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AN IN-DEPTH PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF IXORA POLYANTHA WIGHT

Ms. Saranya S. P.[1]*, Prof. Dr. G. N. Pramodini[2], Prof. Dr. D. Vijay Kumar[3] and Prof. Dr. M. Rajan

[1]Final Year M. Pharm Student of Nehru College of Pharmacy Department of Pharmacognosy,
[2]Professor of Pharmacognosy Department, Nehru College of Pharmacy, [3]Professor.

***Corresponding Author: Ms. Saranya S. P.**
Final Year M. Pharm Student of Nehru College of Pharmacy Department of Pharmacognosy.

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ABSTRACT
Ixora polyantha Wight., a plant belonging to the *Rubiacease* family, is traditionally used in various ethnomedicinal practices. Despite its widespread use, there is limited scientific literature detailing its pharmacognostical and phytochemical properties. This study aims to provide a comprehensive evaluation of the plant's botanical characteristics and chemical constituents. The pharmacognostical investigation includes a detailed examination of the morphological and anatomical features of the plant parts, ensuring accurate identification and quality control. Microscopic analysis reveals key diagnostic traits such as leaf venation patterns, stomatal arrangement, and the presence of specific cellular inclusions. Additionally, physicochemical parameters like moisture content, ash values, and extractive values are determined to assess the and quality of the plant material. The phytochemical screening of *Ixora polyantha* Wight. indicates the presence of significant bioactive compounds, including alkaloids, flavonoids, tannins, and glycosides. These compounds are known for their potential therapeutic properties and contribute to the plant's medicinal value. The findings of this study provide essential baseline data for the future exploration of *Ixora polyantha* Wight. in pharmacological applications.

KEYWORDS: *Ixora polyantha* Wight., Phytochemical, Pharmacognostical, Microscopy, Herbal medicine.

INTRODUCTION
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine.[1] The connection between human and their search for drugs in nature dates from ancient times of which there are enormous evidence from different sources (Written documents, preserved monuments, and even original plant medicines).[2] Plants are an indispensable source of new drugs, offering unparalleled chemical diversity and therapeutic potential that drive modern pharmaceutical innovation.[3] India officially endorses over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are used in traditional, folk and herbal medicine.[4]

In the current scenario herbal medicine has become a popular for healthcare; even though there are some differences between herbal and conventional pharmacological treatments, herbal medicine needs to be tested for efficacy by using conventional methods which were used for trial and specific herbal extracts has been demonstrated which were efficacious for specific conditions. Herbal medicines are gaining interest because they were cost effective and eco-friendly nature18. Recorded uses of plants as medicine from 5,000 years to the Sumerians and the Vedas19. The written text of Rig-Veda came about 2000 years ago.[5]

Numerous standards for quality assurance of herbs have been established by WHO. The identification and safety of a herbal treatment will be based on the standardization of plants. pharmacognostical assessments, preliminary phytochemical screening, and physicochemical studies of plant will help to standardize plant resources.

Ixora polyantha Wight., a member of the *Rubiaceae* family, is a plant recognized for its diverse medicinal properties. The *Rubiaceae* family, commonly referred to as the coffee family, is a diverse group of flowering plants encompassing over 13,000 species. This family is notable for its ecological and economic significance, including key plants such as coffee (Coffea), quinine (Cinchona), and various ornamental species.[6] Among the many genera within the *Rubiaceae* family, Ixora is particularly prominent for its ornamental and medicinal uses. Ixora species are widely cultivated for their striking

appearance, featuring dense clusters of small, colorful flowers that range in shades from red and pink to yellow and white. In addition to their aesthetic appeal, Ixora plants have been used in traditional medicine for their therapeutic benefits. Various species within this genus are reported to possess anti-inflammatory, antimicrobial, and antioxidant properties.[7]

Ixora polyantha Wight. is a notable species within this genus, recognized for its broad spectrum of medicinal and pharmacological activities. This species, native to tropical regions, has been the subject of various pharmacological studies due to its therapeutic potential. One of the most significant properties of *Ixora polyantha* Wight. is its wound healing activity.[8]

***Ixora polyantha* wight. plant**

Fig. 1: *Ixora polyantha* Wight. Plant.

MATERIALS AND METHODS

Collection of plant materials

Leaves of *Ixora polyantha* Wight. were collected at 11.30am, on 21st January 2024 from the local areas of Thrissur district, Kerala. Taxonomically identified and authenticated by the Botanist, Dr. Ranjusha A P, HOD, Department of Botany, N. S. S. College, Ottapalam. Herbarium has been deposited at Nehru College of Pharmacy, Thrissur, for future reference. Dried under shade (30 to 40days) Coarsely powdered and Stored in an air tight container.

Macroscopic evaluation

Evaluation of plant material by color, odour, size, shape, taste; special features including touch, texture and leaf structure like margin, apex, base surface, venation and inflorescence.[9]

Microscopy

A small portion of the midrib region from an entire leaf sample was separated for sectioning. Thin transverse sections of the leaf were manually prepared using sharp razor blades and thermocol pith. Safranin red was used to stain the sections; a small drop of safranin red was diluted in water. The thin sections were immersed in the stain for a few seconds, then removed and washed in water to eliminate excessive stain. The sections were placed on a clean glass slide and mounted with glycerine. A clean coverslip was carefully placed over the section, ensuring no air bubbles were trapped. The slides were then observed under a microscope, initially at low power and subsequently at high power. Clear images were captured and details were noted. The observations and photographs were taken using a Labomed LX-300 binocular microscope equipped with an industrial digital camera.[10]

Preparation of dried powder

The collected leaves of *Ixora polyantha* Wight. were washed with running tap water to remove adhering materials. Then the leaves were dried under shade for about 60-70 days, powderedwith mechanical grinder and stored in an air tight container until use.[11]

Fig. 2: Powder form of *Ixora polyantha* Wight. Leaves.

Powder microscopy

The powdered sample material was placed on slides. A few drops of phloroglucinol solution were added (the phloroglucinol solution was prepared by dissolving a small amount of phloroglucinol in ethanol in a watch glass, with the watch glass covered to prevent ethanol evaporation). Then, 1-3 drops of dilute hydrochloric acid were added, and if necessary, the mixture was stirred

with a fine pointed needle to distribute the testing agent evenly. A coverslip was placed over the sample, and any excess liquid that oozed from under the coverslip was blotted gently with filter paper. Lignified tissues are stained pink with phloroglucinol. The powder slides were observed and photographed using a Labomed LX-300 binocular microscope equipped with an industrial digital camera.[12]

Physicochemical evaluation

Determination of moisture content

2g of the powdered leaves was placed in tarred evaporating dish. Drying was carried out at 105˚C for five hours. The drying was continued with intermittent weighing at half an hour interval until difference between two successive weighing was not more than 0.01gm difference.

$$\text{Moisture content \%} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Ash value

The inorganic content remaining after incinerating a crude drug is called as ash content. An ash value implies the naturally inherent inorganic salts or those imparted from external sources. Official ash values are chiefly used for quality confirmation of powdered drugs. Determination of ash value helps in knowing absence of mineral matter that is accidentally introduced from earth, sand, floor sweepings, absence of other parts of plant, absence of adulterated and exhausted drug, and absence of materials that possess with stone cells, starch which modify the value. Total ash value is used for ensuring quality of drugs which possess with little calcium oxalate. Acid-insoluble ash is referred if calcium oxalate present is high. Treatment of ash with hydrochloric acid leaves only silica which is imparted from soil and hence acid-insoluble ash is preferred. Water soluble ash is the difference between total ash and water insoluble residue. Sulphated ash is usually used for un-organized drugs to control non-volatile inorganic impurities. In the test, Sulphuric acid is used to decompose and oxidize organic matter resulting only with sulphate salt of cations.

Total ash

2g of powdered specimen taken in a previously weighed silica crucible and weighed the silica crucible with powdered specimen. Then the powder was incinerated by 100-105˚C for1 hr. and ignited to constant weight in muffle furnace 450 ˚C, carbon free ash formed.

Acid insoluble ash

The ash was boiled for 10 minutes with 25ml of dil. HCl. It was filtered through on ash less filter paper. Residue was washed twice with hot water. Filter paper with residue was placed together in to the crucible and heated gently until vapour ceases to be evolved, then it was cooled in a desicator and and weighed. Calculated the percentage of water soluble ash with reference to air dried drug.

Water soluble ash

The ash was boiled for 10 minutes with 25ml of water. It was filtered through on ash less filter paper. Residue was washed twice with hot water. Filter paper with residue was placed together in to the crucible and heated gently until vapour ceases to be evolved, then it was cooled in a desicator and and weighed. Calculated the percentage of water soluble ash with reference to air dried drug.[13, 14, 15]

Determination of extractive value

- Coarsely powdered air dried material (5g) and add100 ml solvent in a glass stoppered conial flask.
- Shaken frequently 6 hr and then allowed to stand for 18 hrs.
- Filtered rapidly.
- 25 ml of the filtrate was evaporated in a flat bottom dish at 105˚C cooled and weighed.
- The content of extractable matter in percentage of air dried material was calculated.

Solvents used: Ethanol, Water, Chloroform, Ether.[16, 17]

Phytochemical studies

Extraction

Fig. 3: Soxhlet extraction and dried form of extract.

- Shade dried leaves were coarsely powdered.
- 20 g of coarsely powdered leaves were packed in Soxhlet apparatus and performed. solvent extraction using 250ml of 98% of Ethanol.
- Extract was collected, filtered through Whatman No. 1 filter paper and concentrated. Then calculated the percentage yield (%w/w).[18]

Preliminary phytochemical screening of plant extracts

Preliminary phytochemical screening was done to identify different constituents present in extracts i.e., carbohydrates, proteins, lipids, flavonoids, tannins, glycosides, alkaloids, essential oils etc. All the ELEGS were subjected to preliminary phytochemical screening.[19,20]

Tab. 1: Preliminary phytochemical screening of plant extracts.

Sl. no.	Test		Procedure	Positive observation
1	Alkaloids	Mayer's test	2 ml of the extract + 2 ml of Mayer's reagent	Creamy precipitate
		Hager's test	2 ml of the extract + 1-2 ml of Hager's reagent	Presence of yellow precipitate
		Wagner's test	2 ml of the extract + 1-2 ml of Wagner's reagent	Reddish brown precipitate
2	Glycosides	Baljet's test	Mixed 2-3 ml of sample in 2 ml sodium picrate solution	Yellow to orange colour
		Borntrager's test	To a little quantity of sample solution added H2SO4 and CCl4. Separated the organic layer and shaken with dilute ammonia	Pink to red color
			To a little quantity of sample solution added H2SO4 and CCl4. Separated the organic layer and shaken with dilute ammonia	
3	Phenolic and tannins	Ferric chloride test	Mixed 2 ml of the test solution with few ml of 5% Ferric chloride solution	Presence of blue color
		Lead acetate test	Mixed 2 ml test solution with 1 ml of lead acetate solution	Presence of bulky white precipitate
4	Flavonoids	Shinoda test	2 ml sample solution + Magnesium powder and few drops of Concentrated HCl	Pink scarlet, crimson red or occasionally green to blue color
		Aqueous NaOH test	Aqueous NaOH solution was added to the few ml of the extract	Presence of yellow coloration
5	Volatile oil	Filter paper test	Pressed the powder between filter paper	Filter paper is not permanently stained with volatile oil
6	Carbohydrate	Molisch's Test.	1 ml of the test solution was mixed with 2 ml of Molisch's reagent, shaken the mixture and added 1 ml of concentrated H2SO4 along the sides of the test tube	Presence of violet ring at the junction of two solutions
		Fehling's Test	Boiled 1 ml of test solution with 1 ml Fehling's solution A and 1 ml Fehling's solution B on a water bath	Presence of red residue at the bottom of test tube
7	Saponin	Foam or Froth Test	Shaken few mg of extract with 20 ml of distilled water	Formation of foam
8	Proteins and amino acids	Millon's Test	2 ml of the extract was mixed with 2 ml of Million's reagent and boiled.	Observed for the presence of white precipitate, which on warming turn into a red colored solution
		Ninhydrin Test	Extract + two drops of Ninhydrin solution and heated on a water bath	Presence of violet color was noted
9	Terpenoids	Salkowski's	Dissolved 1-2 mg of sample in 1	Appearance of red

		Test	ml of CHCl3 and added 1 ml of Concentrated H2SO4	color in the chloroform layer and greenish yellow fluorescence in the acid
10	Sterols	Liebermann – Burchard's test	Mixed 2 ml of the test extract with 1 ml CHCl3 of and 1 ml Acetic anhydride. Then added 1 drop of Concentrated H2SO4	Deep red color in the lower portion and green colour in the upper portion which changes to blue and violet
		Salkowski's Test	Dissolved 1-2 mg of sample in 1 ml of CHCl3 and added 1 ml of Concentrated H2SO4	Red colour in the lower layer

RESULT AND DISCUSSION

Macroscopic evaluation

Tab. 2: Macroscopic evaluation.

Sl. No	Features	Observations
1	Color	Leaves- Green
2	Odour	Faint characteristic
3	Taste	Bitter
4	Texture	Smooth
5	Shape	Ovate to obovate, elliptical, coriaceous, glabrous on both sides
6	Petioles	Short (5 mm long)
7	Apex	Shortly acuminate
8	Margin	Entire
9	Base	Broad
10	Venation	Lateral

The leaves of *Ixora polyantha* exhibit a green color with a faint characteristic odour. The taste of the leaves is notably bitter, and their texture is smooth. The leaves are described as having an ovate to obovate or elliptical shape, and are coriaceous, meaning they have a leathery texture. Both surfaces of the leaves are glabrous, indicating they are smooth and without hairs.

The petioles are relatively short, measuring approximately 5 mm in length. The leaf apex is shortly acuminate, giving the tip a slightly pointed appearance. The leaf margins are entire, meaning they are smooth without any serrations or indentations. The base of the leaves is broad, and the venation pattern is lateral, which refers to the arrangement of veins parallel to the leaf's midrib.

Microscopic evaluation

Transverse section of *Ixora polyantha* Wight. leaf

The microscopic evaluation of the transverse section (TS) of *Ixora polyanatha* Wight. leaf reveals several structural details:

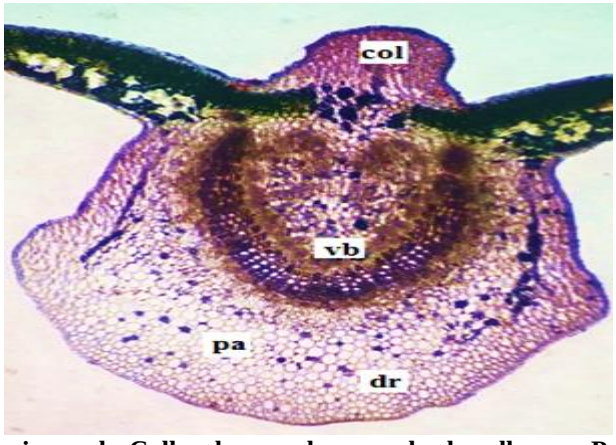

Fig. 1: Ground plan: la.: lamina; col.: Collenchyma; vb.: vascular bundle; pa.: Parenchyma cells; dr.: druse.

Epidermis: Both upper and lower surfaces of the leaf have a uniseriate epidermis covered with a cuticle. The epidermis protects the leaf tissues and is involved in gas exchange and water regulation.

Collenchyma cells: Beneath the epidermis, there is a multi-layered region of collenchymatous cells. These cells provide structural support to the leaf, particularly in areas where flexibility and mechanical strength are needed.

Mesophyll cells: The mesophyll is divided into palisade cells and spongy parenchyma. The mesophyll is involved in photosynthesis. The palisade cells are the primary site of photosynthesis, while the spongy parenchyma facilitates gas exchange.

- Palisade Cells: These are elongated cells located just beneath the upper epidermis, and they are arranged in one or two layers.
- Spongy Parenchyma: Located below the palisade cells, these cells are loosely arranged with air spaces in between.

Vascular bundle: The vascular bundle is embedded within the parenchymatous region. It contains xylem and phloem tissues. The xylem conducts water and minerals from the roots to the leaves, while the phloem transports the products of photosynthesis throughout the plant.

Parenchyma cells: These are general-purpose cells located in various regions of the leaf, particularly surrounding the vascular bundle. They are involved in storage, photosynthesis, and support.

Druse: Druse refers to the presence of rosette crystals of calcium oxalate within the parenchymatous cells. Function: These crystals may serve to detoxify the plant by sequestering excess calcium or to deter herbivory due to their sharp structure.

Lignified vascular elements: Some elements of the xylem within the vascular bundle are lignified, which means they have a thickened, woody cell wall that provides additional structural support and ensures efficient water transport.

Starch grains: Simple, round starch grains are present in the parenchymatous region. These starch grains store energy in the form of carbohydrates, which the plant can use when needed.

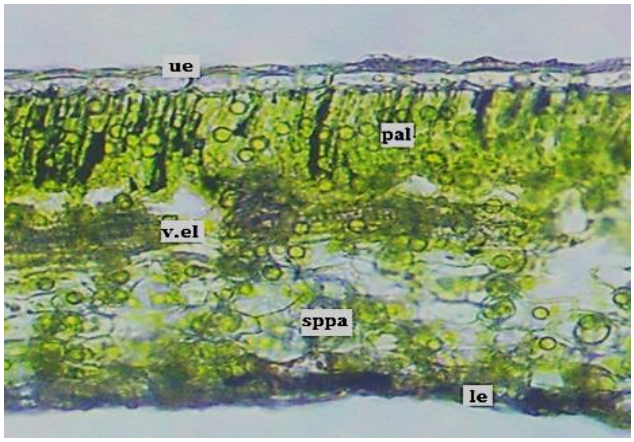

Fig. 5: ue.: upper epidermis; pal.: palisade cells; sppa.: spongy parenchyma; v.el.: vascular elements; le.:lower epidermis.

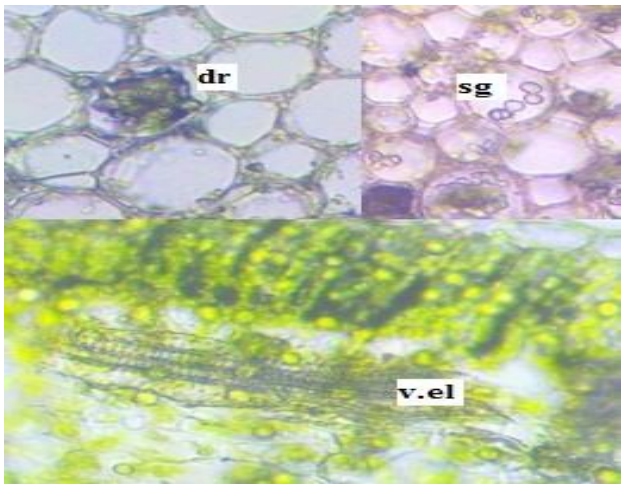

Fig. 6: Cell inclusions of leaf: dr.: druse; v.el.: vascular elements; sg.: Starch grains.

Transverse section of leaf lamina shows uniseriate upper and lower epidermis covered with cuticle. Mesophyll region is composed of one to two layered palisade cells and multilayered spongy parenchyma. Chlorophyll found in this region. Some lignified vascular elements are also observed in this region. Most of the stomata are present.

Powder microscopy

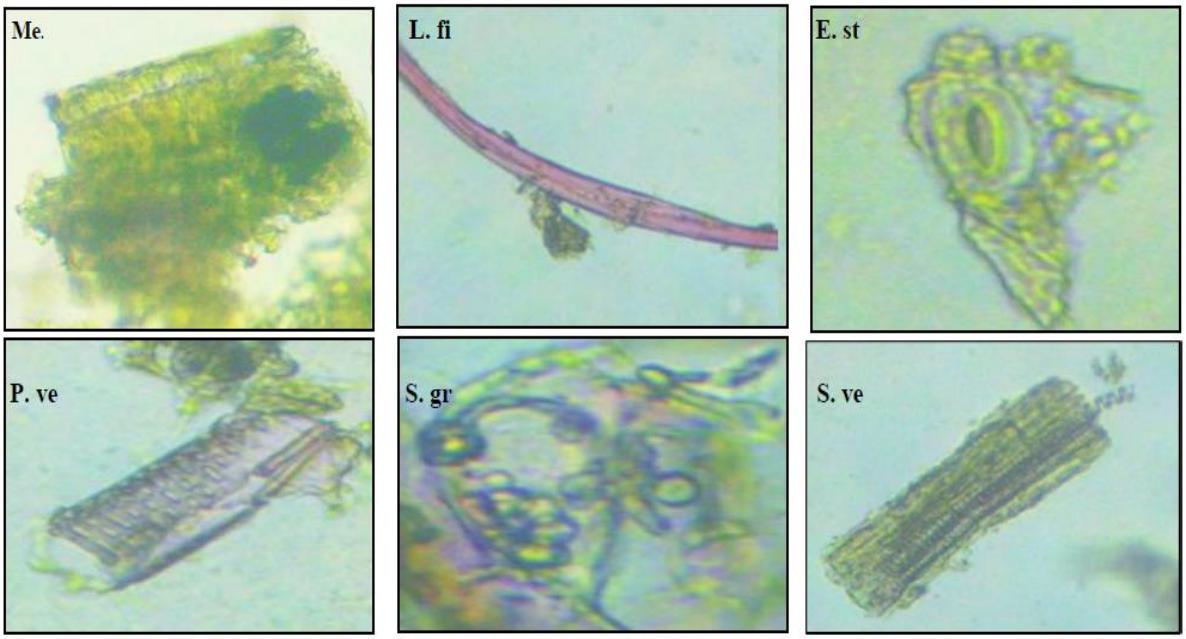

Fig. 7: Powder microscopy: Me.: Mesophyll with sectional view; L. fi.: Lignified fiber; E. st.: Epidermal cells with stomata; P. ve.: Pitted vessel; S. gr.: Starch grain; S. ve.: Spiral vessel.

Mesophyll: Mesophyll fragments are observed in the powder microscopy. These fragments include cells from both the palisade and spongy parenchyma regions. The mesophyll cells are responsible for photosynthesis and gas exchange.

Lignified fiber: Fragments of lignified fibers with narrow lumens are seen under the microscope. Lignification refers to the deposition of lignin in the cell walls, making them rigid and woody. Lignified fibers provide structural support to the plant, helping it maintain its shape and resist mechanical stress.

Spiral vessel: Spiral vessels are specialized xylem vessels characterized by a spiral thickening pattern on their walls. These vessels conduct water and minerals from the roots to other parts of the plant. The spiral structure allows the vessel to be flexible while providing strength.

Starch grain: Simple, round starch grains are present in the powder. These grains are visible as distinct, rounded structures. Starch grains store carbohydrates, which the plant uses as an energy reserve.

Epidermal cells with stomata: Fragments of epidermal cells with stomata are observed. Paracytic stomata with parallel subsidiary cells. Stomata are small openings on the leaf surface that allow gas exchange. The epidermal cells protect the leaf, while stomata regulate the exchange of gases (such as CO_2 and O_2) and control water loss through transpiration.

Pitted vessel: Pitted vessels, which are another type of xylem vessel, are seen. These vessels have pits or small depressions on their walls. Pitted vessels are responsible for water conduction. The pits allow lateral movement of water between adjacent vessels.

While *Ixora polyantha* Wight. shares many anatomical features with other members of the *Rubiaceae* family, such as the paracytic stomata and lignified vascular tissues, it also has some specific characteristics. These include the prominent lignification in its vascular bundles, the structure of its mesophyll, and the type and distribution of calcium oxalate crystals (Rosette crystals are present, embedded in the parenchyma cells). The subtle differences in anatomy can help distinguish *Ixora polyantha* Wight. from other genera within the family and may relate to its specific ecological adaptations.

Physico-chemical evaluation

Tab. 3: Physico-chemical evaluation.

Sl. no	Physico-chemical constants	Result (% w/w)
1.	**Moisture content**	1.45
2.	**Ash value**	
	Total ash	6.95
	Acid insoluble ash	0.95
	Water soluble ash	5.5
3.	**Extractive value**	
	Ethanol soluble extractive value	14.304
	Water soluble extractive value	11.14
	Chloroform soluble extractive value	11.087
	Ether soluble extractive value	9.08

The physico-chemical analysis of *Ixora polyantha* Wight. shows low moisture content (1.45%), indicating stability. The total ash (6.95%) suggests substantial mineral content, with 5.5% water-soluble ash indicating bioavailability. High extractive values in ethanol (14.304%) and water (11.14%) highlight potential for therapeutic use, with diverse bioactive compounds.

Phytochemical studies

Qualitative phytochemical anlaysis of extract.

Tab. 4: Qualitative phytochemical anlaysis of *Ixora polyantha* Wight. Leaves extract. (Note: (++) indicate abundance,(+) indicate presence, (-) indicates absence).

Sl. No.	Chemical constituents	Ethanolic extract
1	Steroids	+
2	Glycosides	+
3	Saponins	-
4	Flavonoids	+++
5	Tannins	+
6	Phenolic compounds	+++
7	Proteins & Amino acids	-
8	Alkaloids	++
9	Carbohydrates	+
10	Terpenoids	++
11	Volatile oil	+

The ethanolic extract of *Ixora polyantha* Wight. reveals a diverse chemical profile, with significant levels of flavonoids and phenolic compounds, indicating a strong presence of these components. Moderate amounts of alkaloids and terpenoids are also noted. The extract contains steroids, glycosides, tannins, carbohydrates, and volatile oils, while saponins, proteins, and amino acids are absent. This composition underscores the plant's complex array of chemical constituents, which could contribute to its various properties.

DISCUSSION

The pharmacognostical and phytochemical analysis of *Ixora polyantha* Wight. significantly enhances our understanding of this traditionally used medicinal plant. Macroscopic evaluation of the plant reveals distinct features such as green, smooth, and ovate to obovate leaves with a bitter taste, which align with its traditional medicinal uses. Microscopic examination shows key anatomical details, including a uniseriate epidermis, collenchyma, and vascular bundles, along with rosette crystals of calcium oxalate and starch grains. These structural features aid in accurate identification and quality control. Physicochemical analysis reveals a low moisture content of 1.45%, indicating good stability, and a total ash content of 6.95%, highlighting substantial mineral presence. High extractive values, particularly in ethanol (14.304%) and water (11.14%), suggest the plant is rich in bioactive compounds. Phytochemical screening identifies abundant flavonoids and phenolic compounds, known for their antioxidant, anti-inflammatory, and antimicrobial properties. The presence of moderate amounts of alkaloids and terpenoids further supports the plant’s therapeutic potential, though saponins, proteins, and amino acids are absent.

These findings underscore *Ixora polyantha* Wight.'s broad spectrum of potential health benefits, aligning with its traditional uses. The diverse chemical profile and high levels of key bioactive compounds suggest significant medicinal value, providing a solid foundation for future pharmacological research and potential therapeutic applications. This comprehensive analysis not only validates traditional uses but also paves the way for the integration of *Ixora polyantha* Wight. into modern therapeutic practices.

CONCLUSION

Ixora polyantha Wight. is a notable species within the *Rubiaceae* family, known for its striking ornamental and medicinal properties. Native to the Indian subcontinent, this plant is characterized by its vibrant, clustered flowers and distinctive foliage, making it a popular choice in gardens and traditional medicine. *Ixora polyantha* Wight. exhibits unique morphological features, including intricate venation patterns and specialized cellular inclusions, which contribute to its botanical interest. This study provides a comprehensive analysis of *Ixora polyantha* Wight., revealing its significant pharmacognostical and phytochemical attributes. The plant exhibits distinctive morphological features and a detailed anatomical structure, including unique venation patterns and cellular inclusions. The physicochemical evaluation shows low moisture content, high extractive values, and substantial mineral presence, indicating stability and potential therapeutic efficacy. Phytochemical screening identifies key bioactive compounds such as flavonoids, phenolic compounds, and alkaloids. These findings establish a solid foundation for future pharmacological studies and underscore the plant's potential for medicinal applications, particularly in wound healing and other therapeutic areas.

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