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Research Article

Pharmacognostic and preliminary phytochemical investigation on bark of *Bridelia retusa Spreng*

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ABSTRACT
Bridelia retusa bark has been traditionally used for the treatment of various ailments such as rheumatism, diarrhoea and dysentery. In view of its medicinal importance and to have taxonomic clarity about the plant the present study was done to investigate the pharmacognostic, microscopical, morphological and various chemical parameters of the bark. The studies will provide referential information for the correct identification of the crude drug.

Keywords: Rheumatism, diarrhoea, *Bridelia retusa.*

INTRODUCTION

Bridelia retusa Spreng (Euphorbiaceae) commonly known as Kasai in India, belongs to the family Euphorbiaceae. The plant is a shrub or a tree up to 18 m height armed with strong conical spines 7 cm long, found through out India up to an altitude of 1,000 m, except in very dry regions. [1] A survey of ethno medicinal records revealed that the bark is pungent, bitter, heating; useful in 'vata' lumbago, hemiplegia. The bark is good for the removal of urinary concretions .The root and the barks are valuable astringents. The bark is used as a liniment with gingerly oil in rheumatism. [2] The bark extract is used by the tribal to develop sterility and it is used as a contraceptive. One or two drops of fruit extract are poured in ear to cure earache. [3] The plant has been used in traditional systems of medicines for treatment of dysentery and diarrhea, diabetes. [4-5] The present investigation was planned with an objective to establish Pharmacognostic standards and to evaluate preliminary phytochemical data's on *Bridelia retusa* that can facilitate the authentification and the isolation of the desired constituent from the correct extract.

MATERIALS AND METHODS

Plant material

Fresh bark of *Bridelia retusa Spreng* were collected in the month of June from Array colony of Goregaon (East) of Mumbai, Mahrashtra, India and authenticated by Dr. Vinayak Naik, Taxonomist and Senior research scientist at Piramal Life Sciences, Goregaon, Mumbai. A voucher specimen no 1967, dated April 2002, is maintained in Piramal life Sciences, Goregaon, Mumbai. The fresh bark was removed and dried in shade. The fresh bark was used for the study of macroscopic and microscopic characters, whereas the dried bark powder was used for determination of ash value, extractive values and phytochemical investigations. All the reagents used were of analytical grade obtained from Qualigens Fine Chemicals, Mumbai, India and Merck Limited, Mumbai, India.

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Microscopy

Fresh barks of *Bridelia retusa* were selected for the microscopical studies. Microscopic sections were cut by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the bark specimen were made and examined microscopically. Histochemical reactions were applied with Hydrochloric acid - Phloroglucinol to reveal the lignified elements, weak iodine solution for starch, Dragendroff's reagent for the alkaloidal substances, ruthenium red for mucilage, 60 % H_2SO_4 for the calcium oxalate and ferric chloride for phenolic compounds on the powdered bark by reported methods. [6-7] Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provide with MOTIC IMAGE PLUS 2.0 software

Powder characteristics

Preliminary examination and behavior of the powder with different chemical reagents was carried out and microscopical examination was carried out. [8-9]

Micrometry

Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provide with MOTIC IMAGE PLUS 2.0 software. The measurement of different cell and cell contents were taken.

Physico-chemical parameters

Percentage of total ash, water soluble ash, acid insoluble ash and sulphated ash were calculated as per the Indian pharmacopoeia. Extract of the powdered bark were prepared with different solvents for the study of extractive values. The total ash of the powdered bark was tested for different inorganic constituents. [10] Fluorescence analysis of the powdered bark was carried out by standard methods. [9, 11]

Table 1: Histochemical color reactions of *Bridelia retusa Spreng* bark

Reagent	Constituent	Colour	Histological zone	Degree of intensity
Phloroglucinol + hydrochloric acid	Lignin	Pink	Xylem, Sclerencyma	++
Aniline Sulphate + Sulphuric acid	Lignin	Yellow	Xylem	+
Weak iodine solution	Starch	--	--	--
Millons reagent	Protein	--	--	--
Dragendorff's regent	Alkalods	--	--	-
Libermann-Burchardt reagent	Steroids	Pink	Cortex, phloem	++
Keddy reagent	Glycoside	--	--	--
Aqs Ferric chloride	Tannins	black	cortex	+++
5% Aq KOH	Anthraquinone glycoside	--	--	-

+++ High, ++ Moderate, + Slight, - Negative

Preliminary phytochemical analysis

For the preliminary phytochemical analysis, 5 gm of the powdered drug was extracted with petroleum ether (60-80), chloroform, methanol, ethyl acetate and water successively. The extracts were dried and weighed. The marc was air dried, and water extract was obtained by boiling with distilled water for 2 h, filtering, concentrating and drying in an oven at 40-50°C. The presence or absence of different phytoconstituents viz triterpenoids, steroids, alkaloids, sugars, tannins, glycoside and flavanoids were detected by usual prescribed methods. [12-1

Table 2: Measurement of cells

S. No.	Type of cells	Size in micron (µm)
1.	Cork cells	59.00 x 38.59 µm
2.	Cortex	12.3 µm in width and 14.5 µm in length
3.	Stone cells	72.5 µm x 29.5 µm
4.	Parencyma	45.31 µm
5.	Phloem fibres	280 µm x 17.2 µm
6.	Pericyclic fibres	382 x 28.2 µm
7.	Phloem Parencyma	27.01 µm
8.	Calcium oxalate crystals	12.3 µm(W) to 14.5 µm (L)

RESULTS

Organoleptic characters (Fig. 1):

Colour: Externally brownish black, internally yellowish brown

Taste- Astringent

Odour- Odourless

Touch- Rough

Size and shape- 7 to 9 cm × 3 to 5 cm × 1 to 2.5cm

Extra feature –The bark shows minute longitudinal wrinkles, and fibrous fracture, internal surface smooth to touch

Histochemical color reactions

Histochemical color reactions were carried out on the bark by reported methods. [7, 12] The results are given in Table 1

Microscopy

A) Transverse section of the bark (Fig. 2)

The transverse section of the bark shows the typical marphoanatomical characteristics as **Cork -**The cork region was found to be well defined, in which the cork cells are rectangular and in 10 to 15 layers. Some of the cork cells are lignified. Phellogen is compactly arranged in 3 to 5 layers, Phellogen and phelloderm are indistinguishable.

Cortex- The periderm and the cortex is separated by a layer of stone cells. The cortex region shows the presence of simple calcium oxalate crystals having average size of 12.3 µm in width and 14.5 µm in length. Few mucilaginous cells are also present. They are solitary, appears brown in colour. In the cortex bundle of lignified bundle of lignified Pericyclic fibers are also present. They are found in a group of 6 to 15. The cortex region is guarded by layers of stone cells.

Table 3: Behavior of the bark powder with different chemical reagents

S. No.	Reagent	Colour/Precipitate	Constituent
1.	Conc. Sulphuric acid	Reddish brown	Steroids present
2.	Picric acid	No change	Alkaloids absent
3.	Aqueous ferric chloride	Blakish	Tannins present
4.	Iodine solution	No change	Starch absent
5.	Aqueous mercuric chloride solution	No change	Alkaloids absent
6.	Magnesium – hydrochloric acid	No change	Flavanoids absent
7.	Aqueous silver nitrate solution	No ppt	Protein absent
8.	Aqueous potassium hydroxide solution (5 %)	No change	Anthraquinone glycoside absent
9.	Spot test	No stain observed	Fixed oils absent
10.	Lieberman's bur chard test	Reddish green	Steroids / Triterpenoids present
11.	Salvoski's test	A yellow ring at the junction	Steroids present
12.	Frothing test	Foam observed	Saponins present
13.	Mollish reagent	Purple colour at the junction	Carbohydrate present
14.	Aq lead acetate	White precipitation	Tannins present
15.	Dragendroff reagent	No precipitation	Alkaloids absent
16.	Aqueous NaOH	No change	Flavanoids absent

Phloem region – Modularly rays are distinct, funnel shaped found to be uniserrate to biserrate.

Phloem- It shows the presence of lignified phloem fibers found in a group of 3 to 8 fibers surrounded by phloem parenchyma and sieve tubes. The phloem region shows trace of calcium oxalate crystals. The phloem region shows the presence of endodermis.

The micrometric analysis was tabulated in Table 2.

Table 4: Ash values of *Bridelia retusa Spreng* bark

S. No.	Types of ash values	% w/w(Mean ± SEM)
1.	Total ash	9.5 ± 89
2.	Acid insoluble ash	2.5 ± 0.69
3.	Water soluble ash	4.5 ± 0.24
4.	Sulphated ash	3.0 ± 0.79

B) Powder characteristics (Fig. 3)

Preliminary examination of powder

Colour- Dark brown

Odor- Odorless

Taste – Astringent

Texture- Rough After pressing a little amount of powder between filter paper, no greasy stain was found, indicates the absence of fatty oils, after shaking powder with water in

Fig. 1: Bark of *Bridelia retusa Spreng*

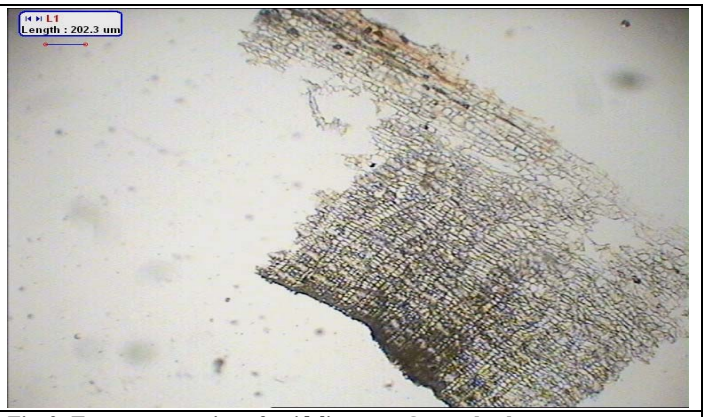

Fig. 2: Transverse section of *Bridelia retusa Spreng* bark

Fig. 3a: Powder characteristic of *Bridelia retusa Spreng* bark (cortex showing calcium oxalates)

Fig. 3b: Non lignified pericyclic fibers

Fig. 3c: Lignified phloem fibers

Fig. 3d: Stone cells

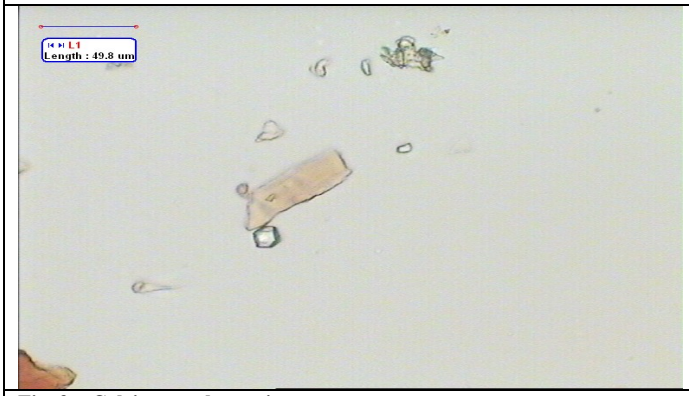

Fig. 3e: Calcium oxalate prisms

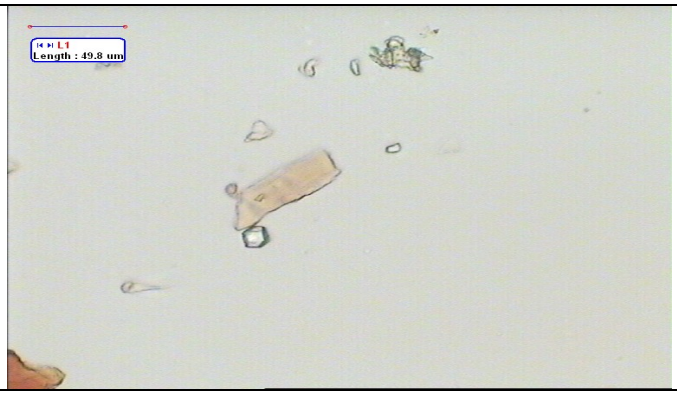

Fig. 3f: Mucilaginous cells

test tube persistent froth was formed, indicates the presence of saponins.

Behavior of the powder with different chemical reagents is shown in Table 3.

Table 5: Extractive values with different solvents

S. No.	Type of solvent	% Extractability(Mean± SEM)
1.	Petroleum ether (60-40)	0.5 ± 0.18
2.	Chloroform	0.9 ± 0.40
3.	Ethyl acetate	0.9 ± 0.31
4.	Methanol	18.0 ± 0.21
5.	Water	10.3 ± 0.39

Table 6: Consistency, colour & fluorescence analysis of different extracts of *Bridelia retusa Spreng* bark

Extract	Consistency	Colour in		
		Daylight	Short UV	Long UV
Petroleum ether	Sticky mass	Light green	Green	Dark green
Chloroform	Resinous	Pale brown	Greenish brown	Dark brown
Ethyl acetate	Semisolid	Brownish green	Yellowish green	Dark green
Ethanol	Solid	Greenish brown	Greenish black	Bluish black
Methanol	Solid	Greenish brown	Greenish black	Greenish black
Water	Semisolid	Light brown	Brown	Light brown

Table 7: Florescence analysis of powdered bark of *Bridelia retusa Spreng*

Sample	Colour in daylight	Colour in Short U V	Colour in long U V
Powder	Light brown	Light brown	Light brown
Powder + sodium hydroxide in methanol	Greenish black	Fluorescent green	Greenish black
Powder + sodium hydroxide in water	Dark brown	Greenish	Dark brown
Powder + 1 N hydrochloric acid	Reddish brown	Greenish	Dark fluorescent green

Microscopical examination of the of the powder

Cortex cells: Simple polygonal parencymatous cells with intracellular spaces were observed. The parenchymal cell shows the presence of simple prism of calcium oxalate (Fig. 3a).

Pericyclic fibers: Lignified long slender tapering towards both the end, Pericyclic fibers of average size 382x 28.2 µm was found in abundance (Fig. 3b).

Phloem fibers: Long cylindrical with cell lumen lignified, phloem fibers were observed. It was frequently adhered to phloem parenchyma having average size 280 µm × 17.2 µm (Fig. 3c).

Stone cells: Thick walled English U shaped lignified stone cells was observed having average size of 72.5 µm × 29.5 µm found in abundance (Fig. 3d).

Calcium oxalate crystals: simple prism of average size 12.3 µm (W) to 14.5 µm (L) was found (Fig. 3e).

Mucilaginous cells: the brown colour mucilaginous cell of average size was found in few numbers (Fig. 3f).

Ash values

Total ash, acid insoluble ash, water soluble ash and Sulphate ash values of the bark powder were done as per the Indian Pharmacopoeia. The results are tabulated in Table 4.

Extractive values

Different extracts of the powdered bark were prepared for the study of extractive values. Percentage of extractive values was calculated with reference to the air dried drug. The results are shown in Table 5.

Fluorescence analysis

Consistency, colour and fluorescence analysis of different extracts and the fluorescence analysis of the powdered drug in daylight, short UV, and long UV were evaluated by reported methods. The observations are given in Table 6 and 7.

Preliminary Phytochemical screening

The presence or absence of different phytoconstituents viz triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavanoids, etc were detected by usual prescribed methods and t results are given in Table 8.

DISCUSSION

Lack of standardization is the major stumbling block in exploiting the potential of traditionally used herbal medicines. *B. retusa* is a plant with old history of use as a traditional medicine and can be well exploited for anti-inflammatory and antiulcerogenic activity. The present investigation has stated important standardization parameters of *Bridelia retusa bark*, qualitative and quantitative microscopic characters, ash values, extractive values, and phytochemical profiles of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of the plant. These standardized parameters would be of immense help in authenticating *Bridelia retusa*

Table 8: Qualitative phytochemical analysis of various extracts of bark of *Bridelia retusa Spreng*

Type of constituent	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
Steroids and sterols	+	+	+	+	+
Carbohydrates	-	-	-	+	+
Alkaloids	-	-	-	-	-
Glycoside	-	-	-	-	-
Reducing sugars	-	-	-	-	-
Flavanoids	-	-	+	+	
Tannins and phenolic	-	-	-	+	+
Proteins and amino acids	-	-	-	-	-
Gums and resins	-	-	-	-	-
Triterpenoids	+	+	+	-	-
Saponins	-	-	+	+	+

+ Present, - Absent

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