IJBPAS, August, 2025, 14(8): 4588-4601

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EXPLORING THE MICRO-MORPHOLOGY, CHEMO-MICROSCOPY, ANALYTICAL STANDARDS AND PHYTOCHEMICAL PROFILE OF BARK OF *IXORA ARBOREA* ROXB., - AN TRADITIONAL MEDICINAL PLANT

KAVIYARASI M[1*], MUTHUSAMY P[1], VIJAYA BHARATHI R[1], THENMOZHI R[1], MONISHA T[1], VANITHA N[1] AND RADHA R[1]

1: Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai – 03, Tamil Nadu, India

***Corresponding Author: Dr. Kaviyarasi M: E Mail: mkaviyarasi2000@gmail.com**

Received 10th April 2024; Revised 4th May2024; Accepted 7th Sept. 2024; Available online 1st Aug. 2025

https://doi.org/10.31032/IJBPAS/2025/14.8.9352

ABSTRACT

Introduction: *Ixora arborea* Roxb., is a garden tree or evergreen shrub, belonging to the Rubiaceae family, which is acclaimed for its therapeutic qualities as mentioned in conventional medicine. **Objective:** Accurate identification and assessment are essential to avoid adulteration due to growing ethno-medicinal significance. Therefore, the purpose of the current study was aimed to explore the Pharmacognostical and physicochemical parameters; phytochemical screening of bark of *Ixora arborea*. **Methods**: Both the macroscopic and microscopic characteristics of the fresh and powdered bark were assessed. Physicochemical characteristics and the phytochemical profile of the ethanolic bark extract of *Ixora arborea* was screened using standard protocols outlined in WHO and Ayurvedic Pharmacopoeia of India. **Results:** Microscopy of mature bark indicated the existence of exfoliating cork cells; cortex consists of parenchymatous cells, stone cells and lignified cortical fibers; phloem consists of starch grains, prismatic crystals, sclereids and lignified walls. The microscopical study of powder unveiled the presence of stone cells, cork cells, fiber bundle, styloid and prismatic crystals. Histochemical analysis revealed the presence of oil globules in phloem; mucilage, phenolic compounds, alkaloids and lignin in cortex. The preliminary phytochemical screening confirmed the presence of glycosides, alkaloids, flavonoids, steroids and triterpenoids. Total phenolic content and total

flavonoid contents of the extract were measured. Using chromatographic techniques like TLC, R_f values were calculated, indicating the presence of the different phytoconstituents. **Conclusion:** The Pharmacognostical standards and physic-chemical parameters of the bark of *Ixora arborea* were established and serve as quality control guidelines for its purity, identity and standardization.

Keywords: *Ixora arborea* Roxb., macroscopy, microscopy, physicochemical studies, and phytochemistry

INTRODUCTION:

According to WHO, ethno-medicines are naturally crop up compounds derived from plants that have undergone little or no industrial processing, and have been utilized in local or regional healing traditions to cure sickness **[1]**. Many medicinal plants relieve symptoms in a way that is akin to that of allopathic medications **[2]**. About 2000 natural medicines are included in Indian Materia Medica, the majority of which are derived from various ancient systems and folklore practices **[3]**. The selection, formulation and application of herbal medications for the control, treatment, and management of wide range of health issues are frequently guided by the useful guidelines found in herbal medicines, which integrate multiple therapeutic experiences and practices of traditional medical systems that may span many previous generations **[4]**. Every plant has a distinct character in terms of its botany, chemistry and therapeutic potency, therefore it is crucial to study pharmacognostic features of a medicinal plant in order to correctly identify it as well as to comprehend its structure and biology **[3]**.

The World Health Organization emphasizes the value of both qualitative and quantitative techniques for sample characterization, biological markers or chemical markers quantification and the fingerprint profiles analysis. A number of official monographs for the standardization of botanicals have been developed in countries across the world. For whole drugs, macroscopic/sensory evaluation is usually enough for the drugs to be identified. Visual inspection in terms of shape, size, surface characteristics, colour, consistency, odour, taste, fracture and appearance of the cut surface of the drug provide the easiest and fastest way to determine its identification, purity and even quality. Establishment of identity of powdered drugs depends on the microscopic recognition of characteristic cell types and cell contents. Microscopic analysis is indispensible for the identification of the correct species and /or the right plant part that is to be present. Physicochemical and

phytochemical studies are also important for the standardization of plant materials **[5]**.

Ixora arborea Roxb. (Syn. *Ixora parviflora* Vahl.), is a blooming garden tree that is a member of the Rubiaceae family, which is rich in flavonoids. It yields bunches of fragrant white blooms. In Siddha medicine, it is known as Shulundukora, Korivi and the local names in Ayurveda include Nevaari, Nepali, Navmalika and Vaasanti **[6]**. In Ayurveda system, Vaasanti is described as being light, bitter, and cooling. It harmonizes the tridoshas such as Vata, Pitta and Kapha. The plant is mostly found growing in dry deciduous forests that are found in Bihar, Western Bengal, Western, Central and South India. Root bark infusion is an traditional remedy used by the tribes of Nellore district in Andhra Pradesh, to treat burning micturition and jaundice. *I. parviflora* flowers was used for catarrhal bronchitis, whooping cough, hemoptysis, and dysmenorrhea. The roots infusion was administered to cure diarrhoea and as a sedative for nausea, hiccoughs, loss of appetite, fever, gonorrhea, as well as to treat dropsy **[7]**. Because of the presence of saponins, flavonoids, tannins, glycosides, phenols and triterpenoids in whole plant of methanolic extract, it has hepato protective and antioxidant properties that significantly raise the levels of the enzymatic antioxidants of liver like catalase, superoxide dismutase, and glutathione levels **[8]**. Anti-obesity property of butenolic fraction of this plant was evaluated in Wistar rat model with obesity induced by high fat diet **[9]**. However, no previous work has been done to evaluate the Pharmacognostical, physicochemical and phytochemical standards of the bark of *Ixora arborea.* This work is aimed at evaluating in detail the Pharmacognostic standards of *Ixora arborea,* hence developing standardization parameters of this plant.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant material *Ixora arborea* was collected in the month of October - 2023 at Tanjore district, Tamil Nadu, India. The plant material was recognized botanically and authenticated (Certificate No: 701.27122303) by Dr. K.N. Sunil Kumar, Head of Pharmacognosy and Research officer at the Centre Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India, Arumbakkam, Chennai – 600106, Tamil Nadu. The procured bark was thoroughly washed, shade dried, ground into coarse powder, sieved through 40 mesh size and stored in a air-tight container. This powder is utilized for physicochemical study and phyto-chemical analysis.

I. Pharmacognostical studies

Macro – morphological examination

The fresh barks of *Ixora arborea* were visually examined. The organoleptic characteristics like taste, odour and colour of the bark were noticed and recorded. Using Nikon D-5600 Digital camera, the morphological features of the bark such as its shape, size, surface, fracture and thickness were recorded.

Microscopic examination

Anatomy of bark

The specimen was kept for more than 48 hours in the fixative FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). Using a sharp blade, the conserved bark specimens were sliced into thin section pieces and the sections were then dyed with 0.8% Safranin and 0.5% Astra Blue. Under bright field light, transverse sections were captured with the help of Axiolab5 trinocular microscope coupled with Zeiss Axiocam208 color digital camera. A scale bar was used to show magnifications.

Powder microscopy

After being cleared with a saturated solution of chloral hydrate, a small amount of the bark powder was placed on a microscopic slide with a drop of 50% glycerol. To confirm the existence of starch grains, sample was treated with an iodine solution. Under bright field light, features were viewed using Nikon ECLIPSE E200 trinocular microscope mounted with Zeiss ERc5s digital camera. Diagnostic characters of photomicrographs were taken and recorded **[10-13]**.

Chemo - microscopic examination

Chemo-microscopic / histochemical study was performed to find out the presence or absence of Phenolic compounds, starch grains, alkaloids, mucilage, oil globules, lignin, cutin and resin using standard techniques **[11-14]**.

II. Determination of analytical standards

Analytical standards / physicochemical constants of the bark such as the percentage of ash values, extractive values, loss on drying, swelling index and foaming index were determined as per the standard procedures mentioned in API and WHO to evaluate the purity and quality of the drug **[15-17]**.

Qualitative analysis of inorganic elements and heavy metals

After turning the powdered bark into ash, 50%v/v hydrochloric acid was added and kept for an hour. After filtering, the filtrate was examined for the presence of inorganic elements such as borate, arsenic, copper, chloride, iron, phosphate, sulphate, mercury, lead, and cadmium.

Quantitative estimation of heavy metals

Quantitative estimation of heavy metals such as arsenic, lead, cadmium and mercury was done by Atomic Absorption Specrtoscopy(AAS) method (Thermo scientific iCE FIOs) which enables elemental analysis at percentage to parts per million (ppm) levels for a wide range of metals. **[18]**.

III. Phytochemical studies

Preparation of extract

The Soxhlet apparatus was filled with dried coarsely ground sample of *Ixora arborea* (70 gm) and ethanol was used as a solvent for extraction. The concentration of the extract was done by using rotary vacuum evaporator. The color, yield percentage, and nature of the extract was noted and proceeded for more detailed Phytochemical screening.

Qualitative phytochemical analysis

The ethanolic extract of bark of *Ixora arborea* was qualitataively analyzed for the secondary metabolites, such as phenolic compounds, flavonoids, tannins, alkaloids, glycosides, steroids, terpenes, and saponins, using conventional procedures **[19-22]**.

Quantitative estimation of phytoconstituents

Total phenolic content

Using the Folin – Ciocalteu's assay technique, the total phenolic content present in the ethanolic extract of *Ixora arborea* bark were been measured. Gallic acid is used as a standard.

Total flavonoid content

Total flavonoid content was determined by calorimetric method, using quercetin as a standard. **[23, 24]**.

Fluorescence analysis

Fluorescence analysis was carried out in day light and in UV light. The bark powder and extracts were treated with different solvents and the fluorescence was observed in day light and in near and far UV light **[25]**.

Thin Layer Chromatography

Chromatography was performed on pre-coated silica gel $60F_{254}$ aluminium sheets. The ethanolic extract of *Ixora arborea* was dissolved in respective solvent. The extract was applied to the plate as band using capillary tube, positioned 2cm from the bottom of the plate. The spotted plates were subjected to development in the TLC developing glass chamber which is pre-saturated with mobile phase (Toluene: Ethyl acetate - 9:1). The developed plate was dried and treated with suitable spraying reagent. The chromatogram was then observed under UV-254 nm and UV-366 nm. And the Rf values were calculated using the following formula. **[26, 27]**.

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

RESULTS AND DISCUSSION

I. PHARMACOGNOSTICAL STUDIES

Macro-morphological study

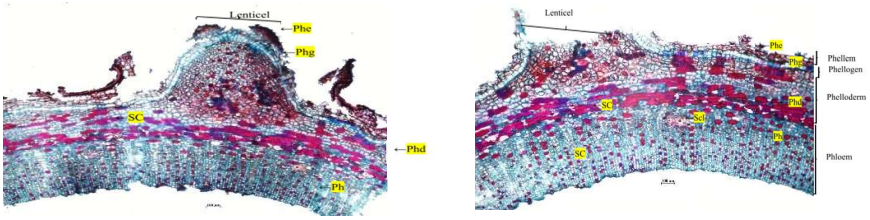
The bark is dark brown coloured on outer surface with circular brownish lenticels, brownish white colour on inner surface, hard and brittle; cut pieces 7 to10 cm long, 1 to 2 cm thick; odour is nil, taste is astringent.

Figure 1: Stem bark of *Ixora arborea* Roxb.

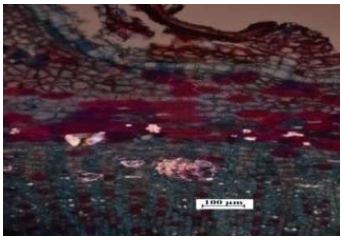
Microscopic examination

Anatomy of stem bark

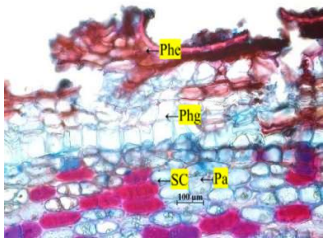
Transverse Section of bark shows outer layers of exfoliating cork cells (phellem) followed by 3 to 4 layers of cork cambium (phellogen) formed of compactly arranged homogenous rectangular thin walled cells; cortex is wide and comprise of 10 to 11 layers of parenchymatous cells filled with plenty of starch grains; both isolated and group of stone cells are scattered throughout the cortical parenchyma in which some of them shows lignified walls; few large thick-walled lignified cortical fibers are also found in cortex; inner most region of the bark is occupied by well-developed broad secondary phloem forming the major part of the section; phloem is formed of sieve elements and phloem parenchyma; radially running phloem rays enriched with starch grain can be seen traversing through the phloem; several stone cells, prismatic crystals and starch grains are distributed throughout phloem; phloem fibers are found associated with thin walled angular shaped group of sclereids with lignified walls.



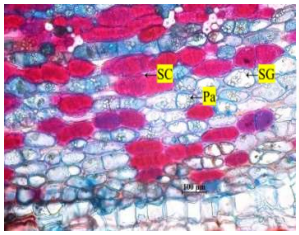
T.S of *Ixora arborea* bark Enlarged view



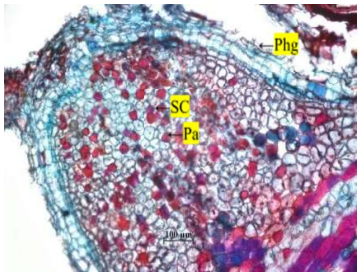
TS of bark under polarized field



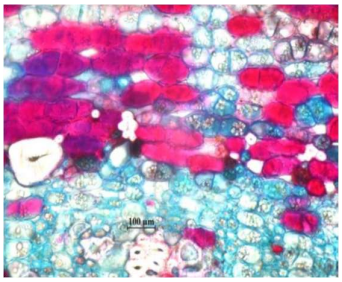
Upper portion enlarged



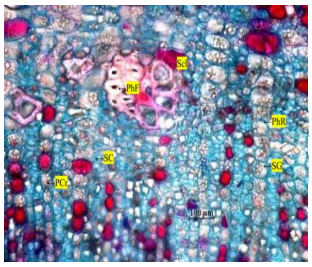
Enlarged view of lenticel



TS view of lenticle



Cortex region enlarged



Phloem region enlarged

Pa - parenchyma; PCr - prismatic crystals; Ph - phloem; Phd - phelloderm; Phe - phellem; PhF - phloem fibre; Phg - phellogen; PhR - phloem ray; SC - stone cell; Scl - sclereid; SG - starch grain

Figure 2: Anatomy of *Ixora arborea* bark

Powder microscopy

The powder is dark brown coloured and odourless with astringent taste; shows the characters like cork cells in surface view, thick-walled fibre with narrow lumen, parenchyma with starch grains, cells with brownish content, sclerieds, stone cells, styloid and prismatic crystals, and cells with brownish content.

Chemo-microscopical studies

Phenolic compounds, starch grains, alkaloids found in cortex; mucilage observed in cortex and phellogen (Cork cambium); oil globules present in phloem region; lignin detected in cortical fibers; cutin and resin absent in bark.

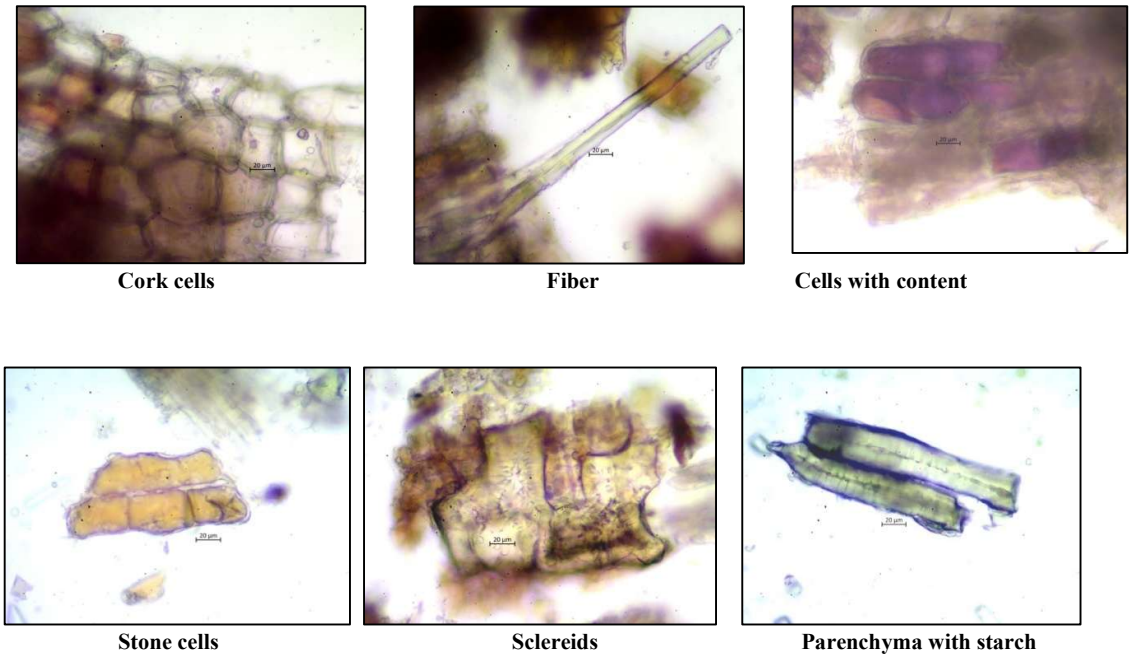
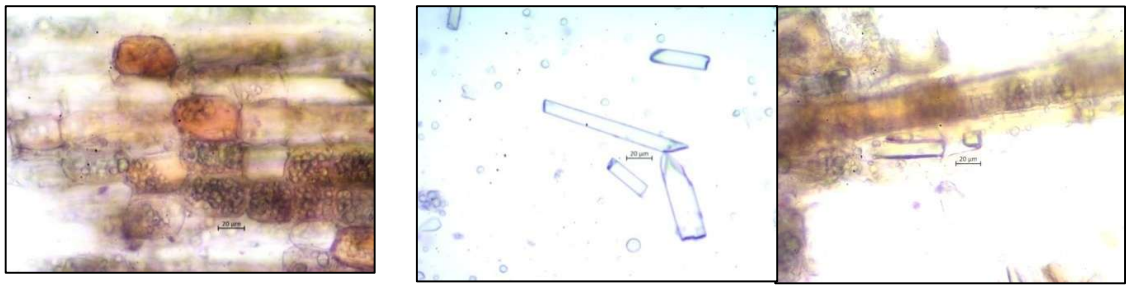
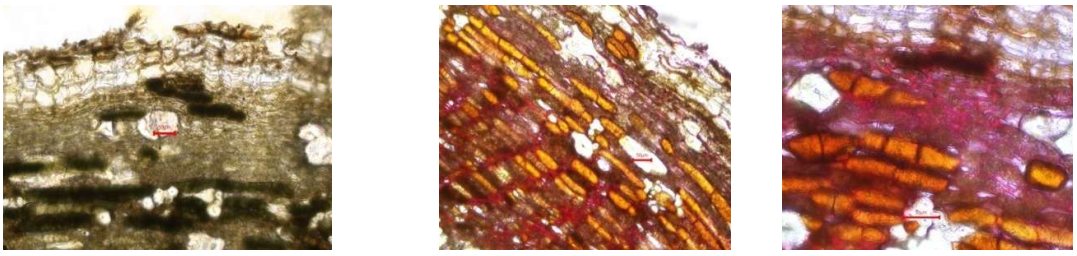
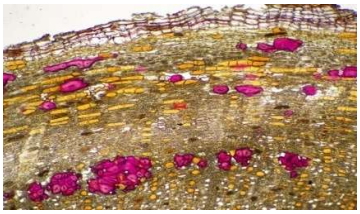


Figure 3: Powder microscopy of *Ixora arborea* Roxb. bark

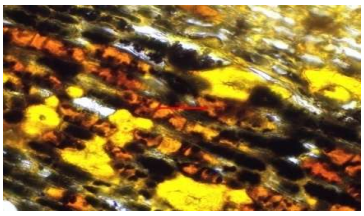


Styloid crystals and Prismatic crystals of Calcium oxalate

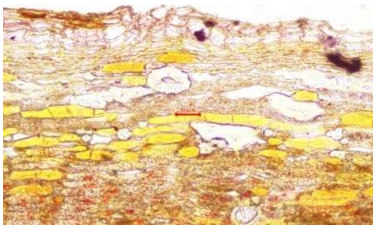




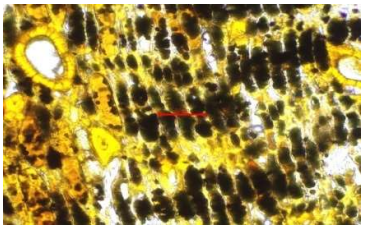
Lignin in cortical fibers



Alkaloids in cortex



Oil globules in phloem region



Starch grains in cortex

Figure 4: Chemo-microscopy of *Ixora arborea* bark

Table 1: Chemo-microscopical test of *Ixora arborea* bark

S. No.	Test for	Reagent	Observations	Region
1.	Starch	Section + Iodine solution	Blue colour	Cortex
2.	Lignin	Section + Phloroglucinol & 50% HCl	Pink to cherry red cell walls	Cortical fibers
3.	Phenolic compounds	Section + alcoholic Ferric chloride	Bluish balck colour	Cortex
4.	Calcium oxalate crystals	Section + Glycerine water & 8 % H_2SO_4	Shiny crystals of prisms and styloid shape seen	Phloem
5.	Alkaloid	Section + Wagner's reagent	Yellow to reddish brown	Cortex
6.	Oil globules	Section + Sudan-IV	Orange to red coloured globules	Phloem
7.	Mucilage	Section + Ruthenium red	Red coloured contents	Cortex and Phellogen

II. ANALYTICAL STANDARDS OF BARK

The bark of *Ixora arborea* powder was studied for the analytical properties as per Ayurvedic Pharmacopoeia of India and expressed in percentage are presented in **Table 2**.

Table 2: Analytical standards of Bark of *Ixora arborea*

S. No.	Parameter	Result (% w/w)
I.	Ash values	
1.	Total ash	11 ± 0.33
2.	Water soluble ash	7.6 ± 0.25
3.	Acid insoluble ash	3.5 ± 0.1
4.	Sulphated ash	9.26 ± 0.15
II.	Extractive values	
1.	Water soluble extractive value	8.8 ± 0.12
2.	Alcohol soluble extractive value	2 ± 0.22
III.	Loss on Drying	2.5 ± 0.17
IV.	Swelling index	Nil
V.	Foaming index	< 100

Note: Each value represents Mean ± Standard deviation (n=3)

Qualitative analysis of inorganic elements and heavy metals

Qualitative analysis of heavy metals and inorganic elements were analyzed in powder sample of *Ixora arborea*. The reports of qualitative analysis are given in the **Table 3**.

Table 3: Qualitative analysis of inorganic elements and heavy metals

S. no	Elements	Observation
1.	Arsenic	+
2.	Borate	+
3.	Chloride	+
4.	Copper	-
5.	Iron	+
6.	Lead	-
7.	Mercury	-
8.	Phosphate	+
9.	Sulphate	+
10.	Cadmium	+

Note: (+) indicates Presence, (-) indicates Absence

Quantitative estimation of heavy metals by Atomic Absorption Spectroscopy method

The findings of the detection and quantification of heavy metals in *Ixora arborea* powder by AAS technique were displayed in **Table 4**. As per this estimation of heavy metals in the sample, the levels are within the recommended limits. It is harmless and safe on consumption.

Table 4: Quantitative estimation of Heavy metals

S. No.	Element	Result (ppm/g)	As per API specification (ppm)
1.	Arsenic	0.012	Not more than 3.0
2..	Lead	Nil	Not more than 10.00
3.	Cadmium	0.03	Not more than 0.30
4.	Mercury	Nil	Not more than 1.00

III. PHYTOCHEMICAL STUDIES

Qualitative and quantitative phytochemical analysis

The percentage yield of ethanolic extract of *Ixora arborea* was found to be 7.1%w/w as dark brown colour semi solid nature. The secondary metabolites present and their various concentrations in the crude extract of *Ixora arborea* are presented in the **Table 5 and 6** respectively.

Table 5: Preliminary phytochemical analysis

S. No.	Tests	Ethanolic extract
1.	Flavonoids	+
2.	Polyphenols	+
3.	Alkaloids	+
4.	Glycosides	+
5.	Steroids and Triterpenoids	+
6.	Carbohydrates	+
7.	Tannins	+
8.	Saponins	-

Note: (+) indicates Presence, (-) indicates Absence

Table 6: Quantitative estimation of phytoconstituents

S. No.	Parameter	Concentration (µg / ml)
1.	Total phenolic content	9.77 ± 0.03
2.	Total flavonoids content	7.49 ± 0.13

Note: Values are expressed as mean ± Standard deviation (n=3)

Fluorescence analysis

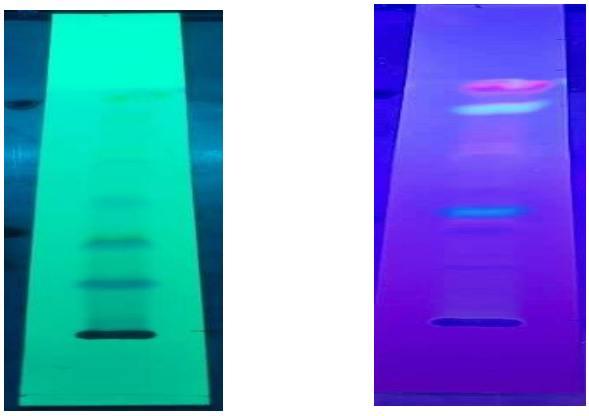
Fluorescence analysis of powder and ethanolic extract of bark of *Ixora arborea* was analyzed in visible and UV light (Short & Long UV) and showed in **Table 7**.

Table 7: Fluorescence analysis of powder and ethanolic extract

S. No	Treatment	Visible light		Short UV (254 nm)		Long UV (366 nm)	
		Powder	Extract	Powder	Extract	Powder	Extract
1.	Distilled water	Pale brown	Brown	Pale green	Brownish green	Pale blue	Dark blue
2.	Ethanol	Pale green	Pale brown	Pale green	Pale green	Pale blue	Pale blue
3.	50% H_2SO_4	Dark brown	Pale yellow	Dark brown	Pale green	Dark blue	Light brown
4.	H_2SO_4	Dark brown	Dark brown	Dark green	Brownish green	Dark blue	Dark blue
5.	10% HCl	Straw yellow	Very light yellow	Light green	Very light green	Pale blue	Pale blue
6.	HCl	Light yellow	Light brown	Light green	Pale green	Very light purple	Dark blue
7.	50% HNO_3	Pale yellow	Light yellow	Pale green	Light green	Purple	purple
8.	1N NaOH	Pale yellow	Pale yellow	Pale green	Pale green	Purple	Blue
10.	5% Iodine	Yellowish brown	Light brown	Greenish yellow	Light green	Dark blue	Pale blue
11.	5% $FeCl_3$	Yellow	Yellowish green	Green	Greenish yellow	Dark blue	Dark blue

Chromatographical studies - Thin Layer Chromatography

To get an excellent resolution, many solvent systems were used. Lastly, the solvents Toluene: Ethyl acetate (9:1) were used as mobile phase. TLC studies of the ethanolic extract of *Ixora arborea*, showed 6 spots with the R_f values 0.98, 0.69, 0.64, 0.56, 0.43, 0.11, and 0.03 **(Figure 5)**.



Short UV-254 nm **Long UV-366 nm**

Figure 5: TLC of ethanolic extract of the bark of *Ixora arborea*

CONCLUSION

Ixora arborea Roxb., an Indian traditional medicinal plant, was taken for the present study. Pharmacognostical standardization helps to ensure proper identification, purity and quality of medicinal plants. In Pharmacognostical study, the morphological features, microscopy, powder microscopy and histochemistry of the bark of *Ixora arborea* were observed. Physicochemical and phytochemical analysis of stem bark of *Ixora arborea* have been evaluated. Those study results can be employed in the preparation of official monographs and standardization of the plant. And also, this work will be useful for correct identification and authentication of the species for the future.

ACKNOWLEDGEMENT

Authors are grateful to the Principal and faculty members of Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for providing all the facilities to carry out this research work. We are also thankful to Dr. K.N. Sunil Kumar, Research officer/Sci-II and HOD, Department of Pharmacognosy, Siddha Central Research Institute, Government of India, Arumbakkam, Chennai-600106, Tamil Nadu for identification of the plant.

AUTHORSHIP STATEMENT

Conceptualization, methodology, writing original draft preparation and writing review – M. Kaviyarasi; Editing – R. Thenmozhi, T. Monisha and N. Vanitha; Supervision – P. Muthusamy, R. Vijayabharathi and R. Radha.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

REFERENCES

[1] Qazi majaz A, Molvi khurshid I. Herbal medicine: A Comprehensive Review. International Journal of Pharmaceutical Research. 2016; 8(2): 01-05.

[2] Mondal S, Raja S, Prasad PN, Suresh P. Investigations of phytochemical, analgesic, anti-inflammatory and antipyretic effects of Ixora pavetta Andrews leaf. Journal of Nepal Pharmaceutical Association. 2014; 27(1): 20-7.

[3] Shastry, R.A., Habbu, P.V., Patil, B.S., Kulkarni, V.H. Pharmacognostical evaluation of *Ixora pavetta* (Andr.) Bark. Journal of Bio Innovation. 2022; 11(1): 01-19.

[4] Chikezie PC, Ojiako OA. Herbal medicine: yesterday, today and tomorrow. Altern Integr Med. 2015; 4(3):195.

[5] Mangathayaru K. Pharmacognosy: an Indian perspective. First edition. Pearson Education India. Chennai. 2013; Pg. No: 20, 199, 201, 202.

[6] CSIR DK. The Wealth of India, Volume-V: H-K. Council of Scientific and

Industrial Research. New Delhi. 1948; 275-276.

[7] Srinivas K, Baboo CR. Antiulcer activity of Ixora pavetta. Int J Curr Pharm Res. 2011; 3(2): 1-2.

[8] Suneeta D, Rao YT. A phyto-pharmacological screening for whole plant of Ixora pavetta, Journal of Medicinal Plants. 2020; 8(6): 142-5.

[9] Kanhere RS, Reddy KR, Jayaveera KN. Evaluation of anti-obesity potential of Ixora pavetta against high fat diet induced obesity in Wistar rats. International Journal of Pharmaceutical Research and Bioscience. 2014; 3(4): 594-605.

[10] Kallingil Gopi Divya, Mattumal Rubeena, Brindha Sundaramoorthy, Erni Bobbili, Remya A, Koppala Narayana Sunil Kumar. Macro-microscopic profiling of Azinjil, Aavaram and Marukkarai ver pattai (root bark) of Siddha. Int J Res Ayurveda Pharm 2018; 9(4): 62-69.

[11] Khandelwal KR, Practical pharmacognosy, 19th edition, Niraliprakashan; 2008, 100-110.

[12] Fahn A. Plant anatomy. Third Edition Pergamon Press, Oxford; 1980, 162-192.

[13] Wallis TE. Analytical Microscopy - Its aims and methods in relation to foods, water, spices and drugs. Third Edition. Boston: Little, Brown and Company; 1965.

[14] Kanakhara RD, Harisha CR, Shukla VJ. Comparative Phyto-pharmacognostical profile of stem of *Ixora coccinea* Linn. and *Ixora arborea* Roxb. Journal of Ayurvedic and Herbal Medicine. 2017;3(2):83-8.

[15] World Health Organization Quality Control Methods for Medicinal plants materials, WHO Geneva, Switzerland. Materials. 1998;128.

[16] The Ayurvedic Pharmacopoeia of India. New Delhi; The controller of publications;2001;p.143

[17] Indian Pharmacopoiea. New Delhi The controller of publications 1996;p 47-60

[18] Ova AK, Ludvikova I. Elemental analysis of nutritional preparations by inductively coupled plasma mass and optical emission spectrometry. J Saudi Chem Soc. 2012; 16: 287-290.

[19] Kokate, C.K.; Purohit, A.P.; Gokhale, S.B. Pharmacognosy; Nirali Prakashan: Pune, India, 2005.

[20] Harborne, A.J. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis, 3rd ed.; Springer: Berlin/Heidelberg, Germany; Amsterdam, The Netherlands, 1998; ISBN 978-0-412-57260-9.

[21] Sadashivan, S.; Manickam, A. Biochemical Methods, 2nd ed.; New

Age International (P) Ltd. Publisher: New Delhi, India, 2005.

[22] Wallis, T.E. Textbook of Pharmacognosy, 5th ed.; CBS Publishers and Distributors: New Delhi, India, 1990.

[23] Barku VYA, Opoku-Boahen Y, Owusu-Anash E, Menash EF. Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of *Amaranthus spinosus.*

[24] John B. Sulaiman CT, George S, Reddy VRK. Total phenolics and flavonoids in selected medicinal plants from Kerala. Int J Pharm Sci.2014; 6(1):406-408.

[25] Kokate CK. Practical Pharmacognosy. 4th ed. Delhi: Vallabh Prakashan; 1994.

[26] Lala PK Lab manuals of Pharmacognosy, 5th ed. Calcutta: CSI Publishers and Distributors; 1993. P.169-175.

[27] Kasture AV, Mahadik KR, Wadokar SG, More HN. Chromatography, Pharmaceutical analysis. 15th ed. Pune: Nirali Publication. 2006; 51-67.