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Pharmacognostic evaluation of stem bark of *Pongamia pinnata* (L.) Pierre

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ABSTRACT

Objective: To perform the pharmacognostic study of *Pongamia pinnata* (L.) Pierre (*P. pinnata*) stem bark. Method: The pharmacognostic studies were carried out in terms of organoleptic, macroscopic, microscopic, fluorescence analysis and physicochemical parameters. Results: The bark consisting of channelled, recurved, slightly quilled, usually 0.2-1 cm thick, lenticellate pieces with outer surface ash-grey to greyish-brown and internal surface yellowish-white to cream coloured having unpleasant odour and bitter taste. The main microscopic characterstics of the bark include phellem (5-20 or more layers of cork), phellogen (2-3 layered) followed by 10-15 layered phelloderm. Among other microscopic components were phloem parenchyma, phloem fibre and stone cells, traversed by wavy medullary rays. Further, physicochemical analysis of the bark power showed total ash, water soluble ash, acid insoluble ash and sulphated ash as 10.94, 1.96, 1.47 and 15.8 % w/w respectively. The alcohol and water soluble extractives values of the stem bark were 9.6 and 18.4 %w/w respectively. Conclusions: Various pharmacognostic characters observed in this study helps in botanical identification and standardization of *P. pinnata* L. in crude form.

1. Introduction

Pongamia pinnata (L.) Pierre(*P. pinnata*) synonyms *Pongamia glabra* Vent. (*P. glabra*), *Derris indica* (Lam.) Bennett (*D. indica*), *Cystisus pinnatus* Lam. (*C. pinnatus*), *Millettia novo-guineensis* Kane & Hat(*M. novo-guineensis*) and *Millettia pinnata* (*M. pinnata*) (*P.)Panigrahi* (family Fabacae) popularly known as 'Karanj' in Hindi, Pongam in Tamil and 'Indian beech' in English, is native to India and widely distributed along Southeast Asia to the West Pacific and North Australia[1-4]. It is a medium-sized tree with a short crooked trunk and a broad crown of spreading or drooping branches. It is naturally distributed along the coasts and river banks in India and Myanmar[5].

Historically, *P. pinnata* is mentioned as folk medicinal plant, particularly in Ayurvedha and Siddha systems of Indian medicine for the treatment of abscess, bronchitis, diarrhea, itches, piles, skin diseases, tumors, painful rheumatic joints, ulcers, whooping cough and quench dipsia in diabetes[6,7]. Traditionally, *P. pinnata* is used in India as an antiseptic, blood purifier, to treat cuts and wounds[8]. The plant has been reported to possess anti-inflammatory, antioxidative, analgesic and antiulcer[9-11], antifungal, antibacterial[12] and antihyperglycaemic[13] activities etc. Various constituents isolated from the bark of this plant include seven flavonoids viz., pongaflavone, karanjin, pongapin, pongachromene, 3,7-Dimethoxy-3′,4′-methylenedioxyflavone, millettocalyxin C, 3,3′,4′,7-tetrame thoxyflavone[14], two prenylated flavonoid derivatives viz., pongaflavanol & tunicatachalcone[15], phenylpropanoids viz., Pongapinone A & B[16], cycloart-23-ene-3 β, 25-diol[17].

Due to its ethnopharmacological importance and the lack of studies on this native medicinal species, the present investigation was sought to perfrom the pharmacognostic study of stem bark of Pongamia pinnata (L.) Pierre, in order to develop pharmacopoeial standards and to contribute to the quality control of this potential plant drug.

2. Materials and methods

2.1. Chemicals

All the chemicals used were of analytical grade and

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were obtained from Rankem Limited India and Hi-Media laboratories, Mumbai, India.

2.2. Procurement of plant materials

Bark of the plant was collected from the campus of Kurukshetra University, Kurukshetra during April 2010 and authenticated by Dr. H.B Singh, NISCAIR under reference number (NISCAIR/RHMD/Consult/-2010-11/1471/69).

2.3. Macroscopic evaluation

Various organoleptic and macroscopic characters of Pongamia pinnata (L.) stem bark like colour, shape, size, tase odour, fracture and configuration etc. were studied[18].

2.4. Microscopic evaluation

In microscopic evaluation, studies were conducted on both grounds qualitatively and quantitatively. The model of microscope used for study of different characters was SKC-400, Suswox Optik, Sudheer Scientific Works, India.

2.4.1. Qualitative microscopy

In this study, transverse sections of stem bark were studied under photomicrograph. Staining reagents (such as phloroglucinol-HCl) were used as per standard procedures[19,20]. The various identifying features of the drug were studied with or without staining and recorded.

2.4.1.2. Stem bark microscopy

The stem bark was dipped in a test tube containing sufficient water and was boiled for few minutes. The softened bark was transversally sliced into fine sections which were subjected to staining reagent 0.1% w/v phloroglucinol followed by concentrated conc. hydrochloric acid. The stained sections were observed under microscope[21,22]. Different layers of cells and identifying characters were observed then photomicrography was done.

2.4.1.3. Powder microscopy

The dried stem bark was powdered and studied under microscope. Different staining reagents (such as iodine for detection of starch grains and phloroglucinol for detection of lignified components) were used. To a little quantity of stem bark powder taken over a microscopic slide, 1-2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope[23]. The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

2.5. Fluorescence analysis

Fluorescence study of stem bark powder was performed as per reported standard procedure[24]. A small quantity of the bark powder was placed on a grease free clean microscopic slide and 1-2 drops of the freshly prepared reagent solution were added, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded.

2.6. Physicochemical analysis

In this study, Air dried material was used for quantitative determination of physiochemical values like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash values and extractive values were determined as per reported method[25].

3. Results

3.1. Macroscopic study of stem bark

Morphological examination of the stem bark shows that the bark consists of channelled, recurved, slightly quilled, usually 0.2-1 cm thick, lenticellate pieces, more or less smooth; outer surface ash-grey to greyish-brown and internal surface yellowish-white to cream coloured; fracture, short and fibrous, odour, unpleasant; taste, bitter.

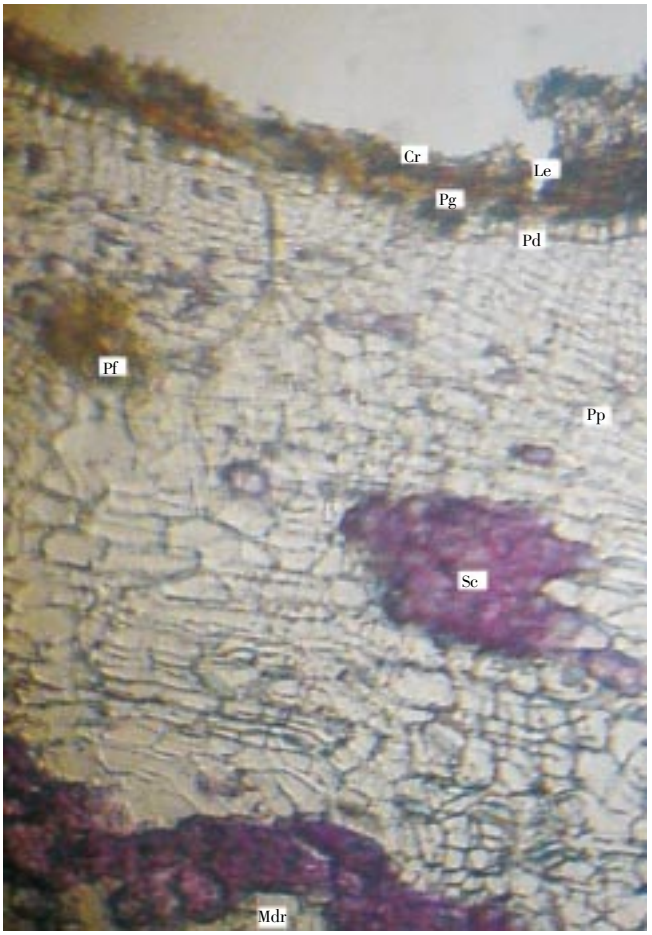

Figure 1. T.S. of of *P. pinnata* (L.) Pierre stem bark
Cr- cork (Phellem), Pg- Phellogen (Cork cambium), Le- Lenticels, Pd- Phelloderm (secondary cortex), Pf- Phloem fibre, Sc- Stone cells, Pp- Phloem parenchyma, Mdr- Medullary rays

Table 1.
Fluorescence analysis of Pongamia pinnata (L.) Pierre stem bark powder

Treatment	Visible light	Under UV light	
		Short Wavelength(254 nm)	Long wavelength(365 nm)
Powder	Yellowish brown	Pale green	Brown
Powder + 1N NaOH (aq.)	Light brown	Green	Brownish green
Powder + 1N NaOH (alc.)	Light yellow	Dark green	Yellowish green
Powder + Ammonia	Light yellow	Green	Yellowish green
Powder + Picric acid	Yellowish brown	Green	Dark brown
Powder + Pet. ether	Light brown	Light green	Pale green
Powder + 50% HCl	Light brown	Green	Pale yellow
Powder + 50% H_2SO_4	Brown	Greenish brown	Brownish

3.1. 2. Microscopic study of stem bark

Transverse section study of the stem bark (Figure 1) shows 5-20 or more layers of cork (phellem), composed of rectangular, thick-walled cells, filled with reddish-brown content, at some places lenticels also appear. Phellogen (cork cambium) is 2-3 layered having polygonal, tangentially elongated, thin-walled, parenchymatous cells whereas phelloderm (secondary cortex) is 10-15 layered having oval to polygonal, tangentially elongated, thin-walled, parenchymatous cells. Beneath secondary cortex, a large group of oval to elongated stone cells, arranged in a tangential manner, occurs forming a continuous or discontinuous band. Secondary phloem is composed of phloem parenchyma, phloem fibre and stone cells, traversed by medullary rays. Phloem parenchyma consists of rectangular to polygonal thin-walled cells, alternating with stone cells. Phloem fibre are small, polygonal, thin-walled and aseptate, a few associated with stone cells and arranged radially. Medullary rays are wavy, usually 2-4 cells wide, radially elongated and rounded to oval in shape. A few stone cells are found scattered in secondary cortex as in secondary phloem.

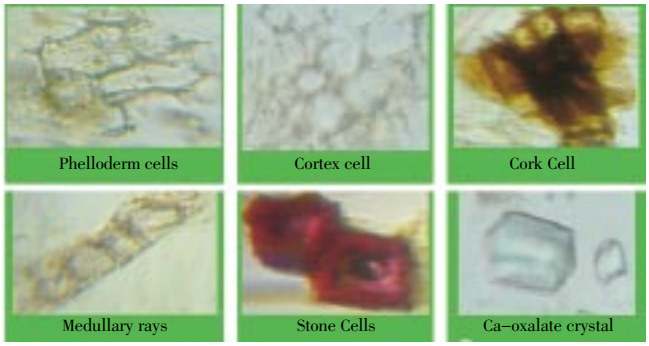

Figure 2. Powder characterstics of *P. pinnata* stem bark

3.1. 3. Powder study

Stem bark powder appears yellowish-cream; showing groups of rectangular to polygonal, elongated, thin walled parenchymatous sieve tube; aseptate fibre and stone cells; rhomboidal crystals of calcium oxalate; rounded to oval, simple and compound starch grains, measuring 3-14 μ in dia. Powder characteristics of the bark have been shown in Figure 2.

3.1.4. Fluorescence analysis

The fluorescence characteristics of the bark powder with different chemical reagents are summarized in Table 1.

Table 2.
Physicochemical analysis of Pongamia pinnata Stem bark

Parameters	Value obtained on dry weight basis (% w/w)*
Loss on drying	12.21±0.16
Total Ash Value	10.94 ±0.23
Acid insoluble ash value	1.47 ± 0.37
Water soluble ash	1.96 ± 0.12
Sulphated ash	15.80 ±0.04
Water soluble extractive	18.40±0.27
Alcohol soluble extractive	9.60±0.04

*Average of three reading ± SEM

3.1.4. Physicochemical analysis

In this study, various physiochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash values and extractive values were determined in triplicate as mentioned in Table 2.

4. Discussion

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus, in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken[26]. The present study reports the pharmacognostical characteristics of Pongamia pinnata stem bark. Morphological and microscopic studies of stem bark acts as a reliable tool for detecting adulteration. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an

important parameter for pharmacognostical evaluation of crude drugs[27-29]. Adulteration of the genuine raw material is the main cause of degradation of desired therapeutic effect of plant species used in various traditional systems of medicine. Thus industries could utilize the scientific background for identification of raw material and this work is not only beneficial to the industries but also enhance the credibility of Indigenous System of Medicine[30].

In the present paper, the extractive value findings will be useful for the further extraction of phytoconstituents from this plant. The other parameters viz. loss on drying, ash value, extractive values and fluorescence analysis add to its quality control and quality assurance. Thus, the above finding will serve in the development of pharmacopoeial standards for the future studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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