha and vata.

**MATERIAL AND METHODS**

**Plant material**

*Myxopyrum smilacifolium* Blume leaves were procured from botanical garden, Department of Botany, University of Kerala, Kerala. It was identified and authenticated by V. Chelladurai, Former Research officer. Central Council of Research in Ayurveda and Siddha, Government Siddha medical College, Tamil Nadu. India.

**Description of the plant**

The plant is twinning which grows in exposed areas like open thickets or seashores. Leaf is elliptical to ovate, petiolate and measures about (9-12-17)-24.5)×4-5.8-10 cm, glabrous, base rounded and apex acuminate. Margin is serrate towards apex region but may be entire or spinose. It shows terminal inflorescence and flowers are yellow in color, sub sessile. Fruit is globose, measures about 5×5-10×13 mm. Seeds are 1-2. In Calyx lobes are ovate-acute and pubescent. Corolla, the lobes are linear openly recurved. Stamens are sub sessile and anthers are elliptic.

**Pharmacognostic evaluation Organoleptic evaluation**

The sensory parameters of leaf of *Myxopyrum smilacifolium* Blume such as size, shape, color, odour and taste were recorded.

**Microscopic evaluation Preparation of sections**

The sections of leaf were made with the help of rotary microtome. Later the sections were made stained with toluidine blue, a polychromatic stain. The cleared sections were then mounted with glycerin for microscopical observations.

**Powder microscopy**

To a little quantity of powder in a watch glass, 1-2 drops of 0.1% phloroglucinol solution and Concentrated HCL was added in a ratio of 1:1. The stained powder was transferred onto a slide, mounted with glycerol and covered with cover slip.³ The prepared slide observed under microscope with 10×10 magnifications. The presence of starch grains were detected by addition of 2-3 drops of 0.01 m Iodine solution.

**Determination of Stomatal Index**

Leaf fragments of about 5×5 mm in size were taken and it was cleared with chloral hydrate solution.⁴ The cleared fragment of leaf was mounted with glycerol on microscopic slide. It is observed under microscope for quantification of Stomatal Number and Stomatal Index, vein islet number and Vein termination number. The slide was examined with 40x objective and 6x eye piece to which a Camera lucida was attached and recorded.

**Physical evaluation Estimation of crude fiber**

2 gm of powdered drug was taken and 50 ml of 10% v/v nitric acid was added. It was heated with constant stirring and strained. To the residue 50 ml of 2.5% v/v sodium hydroxide solution was added, heated and
maintained at boiling point for 30 seconds. After it was strained and the residue is weighed. The percentage of crude fibers was determined.

Moisture content
10 gm of accurately weighed fresh leaves of *Myxopyrum smilacifolium* Blume was placed in a tared porcelain dish and dried at 105°C for 5 hrs and weighed. Drying and weighing is continued at an interval of one hour until two successive weighing is constant.

Total ash Determination of total ash
2 gm of leaf powder of *Myxopyrum smilacifolium* Blume was taken in tared silica crucible and incinerated at a temperature not more than 450°C until free from carbon. The obtained ash was cooled and weighed. The percentage of ash was calculated with reference to the air dried drug.

Acid-insoluble ash
The total ash obtained from 2 gm of leaf powder was boiled with 25 ml of dilute hydrochloric acid for 5 minutes and the insoluble matter was collected on an ashless filter paper. It was washed, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash
The total ash obtained from 2 g of leaf powder was boiled with 25 ml of water for 5 minutes and the insoluble matter was collected on an ashless filter paper. It was washed, ignited and weighed. The percentage of water soluble ash was calculated with reference to the air dried drug.

Determination of alcohol soluble extractive
5 g of accurately weighed leaves was taken and macerated with 100 ml of 95% alcohol for 24 hr. The contents were frequently shaken during the first 6 hr and allowed to stand for 18 hr. After 24 hr, 25 ml of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.

Determination of water soluble extractive
5 g of accurately weighed leaves was taken and macerated with 100 ml of chloroform water for 24 hr. The contents were frequently shaken during the first 6 hr and allowed to stand for 18 hr. After 24 hr, 25 ml of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.

Preparation of extract
500 g of dried coarsely powdered leaf of *Myxopyrum smilacifolium* Blume was packed in soxhlet apparatus and was defatted with petroleum ether (50-60°C). The marc left subsequently extracted with Petroleum ether, chloroform, methanol and distilled water. The extracts obtained were concentrated using rotary evaporator and dried.

Preliminary Phytochemical Screening
The extracts were subjected to preliminary Phytochemical screening for the detection of various plant constituents viz. carbohydrates, fixed oils, alkaloids, glycosides, terpenoids, flavonoids, tannins and phenols.

Fluorescence analysis of the powdered drug
The fluorescence analysis of the powdered leaves was done by placing dry powdered leaves on a slide and observed by treating with several drops of different chemical reagents to detect the color changes under UV and Visible light.
Figure 1: Photograph showing twig of *Myxopyrum smilacifolium* Blume.

Figure 2: Photograph showing vascular bundle arrangement through midrib portion of leaf.
Figure 3: Photograph showing type of trichome in leaf

Figure 4: Photograph showing type of stomata in leaf
Table 1: Quantitative Microscopy of *Myxopyrum smilacifolium* Blume

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal number upper surface</td>
<td>57.16</td>
</tr>
<tr>
<td>Stomatal number lower surface</td>
<td>76.17</td>
</tr>
<tr>
<td>Vein Islet number</td>
<td>11.30</td>
</tr>
<tr>
<td>Vein Termination number</td>
<td>12.40</td>
</tr>
</tbody>
</table>

Table 2: Physicochemical parameters of leaf powder of *Myxopyrum smilacifolium* Blume

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values %w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fiber</td>
<td>32</td>
</tr>
<tr>
<td>Moisture content (Loss on drying)</td>
<td>10</td>
</tr>
<tr>
<td>Total ash</td>
<td>1.74</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.034</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>0.16</td>
</tr>
<tr>
<td>Alcohol soluble extractive value</td>
<td>1.36</td>
</tr>
<tr>
<td>Water soluble extractive value</td>
<td>15.14</td>
</tr>
</tbody>
</table>

Table 3: Phytochemical analysis of various extracts of *Myxopyrum smilacifolium* Blume

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Method</th>
<th>Aqueous Extract</th>
<th>Alcoholic Extract</th>
<th>Pet-ether Extract</th>
<th>Chloroform Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Shinoda Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zn. Hydrochloride test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Lead acetate Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Wagner Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hager’s Test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fcl, Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tanins &amp; Phenols</td>
<td>Potassium dichromate test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foaming Test</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Salkowski test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molish test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Fixed oils</td>
<td>Cuso 4 Test</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Glycoside</td>
<td>Keller Killani Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

-Negative = Absent; +Positive = Present.
leaves of *Myxopyrum smilacifolium* Blume had shown the presence of secondary metabolites which can play a vital role in medicine.

**ACKNOWLEDGEMENT**

We extend our sincere thanks to Dr. P. Jayaraman, Director, Retd, Professor, Presidency College, Chennai-5, for providing authenticated sample of *M. Smilacifolium B*.

**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

**ABBREVIATION USED**

cm: centimeters; mm: millimeters; hr: hour; B.: Blume; nm: nanometers; NaOH: Sodium hydroxide; HCl: Hydrochloric acid; HNO$_3$: Nitric acid; CHCl$_3$: Chloroform.

**REFERENCES**


**PICTORIAL ABSTRACT**

**ABOUT AUTHOR**

Raveesha Peeriga: Is a research scholar at the JNTUA. Her research is focused on the pharmacognostical findings of *Myxopyrum smilacifolium* Blume.

K.B.Chandrasekar: Obtained his Ph. D. degree in 2000 from S.K. University, Anantapur. Currently, he is positioned as Director at JNTUA, Anantapur, Andhra Pradesh (India). He also got *Best research paper award in the discipline of Pharmaceutical Chemistry* 2005 from Indian drugs manufacturers association.