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Pharmacognostical studies on leaves of *Rhynchosia beddomei* Baker

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ABSTRACT

Background: *Rhynchosia beddomei* is a rare and endemic plant belongs to Leguminosae family occurs in moist deciduous forests of Seshachalam hills of Southern Eastern Ghats with great therapeutical importance.

Aim: The present study is meant to authenticate and validate the species *Rhynchosia beddomei* Baker on the premise of the microscopical study.

Materials and Methods:

The main objective of the present work is to evaluate the various pharmacognostic properties like Macroscopical, Microscopical, Physiochemical, and Fluorescence studies. Microscopical studies include cell structure and their arrangement, Physicochemical parameters include loss on drying, total ash value, acid insoluble ash, water-insoluble ash, various extractive values etc. Qualitative tests for various functional groups were also carried out.

Results: The microscopical study on leaf of *Rhynchosia beddomei* revealed the presence of characters like dilated spherical and thin walled trichomes, cylindrical and dark horizontally aligned vermiform cells, small compact thin walled tanniniferous walls are present in the ground tissue, the presence of top shaped discrete bundles in the petiole, the presence of two or three-celled uniseriate, unbranched non glandular abundant trichomes in the petiole. Preliminary phytochemical analysis revealed the presence of Alkaloids, Glycosides, Carbohydrates, Phenols, Proteins, Lignins, Saponins and Tannins.

Conclusion: The pharmacognostical and phytochemical screening on the leaf of *Rhynchosia beddomei* is a helpful data for the identification and to decide the quality and purity of the plant material in future reviews.

KEYWORDS

Pharmacognostical studies, Rhynchosia beddomei, Ethno botany, Microscopical studies, Seshachalam hills

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INTRODUCTION

There are around 7500 wild plants utilized for therapeutic purposes by various tribal inhabitants in India [01]. Unfortunately, the portrayals accommodated the distinguishing proof of these plants/medicates in the writing are inadequate to the best possible comprehension and the usage of multitude vernacular names for the single plant by tribal inhabitants pose problems in identifying the correct botanical names of medicinal plants. And, it is most exceedingly bad dumbfounded with the utilization of various plant species under a similar drug name. Further, these are claimed to possess similar therapeutic efficacy and used by as the same drug. Such drugs are today termed as controversial drugs. In such cases, Pharmacognostical Standardization is the only source to prevent the unscrupulous commercial practice of adulterating and substituting the genuine herbal drugs [02].

Rhynchosia beddomei is a rare and endemic plant belongs to Leguminosae family occurs in moist deciduous forests of Seshachalam hills of Southern Eastern Ghats with great therapeutical importance and locally this plant is named as Vendichettu and Endipodha [03&04]. Traditionally the leaves of *R. beddomei* is used for the treatment of Rheumatic pains, Boils, wounds and cuts [05]. The fresh leaves of this plant are used by the tribes of Chittoor district to treat the Rheumatic pains, fungal diseases, Diabetes and Liver disorders. In spite of its high medicinal value, the pharmacognostical characters of its leaves are not reported till date and also the authentication of this botanical source in dried form is arduous. Hence, the present study planned to evaluate pharmacognostical profiles of its leaves, which includes macroscopical, microscopical, physicochemical, phytochemical and fluorescence studies.

Physicochemical parameters are the important characteristic of a drug and with the help of this; we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values and extract values of different drugs but mostly the difference varies within narrow limits in case of the same drug.

MATERIALS AND METHODS

Collection of specimens [6-15]

The plant specimens, which were subjected to the proposed study, were collected on the way to Japali Theertham at Tirumala Hills of Seshachalam Hill ranges from Rayalaseema region, Andhra

Pradesh. Utmost care was taken in collecting healthy plants. The sample, which we require from different organs, were cut and separated from the plant. Then the separated samples were fixed in FAA. The specimens were allowed to dehydrate with graded series of tertiary-butyl alcohol only after 24 hours of fixation [7]. Then infiltration of specimens was carried out by the gradual addition of paraffin wax (m.p. 58-60^{O}C) until TBA solution attained supersaturation. The specimens were allowed to cast into paraffin blocks.

Sectioning[10-17]

The specimen, which is embedded in the paraffin, was sectioned with the help of Rotary Microtome. The section with thickness of 10-12 µm was de-waxed and stained with Toluidine blue. Lignified cells get blue color, cellulose wall get pink color, suberin gets dark green, mucilage gets violet, protein bodies get blue color etc. and also the necessary sections were stained with safranin, fast green and IKI (for starch). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good, and some cytochemical reactions were also obtained. Jeffery's maceration fluids were prepared for the study of stomatal morphology, venation pattern and trichome distribution and paradermal sections.

Photomicrographs

Microscopic Pictures of different tissues at different magnification were taken with help of Nikon photomicroscopic unit. Polarized light was used for the study of crystals, starch grains, lignified cells and bright field was used for normal observations. Scale bars in pictures indicated the microscopic magnification[8].

Physico-chemical studies

Physical constants like ash values were determined according to the standard methods [6 & 12].

Fluorescence studies

Several crude drugs show a marked fluorescence in ultra-violet light. It is one of the important studies for quality control of drugs and valuable in determining the standards of the quality of powdered drug. The study fluorescence of powdered drugs as a means of identification appears to possess distinct possibilities of practical application [8&14].

Preliminary Phytochemical Studies

Leaves were thoroughly washed and sufficient quantity of the sample was collected and chopped off into small fragments and shade dried. The dried parts were ground to make coarse powder and stored in polythene containers for further analysis [11& 10].

RESULTS

Macroscopical characters of the leaf

Leaves 3-foliolate, leaflets ovate-lanceolate, 2-8 x 1.5 x 2.5 cm, velvety, white pubescent on both surfaces, subcoriaceous, base obtuse, apex acute, margin entire, strongly nerved beneath Petiole to 2.5 cm; petiolule to 3 mm. (**Fig. 01**)

Fig 1 *Rhynchosia beddomei*

Microscopical Studies

1. Leaflet

The leaflets are dorsiventral with prominent, adaxially projecting midrib and lateral veins (Fig.02 & 03). The midrib has a short conical adaxial hump and semi-circular wide adaxial part (Fig. 02 & 03). It is 480 µm thick along the vertical axis and 400 µm along horizontal axis. It has thick and prominent epidermal layer of circular, papillate thick walled cells. Within the adaxial hump occurs a group of thick walled cells. The ground cells in other regions of the midrib are wide, angular, thin walled and compact. The vascular system consists of a bowl shaped main strand situated at the abaxial part of the midrib, two small segments of adaxial accessory strands situated on the upper side and lateral part of the midrib. The vascular strands have thick band of sclerenchyma cells adjacent to the phloem part of the vascular strands (Fig. 04) the lateral vein, though prominent projecting into a conical adaxial structure has simple structure compared to the midrib. The lateral vein has a conical mass of xylem elements and narrow strand of phloem elements and adaxial and abaxial sclerenchyma clusters that subtend the vascular bundle (Fig. 05).

Lamina

The lamina has bilateral symmetry; it is densely pubescent on the abaxial surface. The lamina is 180 µm thick. The adaxial epidermis is cylindrical with thick raised outer tangential walls having the prominent cuticle; the cells are 10 µm thick. The abaxial epidermis has narrow, thin walled rectangular cells. The cells from which the epidermal trichomes originate are dilated, spherical and thin walled (Fig. 02) arrows.

The palisade zone is wide, the palisade cells being cylindrical, less compact and 40 µm high. The second layer and abaxial

zone of spongy parenchyma layers have vertically oriented, cylindrical loosely arranged cells. In the median zone is a single layer of horizontally aligned cylindrical dark vermiform cells.

Venation Pattern

The major lateral veins are thick and straight; the minor veinlets are thin and undulate. The vein-islets are distinct; they are rectangular and squarish.

The islets are disposed of parallel to one another (Fig.06). The vein- terminations are less distinct; they are thin, wavy and unbranched.

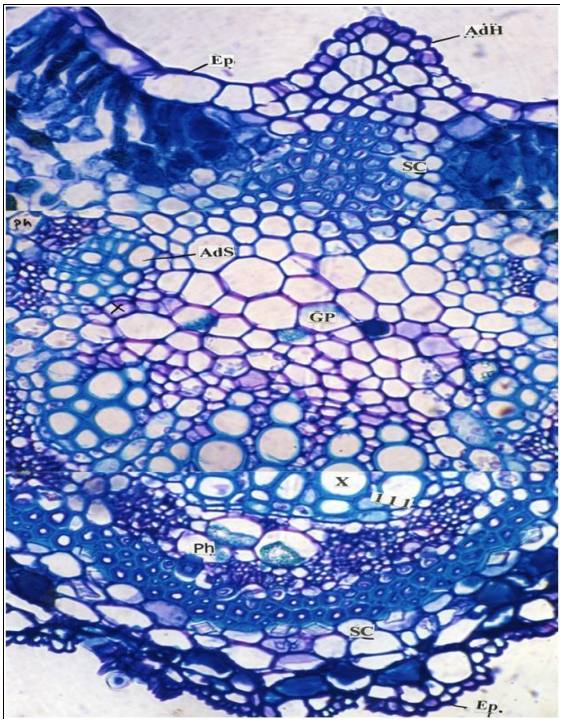

Fig 4 Enlarged – midrib part

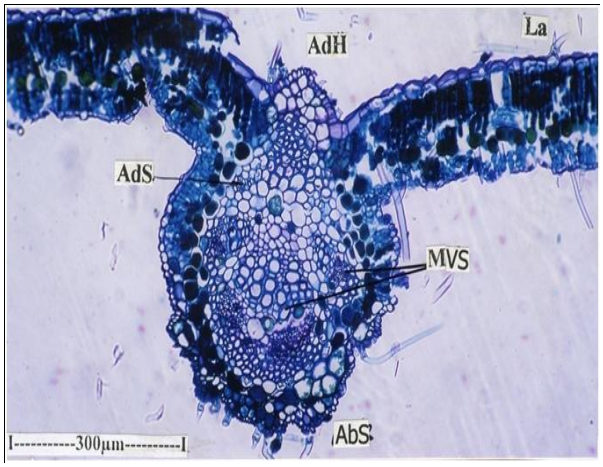

Fig 02 Midrib with lamina

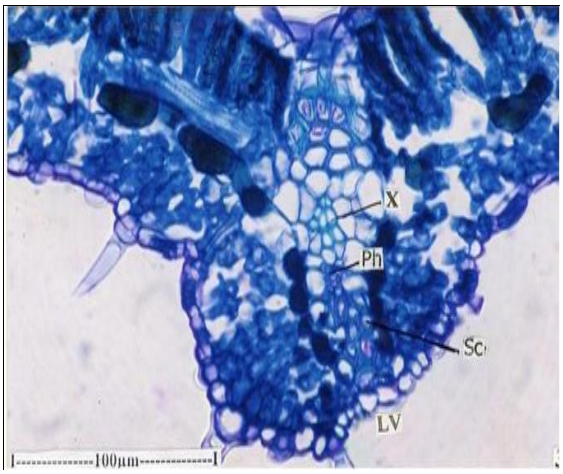

Fig 5 Portion of midrib

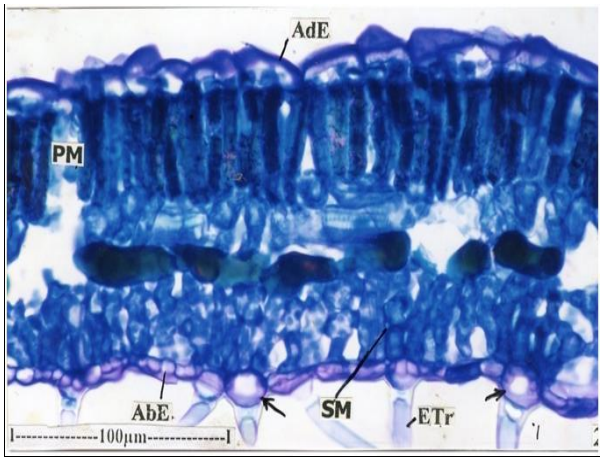

Fig. 03 Portion of lateral vein

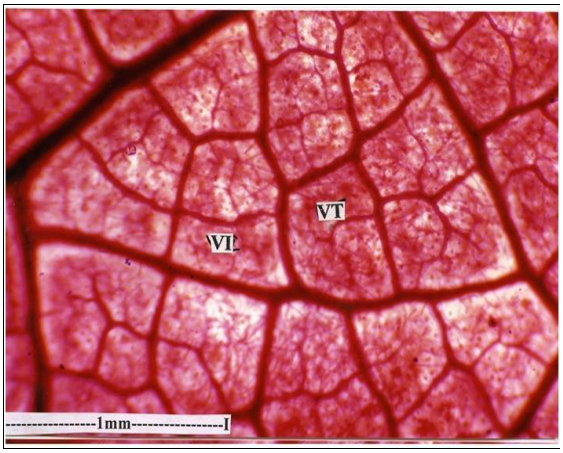

Fig.6 Cleared leaf showing vein islets and vein – termination

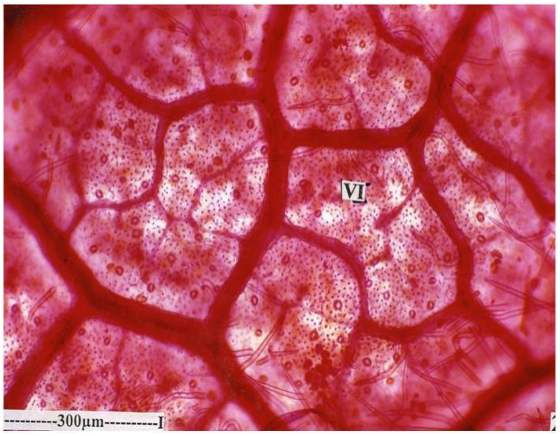

Fig 7 Portion enlarged

Petiole

The proximal (lower) end and the distal (terminal) end of the petiole and the petiolule exhibit distinct structural differences.

The proximal part of the petiole (Fig. 09) is more or less circular in cross sectional view. It is densely pubescent. The epidermis is thin with small squarish cells. The ground tissue consists of small, thin walled compact tannins-filled parenchyma cells. It has heart-shaped vascular strand with a narrow adaxial gap. The xylem tissue has several, thin radial lines of vessels embedded in thick walled filers. Phloem occurs in wide band around the outline of the xylem. A thin layer of small parenchyma cells ensheath the vascular strand.

Distal Parts of the Petiole

The terminal or distal part of the petiole is five-ridged with wide, shallow adaxial groove flanked on either side by two wide short ridges.

It is 1.05 mm along with vertical axis 900 µm along the horizontal axis. The epidermis is thin comprising of small circular thick walled cells. The subepidermal ground tissue consists of three or four layers of parenchyma cells with dense tannin contents.

The vascular system consists of nine discrete bundles, arranged along the periphery of the petiole; the vascular bundles are collateral and top-shaped. Based on their position, they can be classified into:

- adaxial wing bundles (or ridge bundles)
- adaxial bundles
- lateral bundles and
- abaxial bundles

The adaxial wing bundles are two, one in each wing. They have a thick sclerenchyma cap. The xylem elements are one or two rows.

The adaxial bundles are two, lateral in position in the adaxial part. They have three or four closely placed rows of xylem elements and thick are of sclerenchyma cap.

The lateral bundles occur in the lower median part and have three or four lines of xylem elements and sclerenchyma bundle cap.

The abaxial part has three bundles, one median larger and two lateral, smaller

bundles. All three abaxial strands have independent bundles caps.

Dense trichomes are seen all over the surface of the petiole. The trichomes are covering type, two or three celled, uniseriate and unbranched. The cell walls are thin and the cells elongated and narrow (Fig. 10).

T.S. of Petiole and Petiolule

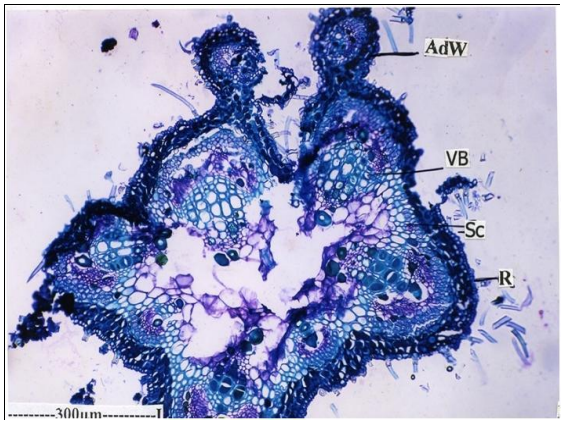

Fig 08 Petiolule - Entire view

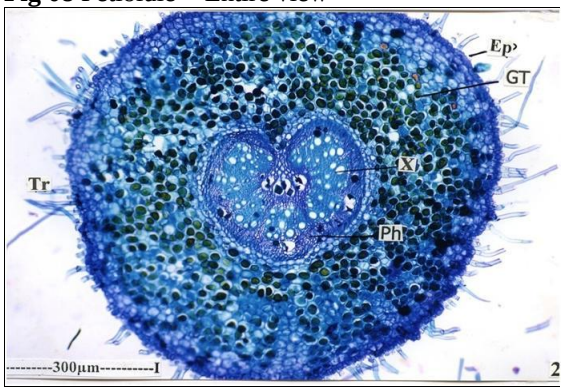

Fig 09 Petiole (proximal part) - ground plan

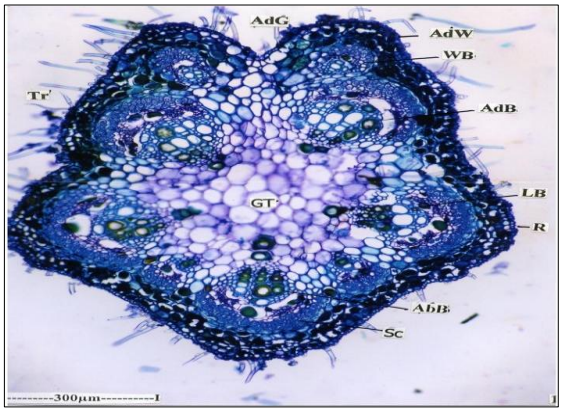

Fig 10 Petiole (Distal part) - Entire view

Powder Microscopic Studies

Leaf

The leaf powder shows broken vascular skeleton and trichomes. The vascular skeleton which represents the venation of the lamina shows distinct vein-islets and vein termination as in the intact lamina (Fig. 11). Epidermal trichomes are abundant in the powder. They are covering-type and nonglandular. They are 2-4 celled, uniseriate and unbranched. They have thin, lignified walls and wide lumen (Fig. 12 & 13). The trichomes are 100 µm long and 5 µm wide.

Diagnostic Characters

A) LEAF

- The presence of dilated, spherical and thin walled trichomes in the lamina.
- Cylindrical and dark horizontally aligned vermiform cells are distinct in the median zone.
- The presence of small, thin walled, compact, tanniniferous cells in the ground tissue.
- The presence of septate, uniseriate, unbranched and glandular trichomes in the petiole.

Powder studies - Leaf

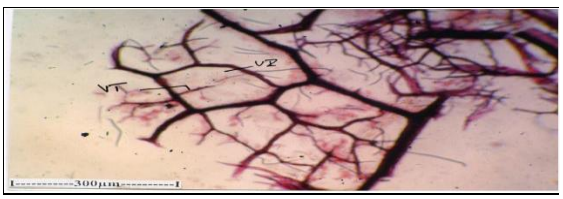

Fig. 11: Venation pattern

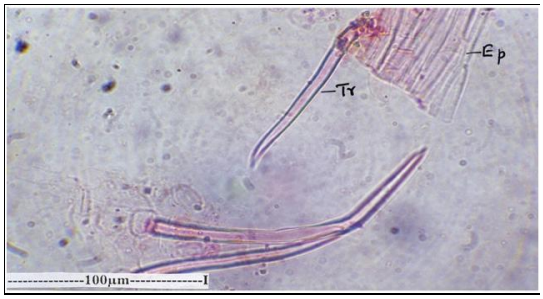

Fig. 12: Non-glandular epidermal trichome

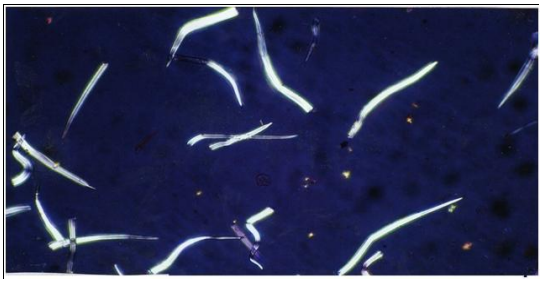

Fig. 13: Non-glandular epidermal trichome (under polarized light microscope)

Table 1 Ash Values of *Rhyncosia beddomei* leaf powder

S. No.	Name of the species	Part used	Alcohol soluble extract (%)	Water soluble extract (%)	Chloroform soluble extract (%)
1.	*Rhyncosia beddomei*	Leaf	5.5	4.0	5.0

Physicochemical Analysis

Ash values of leaf (selected plant species) are provided in Table No. 1

Various extracts values of leaf (selected plant species) are provided in Table No.2.

Table 2 Various extracts values of the leaf powder *R. beddomei*

S. No.	Name of the species	Part used	Extractive values (%)		
			Alcohol soluble extract (%)	Water soluble extract (%)	Chloroform soluble extract (%)
1.	*Rhyncosia beddomei*	Leaf	4.6	0.4	1.2

Fluorescence studies

The results of the above tests are provided in Table No. (S) 3, 4 and 5, respectively.

Table 3 Fluorescence characters with different solvents for leaf powder

Treatment of Powder	Light source used	*Rhynchosia beddomei* (Leaf powder)
Acetone	Visible light	Light green
	UV light	Pink
Benzene	Visible light	Pale yellow
	UV light	Dark green
Chloroform	Visible light	Bright green
	UV light	Light red
Pet ether	Visible light	Light green
	UV light	Whitish pink
Methanol	Visible light	Bright green
	UV light	Brick red

Table 4 Fluorescence studies with different reagents of powdered drugs for selected species

Treatment of Powder		*Rhynchosia beddomei* (Leaf powder)
Powder invisible light		Grayish green.
Powder in UV light		Light grey
Powder + 1N HCl	Visible light	Green
	UV light	Brownish Red
Powder + 1N NaOH in methanol	Visible light	Yellowish green;
	UV light	No fluorescence
Power + 1 N NaOH in H_2O	Visible light	Brownish black
	UV light	No fluorescence
Power + 50% HNO_3	Visible light	Dark brown
	UV light	No fluorescence
Powder + 50% H_2SO_4	Visible light	Pale green
	UV light	Light grey

Table 5 Fluorescence analysis of the Alcoholic extract of the *Rhynchosia beddomei* leaf powder

Treatment of Powder	***Rhynchosia beddomei* Leaf powder**
Color of the extract invisible light	Light Green
Color of the extract in UV light	Orange Red
TEST NO.1	
Step1 Reagent A (1 drop)	Dark Brown
Step 2 Reagent B (3 drops)	Grayish Brown
TEST NO.2	
Step1 Reagent B (2 drops)	Light Green
Step2 Reagent C (1 drop)	Greenish Brown
Step3 Reagent (1 drop)	Light Yellow
Step 4 Reagent C (2 drops)	Dark Brown

Reagent A: Saturated aqueous Silver nitrate solution ($AgNO_3$)

Reagent B: 0.1 N aqueous Sodium hydroxide solution (NaOH)

Reagent C: 5% aqueous mercuric chloride solution ($HgCl_2$)

Preliminary phytochemical studies of selected species

The results of the above tests are provided in Table No. 6

Table 6 Preliminary phytochemical studies of selected species

S. No.	**Tested Part of the Plant**	**Test for**	**Solvents**		
1.	**Leaf**	Saponins	**Alcohol**	**Water**	**Chloroform**
			-	+	-
2.		Alkaloids Mayer's reagent	-	+	-
3.		Dragendorff's reagent	-	-	-
4.		Flavonoids	+	+	-
5.		Cardioglycosides	+	-	-
6.		Glycosides	-	-	-
7.		Saponins	+	-	+
8.		Carbohydrates	+	+	+
9.		Phenols	+	+	+
10.		Proteins	+	+	+
11.		Tannins	+	-	-
12.		Lignins	+	-	-

DISCUSSION

The study is an attempt made to provide detailed pharmacognostical investigation on ***Rhynchosia beddomei,*** an important endemic medicinal species of Rayalaseema region. The pharmocognostical studies comprise of taxonomic studies of the species, macro-and microscopical characters of part used which help in identifying important diagnostic characters for the species. Leaf of *Rhynchosia beddomei* reveals the presence of the characters like

1. Dilated spherical and thin walled trichomes
2. Cylindrical and dark horizontally aligned vermiform cells

3. Small compact thin walled tanniniferous walls are present in the ground tissue

4. The presence of top shaped discrete bundles in the petiole

5. The presence of two or three-celled uniseriate, unbranched nonglandular abundant trichomes in the petiole. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, carbohydrates, phenols, proteins, lignins, saponins and tannins.

CONCLUSION

The pharmacognostical and phytochemical screening on the Leaf of ***Rhynchosia beddomei*** is a helpful data for distinguishing proof and verification of plant. It can likewise help as a critical wellspring of data to guarantee the character and to decide the quality and purity of the plant material in future reviews.

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