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PHYTOCHEMICAL AND PHARMACOGNOSTICAL STUDY OF KARANJA (*PONGAMIA PINNATA* LINN.) SEED

Dr. POONAM SHARMA[1], APOORVA JOSHI[2], Dr. AJAY KUMAR SHARMA[3], Prof. K. N. DWIVEDI[4]

1. Junior Resident, Department of Dravyaguna, I.M.S., B.H.U., Varanasi, India.
2. Ph.D. Scholar, Department of Pharmaceutics, I.I.T., B.H.U., Varanasi, India.
3. Assistant Professor, Department of Shalya Tantra, I.M.S., B.H.U., Varanasi, India.
4. Professor, Department of Dravyaguna, I.M.S., B.H.U., Varanasi, India.

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Abstract: Karanja (*Pongamia pinnata)* an ancient plant described in *Vedas, Samhita* and in many *Nighantu* is a very important medicinal plant. Ethnobotanically it is used in many diseases viz. Diabetes, diarrhea, skin diseases as scabies etc. It is described in Susruta Samhita in Prameha chikitsa and also in many nighantus. So here we are using Karanja for treatment of Prameha and so we are standardizing it for its identity, purity and to prove therapeutic action as well. Pharmacognosy is the study of medicines derived from natural sources. Under this pharmacognosy, pharmacology and phytochemistry are necessary for authentification of crude drug. Research work was carried out to find the phytochemical constituents and its anti-diabetic activity. For this dried seeds of Karanja were taken and then grinded to make fine powder of it, later its ethanolic extract was prepared in Soxhlet apparatus. The results of preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, carbohydrates, flavonoids, steroids, saponins, triterpenoids and tannins. So we may conclude that drug is rationale for anti-diabetic medicinal use.

Keywords: Anti-diabetic, Phytochemistry, Pharmacognosy, Ethnobotanical

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Corresponding Author: Dr. POONAM SHARMA

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INTRODUCTION

Karanja is botanically *Pongamia pinnata* Linn. Pierre belongs to family Fabaceae[1]. *Pongamia pinnata* is a fast-growing tree which reaches 40 feet in height and spread forming a broad, spreading canopy casting moderate shade mostly found near the river side[2]. Ethnobotanical review reveals that different parts like root, stem bark, leaf, flower, seed and seed oil are used by many tribe all over the world to cure various diseases. World Health Assembly (WHA42.43-1989) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards. Assessment of complete and accurate physicochemical value of Ayurvedic herbs not only provides scientific basis of its quality but also helps in globalization of Ayurveda. Roots of *Pongamia pinnata* are bitter, anti-helmintic and used in vaginal and skin diseases[3]. Juice of the root is used for cleansing foul ulcers and closing fistulous sores[4]. Aqueous extracts of stem bark exhibit significant CNS sedative and antipyretic activity[5]. Juice of leaves is used for cold, cough, diarrhea, dyspepsia, flatulence, gonorrhea, leprosy[6]. Seed powder valued as a febrifuge, tonic and in bronchitis and whooping cough[7].

Physical Constituents - Seed powder - Total ash - 4.5%; Acid insoluble ash - 1.37%; Water soluble ash-1.46%

Chemical constituents[8]

Karanjin, pongapin, pongamol-chromeno chromene designated as pongaflavone [2.2-dimethvl pyrano(5,6',8 ,7)-methoxy flavone] (stem); (Glabrin, four furanoflavones viz.. karanjin, pongapin, kanjone and pongaglabrone and a diketone pongamol, fatty acids viz., palmitic, stearic, arachidic, behenic, lignoceric, oleic, linoleic

Pharmacological activities

- Antibacterial, insecticidal, hypoglycaemic, anti-cholinergic, anthelmintic, anti-tubercular, CNS stimulant and depressant, sedative, wound healing activity.

- The oil and its active constituent karanjin posses antibacterial properties. Alcoholic extract of the oil showed activity against both gm. +ve and -ve organisms such as micrococcus pyogenes var citrus, gran C. diptheritus, S. typhosa, S. paratyphi A and E. Coli[9]. Unexploited botanical nitrification inhibitors prepared from Karanja plant[10]. Karanja (Honge) oil proves to be a good biodiesel[11].

Anti-inflammatory Activity[12]

It has been reported that 70% ethanolic extract of *P. pinnata* leaves has potent anti-inflammatory activity against different phases (acute, Sub acute and chronic) of inflammation without side effect on gastric mucosa.

Antioxidant and Anti-hyperammonemic Activity[13]

It has been observed that effect of *P. pinnata* leaf extract on circulatory lipid peroxidation and antioxidant status was evaluated in ammonium chloride- induced hyper ammonium rats. It enhanced lipid peroxidation in the circulation of ammonium chloride-treated rats was accompanied by a significant decrease in the levels of vitamin A, vitamin C, vitamin E-reduced glutathione, glutathione preoxidase, Superoxide dismutase and catalase. It showed that PPET modulates these changes by reversing the oxidant-antioxidant imbalance during ammonium chloride-induced hyper ammonemia and this could be due to its anti-hyperammonemic effect by means of detoxifying excess ammonia, urea and creatinine and antioxidant property.

Anti-Hyperglycaemic and Anti-lipidperoxidative Activity[14]

The treatment of *P. pinnata* seed extract could be used as a safe alternative anti-hyperglycaemic drug for diabetic patients.

Sunscreen Activity[15] **-** Leaves of *P. pinnata* extract used as highly effective sunscreen preparations as it will enhance and effectively contribute to UV absorbing properties of a conventional sunscreen. It will also help in broadening the UV protection in ability of the sunscreens.

Antiviral activity[16] **–** Antiviral property of seed extract of *P. pinnata* against Herpes Simplex virus.

MATERIAL AND METHODOLOGY

Collection and identification of plant

Plant material – Seeds of *Pongamia pinnata* were collected from an authentic shop from the Gola Deenanath Market of Varanasi, U.P., and identified in Department of Dravyaguna. The drug was preliminary identified by the Ayurvedic parameters such as Varna (color), Gandha (odour), Ruchi (taste), Roop (shape) and Parimana (size)[17].

Pharmacognostical Description of plant

1. Macroscopic study of seed :

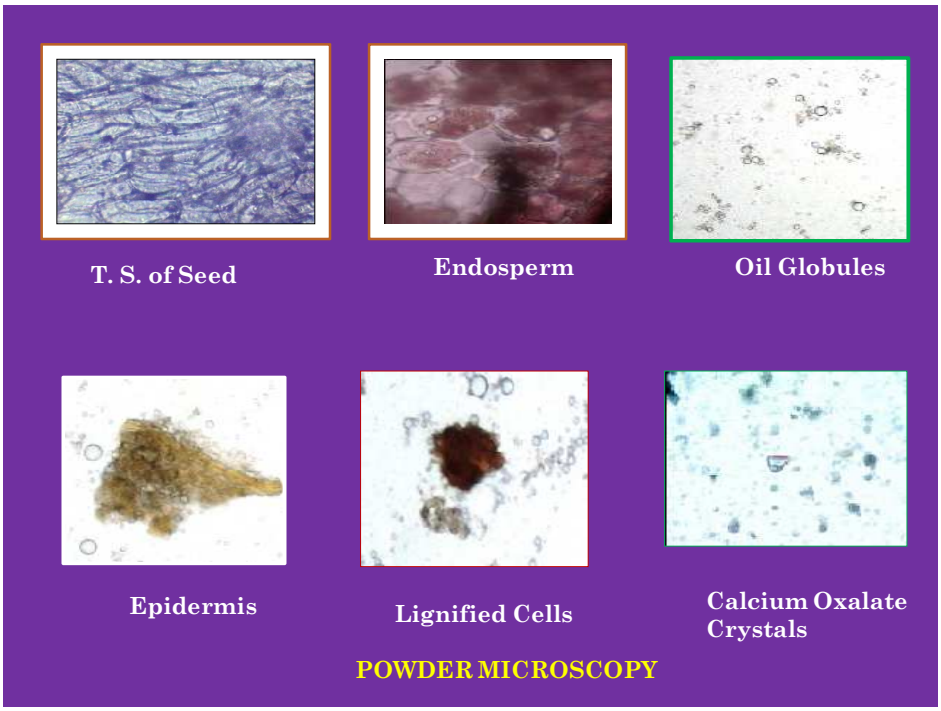
Seeds usually one and rarely two, elliptic or reniform in shape, 1.7 -2.0cm long and 1.2-1.8 cm broad, wrinkled with reddish leathery testa, micropylar end of cotyledons slightly depressed while other side semi-circular in shape.

2. Microscopic study of seed :

Seeds are square like in shape and flat with reddish brown leathery testa which is found compressed around and having wrinkles like vascular strands descending away from the hilum portion like zig-zag veins. The embryo contains two thick oily cotyledons nearly bean-shaped with a depression near the plumule. The seed is exalbuminous. A transection of the seed is elliptical in outline. The testa is a broad zone comprising of cuticle, palisade, single

layered bearer cells and thick parenchymatous layer. The pallisade layer is covered with a thick cuticle, composed of vertical and columnar cells. The cuticle is unsymmetrical and un-uniform in thickness forming depressions at places. Pallisade is followed by a layer of bearer cells and thick zone of parenchymatous cells. The first 2-3 layers of parenchymatous cells consist of small closely arranged, thick walled cells. A next 2-3 layer possesses several air spaces. The cotyledons are protected within the testa. When fresh, they are swollen, fleshy and in close contact with the testa throughout the entire perimeter. On drying, the cotyledons shrink and become detached from the testa at several places. Under a thin cuticular layer, the epidermis of the cotyledon is present, the cells of which are cylindrical. The cells of the mesophyll are filled with round starch grains and rounded to oval, colourless oil globules[18].

Powder study of seed: Colour light yellow through mustard to brown, coarse and free-flowing; bitter in taste. Powder is rich of oil so somewhat sticky. In microscopic powder study it shows oil globules of white colour. Epidermis which is of yellow to light brown in colour, lignified cells of dark red or reddish brown in colour. In this calcium oxalate crystals also seen of light blue colour. Endosperm of light pink colour also seen.



Fluorescence analysis:

Fluorescence study of seed powder was performed as per reported standard procedure. A small quantity of the seed 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 (366 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were noted.

IDENTITY, PURITY AND STRENGTH

METHODOLOGY

Loss on drying at 105° C/Moisture content

Place about 10 gm of drug sample after accurately weighing it in a tared evaporating dish. After placing the above said amount of sample in a tared evaporating dish dry at 105° C for 5 hours and weigh. After drying tared evaporating dish cool in desiccator for 30 minutes and then weight taken.

$$\text{The \% of loss on drying} = \frac{\text{Difference in weight after heating} \times 100}{\text{Weight of sample taken}}$$

Determination of Ash

Determination of Total Ash

About 2.0g of powdered drug was incinerated in a tared silica dish at a temperature not exceeding 450°C until free carbon was left, cooled and final weight was taken. The percentage of ash calculated with reference to the air-dried drug (PASF, 1987).

Determination of Acid Insoluble Ash

The ash obtained as above method was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and collected the insoluble matter on an ash-less filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated (PASF, 1987).

Determination of Sulphated Ash

Heat a silica crucible to redness for 10 minutes; allow cooling in a dessicator and weighing. Put about 2.0g of powdered drug into the crucible; ignite gently at first, until the sample is thoroughly charred. Cool, moisten the residue with 1 ml of *sulphuric acid*, heat gently until white fumes are no longer evolved and ignite at 800° ± 25° until all black particles have disappeared. Allow the crucible to cool; add few drops of *sulphuric acid* and heat. Ignite as before, allow to cool and final weight was taken after two successive operation repetitions.

Determination of Extractable Matter in water and alcohol

About 4.0g of coarsely powdered air dried material, was accurately weighed in a glass stoppered conical flask and macerated with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allowed to stand for 18 hours. Filtered rapidly, taking care not to loose any solvent. The extracted matter was dried at 105°C for 6 hours, cooled in a dessicator for 30 minutes and then weighed. The percentage extractable matter was calculated.

IDENTITY, PURITY AND STRENGTH-

S.No.	Parameters	Result
1.	Foreign matter (w/w%)	1%
2.	Moisture content (w/w%)	2.5%
3.	Total ash (w/w%)	4.5%
4.	Acid insoluble ash (w/w%)	1.37%
5.	Sulphated ash (w/w%)	5.2%
6.	Alcohol soluble extractive (w/w%)	22.5%
7.	Water soluble extractive	15.4%

Preliminary Screening of Phytochemicals

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them [19,20].

Thin Layer Chromatography

Materials used

All the TLC plates used for the analysis were prepared with silica gel containing binder. Most frequently used binder in silica gel is calcium sulphate (Silica gel 60 F_{254}, Merk). Precoated aluminium sheets were also used which is coated with silica gel 60 F_{254} (Merck).

Application of sample

A known quantity of sample was dissolved in a known volume of solvent and the sample applied on precoated TLC plates.

Visualization: TLC plates were visualized under Iodine solution and after spray of Sulphuric acid reagent and heated at 110°C for 5 min.

Detection of spots

Detection of Rf values of spot done by using Iodine exposure and Sulphuric acid reagent.

RESULTS AND DISCUSSION

Macroscopic study of seed shows one rarely two seeds reniform in shape, wrinkled with reddish leathery testa. Microscopic study shows testa composed of a layer of palisade like outer epidermis, filled with brown pigment, covered externally with a thick cuticle.

In powder study it was found that it is rich in oil so somewhat sticky. In microscopic powder study it shows oil globules of white colour. Epidermis which is of yellow to light brown in colour, lignified cells of dark red or reddish brown in colour. In this calcium oxalate crystals also seen of light blue colour. Endosperm of light pink colour also seen.

RESULT:

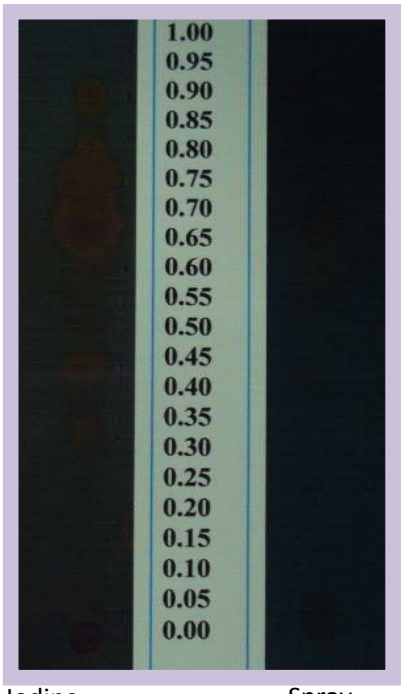
Genuine sample of *Pongamia pinnata* gave the presence of following Phytochemicals:

S.No.	Test Sample	Result
1.	***Test for alkaloids***	
	Dragendorff's test	+
	Hager's test	+
	Wagner's test	+
	Mayer's test	+
2.	***Test for Carbohydrates***	
	Anthrone test	+
	Benedict's test	+
	Fehling's test	+
	Molisch's test	+
3.	***Test for Flavonoids***	
	Shinoda's test	+
4.	***Test for Triterpenoids***	
	Liebermann-Burchard's test	+
5.	***Test for Proteins***	
	Biuret's test:	+
	Millon's test:	+
6.	***Test for Resins***	+
7.	**Test for saponins**	+
8.	**Test for steroids**	+
9.	**Test for Tannins & phenolics**	+
10.	**Test for starch**	+
11.	**Test for glycosides**	-

Fluorescence analysis shows the presence of different light colours in ordinary and UV light:

S.No.	Treatment	Under ordinary light	Under UV-Long (366nm)
1.	Drug as such	Brown	Brown
2.	Drug + Nitrocellulose	Dark brown	Light green
3.	Drug + Picric acid	Yellow	Yellow
4.	Drug + HCl $_{conc.}$	Dark brown	Green
5.	Drug + H_2SO_4 conc.	Black	Blackish brown
6.	Drug + HNO_3 (50%)	Orange	Light green
7.	Drug + 1 N Na OH in Me OH	Light brown	Light green
8.	Drug + 1 N Na OH in Water	Orange	Yellowish green
9.	Drug + NH_4OH	Light brown	Green
10.	Drug + $FeCl_3$	Light green	Dark green
11.	Drug + Acetic acid $_{Glacial}$	Brown	Light brown
12.	Drug + Sudan-III	Dark brown	Light brown

THIN LAYER CHROMATOGRAPHY OF ALCOHOLIC EXTRACT



Iodine Spray

Stationary phase	**Glass plate coated with silica gel**
Mobile phase	Toluene – Ethyl Acetate –Acetic acid (5: 4: 0.5)
Rf value of spots visualized in Iodine	**Seed** 0.30, 0.45, 0.50, 0.65, 0.75, 0.80 and 0.90
Rf value of spots visualized after spray of Sulphuric acid reagent and heated at 110°C for 5 min.	**Seed** 0.40 (Light brown), 0.60 (Yellow), 0.65, 0.75 and 0.90 (Brown)

CONCLUSION

Pongamia pinnata is very important medicinal plant. On preliminary phytochemical and pharmacognostical study it was found that it contains alkaloids, flavonoids, triterpenes, steroids, tannins & phenolics, starch, proteins, resins and carbohydrates.

It has anti-inflammatory, Anti-Hyperglycaemic and Anti-lipidperoxidative Activity, Anti-ulcer Activity, Anti-diarrhoeal Activity, Antioxidant and Anti-hyperammonemic Activity. Seed powder microscopy shows presence of oil globules, epidermis, endosperm, lignified cells and calcium oxalate crystals. Presence of chemical constituents like karanjin, pongamin, pongamol and various other chemicals have anti-hyperglycemic activity.

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