



INDRAVARUNI MOOLA (*Citrullus colocynthis* Schrad) A PHARMACOGNOSTIC RESEARCH

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Abstract : Medicinal plants are one of the sources of natural products for the treatment and management of debilitating diseases. *Indravaruni* is one such medicinal plant botanically known as *Citrullus colocynthis* Schrad belonging to Cucurbitaceae family. Alkaloid and Saponins (in all parts) are the main phytoconstituents present in the plant. Pharmacognostical Evaluation - Both macroscopic and microscopic evaluation of root of *Indravaruni* (*Citrullus colocynthis* Schrad) was carried and the drug was observed for its organoleptic characteristics. The drug *Indravaruni moola* (*Citrullus colocynthis* Schrad) was also subjected to Physico-chemical tests and Phyto-chemical studies. Pale brown colour, Smooth texture, Characteristic odour and Bitter taste were the organoleptic characters observed. Microscopic examination revealed the presence of Epiblema, Cortex, Phloem, Medullary rays, Xylem parenchyma, Xylem vessels (Cavity), Root bark, Starch cells and Pigment cells. Powder microscopy showed the presence of Tracheids (pitted), Fibre, Sclereids, Epiblema cell, Colenchyma cell, Trachea, Vessel, Cork cell, Fragment of trachea, Tracheids thickening, Fragment of vessel, Fragment of fibre, Fragment of vascular bundle. Physicochemical parameters were within permissible limits as per API Standards. Quantification of phytochemical study showed the presence of Alkaloids and Saponins in higher percentage along with the presence of Tannins, Flavanoids and Phenols. All these parameters help in identifying and standardising the plant along with confirming the quality, purity and efficacy of the plant.

Keywords – *Indravaruni*, *Citrullus colocynthis*, Alkaloids, Saponins, Medicinal Plant, Pharmacognosy

INTRODUCTION

Medicinal plants are one of the sources of natural products for the treatment and management of debilitating diseases.¹ *Indravaruni* is one such medicinal plant botanically known as *Citrullus colocynthis* Schrad, belonging to Cucurbitaceae family which is mainly indicated for conditions like *Jaundice*, *Ascites*, *Asthama*, *Cough*, *Skin disorders*, *Tumors*, *Wound*, *Diabetes*, *Thyroid disorders* in *Ayurvedic classics*. *Citrullus colocynthis* is a perennial scabrid prostrate trailing herb found wild in the sandy lands of North west Punjab, Sind and Central

and Southern India. Also found indigenous in Arabia, West Asia, Tropical Africa and in the Mediterranean region.²

Description of the herb

This plant grows in warmer and tropical regions. It grows upto 1500 m above the sea levels on sandy loam, sub desert soils, and sandy sea coasts.²

Leaf - The angular leaves are alternately located on long petioles. Each leaf is almost 5 to 10 centimeters in length and has around 3 to 7 lobes. Sometimes the middle lobe might have an ovate structure. The leaves have a triangular shape with many clefts. The leaves have a rough, hairy texture with open sinuses. The upper surface of the leaves is fine green in color and the lower surface is comparatively pale. **Fruit** - Each bitter apple plant produces around 15 to 30 globular fruits having a diameter of almost 7 to 10 centimeters. The outer portion of the fruit is covered with a green skin having yellow stripes. The fruits may also be yellow in color. The ripe fruits are characterized by a thin but hard rind. The fruits have a soft, white pulp which is filled with numerous ovate compressed seeds. **Flowers** - The yellow-colored flowers appear singly at leaf axils. They are monoecious; the pistils and stamens are present in different flowers of the same plant. They have long peduncles. Each flower is also comprised of a yellow campanulate. The corolla has five lobes and the calyx is parted five ways. The female flowers are easily identified from the males by their villous, hairy ovary. **Seed** - The seeds are around 6 mm in size, smooth, compressed and ovoid-shaped. They are located on the parietal placenta. The seeds are light yellowish-orange to dark brown in color. **Root** - The Bitter Apple plant has a large perennial root that sends out long and slender, angular, tough, rough vine-like stems. The stems are normally spread on the ground and have a tendency to climb over herbs and shrubs by their axillary branching tendrils.

Phytochemistry:

Chemical constituents of *Citrullus colocynthis* Schrad.

Alkaloid I, II & III choline, α -elaterin-2D-glucoside and its aglycone (in all parts); cucurbitacin β and its glycoside, cucurbitacin I & L and their 2-O- β -D glucopyranosides (fruit, leaf, stem); arachidic, linoleic, oleic, myristic, palmitic and stearic acids (also in peels)⁷

Saponin and traces of Alkaloids.⁸

Rasapanchaka⁸

Table No. 1 Rasapanchaka of *Indravaruni moola*

<i>Rasa</i>	<i>Tikta, Katu</i>
<i>Guna</i>	<i>Sara, Laghu</i>
<i>Virya</i>	<i>Ushna</i>
<i>Vipaka</i>	<i>Katu</i>
<i>Karma</i>	<i>Kaphahara, Pittahara, Rechana</i>

Pharmacological Activities⁷

Purgative, Hypoglycaemic, Cathartic, Antipyretic, Diuretic, Anti-Histaminic, Insecticidal, Antiacetylcholine, Cardiac Depressant, Antibacterial, Nematicidal, Anticoagulant, Anti-Inflammatory, Anticancer, Antimicrobial, Antiimplantation.

MATERIALS AND METHODS

Phase 1:

1. Collection of the drug

Root of *Indravaruni* (*Citrullus colocynthis* Schrad) fresh sample was collected from Bamboo forest near Hesraghatta Lake, Bengaluru and Almatti, Bagalkote and was shade dried.

2. Authentication

The sample was botanically identified and authenticated as roots of *Indravaruni* (*Citrullus colocynthis* Schrad) by Senior Scientist (Botany), Department of Dravyaguna at Sri Sri College of Ayurvedic Science and Research, Bengaluru.

Phase 2:

Pharmacognostical Evaluation

Both macroscopic and microscopic evaluation of root of *Indravaruni* (*Citrullus colocynthis* Schrad) was carried out.

1. Macroscopic Evaluation

The organoleptic characters of powdered drug were observed for the following features:

Organoleptic Evaluation - Colour, Texture, Taste, Odour

2. Microscopic Evaluation

The cross section of root of *Indravaruni* (*Citrullus colocynthis* Schrad) was prepared and microscopic characters of the drug was observed under compound microscope and photographs were captured. Similarly, the powder microscopy was also performed and observed for its characteristic features as per the Ayurvedic Pharmacopoeia of India standards

3. Physico and Phytochemical Analysis:

The drug *Indravaruni moola* (*Citrullus colocynthis* Schrad) was subjected to Physico-chemical tests and Phyto- chemical studies at Dept. of Dravyaguna laboratory, Sri Sri College of Ayurvedic Science and Research, Bengaluru; Sri Veda Sattva Pvt. Ltd. (Sri Sri Tattva), Unit Q A & Q C Laboratory, Bengaluru and Azymes Biosciences Pvt. Ltd. Bengaluru

OBSERVATION AND RESULTS

Pharmacognostical Evaluation

1. Organoleptic Evaluation:

Organoleptic evaluation of *Indravaruni moola* (*Citrullus colocynthis* Schrad)

Table No 2. Organoleptic Characters of *Indravaruni moola*

Sl. no	Organoleptic characters	Powder
1.	Colour	Pale brown
2.	Texture	Smooth
3.	Odour	Characteristic
4.	Taste	Bitter

2. Microscopic Evaluation:

- **Transverse section of root of *Indravaruni* (*Citrullus colocynthis* Schrad):**

Root of *Indravaruni* (*Citrullus colocynthis* Schrad) had a triarch primary root anatomy. T.S of root of *Indravaruni* (*Citrullus colocynthis* Schrad.) showed the presence of Epiblema, Cortex, Phloem, Medullary rays, Xylem parenchyma, Xylem vessels (Cavity), Root bark, Starch cells and Pigment cells.

Root:

In transverse section mature root showed wavy outline consisting of 6-10 layers of rectangular, thick walled, tangentially elongated cork cells, a few filled with dark brown contents which are called as pigment cells.

Secondary cortex consists of 10-15 layers of elliptical, tangentially elongated, thin walled, parenchymatous cells. Secondary phloem is a narrow zone, composed of sieve elements, parenchyma and medullary rays. Xylem forms bulk of root, consisting of vessels, fibres, parenchyma and medullary rays; vessels mostly solitary or in groups of two to four having reticulate and spiral thickenings; fibres aseptate, thick-walled, pitted, elongated with pointed ends, lying around vessels; medullary rays poorly developed and uniseriate; starch grains oval to round in shape, 2.5-7.5 u in diameter, mostly simple or rarely compound having 2-3 components, found scattered throughout but more abundantly in phloem parenchyma.

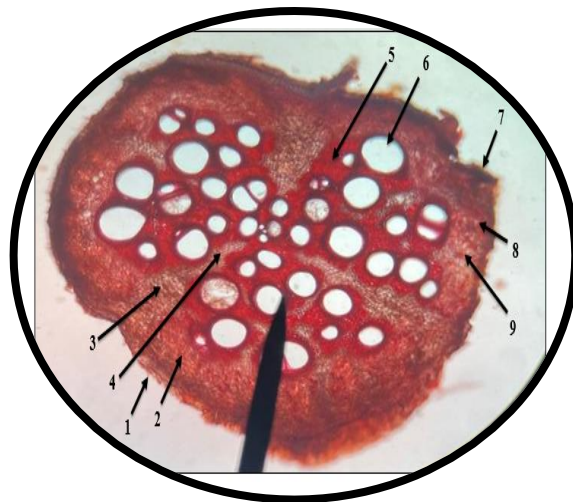


Figure No. 1a: T.S of root of *Indravaruni* (*Citrullus colocynthis* Schrad.) shows 1.Epiblema, 2.Cortex, 3.Phloem, 4.Medullary rays, 5.Xylem parenchyma, 6.Xylem vessels (Cavity). 7.Root bark, 8. Starch cells, 9. Pigment cells

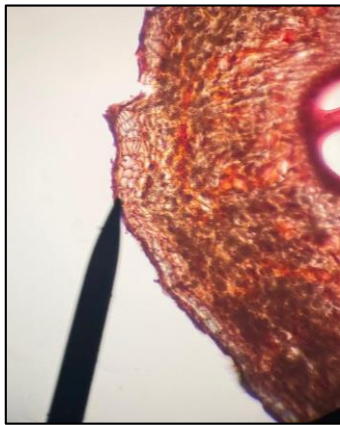


Figure No. 1b: Epiblema of root



Figure No. 1c: Phloem

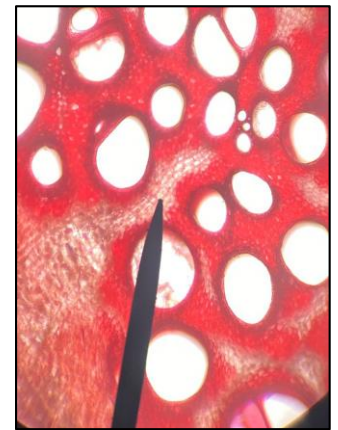


Figure No. 1d: Medullary rays

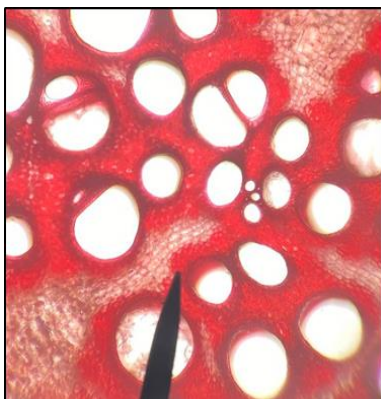


Figure No. 1e: Xylem Parenchyma

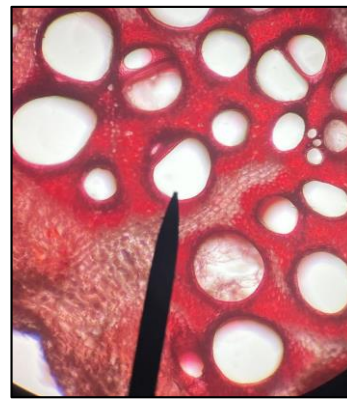


Figure No. 1f: Xylem Vessels

- **Powder microscopy of root of *Indravaruni* (*Citrullus colocynthis* Schrad):**

Powder microscopy of root of *Indravaruni* (*Citrullus colocynthis* Schrad.) showed the presence of: Tracheids (pitted), Fibre, Sclereids, Epiblema cell, Colenchyma cell, Trachea, Vessel, Cork cell, Fragment of trachea, Tracheids thickening, Fragment of vessel, Fragment of fibre, Fragment of vascular bundle.



Figure No. 2a: Tracheids (pitted)



Figure No. 2b: Fibre



Figure No. 2c: Sclereids

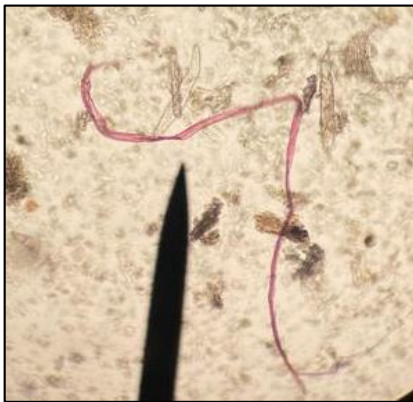


Figure No. 2d: Fibre

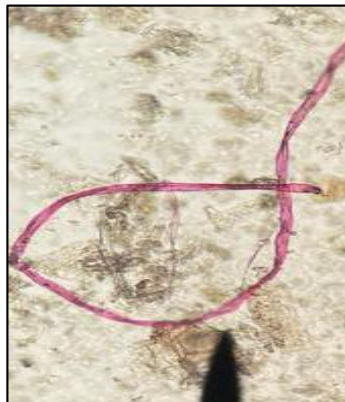


Figure No. 2e: Epiblema cell



Figure No. 2f: Collenchyma cell



Figure No. 2g: Trachea



Figure No. 2h: Vessel



Figure No. 2i: Cork cell



Figure No. 2k: Fragment of trachea



Figure No. 2l: Tracheids thickening

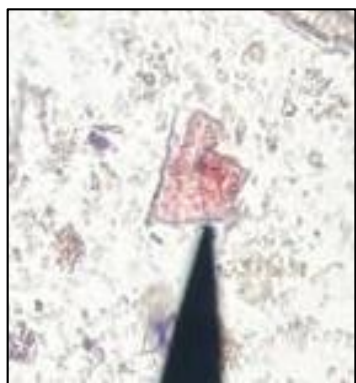


Figure No. 2m: Fragment of vessel



Figure No. 2n: Fragment of Fibre



Figure No. 2o: Fragment of vascular bundle

3. Physicochemical Evaluation:

Table No. 3 - Physicochemical Evaluation of root of *Indravaruni* (*Citrullus colocynthis* Schrad)

Sl No.	Parameters	<i>Indravaruni moola</i> (<i>Citrullus colocynthis</i> Schrad)	Reference standard as per API
1.	Foreign matter	Nil	NMT 2%
2.	Loss on drying	13.8%	-
3.	Total ash	7.55%	NMT 8%
4.	Acid insoluble ash	1.945%	NMT 2%
5.	Alcohol soluble extractive	7.256%	NLT 6.5%
6.	Water soluble extractive	22.44%	NLT 20%

4. Phytochemical analysis

Table No. 4 - Phytochemical analysis of root of *Indravaruni (Citrullus colocynthis Schrad)*

Sl No	Constituents	Tests	<i>Indravaruni moola (Citrullus colocynthis Schrad)</i>
1.	Alkaloids	Wagner's test	+
		Mayer's test	+
		Dragendroff's test	+
2.	Steroids	Salkowski test	-
		Libermann and Burchard test	-
3.	Carbohydrates	Molisch's test	+
		Iodine test (Starch Test)	+
4.	Proteins	Biuret test	-
		Millon's test	-
5.	Tannins	Gelatin test	-
6.	Phenols	Lead acetate test	-
7.	Saponins	Foam test	+
		Froth test	+
8.	Triterpenoids	Salkowski test	+
		Libermann and Burchard test	-
9.	Fixed oils	Stain test	+
10.	Flavonoids	Lead acetate test	+
		Aqueous NaOH solution test	-
		Conc . H ₂ SO ₄ test	+
11.	Glycosides	Liebermann's test	-

Quantification of Phyto-Chemicals

Table No. 5 - Quantification of Phyto-Chemicals of *Indravaruni moola* (*Citrullus colocynthis* Schrad)

SI No.	Constituents	<i>Indravaruni moola</i> (<i>Citrullus colocynthis</i> Schrad)	Reference of the test method
1.	Total Saponin	7.411%	Ayurvedic Pharmacopoeia of India
2.	Total Flavonoid	1.96%	
3.	Total Tannin	0.48%	
4.	Total Alkaloid	42.191%	
5.	Phenols	0.023%	
6.	Triterpinoids	-Ve	
7.	Steriods	-Ve	
8.	Starch	-Ve	
9.	Protein	-Ve	
10.	Carbohydrate	-Ve	
11.	Volatile oil	-Ve	

TLC Profile

Table No. 6 - Rf value of different band colour

Band colour	Rf Value	Reference standard as per API
Blue	0.15	0.16
Greyish Blue	0.27	0.30

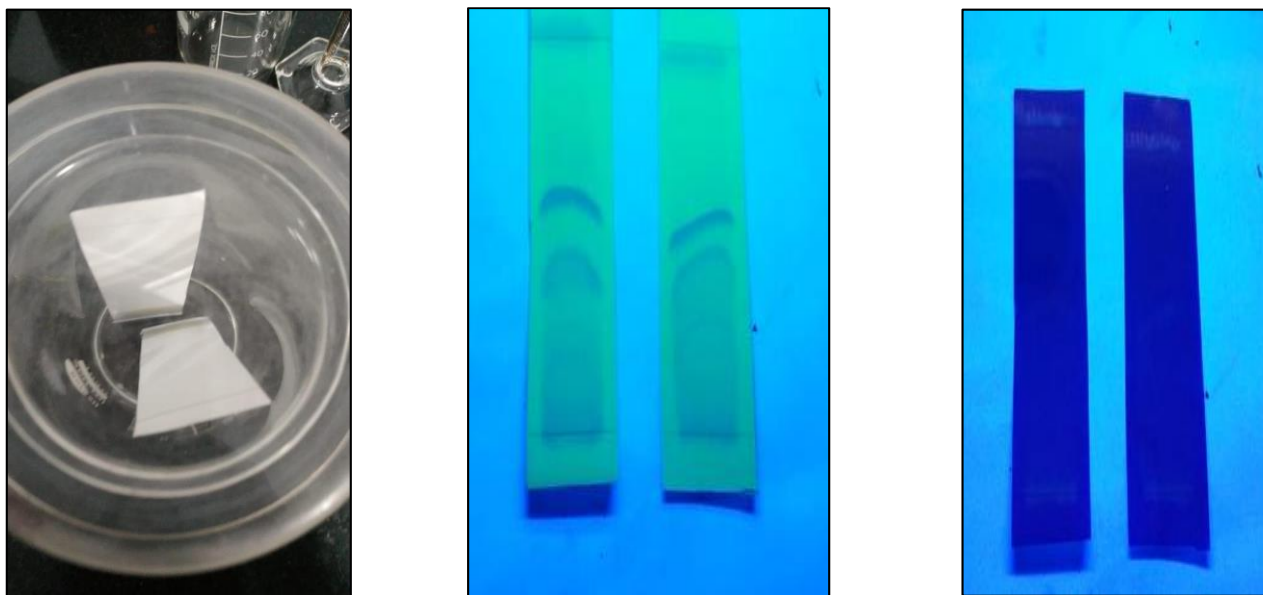


Figure No. 3: TLC Plate showing various colour bands of the drug *Indravaruni moola*

DISCUSSION

The pharmacognostic study of medicinal plants deals with standardization, authentication, and superiority of each crude drug through macromorphological and micromorphological examination along with physicochemical and phytochemical investigation. The pharmacognostic study helped in recognizing the controversial class of plant species and preventing adulteration in dry powder¹⁰

Pharmacognostical Analysis

The macroscopic examination of the root of *Indravaruni* (*Citrullus colocynthis* Schrad) revealed that it is a scabrid perennial twining herb which is a prostrate, spreading on ground with slender stem and bifid hairy tendrils. Tendrils were simple or 2-fid, slender, hairy.

The examination of the organoleptic characters of root of *Indravaruni* (*Citrullus colocynthis* Schrad) revealed that the roots were deep, branched, stout, dull-yellowish or pale-brown, smooth, cylindrical, 2-7 cm long, 0.2-2.5 cm thick, cylindrical, slightly twisted; longitudinal fissures were present; fracture short; taste intensively bitter.

Physico-chemical Analysis

The assessment of physicochemical properties of the raw drugs is essential in identifying adulteration of crude drugs.¹⁰

Physico chemical parameters of *Indravaruni moola* (*Citrullus colocynthis* Schrad) were in permissible limits when compared with API which suggests that the drug is pure, standard and free from adulterants.

Loss on drying - Loss on drying values were within the permissible limits which indicates that the drug was stable and stored properly¹²

Ash values - The ash value is the deposit leftover after heating the plant raw material at $400 \pm 25^\circ\text{C}$ in a muffle furnace, which denotes the presence of inorganic salts that occur naturally or adhering to the plant material from the environment or deliberately added as an adulterant by the humans. There are three

different procedures to determine the ash value as total ash, acid-insoluble ash, and water-soluble ash. The total volume of ash left after ignition is the total ash. This comprises both 'physiological ash' obtained from the plant tissue itself and 'non-physiological ash', obtained from the rest of the extraneous matter sticking to the surface of the plant, and it is particularly important in assessing the purity of the drug based on the presence or absence of foreign inorganic matter such as metallic salts or silica. The acid-insoluble ash is a part of total ash, which measures the amount of silica as sand and siliceous earth.¹⁰

The value of total ash and acid insoluble ash depicts the difference between mineral contaminants and deviations from the natural ash.¹¹

The total ash obtained was 7.55% and this measures the total amount of ash remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash'.¹¹

The acid insoluble ash obtained was 1.945%.

Ash value within the permissible limits indicates that the drug was genuine and pure¹².

Extractive Value - The extractive value is an essential physicochemical parameter to establish the weight of crude extract to detect the exhausted and adulterated plant materials. It reveals the nature of the phytoconstituents of the crude drug and the solvent-soluble chemical constituent¹⁰

The extractive values are valuable to determine suitable solvents for extraction and furthermore assist in the evaluation of definite constituents soluble in a particular solvent. The analytical standards of *Indravaruni moola* (*Citrullus colocynthis* Schrad) were within pharmacopoeia standards and it could be used as a reference guide for the identification and assessment of their quality and purity. The constituents of *Indravaruni moola* (*Citrullus colocynthis* were soluble in both alcohol (7.256%) and water (22.44%) with the highest solubility being in water.

Extractive values within the permissible limits indicates that the drug was pure and free from adulterants.¹²

Phytochemicals Analysis

The preliminary phytochemical qualitative analysis of root of *Indravaruni* (*Citrullus colocynthis* Schrad) revealed the presence of Alkaloids (42.191%), Saponins (7.411%), Flavonoids (1.96%), Tannin (0.48%) and Phenols (0.023 %).

The quantitative estimation of secondary metabolites reveals the concentrations of the various chemical constituents present in the root of *Indravaruni* (*Citrullus colocynthis* Schrad). The Alkaloids had the highest concentration of all the phytochemicals present. Phytochemical analysis uncovers the secondary metabolites and bioactive compounds which are usually responsible for the pharmacological activities of a plant drug.¹¹

Alkaloids and Saponins were present in higher percentage in the drug *Indravaruni moola* (*Citrullus colocynthis* Schrad) when compared to Flavonoids, Tannins and Phenols which were in negligible amount.

The general pharmacological action of Alkaloids and Saponins are –

Table NO. 7 – General Pharmacological Actions of Alkaloids and Saponins

Phyto-chemicals	General Pharmacological Actions ^{13,14}
Alkaloids	Synthesis of hormones, Immunomodulatory activity, Anti-inflammatory, Diuretic, Antitumor, Anti-ulcer, Analgesic, Anti-viral
Saponins	Immunomodulatory activity, Synthesis of hormones, Anti-microbial, Anti-viral, Anticancer, Antioxidant

Thus, the phytochemical screening benefits in identifying the different classes of the natural phytoconstituents leading to the quantification by different analytical methods helps in identifying the pharmacologically active Phytocompounds¹⁰

CONCLUSION

The results of the current study on *Indravaruni moola* (*Citrullus colocynthis* Schrad) provide critical information on pharmacognosy, physicochemical evaluation, phytochemical screening. The macroscopic and microscopic study revealed significant features, which are helpful in the discovery and authentication of this plant. The pharmacognostic, physicochemical and phytochemical analyses of *Indravaruni moola* (*Citrullus colocynthis* Schrad) ensure proper identification, purity and quality of medicinal plant

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