Kumar et al., 2:2
http://dx.doi.org/10.4172/scientificreports.634

Open Access Scientific Reports

Research Article Open Access

Pharmacognostic and Phytochemical Investigation of *Pongamia pinnata*

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Abstract

Pongamia pinnata Linn. (Papilionaceae family) shows potent antidiabetic activity. The plant is also used for the treatment of hypertension and hyperlipidemia, as well as mycobacterium and skin infections. This paper is a detailed pharmacognostic evaluation of the crude drug of *P. pinnata* pods. The detailed pharmacognostic study used physico-chemical, morphological and histological parameters recommended by the WHO and may be used to authenticate *P. pinnata* plants. Total ash, acid-insoluble and water-soluble ash values were 3.377, 0.876 and 1.141%, respectively. Crude drugs showed a hot extractive value in petroleum ether, chloroform, methanol and water of 0.1765, 0.4651, 0.2182 and 0.2155%, respectively while in normal (unheated) petroleum ether, chloroform, methanol and water, the values were 0.2101, 0.1130, 0.1025 and 0.5937%, respectively. Resin content and foaming index were 0.73% (w/w) and <100, respectively.

Keywords: *Pongamia pinnata*; Papilionaceae; HPTLC; Pharmacognosy

Introduction

Pongamia (Papilionaceae) is a monospecific genus, namely *Pongamia pinnata*. *P. pinnata*, commonly known as karanja, is distributed throughout India in tidal and beach forests, often as a mangrove plant. It is used medicinally in India, China, Australia and the Philippines. In the Indian traditional system of medicine Ayurveda, *P. pinnata* has been used in the treatment of bronchitis, whooping cough, rheumatic joints and quench dipsia in diabetes [1]. The flower furnishes an aliphatic waxy matter kaempferol, pongamin ($C_{15}H_{12}O_5$), γ-sitosterol glucoside, quercertin, neoglabrin (A complex amino acids) resembling glabrin and galbrosaponin ($C_{50}H_{84}O_{23}$) [2]. A furanoflavone i.e., pongone has been isolated from flowers [2]. *P. pinnata* contains flavonoids and related compounds including flavones, furanoflavonoids, chromenoflavone, chromenocalchones, coumarins, flavone glycosides sterol, terpenes and modified phenylalanine dipeptide [3]. The seeds contain 13.5% mucilage, traces of essential oil and complex amino acids, termed glabrin. Four furanoflavones karanjin, pongapin ($C_{19}H_{12}O_6$), kanjone ($C_{18}H_{12}O_4$) and pongaglabrone ($C_{18}H_{10}O_5$), identified as 3',4'-methylenedioxy furano [2',3',7,8] flavone, have been isolated from Indian Karanja seed [4]. Three furanoflavonoids (Pongamosides A, B and C) and a flavonol, glucoside Pongamoside D, have been reported from the n-butanol-soluble fraction of the ethanolic extract *P. pinnata* fruit [5]. Pongaglabol, a hydroxyfuranoflavone, and aurantiamide acetate, a rarely occurring modified phenylalanine dipeptide, have been isolated together with four furanoflavones (karanjin, lancheolatin B, kanjone and pinnatin) [6]. Two hydroxychalcones – onganones I and II – have been isolated from bark and characterized. Moreover, two phenylpropanoids – Pongapinone A and B – have been isolated from bark of Indonesian karanja plants [7]. Five flavonoids (Pongamone A, B, C, D and E) have been isolated from *P. pinnata* [8]. Seeds are bitter and acrid, carminative, and purify and enrich the blood, relieve inflammation, cure earache, lumbago, chest complaints and chronic fever. Seed is considered useful in the treatment of scabies, leprosy, piles, ulcers, bronchitis and whooping cough [9]. Seeds are mainly valued for their oil, in cosmetic industry and Ayurvedic herbal medicine [10]. Different parts of *P. pinnata* show different activity anti-inflammatory [11], antihyperglycemic and antilipidperoxidative [12] antiulcer [13], analgesic [14], antimycobacterial [15] and antifilarial activity [16].

Materials and Methods

Plant material

The pods of *P. pinnata* were collected from Jamia Hamdard campus in October 2006 and were identified in the Department of Botany, Jamia Hamdard (Hamdard University), New Delhi. A specimen for further reference has been retained (Voucher No. PRL-001-06).

Morphological properties

P. pinnata is a medium-sized glabrous tree with a short bole and a spreading crown up to 18 m high or sometimes even more and 1.5 m in girth. Pods are compressed, woody, indehiscent, yellowish-gray when ripe, varying in size and shape, elliptic to obliquely oblong, 4.0-7.5 cm long and 1.7-3.2 cm broad with a short, curved beak. Seed usually 1 rarely 2, elliptical or reniform 1.7-2.0 cm long and 1.2-1.8 cm broad, wrinkled with reddish-brown leathery testa. The specimens consisted of pods/fruits of *P. pinnata* with the following morphological characters. The sample consists of 5% shell and 95% oleaginous seed kernels [1].

Powdered microscopic

Pods were ground into a fine powder with the help of a grinding machine and treated with phuloroglucinol and conc. hydrochloric acid and mounted on a glass slide with glycerin. A cover slide was added and samples were examined under a confocal microscope (Fluoview FV-1000; Olympus).

Extraction

Different extracts of *P. pinnata* pods were prepared with a Soxhlet apparatus. Extracts were evaporated and dried to a powder. Dried

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Received January 08, 2013; **Published** February 25, 2013

Citation: Kumar P, Kumar M, Teixeira da Silva JA (2013) Pharmacognostic and Phytochemical Investigation of *Pongamia pinnata*. 2: 634 doi:10.4172/scientificreports.634

extracts were kept in desiccators with calcium carbonate adsorbent to prevent moisture absorption by the dried extract [17].

Preliminary phytochemical and physiochemical analysis

The methanolic extract was subjected to preliminary phytochemical screening to detect major phytoconstitutents using a standard procedure [18,19] and to generate some physiochemical parameters that can be utilized for the identification of plant materials (Table 1). *P. pinnata* pods can be evaluated morphologically but this study will really help in the authentification of *P. pinnata* when supplied in powdered form.

Phytochemical evaluation by thin-layer chromatography

Different extracts like petroleum ether, chloroform and methanolic extract of *P. pinnata* pods were subjected to HPTLC fingerprinting analysis and TLC profile) in different solvent systems (Table 4) and visualized with the help of a UV chamber (254 and 266 nm) and iodine vapor. Moreover, physicochemical parameters have been studied in detail (Table 5).

Results and Discussion

Organoleptic properties

A brown to yellowish-brown powder with a faint characteristic odour and bitter unpleasant taste.

Constituents	Observation
Alkaloids	+
Carbohydrates	+
Glycosides	+
Phenolic compounds and tannins	+
Flavonoids	+
Proteins and free amino acids	+
Saponins	+
Sterols	+
Acidic compounds	+
Mucilage	+
Lipids/fats	+
Resin	+

Table 1: Presence or absence of phytoconstituents in *P. pinnata*.

Chemical Treatment	Observations
Conc. HCl	Yellowish White
Conc. HNO_3	Yellowish Brown
Conc. H_2SO_4	Blackish Brown
Glacial Acetic Acid	Light Yellow
5% NaOH	Dark Brown
5% KOH	Yellowish Brown
5 % $FeCl_3$	Flouricent Yellow

Table 2: Effect of different chemical reagents on *P. pinnata* powder.

Chemical Treatment	254 nm	366 nm
1N NaoH in MeoH	Dark Yellow	Yellowish-Brown
1N NaoH in Water	Light Yellow	Yellowish-White
50% HCl	Fluorescent Green	Yellowish-White
50% HNO_3	Fluorescent Green	Yellowish-Brown
50% H_2SO_4	Dark Green	Yellowish-White
Pet. Ether	Fluorescent Green	Dark Yellow
Chloroform	Dark Green	Light Yellow

Table 3: Effect of different chemical reagents on the fluorescence behavior of *P. pinnata* powder.

Extracts	Solvent system	Number of spots	R_f values
Petroleum ether	Pet.Ether:Ethyl acetate (1:0.5)	6	0.25, 0.38, 0.52, 0.56, 0.67, 0.90
Chloroform	$CHCl_3$:Methanol (5:0.2)	4	0.27, 0.33, 0.77, 0.87
Methanol	Toluene:Ethyl acetate:Methanol (5:1:0.3)	5	0.15, 0.34, 0.47, 0.69, 0.85

Table 4: TLC profile of different extracts of *Pongamia pinnata*.

pH value 1%		6.91
pH value 10%		6.50
Loss on drying		4.7476
Resin content		0.73
Total ash		3.377
Acid-insoluble ash		0.876
Water-soluble		1.141
Foaming Index		<100
Cold extractive value		0.1154
Individual hot extractive value	1. Petroleum ether	0.1765
	2. Choloform	0.4651
	3 Methanol	0.2182
	4. Water	0.2155
Successive extractive values	Petroleum ether	0.2101
	Chloroform	0.1130
	Methanol	0.1025
	Water	0.5000

Table 5: Physicochemical analysis of powder from *P. pinnata* pods.

Powdered microscopic studies of pods

Pods and seeds were powdered. Microscopy revealed the character of pericarp, seeds and attached pedicel.

Epicarp: It consists of a layer of cells that were diametric, non-pigmented, and polygonal in a surface view with a moderately thick wall (Figures 1 and 11) and a thick cuticle (seen on fragments in sectional view).

Testa: The epidermis is composed of a layer of conical and thick-walled palisade cells with a thick cuticle (Figure 2). In surface view, the epidermal cells appeared more regular, polygonal and yellowish-brown (Figure 3).

Bearer cells: Adherent to the palisade epidermis of the testa, a few of stone cells in patches and a layer of bearer cells could be found. The cells were parenchymatous, polygonal with thickened radial walls and slightly contracted in the middle region. When viewed from below the lumen of the central constriction and those on the either side appeared as rings (Figures 2, 4 and 5).

Trichomes: Unicellular, conical, thick, and distinctly warty and bent near the base, trichomes were scattered and rare (Figure 6).

Calcium oxalate crystal: Several clusters and prismatic calcium oxalate crystals were found in the powder (Figure 7).

Fibres of measocarp: Appeared as two crossed layers and covered on one side with thin-walled parenchymatous cells containing prism calcium oxalate crystal. The individual fibers were narrow with moderately thickened walls, occasional pits and a distinct lumen (Figure 8).

Sclereids: Single or cluster or stone cells with an irregular ovoid outline and moderately lignified cell walls with pits were abundantly seen (Figures 9, 12 and 13).

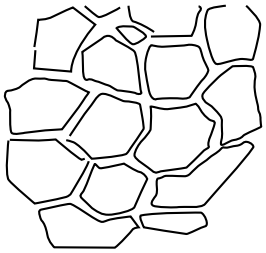

Figure 1: Epicarp (inside face view).

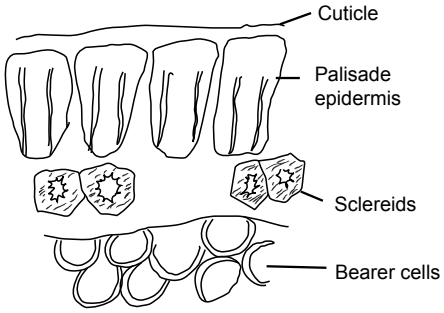

Figure 2: Testa in sectional view.

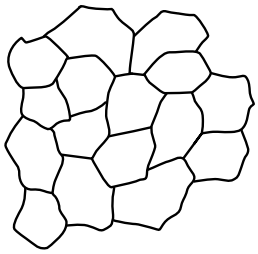

Figure 3: Testa (surface view).

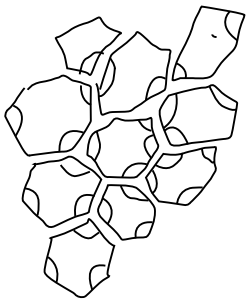

Figure 4: Bearer cells of testa.

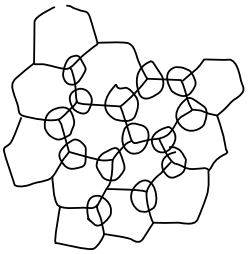

Figure 5: Bearer cells (seen from below).

Figure 6: Trichomes.

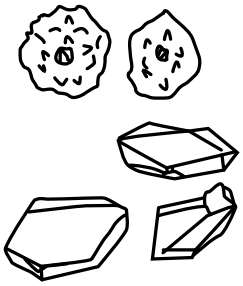

Figure 7: Calcium oxalate crystals (cluster and prism type).

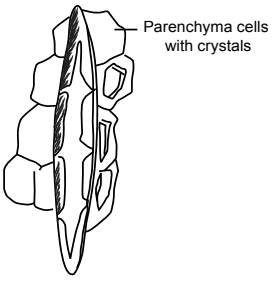

Figure 8: Fibres of mesocarp.

Figure 9: Sclereids.

Vessels: Narrow lignified with annular thickenings were also seen (Figures 10 and 14).

Endosperm: Abundant fragments of stratified food material containing cells were observed. Moreover transverse sections of seeds of *P. pinnata* were performed (Figure 15). The cells were polygonal in outline and with a thick cell wall.

Portion of pedicel

The fibers and sclereids of the pedicel were similar to those found in the pods but were larger. The parenchymatous cell that associated with the fibers was more thick walled and contained bigger cluster-type calcium oxalate crystals. Preliminary phytochemical screening of different extracts showed the presence of alkaloids, carbohydrates,

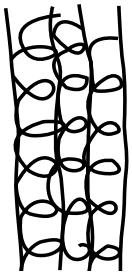

Figure 10: Vessels.

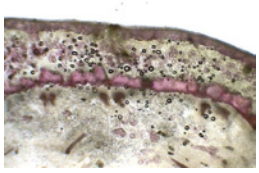

Figure 11: Transverse section (not T.S).

Figure 12: Single sclereid.

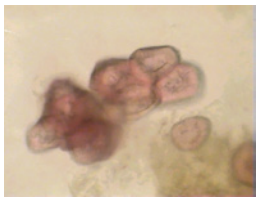

Figure 13: Sclereids in clusters.

Figure 14: Vessels.

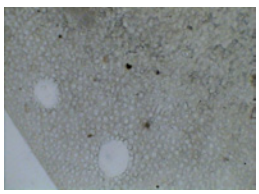

Figure 15: Transverse section of seed.

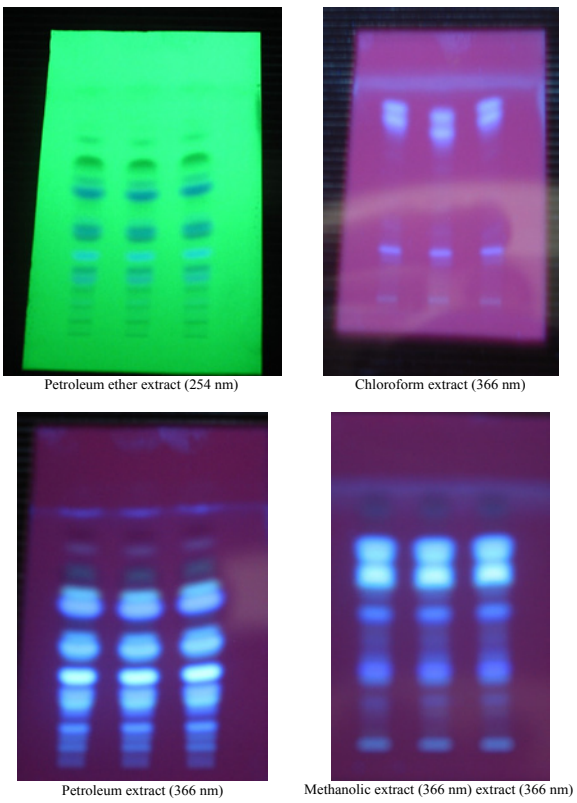

Figure 16: HPTLC fingerprinting of different extracts of *P. pinnata*.

glycosides, phenolic compounds, tannins, flavonoids, proteins and free amino acids, saponins, sterols, acidic compounds, mucilage, lipids, fats and resin (Table 1). The crude powder of *P. pinnata* pods was treated with different chemical reagents and evaluated for fluorescence behavior with the naked eye and UV light (Tables 2 and 3). TLC profiles of different extracts of this powder were obtained in different solvent systems. The methanolic extract in solvent system toluene: ethyl acetate: methanol in a 5:1:0.3 ratio showed five clear spots at R_f = 0.15, 0.34, 0.47, 0.69 and 0.85 (Table 4). Similarly, HPTLC (Figure 16) was performed in the same solvent system (Table 5). The different physicochemical parameters of *P. pinnata* pods assessed in this study would be useful in the authentication of plant material (Table 5). Moisture content of the crude drug is not too high therefore the possibility of bacterial and yeast growth is minimal. The ash value was determined to evaluate the purity of the crude drug. Total ash, acid insoluble and water soluble ash value were 3.377, 0.876 and 1.141%, respectively. Crude drugs showed a hot extractive value in petroleum ether, chloroform, methanol and water of 0.1765, 0.4651, 0.2182 and 0.2155%, respectively while in normal (unheated) petroleum ether, chloroform, methanol and water, the values were 0.2101, 0.1130, 0.1025 and 0.5937%, respectively. Resin content and foaming index were 0.73% (w/w) and <100, respectively.

Acknowledgement

The authors are thankful to Jamia Hamdard (Hamdard University) New Delhi for providing the necessary facility to carry out this study.

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