PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF LEAF OF

**FLEMINGIA STROBILIFERA W.ATION**

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**ABSTRACT**

One among Fabaceae member *Flemingia strobilifera* W.ation is a very useful herbal drug. Leaf of the same is collected, authenticated and used for the study. Morphological study of leaf reveals it as a simple leaf with slightly wavy margin, ovate-lanceolate shape with pulvinous petiole. Leaf microscopy shows blacklids, simple trichome with tannin content. Bundle sheath cells are mostly filled with Calcium oxalate crystal. Ground tissue made up of parenchyma cells heavily deposited by rhomboidal crystal of calcium oxalate, tannin content, simple and compound starch grains and oil globules. Epidermis is covered by cuticle. Lower and upper epidermis is having Trichome. Lower epidermis interrupted by stomata. Mesophyll tissue is rich in oil globules and crystals. Powder microscopy of leaf shows simple unicellular trichome, oil globule, dark brown tannin content, rhomboidal crystal and fragment of paracitic stomata. Physicochemical analysis revealed presence of carbohydrates, saponins, glycosides, steroids and flavonoid. HPTLC study of alkaloid showed the presence of four and two spots in short and long UV respectively. These characteristics will be helpful to identify the plant *Flemingia strobilifera*.

**KEYWORDS:** Fabaceae, Ayurveda, Flemingia strobilifera, blacklid.

**INTRODUCTION**

Family Fabaceae is having many useful herbal drugs. *Flemingia strobilifera* W.ation being one among this family is very useful drug in the treatment of many diseases. It is called as Luck plant in English. Foliaceous bracts helpful to identify plant and also makes the plant very attractive. This plant is used in different parts of the world in different ailments like Haemorrhage, Bronchial Asthma, Hysteria, Fever, Rheumatism, Worm, Scabies etc[1].

**MATERIALS AND METHODS**

**Collection and authenticitation:** *Flemingia strobilifera*, identified by local traditional practitioners, growing in Bangalore, Karnataka, India, was authenticated by expert taxonomist as on the basis of characters given in Indian Medicinal plants[11]. The fresh plant samples were
collected from its natural habitat, Karnataka, in the month of November 2017 and voucher specimen has been preserved in the pharmacognosy laboratory of IPGT and RA, vide no 6210/17-18. The collected plant samples were shaken to remove adherent soil and dirt. The leaves were separated from the stem and then leaves washed with running fresh water and few pieces stored in solution of AAF (Alcohol: Acetic acid: Formalin) in the ratio of (90:5:5) \[III\] to utilize them for microscopic studies. The remaining leaves were shade dried and then powdered with mechanical grinder and passed through mesh no.80# and preserved in an air-tight glass container.

Morphological characters were studied by observing the leaves as such and also with the help of the dissecting microscope. For detailed microscopical observation, free hand thin transverse section passing through the midrib were taken, and cleared with chloral hydrate and observed as such for the presence of any crystals, then were stained with Phloroglucinol and Hydrochloric acid to notice the lignified elements like fibers, vessels etc. of the meristele and other parts\[IV\].

Photographs of the section were taken with the help of Canon digital camera attached to Zeiss microscope. The same procedure is applied for the microscopic study of stem also. Powder characters were observed and histochemical tests carried out, as per the guidelines of Ayurvedic Pharmacopoeia of India. Physicochemical parameters and Phytochemical screening were also carried out as per the guidelines of Ayurvedic Pharmacopoeia of India\[V\]. HPTLC\[VI\] was carried out for the analysis.

RESULTS AND DISCUSSION

**Morphology:** Leaves are slightly aromatic, simple, alternate, 16.8×7.5cm, ovate-lanceolate shape, base slightly cordate, petiole-1.92cm, pulvinous at both ends, midrib with 10pairs of nerves, tip-acute, margin- slightly wavy, both surfaces are rough, midrib and nerves with tomentose hair, upper epidermis- dark green with white patches, lower epidermis - light green without any patches.

**Microscopic description:**

**Mesophyll**

T.S of midrib shows simple Trichome with Tannin content. Blacklids- A special type of glandular trichome which are present at lower epidermis consists greatly the tannin content. Upper Pallisade parenchyma single layered and 1/5 is the Pallisade ratio. Spongy Parenchyma made up of 5-6 layers. Many cells are loaded with tannin.
T.S of the Leaf passing through the midrib

Hypodermis is having 3 layered collenchyma followed by parenchymatous ground tissue. Parenchymal cells lead into bundle sheath. Bundle sheath cells are mostly filled with Calcium oxalate crystal. Bundle sheath leads to formation of ring of pericyclic fibre (3-4 layers). Vascular bundle enclosed in a circular manner with phloem towards outside and xylem towards centre. Centrally located ground tissue is rich in tannin content. Through midrib measures about 5.6x4.6mm. Lamina measures 1mm, Pallisade- 0.3mm, Spongy- 0.6mm, Bundle sheath- 2.1 sq mm. Diagramatic section shows T.S of petiole outermost epidermis followed by ground tissue. Vascular bundles circularly arranged in the ground tissue. Epidermis is single layered with thick cuticle. The epidermal cells are bearing simple trichomes along with blacklids. Trichomes are filled with tannin. Ground tissue is made up of parenchyma cells heavily deposited by rhomboidal crystal of calcium oxalate, tannin content, simple and compound starch grains and oil globules. Some of the parenchyma cells are pitted and lignified towards the centre. Vascular bundles are circularly arranged in the ground tissue. 11-12 groups of vascular bundles are found. Each vascular bundle is covered with arch like pericyclic fibre followed by phloem and xylem. Metaxylem is found towards epidermis and protoxylem towards the centre. Apart from the main vascular bundle, 2 small meristeles are situated at the corner of the section. Black lid and Simple Trichome are found. Cuticularised epidermis is having unicellular simple and glandular trichomes. Lower epidermis is interrupted by stomata. Mesophyll tissue (Lamina) is rich in oil globules and crystals. Through midrib shows 1-3 layered collenchyma tissue which gives the mechanical support to the vascular bundle. Vascular bundle is made up of outer most layer bundle sheath, xylem towards upper epidermis, phloem towards lower epidermis. [Plate A]

Histochemical Test:
Thick sections of sample were subjected to histochemical tests to find starch, tannin, calcium etc. chemical constituent by treating various reagents and result obtained is showed in the table No:1.

Table No:1: Histochemical Test

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Observation</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloroglucinol</td>
<td>Pink colour</td>
<td>Lignin present</td>
</tr>
<tr>
<td>Concentrated HCl(1:1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>Effervescene</td>
<td>Crystal present</td>
</tr>
<tr>
<td>Concentrated HCl(1:1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeCl₃ solution</td>
<td>Black colour</td>
<td>Tannin present</td>
</tr>
</tbody>
</table>
POWDER MICROSCOPY

Organoleptic characters i.e. color, odour, taste, size, shape and feel of drug to touch by sensory observations were noted. Procedure of Microscopy followed to the powder microscopy also.

Organoleptic tests:-

Colour- Parrot green
Taste- Astringent
Odour- Characteristic
Touch- smooth

Microscopic Characters:

Following features are found on microscopic examination of Leaf powder:
Simple unicellular Trichome, fragment of fibre with lumen, dark brown Tannin content, Unicellular Trichome with tannin, Oil globule, fragment of epidermal cells with parasitic stomata, fragment of wavy parenchyma, Rhomboidal crystal, fragment of palisade parenchyma, fragment of spiral vessels. On staining lignified fibres are found.[Plate B]

Physico-chemical Analysis:

Physico-chemical Parameters like loss on drying, total ash, alcohol soluble extractive (90% methanol), water soluble extractive and pH values were determined as per the API guidelines for the powder sample.

Table No: 2 Physico-chemical Analysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Test</th>
<th>F. strobilifera Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss on Drying (%w/w)</td>
<td>8.277</td>
</tr>
<tr>
<td>2.</td>
<td>Ash value (%w/w)</td>
<td>3.479</td>
</tr>
<tr>
<td>3.</td>
<td>Water soluble extractive (%w/w)</td>
<td>4.98</td>
</tr>
<tr>
<td>4.</td>
<td>Alcohol soluble extractive (%w/w)</td>
<td>6.23</td>
</tr>
<tr>
<td>5.</td>
<td>pH value</td>
<td>6</td>
</tr>
</tbody>
</table>
**PLATE B**

- Fibres
- Fragments of epidermal cells with stomata
- Fragment of palisade like cells
- Prismatic crystal
- Simple unicellular trichome
- Spiral vessel

**PLATE C**

- TLC Plate at 254 nm
- TLC Plate at 366 nm
Preliminary Phytochemical Screening

Phytochemical analysis of methanol and water soluble extract of sample drug was carried out for steroids, glycosides, tannins, proteins, flavonoids, alkaloids and saponins.

Table No: 3 Preliminary Phytochemical Screening

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the test</th>
<th>F. strobilifera Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Protein</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Amino acid</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Saponin glycoside</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Alkaloid</td>
<td>-</td>
</tr>
</tbody>
</table>

‘+’ Present, ‘-’ Absent

CHROMATOGRAPHIC ANALYSIS (HPTLC)

Chromatographic techniques were carried out as per the standard protocol. Solvent system which were designed for TLC i.e. Toluene: Ethyl acetate (9:1v/v) was used for HPTLC studies. The results are shown in the (Table no. 4) (Plate C).

Table 4: HPTLC Profile of leaf of F.strobilifera at 254 nm and 366 nm

<table>
<thead>
<tr>
<th>Number of spots</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short UV 254</td>
<td>0.01, 0.71, 0.87, 0.96</td>
</tr>
<tr>
<td>Long UV 366</td>
<td>0.01, 0.96</td>
</tr>
</tbody>
</table>

CONCLUSION

Leaf of Flemingia strobilifera can be identified on the basis of key microscopical characters like single layered paliсадe parenchyma, spongy parenchyma, presence of blacklids, epidermal cells
with simple trichome along with blacklid, tannin, calcium oxalate, oil globule, lignified parenchyma cells, Vascular bundle covered with pericyclic fibre. Powder microscopy of leaf shows simple unicellular trichome, oil globule, dark brown tannin content, rhomboidal crystal and fragment of paracitic stomata. Physicochemical analysis revealed presence of carbohydrates, saponins, glycosides, steroids and flavonoid. HPTLC study of alkaloid showed the presence of four and two spots in short and long UV respectively. These characteristics will be helpful to identify the plant *Flemingia strobilifera*.

**ACKNOWLEDGEMENTS**

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1. Jain S.K., Studies in Indian Ethnobotany- 2, 1963, Plants used in medicine by the tribals of Madhya Pradesh, Bull, pg 126-128