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Research Article

PRELIMINARY PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING OF LEAVES OF *COSMOSTIGMA RACEMOSUM* (ROXB.) WIGHT.

Aparna S G[1]*, A Shahul Hameed[2]

*[1]MD Scholar, [2]Professor & HOD, Department of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India.

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ABSTRACT

Pharmacognostic and phytochemical evaluation are necessary for drug authentication and for prediction and confirmation of pharmacological activities of any plant part. *Cosmostigma racemosum* (Roxb.) Wight, a traditional and folklore drug in Kerala with many reputed usages, locally called as *Vaattuvalli*, is a shrubby twiner of Apocynaceae family. Leaves are the most used plant part. As no scientific data regarding its standards were available, preliminary pharmacognostic, physico-chemical and phytochemical evaluation of the leaves were done as per the guidelines of Ayurveda Pharmacopoiea of India and WHO. The study revealed that leaves of *Cosmostigma racemosum* (Roxb.)Wight are simple, opposite, exstipulate, apex caudate, base cordate and with a characteristic chilly odour. Microscopic examination of leaves revealed the presence of characteristic features such as lacticifers, secreting cells, absence of stomata on the upper epidermis, presence of paracytic stomata on the lower epidermis and presence of calcium oxalate crystals especially druse crystals. The preliminary phytochemical evaluation revealed the presence of tannins, alkaloids, steroids, saponins which indicates the wide range of pharmacological activities of the plant. The 13 peaks in the HPTLC profile indicate a wide pharmacological prospect of the leaves. The ICP-MS analysis confirmed that heavy metals like Cd, Cr, Zn, Cu, Pb and As present in the leaves are within permissible limits. Physicochemical parameters such as moisture content, different ash values, volatile oil content, different extractive values, ph, fibre content, and sugar content were also determined. All these findings can serve as standards for assuring the safety, quality and purity of the drug.

INTRODUCTION

Cosmostigma racemosum (Roxb.) Wight (abbreviated as CR), known as *Vaattuvalli* in Malayalam is a twiner belonging to family Apocynaceae. Globally it has distributions in the Indo-Malaysian regions. In India it is found in Kerala, Tamilnadu, Maharashtra and Karnataka (Fig 1). Eventhough not described in Ayurvedic classics, it is a plant of ethnobotanical importance. The plant is used as food and medicine by the tribal people of different parts of India.

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Various therapeutic uses are reported for different parts of the plant in many ethnobotanical surveys. The botanical treatise Hortus Malabaricus also have descriptions about the plant[1]. The leaves are said to be effective in healing wounds and inflammation. The bark is useful in fever and root barks have chologogic activity.[2,3] Standardization of such medicinal plants are essential for the scientific validation of traditional and folklore medicinal practices. As no scientifically validated data regarding the standardization is available, preliminary pharmacognostic and phytochemical screening of leaves of CR is done as a primary step.

Scientific name: *Cosmostigma racemosum* (Roxb.) Wight

Family: Apocynaceae (according to APG system III)

Synonyms

Asclepias racemosa Roxb.[4]

Cosmostigma acuminata Wight[4]

Plant taxonomy of *Cosmostigma racemosum* (Roxb.) Wight

Taxonomical positioning of the plant is as follows[4]

Kingdom	: Plantae
Phylum	: Tracheophyta
Class	: Magnoliopsida
Order	: Gianales
Family	: Apocynaceae
Subfamily	: Asclepiadoideae
Genus	: Cosmostigma
Species	: racemosum

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Material

The whole plant of CR was collected from Nagalassery Hills, Vavanoor, Palakkad and from Sicilipuram Uchakkada, Thiruvananthapuram. The samples were authenticated for its botanical identity at the Pharmacognosy Unit, Govt Ayurveda College, Thiruvananthapuram. The voucher specimen was kept at the department of Dravyaguna Vigyana, Govt. Ayurveda College, Thiruvananthapuram. The leaves of CR were dried in shade and stored in air tight container

Macroscopic Examination of Leaves of *Cosmostigma racemosum* (Roxb.) Wight

Fresh leaves of the plant were taken for macroscopic examination. Macroscopic examination of leaf included the evaluation of different characters such as type of leaf, arrangement, size, shape, colour, surface characters, margin, apex, base, venation, petiole, stipule, odour and taste

Microscopic Examination of Leaves of *Cosmostigma racemosum* (Roxb.) Wight

The microscopic evaluation of leaves of *Cosmostigma racemosum* (Roxb.) included:

- Qualitative microscopy
  Leaf microscopy
  Powder microscopy
- Quantitative microscopy
  Determination of stomatal index.
  Determination of palisade ratio

Qualitative Microscopy

Leaf Microscopy

Fresh leaves were taken for microscopic examination. Fine transverse sections of the leaves were taken and stained with safranin. It was mounted on glass slide with help of glycerin and observed under microscope. Various identifying characters and cell composition were recorded and microphotography was taken.

Powder Microscopy

Sufficient amount of leaf powder of CR was mounted on a glass slide. One drop of glycerine was added. The slide was then examined under digital microscope. Ocular 40x and 100x objectives was used for all observations and diagnostic features were photographed.[5]

Quantitative Microscopy

The following features were studied.

Determination of Stomatal Index

Stomatal index (SI) is the ratio of stomata to the total number of epidermal cells in 1mm square area expressed in percentage. A piece of leaf was cleaned and the upper and lower epidermis was peeled out separately. Peeled out piece was kept on slide, stained with safranin and mounted in glycerine and examined under 100x microscope. The number of stomata and epidermal cells in each field were counted. Stomatal index on both surfaces of leaves were calculated by using the formula,

Stomatal index (%) = (S / S+E) x 100

Where S denotes the number of stomata and E, the number of epidermal cells.[6]

Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells below a single upper epidermal cell. Small pieces of leaves were mounted and examined under microscope. A number of groups of each of four epidermal cells were first focused. Palisade ratio was then obtained by dividing the total number of palisade cells under 4 epidermal cells and then dividing it by 4. Readings were taken for 5 times from different pieces and average was calculated.[6]

Preliminary Physicochemical Analysis

Preliminary physicochemical analysis of leaves of *Cosmostigma racemosum* (Roxb.) Wight was done as per standard procedures mentioned in Ayurveda Pharmacopoeia of India and WHO guidelines.[6] The parameters such as moisture content, different ash values, volatile oil content, different extractive values like water soluble extractive and alcohol soluble extractive, fibre content, and sugar content were determined.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis was done with the methanolic extract of CR leaf powder for detection of presence of various secondary metabolites as alkaloids, starch, tannins, phenols, flavanoids, saponins and steroids.

Qualitative Analysis of Phytochemicals

Test for Steroids

Steroids were detected by evaporating the methanolic extract of leaves of CR in a watch glass and to the residue; acetic anhydride and conc. H_2SO_4 were added through the sides. The change of colours from yellow to brown indicates the presence of steroids.[7]

Test for Alkaloids

Methanolic extract of leaves of CR was evaporated and to the residue, dil. HCl were added and filtered. To the filtrate Dragendroff's reagent (Potassium bismuth iodide solution) was added, an orange brown precipitate indicate the presence of alkaloids.[7]

Test for Tannins

10g of the CR leaf powder was weighed and transferred into a 250ml round bottomed flask. 100ml of distilled water was added and refluxed for one hour. The solution was filtered hot using an ordinary filter paper into a conical flask and made up to 100ml. The made up 100ml filtrate was then transferred into a standard flask and 2ml of lead acetate solution was added, appearance of a precipitate indicate the presence of tannins.[7]

Test for Phenols

Methanolic extract of leaves of CR was evaporated and the residue of extract was dissolved in alcohol and neutral $FeCl_3$. A violet color indicates the presence of phenols.[7]

Test for Flavanoids

The residue of methanolic extract of leaves of CR was dissolved in alcohol. Magnesium ribbon and conc. HCl were added to it. A reddish brown colour indicates the presence of flavanoids[7].

Test for Saponins

A few drops of sodium bicarbonate solution was added to the methanolic extract of leaves of CR and shaken well. Formation of a honey comb like frothy appearance confirms the presence of saponins.[7]

Chromatography - High Performance Thin Layer Chromatography

The hydromethanolic extract of the leaves of CR was used as the test solution for High Performance Thin Layer Chromatography (HPTLC) analysis. 2μl of each of hydromethanolic extract solution was applied as 8mm band length in the 10×200 silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The sample applied plate was kept in TLC twin through developing chamber (after saturated with solvent vapour) with respective mobile phase up to 70mm.To evaporate solvents from the plate, the developed plate was dried by hot air. The plate was kept in photo documentation chamber (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV254nm and UV366nm. The peak table, peak densitogram were noted using the WinCATS 1.3.4 version.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The determination of Lead, Cadmium, Iron, Zinc, Copper, and Chromium in ppm levels in test drug were carried out using ICP-MS. Leaf powder of CR was taken and addition of hydrogen peroxide solution and concentrated nitric acid was carried out. Milli-Q water was added to each acid and the vessels were slowly shaken and sealed after 30 minutes of pre-digestion. The plant sample was then digested by using Mars Protein Analyser Microwave Digester. Finally the entire digested sample was transferred into volumetric flask and made up the volume with Milli Q water. Blanks were also prepared in the same manner by excluding the samples. The solutions were subjected to quantitative analysis for determination of elements by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrument. The standard solution and sample solution was introduced in ICP-MS. Then 5% acidifying water (freshly prepared) was added in ICP-MS followed by standard solutions of different linearity. Again addition of freshly prepared 5% acidifying water was carried followed by blank sample. Then sample solution was added and the data analysis was done by using Agilent 7700 Series ICP –MS Mass Hunter Workstation data analysis software.

RESULTS AND DISCUSSION

Results of Macroscopic Evaluation (Fig 2)

Macroscopic evaluation is simple and important method for the standardization of any drug after its identification and authentication. The results of macroscopical evaluation of CR are summarized in the Table 1.

Table 1: Macroscopic evaluation of *Cosmostigma racemosum* (Roxb.) Wight leaf

S. no	Characters	Observations
1	Type	Simple
2	Arrangement	Opposite
3	Petiole	Petiolate. 3cm–5cm long
4	Stipule	Exstipulate

5	Size	Length– 7cm -12.5cm, Breadth– 5cm – 7.5cm
6	Shape	Cordate
7	Colour	Adaxial surface – Dark green Abaxial surface – Light green
8	Surface characters	Adaxial surface – Glabrous Abaxial surface – veins are more prominent
9	Margin	Entire
10	Apex	Caudate
11	Base	Cordate
12	Venation	Unicostate reticulate
13	Odour	Characteristic chilly like
14	Taste	Bitter, slight sweet

Results of Microscopic evaluation of leaves of *Cosmostigma racemosum* (Roxb.) Wight

Transverse section of leaf of *Cosmostigma racemosum* (Roxb.) Wight (Fig 3)

The transverse section of leaf of CR was taken. The findings are described under 3 headings

a. Midrib
b. Lamina
c. Petiole

Midrib (Fig 4)

- The transverse section of midrib was broader on the abaxial side.
- Single layer of epidermis was seen on the upper and lower side of the midrib.
- Multicellular, uniseriate trichomes and glandular trichomes were seen on both surfaces.
- Epidermal layer was covered by a layer of cuticle.
- Inner to the epidermal layer 3-5 layers of collenchyma cells can be seen on both upper and lower surface.
- Inner to the collenchymal zone, thin walled, isodiametric or circular parenchymal cells can be seen. Intercellular spaces were present in between the parenchymal cells.

Vascular bundle

- Crescent shaped
- It was of bicollateral open type

b. Lamina (Fig 5)

- Dorsiventral type
- Upper and lower epidermis covered by cuticle
- Mesophyl tissue composed of palisade parenchyma and spongy parenchyma.

Upper Surface

- Epidermal cells on the adaxial surface were larger than the lower epidermal cells.
- Palisade tissue was present only on the adaxial surface. Single layer of palisade cells filled with chlorophyll were seen.
- Inner to the palisade cells, spongy parenchyma cells were seen.

Lower Surface

- Epidermal cells were smaller than the adaxial surface
- Palisade cells were absent in the lower surface

Prismatic and druse crystals of Calcium oxalate can be seen in midrib and lamina. Plenty of lacticifers were present in the mid rib and lamina. Secreting cells with tannins were also seen

Stomata (Fig 6)

- Stomatal cells were found only on lower epidermis
- Rubiaceous or paracytic stomata were seen.

c. Petiole (Fig 7)

The transverse section of petiole of CR showed following characters

- The diagrammatic transverse section of petiole had a slight concave groove on the adaxial surface and was broad circular on the abaxial surface.
- Outer epidermal layer was covered by a thick cuticle. Multicellular trichomes and glandular trichomes were seen on the both surfaces.
- Inner to the epidermis 2-4 layers of cholenchyma cells were seen, followed by 2-3 layers of chlorenchyma cells.
- Inner to this a broad area of parenchyma cells was seen.
- Plenty of lacticifers, druse crystals, prismatic crystals were seen in the parenchymal cells.
- 3 sets of vascular bundles were seen in the petiole. Two circular shaped near the adaxial surface on both sides and one crescent shaped in the central region.

- In the vascular bundles radially arranged xylem vessels can be seen. Phloem fibres were seen as patches encircling the xylem.
- Parenchymal cells surrounding vascular bundles had plenty of prismatic crystals.
- The region above the concave side of crescent shaped vascular bundle appears to be hollow in transverse section due to the presence large number of lacticifers.

Results of powder microscopy of leaf of *Cosmostigma racemosum* (Roxb.) Wight (Fig 8)

The powder microscopy of leaf of CR revealed the presence of fibres, annular vessels from vascular bundles, druse and prismatic crystals, trichomes and group of sclereids.

Determination of stomatal index and palisade ratio

The stomatal index and palisade ratio are important features for identification of plant species. The stomatal index of CR calculated was 25.67%.The palisade ratio of CR was calculated as 5.8

Results of preliminary physicochemical evaluation of leaves of *Cosmostigma racemosum* (Roxb.) Wight

The results of physicochemical evaluation are enlisted in the Table no 3

Table 2: Results of Preliminary Physicochemical Evaluation of Leaves of *Cosmostigma racemosum* (Roxb.) Wight

S.No.	Parameter	Observations
1	Foreign matter	Nil
2	Moisture content	8.2
3	Volatile oil	Nil
4	Water soluble extractive	11.12
5	Alcohol soluble extractive	28.65
6	Total ash	8.21
7	Acid insoluble ash	4.75
8	Total sugar	7.03
9	Reducing sugar	5.27
10	Fibre content	12
11	ph of leaf powder	6.95 at 28.9°C

Results of Preliminary phytochemical analysis of leaves of *Cosmostigma racemosum* (Roxb.) Wight

The results of preliminary phytochemical analysis of leaves of CR are summarized in theTable no 3

Table 3: Results of preliminary phytochemical analysis of leaves of *Cosmostigma racemosum* (Roxb.) Wight

S.no	Qualitative analysis	Observation
1	Steroid	+++
2	Flavanoid	+
3	Phenol	++
4	Alkaloids	+++
5	Tannin	+++
6	Saponin	+++

+++ - High presence, ++ - Moderate presence, + - Less presence.

The presence of these secondary metabolites confirms a wide pharmacological profile for the drug.

Results of High Performance Thin Layer Chromatography analysis (Fig 8, 9)

HPTLC analysis of hydromethanolic extract of leaves of CR was done with the solvent system Toluene: ethyl acetate: methanol in the ratio 8: 3: 1. Track 1, 2 and 3 were applied at 12.5mm 25mm 35.5mm respectively (10µl of the sample)

- The chromatogram scanned for track 1 showed 13 peaks with Rf values ranging from -0.0 to 0.70 with areas 11.33% and 22.62% respectively
- The chromatogram scanned for track 2 showed 12 peaks with Rf values ranging from -0.08 to 0.69 with areas 11.59% and 21.32% respectively

- The chromatogram scanned for track 3 showed 11 peaks with Rf values ranging from -0.08 to 0.71 with areas 13.04% and 15.64% respectively

The presence 11- 13 peaks indicates wide range of biologically active components in the leaf extract of CR.

Results of Heavy Metal Analysis IC-PMS

According to WHO, heavy metal analysis is a must for herbal drug standardization. Heavy metal analysis of leaves of CR was done using ICPMS. The results observed are summarized in Table 4.

Table 4: Results of Heavy metal analysis of *Cosmostigma racemosum* (Roxb.) Wight by IC-PMS

Heavy metals analysed	Concentrations in ppm	Maximum permissible limit
Cadmium	Below detectable limit	0.3
Chromium	1.1	2
Copper	8.5	20
Zinc	13.4	20
Lead	0.43	10
Arsenic	0.11	3

The results confirms that the heavy metals Cadmium, Chromium, Copper, Zinc, Lead and arsenic are present within permissible limits in leaf powder of CR.

CONCLUSION

Cosmostigma racemosum (Roxb.) Wight is an extrapharmacopoeial plant having many traditional and folklore usages. The preliminary pharmacognostical and phytochemical analysis of the leaves of the plant are done according to the guidelines of WHO and Ayurveda Pharmacopoiea of India. The findings may serve as a standard reference for future research.

REFERENCES

1. K S Manilal. Van Rheede's Hortus Malabaricus (Malabar Garden). Vol-7. English Edition. Thiruvananthapuram; University of Kerala; 2003.p 117-118
2. Kritikar KR, Basu BD. Indian Medicinal Plants Vol-3. Dehradun; International Book Distributors; 2008. p.1633-1634
3. Nadkarni KM. Dr. K. M. Nadkarni's Indian materia medica Vol- 1. Bombay; Popular Prakashan; 2007. p.385
4. Cosmostigma racemosum (Roxb.) Wight. 2008. Available from: https://indiabiodiversity.org/species/show/229341
5. Kumar S, Kumar V, Prakash OM. Microscopic evaluation and physiochemical analysis of Dillenia indica leaf. Asian Pacific journal of tropical biomedicine. 2011 Oct 1; 1(5): 337-40.
6. Shafqat Ali Khan, Muhammad Ibrar, Barkatullah. Pharmacognostic Evaluation of the Leaf of Rhus succedanea var. himalaica. J. D Hooker. Afr J Tradit Complement Altern Med. 2016 Sep 29; 13(6): 107-120
7. Department of AYUSH. Ayurvedic Pharmacopoeia of India, Part II (Formulations), New Delhi; Government of India, Ministry of Health and Family Welfare, 2008.

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***Address for correspondence**
Dr. Aparna S G
MD Scholar,
Department of Dravyagunavijnanam,
Govt. Ayurveda College,
Thiruvananthapuram, Kerala,
Ph no: 9495181793
Email:
dr.aparnanikhil@gmail.com

FIGURES

Figure 1: *Cosmostigma racemosum* (Roxb.) Wight

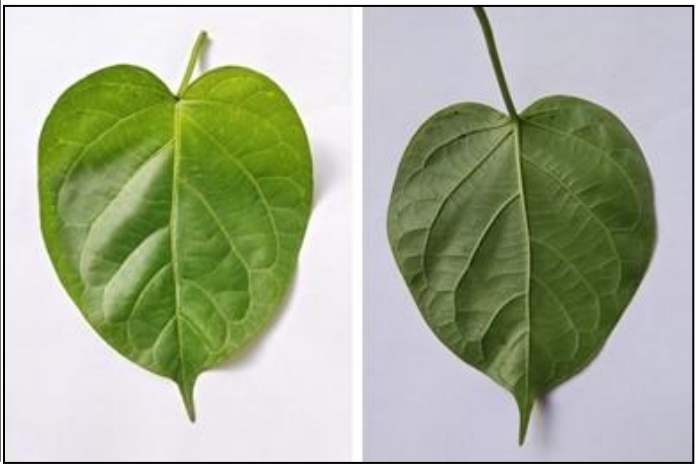

Figure 2: Morphology of *Cosmostigma racemosum* (Roxb.) Wight leaves

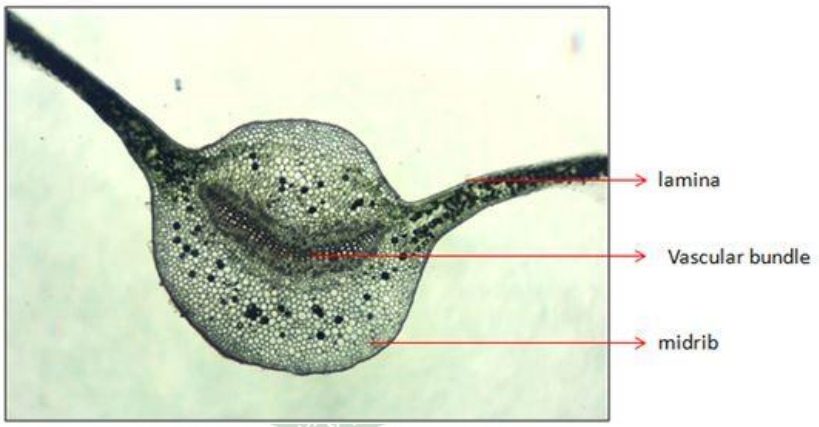

Figure 3: Transverse section of leaf

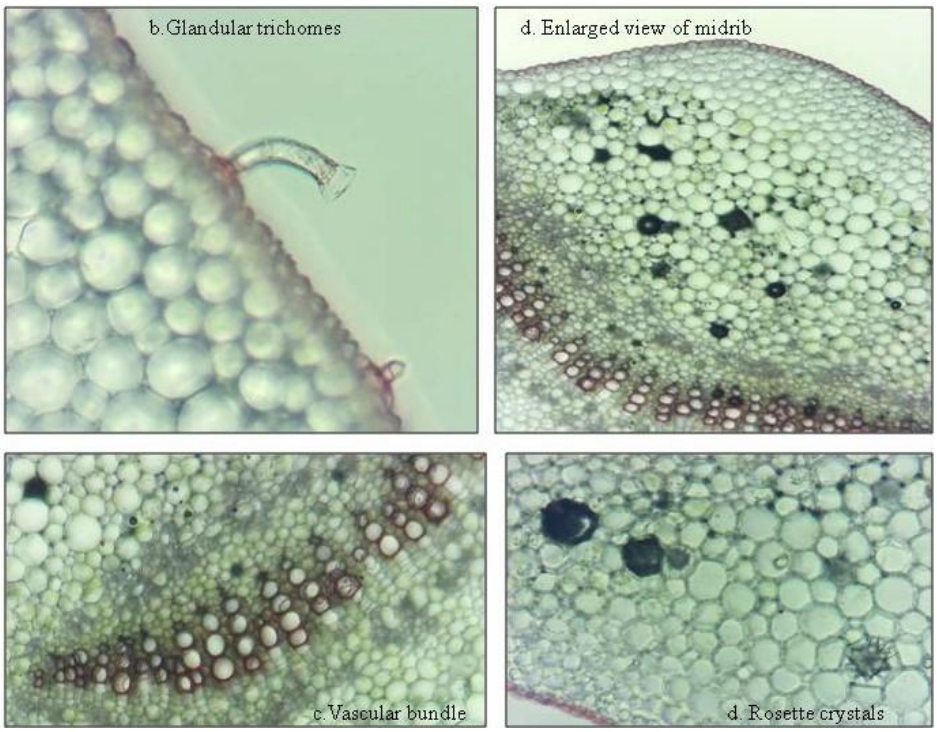

Figure 4: Enlarged view of midrib and its components

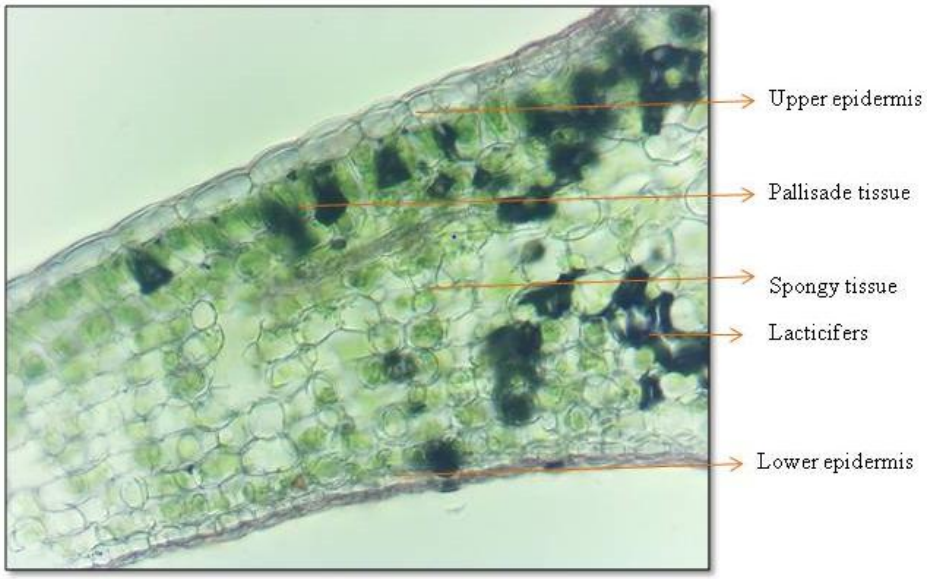

Figure 5: Lamina of *Cosmostigma racemosum* (Roxb.) Wight

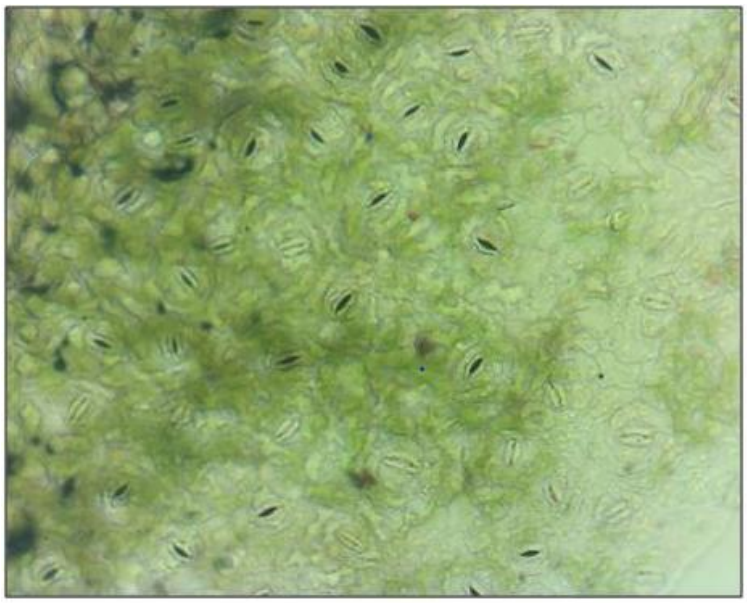

Figure 6: Paracitic Stomata on the lower epidermis

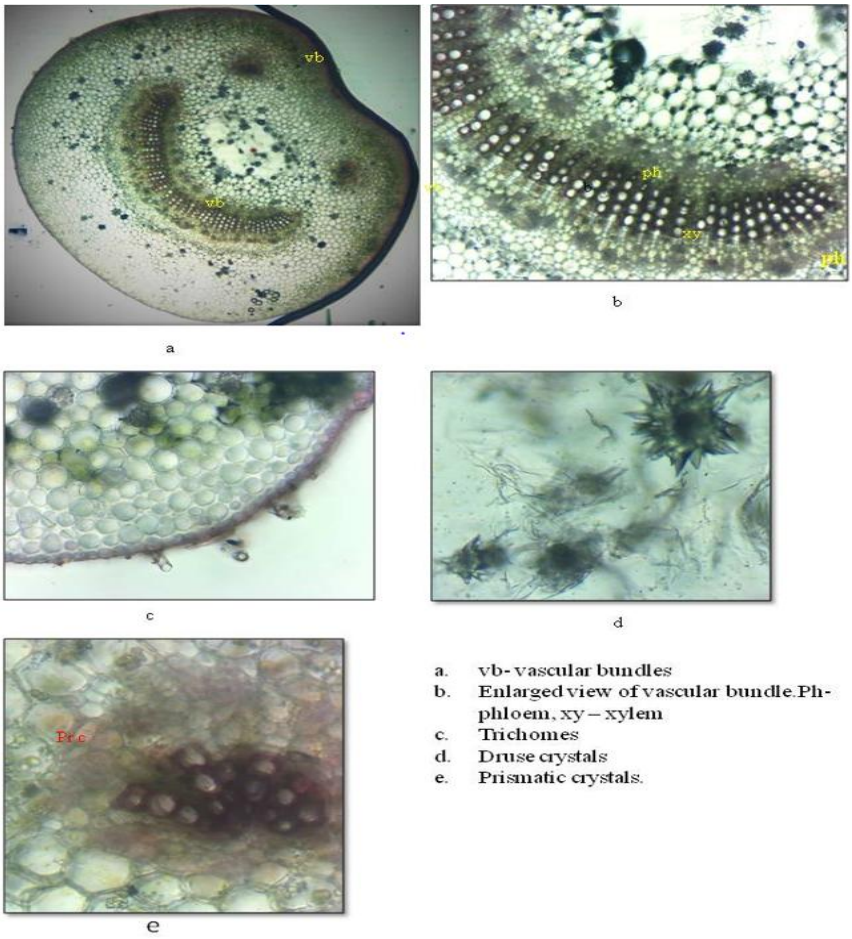

Figure 7: Petiole of *Cosmostigma racemosum* (Roxb.) Wight. a. Transverse section b, c, d, e enlarged view

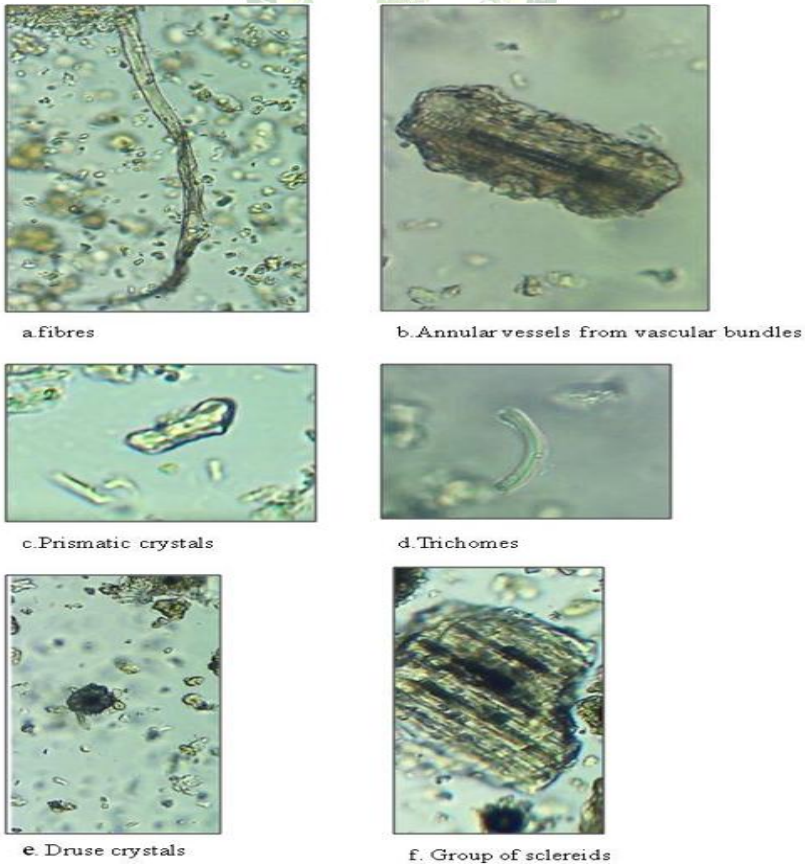

Figure 8: Powder microscopy of leaves of *Cosmostigma racemosum* (Roxb.) Wight

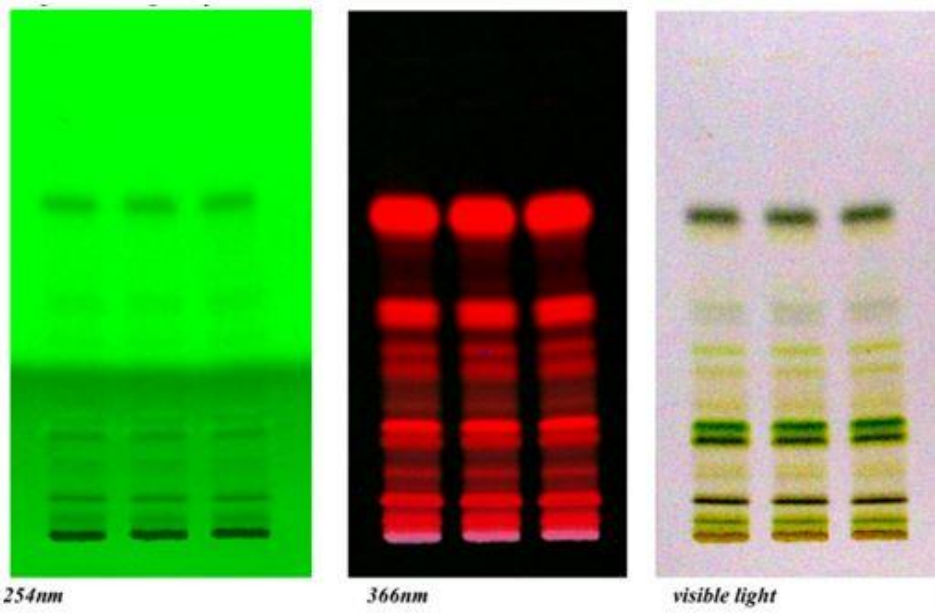

Figure 9: HPTLC plate visualized under (a) UV 254 nm (b) UV 366 nm (C) White light

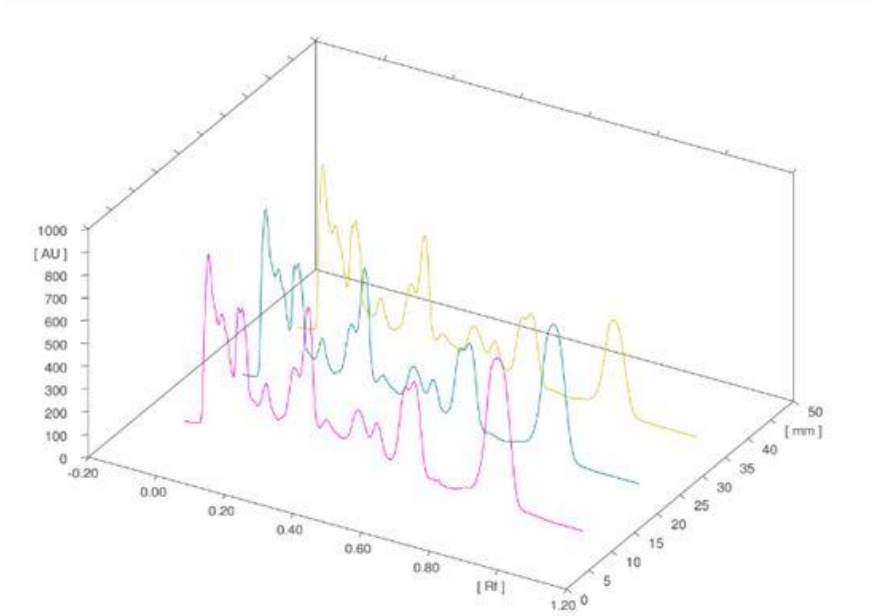

Figure 10: Graphical representation of 3 tracks in HPTLC