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Research Article

Phytochemical and pharmacognostic evaluation of leaves of *Pongamia pinnata* L. (Fabaceae)

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ABSTRACT: **Objective:** The objective of this study was to carry out phytochemical and pharmacognostic evaluation of leaves of *Pongamia pinnata* L. (Fabacea). **Method:** The present study provides pharmacognostic, physicochemical and phytochemical details of the leaves of *P. pinnata.* Macro and microscopic evaluation and WHO recommended parameters were followed in the study. **Results:** The macroscopic study showed that the leaf was ovate or elliptic with smooth margins, short petiole, alternate imparipinnate, hairless, acuminate at apex, rounded to cuneate at base and slightly thickened. Microscopic study revealed collateral, closed vascular bundles, trichomes, paracytic stomata, xylem vessels and prismatic calcium oxalate crystals. Physiochemical analysis of leaf showed total ash, water soluble ash and acid insoluble ash as 8.33 ± 0.31, 2.33 ± 0.36 and 0.5 ± 0.0 %w/w respectively. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, tannins, triterpenes, cardiac glycosides, steroids and saponins. **Conclusions:** The results of this study can serve as valuable source of information for identification of this plant for future investigation and applications.

KEYWORDS: *Pongamia pinnata,* pharmacognostic, phytochemical, physicochemical, leaf

INTRODUCTION

Plants have been the foundation of traditional medicine system throughout the world and continue to nurture mankind with new remedies. The research pertaining to medicinal plants is rapidly increasing at national and international levels.[1] Further investigation of traditional systems of medicine with emphasis on safety, efficacy and quality will help to rationalize the use of natural products in healthcare.[2] In the Indian systems of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and an understanding of the medicinal components and their effects. Pharmacognosy is the study of medicines derived from plants and it is the preliminary step in the standardisation of crude drugs. Authentication and standardisation are prerequisite steps for herbal drugs and their formulations in traditional systems of medicine.[3] Erroneous identification is the elemental step where the false uses of herbal medicines begin. In the records of the majority of traditional systems, one common vernacular name is often given to two or more completely different species. For example, *Aristolochia mollissima* and *Solanum lyratum* share a common Chinese name 'Baimaoteng'. This lead to erroneous usage of *A. mollissima* instead of *S. lyratum*.[4]

Pongamia pinnata L. belongs to family Fabacea. It is native to India and widely distributed along Southeast Asia to the West Pacific and northern Australia.[5] It is also called *Deris indica, Pongamia glabra* and *Milletia pinnata*. The plant has been documented in Ayurveda, an alternative medicine in India. All parts of this plant are used in the treatment of abscess, bronchitis, diarrhea, itches, piles, skin diseases, tumors, painful rheumatic joints, ulcers, whooping cough quench dipsia in diabetes, blood purifier and as an antiseptic to treat wounds and cuts.[6,7] Some of the reported activities of this plant include antioxidant,[8–10] antimicrobial,[11,12] anti-inflammatory,[13] antiulcer,[14] antihyperglycemic[15] amongst others.

In spite of the numerous medicinal uses attributed to *P. pinnata*, there are inadequate accounts of its morphological, microscopical, physicochemical and phytochemical aspects. Hence this study was undertaken to address this need.

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MATERIALS AND METHODS

Procurement of Plant Material

The fresh leaves of *P. pinnata* were collected from Rajkot, Gujarat, India in August 2013. The leaves were washed under tap water, air dried, homogenised into a fine powder and stored in air tight bottles.

Pharmacognostic studies

Macroscopic characteristics

Visual observation provides the simplest and quickest means to recognize identity and possibly also the quality of the plant material. Various macroscopic characters of fresh leaves including shape, venation, margin, presence or absence of petiole were recorded.

Microscopic characteristics

The microscopic evaluation was performed by taking free hand sections of fresh leaves and staining with safranine to confirm lignifications. Various identifying characters such as trichomes and cell composition were recorded and then pictomicrography studies were undertaken.[16] Powder microscopy of the dried leaf powder was studied under microscope (Magnus MLM - Olympus India Pvt. Ltd., at 10X and 40X). The characteristic structures and cell components were observed and their photographs were taken.

Physicochemical and Phytochemical evaluation

The dried powdered leaves were subjected to physicochemical analysis. Various physicochemical parameters including loss on drying, total ash value, water soluble ash, acid insoluble ash, petroleum ether, acetone, methanol and water soluble extractive values were determined. The preliminary phytochemical screening of the leaf powder was performed with standard qualitative chemical tests as per WHO guidelines[17] in order to detect the presence or absence of major secondary plant metabolites of pharmacognostic importance. The classes of phytoconstituents tested include; alkaloids, flavonoids, tannins, phlobatannins, triterpenes, steroids and saponins.

Fluorescence analysis

Fluorescence study of leaf powder was performed as per standard procedure.[18,19] Powdered leaf was treated with various chemical reagents and exposed to visible and ultraviolet light to study their fluorescence behaviour.

Statistical analysis

All experiments were repeated at least three times. Results are reported as Mean ± S.E.M. (Standard Error of Mean).

RESULTS

Macroscopic characteristics

The macroscopic study involved visual examination of the morphological characters of the leaves. The fresh leaves of *P. pinnata* were glossy dark green above and dull green with prominent veins beneath when mature. The size of leaves varied from 6- 16.5 cm in length and 4-8.3 cm in breadth. The leaf was ovate or elliptic with smooth margins, short petiole, alternate imparipinnate, hairless, acuminate at apex, rounded to cuneate at base and slightly thickened (Figure 1).

Microscopic characteristics

Leaf microscopic study

The transverse section of *P. pinnata* leaf showed the presence of single layered epidermis, consisting of tubular cells (Figure 2). The epidermis was covered with a single layer of cuticle. Epidermis was followed by 3-4 layers of collenchymatous hypodermis. The vascular bundle was surrounded by 4-6 layers of cortex. Cortex

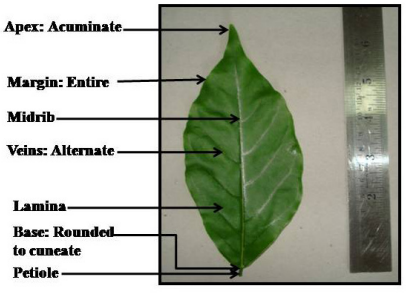

Figure 1. Macroscopic characteristics of *P. pinnata* leaf.

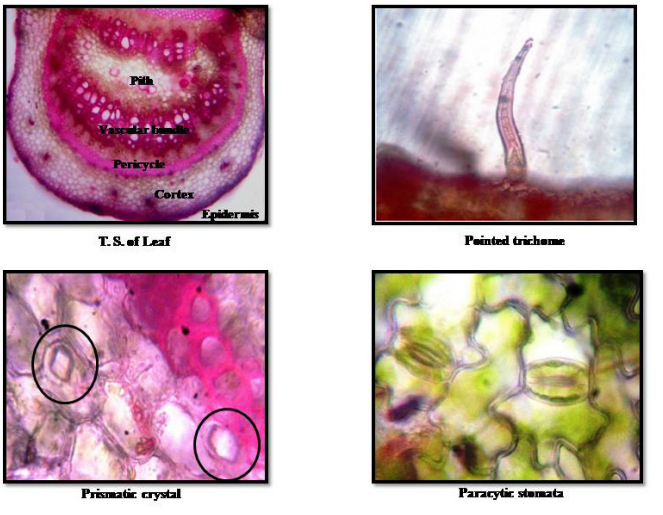

Figure 2: Photomicrographs of microscopic characteristics of *P. pinnata* leaf.

consisted of oval shaped parenchymatous cells. Pericyle can be observed in the form of sclerenchymatous sheath. Vascular bundles were collateral, closed and arranged in discontinuous ring. Xylem was lignified, phloem was non-lignified. In the central portion, compacted parenchymatous pith was present. Prismatic crystals of calcium oxalate, pointed trichomes and paracytic stomata were found (Figure 2). Thus the salient diagnostic features of leaf were collateral, closed vascular bundle, paracytic stomata, xylem vessels and prismatic calcium oxalate crystals. These characters can be used for standardization of drugs and also used for preparation of plant monographs.

Powder microscopic study

Powdered leaf of *P. pinnata* was dark green in colour with a characteristic bitter or pungent smell and bitter taste. Microscopic investigation showed the presence of xylem vessels with spiral thickening, pointed trichomes and epidermal cells. The calcium oxalate crystals of prismatic type were observed. The fragments of epidermis were embedded with paracytic stomata (Figure 3).

Physicochemical analysis

Various physicochemical parameters of powdered drug like loss on drying, total ash value, water soluble ash, acid insoluble ash, petroleum ether, acetone, methanol and water soluble extractive values were determined in triplicate and are shown in Table 1.

Fluorescence analysis

The fluorescence characteristics of the leaf powder with different chemical reagents are summarized in Table 2. The leaf powder showed different colours and fluorescence under visible light and UV light (254nm and 365nm) with various reagents.

Phytochemical analysis

The results of qualitative phytochemical analysis of the crude powder are shown in Table 3. Preliminary phytochemical analysis revealed the presence of medicinally important secondary metabolites. Steroids, saponins and

Table 1: Physicochemical parameters of *P. pinnata* leaf (% w/w*).

No.	Parameters	Leaf
1.	Loss on drying	9.16±0.36
2.	Total ash	0.9108.33±0.31
3.	Water soluble ash	2.33±0.36
4.	Acid insoluble ash	0.5±0.0
5.	Petroleum Ether extractive value	0.82±0.0
6.	Acetone extractive value	1.51±0.02
7.	Methanol extractive value	7.20±0.19
8.	Water soluble extractive value	8.74±0.28

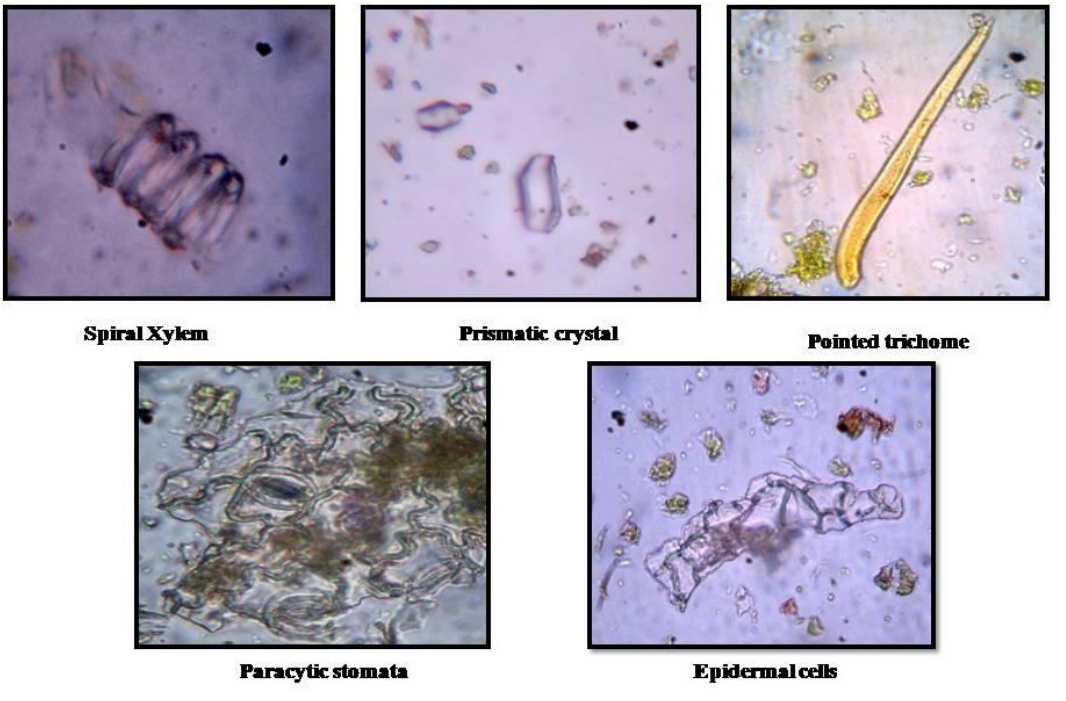

Figure 3: Photomicrographs of microscopic characteristics of powder of *P. pinnata* leaf..

Table 2: Fluorescence analysis of *P. pinnata* leaf powder.

No.	Extractives	Visible light	Short UV Wavelength (254 nm)	Long UV wavelength (365 nm)
1.	Petroleum ether	Dark green	Black	Light green
2.	Ethyl acetate	Dark yellow	Black	Green
3.	Methanol	Green	Black	Light green
4.	Ethyl alcohol	Dark green	Black	Light yellow
5.	Aqueous NaOH	Light green	Black	Dark green
6.	Alcoholic NaOH	Light green	Black	Yellow
7.	50% H_2SO_4	Yellow	Black	Green
8.	50% HCl	Green	Black	Dark green
9.	Picric acid	Green	Black	Green
10	Ammonia	Light green	Black	Yellowish green

Table 3: Qualitative phytochemical analysis of *P. pinnata* leaf.

Phytochemicals	Test	Crude powder
Alkaloids	Dragendroff's test	++
	Mayer's test	+
	Wagner's test	++
Flavonoids	Alkaline reagent	++
Tannins	$FeCl_3$	+
Phlobatanins	HCl test	-
Triterpenes	H_2SO_4 test	+
Steroids	Liebermann-Burchard test	+++
Saponins	Frothing test	+++
Cardiac Glycosides	Keller- Kilianni test	+++

Phytochemicals present in less (+), moderate (++) and high (+++) amounts; absent (-)

cardiac glycosides were present in relatively high amount. Alkaloids, flavonoids, tannins and triterpenes were present in moderate amount whereas phlobatanins were absent in crude powder.

DISCUSSION

Evaluation of crude drugs is an integral part of establishing correct identification of plant material. Pharmacognostic and physicochemical parameters are the primordial steps for this evaluation.[20] The present study reports the pharmacognostic characteristics of *P. pinnata* leaf. Morphological and microscopic studies are reliable, simple and cheapest in establishing the identity of source materials.[21,22] To detect adulteration, ash values and extractive values are unambiguously useful.[23] Chemical constituents of crude drug that are soluble in particular solvents can be known by extractive values.[24] Ash values are less, which indicates that foreign inorganic matter is present in fewer amounts in the *P. pinnata* leaf powder. The extractive value of water was highest followed by methanol. The fluorescent analysis under visible light and UV light by treatment of different chemical reagents showed different colour. This is attributed to the ultra violet light which produces fluorescence in many natural products that do not visibly fluoresce in daylight. Thus fluorescence is used for qualitative assessment of crude drug.[25,26] The analysis of leaf of *P. pinnata* suggests that it may contain active agents and this provides the basis for its folkloric use as a cure for some human ailments. There are many reports in the literature where pharmacognostic study of leaves of different plants is reported eg. *Melaleuca leucadendron* [27], *Nelumbo nucifera,*[28] *Manilkara zapota,*[29] *Eucalyptus globulus,*[30] *Cinnamomum verum,*[31] *Terminalia catappa*,[32] *Psidium guajava*[33] and *Polyalthia longifolia.*[34]

In conclusion, these parameters are reported for the first time for *P. pinnata* leaf. These would not only help in setting indices for identification of raw material and preparation of plant monograph but also will serve in development of pharmacopoeial standards for the future studies.

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