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PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF *TABEBUIA PALLIDA (LINDL.)MIERS* LEAVES

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Abstract:

The study's primary goal was to perform preliminary phytochemical and pharmacognostic investigations to look into the therapeutic qualities of *Tabebuia pallida* leaves. For pharmacognostic studies, organoleptic, microscopic, and physical characteristics were evaluated. Methanol Maceration and Soxhlet extraction techniques were used to extract dried leaves. After that, the extracts were analysed for a variety of chemical components, including proteins, carbohydrates, glycosides, steroids, alkaloids, tannins, and phenolic compounds. The results finally showed that steroids and carbohydrates were present.

Index terms: Pharmacognostic, Soxhlet, Maceration, Methanol

INTRODUCTION:

We find plants fascinating in all aspects, and each plant has at least one therapeutic quality. Since the dawn of humankind, medicinal plants have been used extensively to cure a wide range of illnesses. The chemical components found in medicinal plants are what give them their potential health advantages. While there are numerous options available today for treating different illnesses, goods containing plant-based therapeutic ingredients are of utmost importance.

Bignoniaceae is the family to which *Tabebuia pallida* belongs. The genus *Tabebuia pallida* is diversified, containing a range of flowering plant species. Their distinctive deciduous characteristics are well-known, and they are primarily favoured as decorative plants. The Caribbean is home to this plant. It often reaches a height of 25 to 40 feet. The trumpet-shaped flowers range in colour from pink to lavender. Despite the fact that *Tabebuia pallida* has been shown to have antibacterial, antioxidant, and anti-cancer properties. It is thought to have anti-inflammatory and analgesic qualities, as well as immune system support and blood purifying properties, based on its traditional applications(https://en.wikipedia.org/wiki/Tabebuia_pallida).

In order to fully understand *Tabebuia pallida's* physical, chemical, microscopical, and chemical ingredients, a thorough assessment of the plant's pharmacognostic and phytochecimal properties is being carried out in this work.

Materials and Methods

Chemicals

All the chemicals were of highest available purity and were Procured from E. Merck, Mumbai, India, HiMedia Laboratories, Mumbai, India and SD fine chemicals, Mumbai, India.

Procurement of plant material

The leaves of a wild developing *Tabebuia pallida* tree were collected from the botanical garden within the pharmacy department of Vaageswari College of Pharmacy located in Thimmapur, Karimnagar, Telangana, India. A certified taxonomist completed the identification and validation process. After that, a specimen was added to the institutional herbarium. The obtained plant material was carefully cleaned to remove any organic debris that wasn't native to the area. After that, the leaves were divided, dried in the shade, and ground into a fine powder using a lab mixer before being sieved. Studies on pharmacognostic use of both fresh and powdered leaves were carried out.

Pharmacognostic Evaluation

Organoleptic evaluation

The size, shape, colour, and flavour of the leaves, among other sensory characteristics, are carefully recorded while evaluating the organoleptic attributes of plant materials. In research, conclusions are drawn from impressions gleaned from these sensory observations.

Microscopic assessment

Examining powdered crude pharmaceuticals under a microscope is essential because it gives light on identifiable tissue shapes and cell fragments. Furthermore, using the eyepiece to examine surface constants as stomatal number, stomatal index, and Palisade ratio has diagnostic value. These constants aid in the identification of flaws and the verification of leaf capsules. Microscopic examinations, with or without staining, are frequently used to investigate the diagnostic properties of *Tabebuia pallida* leaves and leaf powder (Goyal R.K. and Shah B.S 2005).

1. Powder analysis of leaf

On a microscopic slide, a small amount of powder was added, and then 1-2 drops of a 0.1% phloroglucinol solution and a drop of strong hydrochloric acid were added. After that, the apparatus was submerged in glycerol, placed

beneath a coverslip, and viewed at a 10x magnification using a microscope. Vascular tissues, xylem fibres, and phloem fibers—distinctive characteristics of the powder—were noted and recorded.

2. Determination of stomatal index

A test tube filled with 5 ml of chloral hydrate solution was used to hold the leaf fragments (5 x 5 mm), which were then cooked in a water bath for around 15 minutes until they became transparent. Following boiling, these pieces were placed on a microscopic slide, covered with glycerol, and viewed under a microscope to measure and count the number of epidermal cells, vein islet variety, stomata (type and distribution), and palisade cells. After that, the slide was adjusted to a 45x objective and a 10x eyepiece with a camera lucida connected so that epidermal cells and stomata in a chosen region could be recorded. By treating each stoma as a separate cell and computing the percentage of stomatal number per number of epidermal cells, the stomatal index was found.

Extraction methods:

Maceration

The maceration process yields a methanolic extract of the leaves of the *Tabebuia pallida* plant. Of all the techniques, maceration is the easiest. The extract underwent an initial phytochemical analysis by the maceration procedure in order to identify the alkaloids, glycosides, flavonoids, carbohydrates, proteins, amino acids, saponins, and sterols. Weigh out 100 grammes of plant powdered leaves, then fill the conical flask with the necessary amount of solvent (methanol) powderThe powder was thoroughly combined with the solvent and stirred with a glass rod. After that, the beaker is sealed with aluminium foil and stored for a minimum of seven days. Periodically shaking it is necessary to make sure all of the material is extracted. Following the extraction process, the extracts were concentrated and filtered before being evaporated by sunlight for more research. Weighing the leftover extract after evaporation allows us to determine whether any alkaloids or carbohydrates are present (Mukherjee P.K 2002).

Soxhlation

The main step in the soxhlation process is the extraction of leaf powder. The procedure is as follows: first, we arranged the thimble equipment with the soxhlet setup, and then we took 100g of leaf powder. Next, a soxhlation apparatus was installed, along with porcelain pieces and a running water system connection for the condensation process. A round-bottom flask was also connected. The solvent used in this apparatus was methanol. The procedure involves heating the extract for six hours at 60 degrees Celsius on a hob. The extract is then collected and allowed to dry in a china dish. Once dried, the extract is weighed and the yield is recorded.

Subsequently, the dehydrated extract is employed in chemical analyses and the screening of phytochemical outcomes. Molish, Benedict's, Fehling's, and biuret tests are among the chemical tests used to determine or estimate the amount of carbohydrates. The Libermann-Burchard and Salkowski tests are used to determine the amount of steroids. The Alkaline and Shinoda tests are used to determine the amount of flavanoids. The Flavonoids, Alkaloids, and Tannins tests are used to observe the presence of the compounds in the leaf through the extraction process of the soxhlation process of tests performance (Evans W.C 2002).

Physical Evaluation

Crude fibre (acid detergent fibre), moisture content, extractive values (alcohol soluble, water soluble, and ether soluble), and ash values (total, acid insoluble, and water soluble) have all been determined in the physical examination (Harborne J.B 2005). The drug's inorganic salts are what make up the ash values. The variability in chemical nature and qualities of the drug's contents is indicated by the approximate measures of specific chemical components found in extracts obtained through the exhaustion of crude pharmaceuticals(Heinrich M and Barnes J 2004). The findings are shown as mean ± SD, and the calculations were done in triplicate. The percentage of w/v values is the same as that of the medication that has been air-dried (Joshi S.G 2000).

1. Estimation of crude fiber (Acid detergent fiber, ADF)

Alkali-soluble lignin, cellulose, and lignified nitrogen make up the leaf's ADF. Two grammes of leaf material were refluxed with 50 millilitres of ADS (20 grammes of cetyl trimethyl ammonium bromide, or cetrimide) in one litre of previously standardised N sulfuric acid) in a 500 millilitre Berzelius beaker. The refluxing was done by first heating the mixture vigorously and then more gradually. The beaker's contents were moved to a tared crucible and allowed to percolate through the sintered glass plates following a one-hour reflux distillation period. Boiling water was used to repeatedly wash the residue until the filtered solution had no more foam. After being sucked dry, the residue was cleaned with 3 x 20 ml of acetone and then sucked dry once more. After being held overnight at 100 °C in a hot air oven, the crucibles were weighed after being cooled in a desiccator. The amount of ADF in the sample was the residual that remained insoluble in the heated ADS. Leaf powder's ADF content was computed(Bruneton J 1999).

2.Determination of moisture content (Loss on drying):

Two grammes of *Tabebuia pallida* (Lindl.) miers powder are taken, put in a tarred evaporating dish, and baked at 105˚ C for five hours. The weight of the amount we obtained is then calculated. Weighing and drying should be done in sequence every hour until the difference between the two subsequent weights is computed and the corresponding value is less than 0.25%. once successive weights of at least 0.01 g are obtained following 30 minutes of drying and 30 minutes of desiccator cooling (Khandelwal K.R 2002).

Ash Values:

The substance left over after burning is called the drug's ash content, which is made up of inorganic salts that are either naturally present in the drug or adhere to it. Three different kinds of ash values can be found for a crude drug: total ash, acid insoluble ash, and water-soluble ash (Mukherjee P.K 2002).

3.Determination of Total Ash:

First, measure out two grammes of *Tabebuia pallida* (lindl.) Miers leaf powder and put it in a crucible made of silica that has been tarred. The medication in powder form was burned at a temperature of no more than 450˚ C until it was carbon-free. After cooling, the resulting ash was weighed. The proportion of ash was computed using the medication that had been air-dried. Typically, silicates, phosphates, and silica make up total ash.

4.Determination of Acid-insoluble ash:

Two grammes of crude drug leaf powder with a total ash value were taken, and it was boiled in 25 millilitres of diluted hydrochloric acid solution for five minutes. Next, the insoluble matter was collected using ash-less filter

paper, and it was then twice washed with hot water, ignited, cleaned, and weighed. Finally, it was allowed to air dry for a while, after which the percentage of the air-dried insoluble ash value was calculated (Parotta J.A 2001).

5.Determination of water-soluble ash:

Two grammes of crude drug made from leaf powder with a total ash value were taken, and it was boiled in 25 millilitres of water for five minutes. Filter paper was then used to collect the insoluble matter from the ash-less filter paper, and this washed twice with hot water, ignited, cleaned, and weighed before being allowed to air dry for a while and the percentage of water-soluble ash was calculated (**Rangari V.D 2004)**.

Extractive values:

6.Determination of alcohol soluble extractive:

A separate 5 g portion of precisely weighed leaf powder was macerated for 24 hours in 100 ml of 95% alcohol. For the first six hours, the contents were shaken often, then they were left for eighteen hours. The extract was filtered after 24 hours, and 25 millilitres of the filtrate were evaporated. At 105°C, the extract was dried to a consistent weight.

7.Determination of water-soluble extractive:

With the exception of utilising chloroform water for maceration, the method for determining the water-soluble extractive value was the same as that for alcohol soluble extractive.

8.Determination of ether soluble extractive:

Five grammes of precisely weighed leaf powder were extracted, and a thimble pack was made. For six hours, the crude medication in the pack was extracted using solvent ether in a continuous extraction (Soxhlet) device. After the extract was filtered, the filtrate was dried to a constant weight at 105°C by evaporating it.

Fluorescence characteristics

By using this technology, we are able to investigate the drug's unknown specimen and identify adulterants in the plant material based on their fluorescence (Alabi D.A and Alausa A.A 2006).

RESULTS AND DISCUSSIONS:

Pharmacognostic Evaluation:

Organoleptic and microscopic evaluation

Appropriate characteristics, such as leaf powder and leaf size, shape, colour, and odour, were examined during the organoleptic examination. From a macroscopic perspective, the leaf exhibits a simple composition, opposing arrangement, acute apex and base, a complete edge, and an average dimension of 8.2 ± 0.4 cm for length and 4.6 ± 0.2 cm for width. Fresh leaves have a distinctive smell, are green in appearance, and have a little bitter flavour. The leaf powder had a distinctive smell, was bitter to the mouth, and was green in colour.

Micromorphological characteristics showed that the leaf powder contains a large number of simple and compound starch grains as well as calcium oxalate crystals in the shape of raphide. Phloem and xylem were also visible in the

powder. Lignified were the multicellular, lengthy, and covered trichomes seen.

The findings of the quantitative microscopic analysis of leaf powder and fresh leaves are displayed in Table 1 and Figures 1-8. Fresh leaves have Anomocytic arrangement in their lower epidermal layers, where the stoma is surrounded by a definite number of cells that are not different from the remainder of the epidermis. The top layers of the epidermis have no stomata.

Table:1 Quantitative Microscopic characteristics of leaves *Tabebuia pallida (lindl.) miers*

Parameters	**Value**
Stomatal number (lower epidermis)	220/mm^2
Stomatal index (lower epidermis)	13
Phloem fibres (length)	6-40 µ
Phloem fibres (width)	2-10 µ
Xylem (length)	12-50 µ
Xylem (width)	5-20 µ
Value islet number	15/mm^2
Vein termination	7/mm^2
Palisade ratio	4.55/mm^2

Physical evaluation:

The different physical characteristics of leaves and leaf powder, such as moisture content, ash values (total, acid insoluble, and water-soluble), extractive values (alcohol soluble, water soluble, and ether soluble), and crude fibre (acid detergent fibre) were measured. Table 2 displayed the study's findings.

The outcomes of these studies may provide a foundation for accurate plant identification, collecting, and research.

Table:2 Physiochemical parameters of leaf powder *Tabebuia pallida (lindl.) miers*

Parameters	**Value %w/w**
Acid detergent fiber	31.5
Total ash	6.32
Acid insoluble ash	0.54
Water soluble Ash	3.57
Alcohol soluble extractive value	7.2
Water soluble extractive value	14.2
Ether soluble extractive value	7.5
Moisture content	68.5

Fluorescence characteristics:

When alternative approaches yield unsatisfactory results, this quick procedure can be used to develop research on a crude drug of an uncertain specimen. Based on the nature of their fluorescence, the plant material can be distinguished from their adulterants. Table 3 provides a description of the findings.

Table 3: Fluorescence characteristics of drug with different chemicals under UV

	Observation (color developed)		
	Visible Light	**Ultraviolet light**	
		254nm	**365nm**
Powder alone	Light green	Light green	Light yellow
Powder + Picric acid	Lime green	Light green	Black
Powder + NaOH	Lime green	Lime green	Black
Powder + Glacial acetic acid	Green	Brown	Black
Powder + HCL	Lime green	Light brown	Black
Powder + HNO3	Colorless	Tea green	Black
Powder + iodine	Green	Brown	Colorless
Powder + FeCL3	Green	Brown	Black
Powder + H2SO4	Colorless	Brown	Navy blue
Powder + Methanol	Brown	Dark Brown	Dark blue

Preliminary Phytochemical Evaluation:

The type and yield of the extracts were monitored after *Tabebuia pallida* leaf powder was extracted with methanol using the processes of Soxhlation and maceration extraction. Resinous extracts were created via methanol. Dark, green-colored extracts were obtained from the leaf powder using both extraction techniques. It was discovered that the extracts had yields of 20.63 and 15.36% w/w, respectively.

Testing for flavonoids, steroids, terpenoids, phenolics, tannins, alkaloids, and glycosides gave positive results when the leaves were subjected to methanolic extraction for the Soxhlation and Maceration extracts.

These secondary plant metabolites may be the cause of *Tabebuia pallida* 's diverse functions, as they are known to have a range of pharmacological effects.

Many medical disorders are treated using *Tabebuia pallida*. A crude drug's standardization is essential to determining its accurate identity. Pharmacognostic metrics and standards must be established prior to the inclusion of any crude medication in a herbal pharmacopoeia. The outcomes of the current studies may provide a foundation for accurate plant identification, collecting, and research. The leaf described has both macro- and micro-morphological characteristics that set it apart from other members of the genera. Quantitative leaf microscopy and numerical data are specific to the plant and are needed for standardization. Numerous secondary plant metabolites that have been attributed to a range of pharmacological actions were found during phytochemical analysis and results showed in table 4.

Table:4 Phytochemical and preliminary evaluation:

Test	**Soxhlation observation**	**Maceration observation**
Carbohydrates	+ve	+ve
Glycosides	+ve	+ve
Proteins And Amino Acids	+ve	+ve
Steroids	+ve	+ve
Flavanoids	+ve	+ve
Alkaloids	+ve	+ve
Tannins And Phenolic Compounds	+ve	+ve

FIG 1:TRANSVERSE SECTION OF LEAF

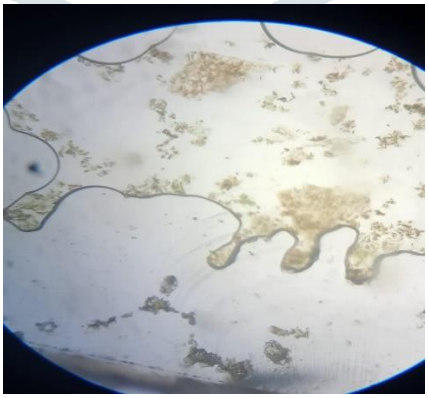

FIG 2: EPIDERMAL CELLS WITH STOMATA

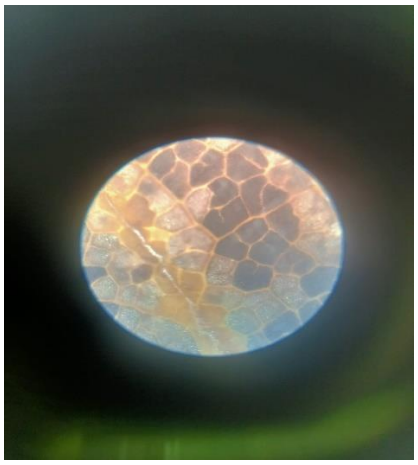

FIG:3 VEIN-ISLET AND VEIN-TERMINATION

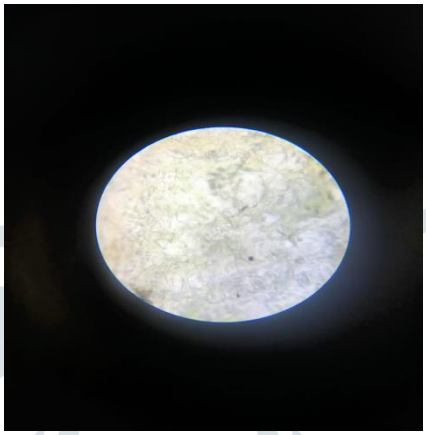

FIG 4: STOMATA

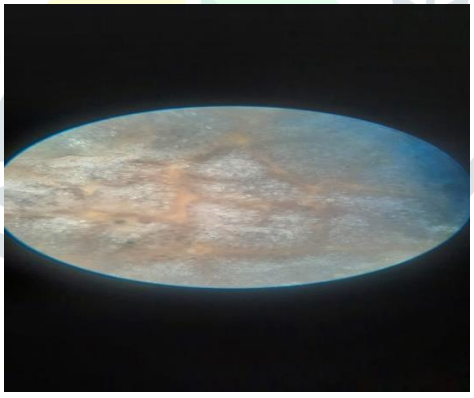

FIG 5: PALISADE CELLS

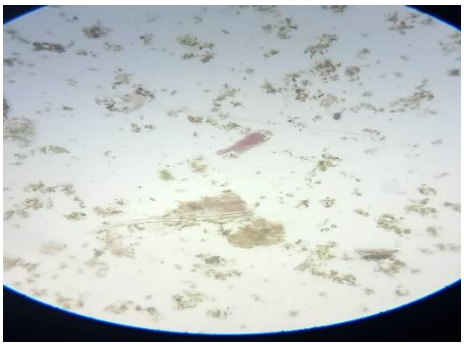

FIG:6 PHLOEM FIBERS

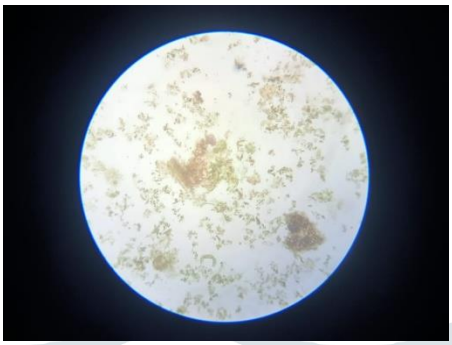

FIG:7 XYLEM VESSELS

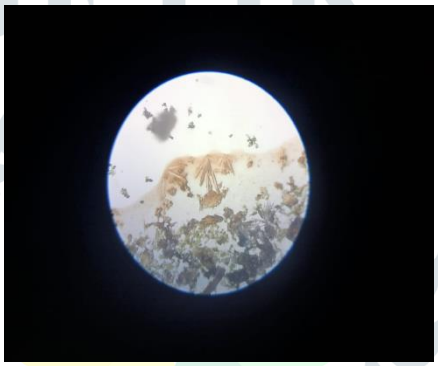

FIG 8:TRICHOMES

CONCLUSION:

For the first time, the pharmacognostic parameters are being provided, and they may help identify and standardize a crude medication. The information gathered during this inquiry is also useful for creating the monograph for the crude drug and adding it to different pharmacopoeias.

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