EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 8
363-368
Year: 2019

PHYTO-PHARMACOGNOSTICAL STUDIES OF *INDRAVARUNI MOOL (Citrullus colocynthis Root)*

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Article Received on 10/06/2019 **Article Revised on 30/06/2019** **Article Accepted on 21/07/2019**

ABSTRACT
World Health Organization survey indicated that about 70-80% of the world's population rely on nonconventional medicine, mainly of herbal sources, in their primary healthcare. In *Ayurveda*, medicinal plants as wholesome considered as a drug which contains chemical constituents in such a manner which acts via counter acting mechanism. *Indravaruni* is a well known plant of ancient medical sciences. It is described in almost all Samhita's and *Nighantus* of Ayurveda. *Indravaruni* is *Citrullus colocynthis* Schrad belongs to family Cucurbitaceae, mostly found throughout India, both wild and cultivated. All the parts of this plants are reported for various ethnobotanical and therapeutic uses. In present study roots of the plant were taken for phrmacognostic & photochemical study. Microscopically roots showed presence of thick wall epidermis, 5-8 layer cortex, pericycle & vascular bundles in between medullary rays and pith present. In Phytochemical study, on the basis of qualitative analysis phenols, tannins, flavonoids, proteins, carbohydrates, lipids, saponin, alkaloids were estimated. In Physicochemical analysis foreign matter found 0%, moisture content was found 6.47%, pH value found 5.1%, total ash found 12.98%. All these parameters will help in confirming identity, quality, purity, potency, safety and efficacy of the plant.

KEYWORDS: *Indravaruni, Saponin, Epidermis, Flavonoids, Alkaloids.*

INDRODUCTION
Medicinal plants have been used as a source of medicine since ancient time. In *Ayurveda*, medicinal plants as wholesome considered as a drug which contains chemical constituents in such a manner which acts via counter acting mechanism. Drugs derived from medicinal plants, either single or polyherbal, have been subjected to preclinical testing and promising results have been published in indexed journals. A fraction of plants described in *Ayurvedic* texts has been scientifically tested. *Indravaruni* is a well known plant of ancient medical sciences. It is described in almost all Samhita's and *Nighantus* of Ayurveda. *Indravaruni* is *Citrullus colocynthis* Schrad belongs to family Cucurbitaceae, mostly found throughout India, both wild and cultivated.[1] *Indravaruni* is a trailing herb; It has angular stems and bifid tendrils; leaves are sinuately pinnatified, flower are nomoecoius, solitary, axillary, yellow; fruits are globose pepo, 3.5-10.5 cm in diameter, smooth, green mottled with yellow blothes, pulp spongy, bitter and its seeds are many with white or light brown color.[2] Phytochemistry of C.colocynthis belongs to many classes like alkaloids, amino acids, saponin, flavonoids, glycosides, tannin etc. *C. colocynthis* is extensively used by the Ayurvedic practitioners to treat wide number disease. It is traditionally Practiced by various ethnic groups especially to treat Diabetes, Constipation, Abdominal Pain, Amennorhoea, Deafness, Rheumatism etc. Details of various important aspects such as its vernacular names and its important ethnobotanical, Ayurvedic properties, Pharmacological actions have already been published. In the present study roots of the plant were investigated pharmacognostically by using macro-microscopical characters, physico-chemical analysis and phytochemical evalution along with TLC fingerprinting.

METHODOLOGY
The Methodology has been grouped in sections
1. Collection of Drug.
2. Identification and Authentication of Drug.
3. Pharmacognostical Study.
4. Phytochemical Analysis.

COLLECTION OF DRUG
- ***Indravaruni Roots:*** The original plant have been collected from fields of Bawani Khera, Disst.-Bhiwani, Haryana, in the month of April 2018.

- ***Identification and Authentication of Drug:*** The plant was taxonomically identified and authenticated by Botanical Survey Of India (B.S.I.) Dehradun. Vide reference number –BSI/NRC Tech./Herb(Ident.)/2018-19/33, Acc. No-118191. As (Citrullus colocynthis (L.)Schrad) family Cucurbitaceae dated on 10-4-2018. Herbarium was prepared for identification and authentification of drug was done and the drug was found genuine.

- **Details of collection of genuine sample *Indravaruni* (*Citrullus colocynthis*)**

S.N	Name of the Plant	Time of Collection	Place of Collection	Authentication of Herbarium
1.	*Indravaruni (Citrullus colocynthis)*	April 2018	Bawani khera, Haryana	BSI, Dehradun

Pharmacognostical & Phytochemical evaluation[3,4]

For macroscopic study All the collected genuine samples of Root of Citrullus colocynthis were dried and studied macroscopically with naked eye, magnifying lens and measuring tape with the help of Pharmacognostical parameters i.e shape, size, surface, colour, odour and taste and findings were recorded. For microscopic study Sample was soaked in water for overnight. After sample was softened, it was holded between index finger & thumb and was cut in very thin slices with the help of blade and slices were dipped in water for some time.The sections were transferred to a watch glass containing water with the help of a brush. Thick & oblique sections were rejected.

Then the sections were soaked in Safranine stain for 5 min. & then transferred to a watch glass with plain water, so that excess stain gets washed away. The sections were transferred on a glass slide & few drops of glycerin water was added on it. With the help of a needle, a cover slip was placed on the section gently & then the slides were observed in microscope & photos were taken.

For examining the characters of the powder take sufficient amount of Root powder of Citrullus colocynthis different chemical reagents on a slide and warm over a low flame for a short time. Put drop of glycerin on the slide, cover it with the cover slip and observe under the microscope.

Chemical reagents used for staining of the powder samples were as follows

Safranin, Dilute Ferric chloride, Eosine, Methylene Blue, HCl Phlorogucinol, Iodine solution. Various physicochemical parameters were calculated as per Ayurvedic pharmacopeial methods using the powdered drug. Drug samples were tested for the presence of various active phyto compounds like phenols, tannins, flavonoids, proteins, reducing sugar, carbohydrates, lipids, saponins, alkaloids. For TLC study following methods were used:

a) Chromatography plates

T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width).

b) Activation of pre-coated Silica gel 60 F_{254}

Plates were dried in hot oven at 105^0 C for one and half hour.

c) Preparation of mobile solution

Toluene: Ethyl Acetate: Formic Acid (6: 3: 1).

d) Preparation of test solution

4 gm powdered drugs were extracted with 100 ml of ethanol (90 percent) in a Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

e) Sample application

Samples were applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate.

f) Visualization

Ultraviolet flor/Iodine Vapour/p-Anisaldehyde Sulphuric acid.

g) R_f Value

Measured and recorded the distance of each spot from the point of its application and calculated Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

h) Calculation of R_f Value

$$R_f = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

OBSERVATION AND RESULTS

A) PHARMACOGNOSTICAL STUDY

1. MACROSCOPIC STUDY

(i) Macroscopic characters of different parts of *Indravaruni (Citrullus colocynthis)*

a) Leaf: Leaves very variable, 3.6-6.3 cm long, 2.5-5.0 cm wide, pinnately lobed in outline, generally 3 lobed, sometimes 3-7 lobed, middle lobe largest, each lobe deeply pinnatifid; petiole 1.3-2.5 cm long, entire leaf densely hirsute.

b) Root: Root available in cut pieces of 2-7 cm long, 0.2-2.5 cm thick, cylindrical, slightly twisted; dull yellow; longitudinal fissures present.

c) Fruit: White or pale yellowish-white, light, pithy fragments upto about 6 cm long and 2 cm thick; externally convex with ridges and flattened areas 5-10

mm wide resulting from peeling with a knife.
d) Flower: The flowers are yellow and solitary in the axes of leaves and borne by yellow greenish peduncles.
e) Seed: Seeds are flattened, ovoid, yellowish white to dark brown, 5mm long & 3mm wide.

2. ORGANOLEPTIC STUDY

Organoleptic characters of Roots of *Indravaruni (Citrullus colocynthis)*

S. No	Test sample	Citrullus colocynthis Root
1	Taste	Bitter
2	Odour	Characteristics
3	Colour	Light Brown
4	Shape	Cylindrical, Slightly twisted
5	Size	2-7 cm

3. MICROSCOPIC STUDY

(i) Microscopic characters of Roots of *Indravaruni (Citrullus colocynthis)* in transverse section (T.S)

- Tick walled epidermis was present.
- 5 to 8 Layer cortex was found.
- Single layer endodermis was present.
- Pericycle and Vascular bundle in between medullary rays was found.
- Pith was absent.

4. POWDER MICROSCOPY STUDY

Features seen in the Powder microscopy of Roots of *Indravaruni (Citrullus colocynthis)* are given in tabulated form.

S.No.	Features	Present/Absent
1.	Pitted vessels	Present
2.	Trichomes	Present
3.	Cork cells	Present
4.	Fibers	Present
5.	Starch Grain	Present
6.	Cork cells	Present

B) PHYSICOCHEMICAL STUDY

S. No.	Test	Citrullus colocynthis Root
1.	Foreign Matter(%)	**0**
2.	Moisture content (%)	6.47
3.	pH value	5.1
4.	Aqueous soluble extract (%)	18.74
5.	Alcohol soluble extract (%)	12.57
6.	Petroleum ether soluble extract (%)	1.10
7.	Total ash (%)	12.98
8.	Acid insoluble ash (%)	5.48
9.	Water soluble ash (%)	7.61

C) PHYTOCHEMICAL STUDY

1. Carbohydrate test

S. No.	Name of test	Citrullus colocynthis Root	
		Aqueous extract	Alcohol extract
A.	Molisch test	+ ve	+ve
B.	Benedict test	+ ve	- ve
C.	Barfoed's test	- ve	- ve
D.	Fehling test	+ ve	+ ve

2. Alkaloids

A.	Dragondrof test	+ ve	+ ve
B.	Wagner's test	- ve	+ ve
C.	Hager's test	+ ve	- ve

3. Amino acids

A.	Ninhydrine test	+ ve	+ ve

4. Proteins

A.	Biuret test	+ ve	- ve
B.	Xanthoprotic test	+ ve	+ ve
C.	Millon's test	- ve	- ve

5. Saponin

A.	Foam test	+ ve	- ve

6. Glycosides

A.	Borntragor's test	+ ve	- ve

7. Phenolic compound

A.	Phenolic test	- ve	- ve

8. Steroids

A.	Salkowaski reaction	+ ve	- ve

9. Tannin

A.	$FeCl_3$test	- ve	- ve
B.	Lead acetate test	+ ve	+ ve
C.	Potassium dichromate test	- ve	- ve

D) CHROMATOGRAPHIC STUDY

A. TLC (Thin Layer Chromatography) analysis for Root of Citrullus colocynthis

Mobile solution: Toluene: Ethyl acetate: Formic acid (6:3:1 ml) for given sample.

Visualization: Ultra Violet Flor, p-Anisaldehyde Sulphuric Acid.

- **TLC Profile of samples on Silica Gel G60F254.**

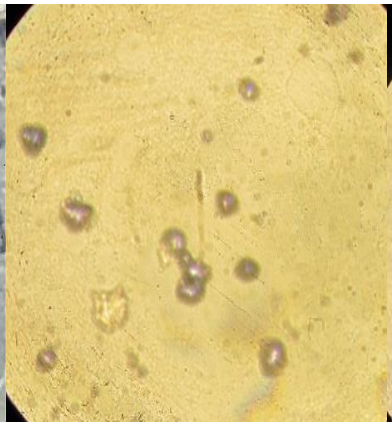
S.No.	Sample	Visualization	No of Spots	Rf Values
1.	Root	Uv Flor	5	0.16,0.19,0.24,0.36,0.45
2.	Root	p-Anisaldehde Sulphuric Acid	5	0.16,0.19,0.24,0.36,0.45

STUDY PHOTOGRAPHS: 1. Macroscopic Characters of Indravaruni
DIFFERENT PARTS OF INDRAVARUNI

2. Powder microscopy of Root of Indravaruni

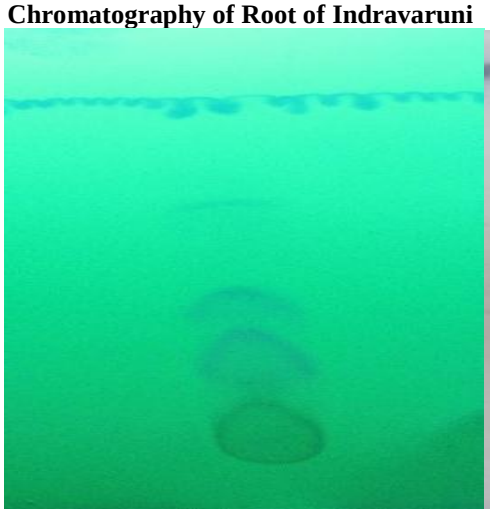
Cork cells **Trichomes** **Pitted vessels**

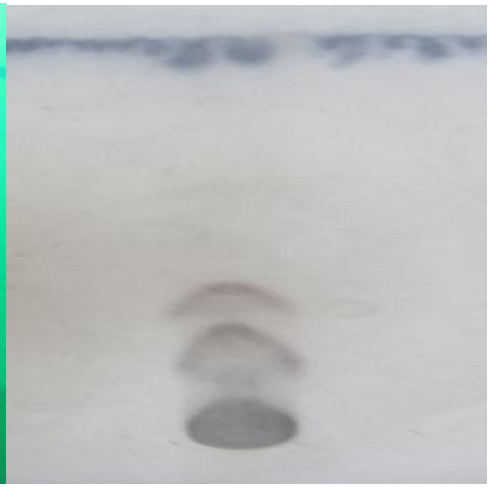

Fibers


Starch grain


Cork cells

3. Thin Layer Chromatography of Root of Indravaruni


UV
(R_f Value: 0.16, 0.19, 0.24, 0.36, 0.45)


p anisaldehydesulphuric acid
(R_f Value: 0.16, 0.19, 0.24, 0.36, 0.45)

CONCLUSION

Finally it has been concluded that Pharmacognosy of medicinal herbs is very important as to confirm their identity, quality, purity, potency, safety and efficacy. In this study Physicochemical study showed result that foreign matter was 0% in sample. **Moisture content** is a water holding property of test substance and Moisture content of test sample was found 6.47% .**pH** value play significant role in compatibility of drug with body fluid, site of action and stability of drug. And pH value was found in test sample is 5.1.

Extractive value is directly relative to strength or potency of drug which estimates in different solvents. Water soluble extract value was found in test sample is 18.74 %, Alcoholic soluble extractive value was found 12.57 %. Petroleum ether extractive value was found 1.10 %. **Ash value** is the indicator of the presence of inorganic & earthy matter in the plant. Total ash in test sample was found 12.98 %. The acid insoluble content indicates the presence of siliceous matter and heavy metals test sample found 5.48 %. Water soluble ash estimate the inorganic water soluble salt in sample was found 7.61 %. The Phytochemical study showed the presence or absence of different phytochemicals: In Root of Citrullus colocynthis **Molisch test** is positive in aqueous and alcoholic extract it indicate that monosaccharides /disaccharides / polysaccharides are present. **Benedict test** is positive in aqueous extract of samples which indicates the simple sugar may be present. **Fehling test** was positive in alcoholic and aqueous extract of sample that indicates reducing sugar were present. Alkaloids was identified in aqueous and alcoholic extract due to positive of **Dragondrof test**. **Wagner's test** are positive in alcoholic extract, **Hager's test** are positive in aqueous extract. Amino acid and protein are present in test sample. **Foam test** was positive in aqueous extract of sample that indicates saponin was present. **Borntragor test** was positive in aqueous extract of sample that indicates glycoside were present in the sample. **Salkowaski reaction** was positive in aqueous extract of sample that indicates steroids were present. **Lead acetate test** was positive in test sample that's indicate that tannin is present in sample. In Root of Citrullus colocynthis T.L.C. shows under UV and p-Anisaldehyde sulphuric acid. Rf values are - **R_f. 0.16, 0.19, 0.24, 0.36, 0.45**.

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