ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Pharmacognostical evaluation of the stem and root of *Rhynchosia beddomei* - An endemic plant of Andhra Pradesh

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Article History:

Received on: 10 Apr 2021
Revised on: 15 May 2021
Accepted on: 29 May 2021

Keywords:

Rhynchosia beddomei, Endemic Plant, Ethnobotany, Pharmacognostical Approaches

ABSTRACT

Rhynchosia beddomei (Fabaceae) is an endemic plant to the Eastern Ghats of Andhra Pradesh, India. The plant is widely used by Yanadi and Chenchu tribes for the treatment of rheumatic pains, wound healing, cuts, boils, sprains, and also as an antidote for insect bites. For the worldwide acceptance of the traditional system of medicine, documentation and standardization of the raw materials used for the preparation of herbal medicine is of primary concern. Pharmacognostical studies play an important role in the identification of plants. Standardization of plant materials is mainly done by detailed microscopic evaluation. The present study aims to evaluate and establish the various pharmacognostical approaches like macroscopical, microscopical (cell structure and their arrangement), physicochemical (including different ash values, loss on drying and extractive values), fluorescence, preliminary phytochemical profiles of the stem and root parts. The microscopical studies of stem revealed the presence of prominent tanniferrous circular idioblasts in the phloem region and prismatic, rectangular, and cuboidal-shaped calcium oxalate crystals in the pith, cortex, and phloem parenchyma regions. In the roots, thin and thick roots were studied. The thin root has a wide fissured periderm and a narrow cortex with a discontinuous layer of sclerenchyma elements inner with periderm. The thick root has a wider, fissured superficial periderm followed by a cortical zone where parenchyma cells and sclereids are mixed at random. The current research report on pharmacognostical evaluation provides an important diagnostic tool for the correct identification and documentation of the endemic plant material of *R. beddomei* in dried form.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i3.4822

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INTRODUCTION

India is a treasure house for endemic flora with various climatic conditions. Nearly 5725 species of flowering plants are widely recognized as endemics plants. Among this endemic flora, 3471 species are found in the Himalayas, 2051 in Peninsular India and 239 in Andaman & Nicobar Islands (Nayar, 1996). *Rhynchosia beddomei* Baker (Fabaceae) is an endemic plant of the Eastern Ghats and its distribution is recorded in Andhra Pradesh, Tamil Nadu and Karnataka states (Aluri and Kunuku, 2018; Rao and

Henry, 1996). In Andhra Pradesh, its distribution is restricted to moist deciduous forests of Seshachalam hill ranges. *R. beddomei* is a source for the tribal inhabitants of the Seshachalam hill ranges for the treatment of sprains, healing wounds and as an antidote for insect bites (Rao and Henry, 1996; Reddy *et al.*, 2006). In addition to its endemism, rare distribution and high therapeutic importance, its identification in dried form is a big challenge (Verma *et al.*, 2020). For the identification of an authentic botanical source, pharmacognostic approaches are the only choice (Pratap *et al.*, 2016; Balasubramaniam *et al.*, 2020). The pharmacognostic characteristics of *R. beddomei* are not reported yet. The present study was conducted with an aim to establish pharmacognostical data of stem and roots with the scope of macroscopical, microscopical, physicochemical, phytochemical and fluorescence studies.

Description and distribution

Rhynchosia beddomei Baker in Hook. f. Fl. Brit. India. 2.222.1876; Gamble 1: 374.1981.

Erect undershrubs, up to 1.5 m tall, branches white – covered with whitish or greyish hairs. Leaves 3-foliolate, leaflets oblong or oblong-lanceolate, velvety, white pubescent on both surfaces, subcoriaceous, margin entire, acute (Figure 1 A-C; Figure 2 A-B). Flowers in subsessile congested racemes Calyx to 1 cm long, lobes oblong, obtuse, longer than the corolla and silky. Corolla bright yellow. Pod suborbicular, thinly canescent; seed 1, strophiolate. *R. beddomei* distributed in moist deciduous forests of Seshachalam hills in Southern Eastern Ghats.

Phenology of the plant

Leaf fall is noticed from May to June, followed by new foliage formation from August to October. Flowering is started in November and ends in January, followed by fruiting from February to April.

MATERIALS AND METHODS

Collection of specimens

The whole plant material was collected from the Seshachalam hill ranges and the plant was identified with the help of Flora of the Presidency of Madras (Gamble, 1936) and crosschecked (Voucher Specimen Number: CR119). A specimen of the herbarium was deposited in the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. Different parts were collected and fixed in FAA (Formalin+ Acetic acid + 70% Ethyl alcohol) (Ratio of 5:5:90). After 24 hours, the specimens were dehydrated with graded series of tertiary-butyl alcohol (Sass, 1940), followed by infiltration of the specimens by progressive addition of paraffin wax (Melting point 58-60^{o}C) until TBA solution attained supersaturation. The specimens were cut into paraffin blocks.

Ethnobotany of the plant

The ethnopharmacological importance of *R. beddomei* was obtained through conversation in the local Telugu language with nearly 130 tribal informants from Seshachalam hill ranges between the age groups of 40-70 years.

The studied areas are inhabited by Yanadis and Chenchus. The majority of the tribal inhabitants are Chenchus. The survey is aimed to document the data on the local names of the plant, useful plant parts, method of drug preparation, mode of drug administration, dosage, the form of usage and whether the plants used either singly or in combination with other plants, minerals and salts.

Microscopical studies

For microscopical observation, plant specimens were subjected to dehydration, infiltration, supersaturation, sectioning, de-waxing and staining. It is followed by the preparation of microphotographs at different magnifications (Johansen, 1940; Pratap *et al.*, 2014).

Physico-chemical studies

Loss on drying, total ash values, acid insoluble ash values, water-insoluble ash, various extractive values were calculated (Kumari *et al.*, 2020; Yadav and Singh, 2018).

Fluorescence studies

Fluorescence studies used to develop the standards for the measurement of the purity of the powdered drug (Jyothi *et al.*, 2020; Pratap *et al.*, 2014).

Preliminary phytochemical studies

The root and stem powders were subjected to various types of analysis for the identification of secondary metabolites like alkaloids (Dragendorff's and Mayer's tests), triterpenes (Libermann Burchard's test), flavonoids (Aluminium chloride test), anthraquinones (Borntrager's test), polyphenols (Ferric chloride test), sterols (Salkowski's test), coumarins (Lacton test), saponins (Foam test) and tannins (Gelatin test) (Harbone, 1973; Kokate *et al.*, 2008).

RESULTS AND DISCUSSION

Ethnopharmacological medications of the plant

The ethnopharmacological uses of *R. beddomei* root, stem and leaf was collected from the Yanadi and Chenchu tribes from Seshachlam hills (Table 1).

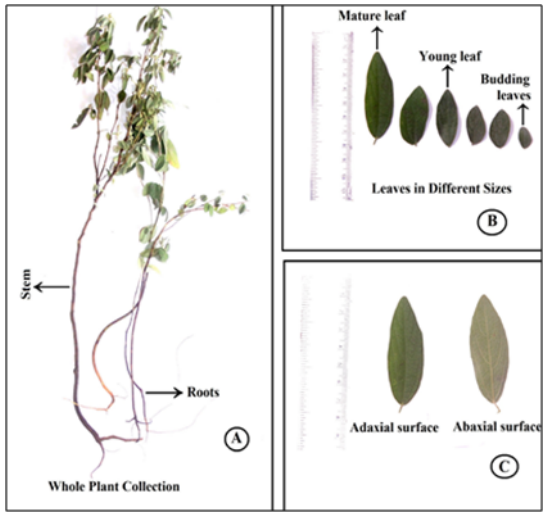

Figure 1: *Rhynchosia beddomei*; A. Whole plant; B. Leaf morphology; C. Leaf adaxial and abaxial surface

Table 1: Ethnobotanical information

Part Used	Claimed by	Method of Preparation and Application
Root	Yanadis	1. The root decoction is applied on chronic sores to keep off infection due to airborne diseases. 2. A liniment prepared from the root is applied to reduce the pain near swollen wounds.
Stem	Yanadis and Chenchus	The crushed stem parts are boiled in the sesame oil and used externally to cure sprains.
Leaf	Chenchus	The leaf paste is used as an antidote to treat insect bites (Y). Bruished leaves are also applied to ulcers.

Figure 2: Stem Part; A. Mature stem; B. Young stem

Table 2: : Ash values

S.No	Part used	Alcohol soluble extract (%)	Water soluble extract (%)	Chloroform soluble extract (%)
1	Root	5.0	4.5	1.0
2	Stem	5.5	1.0	2.5
3	Leaf	5.5	4.0	5.0

Microscopical studies of stem

The stem is angular in transactional outline with short ridges of varying length (Figure 3). The stem has a thin, uninterrupted epidermal layer of small thick walled cells with prominent cuticle. The cortical zone is narrow, comprising of four or less layers of parenchyma cells with dense tannin contents. Inner to the cortex is a thick, undulate continuous cylinder of sclerenchyma cells (fibers) enclosing the vascular cylinder (Figure 4).

The vascular tissue consists of secondary phloem and secondary xylem. Secondary phloem is wide and continuous, the phloem elements being diffuse in distribution. Wide, circular tanniniferous

idioblasts are frequently seen in the phloem. Secondary xylem occurs in the thick, hollow cylinder; it has narrow, angular or circular, thick walled vessels and libriform fibres. The vessels are in radial multiples and are 20 μm wide. Along the inner circumference of the xylem cylinder, these are triangular clusters of small, compact cells abutting the primary xylem strands.

Table 3: Extractive Values

S.No	Part used	Extractive values (%)		
		Alcohol	Water	Chloroform
1	Root	2.2	1.2	0.8
2	Stem	2.2	1.2	1.0
3	Leaf	4.6	0.4	1.2

When the sections of the stem is viewed under the polarized light microscope, calcium oxalate crystals were evident in a different part of the stem (Figures 5 and 6) as well as in the midrib. The crystals are mostly prismatic type of rectangular and cuboidal shape. Less frequently, they are druses. In the midrib, rhomboidal type crystals are located along the outer margin of the sclerenchyma cylinder (Figure 5). In the stem, the crystals occur in the pith and cortex; they are also seen in the phloem parenchyma (Figures 6 and 7).

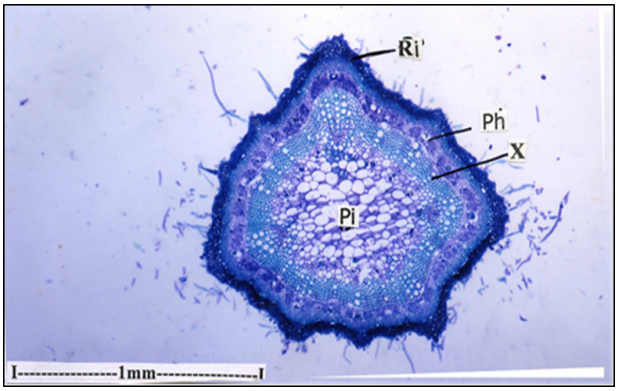

Figure 3: Stem- Ground plan (Ri: Ridges; Ph: Phloem; X: Xylem; Pi: Pith)

Microscopical studies of root

Thin and thick roots were studied and both the roots have secondary thickening.

The thin root (Figures 8 and 9) has a fairly wide fissured periderm and a narrow cortex with a discontinuous layer of sclerenchyma elements inner with periderm. The secondary phloem is fairly wide and continuous. Secondary xylem is a solid, wide circular cylinder, comprising of xylem fibres wide dilated xylem rays, wide, thin walled, circular, solitary vessels which diffusely radiate from the centre towards

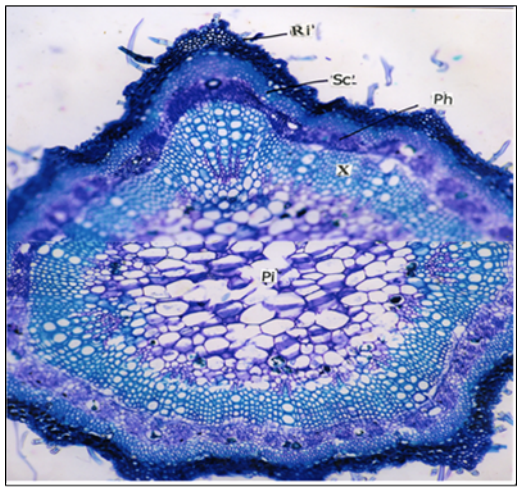

Figure 4: Section – Enlarged (Ri: Ridges; SC: Sclerenchyma cylinder; Ph: Phloem; X: Xylem; Pi: Pith)

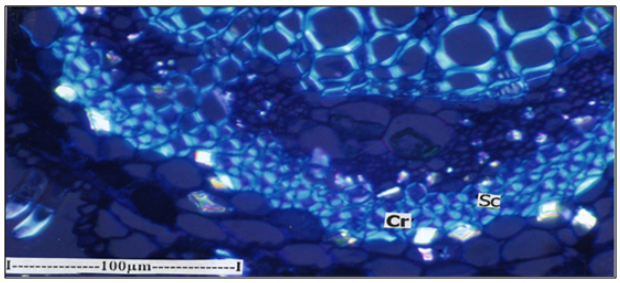

Figure 5: Rhomboid crystals in the sclereid band of the midrib (Cr: Crystals; SC: Sclerenchyma cylinder)

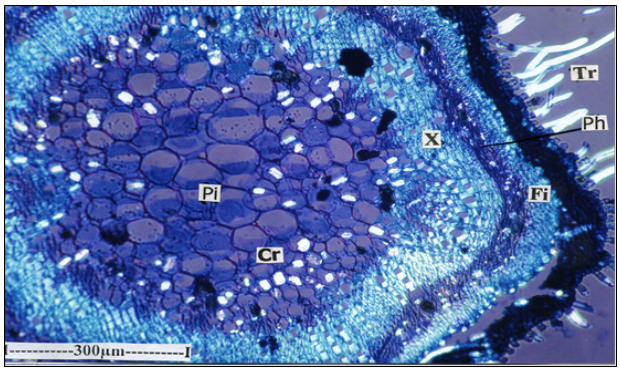

Figure 6: Rhomboid crystals in the pith and phloem (Tr: Trichomes; Ph: Phloem; X: Xylem; Fi: Fibres; Pi: Pith; Cr: Crystals)

the periphery. The diameter of the vessels is narrow in the centre and wider towards the periphery (Figure 9). The wide vessels are 70 μm in diameter.

The thick root measuring 2.5 mm is similar to the thin root, but there are some differences due to more amount of secondary tissues (Figures 10, 11 and 12). The thick root has a wider, fissured super periderm followed by a cortical zone where parenchyma cells and sclereids are mixed at ran-

Table 4: Preliminary phytochemical studies

S.No	Test for	Solvent	Part used	
			Stem	Root
1.	Saponins	Alcohol	-	-
		Water	+	-
		Chloroform	-	-
2.	Alkaloids	Alcohol	-	-
	Mayer's reagent	Water	-	-
		Chloroform	-	-
	Dragendorff's reagent	Alcohol	-	-
		Water	-	-
		Chloroform	-	-
3.	Flavonoids	Alcohol	-	+
		Water	-	+
		Chloroform	-	+
4.	Cardioglycosides	Alcohol	+	+
		Water	-	-
		Chloroform	-	-
5.	Glycosides	Alcohol	-	-
		Water	-	+
		Chloroform	-	-
6.	Saponins	Alcohol	+	+
		Water	-	-
		Chloroform	+	-
7.	Carbohydrates	Alcohol	+	+
		Water	+	+
		Chloroform	+	+
8.	Phenols	Alcohol	+	+
		Water	-	+
		Chloroform	-	+
10.	Tannins	Alcohol	+	-
		Water	-	-
		Chloroform	-	-
11.	Lignins	Alcohol	+	+
		Water	-	-
		Chloroform	+	-

"+" Presence of phytochemicals and
"-" Absence of Phytochemicals

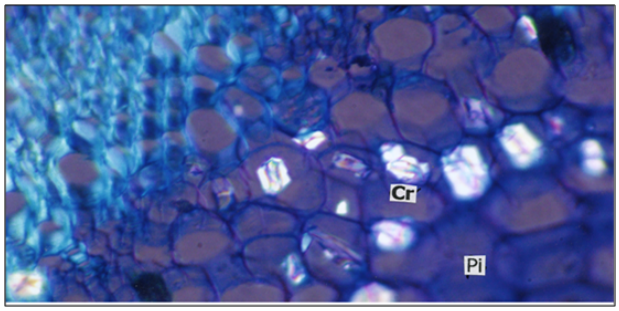

Figure 7: Crystals enlarged (Pi: Pith; Cr: Crystals)

dom.

Secondary phloem consists of regular radial files of phloem elements. The phloem rays are narrow and less conspicuous. The secondary xylem has more vessel frequency, the vessels are diffuse and spread towards the periphery with an increasing diameter (Figure 12). Xylem fibers of thin walled and thick walled cells occur in alternating circles. The wide vessel elements are 50 μm in diameter.

Powder microscopic studies of stem

Stem powder (Figures 13, 14 and 15) contains an

Table 5: Fluorescence analysis of the alcoholic extract of the powdered drugs of root and stem of *R. beddomei*

Treatment of Powder	Part used	
	Root	Stem
Color of the extract in visible light	Pale yellow	White
Color of the extract in UV light	Dark brown	Light yellow
Test No1		
Step1 Reagent A (1 drop)	Blackish brown	Light green
Step 2 Reagent B (3 drops)	Dark brown	Dark green
Test No.2		
Step1 Reagent B (2 drops)	Pale green	Light green
Step2 Reagent C (1 drop)	Dark green	Greenish brown
Step3 Reagent (1 drop)	Light green	Black
Step 4 Reagent C (2 drops)	Light black	Dark black

Table 6: Fluorescence characters with different solvents of the powdered drugs of *R. beddomei*

Treatment of Powder	Light source used	Part used	
		Root	Stem
Acetone	Visible light	Brown	Pale brown
	UV light	Greenish brown	Greenish brown
Benzene	Visible light	Blackish brown	Brown
	UV light	Brown	Pale green
Chloroform	Visible light	Brown	Light brown
	UV light	Green	Light green
Pet ether	Visible light	Brown	Light brown
	UV light	Light green	Light green
Methanol	Visible light	Dark brown	Brown
	UV light	Light brown	Pale green

abundance of fibers and a few vessel elements. The fibers are all narrow, thick walled and lignified. The fibers 600-750 μm long and 10 μm wide. No pits are evident on the fibers.

In addition to fibers, vessel elements are also seen in the powder. They are short and cylindrical. They are 90 μm wide and 130 μm long. They have dense elliptical pits on the lateral walls (Figure 15). The perforation is simple and horizontal.

Powder microscopic studies of root

The root powder has fibers similar to those of the stem. Vessel elements are more frequent in the root than in the stem. The vessel elements vary from narrow to wide; some of the narrow elements have a short tail. The perforation plate is simple and oblique (Figure 16). The wider vessel elements have no tails; their perforation plate is horizontal (Figure 17). Lateral wall pits are circular, alternate and densely crowded. The narrow vessel elements are 120-150 μm long. The wider elements are 150 μm long.

Diagnostic Characters

Stem

1. Tanniniferrous circular idioblasts are prominent in the phloem region.

Table 7: Fluorescence with different reagents of powdered drugs of root and stem of *R. beddomei*

Treatment of Powder		Part used	
		Root	Stem
Powder in visible light		Greyish brown	Yellowish brown
Powder in UV light		No fluorescence	Grey
Powder + 1N HCl	Visible light	Yellowish brown	Pale brown
	UV light	Light green	Brown
Powder + 1N NaOH in methanol	Visible light	Brown	Chocolate
	UV light	Pale green	Dark green
Power + 1 N NaOH in H_2O	Visible light	Reddish brown	Blood red
	UV light	No fluorescence	No fluorescence
Power + 50% HNO_3	Visible light	Brown	Dark brown
	UV light	No fluorescence	Chocolate
Powder + 50% H_2SO_4	Visible light	Greyish brown	Pale brown
	UV light	Light green	Grey

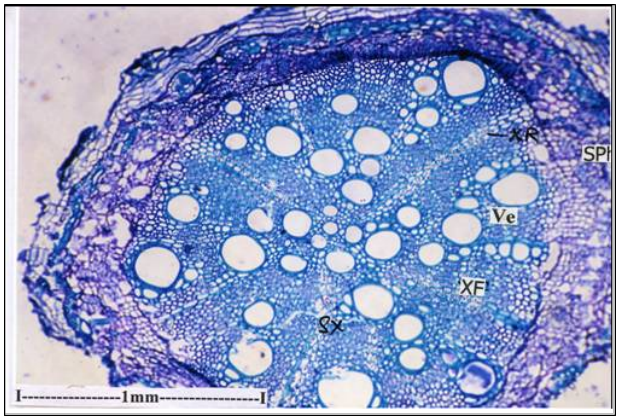

Figure 8: Root - Ground plan (T.S. of the Young Root) (Sph: Secondary phloem; XR: Xylem rays; Ve: Vessels; XF: Xylem Fibres;Sx: Secondary Xylem)

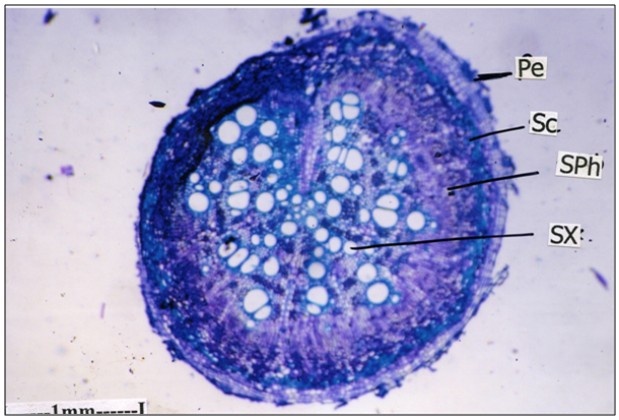

Figure 10: Root - Ground plan (T.S. of the Mature Root) (Pe: periderm; Sc: Secondary cortex; Sph: Secondary phloem; Sx: Secondary Xylem)

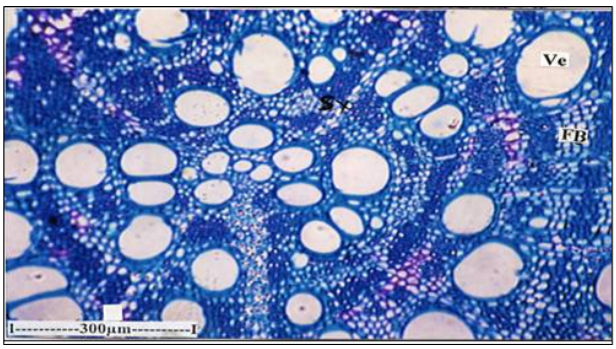

Figure 9: Root - Secondary xylem enlarged (Ve: Vessel)

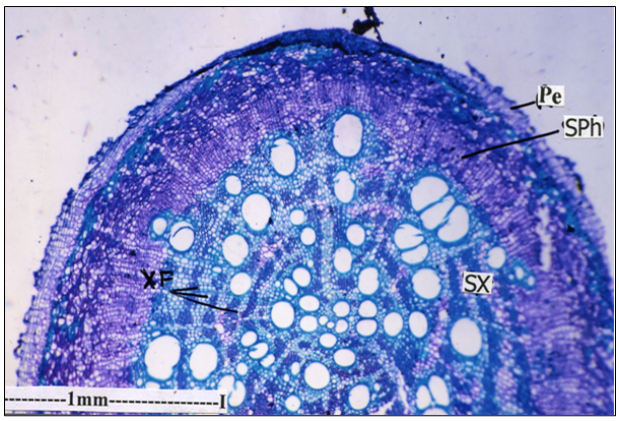

Figure 11: Root - Portion enlarged (Mature Root) (Pe: periderm; Sph: Secondary phloem; Sx: Secondary Xylem; XF: Xylem Fibres)

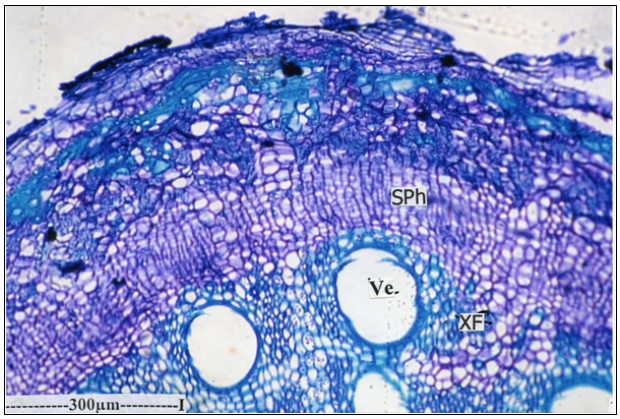

Figure 12: Root - Sector enlarged(Mature Root) (Sph: Secondary phloem; XF: Xylem Fibres; Ve: Vessel)

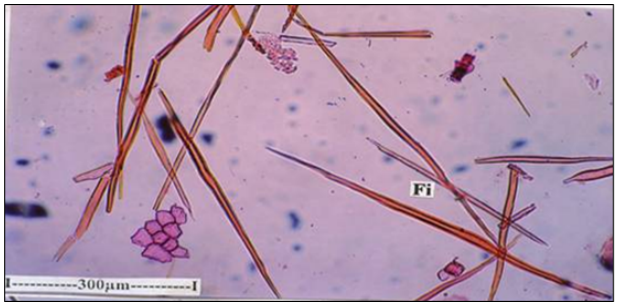

Figure 13: Fibres (Fi: Fibres)

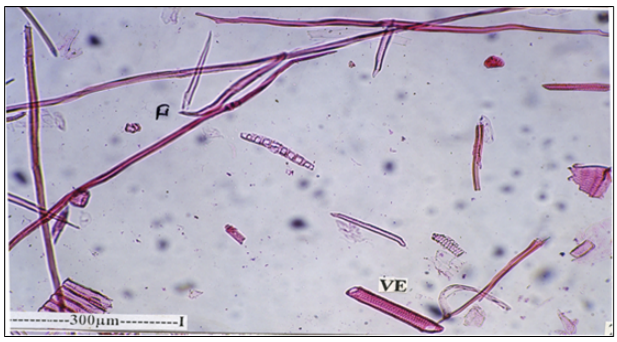

Figure 14: Vessels and Fibres (Ve: Vessel)

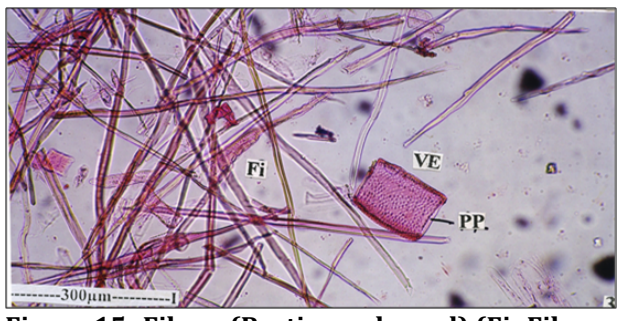

Figure 15: Fibres (Portion enlarged) (Fi: Fibres; VE: Vessel; PP: Perforation plates)

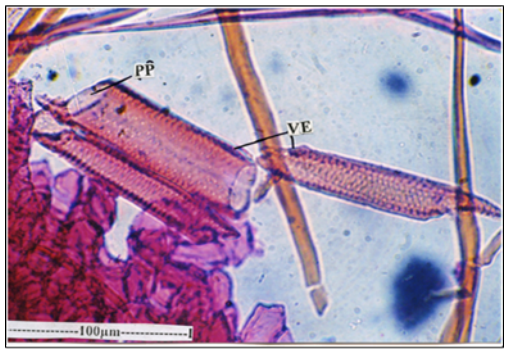

Figure 16: Tailed vessel with parenchyma cells (VE: Vessel; PP: Perforation plates)

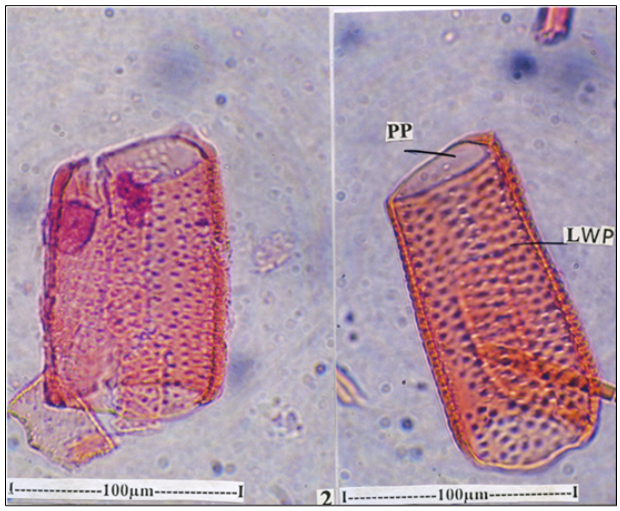

Figure 17: Vessels with lateral wall pits (PP: Perforation plates; LWP: Lateral wall pits)

2. Prismatic, rectangular and cuboidal shaped Calcium oxalate crystals are found in the pith, cortex and phloem parenchyma.

3. Vessels are short, cylindrical and with elliptical pits on the lateral wall

Root

1. Presence of secondary growth.

2. Narrow vessels in the centre and wider (7μm) towards the periphery in the thin root, whereas wider vessels (50 μm) in the thick root.

3. Narrow vessel elements have short drawn out ends and wider elements without drawn out ends.

Ash and extractive values of different parts of *R. beddomei*

Ash and the Extractive values of a drug provide an opinion on the inorganic composition and other

impurities present along with the drug. The results are given in Tables 2 and 3.

Preliminary phytochemical Studies

The present study is intended to unveil the preliminary phytoconstituents from aqueous, ethanol and chloroform extracts of root and stem parts from *R. beddomei*. The study revealed alkaloids, carbohydrates, cardio glycosides, flavonoids, glycosides, lignins, phenols, saponins and tannins (Table 4).

Fluorescence studies

Fluorescence analysis of the powder produced from the root and stem parts was studied and recorded with different extracts, solutions and reagents (Tables 5, 6 and 7).

CONCLUSIONS

The study signifies the pharmacognosy of an endemic medicinal plant, *R. beddomei,* with important diagnostic characters for the authentication of species. These findings are the source for the development of quality control parameters to overcome substitutes and adulterants. The present study is also aimed to document the ethnomedicinal uses on *R. beddomei*. It will be a great asset if this knowledge of ethnomedicinal and pharmacognostic evaluation is subjected to scientific validation by advanced pharmacological and clinical studies. Such investigations will bring to light the newer drugs of plant origin for the treatment of various ailments.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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