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Ethno-pharmacognostical Studies on Root Bark of *Rubus ellipticus* Smith. from Manipur

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Rubus ellipticus Smith. belongs to family Rosaceae. It is one of the important ethnomedicinal plants of Manipur. The Naga tribe of Manipur uses the root bark of the said plant for curing fever since ancient times. The present study deals with comprehensive pharmacognostical studies on root bark of this plant, it includes macroscopy, microscopy, preliminary phytochemical analysis and physicochemical parameters. Some diagnostic characters are presence of uniserriate root hairs, endodermal cells, stone cells and calcium oxalate crystals. Physicochemical studies revealed total ash (3.35%), acid insoluble ash (1.0%), water soluble ash (0.9%), alcohol soluble extractive (21.12%) water soluble extractive (25.3%) and chloroform extractive (1.3%). This will help in laying down pharmacopeial parameters.

Keyword: *Rubus ellipticus,* Rosaceae, Pharmacognosy

1. Introduction

Manipur is blessed with amazing varieties of flora and fauna. There are various tribes who depend on this natural wealth. One among them is the Naga tribe of Manipur. They dwell in the hilly terrains and totally depend on nature for their livelihood. For curing various ailments they use the medicinal plants from the wild since ages. For the present investigation interviews and direct interactions were made with the local and the medicine men in relation to the plants used in treating fever. *Rubus ellipticus* Smith.was one among the shortlisted plants used as antipyretics.

Rubus ellipticus Smith. belongs to family Rosaceae. It is commonly known as 'Kavathipu' by the Nagas [1]. It is sub erect straggling shrub with prickles on the branches. The leaves are pinnately trifoliate, flowers white with drupe type of edible fruit (Fig. 1). Besides Manipur the plant is well distributed in Nilgiri hills, Deccan, Burma etc [2]. The decoction of root bark is recommended twice a day for curing fever by the Nagas. The root bark is also used in diarrhea, dysentery, as abortificient, emmenagogue and in fractured bones [3].

Fig 1: Entire plant of *Rubus ellipticus* Smith.

2. Material and Methods

2.1 Plant Material: Ethno-medicinal survey using the suitable questionnaire was conducted. Fresh root bark was collected from a matured plant with prior permission from the forest department of Manipur. The specimen was authenticated from Botanical Survey of India (BSI), Shillong. A voucher specimen has been deposited in Botany Research Laboratory of K.V. Pendharkar College, Thane, India. The accession no. of the sample is (KVP 790). After collection some of the root bark was preserved in FAA solution. Materials were dried in oven at 37 ^{0}C and stored in airtight container.

2.2 Macroscopy: The root barks were studied for its morphological characters using the standard techniques [4].

2.3 Microscopy: Transverse hand cut sections were taken and made permanent with suitable stains. Quantification and photomicrographs were taken of the permanent preparations. The cell contents were measured using stage and occular micrometer [5, 6].

2.4 Histochemistry: The histochemical studies for the cell contents were performed using standard methodology [7].

2.5 Powder Study: The powdered drugs were soaked in aqueous solution of chloral hydrate and mounted in 50% glycerin for microscopical studies [8].

2.6 Proximate Analysis: The physicochemical parameters like ash values and extractive values were done [9].

2.7 Fluorescence Analysis: The fluorescence response of powdered drugs exposed to U.V. radiations was studied using the standard procedure [10].

2.8 Preliminary Phytochemical Screening: A known quantity of dried powder was extracted with chloroform, alcohol and water. These extracts were tested for different constituents [11].

3. Results and Discussion

3.1 Macroscopy:

The root bark is 0.8 – 1.0 cm in thickness. It is longitudinally, slightly curved or at times single quilled in shape. Outer surface is grey to dark brown while inner surface is grey to light brown to dark brown or slightly black in colour. It is fibrous in fracture, aromatic odour with strongly bitter and astringent in taste (Figs. 2 and3).

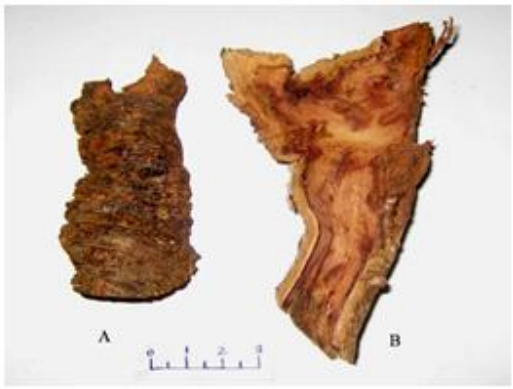

Fig 2: Entire root of *Rubus ellipticus* Smith **Fig 3:** Root bark (A – Outer surface; B – Inner surface)

3.2 Microscopy:

T.S. of root bark shows the following parts-

3.2.1 Epiblema: It is the outermost region with single layer tangentially elongated cells, compactly arranged measuring (4.8 – 5.2 – 6.3 µm in length and 10.7 – 11.5 – 11.9 µm in breadth). These cells are interrupted with lenticels and numerous uniserriate root hairs.

3.2.2 Polyderm: Just below the epiblema there are 3 – 4 layers of thin walled non suberized cells measuring (7.2 – 10.0 – 11.8 µm in length and 12.2 – 17.4 – 20.1 µm in breadth).

3.2.3 Endodermis: Polyderm is followed by a layer of tangentially elongated cells called endodermal cells measuring (5.2 – 6.4 – 6.9 µm in length and 10. 6 – 11.1 – 12.4 µm in breadth). Casparian band alternate with this cells, measuring (5.7 – 6.2 - µm in length and 10.7 – 11.5 µm in breadth).

3.2.4 Cork: consists of tangentially elongated suberized compactly arranged cells measuring (7.54 - 10.60 - 12.57µm in length and 12.57 - 17.56 - 20.58 µm in breadth). Few layers of cells are thick walled interrupted with thin wall cells.

3.2.5 Phellogen: is single layer, tangentially elongated cells measuring (10.60 - 12.52 - 17.51 µm in length and 5.60 - 17.56 - 20.25 µm in breadth).

3.2.6 Phelloderm: is 9 - 10 layers, thin walled polyhedral compactly arranged cells, measuring (10.22 - 12.51 - 15.60 µm in length and 22.51 - 25.70 - 30.11 in breadth) filled with simple and compound type of starch grains and tannin filled cells. Each cell contain nucleus.

3.2.7 Secondary Phloem: consists of compactly arranged parenchymatous cells measuring (25.50 - 37.52 - 45.56 µm in diameter). Uniserrate to biserrate to multiserrate medullary rays passing through the secondary phloem, each cells measuring (22.54 - 25.55 - 27.57 µm in length and 20.77 - 25.12 - 32.55µm in breadth). Stone cells are embedded in patches within this region (10.76 - 15.12 - 22.50 µm in diameter). Parenchyma cells are filled with simple and compound starch grains, tannin filled cells and few prism shaped calcium oxalate crystals are also found (Figs. 3, 4 and 5).

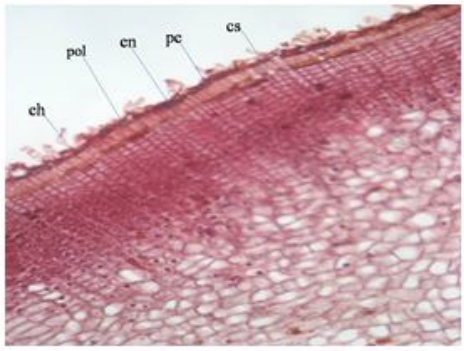

Fig 3: T.S. of *Rubus ellipticus* bark passing through epiblem & periderm
(eh-epidermal hair; pe-periderm; pol-polyderm en- endodermis; cs- casparin band)

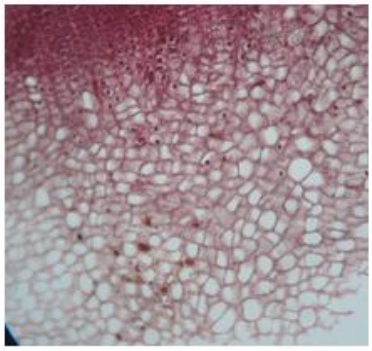

Fig 4: T.S. of *Rubus ellipticus* bark passing through phelloderm

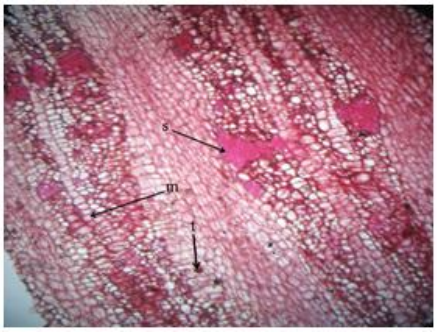

Fig 5: T.S. of Rubus ellipticus bark showing secondary phloem (s-stone cells; t- tannin filled cells and m-medullary rays)

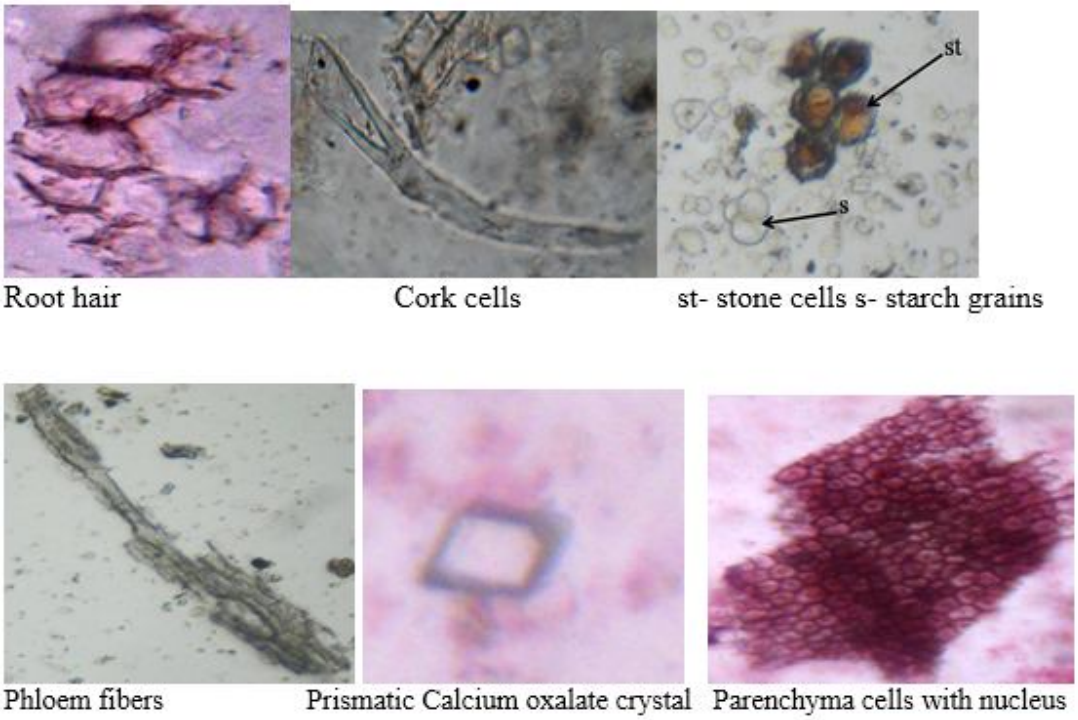

Fig 6: Powder study of *Rubus ellipticus* Smith. root bark

3.4 Histochemical Analysis: the study shows presence of starch, lipids, proteins, tannins, saponins, glucosides and mucilage. (Table 1).

Table 1: Histochemical analysis

Sr. No.	Plant constituent Test	Observations
1	Test for Starch	+
2	Test for Lipids	+
3	Test for Proteins	+
4	Test for Tannins	+
5	Test for Alkaloids	+
6	Test for Saponins	+
7	Test for Glucosides	+
8	Test for Mucilage	+
9	Test for Calcium oxalate crystals	+

3.5 Proximate Analysis: The physicochemical parameters like ash values and extractive values are summarized in (Table 2).

Table 2: Physicochemical evaluation

Ash values	Total ash	Not more than 3.35%
	Acid insoluble ash	Not more than 1.0%
	Water soluble ash	Not more than 0.9%
Extractive values	Ethanol	Not less than 21.12%
	Water	Not less than 25.3 %
	Chloroform	Not less than 1.3%

3.6 Fluorescence Analysis: data are summarized in (Table 3).

Table 3: Fluorescence analysis

Test	i	ii	iii	iv	v	vi	vii	viii	ix
Fluorescence	3fG	3fG	3pF	1yF	3gF	2rF	3pF	1fF	1fF

Keys to the letters and numbers used-

Predominant colours:	Modifying colours:	Quality of colours:
G- Green	y- Yellowish	1 Very light
F- Brown	f-brownish	2 Light
	p-purplish	3 Dark
	g- greenish	
	r - reddish	

3.7 Preliminary Phytochemical and Physicochemical Evaluation: data are summarized in (Table. 4)

Table 4: Preliminary phytochemical screening

Test for phytoconstituents	**W**	**C**	**E**
Test for Starch	+	+	+
Test for Terpenoids	+	+	+
Test for Proteins	+	+	+
Test for Amino acid	+	+	+
Test for Mucilage	+	+	+
Test for Alkaloids	+	+	+
Test for Anthraquinone glycoside	+	+	+
Test for Cardiac glycoside	+	+	+
Test for Saponin	+	-	-
Test for Tannins	+	+	+
Test for Steroids	-	-	-
Test for Flavonoids	-	-	-

Key: W- water extracts, C- Chloroform extract, E- Ethanol extract, + Present, - Absent

4. Conclusion

In nutshell, the above pharmacognostical studies will help in laying a pharmacopeial standard. The ethnomedicinal known plant drug of Manipur will be known in manifold. Detailed phytochemical and pharmacological studies are in progress.

5. Acknowledgments

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