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DETAILED PHARMACOGNOSTICAL EVALUATION OF ROOT OF *RHYNCHOSIA MINIMA* DC. WITH ROOT POWDER MICROSCOPY

Hemlata Patel^{*1}, Gautam Fichadiya², Harisha C.R.³

^{*1} MPharm Scholar, Department of Pharmacognosy, Gujarat Ayurveda University, India

² Ph.D. Scholar, Department of Pharmacognosy, Gujarat Ayurveda University, India

³ HOD, Department of Pharmacognosy, Gujarat Ayurveda University, India

Address for correspondence:

Hemlata Patel, MPharm Scholar, Department of Pharmacognosy, Gujarat Ayurveda University, India. hemlatapatel2112@gmail.com

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ABSTRACT:

India is one of the highest resource of medicinal plant in the world. Many plants are studied well for its medicinal importance which are written in Ayurved. But there are some plant which are not written in Ayurved but used by traditional medicinal practitioners. These drugs are called as anukta dravya i.e. scientifically unexplored. So in this category one plant i.e. *Rhynchosia minima* DC belonging to family fabaceae which is used in Gujarat. The aim of the study focuses on detailed microscopy of root along with root powder and micrometry of *Rhynchosia minima* DC. *Rhynchosia minima* DC. was collected from natural habitat which was free from pollution out skirts and from the edge of cultivated region of Jamnagar in month of September-November. Diagrammatic T.S. of root is circular in outline. Outer cork is followed by cortex, endodermis, pericyclic fiber & vascular bundles. Root is tap root with secondary and tertiary root along with root nodules. Dark brown in color, rounded, hard inner creamish, fracture fibrous.

KEYWORDS:

Rhynchosia minima DC, papilionatae, Root, pharmacognosy

INTRODUCTION:

Rhynchosia minima DC. belonging to sub-family papilionatae, locally known as *Nahni kamal vel* in Gujarati¹ and *Chittavarai* in Tamil. The plant is available in the rainy season throughout India ascending up to All over plains and in the Himalayas up to 1,200 m.² Sierra Leone, Senegambia, Nubia, Abyssinia, Arabia, Cape. (Everywhere in the tropics).³ All over the tropics, and extending to temperate regions as in south Africa.⁴ Grows wild in Southern India.⁵ Bangalore, Bellary, Bijapur, Chikmagalur, Dharwar, Gulbarga, Mysore, Raichur. India.⁶ Throughout India. Pantropic.⁷

The plant is found as erect or diffuse herb or under shrub having curved hairs on stem, green in colour with proper node and internodes. Root is tap root with secondary branching. Stem angled and puberulous. Leaves compound, 3 foliolate, alternate, median large than laterals, broadly ovate, median, laterals, Pubescent above, glandular hairy below, apex obtuse, base cuneate, margin entire, petioles long more or less pubescent, striate, stipules, linear lanceolate. Leaflets orbicular or ovate rhomboid, obtuse-round, gland dotted beneath. Inflorescence racemes axillary 6-12 flowered lax racemes usually exceeding the leaves in yellow color pedicels very short. Calyx silky, teeth subulate-acuminate. Corolla about twice as long as the upper teeth in yellow colour. Fruit pods oblong-oblongate, turgid, slightly recurved, flattened, slightly curved. Seed brown, mottled black, 0.3-0.2 cm long. Flowering and Fruiting in September-November.^{1,2,3,6,7}

Review of literature revealed many folklore claims are reported, the root of this plant is used by the folk people in *grahi*. Root and *Marich* powder given in *sangrahi* (sprue). Root is also useful in case of diarrhea, diabetes, and leucorrhoea. In diarrhea and *sangrahi* decoction of root is given with hot milk and sugar.⁸ Isolated scientific information is available regarding the phytochemical profile of this drug. Moreover, to standardize as per monographs, physico-chemical parameters are to be studied to get list of the physical and chemical nature of the plant. Hence in this research article an effort has been made to obtain detailed microscopy of root along with root powder microscopy and micrometry and studying phytochemical profile of *R.minima*.

MATERIALS AND METHOD:

COLLECTION AND AUTHENTICATION

Rhynchosia minima DC. was collected from natural habitat which was free from pollution out skirts and from the edge of cultivated region of Jamnagar in month of September-November. Pharmacognostical identification and authentication was done in pharmacognosy lab., IPGT & RA. (Phm/6257/17-18) (Fig.1)

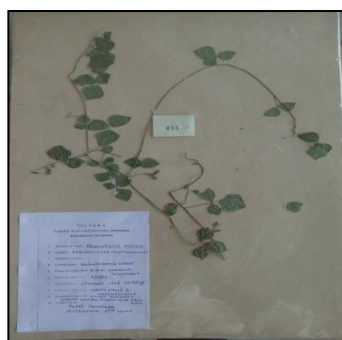


Fig.1

ROOT POWDER PREPARATION

Root was separated, shade dried, powdered by mechanical mixer grinder and sieved through 80 # sieve and stored in air tight glass container for further powder microscopy.

PHARMACOGNOSTICAL EVALUATION

MORPHOLOGY OF R. MINIMA

Root, tap root with secondary and tertiary root along with root nodules. Dark brown in color, rounded, hard inner creamish, fracture fibrous.^{7,8,9}



Fig.2

MICROSCOPICAL EVALUATION OF ROOT

Thin free hand transverse sections of root of *R. minima* were taken and sections were first observed in distilled water with help of Quasmo binocular microscope. Histochemical test were performed on thick sections of root was treated with various reagents to locate chemical constituents *i.e.* Tannin, lignin and calcium etc.^{10 to 13}

ORGANOLEPTIC CHARACTERS OF ROOT POWDER

The color, odour and taste of root powder through visual and sensory observation following standard protocol.¹⁰

MICROSCOPIC EVALUATION OF ROOT POWDER

The powder samples were studied under the microscope with distilled water and also examined after staining with different suitable reagents *i.e.* phloroglucinol along with hydrochloric acid and ferric chloride.¹⁰

MICROMETRIC EVALUATION ROOT

Micrometric measurements of T.S. of root and different characters of root powder were noted down by preloaded micrometric scale.¹⁰

PHARMACEUTICAL EVALUATION

The colour, odour and taste of whole plant powder were recorded separately through visual and sensory perceptions.¹⁰ Physicochemical parameters¹⁴, preliminary phytochemical investigations¹⁵, Quantitative estimation of Tannin¹⁶ and HPTLC¹⁷ were conducted on sample. All determinations were performed in triplicate and the results are presented as mean value.

RESULTS AND DISCUSSION:

MORPHOLOGY OF *RHYNCHOSIA MINIMA* DC

In natural habit, the plant is found as erect or diffuse herb or under shrub having curved hairs on stem, green in colour with proper node and internodes. Root is tap root with secondary branching. Stem 5–8 cm long, angled and puberulous. Leaves compound, 3 foliolate, alternate, median large than laterals, broadly ovate, median 5-4.3×1-1.5 cm, laterals 1.4-2×1-1.2 cm, Pubescent above, glandular hairy below, apex obtuse, base cuneate, margin entire, petioles upto 3 cm long more or less pubescent, striate, stipules 4 mm long, linear lanceolate. Leaflets orbicular or ovate rhomboid, mostly under 5 cm long, obtuse-round, gland dotted beneath. Inflorescence racemes axillary 6-12 flowered lax racemes usually exceeding the leaves in yellow color pedicels very short. Calyx 7.5 mm long silky, teeth subulate-acuminate. Corolla about twice as long as the upper teeth in yellow colour. Fruit pods oblong-oblancheolate, turgid, slightly recurved, and glabrescent measuring about 1.2-1.8 cm long, flattened, slightly curved, 1 or 2 seeded. Seed brown, mottled black, 0.3-0.2 cm long. Flowering and Fruiting in September-November.

MACROSCOPY OF ROOT

Root, tap root with secondary and tertiary root along with root nodules, measures about 7-11 cm. Dark brown in color, rounded, hard inner creamish, fracture fibrous.

TRANSVERSE SECTION

Diagrammatic T.S. of root is circular in outline. Outer cork is followed by cortex, endodermis, pericyclic fiber & vascular bundles. (Plate no. 1)

CORK

Detailed T.S. shows the outer most 3-5 layers of tangentially elongated lignified cork cells filled with brown content.

CORTEX

Cortex region is composed of loosely arranged 10-12 layers of parenchyma cells, which are embedded with isolated simple starch grains, rhomboidal crystals of calcium oxalate and tannin content. Some of the circularly arranged pericyclic fibers ring is observed in the cortical region. Cortex ends with single layer of endodermis.

VASCULAR BUNDLE

Vascular bundles are open collateral type and radially arranged. Phloem is situated above the xylem, made of sieve elements and phloem fibers. Xylems consist of xylem parenchyma and fibers. Some xylem vessels are filled with yellowish brown content.

MEDULLARY RAYS

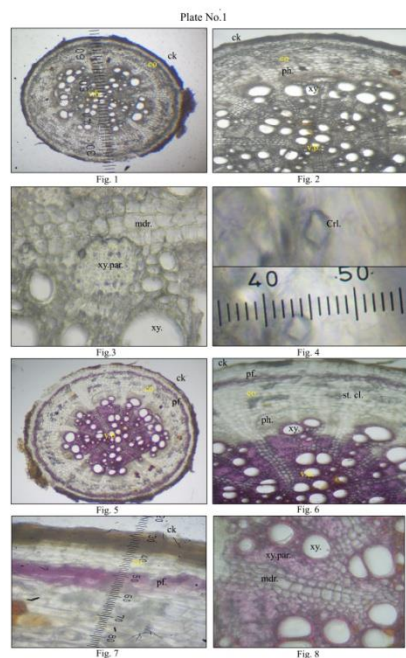
Medullary rays consist of rectangular to oblong shaped cells, uni-multiseriate arising from central region and extended up to inner layers of the cortical loaded with some starch grains, brown content and rhomboidal crystals of calcium oxalate.

MICROMETRIC EVALUATION: Micrometric measurements of T.S. of root shows $6.2 \mu\text{m}^2$, other characters are given in table no.1.

Table no.1: Micrometric measurements of T.S. of root

Sr. No.	Character	Measurement
1.	Diameter	$6.2 \mu\text{m}^2$ (40 \times)
2.	Cork	$1.2 \mu\text{m}$ (100 \times)
3.	Cork cells	$0.5 \times 0.4 \mu\text{m}$ (100 \times)
4.	Cortex	$4.2 \mu\text{m}$ (100 \times)
5.	Pericyclic region	$2.3 \mu\text{m}$ (400 \times)
6.	Phloem	$2.4 \mu\text{m}$ (400 \times)
7.	Medullary rays	$5.8 \mu\text{m}$ (100 \times)

(\times Magnification)



POWDER MICROSCOPY:

ORGANOLEPTIC CHARACTERS

Results of organoleptic characters of *R. minima* root powder are depicted in the table no. 2.

Table no. 2: Organoleptic characters of Root powder

Sr. No.	Characters	Observation
1.	Color	Creamish
2.	Taste	Sweet/Woody
3.	Odor	Characteristic
4.	Nature of powder	Fibrous

POWDER MICROMETRY:

Diagnostic characters are rhomboidal crystals, brown content, silica deposition, starch grains, compound starch grains, group of stone cells, fragment of cork cells in tangential view, trichome, fragment of annular, pitted and bordered pitted vessels, fragment of fiber and fragment of crystal fiber.

MICROMETRIC EVALUATION: Starch grain measures about $0.84 \times 0.4 \mu\text{m}$, measurements of other characters are denoted in table no. 3.

Table no. 3: micrometry value of root powder

Sr. No.	Character	Measurement (400×)
1.	Silica deposition	$1.5 \times 1.2 \mu\text{m}$
2.	Starch grain	$0.4 \times 0.4 \mu\text{m}$
3.	Bordered pitted vessel	$1.6 \times 0.9 \mu\text{m}$
4.	Brown content	$1.3 \times 1.2 \mu\text{m}$
5.	Width of crystal fiber	$3.9 \mu\text{m}$
6.	Trichome	$1.3 \times 0.4 \mu\text{m}$
7.	fragment of fiber	$0.8 \mu\text{m} \times 0.2 \mu\text{m}$
8.	Rhomboidal crystals	$0.5 \times 0.3 \mu\text{m}$

(× Magnification)

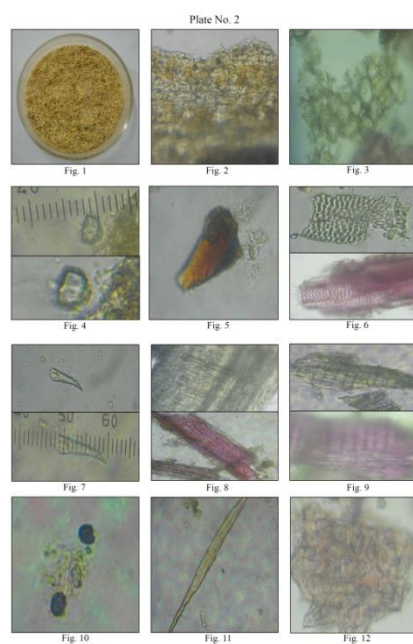


Fig.2

**PHARMACEUTICAL EVALUATION
PHYSICOCHEMICAL PARAMETERS:**

Physicochemical parameters of *R. minima* root powder included various physicochemical analysis. The moisture content in the sample is 2.50 %w/w. The other observed results are shown in the table no. 4

Table : 4 showing results of physicochemical parameters

Sr. No.	Parameters	Root
1.	Loss on drying	2.50 %w/w
2.	Total ash	4.445%w/w
3.	Acid insoluble ash	0.199%w/w
4.	water soluble extractive value	11.22%w/w
5.	Alcohol soluble extractive value	5.93%w/w
6.	pH (Aqueous 5%)	5.25

PRELIMINARY QUALITATIVE TESTS:

R. minima root powder was qualitatively tested for the presence of different phytoconstituents. Carbohydrate, Cardiac glycoside, Flavonoid and Tannin are present in both the extracts. The other observed results are shown in the table no. 5

Table no. 5: Qualitative chemical screening of *R. minima* Root powder

Phytoconstituents of tests	Root	
	W.E	A.E
Carbohydrates (Fehling's test)	+	+
Proteins (Biuret test)	-	-
Amino acids (Ninhydrin test)	+	+
Steroid (Salkowski reaction)	+	-
Glycosides (Saponin)	+	-
Flavonoid (Lead acetate solution)	-	-
Alkaloids (Dragendroff's test)	-	-
Tannins (FeCl ₃ solution)	+	+

QUANTITATIVE ANALYSIS:

The sample powders were subjected to quantitative estimation for total tannin & total alkaloids. The result of total tannin & total alkaloids content is described in table no. 6

Table no. : 6 Quantitative estimation of *R. minima* powder

Phytoconstituents	Root
Total Tannin content (% w/w)	21.1928
Total Alkaloids Content (% w/w)	0.7591

HPTLC:

Solvent system which was designed for HPTLC *i.e.* Pet ether: Diethyl ether: Acetic acid (9:1:0.1) v/v was used. The results are shown in the table 7.

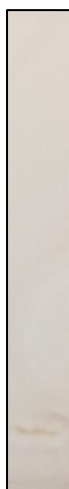


Fig.3



Fig.4

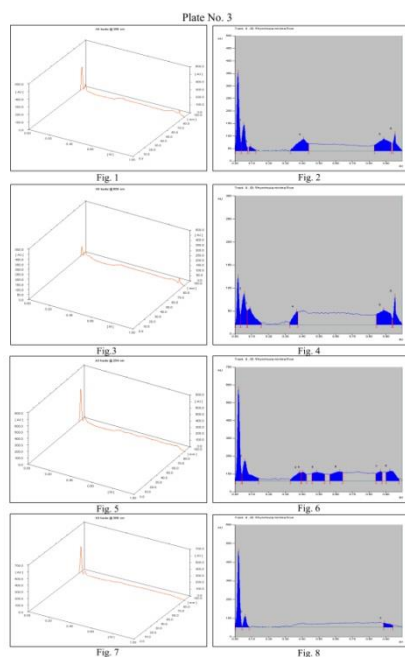


Fig.5



Fig.6

Before spray				After spray			
254 nm		366 nm		Visible		366 nm	
Peak	R _f value	Peak	R _f value	Peak	R _f value	Peak	R _f value
1	0.02	1	0.02	1	0.02	1	0.02
2	0.06	2	0.06	2	0.06	2	0.05
3	0.39	3	0.89	3	0.09	3	0.09
4	0.40			4	0.37	4	0.41
5	0.49			5	0.89	5	0.89
6	0.62			6	0.95	6	0.95
7	0.87						
8	0.92						



DISCUSSION:

MACROSCOPY:

Taxonomically the plant was placed under the family Fabaceae, available all over India on the hedges of cultivated or non cultivated area. The plant is trailing or twining, glabrous annual herb with small obtuse leaflets measured by $1.5-4.5 \times 0.4-2.2$ cm, broadly ovate or nearly orbicular, acute or acuminate at apex, glabrous. Flowers yellow, in 5-12 cm long, axillary, lax racemes. Pods $1-1.5 \times 0.4-0.6$ cm, obliquely oblong, pubescent, compressed, 2-3 seeded. Seeds globose, dark-brown, somewhat compressed, estrophiolate. Season of flowering and fruiting is august to april. Genus *Rhynchosia*, *Atylosia* and *Dunbaria* bears creamy-yellow, yellow or tinged red coloured flowers. *Rhynchosia minima* also possess yellow coloured flowers in which corolla not more than 1 cm long, exhibit the dominant character of the family.

MICROSCOPY:

Root anatomy of plants from papilionaceae family shows well developed cork, xylem and phloem, pericyclic ring and medullary rays. Similar characters were observed in *R. minima* root's transverse section.¹⁸

PHARMACEUTICAL EVALUATION

The physicochemical parameters shows that loss on drying is more in drug means the powder has less capacity to absorb the moisture. Moreover, due this reason the stability of powder increase. After incineration of the drug powder, the quantity of inorganic residue is more than the mineral composition. The solubility of active constituents in methanol extract is less than the water extract. The pH of root powdered drug depicts slightly acidic nature of the plant but strongly towards neutral pH. Among the qualitative assessment, carbohydrates, cardiac glycosides, flavonoids and tannin content are present in both the extract of sample while steroid is only present in methanol extract and saponin glycoside is present only in water extract. Qualitative analysis output showed an overall profile of chemical moieties present in plant extract. Natural products with reservoirs of structural and chemical entities will have definite therapeutic relevance. Quantitative estimation of sample powder showed presence of tannin and alkaloids.

The number of bands obtained at 254 nm is 8 and at 366 nm are 3. The result obtained, study showed the chemical profile of drug.

CONCLUSION:

Root is tap root with secondary and tertiary root along with root nodules. Dark brown in color, rounded, hard inner creamish, fracture fibrous.

Transverse section of root shows circular in outline. Outer cork is followed by cortex, endodermis, pericycle & vascular bundles. Cortex region is embedded with isolated simple starch grains, rhomboidal crystals of calcium oxalate and tannin content. Vascular bundles are open collateral type and radially arranged. Medullary rays are uni-multiseriate, consist of rectangular to oblong shaped cells.

Root powder is creamish in colour with characteristic odour and sweet/woody taste. Diagnostic characters of root powder shows rhomboidal crystals, brown content, silica deposition, starch grains.

physicochemical parameters, qualitative analysis and quantitative analysis can be used for standardization of *R. minima* identity and purity. The HPTLC profile can be helpful for identification of the plant. The standard results may help for the further research works.

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