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# Development of pharmacognostic parameters for the leaf of *Bridelia scandens* (Roxb.) Willd

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## ABSTRACT

**Background:** Pharmacognostic study of medicinal plants is an important parameter for standardization and authentication of plants, with the help of which adulteration and substitution can be prevented. The present study deals with pharmacognostic profile of leaf of *Bridelia scandens* (Roxb).Willd. an important traditional plant, belonging to family Euphorbiaceae used to treat various ailments. **Methods**: The present study includes macroscopic and microscopic studies, quantitative microscopy, and physiochemical characters such as ash value, extractive values, fluorescence analysis, and total phenol and flavonoid content. **Results**: Macroscopically, the leaves are *B. scandens* are elliptic –oblong or obavate, dark green above, pale green below lateral veins. Microscopically, leaf consists of thick semicircular midrib and the lamina, cortical zone ending with thick continuous cylinder of sclerenchyma cells. Sclerenchyma cylinder completely enclosed the vascular cylinder of the midrib, consists of only continuous thick layer of phloem. Xylem cylinder consists of numerous short or long radial chains of vessels. The lateral vein is flat on the adaxial side and prominently projecting hemispherical body on the adaxial side. Powder microscopy of leaf revealed the presence of spiral xylem vessels, rosette and prismatic calcium oxalate crystals and trichomes. A Physiochemical characteristic was also determined. **Conclusion**: Existing literature revealed that so far, no Pharmacognostic study has been reported on the leaf of *B. scandens*. Findings from this investigation can be used for its identification and determination of quality and purity of medicinally important plant. Thus exploring the usefulness of pharmacognostic evaluation to validate and authenticate drug.

**Keywords:** *Bridelia scandens*, Cyclocytic, Trichome, Euphorbiaceae, Fluorescence.

## INTRODUCTION

India is a richest source for herbal medicine and has spread almost in all parts of the country. These medicinal plants are still effective source as a healing agent and it is because of their efficacy, low cost and lack of lethal effects on humankind. Synthetic drug usage has led to serious issue all over the world and therefore there is increase demand of plant based raw materials for medications. WHO has also emphasized on the practise of indigenous systems of medicine because of the availability of raw material – medicinal plants [1].

As the demand for natural drugs increases, availability becomes problematic. To meet the growing demand, natural drug is easily adulterated or substituted with low grade material or other plant species. Intentionally adding foreign substances to increase the weight and decrease the cost will lead to loss of therapeutic efficacy of medicinal plants [2]. So, it turns out to be essential for making a pharmacognostic specifications of the medicinal plants which are used as drugs. These studies help in correct identification and standardization of the plant material, which is required for reproducible quality of herbal medicine contributing to its safety and efficacy [3].

*Bridelia scandens* is one among the 60 species of genus Bridelia under the family Euphorbiaceae. In India, it has spread throughout in the dense evergreen forests of Western Ghats as a large woody evergreen climber or straggling shrub with pendent branches armed with large deflexed spines [4]. Decoction of bark is used treat for cough, fever and asthma, and also has hypotensive and hypoglycaemic action on animals. Stem bark and leaf are used to cure jaundice [5, 6]. Fruits are edible and seeds possess hemagglutinating properties [7-9]. Decoction of wood of *B. Scandens* administered orally to treat malaria disease [10]. Leaf extract is used to cure allergy and to heal oral problems [11, 12]. Leaf and root are used to treat inflammation, scabies, dermatitis [13]. Root of *B. scandens* is used to treat herpes [14]. *B. scandens* reported to possess antimicrobial [15. 16], antioxidant [17], hepatoprotective [18] and antidiabetic property [19]. However existing literature reveals that no pharmacognostic study for leaf of the *B. scandens* has been carried out yet, which may help in the standardization and monograph development.

## MATERIALS AND METHODS

### Sample collection and processing

The fresh leaf of *Bridelia scandens* was collected from Biligiri Rangana hills (BR hills) of Chamarajanagar district, Karnataka, India. The plant material was authenticated by Dr. Shiddamallayya. N, at National Ayurveda Dietetics Research Institute, Department of AYUSH, Govt. of India, Bangalore and the voucher Specimen (No: RRCBI-MUS-10121) was deposited for future references. The collected sample was air dried under shade and powdered using pestle and mortar, stored in air tight container, used for the present study.

### Macro morphology

Fresh leaf of *B. scandens* was studied for macromorphlogical characters like shape, size, colour, texture, margin type, apex, odour and taste.

### Microscopic characters

Epidermal layer and transverse sections of leaf of *B.scandens* was microscopically observed.

### Epidermal layers of leaf

Leaf of the *B. scandens* was sectioned parallel to the surface of the leaf. Epidermal peeling was done by partial maceration by Jeffrey's maceration [20]. Glycerine mounted temporary preparations were made for macerated materials.

### Transverse section of leaf

Section was obtained by free hand sectioning using a clean razor blade. It was first cleared with chloral hydrate solution and stained with Toluidine blue and mounted with glycerine [21].

### Quantitative microscopy

In this proximate analysis, stomata number, stomatal index, vein islet and vein termination number were determined by using appropriate formula [22].

### Powder Microscopy

A pinch of powder of leaves treated with chloral hydrate solution, warmed to clear chlorophyll mounted with glycerine observed under microscope for different characters [23]. Quantitative microscopy of the leaf powder was performed to determine the size and dimension of different features present.

### Florescence Analysis

Dried leaf powder of *B. scandens* were treated with various chemical reagents and then exposed to visible, ultraviolet light to study their fluorescence behaviour [24.25].

### Physicochemical parameters [23]

### Extractive values

Extractive values denote the nature of the constituents present in a crude drug. There are two types of extractive values.

- **Hot extraction**

**Water soluble extractive value**: About 4.0g of sample in 100 ml of water was used for the study. The content of extractable matter was calculated in mg /g of air-dried material.

**Alcohol soluble extractive value**: Here, instead of water, 95% ethanol was used and remaining procedure was followed as mention in the water soluble extractive value.

- **Cold extraction**

**Water soluble extractive value**: About 4.0g of 100ml of chloroform was used. The content of extractable matter was calculated in mg per g of air-dried material.

**Alcohol soluble extractive value**: Same method as described above was followed. But instead of water 95% ethanol was taken.

### Moisture content

Weigh accurately the leaf powder of *B.scandens* and heated at 105º C in an oven and again weighed. The procedure was repeated till a constant weight was obtained and percentage with reference to leaf powder was calculated.

### Total ash

Accurately 2gm of dried powdered leaf of *B. scandens* weighed in a tarred silica crucible. It was incarnated to a temperature up to 450 ºC, till it becomes free from carbon and further cooled and weighed. Shade dried leaf was taken as reference to calculate percentage of total ash.

### Acid-insoluble ash & Water-soluble ash

Dilute hydrochloric acid of about 25ml was added to the total ash obtained and boiled for five minutes. Ash less filter paper was used to collect the insoluble matter and further washed with hot water, ignited, cooled and weighed accurately. Same procedure was followed for water-soluble ash values and the values obtained were subtracted by the weight of the insoluble ash. Shade dried leaf was taken as reference to calculate percentage of acid insoluble ash and water insoluble ash.

### Estimation of Total Phenol and flavonoid content of plant extracts.

Water extract and alcohol extract of leaf of *B. scandens* was estimated for their total phenol [26] and flavonoid content [27].

## RESULTS

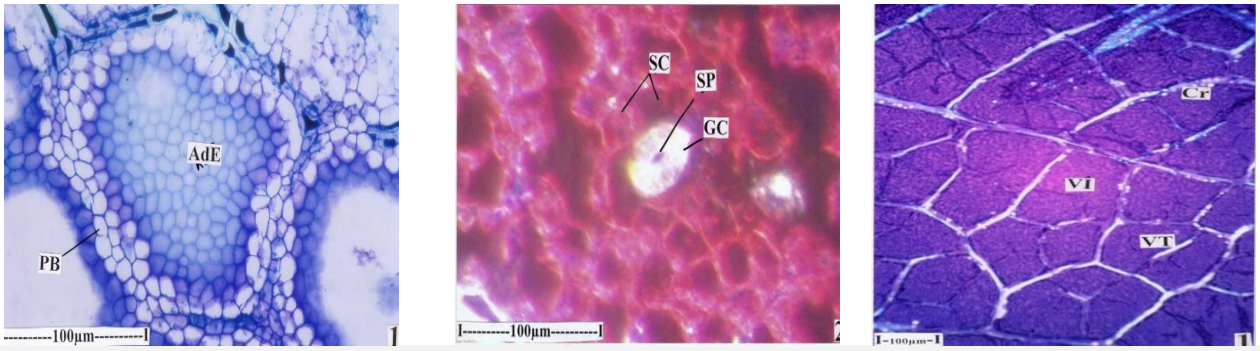
### Macro morphology character

Leaves of *B. scandens* are elliptic – oblong or obavate, dark green above, pale green below lateral veins often, yellowish running straight to the margin. Apex is tapering to rounded, margin is entire. It is tasteless with characteristic odour.

**Microscopy studies**

**Epidermal layer**

Leaf of *B. scandens* was studied for their epidermal features. Epidermal cells were polygonal in shape, thin walled and compactly arranged. They were located within shallow pits surrounded by slightly raised parenchymatous borders (Fig.1 A). In our stomatal studies, Adaxial epidermis was apostomatic, whereas abaxial epidermis found to be stomatiferous, Cyclocytic type of stomata was revealed on the abaxial side, each stomata was surrounded by 4 or more semi-circular subsidiary cells. The stoma was surrounded by a ring of about eight squarish thin walled subsidiary cells. The guard cells were broadly elliptic (Fig 1 B). The venation was widely reticulate and the vein islets were wide rectangular and squarish with thin borders. Calcium oxalate prismatic crystals are densely distributed all along the veins (Fig 1 C). The crystals showed vary in their size and shape and were arranged in vertical file.




**A. Paradermal section of the adaxial epidermis.** ADE - Adaxial epidermal cells, PB- Pit Border

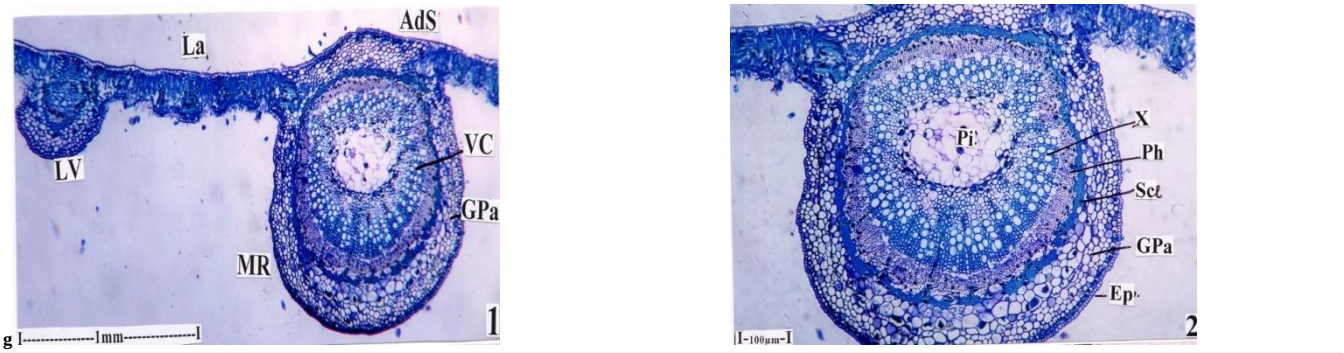
**B. Paradermal section of the Abaxial Epidermis showing sunken cyclocytic stoma.** SC- Subsidiary cells, SP-Stomatal Pore

**C. Lamina showing venation pattern** Cr- crystals, VI- Vein Islet, VT-Vein termination

**Figure 1:** Epidermal layer studies on the leaf of *B. scandens*

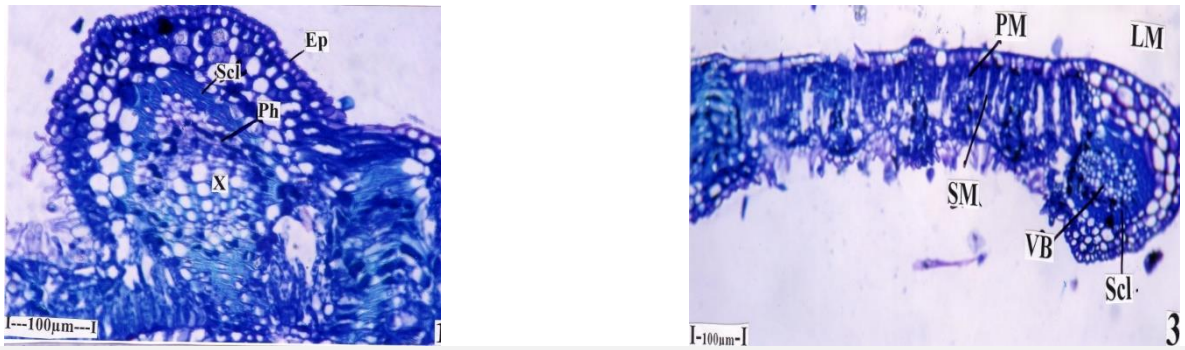
**Transverse section**

Transverse section of leaf of *B. scandens* consists of thick semicircular midrib and the lamina. The lateral vein was found to be small and semi-circular (Fig. 2 A).Thickness of the midrib exhibited 1.15mm thick and 1.25mm wide. The adaxial part of the midrib was lightly raised into short hump, the abaxial part form wide prominent semi-circular midrib. Inner to the epidermis is a thick cortical zone, where there were about eight layers of fairly thick walled, angular compact cells. The cortical cells were small in the peripheral region and become wider towards interior. At the end of the cortical zone, there was thick continuous cylinder of sclerenchyma cells. The sclerenchyma cylinder having two or three layer thick lignified walled and narrow lumen. The sclerenchyma cylinder completely enclosed the vascular cylinder of the midrib (Fig. 2 B).




**A. T.S of leaf.** La-Lamina, Lv-Lateral vein, MR-Midrib, Ph-Phloem, VC-Vascular cylinder, X-xylem.(4X)

**B. T.S of midrib enlarged**. Ep-Epidermis, Gpa-Groundparenchyma, Ph-Phloem,Pi-Pith,Scl-Sclerenchyme. (10X)




**C. T.S. Lateral Vein**. X-Xylem, Scl-Sclerenchyma, Ph-Phloem, Ep-Epidermis. (20X)

**D. T.S.** Leaf Margin.PM-Palisade Mesophyll, LM-Leaf margin, SM-Spongy Mesophyll, VB-Vascular Bundle, X-Xylem. (10X)

**Figure 2:** Transverse section of leaf of *B.scandens*

The vascular system of the midrib consists of a wide hollow cylinder with small central core of parenchymatous pills. The cylinder was thicker on the abaxial part and thinner within the adaxial part. The adaxial part of the vascular cylinder is 150µ m thick, abaxial part is 200 µm thick. The vascular cylinder of the midrib consists of only continuous thick layer of phloem, the phloem elements were small squarish thin walled cells. They were arranged in a compact radial row containing cells which possessed dark inclusions (Fig. 2 B).

Xylem cylinder consists of numerous short or long radial chains of vessels. The vessels were circular, wide and are thick walled. The xylem cylinder also includes xylem fibres. The fibres were dense with peripheral part and found to be reduced towards the inner part. The pith of Parenchyma cells located along the inner boundary of the xylem cylinder has tannin contents (Fig. 2 B).

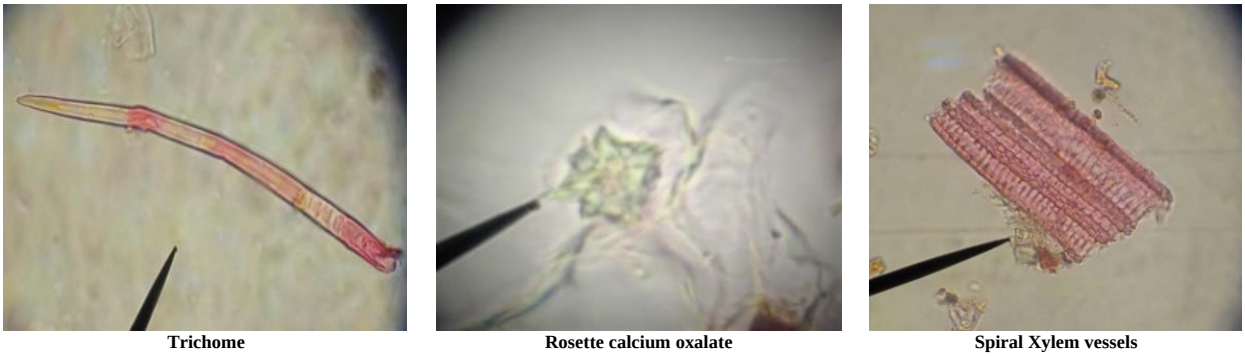
The lateral vein was flat on the adaxial side and prominently projecting hemispherical body on the adaxial side. It consists of single collateral vascular bundle which includes a few rows of xylem elements and thick rows of phloem. A thick layer of sclerenchyma cells occur adjacent to the phloem. Lamina was distinctly undulate on the abaxial side with prominent ridges and furrows. The adaxial side of the lamina is slightly undulate. The lamina is 130 µm thick. The abaxial surface was densely covered by non-glandular short trichomes. The adaxial epidermal cells were found to be thick rectangular and circularized. The abaxial epidermal cells were small. The mesophyll tissue consists of adaxial horizontal layer of thin, high compact palisade cells. The spongy parenchyma cells were small, loosely arranged forming wide air chambers (Fig. 2 C).The leaf marginal part was straight and the extreme end was dilated. It was 270 µm thick and 200 µm long. The epidermal cells of the leaf margin were small thick walled and squarish. Within the dilated end of the leaf margin occur three circular vascular and the bundles are unsheathed by thick sclerenchyma bundle sheath (Fig. 2D).

**Quantitative microscopy**

Microscopic examination of leaf led to the identification of cyclostatic stomata on the abaxial side of the leaf. Stomata number was found to be 2 and stomatal index 4.3. Vein islet and termination was observed to be 7.16±0.72 and 3.06±0.63 respectively.

**Powder microscopy**

The microscopic examination of the powder of *B. scandens* shows fibres, spiral xylem vessels, rosette and prismatic calcium oxalate crystals, stone cells or sclerides, starch granules and multicellular unicellate covering trichomes (Figure 3). These characteristic features of powder may help in identification for future works. The length of the trichome was found to be minimum of 92.4µ, maximum of 194.5µ and average of 143.32 µ.



**Trichome** **Rosette calcium oxalate** **Spiral Xylem vessels**

**Figure 3:** Powder studies of leaf of *B. scandens* (40X)

**Fluorescent studies**

Powder of *B. scandens*, when treated with different chemical reagent showed different colour reactions, in accordance to the nature of the constituents present in it. Many plants constituents show fluorescence in visible light and some of the metabolites shows fluorescence only when they are exposed to ultraviolet light. When treated with different chemical reagent results in different colour reactions (Table.1).

**Table 1:** Fluorescence analysis of leaf powder of *B. scandens*

| Treatment | Visible | Short UV (254 nm) | Long UV (365nm) |
|---|---|---|---|
| Powder | Light olive green | Brown | Black |
| Powder + water | Pale brownish green | Brownish | Dark Green |
| Powder +1NHCl | Pale brown | Magenta Brown | Dark Green |
| Powder +1$NHNO_3$ | Pale brown | Magenta Brown | Dark Green |
| Powder +1$NH_2SO_4$ | Pale brown | Dark brown | Dark Green |
| Powder +1NaOH | Dark brown | Dark brown | Dark Green |
| Powder +1N Alc NaOH | Magenta brown | Dark brown | Orange Yellow |
| Powder +1N KOH | Magenta brown | Dark brown | Dark Green |
| Powder +1N Alc KOH | Magenta brown | Dark brown | Mustard Yellow |
| Powder + Ammonia | Dark brown | Dark brown | light Green |

**Physicochemical parameters**

Extractive values (Table.2) determine the active constituents present in a given amount of plant material. Nature of drug and solvent used governs the composition of phytoconsituent present. Yield obtained for hot extraction of leaf of *B. scandens* was more than cold extract. Alcohol extract in hot extraction yielded 12.56% and cold extract was 10.75%. Whereas aqueous extract yield in hot extraction was 13.7 % and cold extract were 11.57.

The Percentage of total ash, acid insoluble ash, water soluble ash, moisture content are presented in Table.3.These parameters helps to determine the quality and purity of the drug.

**Table 2:** Extractive value of crude drug in different solvent

| Extracts | Hot extraction | Cold extraction |
|---|---|---|
| Alcoholic extract | 12.56±0.35 | 10.75± 0.03 |
| Aqueous extract | 13.7±0.2 | 11.57±0.04 |

All values are % W/W means ± S.D

**Table 3:** Physiochemical characteristics of *B. scandens*

| Parameters | Values |
|---|---|
| Moisture content (%w/w) | 5.382% |
| Total ash (%w/w) | 3.019% |
| Acid Insoluble ash(%w/w) | 0.521% |
| Water soluble ash(%w/w) | 1.34% |

**Total Phenol and Flavonoid content**

Total phenol and flavonoid content determined is mentioned in the table.4,which shows that alcoholic extract of both the hot and cold extraction possess higher values than aqueous extracts.

**Table 4:** Total Phenol and Flavonoid Content

| Extracts | Total Phenols (mg of GAE)/ gm) | Total flavonoids (mg of QUE/gm) |
|---|---|---|
| Alcoholic (Hot extraction) | 57.13±0.17 | 49.7±0.9 |
| Aqueous (Hot extraction) | 5.7±0.11 | 2.3±0.4 |
| Alcoholic (cold extraction) | 35±0.34 | 22±0.56 |
| Aqueous (cold extraction) | 1.5±0.5 | - |

## DISCUSSIONS

Medicinal plant material with therapeutic potential are utmost important for herbal industry and represent a substantial proportion of the global drug market. Despite of modern techniques, recognition, assessment and documentation of these plant drugs by pharmacognostic studies is more precise, dependable, and economical. The current study on leaf of *B. scandens* was undertaken to lay down the standards that could be useful for establishing the authenticity of the drug material.

The macroscopic and microscopy characters of leaf of *B. scandens* will help in the identification of the crude drug. Epidermal studies reveal a cyclocytic type with 4 or more distinguished subsidiary cells present in abaxial surface of the leaf of *B. scandens*. Presence of cyclocytic type of stomata individualizes the leaf of *B. scandens.* Quantitative determination like stomatal number and index, vein islet and termination values are important in the evaluation of crude material. Trichome present on both side of the leaf and its intended length will help in the recognition of leaf for sourcing it as a drug. Loss on drying or moisture content of leaf of *B. scandens* determined helps to known the water content [28]. thereby helps in storing the material for extended period of time with a fewer possibilities' microbial contamination. Incineration of leaf residue or ash content represents inorganic salts in the material. Acid insoluble ash is a part of total ash and measures the amount of silica present, water soluble ash is the water-soluble portion of the total ash [29]. These ash values determined for leaf of *B. scandens* are important pharmacognostic tool to standardize the crude drug. Fluorescence study of powder of *B. scandens* exhibited by various colour reactions, because of chemical constituent present in it. Visible and long UV studies showed characteristic colour which can be used as a fluorescence pattern for leaf of *B. scandens.* Phytoconsituent like phenols and flavonoids are able to scavenge free radicals by donating hydrogen atom to free radicals, initiating the defensive mechanism against various illnesses. Alcoholic and aqueous extract possess the phenol and flavonoid compounds, which are known for the antioxidant nature [30]. This show that this extract may be pharmacologically active, which needs detail study.

## CONCLUSIONS

The pharmacognostic study of the leaf of *Bridelia scandens* has been carried out for the first time. This may be helpful for the selection of raw material for its correct usage. It can be concluded that the pharmacognostic constants of leaf, diagnostic microscopic features and all numerical standards attained from the study would be useful for the identification, authentication and standardization of the plant specimen for quality assurance and also to prepare a monograph of the plant.

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