International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(6): November-December 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Paper……!!!**

Received: 01-09-2016; Revised: 28-11-2016; Accepted: 06-12-2016

PHARMACOGNOSTIC STUDY OF BARKS AND LEAVES OF *BRIDELIA RETUSA*

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Keywords:

Bridelia retusa, Pharmacognosy, bark, leaves

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ABSTRACT

Bridelia retusa is a traditional medicinal plant with diverse therapeutic ability. The current study was therefore carried out to provide requisite pharmacognostic details about the plant. Pharmacognostic investigation of the fresh, powdered and anatomical sections of the bark and leaves of *Bridelia retusa* was carried out to determine its morphological, microscopical, and phytochemical diagnostic features. Quantitative diagnostic characteristics, physicochemical properties and qualitative phytochemical measures were established. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

INTRODUCTION

Bark of *Bridelia retusa* (Euphorbiaceae) commonly known as Asana, Khaja found to be distributed throughout the hotter parts of India. It is a medium-sized to large deciduous tree with stout conical spines when young. Traditionally, bark is used as astringent and for rheumatism, topically[1]. Pharmacologically bark has antiviral, hypoglycemic, hypotensive, antifertility anti-inflammatory properties [2, 3]. Furthermore, literature revealed that the plant has contraceptive and antibacterial properties [4, 5]. Despite of strong traditional and pharmacological validity, the plant parts has not extensively studied for pharmacognostic study. Thus objective of the present study is to lay down pharmcognostical standards for leaves and bark of the *Bridelia retusa*.

MATERIALS AND METHODS

Plant collection

The *Bridelia retusa* bark and leaves were collected from Western Ghat of Maharashtra and authenticated by Dr. J. Jayanthi Scientist, Botanical Survey of India, Pune (voucher no. BRIRVIGI) and herbarium was deposited in BSI Pune.

The bark and leaves were washed, cleaned, shade dried, powdered, and passed through a 40-mesh sieve. It was stored in a tightly closed container. The fresh bark and leaves were used for macroscopy and microscopical study.

Macroscopy

The bark and leaves was separated from other parts, washed, cleaned, and dried for further use. Macroscopic characters of the fresh barks and leaves like color, odor, taste, size and shape, and other diagnostic characteristics were noted (Figure 1).

Microscopy

The free hand thin transverse sections of the fresh bark and leaves were taken and treated with different staining agents[6] and observed for the general and specific microscopic characteristic (Figure 2 and 3). Small quantity of the powdered leaves was cleared, mounted, and observed for diagnostic powder characteristics (Figure 4). Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provided with Motic Images plus 2.0 software. Furthermore, small quantity of the powdered leaves was cleared, mounted and observed for diagnostic powder characteristics[7].

Physicochemical and *Fluorescence analysis*

Bark and leaves powder was analyzed for their fluorescence analysis according to Kalaskar and Co(2012). The dried bark and leaves material was analyzed under visible light, short, and long UV light after treatment with various acids, alkalis, other reagents (Table 1and 2).

Physicochemical parameters such as percentage of ash, extractive values, foreign matter content, and loss on drying were determined[8] (Table 3).

RESULTS AND DISCUSSION

Macroscopy

The leaves are green in color on both the surfaces with characteristic odour. Leaf blade obovate, sometimes elliptic, 8-25 × 4-13 cm. The adaxial surface is glabrous while abaxial leaf surface is pubescent to pilose having bracts on both surfaces. Stipules are ovate-triangular, caducous, but linear stipular traces persistent at lateral base of petiole; petiole 0.7-1.2 cm, slightly stout;, papery or thinly leathery, base obtuse, rounded, or shallowly cordate, apex rounded or truncate, rarely acute, sometimes with short acumen; lateral veins 16-23 pairs, subparallel, reticulate veins prominent, subparallel, anastomosing lateral veins. Bark is dark brown to blackish, thick, with astringent taste, roughly cracked, flaking in scales. The bark has rough surface with splintery and fibrous fracture.

Microscopy

The stem bark shows presence of thin periderm and wide secondary phloem. Periderm is outermost layer showing thin walled, tubular cork cells and collopsidal phelloderm and less prominent. Secondary phloem is the major portion of the bark. It is differentiated into two zones, namely outer and wider collapsed phloem, while inner is narrow non-collapsed. The outer collapsed phloem consists of horizontal dark lines which are formed due to crushing and collapsing of the sieve elements. The cells are not in radial alignment rather they are tangentially arranged. Thin tangential lines of phloem sclerenchyma (fibres) in regular order alternating with thick bands of tannin containing parenchyma cells are present. Calcium oxalate crystals are accumulated in paranchymatous cells. Non-collapsed phloem consists of sieve-tubes, parenchyma cells, companion cells and phloem fibers; the tannin-containing cells are also present. The phloem rays are prominent and found to be uniserrate to biserrate (Figure 2).

The microscopy of the leaves showed all the general characteristics with some distinguishing features. The cells of the epidermis are cuticulized. The polygonal epidermal cells observed with anticlinal walls. The upper epidermal cells are comparatively larger than lower one, while the lower epidermis has a thick cuticle compared to upper epidermis.The leaf lamina is found to be dorsiventral showing 2-3 layers of palisade cells. The upper epidermis showing negligible trichomes, while plenty of bicellular to multicellular covering trichomes on lower epidermis.

The midrib region is biconvex. Upper and lower epidermis layer is continuous over the midrib, the epidermal cells shown similar features as seen in the lamina region. Adjacent to the epidermis,

angular collenchyma occur, comprising approximately three to five rows on the ventral side and two to four on the dorsal side one embedded in the ground parenchyma. The collateral vascular bundles arranged nearly as a closed arc showed lignified spiral xylem vessels measuring 28 - 46 µ in diameter and non lignified phloem as sieve tubes. This exhibits an evident cambial zone and sclerenchymatic fibers surrounding the vascular bundle(Figure 3).

Powder characteristics

Leaf: The ranunculaceous stomata means stomatal pores are surrounded by irregularly arranged gaurd cells. Numerous bi-cellular to multi-cellular covering trichomes are present. Fibers are few, lignified well-developed sclerenchymatous fibers from the vascular bundle region (Figure 4). Fragments of mesophyll tissue containing vascular strands i.e. Spiral xylem vessels are seeing a good many in number (Figure 4).

Bark: Microscopical powder analysis showed presence of lignified phloem fibers, prisms of calcium oxalate and abundant starch grains in parenchymatous cells observed (Figure 4).

Table 1: Fluorescence analysis of powdered bark of *B. retusa*.

Sample	Colour in daytime	Colour in short UV	Colour in long UV
Powder	Brown	Brown	Black
Powder + Sod. hydroxide in methanol	Green	Green	Blackish green
Powder + Sod. Hydroxide in water	Brown	Green	Brown
Powder + 1 N Conc. HCl	Brown red	Green	Fluorescent green
Powder + Conc. HNO_3	Reddish	Fluorescent green	Blackish brown
Powder + Conc. H_2SO_4	Black	Blackish brown	Black

Table 2: Fluorescence analysis of powdered leaves of *B. retusa*.

Sample	Colour in daytime	Colour in short UV	Colour in long UV
Powder	Green	Green	Green
Powder + Sod. hydroxide in methanol	Green	Fluorescent green	Yellow
Powder + Sod. Hydroxide in water	Light brown	Greenish black	Dark brown
Powder + 1 N Conc. HCl	Light brown	Light green	Light green
Powder+ Conc. HNO_3	Blackish	Blackish	Fluorescent green
Powder + Conc. H_2SO_4	Yellowish red	Fluorescent green	Brown

Table 3. Physicochemical analysis of *B. retusa* barks and leaves.

Types of ash value/extractive values	% w/w	
	Bark	Leaves
Ash values		
Total ash	9.1	8.9
Acid insoluble ash	2.6	2.3
Water soluble ash	5.1	6.0
Sulphated ash	4.3	3.8
Extractive values		
Alcohol	15.5	16.2
Water	9.3	10.1
Foreign organic matter	3.2	4.1
Loss on drying	3.8	4.6

Figure legends

Figure 1. *B. retusa:* a. External features of bark, b.External features of leaf.

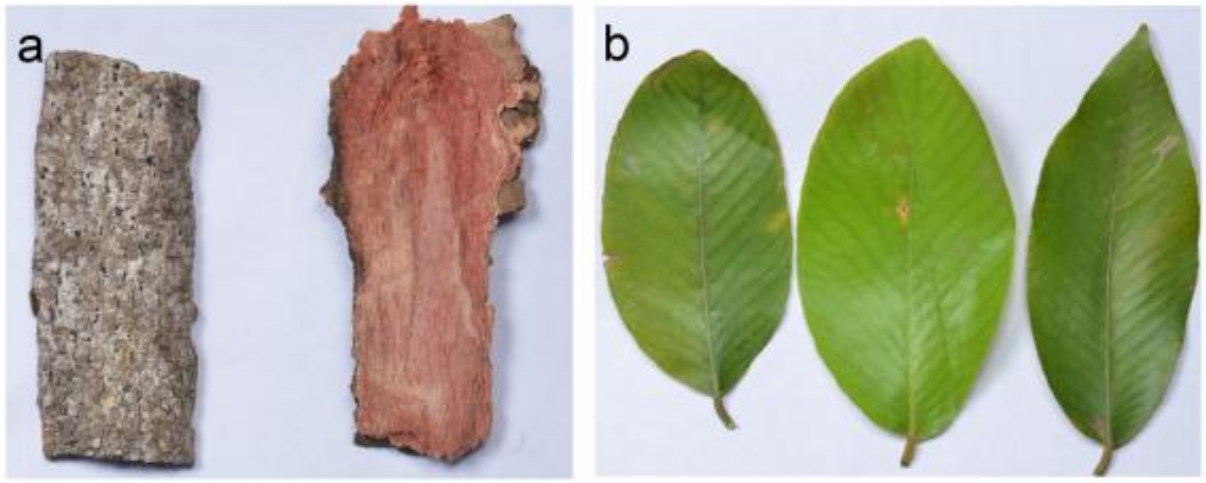


Figure 2. Transverse section of *B. retusa* bark

Figure 3. Transverse section of *B. retusa* leaves

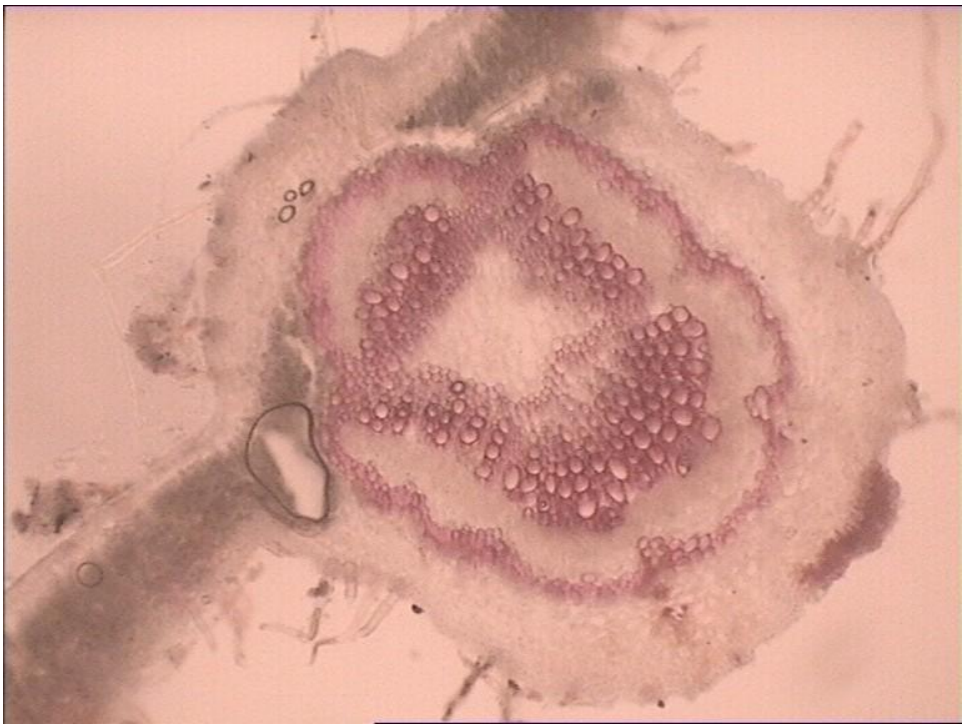
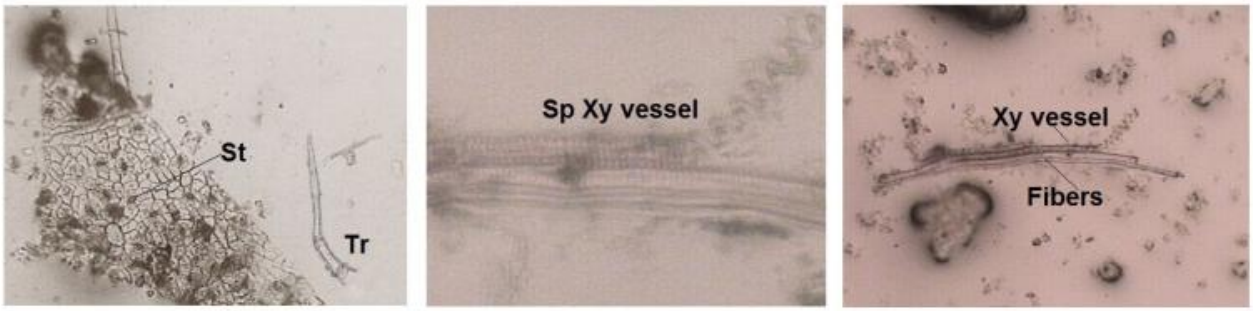
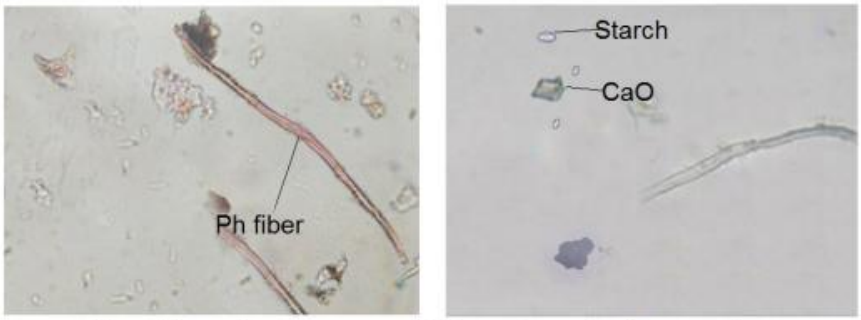


Figure 4. Powder microscopy of *B. retusa* barks and leaves



Powder microscopy of *B. retusa* leaves powder



Powder microscopy of *B. retusa* bark powder

St- Stomata; Tr-trichomes; Spxy vessel- spiral xylem vessel; Ph fiber- Phleom fiber; CaO- Calcium oxalate

Physicochemical analysis

The physicochemical standards are important to check the quality, purity, and adulteration of given crude drug. The foreign matter, LOD, ash, and extractive values were determined and summarized in table 3.

These distinguishing morphological and microscopical features of leaves and bark would be source of correct identification of *B. retusa*. The ash values of a drug gives an idea of the earthy matter or inorganic composition and other impurities present along with the drug. Extractive values are preliminary useful for determination of exhausted or adulterated drug (Kalaskar et al., 2012). Thus ash values, extractive values and fluorescence analysis will be helpful in identification and authentication of plant material. The preliminary phytochemical screening showed presence of different phytoconstituents groups such as steroids, triterpenoids, flavonoids, polyphenolic, and tannin.

The present study provides in-depth microscopical features, and physicochemical characteristics, will definitely provide pharmacopoeial standards for easy identification of the *B. retusa* hence, differentiating it from closely related species.

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