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RESEARCH ARTICLE

Pharmacognostic Standardisation and Phytochemical Profile of the Leaves and Roots of *Xanthium indicum* J. Koening. Ex. Roxb

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Abstract

Background: *Xanthium indicum* Roxb is a tiny mangrove tree or shrub belonging to the family of *Asteraceae*. The various parts of the plant are used traditionally in the treatment of fungal infection, urticaria, arthritis, salivation, epilepsy, TB, snake bite, insect bite, lumbago, malaria, diarrhoea, tooth pain and diabetes. Despite its extensive use in traditional healing practices, there remains a significant lack of scientific data regarding its pharmacognostic and phytochemical characteristics. **Aim:** Despite its widespread use in traditional medicine, comprehensive scientific data on the pharmacognostic and phytochemical properties of *X. indicum* remains limited. This study aims to systematically investigate these characteristics of the plants roots and leaves to facilitate accurate identification and establish quality control standards. By addressing the current gap in literature, it also gives a basic understanding for upcoming pharmacological and phytochemical studies of this under-researched mangrove species. **Methods:** Macroscopic and microscopic characters, physicochemical parameters, and fluorescence analysis were carried out for pharmacognostic evaluation. The purity profile of plant materials was assessed; physicochemical characteristics like moisture content and ash value were used. Extractive values for both root and leaf powder were also used. Additionally, various phytochemical tests were performed upon that ethanolic root extract so as to detect the presence of possible phytocompounds. **Results:** Preliminary phytochemical screening revealed that the presence of several alkaloids, cardiac glycosides, proteins, carbohydrates, phenolic compounds, flavonoids, steroids, saponins, and amino acids. TLC profiling confirmed the existence of β-sitosterol. These findings do contribute to the correct identification, also to comprehension of the plant's therapeutic potential with its chemical composition. **Conclusion:** The present study offers necessary pharmacognostic, physicochemical, as well as phytochemical understandings into *Xanthium indicum*. These characteristics, for the first time, are being reported and then serve as such important baseline data. These findings will certainly aid in the correct identification, in addition to the quality control, of this unexplored mangrove species, as they also support future research in addition to potential pharmaceutical applications.

Major Findings: The study presents the first detailed pharmacognostic and phytochemical profile of *Xanthium indicum* leaves and roots. It identifies key diagnostic features and establishes physicochemical standards for proper identification. Bioactive compounds such as alkaloids, flavonoids, and β-sitosterol were confirmed, supporting its traditional medicinal uses and potential pharmacological applications

Keywords: Fluorescence Analysis, Pharmacognostic Study, Phytochemical Screening, TLC, *X. indicum*

1. Introduction

Many indigenous tribes have traditionally employed medicinal plants as effective therapies for a variety of ailments[1]. Traditional medical approaches are based on ideas and observations that precede the development and diffusion of modern medicine for hundreds of years[2]. In ancient civilizations, in nations such as India,

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Africa, Egypt, China, and South America, 80% of the population relies on herbal medicine to cure a variety of deadly illnesses such as AIDS, cancer, and malaria[3]. Several local projects declare the presence of over 800 medicinal plants, including *Ayurvedic*, *Siddha*, and *Unani* remedies[4]. In the 20th century, the production of conventional medications contributed to a decrease in the use of herbal plant products[5]. Herbal treatments have slowly gained major popularity and reputation in recent years, owing to extensive investigations on their pharmacological influence on human health care. Although herbal medicine is beneficial to mankind, there is still a lack of proper standardized tools for establishing its identity, uniformity, number of active constituents, and efficacy[6]. Despite much concentration and research in recent years, there is still a lack of understanding of herbal medicine's phytochemistry and its original quality, which has created a significant problem in standardization procedures[7]. As a result, a system was developed for the quality management and standardization processes of herbal medications[8]. Observational studies are used to assess the best quality of herbals, as documented in the literature. Research in physical, chemical, microscopy, and macroscopy standardization techniques utilising non-conventional analytical practices is thus necessary for the validation of herbals. Preliminary investigation of biologically susceptible marker agents is currently very successful in herbal medication verification and thus helps to decrease adulteration[9].

Xanthium indicum J. Koenig, Ex Roxb, is an annual herb widely distributed in Asia, North America, and European countries and belongs to the family *Asteraceae*[10]. The lower leaf and flower tips are used as edible plants in Assam and are cooked in water. Typically, the herb and fruit are properly cleansed before being used in cooking since they are thought to be toxic and dangerous, respectively. *X. indicum* is regarded as an animal-poisonous herb in Western culture. The base of each leaf of the plant has long thorns and thin spines. The pharmacological profile of *X. indicum* has also been detailed in a comprehensive review, which summarized its wide-ranging therapeutic potential. This includes activities such as anti-tussive, anti-cancer, anti-fungal, CNS depressant, diuretic, antioxidant, insecticidal, herbicidal, and anti-allergic rhinitis properties. Specific bioactivities have been supported by studies such as antifungal effects against *Phytophthora drechsleri* and anticancer potential with cytotoxic extracts. The plant also shows promise in treating dermatitis, arthritis, lipid disorders, yeast infections, and allergic responses, as well as displaying notable antinociceptive and antihyperglycemic actions in animal models. These findings significantly reinforce the traditional use of *X. indicum* in ethnomedicine[11]. This plant supports evidence on its antinociceptive and antihyperglycemic effects. The methanolic stem extract showed a dose-dependent reduction in blood glucose levels in glucose-loaded Swiss albino mice (up to 31.2% at 400 mg/kg), compared to 46.2% by glibenclamide. In antinociceptive assays, the extract reduced acetic acid-induced gastric pain up to 61.0% at the highest dose, exceeding the standard aspirin response (37.5%). These bioactivities likely stem from mechanisms involving enhanced insulin secretion or glucose uptake and inhibition of prostaglandin pathways, respectively. Furthermore, the stem extract was found more potent than the leaf extract, aligning with traditional practices[12].

In another study by Yeasmen and Chowdhury, *X. indicum* offers many health benefits due to its powerful antioxidant and pain-relieving qualities. It plays a key role in fighting free radicals and lowering oxidative stress, which helps shield cells from damage. Additionally, this plant has strong pain-killing effects by influencing inflammation and dulling pain signals[13]. Traditionally, it has been used to alleviate swelling, infections, and diabetes-related conditions. The plant is known to fight germs, reduce inflammation, lower blood sugar, and ease pain. People, especially those with diabetes, often use it to manage rheumatic fever and other inflammatory ailments. This makes it valuable in both traditional and contemporary medical systems[14].

Xanthium indicum demonstrates significant medicinal value, backed by traditional use and scientific studies confirming its antinociceptive, antihyperglycemic, antioxidant, and anti-inflammatory properties. Despite its potential, proper standardization and quality control remain essential for its safe and effective integration into modern medicine. An exclusive study of the plant morphological findings, emphasising quantitative and qualitative microscopy, ensures accurate authenticity and prevents easy adulteration[15]. The current study aims to investigate

the morphological and microscopical characteristics, physicochemical parameters, and phytochemical screening of *X. indicum* leaves and roots.

2. Materials and Methods

2.1 Collection and Identification of Plant Material

The fresh roots and leaves of *X. indicum* were collected separately from west Karbi Anglong in May, 2021. The roots and leaves were washed thoroughly with water and shade-dried for 20 days before being used for the study. A herbarium was prepared and sent to the Botany Department, Guwahati University. The plant was identified and authenticated as *X. indicum* J. Koenig, Ex Roxb., by Mr Sourav Jyoti Borah, Curator, Department of Botany, Guwahati University, Assam, with reference no. Herb/GUBH/2019/125.

2.2 Chemicals and Instruments

Microscopy (TS) and powder characters of leaf and root drugs were studied using a digital light microscope (Primo Star Zeiss), and photographs were taken using T-capture software. All the reagents, solvents, and chemicals used are of analytical grade (Merck, Loba, Qualigens) and purchased from a local supplier in Mizoram and β-sitosterol has been purchased from Natural Remedies Private Ltd, Bangalore, Karnataka.

2.3 Macroscopic Evaluation

Macroscopic evaluations of leaves and roots were carried out on 5 samples separately and mean values were taken. The taxonomical description of *X. indicum* was determined according to the data given in different standard books. The type of leaf base, presence or absence of petiole, and lamina characteristics of fresh *X. indicum* leaves and roots were all recorded. Composition, incision, shape, venation, margin, apex, base, surface, and texture are all characteristics of the lamina. The size, shape, surface, fracture, and configuration of the root and leaf were studied morphologically[16].

2.3.1 Organoleptic Evaluation

The different organoleptic parameters, such as colour, odour, taste, size, and shape of *X. indicum* roots and leaves, were studied according to the data given in different books[17].

2.4 Microscopic Analysis

2.4.1 Study of Transverse Sections

Leaf and root transverse section: The leaf and root were transversely cut by a razor blade to observe the microscopic structures.

The leaf and root sections were placed on a glass slide, mounted with a few drops of glycerine, and covered with a coverslip. Each sample was evaluated under a digital light microscope with an objective lens (magnification of 4X, 10X, and 40X power), and the characteristics of the midrib and root were illustrated with the help of T-capture software[18].

2.4.2 Quantitative Microscopy

Quantitative microscopy of the plant such as stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio were determined by the standard procedure described in the research journal as follows[19]:

2.4.2.1 Stomatal Number

The stomatal number which is the average number of stomata per millimeter square area of the upper and lower epidermisof the leaf has been calculated.

2.4.2.2 Stomatal Index

The stomatal index is the ratio of the number of stomata to the total number of stomata and epidermal cells. The stomatal number and the epidermal cell number per millimetre square area of the upper and lower epidermis were recorded. The stomatal index was calculated using the following equation:

$$\text{Stomatal index} = \frac{S}{E+S} \times 100 \quad \text{.....(1)}$$

Where,

S = number of stomata per unit area; E = number of epidermal cells in the same unit area, including trichomes or cicatrices.

2.4.2.3 Vein-islet and Vein Termination Number

Vein islet number is defined as the average number of vein islets per square millimeter of a leaf surface midway between the midrib and margin.

The leaf portion between the midrib and margin was macerated in a 5% sodium hypochlorite solution for 24 h. The cleared lamina portion was mounted on a glass slide, and vein islets and vein terminations were traced with the help of T-capture software. The measurement was done in a 20 mm square using low power (4x). The process was repeated, and values were determined per square millimetre of leaf area between the midrib and margin.

2.4.2.4 Palisade Ratio

The palisade ratio is the average number of palisade cells beneath each epidermal cell. The small leaf samples were treated with 5% sodium hypochlorite for 24 hours. The cleared leaf surface was then mounted on a glass slide. 4 sets of epidermal cells were traced using T-capture software in a 4x objective lens, and spherical palisade cells were counted in each cell. The average number of palisade cells was calculated, and the palisade ratio was determined.

2.4.3 Powder Microscopy

Fine sample powders of the root and leaves of *X. indicum* were mounted on a clean glass slide and clarified with NaOH as the clearing solution. The powder sample was then treated with the different chemical reagents mentioned in Table 1. Stained samples were then mounted with glycerine and observed for the identification of diagnostic characters. Different chemical reagents are used for the identification of diagnostic cellular characters and cell inclusions[20].

2.5 Physicochemical Analysis

2.5.1 Loss on Drying (Gravimetric Determination)

10 g of plant material was placed (without preliminary drying) after accurately weighing it in a tared evaporated dish. This was dried at 105° C for 5 hours and weighed. Drying and weighing were continued at a 1-hour interval until a constant weight was obtained. A constant weight was reached after two consecutive weighing and drying for 30 min and cooling for 30 min in desiccators that showed not more than a 0.1 g difference[21].

Table 1. Chemical reagents for diagnostic characters

S. No.	Diagnostic character	Reagent used
1	Lignified tissues	1: 1 Phloroglucinol and conc. HCl
2	Calcium oxalate crystal	Chloral hydrate/ acetic acid

2.5.2 Determination of pH

pH of 1% solution: 1 g of air-dried, coarsely powdered root material was accurately weighed and diluted in 100 ml of distilled water. The pH of the water-soluble component was determined using a calibrated pH meter at room temperature[22].

2.5.3 Determination of Total Ash

About 2 g of both root and leaf powdered drugs were weighed accurately in a tared silica crucible separately. The powder was incinerated in a muffle furnace at 450° C until it became carbon-free, and it was kept in a desiccator for cooling. The remaining ash was weighed, and the total ash percentage was calculated by the following formula[23]:

$$\text{The total ash value of the sample} = \frac{Z - X}{Y} \times 100 \quad \ldots\ldots (2)$$

Where,

X = Weight of the empty dish; Y = Weight of the drug taken; Z = Weight of the dish + powder sample (before incineration); V = Weight of the dish + ash (after complete incineration).

2.5.3.1 Acid Insoluble Ash

The obtained total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble matter was filtered and collected on ash-less filter paper, washed with hot water, ignited in a trace crucible, cooled, and kept in a desiccator. The remaining residues were weighted and the acid-insoluble ash of both the root and leaves of *X. indicum* was calculated by the following formula[24]:

$$\text{The total Ash value of the sample} = \frac{A - B}{C} \times 100 \quad \ldots\ldots (3)$$

Where,

B = Weight of the empty dish; C = Weight of the drug taken divided by two; A = Weight of the dish + Filtered acid insoluble ash sample (before incineration); V = Weight of the dish + ash (after complete incineration).

2.5.3.2 Water Soluble Ash

The obtained total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was filtered and collected on ash-less filter paper, washed with hot

water, ignited in a trace crucible, cooled, and kept in a desiccator. The remaining residues were weighted and the water-insoluble ash of both the root and leaves of *X. indicum was* calculated by the following formula[25]:

$$\text{The total Ash value of the sample} = \frac{Z - X}{Y} \times 100 \ldots\ldots (4)$$

Where,

X = Weight of the empty dish; Y = Weight of the drug taken divided by two; Z = Weight of the dish + filtered water insoluble ash sample (before incineration); V = Weight of the dish + ash (after complete incineration).

2.5.4 Extractive Values

5 g of each powdered root material was accurately weighed and transferred into a 250 ml conical flask. Then, 100 mL of each solvent (95% ethanol and petroleum ether) was added separately in the conical flasks and the material was macerated for 24 hours by closing the flask with a lid and using a mechanical shaker for 6 hours. After that, the extract was filtered. 25 mL of filtrate from each sample was taken in a dried and pre-weighed petri-dish, evaporated in an oven at 105° C until it reached a constant weight, and kept in a desiccator for cooling. The % w/w extractive values of both solvents were calculated in relation to the drug[18].

$$\text{Percentage extractive value} = \frac{Weight\ of\ residue\ (g)}{Weight\ of\ Sample\ (g)} \times 100 \ldots (5)$$

2.5.5 Fluorescence Analysis of Powder Sample

1 g of the powdered crude drug was taken on a glass slide and a few mL of each reagent (Table 2) was added. It was allowed to stand for a few minutes. Each test tube was then examined under ordinary and UV light (a short wavelength of 254 nm and a long wavelength of 366 nm), and the colours were recorded[19].

2.6 Preliminary Phytochemical Screening

500g of dried plant material of roots of *X. indicum* was extracted by Soxhlet method of extraction by using ethanol as solvent and the extract was screened for the presence of phytochemical constituents such as alkaloids, cardiac glycosides, proteins and amino acids, carbohydrate, saponin, phenolic compounds, tannins, flavonoid compounds, and phytosteroids following standard procedures[26,27].

Table 2. Different reagents for fluorescence analysis

S. No.	Reaction Mixture	S. No.	Reaction Mixture
1	Powder as such	11	Powder + Pet. Ether
2	Powder+ 1N Aqueous NaOH	12	Powder+ Acetonitrile
3	Powder + 1N HCl	13	Powder + Chloroform
4	Powder + Conc. H_2SO_4	14	Powder + Methanol
5	Powder + Conc. HNO_3	15	Powder + Ethanol
6	Powder + Conc. HCl	16	Powder + 5% KOH
7	Powder+ Conc. 50% HNO_3	17	Powder + Picric acid
8	Powder + Acetic acid	18	Powder + Distilled water
9	Powder + Ferric chloride	19	Powder + Acetone
10	Powder + NH_3		

2.7 Thin Layer Chromatography

5 g of the powdered plant materials were cold-extracted with 30 mL of ethanol for 24 h. The filtrate was concentrated under a vacuum of 5 mL and used for the experiment. Three spots were given on the TLC plate (Analytical TLC on silica gel G_{60} F_{254}, 0.25 mm layer), one for standard β-sitosterol (purchased from Natural Remedies Pvt. Ltd.) and others for extract just 2 cm above the bottom, and a chromatogram was developed using mobile phase Chloroform: Petroleum ether (9.5:0.5) as the mobile phase. Separated compounds were detected with 1N H_2SO_4 solution, and characteristic fluorescence was investigated under visible light.

3. Results

3.1 Macroscopical Study

3.1.1 Macroscopical Characteristics of Leaves

The colour of the leaf is green on both sides. The upper and lower surfaces of the leaf are hairy with rough lamina. The base is mostly cordate or cuneate, while the apex is acute or obtuse, broadly deltoid or ovate shape, irregular margin, and 3-5 lobes as shown in Figure 1 (b) and described in Table 3. Leaf powder is green in

Table 3. Macroscopic characteristics of leaf and roots

Organoleptic Parameter	Leaf	Root
Colour	Green	Brown to Light Brown
Odour	Characteristic	Aromatic
Taste	Unpalatable	Unpalatable
Size	Length-5.1 cm to 15.2 cm and width-4.5 cm to 13.1 cm	2 to 12 cm long
Shape	Broadly deltoid or ovate, irregular with 3-5 lobes.	Round, hairy, irregular

colour, gritty in texture, and has a characteristic odour and unpleasant taste.

3.1.2 Macroscopical Characteristics of the Root

The colour of the root is brown with a round, hairy,

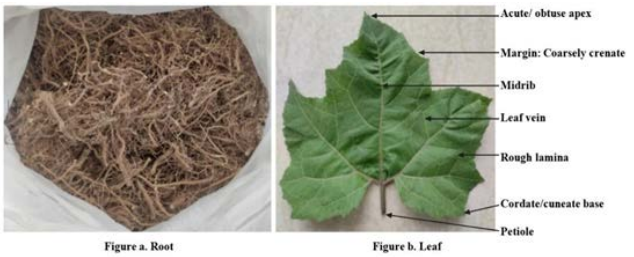


Figure 1. Morphological Characteristics of root and leaf.

irregular shape as shown in Figure 1 (a) and described in Table 3. The root powder is dark to light brown in colour, aromatic in odour, and has a bitter taste.

3.2 Microscopic Analysis

3.2.1 (a). Transverse Section of the Leaf

The transverse section of the leaf was seen under the Primo Star Zeiss microscope. Different types of microscopical characters were observed in the T.S. section. At the centre, 4-6 lobes of fibrous tissue (xylem and phloem) were observed. The epidermis was observed at the upper and lower lamina of the leaf, along with palisade cells just beneath the epidermis. Hairy, non-glandular trichomes were observed on the whole outer surface. Parenchyma and collenchyma tissues were clearly observed, as shown in Figure 2.

3.2.1 (b). Transverse Section of the Root

In the transverse section of *X. indicum*, the root vascular bundle and medullary rays were mostly observed, along with cork and cortex at the outer part of the root section (Figure 3).

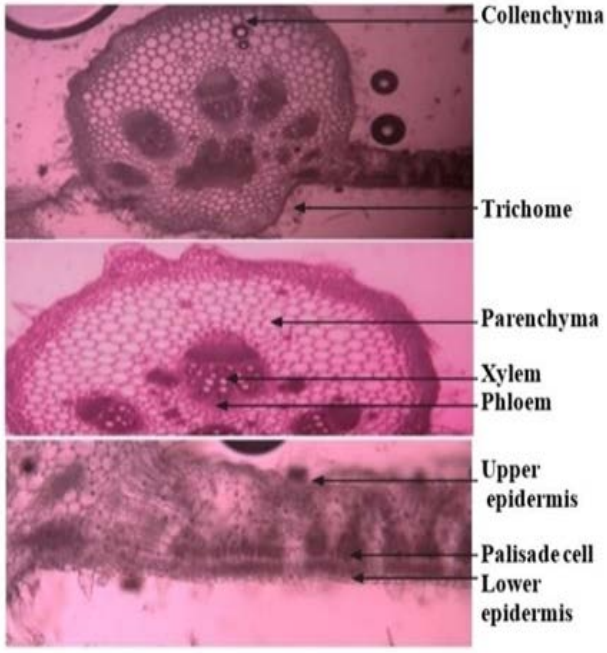


Figure 2. T.S. of the leaf midrib.

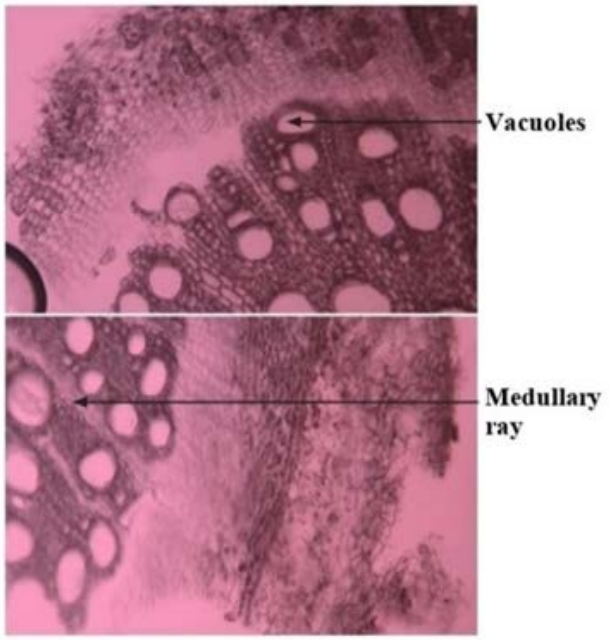


Figure 3. T.S. of the root.

3.2.2 Leaf Constants or Diagnostic Characters of Leaves

Leaf diagnostic characteristics such as stomatal number, stomatal index (for both upper and lower surface), vein-islet number, vein-termination number, and palisade ratio were determined as shown in Figures 4, 5, 6 and 7 and summarised in Table 4 using a Primo Star Zeiss microscope. Anomocytic stomata were observed on both the upper and lower surfaces of the leaf, as shown in Figures 4 and 5.

3.2.3 Powder Microscopic Evaluation

3.2.3.1 Powder microscopy of leaves

Powder microscopy of *X. indicum* leaves revealed the presence of scalariform vessels, elongated sclerites, glandular and non-glandular trichomes, anomocytic stomata, and calcium oxalate crystals (Figure 8).

Table 4. Diagnostic characteristics of the leaf

S. No.	Parameter	Result (1 mm sq.)
1	Stomata number (40 x)	U-80, L-140
2	Stomata index	U-13.33, L-17.94
3	Vein-islet number (4 x)	440
4	Vein-termination number (4 x)	120
5	Palisade ratio (40 x)	6.5

3.2.3.2 Powder Microscopy of the Root

Powder microscopy of the root of *X. indicum* revealed the existence of a bordered pitted vessel fragments, cork in surface view, stone cells, phloem parenchyma with pits, cork cell, and calcium oxalate crystals. (Figure 9).

3.3.1 Physicochemical Examination of Leaves

A physicochemical examination for leaf powder was determined, and the results are presented in Table 5 with a standard deviation based on triplicate values. The parameters included ash values (total ash, acid-insoluble ash, and water-insoluble ash), loss on drying, extractive value, and pH value. The leaves had the highest total ash value compared to water-insoluble and acid-insoluble ash. The loss on drying result shows the total moisture content of the sample. The extracts pH value suggested that the material is somewhat basic in nature. Successive extractive values were determined using chloroform, methanol, and petroleum ether. The sample had a higher maximum extractive value for petroleum ether than with methanol and chloroform, which had the lowest extractive values.

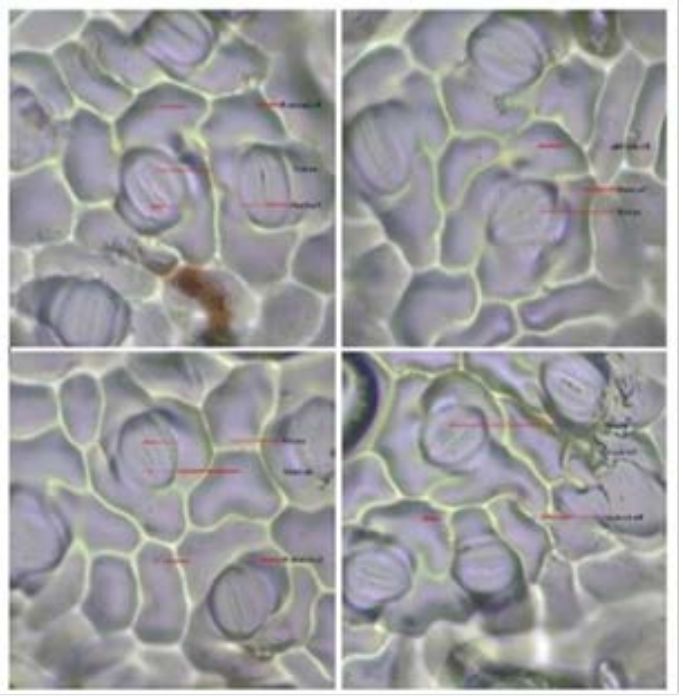


Figure 4. Upper leaf surface stomata.

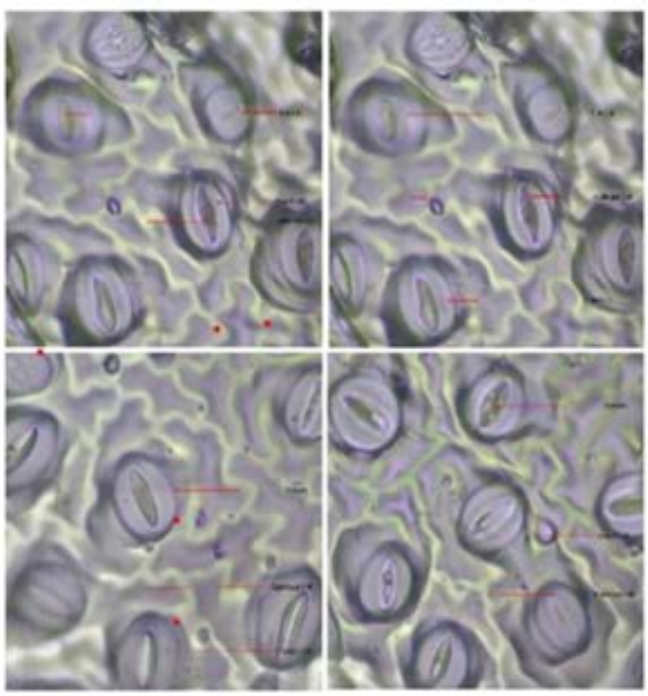


Figure 5. Lower leaf surface stomata.

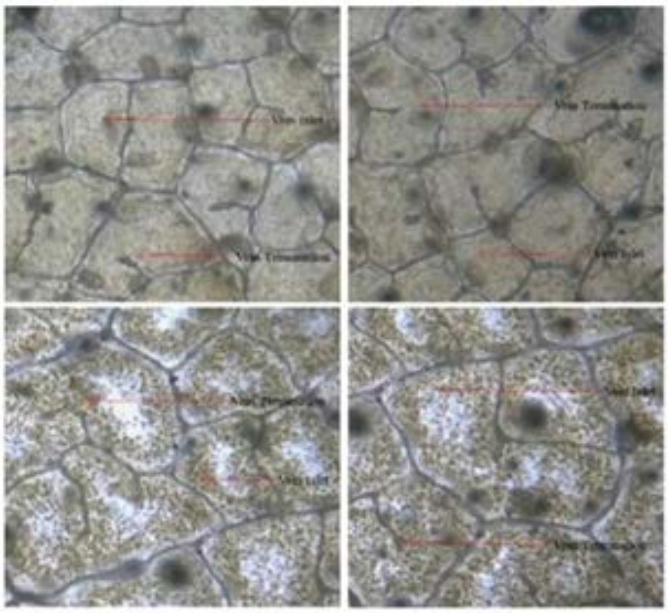


Figure 6. Vein-islet and termination number.

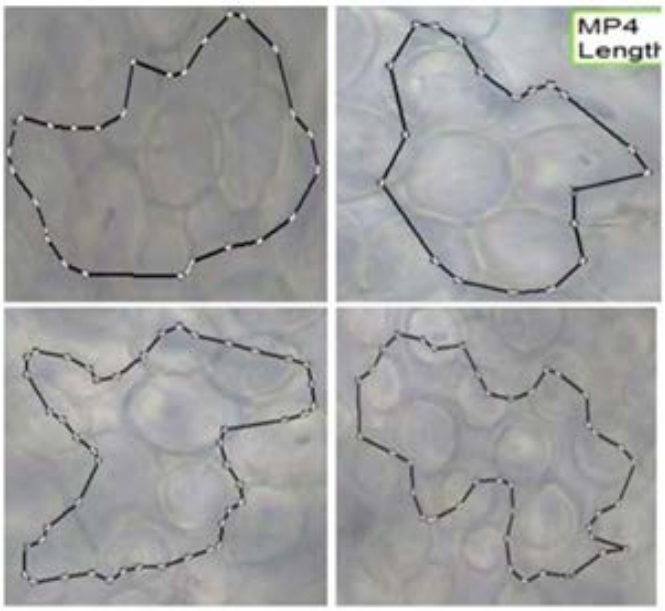
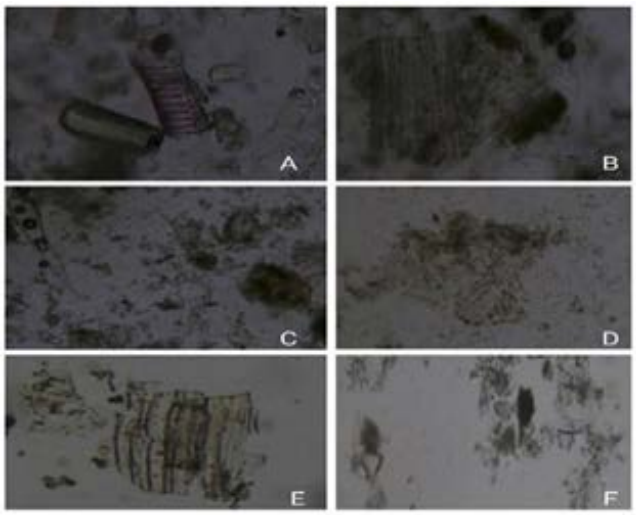
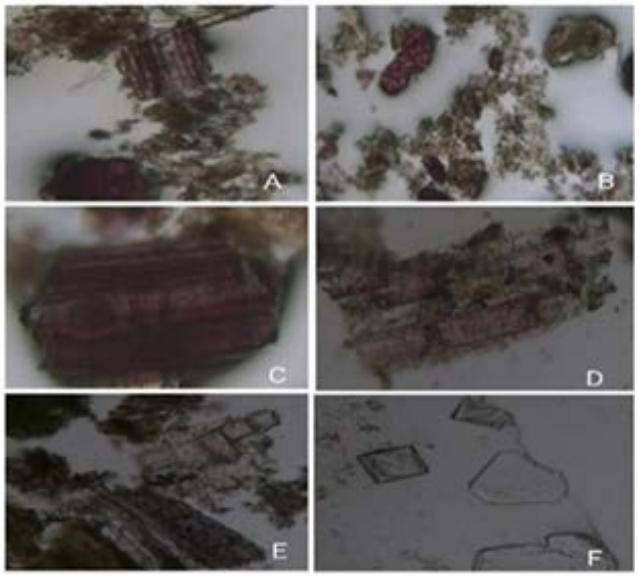


Figure 7. Palisade cell.



A. Scalariform
B. Elongated sclerids
C. Glandular trichome
D. Stomata
E. Elongated sclerids
F. Ca. oxalate crystal

Figure 8. Powder microscopy of the leaf of *X. indicum*.



A. Fragment of bordered pitted vassal
B. Cork in surface view
C. Stone cell
D. Phloem parenchyma with pits
E. Cork cell
F. Ca. oxalate crystal

Figure 9. Powder microscopy of the root of *X. indicum*.

3.3.2 Physicochemical Examination of the Root

A physicochemical parameter of root powder was determined, and the results are presented in Table 5 with a standard deviation based on triplicate values. Ash values (total ash, acid-insoluble ash, and

water-insoluble ash), loss on drying, extractive value, and pH value were among the parameters measured. The roots had a higher total ash value than the water and acid-insoluble ash. The loss of drying result indicates the overall moisture content of the sample. The extract's pH value indicated that it is fairly basic in nature. The extraction values of chloroform, methanol, and petroleum ether were determined in order. Petroleum ether had the highest extractive value in the sample, followed by methanol and chloroform, which had the lowest extractive values.

All results were calculated using triplicate values. SD= Standard Deviation.

3.3 Fluorescence Analysis

The powdered roots were exposed to daylight and short and long-wavelength UV light after being treated with various chemicals. Different colours were noticed in all environments, recorded, and summarised in Table 6.

The findings were observed using triplicate values.

3.4 Preliminary Phytochemical Screening

A preliminary phytochemical screening was performed on the root to identify diverse phytochemical groups. Several phytochemicals were detected in the ethanolic extract of *X. indicum* roots. The roots were found to be a rich source of many phytochemical groups. Alkaloids, cardiac glycosides, proteins, carbohydrates, phenols and tannins, flavonoids, steroids, saponins, and amino acids were the most abundant phytoconstituents identified and summarised in Table 7.

3.5 Thin Layer Chromatography

The results of TLC profiling of ethanolic extract of *X. indicum* roots showed the presence of β- Sitosterol with Rf value 0.44 as compared to standard. The chromatogram of TLC profiling of standard β- Sitosterol and ethanolic extract have shown together in Figure10.

4. Discussion

Because of their lower toxic and damaging effects, phytopharmaceuticals are gaining favour as an alternative to allopathic treatments. Due to the increased demand for herbal medicine and plant-derived pharmaceuticals, WHO has emphasised the need to take appropriate steps to ensure the quality of medicinal plants and their derivatives *via* the use of diverse methodologies and standard processes[28]. Pharmacognostic characteristics and standards must be developed before any crude medication is included in herbal pharmacopoeia because the standardisation parameter of a crude medicine is essential for determining its true identity[29].

According to the present literature, the distinct pharmacognostic parameters of *X. indicum* have not been established. Thus, this information will serve as the foundation for future identification and validation of this scientifically unknown herb, which local healers in West Karbi Anglong claim to possess a great analgesic effect on tooth pain.

Table 5. Physicochemical parameters of root and leaf

S. No	Proximate Composition Root		Value (% w/w) Mean (n=3) ±SD	
			Leaf	Root
1	Loss on drying		1.3 ± 0.2013	1.05 ± 1728
2	pH		7.493±0.0524	7.502±0.0892
3	Ash value		5% ± 0.3412	18% ± 0.2417
4	Acid insoluble ash		0.5% ± 0.2211	5% ± 0 .1380
5	Water insoluble ash		1.5% ± 0.1735	10% ± 1503
6	Extractive value	Ethanol soluble extract	3.46%±0.0521	11.74%±0.9188
		Chloroform soluble extract	2.02%±0.0125	7.57%±0.5090
		Petroleum benzene soluble extract	2.97%±0.0257	9.21%±0.4912

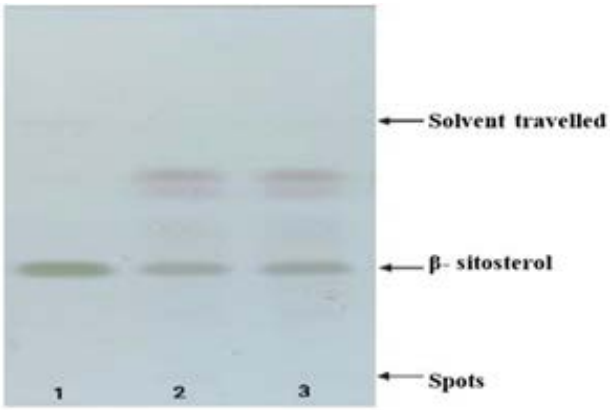
The findings were noted using triplicate values.

Table 6. Fluorescence analysis of root powder with different reagents

S. No.	Reaction Mixture	Daylight	UV (254nm)	UV (365nm)
1	Powder as such	Brown	Black	Brown
2	Powder+ 1N Aqueous NaOH	Yellowish Brown	Black	Black
3	Powder + 1N HCl	Old gold	Greenish black	Light Broun
4	Powder + Conc. H_2SO_4	Black	Black	Dark green
5	Powder + Conc. HNO_3	Orange	Black	Dark black
6	Powder + Conc. HCl	Light brown	Black	Dark black
7	Powder + 50% HNO_3	Devil red	Crimson black	Black
8	Powder + Acetic acid	Bronze	Brownish black	Blackish brown
9	Powder + Ferric chloride	Shark teal	Black	Black
10	Powder + NH_3	Greenish brown	Dark brown	Black
11	Powder + Pet ether	Light brown	Greenish	Light green
12	Powder+ Acetonitrile	Light brown	Greenish	Light green
13	Powder + Chloroform	Light brown	Dark green	Light green
14	Powder + Methanol	Light brown	Greenish	Light green
15	Powder + Ethanol	Light brown	Dark green	Light green
16	Powder + 5% KOH	Metallic gold	Dark green	Black
17	Powder + Picric acid	Raven gold	Brownish black	Black
18	Powder + Distilled water	Bronze	Greenish	Dark green
19	Powder + Acetone	Light brown	Greenish	Light green

Table 7. Phytochemical screening of ethanolic root extract of *X. indicum*

S. No	Phytoconstituents	Ethanol Extract
1	Alkaloids	+
2	Cardiac glycosides	+
3	Proteins	+
4	Carbohydrates	+
5	Phenols and tannins	+
6	Flavonoids	+
7	Steroids	+
8	Saponins	+
9	Amino acids	+
10	Quinones	-



1. Standard-sitosterol
2 and 3- Extract

Figure 10. TLC chromatogram.

The macroscopic study is the morphological examination of the parts of the plant that can be seen with the naked eye. It aids in determining the basic distinguishing characteristics of several species within a single genus[30]. All macroscopic characteristics of the leaves and roots were recorded for rapid and accurate identification of the plant in its native habitat, which revealed specific characteristics that may be used as particular diagnostic factors for the *X. indicum* plant[31]. The colour, odour, taste, size, shape, and other parameters of leaves and roots were examined, followed by root macroscopy, as mentioned in Table 3.

The results from microscopic examination are valuable tools for defining the Pharmacobotanical parameters that are regarded as standard features of certain plants. Microscopical examination revealed many covering trichomes, xylem, and phloem bundles in the leaf midrib T.S. section. Moreover, it has collenchyma, parenchyma, palisade cells, and upper and lower epidermis in the leaf. Vacuoles and medullary rays were found in the roots of the T.S. section. Leaf constants such as palisade ratio, vein islet number, vein termination number, stomatal number (upper and lower surface), and stomatal index (upper and lower surface) were investigated, as these are important for settling standards for crude drugs because the values are relatively constant for a specific plant species and shown in Table 4. Microscopic powder analysis revealed the presence of scalariform vessels, elongated sclereids, glandular trichomes, anomocytic stomata, calcium oxalate crystals in the leaf and fragments of bordered pitted vessels, cork in surface view, stone cells, phloem parenchyma with pits, cork cells, and calcium oxalate crystals in the roots, which are distinguishing microscopic characteristics and serve as specific anatomical identifiers[32].

Different ash values, such as total ash, acid-insoluble ash, and water-soluble ash, were examined in the investigation of the physicochemical properties of fine powder and are listed in Table 5. These ash assessments are a measure of the drug's inorganic content[33]. When genuine or pure pharmaceuticals are contaminated by adulterants, their values tend to decrease or increase[34]. As a result, an ash value indicates the presence of earthy elements and other impurities in the medicine and can be used as a reliable tool for detecting adulteration[33].

The amount of water and volatile substances present in the herbal material is determined by the loss of drying parameters. The presence of moisture in the plant material serves as a suitable medium for bacterial and fungal growth and also causes the degradation of moisture-sensitive phytoconstituents, implying that powdered plant material may be susceptible to microbial growth if proper precautions are not taken during storage[35]. To determine the purity of the test powder, other physicochemical constants such as pH were tested and documented in Table 5.

Extractive values are regarded as a significant criterion for identifying exhausted and contaminated herbal pharmaceuticals[36]. The type of chemical ingredients contained in the crude medicament, as well as the solubility of certain chemical constituents, may be identified using the extractive values[37]. In our investigation, ethanolic extract had the maximum extractive value in both the root and the leaves, followed by petroleum benzene and chloroform. As a result, the majority of the phytochemicals in the test extract are more soluble in polar solvents than non-polar solvents.

When various phytochemicals are handled with various solvents and chemical reagents, they produce radiation of varying wavelengths[38]. In our fluorescence assessment, the root powder of *X. indicum* plant displayed particular fluorescence characteristics in diverse scenarios of daylight, short wavelength, and long wavelength, as shown in Table 6, which may be used to determine the right identity of the drug.

The presence of alkaloids, cardiac glycosides, proteins, carbohydrates, tannins and phenolic substances, flavonoids, steroids, saponins, and amino acids was detected in the preliminary phytochemical examination of ethanolic extracts. Thus, basic phytochemical analysis aids in defining the kind of phytochemical components present in the extract, which may lead to quantitative estimation, as well as identifying the source of pharmacologically active phytocompounds[39].

Thin-layer chromatography is usually done for better identification of the bioactive compounds. In the present study, the TLC profiling of ethanolic extracts was done. The study revealed the presence of β-Sitosterol when compared with standard β- Sitosterol (Natural Remedies Pvt. Ltd.). The Rf values of the compounds provide an idea about polarity that may also help in selecting a particular solvent system for further isolation and quantification with sophisticated instruments such as HPLC, HPTLC *etc*. Compounds showing high Rf value in less polar solvent systems have low polarity while those with a low Rf value have high polarity[40].

5. Conclusion

To establish minimum standards for the correct identification of plants or plant parts, particularly for *X. indicum* Roxb. (a plant whose only recognized therapeutic use is traditional and for which not much

pharmacogenetic data are provided), becomes the very necessary. To fill this gap, in order to unambiguously prevent confounding with relaxation, extensive pharmacognostic analysis was carried out based on macroscopic, and microscopic studies on leaves and roots of the plant. Such observations prevent misidentification and adulteration through recognition of unique anatomical features of the proper plant parts such as trichomes, xylem, and phloem bundles. Then, quantitative macroscopical techniques also obtained species-specific values of key importance in the standardization of raw herbal materials *e.g.* of stomatal count, vein islet count, and palisade ratio. Natural source physicochemical evaluation such as ash value, moisture content, pH, and extractive values are further studied to ensure the purity and quality of the plant. The assessment provides an indication for contamination or degradation possibilities, suggesting that when ash content and moisture increases, this is a sign of adulterant presence and a decrease in shelf life. Fluorescence analysis under visual and UV light, after treatment with various reagents, provided a fast and clear means to authenticate plant identification through recognizable fluorescence patterns. Preliminary phytochemical screening has indicated the presence of important bioactive classes such as alkaloids, flavonoids, and steroids, which might be responsible for the drug potential of the plant, especially its use as a toothache remedy as found in traditional medicine, perhaps due to phenylpropanoids worth isolating. Further Thin Layer Chromatography (TLC) ran alongside to confirm the existence of β-sitosterol, a phytochemical marker useful for standardization and for drug development in the future. In all, the multidisciplinary outcomes collectively provide preliminary pharmacobotanical, physicochemical, and phytochemical data on *X. indicum* and constitute a platform for its standardization towards quality and call for further investigations into its phytochemistry and pharmacological potential.

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7. Author Contribution

Surender Prasad and P. C. Lalawmpuii: Conceptualization, methodology, laboratory work, formal analysis, investigation, data curation, writing-original draft preparation; Surender Prasad, P. C. Lalawmpuii, Bikash Gupta and Yashdeep Pathak: Writing- review and editing, validation, data curation, supervision, project administration. All authors have read and agreed to the published version of the manuscript.

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