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PHYTOCHEMICAL SCREENING AND PHARMACOGNOSTICAL EVALUATION OF COLLECTED CRUDE DRUG OF *PONGAMIA PINNATA* (FABACEAE) LEAF

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ABSTRACT

The present research work was carried out the standardization of *Pongamia pinnata* Leaf, the Pharmacognostical and phyto chemical screening were performed and reported. The Pharmacognostical screening of macroscopy, microscopical character, fluorescence analysis, physio chemical parameters, and phytochemical screening like extraction and identification of phyto constituents from the crude drug. More than ten Pharmacological activities were done in this plant. But proper documentation of this plant is not available from any research papers. So we carried out and concentrate only on Standardization of *Pongamia pinnata* (Leaf). This research based work on most useful in future research scholars for continuing the studies. Well developed countries also now-a-days adopting to follow the herbal medicines, for the reason risk, side effect less when compared to Allopathic system of medicine. India like developing countries poor sector of people more, they are easily available herbal medicine used day by day activities.

KEYWORDS: *Pongamia pinnata*, Microscopical, fluorescent, physio chemical, extraction.

INTRODUCTION

Pongamia pinnata can cultivate on most soil types; finest development is found on deep well-drained sandy loams with secure moisture, but it grows on sandy soils and heavy swelling clay soils. It does not do fine on dry sands, even though it stands saline conditions, alkalinity and waterlogged soils. The natural allocation of Pongamia *pinnata* is along coast and river banks in lands and native to south-east Asia and India. The tree is introduced in moist humid regions all over the world including the US and Australia. It is cultivated along road sides, canal banks and open farm lands. Grafting is necessary because it can take as long as ten years for a tree in the wild to reach full seed-bearing maturity. The researchers studied thousands of Pongamia variants, identifying and collecting the best sources of high quality grafting stock. The tree will increase yield until age 15 and will continue having reliable yields until age 50.The total life span of the tree is between 85 and 100 years. The theoretical projected yields depend on many factors including rainfall, temperature, soil conditions, age of tree, density of trees, irrigation, specific variety (seed weight) and many others.

SCIENTIFIC CLASSIFICATION (Arote, s.r., et al 2010).

Kingdom : Plantae
Phylum : Magnoliphyta
Class : Magnoliopsida
Order : Fabales
Family : Fabaceae
Genus : Pongamia
Species : pinnata

Synonyms

Derris indica (Lam.) Bennett, *Millettia novo* - guineensis Kane, *Pongamia glabra* Vent, *Pongamia pinnata* Merr.

Vernacular names (Yadav, R. V., et al 2011)

English : Indian beech, karum tree. oil tree, pongam, pongam oil
Telugu : Kanuga
Hindi : Karanj
Tamil : Pungai
Kanada : Honge
Malayalam : Punnu
Sanskrit : Naktamala
Arabic : Um al shuur
Bengali : Karanj, karanja

Common constituents of *Pongamia pinata*: (Carcache-Blanco EJ et al 2003, Yadav PP, et al 2004, Yin H, et al 2005, Natanam R et al 1989, Nagaraj G et al 2004).

Karanjin, Pongaglabrone, Pongapin, Pongachromene, Glabrachromene -1, Friedeline, Phytochemical investigation of *Pongamia pinnata* indicated the presence of abundant prenylated flavonoids such as furanoflavones, furanoflavonols, chromenoflavones, furanochalcones and pyranochalcones. *Pongamia pinnata* contain 30 to 40% pongam oil and also called pongomol or Hongay oil.

Common uses of *Pongamia pinnata* (Subramoniam P et al 1999, Dahanukar SA et al 2000, Aliyu R. 1994, Meera B, et al 2003, Allen ON et al 1981, Satyavati GV et al 1987, Tanaka T, et al 1992, Srinivasan K et al 2001, Punitha R etl al 2006, K. Srinivasan et al 2001).

Dried pongamia leaves are used in stored grains to repel insects.

Leaves of pongamia are Used for cold, cough, diarrhea, dyspepsia, flatulenence, leprosy, antihelmentic, digestive and laxative, inflammation, piles, wounds, relieve rheumatism, treat itches and herpes.

In the traditional systems of medicines, such as Ayurveda and Unani, the P. pinnata plant is used for anti-inflammatory, anti-plasmodial, anti-nonciceptive, anti-hyperglycemic, anti-lipid oxidative, anti-diarrhoeal, anti-ulcer, anti-hyper ammonic and antioxidant. Its oil is a source of biodiesel. It has also alternative source of energy, which is renewable, safe and non-pollutant.

MATERIALS AND METHODS

The plant material was collected from the surrounding area of Narayana Medical College Hospital, Chinthareddypalem. It is 7 km away from Nellore. The plant was identified initially by local people and authenticated by Dr. Venkata Ramanaiah, V.R College, Nellore. The voucher specimen was kept in this college.

PHARMACOGNOSTICAL SCREENING

(Khandelwal K.R 2008).

Macroscopical character was identified by using sensory organs, like colour, odour, taste,size,shape and texture noted on the results.

Microscopical examination (Khandelwal K.R 2008).

Microscopical examination was carried out by using Transverse section techniques. The staining reagents are Phloroglucinol and Hydrochloric acid (1:1), Safranin and Methy red. Finally conclude the microcopical character under the microscope and reported on results.

PHYSIOCHEMICAL PROPERTIES

Determination of petroleum ether soluble extractives

Weigh about 5g of powder drug in a weighing bottle and transfer it to a dry 250ml Conical flask. Fill a100ml graduated flask to the delivery mark with the solvent (Petroleum Ether). wash out the Weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hrs, shaking frequently (maceration).

Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of filtrate to a weighed, thin porcelain dish, as used for the ash values determination.

Evaporate to dryness on a water bath and complete the drying in an oven at 100^0C.Cool in a desiccators and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

Determination of chloroform soluble extractives

Weigh about 5g of powder drug in a weighing bottle and transfer it to a dry 250ml conical flask. Fill a100ml graduated flask to the delivery mark with the solvent (Chloroform).wash out the Weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hrs, shaking frequently (maceration).

Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of filtrate to a weighed, thin porcelain dish, as used for the ash values determination.

Evaporate to dryness on a water bath and complete the drying in an oven at 100^0C.Cool in a desiccator and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

Determination of methanol soluble extractives

Weigh about 5g of powder drug in a weighing bottle and transfer it to a dry 250ml conical flask. Fill a100ml graduated flask to the delivery mark with the solvent (Methanol).wash out the Weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hrs, shaking frequently (maceration).

Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of filtrate to a weighed, thin porcelain dish, as used for the ash values determination.

Evaporate to dryness on a water bath and complete the drying in an oven at 100^0C. Cool in a desiccators and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

Determination of ethanol soluble extractives

Weigh about 5g of powder drug in a weighing bottle and transfer it to a dry 250ml conical flask. Fill a100ml graduated flask to the delivery mark with the solvent (Methanol).wash out the Weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hrs, shaking frequently (maceration).

Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of filtrate to a weighed, thin porcelain dish, as used for the ash values determination.

Evaporate to dryness on a water bath and complete the drying in an oven at 100^0C.Cool in a desiccators and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

Determination of water soluble extractives
Weigh about 5g of powder drug in a weighing bottle and transfer it to a dry 250ml conical flask. Fill a100ml graduated flask to the delivery mark with the solvent (Chloroform Water).wash out the Weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hrs, shaking frequently (maceration).

Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of filtrate to a weighed, thin porcelain dish, as used for the ash values determination.

Evaporate to dryness on a water bath and complete the drying in an oven at 100^0C.Cool in desiccators and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

Determination of acetone soluble extractives
Weigh about 5g of powder drug in a weighing bottle and transfer it to a dry 250ml Conical flask. Fill a100ml graduated flask to the delivery mark with the solvent (Chloroform Water).wash out the Weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hrs, shaking frequently (maceration).

Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of filtrate to a weighed, thin porcelain dish, as used for the ash values determination.

Evaporate to dryness on a water bath and complete the drying in an oven at 100^0C.Cool in desiccators and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

Crude fiber content
Weigh 2g of powdered drug in a beaker. Add 50ml of 10%v/v Nitric acid. Heat to boil with constant stirring (till about 30 seconds after boiling starts). Strain through fine cotton cloth on a Buchner funnel. Give washing to the residue with boiling water. Transfer residue from the cloth to a beaker. Add 50ml of 2.5% v/v sodium hydroxide solution. Heat to boil. Maintain at boiling point for 30 seconds, stirring constantly. Strain and wash with hot water as mentioned earlier. For quantitative determination transfer the residue in a cleaned and dried crucible. Weigh the residue and determine percentage crudefibres.

Foreign organic matter
Weigh 100-500 g of the sample. Spread the sample on a white tile or a glass plate uniformly without overlapping. Inspect the sample with naked eyes or by means of a lens (5x or above). Seperate the foreign organic matter manually. After complete separation, weigh the matter and determine % w/w present in the sample.

Loss on drying
Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100^0C or 105^0C.Cool in a desiccators and watch the loss in weight is usually recorded as moisture.

Vein islet number
Clear a piece of the leaf by boiling in Chloral hydrate solution for about 30 minutes. Arrange Camera lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16 mm objective. Draw a line equivalent to 1 mm as seen through the microscope. Construct a square on this line. Move the paper so that the square is seen in the eye piece, in the centre of the field. Place the slide with the cleared leaf (epidermis on the stage).Trace of the veins which are included within the square, completing the outlines of those islets which overlap two adjacent sides of the square. Count the number of vein islets in the square millimeter. Where the islets are intersected by the sides of the square including those on the two adjacent sides and exclude those islets on the other sides. (to obtain a critical result for a leaf,4sq.mm. should be used, preferably in one large area of 4 sq.mm.) Find the average number of vein islets from the four adjoining squares, to get the values for 1 sq.mm.

Determination of vein islet termination number
Clear a piece of the leaf by boiling in Chloral hydrate solution for about 30 minutes. Arrange Camera lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16 mm objective. Draw a line equivalent to 1 mm as seen through the microscope. Construct a square on this line. Move the paper so that the square is seen in the eye piece, in the centre of the field. Place the slide with the cleared leaf (epidermis on the stage).Trace of the veins which are included within the square, completing the outlines of those islets which overlap two adjacent sides of the square. Count the number of vein islets in the square millimetre. Where the islets are intersected by the sides of the square including those on the two adjacent sides and exclude those islets on the other sides. (to obtain a critical result for a leaf,4sq.mm. should be used, preferably in one large area of 4 sq.mm.)Find the average number of vein islets from the four adjoining squares, to get the values for 1 sq.mm.

Count the number of veinlet terminations present within the square. Find the average number of vein let termination number from the four adjoining squares to get the value for 1 sq.mm.

Fluorescent analysis
The plant powdered drug was added various chemical reagents under the U.V and Visible light and reported on the table.

Extraction of plant leaf material

The plant material was extracted by using various chemicals like Pet ether, Chloroform and Ethanol by Successive Solvent Extraction Method. These are all the chemicals selected depending upon the order of polarity.13g of the plant powder material was taken in a Sox let apparatus and added different solvents (order of polarity-pet ether, chloroform and ethanol). At the time of process ended 14hrs of duration.

PHYTO CHEMICAL SCREENING

Detection of alkaloids

The small portions of solvents of solvent free chloroform, alcoholic and water extracts are stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloid reagents; Mayer's reagent (cream precipitate), Dragendroff's reagent (orange brown precipitate), Hager's reagent 9tellow precipitate) and Wagner's reagent (reddish-brown precipitate).

Detection of carbohydrates and glycosides

Small quantities (200mg) of alcoholic and aqueous extracts are dissolved separately in 5ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to detect the presence of carbohydrates.

Another small portion of extract is hydrolyzed with dilute hydrochloric acid for few hours in water-bath and is subjected to Libermann-Buchard's tests to detect presence of different glycosides.

A small amount of extact is dissolved in water and treated with Fehling's, Barfoeds and Benedict's reagents to detect presence of different sugars.

Detection of phyto sterols

The petroleum ether, acetone and alcoholic extracts are refluxed separately with solution of alcoholic potassium hydroxide till complete saponification takes place. The saponification mixture is diluted with distilled water and extracted with ether. The ethereal extract is evaporated and the residue (un saponifiable matter) is subjected to Liebermann's and Burchard's tests.

Detection of fixed oils and fats

A small quantity of petroleum ether and benzene extracts is pressed separately between two filter papers. Oil stains on the paper indicate the presence of fixed oil. A few drops of 0.5N alcoholic potassium hydroxide is added to a small quantity of petroleum ether or benzene extract along with a drop of phenolphthalein. The mixture is heated on water bath foe 1-2 hr. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Detection of saponins

About 1ml of alcoholic and aqueous extracts is diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 15min.One cm layer of foam indicates presence of saponins. The test solution may be subjected to test for haemolysis.

Detection of phenolic compounds and tannins

Small quantities of alcoholic and aqueous extracts in water are tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%), 1% solution of gelatin containing 10% sodium chloride,10% lead acetate and aqueous bromine solutions.

Detection of proteins and free amino acids

Small quantities of alcoholic and aqueous extracts are dissolved in a few ml of water and subjected to Millen's, Biuret and Ninhydrin tests.

Detection of gums and mucilage

About 10ml of aqueous extract is added to 25 ml of absolute alcohol with constant stirring. The precipitate is dried in air. Then the precipitate is examined for its swelling properties and for the presence of carbohydrates.

Detection of volatile oil

About 50gm of powdered material is taken in a volatile oil estimation apparatus and subjected to hydro-distillation for the detection of volatile oil. The distillate is collected in the graduated tube of the assembly in which the aqueous portion is automatically separated from the volatile oil, if it is present in the drug, and returned back to the distillation flask.

RESULTS

Macroscopy

Fig: 1 *Pongamia pinnata*

Colour : Pale green to Dark green
Odour : Characteristic
Taste : Slightly bitter and aggreable
Shape : Lanceolate, ovate
Size : Length- 6cm, Width -2.5 cm
Fracture : Short
Petiole : Present and short
Pubescent : Absent
Base : Asymmetrical in nature
Venation : Reticulate
Veins are promptly seen in lower surface of the leaf.

Microscopy character of *Pongamia pinnata* leaf

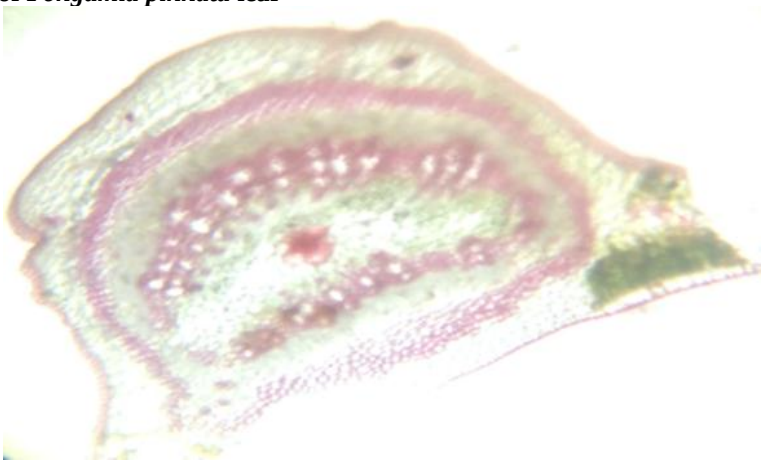

Fig: 2 Transverse section of mid rib region of *Pongamia pinnata* leaf

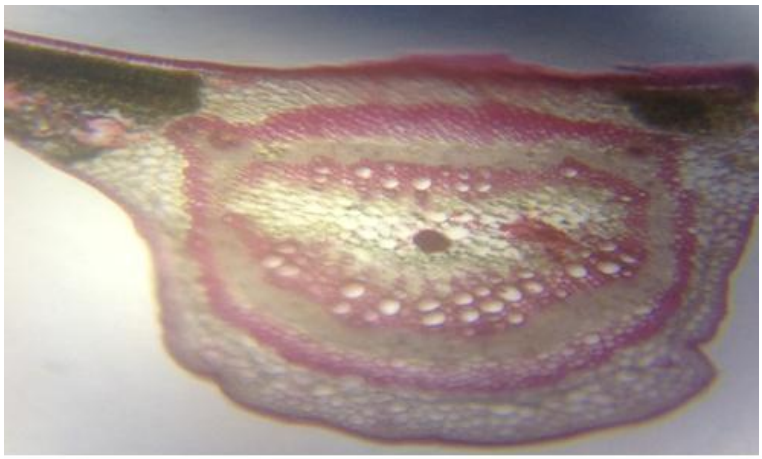

Fig: 3 Transverse section of *Pongammia pinnata* leaf- lamina

Upper and lower epidermis is single made up of single layered cells, it is rectangular in shape, Palisade cells present below the upper epidermis, collenchymas present below the upper epidermis. Vascular bundle contains xylem and phloem. Phloem cells un lignified, Xylem lignified, calcium oxalate crystals present in the midrip region of parenchyma cells. Lignified sclrenchyma present in the midrip region of the epidermis. Thick cuticle is covered the upper and lower epidermis. Collenchyma followed remaining all the places occupied by parenchyma cells. Lignified sclerenchymatous layer present. Starch grains present.

Vein islet number

Number of vein islet is present in the given sample of the leaf is 31.

Vein termination number

Number of vein termination present in the sample of the leaf is 21.

Physiochemical parameters of *Pongamia pinnata* leaf powder

Table No: 1 physiochemical parameters of *Pongamia pinnata* leaf powder

SL.NO	PHYSIOCHEMICAL PARAMETERS	% W/W
1	Crude Fibre Content	43
2	Foreign Organic Matter	1.860
3	Loss On Drying	10
4	Pet eher	0.78
5	Acetone	3.644
6	Chloroform	4.4
7	Methanol	18
8	Ethanol	10
9	Water	14.22

Fluorescence analysis of Pongamia *pinnata* leaf powder

Table No: 2 Fluorescence analysis *Pongamia pinnata* leaf powder

SL.NO	CONTENTS	DAY LIGHT	U.V LIGHT
1	Powder	Green	Dark Green
2	50 % H_2SO_4 + Powder	Slight brown	Pale Green
3	10 % NaoH (Aquoeus)	Pale Yellow	Green
4	10 %NaoH (Alcohol)	Dark Yellow	Dark Green
5	Pet Ether	Pale Green	Green
6	Benzene	Dark Green	Dark Brown
7	1N Hcl	Slight Yellow	Pale Green
8	Chloroform	Green	Green
9	Ethanol	Slight Brown	Pale Green
10	Acetone	Brown	Dark Green

Extraction

The extraction was carried out by using different solvents, continuous hot percolation (Sox let Apparatus).We were selected the solvent based on the order of polarity like pet ether, chloroform and ethanol.

1. The percentage yield of pet eher extracts 2% w/w.
2. The percentage yield of chloroform extracts 2.7 %w/w.
3. The percentage yield of ehanol extracts 8.6% w/w.

Table No: 3 Phytochemical screening of alcoholic extract *Pongamia pinnata* leaf

S.NO	PHTOCONSTITUENTS	INFERENCE
1	Saponins	Present
2	Tannins	Present
3	Phenolics	Present
4	Glycosides	Absent
5	Alkaloids	Present
6	Carbohydrates	Present
7	Reducing Sugars	Present
8	Proteins	Absent
9	Amino Acids	Absent

Table No: 4 Phytochemical screening of pet ether extract *Pongamia pinnata* leaf

SL.NO	PHYTOCOSTITUENTS	INFERENCE
1	Steroids	Present
2	Flavanoids	Present
3	Fats	Present
4	Volatile Oils	Present

Table No: 5 phytochemical screening of chloroform extracts *Pongamia pinnata* leaf

SL.NO	PHYTOCOSTITUENTS	INFERENCE
1	Alkaloids	Present

DISCUSSIONS

Pongamia pinnata (L.) Pierre [family: Leguminosae] is a medium –sized glabrous tree popularly recognized as Karanja in Hindi, Indian beech in English and Pongam in Tamil. It is adjustable tree for tropical and sub-tropical regions which requires excellent drainage and a sunny location. It grows easily from seed. Historically, this plant has long been used in India and neighboring regions as a source of traditional medicines, animal fodder, green manure, timber, fish poison and fuel. Various parts of this plant are habitually claimed to be used for the treatment of broad spectrum of ailments including bronchitis, whooping cough, rheumatism, diarrhea, dyspepsia, flatulence, gonorrhea and leprosy to list a few. The present research work was carried out the Standardisation Of Pongamia pinnata Leaf, it is cultivated species its not collected from anywhere, it is present in our campus of Narayana Medical College Hospital, Chinthareddypalem, Nellore. Above mentioned various diseases were cured by this plant.But proper standardization of Pharmacognostical screening work were performed and reported. This Pharmacognostical screening work including the macroscopy (Colour, Odour, Taste, Size and Shape), microscopical character (Transverse section of mid rib region, leaf constants-Vein islet number and Vein termination number).The average vein islet number is 55,The average vein termination number is 28.Both the vein islet and termination was performed triplicate.

Fluorescent analysis was reported by using UV 378 nm and day light. physio chemical parameters (The different extractive values were calculated by order of polarity.18 %w/w of a methanol soluble extractive value is high,0.78 %w/w of a pet ether soluble extractive value is least. The loss on drying value of the leaf is 10 %w/w.The percentage of crude fibre content is 43 %w/w. The foreign organic matter is 1.860 %w/w).The extraction wasperformed by using Soxhlet Apparatus. (The extractive value of pet eher-2% w/w, chloroform-2.7%w/w, and ethanol-8.6 % w/w). The different extracts was screened for prenting the phytochemicals by using colour reaction tests. (pet eher-steroids, flavanoids, fats, oils was reported. Alkaloids only reported by Chloroform extract. Alcohol extract having the phyto constituents like Alkaloids, Carbohydrates, Tannins and Saponins).

CONCLUSIONS

The present research work was carried out the Standardisation Of Pongamia pinnata Leaf, the Pharmacognostical screening work were performed and reported. This Pharmacognostical screening work including the macroscopy, microscopical character,

fluorescent analysis, physio chemical parameters, extraction and identification of phytoconstituents. This research work on most useful in future research scholars for future studies. Well developed countries also now-a-days adopting to follow the herbal medicines, for the reason no risk, side effect less, when compared to Allopathic system of medicine. India like developing countries poor sector of people more, they are easily available herbal medicine used day by day activities.

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