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Pharmacognostic Studies on Indian Madder (*Rubia cordifolia* L.)

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'Indian Madder' (*Rubia cordifolia* L.) has wide range of pharmacological properties. In the present study, an attempt was made to identify the pharmacognostic features of various parts of *R. cordifolia* to differentiate it from adulterants. The organoleptic characters, proximate analysis, physio-chemical behavior of powders were recorded. The stomatal index, palisade ratio and ash value were shown to be characteristic under standard conditions. Optical activity of various *R. cordifolia* extracts under visible and UV light (254 nm and 366 nm) were recorded. Phytochemical studies revealed the presence of anthraquinone and other metabolites in different plant parts. Powder analysis showed trace red colour in root powder under most of the standard test conditions. Phytochemical and pharmacognostic records evolved in the present study can be used for framing standard parameters for the proper identification of raw materials of *R. cordifolia*.

Keyword: *Rubia cordifolia*, Phytochemical, Powder, Extract

1. Introduction

Rubia cordifolia L. is a climbing or scrambling herb, with red rhizomatous base and roots. It is an essential raw drug for the traditional herbal formulations such as aswagandharistam, gulguluthikthkarishtam, jaatyaadi ghrita, madhookasavam, majishthaadi taila, useerasavam etc. It is a member of Rubiaceae family, distributed in hilly tracts of India up to 3750m. The plant (Fig. 1A) is commonly known as 'Indian Madder' and sold under the trade name 'manjistha'. The plant has other vernacular names such as manjit in Hindi, chitravalli in Kannada, ceevalli in Tamil, manchatti and manjatti in Malayalam and manderti in Telengu[1].

Fig 1A: *Rubia cordifolia* Habit

The mature roots are chiefly valued plant part of *R. cordifolia*. The p0lant may be used internally or externally to treat disorders. As per 'Charaka Samhitaa', the powdered dried roots and fruits are taken internally for the treatment of skin diseases and disorders of spleen. In 'Sushruta samhitaa'

preparations based on `manjistha` are prescribed for treatment of major burns, bone fractures and dysentery. Manjistha is considered as tonic, antitussive and useful in chronic low fevers. Decoction from roots is prescribed to cure jaundice, paralytic affections, urinary troubles, amenorrhea and to the mother after delivery for cleansing and shrinking of the uterus.[2] The root decoction is effective to regulates menstruation cycles.. It is proved that various root extracts of *R. cordifolia* are has astringent, themogenic, febrifuge, antidysenteric, antihelmintic, galacto-purifier, ophthalmic, and rejuvenating effect.[3,4] It is used to cure tuberculosis and intestinal ulcer. In modern pharmacopeia, *R. cordifolia* is reported to be active against a diversified panel of cancer cell lines, such as P388, L1210, L5178Y, B16 melanoma, Lewis lung carcinoma, and sarcoma-180[5].

The macroscopic and microscopic description of a medicinal plant is the first step towards identification and determination of purity. However, such an attempt has not been made on *R. cordifolia* so far. In the present communication we devoted our effort to document the pharmacognostic features of the entire plant to identify the plant in its crude form.

2. Materials and Methods

The plants were collected from the natural habitat, Ellappara, (Latitude 9º36', 49.89" N, Longitude 77º00', 6.59"E Elevation 1158m)), Idukki Distt, Kerala and was positively identified and confirmed by the herbarium in the Department of Botany, University of Kerala.

Morphological studies were performed by using simple, binocular, light microscope. For micro-characterization, free hand sections of about 10-20 µm thickness of stem, root, petiole and leaves were made and stained with aqueous safranine (0.5%) solution. After washing, the stained sections were mounted on clean micro slides and examined. The preparation was further observed in an image analyzer (Olympus BX 51) and the anatomical peculiarities were photo documented. As a part of quantitative microscopy, stomatal number, stomatal index and vein islet number were determined by using fresh leaves of the plant.[6]

Shade dried, coarsely powdered raw drug was used for powder analysis, which includes the identification of organoleptic characters, determination of physicochemical characters, behaviour of the powder with different chemical reagents/solvents according to methods described in Indian pharmacopeia[7] and fluorescence as suggested by Chase and Pratt[8,9].

Preliminary phytochemical studies were carried out using 10 g powdered material and subjecting it to successive extraction in a Soxhlet apparatus with 150 ml solvents *viz*., hexane, chloroform, acetone, methanol, and water. The extraction was continued until the solvent became colourless. The extracts were collected; concentrated using a rotary evaporator and the obtained extracts were air dried. Presence of various phytoconstituents *viz*., alkaloids (Dragendorff's test, Mayer's test, Wagner's test), anthraquinones (Borntrager's test, Modified Borntrager's test), carbohydrates (Anthrone reagent test, Benedict's reagent test, Molish's test), coumarins (Sodium hydroxide test), cardiac glycosides (Kellar kiliani test, Potassium Hydroxide test), flavonoids (Shinoda test, Ammonia test), glycosides (Benedict's reagent test, Fehling solution test), phenols (Ferric chloride test, Phosphomolybdic acid test), phlobatannins (Hydrochloric acid test), protein (Biurete test, Millon's test, Xanthoprotein test), quinines (Sodium hydroxide test), reducing sugar (Benedict's test), resins (Turbidity test), saponins (Foam test), steroids and terpenoids (Lieberman burchard test, Salkowski test) and tannins (Braemer's test) were tested. [10, 11]

3. Result and Discussion

3.1 Pharmacognostic Features

a. Stem: is quadrangular, divaricately branched, glabrous or prickly-hispid, especially on the angles. C.S of the stem showed rectangular (Figure 1B) outline. It has a single layered epidermis covered with cuticle. Pyramidal hairs are present on the epidermis. Sclerenchymatous hypodermis is present only at the corners of the stem. The cortex is chlorenchymatous, hence photosynthetic. Phloem is arranged in 4-6 layers

and is composed of sieve tubes and phloem parenchyma. Cambium ring is represented in two layers. Secondary xylem forms a continuous cylinder, which is made of vessels, tracheids, fibres and xylem parenchyma. In secondary xylem, vessels are large and uniformly arranged. Uniseriate medullary rays are also present. In older stem, large central parenchymatous pith is present.

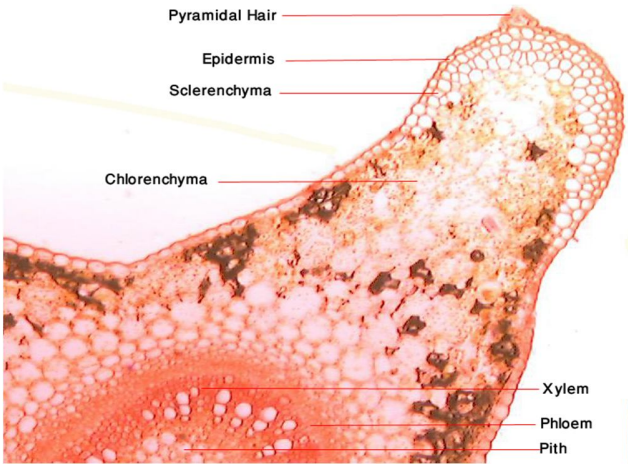
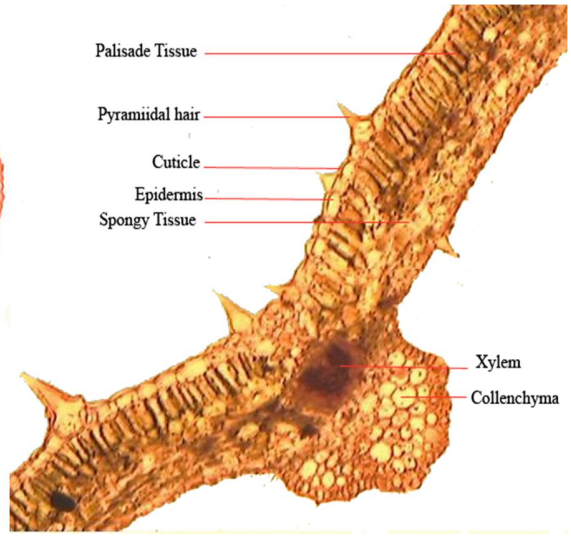


Fig. 1B: C.S. of Stem Fig.



1C: T.S. of Leaf

b. Leaves: are 3.8-9 X 1.6-3.5 cm long, arranged in a whorl of four, cordate-ovate to ovate-lanceolate, 3-9 palmately veined, upper surface mostly glabrous and rough. Lower leaves are larger than the upper, and all are scabrous above. Leaf base is slightly cordate. The margins are with minute white prickles. Leaf section showed single layered epidermis, covered with cuticle and possesses pyramidal hairs. Palisade cells are single layered and compactly packed and the spongy cells are multilayered and loosely arranged. In the lower portion of the midrib 2-4 layers made of collenchymatous cells. Vascular bundles are definite in number, conjoint, collateral and closed (Fig. 1C).

c. Petiole: is 5-10 cm long with sharp, recurved prickles. Stipules are completely absent or modified into leaves. The T.S. of the petiole showed a 'V' shaped prominent median groove. It had a single layered epidermis provided with pyramidal hairs. Below the epidermis at the lobes and base, 3-4 layers of sclerenchymatous cells were present. The cortical cells were made of thin walled chlorenchyma. Vascular bundle is 'C'- shaped (Fig. 1D).

d. Root: *Rubia cordifolia* has long, cylindrical, flexuose, smooth and reddish roots. The cross section of well-developed roots showed an outer 5-7 layer of cork tissue, which occasionally contains tannin. Phellogen is not distinct (Fig. 1E). Secondary cortical cells are thin walled, red in colour and polygonal in shape. Secondary xylem forms a continuous cylinder of reddish colour, consists of mainly vessels and tracheids. Vessels are numerous and distributed uniformly. Secondary phloem composed of thin walled cells like sieve elements and phloem parenchyma but lacks phloem fibres, which forms a wide zone of reddish colour. Cambium is distinct and characterized by absence of medullary rays. The entire portion of root is reddish in colour, indicates the presence of anthraquinone.

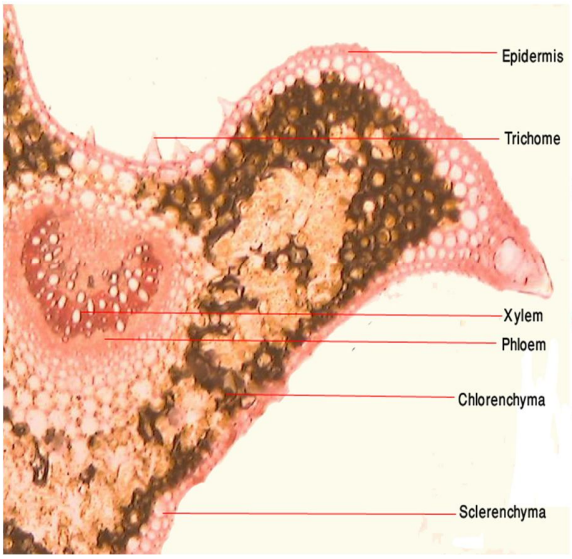


Fig. 1D: T.S. of Petiole

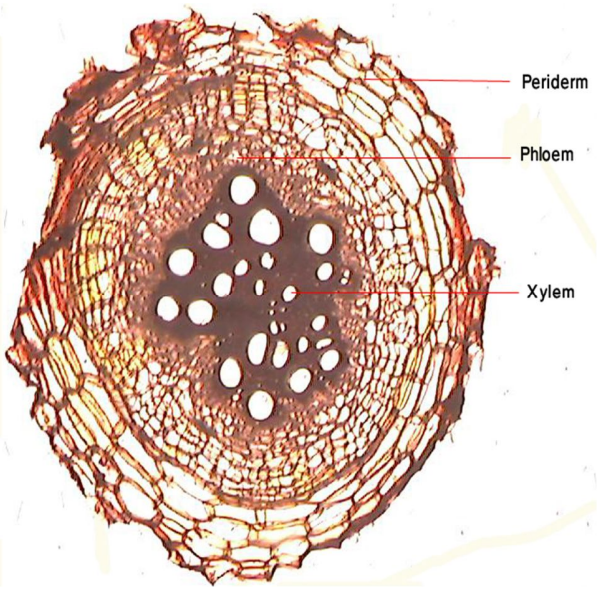


Fig. 1E: C.S. of Root

3.2 Quantitative Microscopy

Leaf peelings from both abaxial and adaxial side showed paracytic (rubiaceous) type of stomata where the stoma is surrounded by two subsidiary cells, the long axis of which are parallel to the stoma. The palisade ratio (1.78), stomatal index (16.67) and vein-islet number (1.48) were calculated as a part of quantitative microscopy (Table 1).

Table 1: Quantitative microscopic examinations of *R. cordifolia* leaf peelings

Determinations	Range
Palisade ratio	1.78
Stomatal index	16.67
Vein-islet number	1.48

3.3 Powder Analysis

Powder Microscopy: Organoleptic (morphological) characters refers to evaluation of drugs by colour, odour, taste, size, shape and consistence (touch and texture). These were identified by using the crude powder samples. The root powder was smooth, moderately fine with coral pink colour (Table: 2).The stem and leaf powders were somewhat grayish. Stem powder was moderately coarse and fibrous. All the samples were with mouldy smell. The root samples were acrid and bitter in taste.

Table 2: Organoleptic characters of root, stem and leaf powders of *R. cordifolia*

Character	Root	Stem	Leaf
Colour	Coral Pink	Gray	Ivy Green
Odour	Mouldy	Mouldy	Mouldy
Size	Moderately fine	Moderately coarse	Fine
Taste	Acrid and bitter	Bitter	Acrid
Texture	Smooth	Fibrous	Smooth

3.4 Proximate Analysis: As the part of physico-chemical studies, ash value determination was carried out and complied (Table 3). These were found to be within the limit and were observed slightly higher as reported earlier.[12]The official ash values are of prime importance in determination of the purity of powdered drugs.

Table 3: Physico-chemical analysis of coarse powdered samples of *R. cordifolia*

Ash value	Root	Stem	Leaf
Total Ash (%)	8.5	12.0	24.5
Acid insoluble ash (%)	3.5	2.75	7.0
Water insoluble ash (%)	3.0	3.5	8.0

Table 4: Behaviour of the *R. cordifolia* drug with different reagents/ solvents

Treatment	Root	Stem	Leaf
Powder+ Distilled Water	Light orange	Brilliant yellow	Brilliant yellow green
Powder+ 5% $FeCl_3$	Moderate olive	Greenish yellow	Light Greenish yellow
Powder+ l Acetic acid	Light orange yellow	Strong yellow brown	Strong Yellowish green
Powder+ 5% KOH	Strong Pink	Strong Brown	Light Greenish yellow
Powder+ 5% NaOH	Cardinal red	Dark brown	Strong Brown
Powder+ Conc. HCl	Greenish yellow brown	Light Reddish black	Moderate Brown
Powder+ Conc. H_2SO_4	Reddish black	Reddish black	Reddish black
Powder+ Conc. HNO_3	Brilliant yellow	Orange buff	Barium yellow
Powder+ N/10 Iodine soln.	Cadmium Orange Mandarin red	Yellow brown	Primrose yellow
Powder+ Ammonia soln.		Mimosa yellow	Canary yellow

Table 5a. Fluorescence analysis of extracts prepared by using various solvents

Treatment	Plant Part	Observations		
		Short UV light (254nm)	Long UV light (366nm)	Visible light
Hexane	Root	Coffee Brown	Dark Brown	Indian Orange
	Stem	Cyprus Green	Dull Black	Primrose Yellow
	Leaf	Cyprus Green	Dark Brown	Uranium Green
Chloroform	Root	Chocolate	Brown	Orange
	Stem	Fern Green	Black	Dark Yellow Brown
	Leaf	Greenish Brown	Coffee Brown	Willow Green
Acetone	Root	Cyprus Green	Light Brown	Nasturtium Orange
	Stem	Agathia Green	Colourless	Pea Green
	Leaf	Cyprus Green	Light Brown	Agathia Green
Methanol	Root	Yellow Brown	Brown	Fire Red
	Stem	Agathia Green	Brown	Yellow Brown
	Leaf	Cyprus Green	Dull Black	Agathia Green
Water	Root	Lavender Green	Blue	Light Brown
	Stem	Fern Green	Black	Brownish Yellow
	Leaf	Fern Green	Black	Dark Brownish yellow

3.5 Behaviour of powder with chemical reagents: Detection of colour variation of the powdered samples under day light is a way to identify its purity. The root samples showed trace of red colour with almost all reagents except with Conc. HCl and $FeCl_3$ (Table 4). Stem and leaf powder showed yellowish to reddish black colour with various reagents.

3.6 Fluorescence Analysis: The optical activity of the powdered samples (Fig. 2A, B & C) and the

plant extracts under different optical regimes *viz*. visible, UV (254 nm) and UV (366 nm) were recorded by the reported method (Table: 5a &5b). Various extracts and powder showed yellow, green and brown fluorescence in visible and short UV light (254nm) and brown and black fluorescence in long UV light helped to identify, authenticate and differentiate *R. cordifolia* from other related species.

Fig. 2A: Root Powder

Fig. 2B: Stem Powder

Fig. 2C: Leaf Powder

Table 5b: Powder analysis of *R. cordifolia* at short, and long UV and visible light

Treatment	Plant Part	Observations		
		Short UV light	Long UV light	Visible light
Powder alone	Root	Light Gray	No Fluorescence	Coral Pink
	Stem	Light Gray	No Fluorescence	Gray
	Leaf	Light Gray	No Fluorescence	Ivy Green
Powder + 50% H_2SO_4	Root	Willow Green	No Fluorescence	Light Brown
	Stem	Agathia Green	No Fluorescence	Yellowish brown
	Leaf	Agathia Green	No Fluorescence	Pea Green
Powder + 50% HNO_3	Root	Pea Green	No Fluorescence	Pea Green
	Stem	Agathia Green	No Fluorescence	Canary Yellow
	Leaf	Agathia Green	No Fluorescence	Dresden Yellow
Powder + 50% HCl	Root	Willow Green	No Fluorescence	Sage Green
	Stem	Agathia Green	No Fluorescence	Light Cadmium
	Leaf	Agathia Green	Blue	Sap Green
Powder + 1N HCl	Root	Agathia Green	Colorless	Apricot Yellow
	Stem	Agathia Green	Light Brown	Greenish yellow
	Leaf	Pod Green	Sepia	Naples Yellow
Powder + 5% KOH	Root	Dark Green	Sepia	Light Coral Pink
	Stem	Cyprus Green	No Fluorescence	Buttercap Yellow
	Leaf	Pod Green	No Fluorescence	Empire Yellow
Powder + 1N NaOH (Methanol)	Root	Sage Green	No Fluorescence	Prawn Red
	Stem	Agathia Green	No Fluorescence	Empire Yellow
	Leaf	Agathia Green	No Fluorescence	Empire Yellow
Powder + 1N NaOH (in water)	Root	Leek Green	No Fluorescence	Porcelain Rose
	Stem	Agathia Green	No Fluorescence	Chrome Yellow
	Leaf	Agathia Green	No Fluorescence	Lemon Yellow

3.6 Phytochemical Analysis

The pharmacological action of the crude drug is largely depends on the metabolites present in it. In the present investigation, the qualitative screening by using prepared extracts revealed the presence of a wide range of phytoconstituents (Table 6). Borntrager's test for anthraquinone was found to be positive for all the studied samples.[13, 14] More intensive colour was noticed in root samples compared to stem and leaves indicating presence of more amount of anthraquinones in the roots. Glycosides other than cardiac glycosides were present only in all root extracts. Absence of flavonoids indicates the purity of the samples.[15] In addition to these, metabolites like saponins, resin and steroids/ terpenoids were present in *R. cordifolia* extracts. Coumarins, cardiac glycosides and flavanoids, phenol, phlobatannin, protein and quinines were absent. Steroids were also noted in root samples only.

Table 6: Phytochemical analysis of extracts of *R. cordifolia*

Compounds	Name of Test	Root extracts					Stem extracts					Leaf extracts				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Alkaloids	Dragendorff's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mayer's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Wagner's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinone	Borntrager's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Modified Borntrager's test	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	Molisch's test	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+
	Anthrone reagent test	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
	Benedict's reagent test	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
Coumarins	Sodium Hydroxide test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac Glycosides	Kellar Kiliani test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Potassium Hydroxide test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavanoids	Shinoda test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ammonia test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	Benedict's reagent test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fehling Solution test	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Phenols	Ferric chloride test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Phosphomolybdic acid test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	Hydrochloric acid test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteins	Xanthoprotein test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Biurete test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Million's reagent test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinines	Sodium Hydroxide test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ReducingSugar	Benedict's reagent test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Resins	Turbidity test	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+
Saponins	Foam test	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+
Steroids/ Terpenoids	Salkowski test	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	Limberman-Buchard test	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	Braemer's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1: Hexane; 2: Chloroform; 3: Acetone; 4: Methanol; 5: Water; +: Present ; -: Absent

4. Conclusion

It is evident that plants having therapeutic value usually contain diverse groups of secondary metabolites and *R. cordifolia* proved no exception. Apart from the wide range of health care uses it is being used as a textile dye from ancient times. However, due to the endemic distribution, the plant is not familiar to the populace of the modern world or even neglected where it flourishes well. The plant play a vital role in the development of novel drugs and well defined pharmacognostic parameters and standards must be established before the inclusion of any crude drug in a herbal pharmacopoeia. The studied pharmacognostical and phytochemical characters of *R. cordifolia* will be useful to identify the plant in the powder form and in the elimination of adulterants. So if any crude drug which is claimed to be *R. cordifolia* but whose characters significantly vary from the accepted standard should be rejected irrationally.

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