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STANDARDIZATION OF *POUTERIA CAMPECHIANA* LEAF FOR ITS PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES

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ABSTRACT

Medicinal plants have been used to treat various illnesses for decades. The present study is primarily focused on the Pharmacognostical and phytochemical analysis of the *Pouteria campechiana* leaves in order to authenticate plant material suitable for traditional use. Pharmacognostic study mainly covers the macroscopic and microscopic features of the leaves including powder microscopy and revealed the presence of epidermal cells, palisade cells, trichomes, rod shaped calcium oxalate crystals, stomata, phloem and xylem vessels. Preliminary phytochemical screening showed the presence of various phytoconstituents like alkaloids, glycosides, tannin, flavonoids, steroid, phenols,carbohydrate and protein, and absence of saponins. Determination of moisture content, ash value,extractive value were performed.Estimation of total phenoplic content and total flavanoidal content were done which will help in qualitative studies of the plant.The proposed study is usefull to identify the plant by physicochemical, macroscopical and microscpical techniques.

Keywords: *Pouteriacampechiana*, Pharmacognostical, Macroscopic, Microscopic,physicochemical, phytochemical

INTRODUCTION

The biodiversity of India has numerous potential in the fields of medicine and related sciences. This biodiversity is essential to both conventional and folk medicine .The only method through which the ancestors met their medical needs was through traditional medicine. Although it was developed through personal and ethnic experiences, it still requires additional research under the guidelines of several disciplines of advanced scientific methodologies to address issues like identification, standardisation, safety profiles, etc. According to a factual study conducted by the World Health Organisation (WHO), 70–80% of all communities worldwide rely on eastern herbal remedies for their health care systems.Due to is use in treating a number of ailments with less hazardous side effect.

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 .

Pouteria campechiana (Kunth) Baehni is an evergreen tree which is widely found around the world, belonging to the family Sapotaceae. It is commonly known as canistel or egg fruit. Canistel is a native of Central America. The edible part of the tree is its fruit which resembles a hard-boiled egg yolk giving its common name egg fruit [3]. *Pouteria campechiana* was found tobe rich in secondary metabolites and may have used for treating several diseases. Canistel is used in folk medicine to treat fever, skin eruptions and ulcers. From literature review, it was found that *Pouteria. campechiana* possess numerous biological activities such as hepatoprotective, anti-inflammatory, anti-mitotic, antioxidant, and anti-diabetic properties [4,5].Several chemical substances from the Pouteria genus have been identified, including phenolic acid, flavonoids, and derivatives of terpenoids [6]. From the leaves of *Pouteria campechiana*, six stilbenes and six flavonoid glycosides were isolated and identified. The leaves also contain phenolic and flavonoid substances like protocatechuic acid, quercetin, myricetin, myricetin-3-O-galactoside and myricetin-3-O-L-rhamnoside[7].Therefore, the present study aims to carry out the pharmacognostical and phytochemical analysis of *P. campechiana* leaf to ascertain the chemical standards and constituents, in order toidentify and authenticate the plant material for future use.

MATERIALS AND METHODS

(1) collection of plant materials

The plant *Pouteria campechiana* (Kunth) Baehni were collected in September month 2023 from local area of Malappuram district. The plant material was taxonomically identified and authenticated by the Botanist, Dr. N. Pramod Kumar, Department of Botany, NSS College, Ottapalam. The plant material was then dried under shade for about 50-60 days, powdered with mechanical grinder and stored in an airtight container until use. pharmacognostical studies

Macroscopic Studies [8]

Table no: 1 Macroscopic studies

Features	**Observations**
Colour	Light to dark green
Size	Approximately 16-25 cm long and 3.5-6 cm wide
Odour	characteristic
Taste	Astringent taste
Texture	Glossy
Shape	Lanceolate-oblong or obovate
Petioles	1-2 cm long
Apex	Bluntly pointed
Margin	Entire
Base	Sharply tapered
Venation	Pinnate

Microscopic Studies [9]

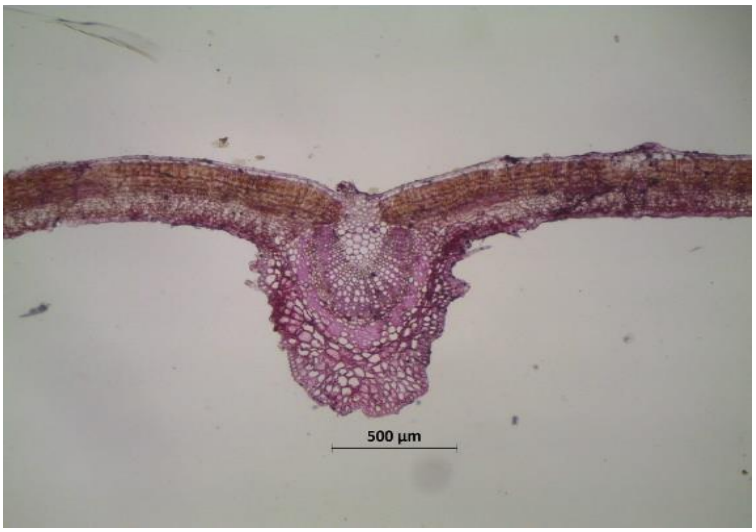

Fig no:1 Ts of *pouteria campechiana* leaves

Ts studies revealed that the epidermis is found with oval rectangular and square shaped cells covered with thin cuticle .It shows covering trichomes and anomocytic stomata .3,4 layers of elongated *pouteria campechiana* cells present in below upper epidermis of lamina .For the diagram of TS of *pouteria campechiana* leaf in figure no1 .

(2) Preparation of dried powder

The collected leaves of *pouteria campechiana* Linn were washed with running tap water to remove adheringmaterials. Then the leaves were dried under shade for about 25-30 days, powdered with mechanicalgrinder and stored in an air tight container until use [10].

(3) Powder Microscopy

Take sufficient amount of powder on a microscopic slide. Add 1-2 drops of saffranin.Spread the sample evenly over the slide. Mount in glycerin. Observe through the microscope. Repeat the procedure in 2-3 slides to get maximum characters. Transfer the images using the attached camera and software [11].

(4) physicochemical standardization

The physico-chemical parameters such as loss on drying, extractive values (water-soluble and alcohol soluble), foreign matter, ash values were determined. Physico-chemical parameters wereanalyzed in accordance with the Ayurvedic Pharmacopeia of India.

(A) 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. Cooled in desiccators and calculated the loss in weight which is usually recorded as moisture.

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

(B) Determination of Ash Value

a) Total ash

About 2gm of powdered drug was weighed accurately into a tarred silica crucible. Incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of total ash was calculated with reference to air-dried substance.

b) Acid insoluble ash

The total ash obtained was boiled with 25 ml of 2 M HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper washed with hot water. Transferred the filter paper containing insoluble matter to the original crucible, ignited and weighed. Calculated the percentage acid-insoluble ash with reference to the air-dried drug.

c) Water soluble ash

To the crucible containing the total ash, 25 ml each of water was added and boiled for 5 minutes. The insoluble matter was collected in sintered glass crucibles. Washed with hot water and ignited in a crucible for minutes at a temperature not exceeding 450˚C. The weight of that residue was subtracted from the weight of total ash. The content of water-soluble ash was calculated per gram of air-dried material.

d) Sulphated ash

To the crucible containing the total ash, a few drops of sulphuric acid was added again and ignited as before, cooled and weighed to get a constant weight.Then calculated the percentage of sulphated ash with reference to the air-dried drug [12,13,14].

(C) Determination of Extractive Value

a) Water soluble extractive value:

Macerated 5 grams of coarsely powdered air-dried plant with 100 ml of water in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

b) Ethanol soluble extractive value:

Macerated 5 grams of coarsely powdered air-dried plant with 100 ml of ethanol in a stoppered flask for 24 hours, with occasional shaking during the 1st 6 hours and allowed to stand undisturbed for another 18 hours. Thereafter it was filtered rapidly taking precautions against loss of the solvent. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. The %of the alcohol extractive value was calculated with reference to the air-dried drug [15, 16].

Parameter	**Results (%w/w)**
Loss on drying	5.5±.3
Total ash	6.7±.2
Acid insoluble ash	4.3±.3
Water soluble ash	2.1±.3
Sulphated ash value	1.2±.2
Water soluble extractive value	16.2±.5
Alcohol soluble extractive value	12.1±.4

(5) Preparation of plant Extract

The 20g of dried leavesa was weighed accurately and heated between 50 -60 with hydroethanol for Half and hour. It was cooled and then concentrated under vacuum.The percentage extractive value obtained on dried extract is 12.3% w\w.

(6) Phytochemical analysis

a) Qualitative phytochemical Analysis

The ethanolic extract was subjected to preliminary phytochemical screening usingreagents.The results are given in the table no:3 [18,19]

Qualitative parameter	Results
Phenols	++
Flavonoids	++
Alkaloids	+
Steroids	+
Carbohydrates	+
Protein and amino acid	+
Glycosides	+
Saponin	-
Tannins	+

Note:

(+) presence

(++)abundance

(-) absence

b) Quatitative phytochemical analysis

Estimation of total phenolic content

The total phenolic content (TPC) of the extract was determined by using Folin-Ciocalteu assay. 1ml of the sample (1 mg/ml) was mixed with 1 ml of Folin –Ciocalteu phenol reagent. Then 10 mlof 7 % sodium carbonate solution was added to the mixture followed by the addition of 13 ml of deionized water and mixed thoroughly. The mixture was kept in the dark for 90 minutes. After which the absorbance was read at 760 nm. Standard Gallic acid solutions (20, 40, 60, 80 and 100μg) were done mentioned as above. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The total phenol content was expressed as μg/ mg of extract [20].

Estimation of total flavonoid content

The total flavonoid content of the extract was determined by using aluminium chloride colorimetric method. 1 ml of extract was mixed with 4 ml of distilled water in 10ml volumetric flask. To the flask was added 0.3 ml of sodium nitrite (5 %). After 5 minutes, 0.3 ml aluminium chloride solution (10 %). After 5 minutes, 2 ml of I M sodium hydroxide was added. The volume was made upto10ml with distilled water and mixed. Standard solutions of quercetin (20, 40, 60, 80 and 100 μg) were prepared in the same manner. Absorbance of both test and standard were determined against the blank at 510 nm with an UV/visible spectrophotometer. The total flavonoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution. The total flavonoid content was expressed as μg/ mg of extract [21].

Table no: 4 Quantitative phytochemical analysis of *pouteria campechiana e*xtract

Phytochemical constituents	**Result**
Total phenolic in μg/ml content	61.3
Total flavonoid in μg/ml content	46.4

RESULT AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

Macroscopic Evaluation

Organoleptic characters revealed that the leaf was light to dark green in color, Astringent in taste,and characteristic in odour.

The macroscopic analysis revealed that the leaves of are lanceolate-oblong or obovate in shape, measuring about approximately 16-25 cm long and 3.5-6 cm wide with Pinnate venation.

Microscopic Evaluation

The epidermis is found on both upper and lower surfaces of the leaf. Epidermal layer is single- layered oval, rectangular or squarish shaped cells covered with thin cuticle. covering trichomes and anomocytic stomata are present at both the sides. Below the epidermal layer, showed the presence of3-4 layers of elongated palisade cells followed by loosely arranged spongy parenchymatous cells. Inthe midrib section, the epidermis is followed by an irregular shaped lower and upper collenchyma, which is responsible to give mechanical strength to the leaf. The vascular bundle present at the center containing lignified xylem arranged in vertical layers surrounded by phloem responsible for conduction of water and food. Rod shaped crystals of calcium oxalate are present in the parenchymatous cells.

Powder microscopy

powder microscopy of *Pouteria campechiana* leaves reveals the occurrence of epidermal cells, palisade cells, trichomes, rod shaped calcium oxalate crystals, stomata, phloem and xylem vessels. This helps in identification and authentication of the plant material in future works.

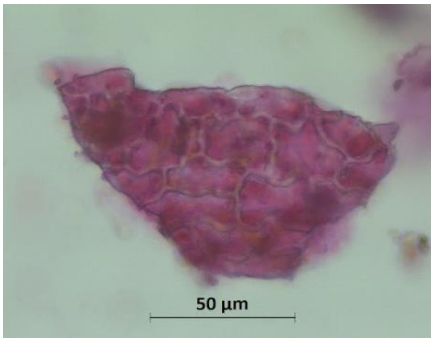

Fig 2 : Epidermal cell fragments in surface view

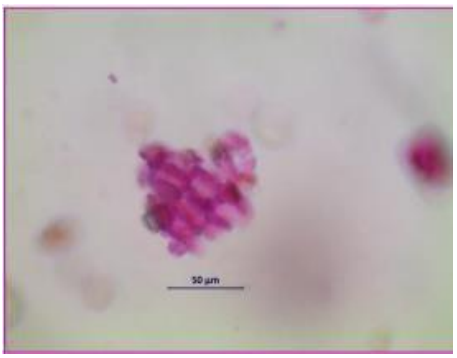

Fig 3: Parenchyma

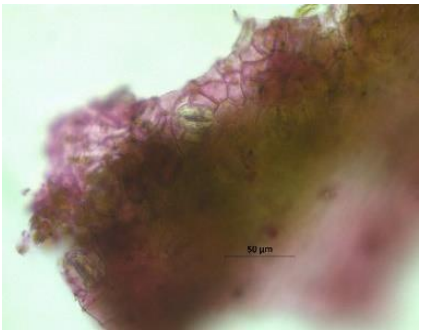

Fig 4 :Epidermal cells in surface view with stomata

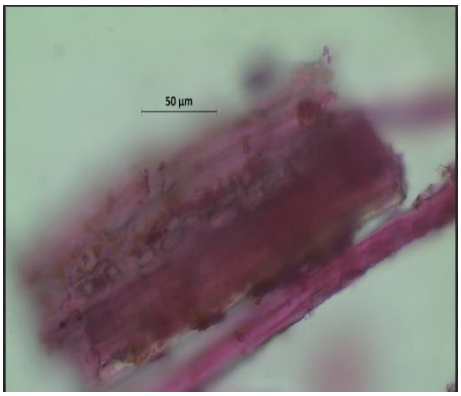

Fig 5: Cells with calciumoxalate crytals

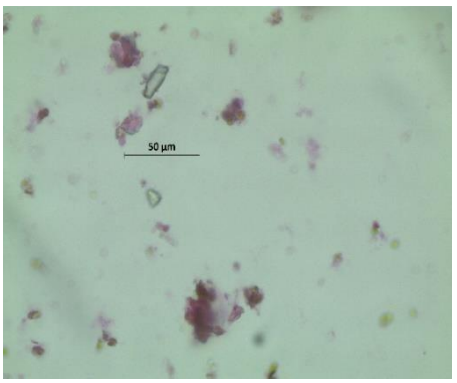

Fig 6: Rod shaped crystals of calciumoxalate cells

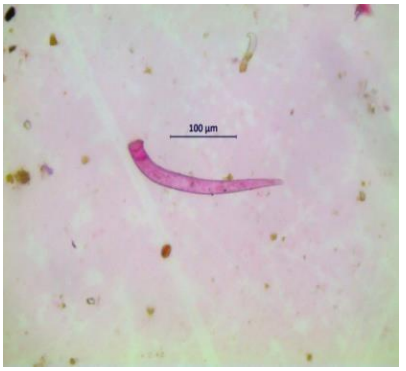

Fig:7: Trichome

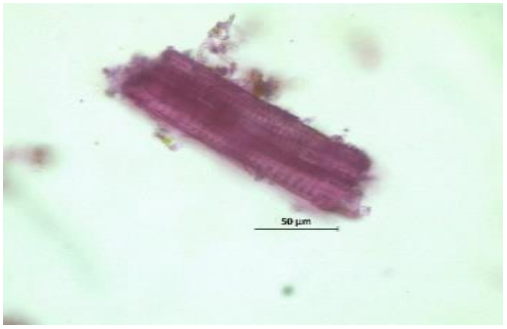

Fig 8: Fragments of vessels

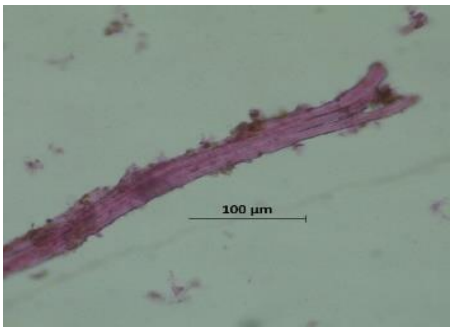

Fig 9: Fragments of fibers

Physico-chemical analysis

The Physicochemical characteristics of dried leaves of *Pouteria campechiana* were measured. The use of physicochemical factors helps to identify medications that have been adulterated or handled improperly. A drug's purity, or the presence or absence of foreign organic materials, such as metallicsalts and/or silica, is mostly determined by the amount of ash contained in the plant material. The amount of total ash reveals the presence of residue after ignition, which is equivalent to plant residue, as well as foreign materials. The amount of inorganic elements is determined by water- soluble ash. The findings from different types of ash values could serve as a basis for determining the drug's quality and purity. The low moisture content (%) of the leaf aids in limiting the growth of microorganisms during preservation. The extractive value can be used to determine the nature of powder and also determine the solubility of individual elements in a given solvent.

PHYTOCHEMICAL STUDIES

Extractive Yield

The percentage yield of the ethanolic extract of leaf was found be 12.3%w/w

Qualitative Phytochemical analysis

The qualitative phytochemical investigations of ethanolic extract of *Pouteria campechiana* gave beneficial information and ideas about the different phytoconstituents present in the plant. The extractswere subjected to preliminary phytochemical screening to identify the phytoconstituents present in theplant extract using chemical reagents. The phytochemical tests of the ethanolic extract of leaf revealed the presence of phytoconstituents, such as alkaloids, glycosides, tannin, flavonoids, steroid, phenols, carbohydrate and protein, and absence of saponin.

Quatitative phytochemical analysis

quantitative estimation of phytoconstituents was carried out by various standard methods. quantitative analysis shows the presence of a significant amount of phenols and flavonoids in theethanolic extract of *Pouteria campechiana* leaf. The results of total phenolic and flavonoid are presented in Table 4.

CONCLUSION

There is extremely little and insufficient textual material available about the plant Pouteria campechiana. As the drug is widely used in used in traditional medicine to treat various ailments It is essential to carry out the pharmacognostical and phytochemical analysis, which covers the initial stages of standardisation. In order to establish identification and to explore the physico-chemical and phytochemical analysis of Pouteria campechiana leaf, this study was conducted. The confirmation and diagnosis of this plant can be made using the macroscopic parameters, microscopic parameters, physico-chemical reports, and phytochemical results that were collected in this work.Hope that this study and the information obtained here will serve as a basis for more in-depth investigations into Pouteria campechiana in the fields of plant science and medicine.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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