



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)

Document heading

# Morpho–anatomy, physicochemical and phytochemical standardization with HPTLC fingerprinting of aerial parts of *Rivea hypocrateriformis*

Saboo Shweta<sup>1</sup>, Tapadiya Ganesh<sup>2\*</sup>, Khadabadi Somshekhar<sup>1</sup><sup>1</sup>Department of Pharmacognosy, Govt. College of Pharmacy, Kathora Naka, Amravati (M.S.), India<sup>2</sup>R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur

## ARTICLE INFO

## ABSTRACT

**Article history:**

Received 25 June 2012

Received in revised form 5 July 2012

Accepted 7 August 2012

Available online 28 August 2012

**Keywords:***Rivea hypocrateriformis*

Morpho–anatomy

Quantitative estimation

HPTLC fingerprinting

Standardization

Physicochemical

**Objectives:** To study the morpho–anatomy of the aerial parts of *Rivea hypocrateriformis* (Convolvulaceae) to increase the knowledge and standardization parameters of these plants and its family. **Methods:** Morpho–anatomical studies of aerial parts of have been carried out by free hand. The different types of histochemical tests were performed for the identification of micro–chemical by using staining reagents. Preliminary phytochemical and quantitative estimation has been determined along with HPTLC fingerprinting. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS–4 software was used for fingerprinting. **Results:** The morpho–anatomical structure of plant revealed the presence of thick wall collenchyma cell and parenchyma cells along with spheraphides and columnar palisade cells. Presence of numerous covering trichomes, parasitic stomata, oval shaped vascular bundles and calcium oxalate crystals were observed. In powder microscopy lignified xylem vessels, and covering trichomes were clearly seen. Physicochemical parameters, ash value, inorganic elements, moisture content and extractive value were determined to develop the stringent pharmacognostic standards. Qualitative standardization and HPTLC fingerprinting of phytochemical revealed the presence of various plant secondary metabolites. **Conclusions:** These findings will be useful in establishing pharmacognostic and phytochemical standards for identification, as well as assessment of purity and quality of this plant, which definitely gaining the relevance in plant drug research and establishment of plant monograph.

## 1. Introduction

Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant or parts of plants to be potential sources of medicinal substances[1]. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines[2]. With

this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies, include morphological, anatomical study and biochemical characterization by qualitatively as well as quantitatively. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy[3].

*Rivea hypocrateriformis* (*R. hypocrateriformis*), a large climbing shrub which is found almost throughout the India. Leaves are orbicular–cordate mucronate; flowers shows fragrance and white in colour. Flowers are used for different perfume preparations. Root is given in Bihar to

\*Corresponding author: Ganesh Tapadiya, Department of Pharmacognosy, Govt. College of Pharmacy, Kathora Naka, Amravati (M.S.), India  
Tel–0721–2567020  
Fax–0721–2566983  
Email: [ganeshatapadiya@gmail.com](mailto:ganeshatapadiya@gmail.com)

woman after childbirth, in tooth ache, treatment for piles and inflammation[4]. Leaf pastes are used for treatment for skin disorders and ethanol extract shows antiimplantation and pregnancy interruption action[5]. Phytochemically it contains amino acids, sugars, lupeol and gallic acid[4].

In the present study, an attempt has been made for morpho-anatomical as well as physicochemical and phytochemical standardization of this plant for contribution in the quality control herbal drug and increase in the knowledge of plant and its family.

## 2. Material and method

### 2.1. Plant material and extraction

Fresh leaves of *R. hypocrateriformis* was collected in the month of August to September from Amravati district, Maharashtra, and authenticated by Dr. Prabha Bhagoakar, VIHS, Amravati. The fresh leaves were dried under the shade and powdered in a mixture grinder. The powdered leaves packed in a paper bags and stored in air tight container until use. The powder material of *R. hypocrateriformis* extracted successively by petroleum ether, chloroform and ethanol solvent[4]. The extracts were concentrated under reduced pressure (17 mm Hg, 45 °C, 10–20 min depending on the solvent). The extracts were stored at cool place in the dark until use. Each time before extraction with next solvents, the powder material was dried in hot air oven below 50 °C.

### 2.2. Organoleptic evaluation

Organoleptic evaluation was evaluated by means of organs of sense or appearance of the drug. External morphological characters of freshly collected leaves were study by Singh *et al*[6].

### 2.3. Microscopical evaluation

For anatomical description material was sectioned free-hand, in transverse direction. Various types of histochemical tests were performed for the identification of starch grain, globules and xylem with the following solutions: hydrochloric and phloroglucinol, for the detection of lignin; iodine solution for starch, and sulphuric acid to confirm the chemical nature of the crystals of calcium[7].

### 2.4. Leaf constant and physicochemical investigation

For establishing standardization parameter various leaf constants, palisade ratio, vein islet number, vein termination number and stomatal index evaluated by Khandelwal[8] and

physicochemical parameters such as ash value, inorganic substance identification, extractive values and moisture content[9].

### 2.5. Qualitative and quantitative phytochemical investigation

The preliminary qualitative phytochemical identification had been carried out by using various phytochemical tests[9] and phytoconstituents total steroids, total flavonoids and total phenolic had been quantitatively estimated by methods of Kulkarni *et al*[10] and Sudarsing *et al*[11].

### 2.6. HPTLC fingerprinting

HPTLC study of different extracts was carried out by the method of Harborne[12] and Wagner *et al*[13].

### 2.7. Development of solvent system

A number of solvent were tried individually as well as in combination for separation of different components of extract, but the satisfactory resolution was obtained in the solvent system benzene:chloroform (1:1 v/v) for pet ether extract, chloroform:ethyl acetate:n-propanol:glacial acetic acid (45:4.5:4.5:0.5 v/v) for chloroform extract and chloroform:glacial acetic acid (9:1 v/v) for ethanol extract.

### 2.8. Sample application and development of chromatogram

The samples (5 µL) were spotted in the form of bands of width 6 mm with a 100 µL Hamilton syringe on precoated HPTLC aluminum silica gel GF-254 plates (20 cm×10 cm) with the help of Linomat 5 applicator. Prepared plates were developed in previously saturated twin trough chamber (20 cm ×10 cm) in linear ascending direction.

### 2.9. Detection of spots

The developed plates were dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with anisaldehyde sulphuric acid and vanilline sulphuric acid reagent as spray reagent and dried at 100 °C in hot air oven for 3 min. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 nm and 334 nm, respectively. The R<sub>f</sub> values and finger print data were recorded by WIN CATS software.

## 3. Results

### 3.1. Morphological study

Morphologically leaves are green in color while flowers are white. Leaf is orbicular–cordate shape and bitter taste.

### 3.2. Microscopical study

#### 3.2.1. Study of midrib

Epidermis layer showed presense of cuticle as well as 6 to 7 layers of thick collenchyma cell and parenchyma cells (Figure 1a). The upper epidermis indicated presense of several thick walled cells containing spheraphides and columnar palisade cells. Palisade cells were relatively short somewhat loosely placed (Figure 1a). While lower epidermis contained thin walled cell and paracytic stomata (Figure 1b). It also showed presense of covering trichomes, unicellular long simple trichomes with long arms (Figure 1c). Vascular tissues were collateral conjoint and arranged in half circle (Figure 1d). The pith was large containing several large resin canals and isodimetric irregularly shape parenchymatous thin walled cells. Vascular bundles were scatters in the pith.

#### 3.2.2. Study of petiole

In the T.S. of petiole, epidermal layer showed composed of two to three layer of thick walled collenchymatous cell followed by 5 to 6 layer of cortical parenchyma. Endodermis and pericycles were not distinct. The inner cortex of petiole was about three to four layered containing regular isodimetric cell. Opening of vascular tissues were conjoint and appears as collateral. Open vascular bundles were arranged in ring. Each bundle was capped by sclerenchyma. Vascular bundle showed secondary growth but growth restricted only up to the vascular bundle. Secondary conjunctive tissues were lignified. Polygonal rectangular shapes of calcium oxalate crystal were observed in pith (Figure 1e).

#### 3.2.3. Microchemical test

Chemo–microscopy revealed the presence of tannins, starch grains, proteins and calcium oxalate crystals (Table 1 and Table 2).

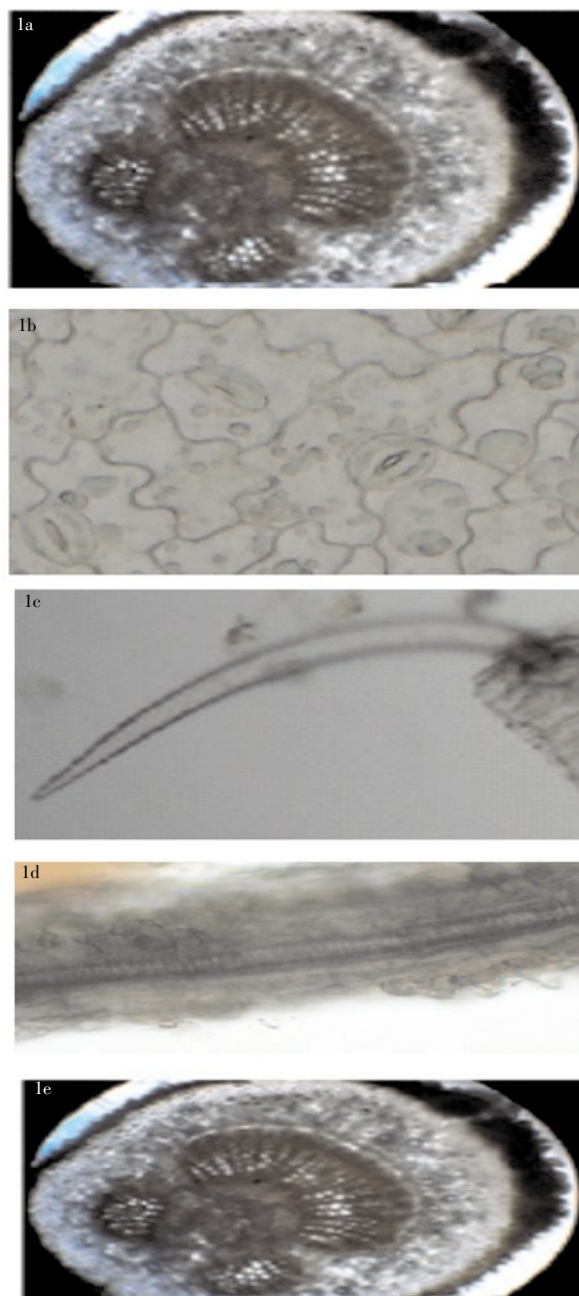


Figure 1. Microscopy of leaves and petiole.

Table 1

Results of reagents tests.

Reagents	Observation	Inference
T.S. + phloroglucinol + concentrated HCL	Lignified fibers and sclerides	Fibers and sclerides present
T.S. + Iodine	Blue or violet colored	Starch granules present

Table 2

Results of powder characterization tests.

Powder characterization	Observation	Inference
Powder drug + few drops of water	No swelling	Mucilage absent
Powder drug + Phloroglucinol + Conc. HCL	Lignified fibers	Lignified sclerides
	Fibers present	Sclerides present
Powder + dil. Iodine solution	Blue starch grains observed	Starch grains present

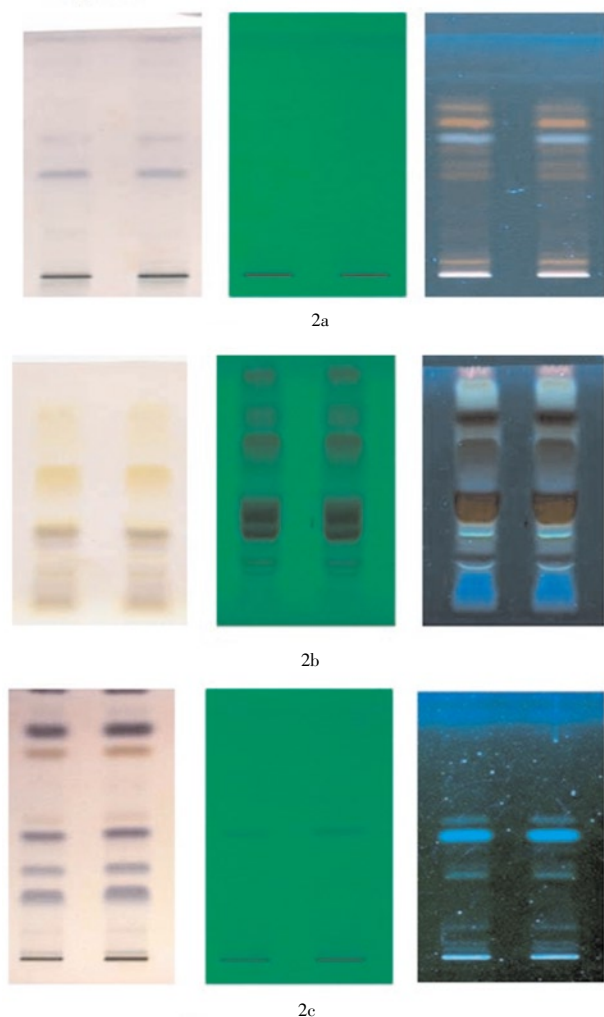
### 3.3. Qualitative microscopy and physicochemical study

Various types of leaf constants, ash values, inorganic element, extractive values and moisture content were important to determine purity of the drug (Table 3). In leaf constants stomatal index and palisade ratio were found to be 25.2–26.6 and 1–6 respectively. Whereas vein islet and vein termination number were ascertained as 28–30 and 60–65 respectively. The ash content of drug also showed presence of calcium, magnesium and sulphate while absence of sodium, potassium and phosphate types of inorganic compounds.

**Table 3**

Physicochemical study.

Parameters		% (w/w)
Ash Values	Total ash	6.54
	Acid insoluble ash	4.74
	Water soluble ash	2.76
	Sulphated ash	0.21
	Alcohol	7.27
Extractive values	Water	5.54
	Ether	2.31
Moisture content		9.09



**Figure 2.** HPTLC profile of three extracts under Day and UV light at 254 nm and 366 nm.

2a: petroleum ether extract; 2b: chloroform extract; 2c: ethanol extract.

### 3.4. Qualitative and quantitative phytochemical investigation

Preliminary phytochemical investigation revealed the presence of plants secondary metabolites such as carbohydrates, protein, tannins, glycosides, flavonoids and steroids. Quantitatively steroids, flavonoids and carbohydrates were determined and found to be 15.67% w/w, 25.78% w/w and 12.11% w/w respectively.

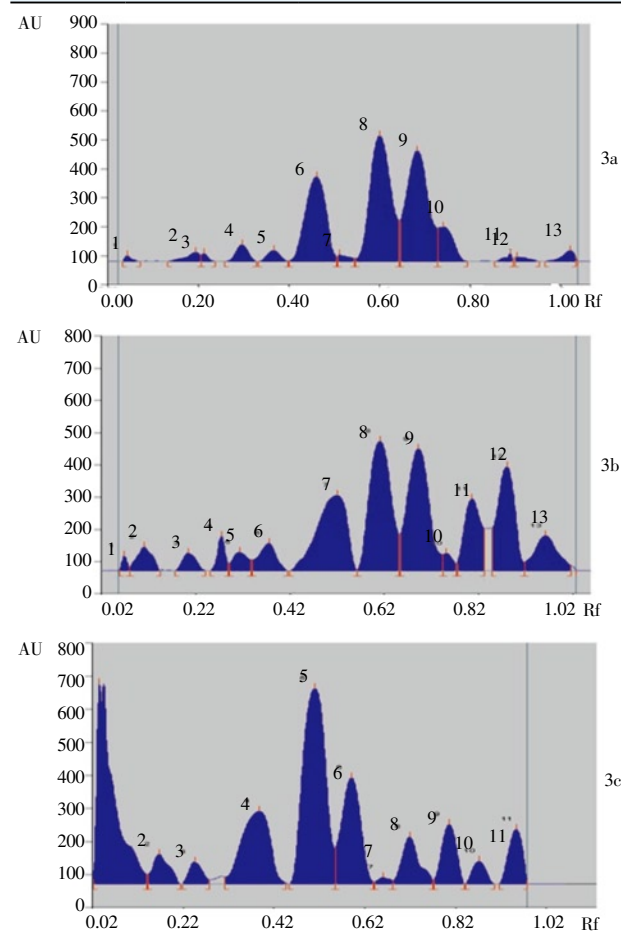
### 3.5. HPTLC fingerprinting

HPTLC fingerprinting of different extracts of *R. hypocrateriformis* had been carried out by using various types of solvent system for separation of as many as phytochemicals. Results revealed that the presence of several constituents in the extracts. The number of constituent in the extract and their retention factor (Rf) are summarized in Table 4 and chromatographic profile had been shown by Figure 2 and Figure 3.

**Table 4**

HPTLC profile of different extracts of *R. hypocrateriformis*.

Extract	No. of constituents	Rf value
Pet Ether	13	0.10, 0.18, 0.20, 0.31, 0.38, 0.43, 0.57, 0.60, 0.70, 0.76, 0.83, 0.85, 0.98
Chloroform	13	0.10, 0.16, 0.22, 0.28, 0.32, 0.40, 0.52, 0.63, 0.72, 0.78, 0.82, 0.90, 0.95
Ethanol	11	0.15, 0.19, 0.25, 0.37, 0.49, 0.53, 0.56, 0.70, 0.83, 0.89, 0.97



**Figure 3.** HPTLC chromatogram of three different extracts.

3a: Petroleum ether extract; 3b: chloroform extract; 3c: ethanol extract.



#### 4. Discussion

The present study was undertaken with the aim of developing the stringent pharmacognostic and physicochemical standards of *R. hypocrateriformis*. Anatomical characteristics are relevant in pharmacognosy.

Microscopical study revealed the presence of abundant covering trichomes, parasitic stomata, and oval shaped conjoint collateral vascular bundles. Presense of columnar palisade cell, which is the important distinguish characters, is also observed. Pharmacognostical parameters including HPTLC are helpful for the future identification and authentication of this plant in the herbal industry. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The stomatal index, palisade ration, vein islet and vein termination numbers was determined in the quantitative microscopy and they can be used to differentiate closely related other *Rivea* species. The physicochemical investigation of the drugs is an important task in detecting adulteration or improper handling of drugs. The estimation of moisture content of the drug is essential requirements in evaluation, as it supports bacteria, fungi or yeast growth. Also determinations of ash value and acid-insoluble ash value have equal importance in the evaluation and identification of inorganic impurities in crude drugs<sup>[10]</sup>. Historically plant considered as biosynthetic laboratory for a variety of compounds (secondary metabolites) that exert physiological effects. Presense of important plant secondary metabolites such as tannin, phenolic substance, steroids, carbohydrates, protein in *R. hypocrateriformis*, could make the plant useful for treating different ailments of living organism because therapeutic efficiency of any plant is usually traced by their chemical compound(s)<sup>[6]</sup>. Thus the preliminary screening tests may be useful in the detection of bioactive principles. HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug. More phytochemical research work is required for isolation, purification and characterization of biologically compounds<sup>[14]</sup>.

Since the plant, *Rivea hypocrateriformis* is useful in traditional medicine for the treatment of various ailments; it is need of time to standardize the plant for development of quality control parameters. The Pharmacognostic constants of this plant and diagnostic microscopic features reported in this work could be useful for the compilation of a suitable monograph and proper identification as well as distinguishing between closely related species.

#### Acknowledgements

Authors are thankful to Principal, Govt. College of Pharmacy Amravati for providing the necessary facilities to carry out this research work.

#### References

- [1] Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. Fruit. *J Herbal Med Toxicol* 2008; **2**: 51–54.
- [2] Dahanukar SA, Kulkarni A, Rege NN. Pharmacology of medicinal plants and natural products. *Indian J Pharmacol* 2000; **32**: 81–118.
- [3] World Health Organization. Macroscopic and microscopic examination: quality control methods for medicinal plant materials. Geneva: WHO, 1998.
- [4] Saboo SS, Taapadiya GG, Khadabadi SS. Antioxidant and hepatoprotective potential of isolated fraction of *Rivea hypocrateriformis*. *J Bio Sci* 2011; **11**(6): 428–432.
- [5] Shivalingappa H, Birader J, Srudresh K. Antiimplantation activity of alcoholic extract of *Rivea hypocrateriformis*. *Indian J Pharm Sci.* 1999; **61**: 1309–1310.
- [6] Singh S, Machawal L, Chauhan MG. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on micrtechnique. *J Pharm Bioallied Sci* 2010; **2**(5): 71–75.
- [7] Sanghvi GV, Koyani RD, Patil VS, Rajput KS. Morpho-anatomy of solanum pseudocapsicum. *Rev Bras Farmacogn* 2011; **21**(1): 11–15.
- [8] Khandelwal KR. *Practical pharmacognosy, techniques and experiments*. Pune: Nirali Prakashan; 2010.
- [9] Mukherjee PK. Quality control of herbal drugs. New Delhi: Business Horizons; 2002, p. 390–403.
- [10] Kulkarni YA, Ghokhale SB, Yele SY, Surana SJ, Tatiya AU. Pharmacognostic studies and preliminary phytochemical investigation on the bark of *Persea macrantha* (Nees) Kosterm (Lauraceae). *Indian J Nat Prod Pro Resour* 2011; **2**(2): 211–217.
- [11] Sutharsingh R, Kavimani S, Jayakar B, Uvarani M, Thangathirupathi A. Quantitative phytochemical estimation and antioxidant studies on aerial parts of *Naravelia zeylanica* DC. *Int J Pharma Studies Res* 2011; **2**: 52–56.
- [12] Harborne JB. *Phytochemical methods : a guide to modern techniques of plant analysis*. London: Chapman and Hall; 1998.
- [13] Wagner H, Baldt S, Zgainski EM. *Plant drug analysis*. Berlin: Springer; 1996.
- [14] Bhat JU, Nizami Q, Parray S, Aslam M, Fahamiya N, Siddiqui A, et. Pharmacognostical and phytochemical evaluation of *Melissa parviflora* and HPTLC finger printing of its extracts. *J Nat Prod Plant Resour.* 2012; **2**(1): 198–208.

#### Conflict of interest statement

We declare that we have no conflict of interest.

