

Pharmacognostic Evaluation of an Ethnoplant *Bridelia crenulata* Roxb.

Gabhe S Y^{1*}, Tatke P A², Punjabi R K²

¹Poona College of Pharmacy, Bharati Vidyapeeth, Paud Road, Pune-411038

²C. U. Shah College of Pharmacy, S.N.D.T. Women's University, Juhu Campus, Santacruz (West), Mumbai- 400049, India

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ABSTRACT

India is one of the world's twelve leading biodiversity centers having over 45,000 different plant species. Many of these plant species have beneficial values and are used for several years by our ancestors as effective home remedies or remedies suggested by the vaidya's or hakim's. These ethnoplants can be considered as a great source of therapeutic agents. However, the biggest constraint is that they have not been studied systematically and scientifically. Thus, an understanding of the pharmacognostical, pharmacological and phytochemical potential of these ethnomedicinal plants would provide an authentic data which would enable such precious source of medicinal plants to fulfill demands of the global market. *Bridelia crenulata* Roxb belonging to family Euphorbiaceae is one such ethnoplant, which is known to be used by the inhabitants of Orissa to prevent pregnancy. In this research article, pharmacognosy of *Bridelia crenulata* is studied, which may serve as a valuable source of information and provide suitable standards to determine the quality, purity and authenticity of *Bridelia crenulata* in future investigations or applications.

Keywords: *Bridelia Crenulata* Roxb., pharmacognostical evaluation, ethnoplant, abortifacient.

INTRODUCTION

Bridelia crenulata Roxb belonging to family Euphorbiaceae is an ethnoplant, which is used by inhabitants of Mayurbhanj district in Orissa to prevent pregnancy. The liquor of stem bark is given after menstruation¹. The women of the Paliyan tribes in Tirunelveli district of Tamil Nadu in India consume the stem bark extract to cure menorrhagia². Very less information is available on this ethnoplant regarding its pharmacognosy, pharmacology or phytochemistry in common medicinal plant literature.

Despite the modern techniques, identification of the plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such material and should be carried out before any tests are undertaken.

Scientific parameters to identify the true plant material of *Bridelia crenulata* and to ensure its quality were not available in any standard literature. Thus, there was ample scope to work on this plant for various pharmacognostical parameters. Comprehensive information on morphological characters of whole plant and microscopical characteristics of the stem bark of *Bridelia crenulata* has been provided in the study.

MATERIALS AND METHODS

For morphological characters, different parts of *Bridelia crenulata* (Family- Euphorbiaceae) were studied. Photographs of the whole plant and its different parts were



Figure 1: Photograph of *Bridelia crenulata* in its natural habitat.

obtained from the forest of Tirunelveli, Tamilnadu [Fig 1-3].

For microscopical studies, stem bark of *Bridelia crenulata* was collected from forest area of Tirunelveli, Tamil Nadu in the month of July and August. The plant was authenticated at Survey of medicinal plants unit - Siddha Govt. Siddha Medical college campus, Palayamkottai, Tirunelveli-627002, Tamil Nadu [SMPU Spec.No.8324 February 2004]. The stem bark was separated, air-dried and was powdered by hammer mill to obtain a coarse material for powder microscopic studies. Microscopic characteristics of stem bark of *Bridelia crenulata* Roxb and its powder were studied at Plant anatomy research centre, Chennai.



Figure 2: Leaves of *Bridelia crenulata*.



Figure 3: Bark cut off from the stem of *Bridelia crenulata*.

Procedure for microscopic studies

Collection of the specimen

The fresh stem bark of *Bridelia crenulata* was collected for microscopic evaluation of histological sections. Course powder of the fresh stem bark was used for powder microscopy. For histology, stem bark was fixed in FAA (formalin+ acetic acid+ 70% ethyl alcohol in the ratio of 0.5: 0.5: 9). After 24 hrs fixation, the specimen were dehydrated with graded series of T-butyl alcohol as per the schedule given by Saa, 1940³. Infiltration of the specimen was carried by gradual addition of paraffin wax (melting point 58- 60° C) until thiobarbituric acid solution attained super saturation. The specimens were then cast into paraffin blocks.

Preparation of sections

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done by standard procedure⁴. The sections were later stained with toluidine blue by procedure⁵. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and fast-green. For powder microscopy, powdered stem bark was cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and measured.

Photomicrographs

Photographs of different magnifications were taken with Nikon labphot 2 microscopic units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was used. However since these features have birefringent property under polarized light they tend to appear bright against dark background. Magnifications of the features are indicated by the scale bars on the images. The descriptive terms used were as given in the standard anatomy books⁶. Results are given in fig 4 [a-h] for TS of

stem bark & fig 5 [a-f] for powder microscopy of stem bark.

RESULTS AND DISCUSSION

Morphological characteristics of Bridelia crenulata

The plant is a profusely branched medium sized deciduous tree. The habitat of the plant is grass land [Fig 1]. Leaves are compound and glabrous. Colour of the leaves is green and pale grey beneath. Leaf type is simple with alternate distichous arrangement. Leaf shape is ovate, elliptic, and oblong with obtuse apex, rounded base and entire margin [Fig 2]. The branchlets are densely brownish. Each leaf has 15-20 pairs of strong paired veins [Fig 2]. The bark is 1.8 mm thick. It has smooth surface with membranous exfoliating flakes [Fig 3].

Microscopic study of stem bark of Bridelia crenulata

Microscopic characteristics of stem bark of *Bridelia crenulata* Roxb and its powder were studied at *Plant anatomy research centre, Chennai*

Microscopic features of the stem bark sections

The stem bark showed presence of thin periderm and wide secondary phloem.

Periderm is superficial and less prominent. It consists of thin walled, tabular cells.

Secondary phloem is the major portion of the bark. It is differentiated into two zones, namely outer and wider collapsed (crushed) phloem [CPh] and inner & narrow non-collapsed (intact) phloem [NCPH].

Collapsed phloem [CPh]: It is formed due to tangential pressure of newly formed phloem during growth of bark tissue [fig 4a]. It consists of horizontal dark lines which are formed due to crushing and collapsing of the sieve elements. The cells are not in radial alignment. The collapsed phloem is nearly 1.4 mm wide. Along with collapsed phloem, thin tangential lines of phloem sclerenchyma (fibres) in regular order, alternating with thick bands of tannin containing parenchyma cells are present [fig 4c]. Calcium oxalate crystals are accumulated along with the sclerenchyma bands. The crystals occur on

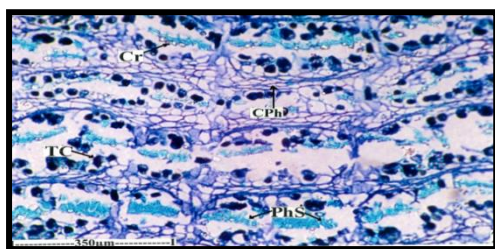


Figure 4[a]

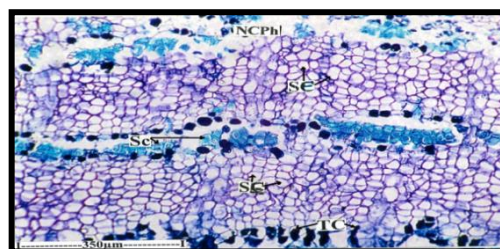


Figure 4 [b]

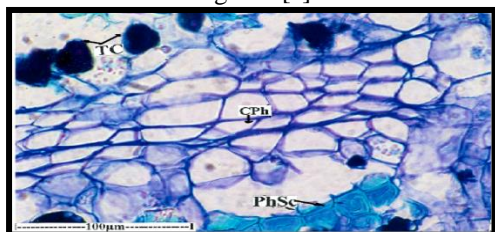


Figure 4[c]

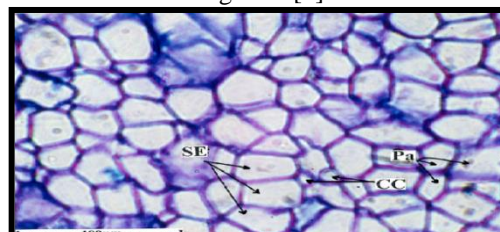


Figure 4 [d]

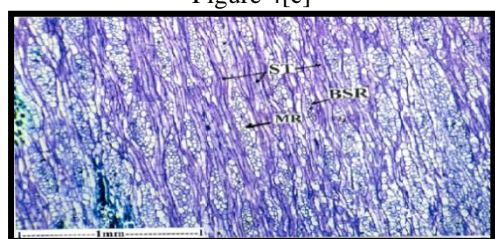


Figure 4[e]

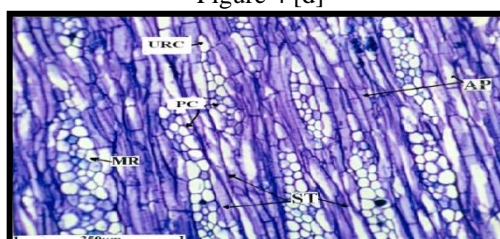


Figure 4 [f]

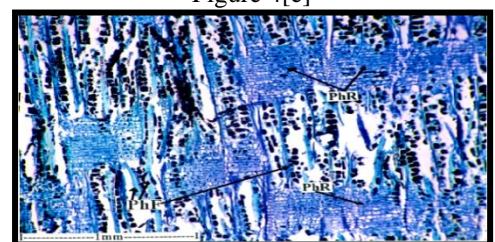


Figure 4[g]

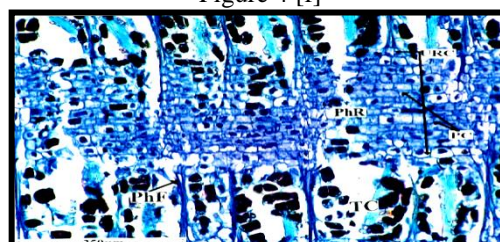


Figure 4 [h]

Figure 4: [a –h] TS and powder microscopy of stem bark

[a] TS showing collapsed phloem; [b] TS showing non-collapsed phloem [c] Magnified collapsed phloem and tanniniferous cells [d] Magnified non-collapsed phloem showing companion cells and sieve elements [e] TLS of phloem - under low magnification [f] Magnified TLS of phloem [g] Radial longitudinal section [RLS] of phloem [h] Magnified RLS of phloem.

Abbreviations: Cr-Crystal; CPh-collapsed phloem; PhS-phloem sclereids; TC- Tanniniferous cells i.e. tannin containing cells; NCPh-Non-collapsed phloem; Sc- Sclereids; SE- Sieve elements; CPh- collapsed phloem; PhSc Phloem Sclereids; TC - Tanniniferous cells; CC- companion cell; Pa- Parenchyma cells; SE - Sieve elements; BSR- Biseriate rays; MR- Multiseriate rays; ST- Sieve tube member; AP- Axial Parenchyma; ST- Sieve tubes; URC-Upright cells; PC Procumbent cell; PhF- Phloem fibres; PhR- Phloem ray PhR- Phloem ray;

the outer and inner borders of the sclerenchyma bands [fig 4a].

Non-collapsed phloem [NCPH]: The intact or non-collapsed phloem has well preserved sieve-elements [SE], parenchyma cells and companion cells of the sieve-elements. The sieve elements [SE] are angular in cross-sectional outline and thick walled. The companion cells are prominent and occur along the corners of the sieve elements. Along with the non-collapsed phloem, phloem sclerenchyma cells [sclereids (Sc)] and tannin-containing cells [TC] are also present [fig 4b & 4d].

Phloem in TLS and RLS sectional view:

The structure and organization of the phloem rays, sieve elements and axial parenchyma cells were studied in LS view. In TLS view, the phloem part of the bark exhibits the characters of the phloem rays and sieve tubes [fig 4e & 4f]. The phloem rays are non-storied, but long and narrow. The rays range from biseriate to multiseriate structures. The biseriate rays have two vertical rows of cells. The multiseriate rays have more than three vertical rows of cells [fig e]. The phloem rays are heterocellular. The central portion of the rays consists of polyhedral cells called procumbent cells and those along the ends of the rays are vertically elongated cells called the upright cells. The sieve tube members are straight, narrow and have

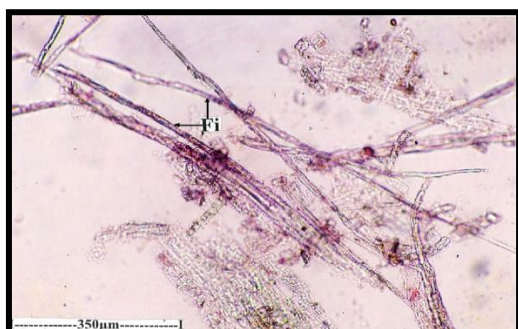


Figure 5[a]

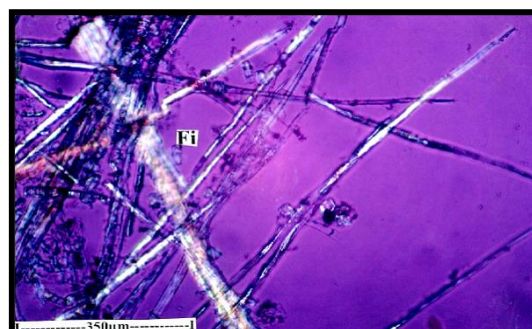


Figure 5[b]

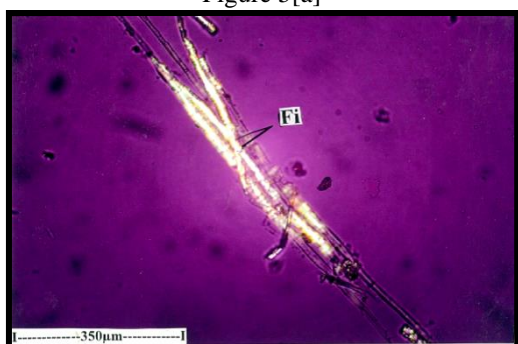


Figure 5[c]



Figure 5 [d]

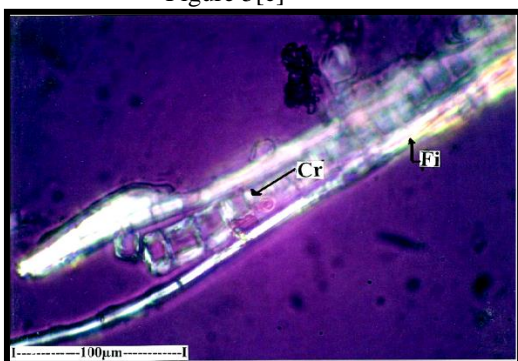


Figure 5[e]

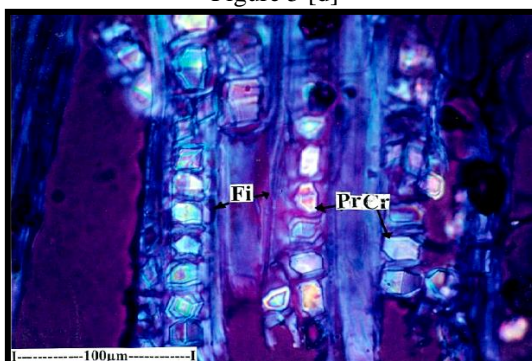


Figure 5 [f]

Figure 5: [a –f] Powder microscopy of stem bark

[a] Powder microscopy showing fibres [b] Powder microscopy showing fibres under polarized light microscope [c] Powder microscopy showing fibres under polarized light microscope [d] Powder microscopy showing fibres and crystal strand [e] Powder microscopy showing fibres and crystal strand under polarized light microscope [f] Powder microscopy showing fibres and prismatic crystals under polarized light microscope.

Abbreviations: Cr-Crystal; Fi- fibres; Crs- Crystal strand, PrCr- Prismatic crystals.

simple and oblique sieve plates. They are 370-480 μm in height and 20-30 μm wide. Their walls are thick. Axial parenchyma cells [AP] are vertically running cells. They occur in vertical strands. They are narrow and rectangular in shape [fig f]. In radial longitudinal section [RLS], the procumbent cells occur in horizontal rows. The cells at the terminal part of the rays are called the upright cells and appear in vertical orientation [fig 4g & 4h]. The rays are 300-900 μm in height and 40-100 μm thick. Ray frequency is 5 mm.

Powder microscopy of stem bark Microscopic evaluation of stem bark powder showed presence of phloem fibres and calcium oxalate crystals. Fibres [Fi] are libriform type, having thick lignified wall, narrow lumen and tapering ends. They are 1mm to 1.5 mm long and less than 10 μm wide [fig 5a]. When the fibres were viewed under the polarized light microscope, the walls of the fibres appeared

bright under dark background, which suggested that the walls contain lignin. This is a characteristic property of lignified cells [fig 5b & 5c]. The stem bark powder also showed abundant calcium oxalate crystals [fig 5d]. They are prismatic type and cuboidal in shape. They occur in vertical row within the xylem parenchyma and are always observed to be present along with the fibres. The structures are called crystal-strands, since they occur in vertical strands in the bark. Like the lignified fibres, calcium oxalate crystals also appear as bright bodies under the polarized light microscope [fig 5e & 5f].

CONCLUSION

The present study involved pharmacognostic evaluation of *Bridelia crenulata*, which may serve as a valuable source of information and provide suitable standards to determine the quality, purity and authenticity of *Bridelia crenulata* in

future investigations or applications. It could help in differentiating the plant material of *Bridelia crenulata* from other closely related species of *Bridelia* and may also be useful for the compilation of a monograph in suitable pharmacopoeia for its proper identification and quality control.

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