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A STUDY ON PHYTOCHEMICAL INVESTIGATION OF *PONGAMIA PINNATA* LINN. LEAVES

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ABSTRACT

The present work deals with development and standardization of phytochemical screening for quantification of ethanolic extract of medicinal plant leaves of *Pongamia pinnata Linn.* The scientific parameter is necessary to identify the exact plant material and to find its quality and purity. The present study deals with various, physical evaluation and preliminary phytochemical screening of various successive extracts such as qualitative chemical analysis and HPTLC of extract shows the twelve different peaks confirming that the twelve compounds present in ethanolic extract of *Pongamia pinnata*. These studies indicated the possible information for correct identification and standardization of this plant material.

INTRODUCTION: The 'Pongam Tree' is known as one of the richest and brightest trees of India. The tree is named as 'Pongamia pinnata' in science. The name 'Pongamia' has derived from the Tamil name, 'pinnata' that refers to the 'Pinnate leaves'. The tree is a member of the 'leguminosae' family. Its sub family is 'Papilionaceae'. In the Tamil, this is generally known as 'Ponga', 'Dalkaramacha', 'Pongam' and 'Punku'.

In both the languages of Hindi and Bengali, the people named it as 'Karanj' or 'Papar' or 'Kanji'. It is called 'Karum Tree' or 'Poonga Oil Tree' in English. It is an Indo-Malaysian species, a medium-sized evergreen tree, common on alluvial and coastal situations from India to fiji, from sea level to 1200 m. Now found in Australia, Florida, Hawaii, India, Malaysia, Oceania, Phillippines and Seychelles [1]. In the months of March and April, the 'Pongam Tree' stands as painted in crimson colour for a week or so as the buds develop into wilted, new leaves and just after the leaves begin to grow mature, the tree attains a gorgeous glowing lime-green colour. The 'Pongam Tree' is being cultivated in a large number of gardens and along the countless roads in India and is becoming the one of the most admired city trees [2].

It grows wild in the coastal forests throughout India and beside the streams and rivers. The 'Pongam Tree' is a medium-sized tree that grows rapidly. It contains a rough and grey-brown bark. The new leaves develop and the flower bloom in the great numbers almost simultaneously in this tree. They remain half hidden in the midst of the leaves. The blossoms are 1.3 cm in length and they mass along the ends of the long stems. These stems rise from the upper angle of the leaves. The flowers have a minute stem. They are loose and brown in colour and also bear a calyx that is shaped as cups.

There are five white petals as well as that are traced with the pink or violet colour [3]. The fruits of 'Pongam Tree' are some timber-like pods that grow about in length. They are dark grey in colour and get matured just before the next lot of new leaves appears. Each of the seeds of this tree is covered with a strong raft. The raft looks like a rubber ship. The ground underneath the tree always remains covered with a crackling carpet. The leaves of the 'Pongam Tree' have five, seven, or nine oval-shaped leaflets that have pointed tips [4].

The leaves are around 15 cm to 30 cm in the length and each of the leaflets is short stalked. The leaf stems and the flower stems are normally puffy at their bases. It is one of the few 'Nitrogen Fixing Trees' producing seeds containing 30-40% oil. The present review will possibly help to the bridge between traditional claims and modern therapy on Pongamia pinnata [5].

SYNONYMS

Pongamia glabra vent, Derris indica bennet, Pongam, Indian beech, Karum tree, Poonga oil tree.

MATERIALS AND METHODS:

Collection of Plant Material: The leaves of *Pongamia pinnata* linn (Family: Fabaceae) were collected from Bareilly District in the month of July 2010 and authenticated by Dr. Tariq Hussain, Head & Scientist Biodiversity & Angiosperm Taxonomy, National Botanical Research Institute (N.B.R.I) Lucknow, Uttar Pradesh, India, and accession number is 97841. The plant material was dried, powdered and stored in airtight containers until further studies.

FIG. 1: PLANT, FRUITS AND LEAVES OF *PONGAMIA PINNATA* LINN.

Morphological examination of *Pongamia pinnata* leaves is summarized in **table 1.**

TABLE 1: MORPHOLOGICAL EXAMINATION OF *PONGAMIA PINNATA* LEAVES

S. NO	Feature	Observation
1.	Colour (upper surface)	Glossy dark green
2.	Colour(lower surface)	Dull green
3..	Odour	Characteristic
4.	Shape	Ovate or elliptical
5.	Size	6.2 - 11.5 cm long and 3.9-8.3 cm wide
6.	Arrangement	Alternate
7.	Texture	Smooth

Preparation of Extract:

Preparation of *Pongamia pinnata* leaf extracts (pple): Fresh leaf was collected and air dried in shade at room temperature. Dried leaves were powdered mechanically through mesh sieve. 500 gm of freshly powdered leaves were evenly packed in Soxhlet's apparatus and the extraction was done with 70% ethanol. Then solvent was evaporated at low temperature under reduced pressure. In the preliminary phytochemical screening, the ethanolic extract of PPLE gave positive tests for glycosides, sterols, tannins, terpenoids and flavones. The residual extract was dissolved in sterile water and used for further investigation [6].

Proximate Analysis:

Determination of Total Ash Value: Accurately weighed 5 gms of powdered leaves of *Pongamia pinnata* Linn. (Fabaceae) was taken in a dried silica crucible. It was incinerated at temperature 450°C, until free from carbon and then cooled. The weight of total ash was taken and the percentage of it was calculated with reference to the air-dried sample.

Determination of Acid Insoluble Ash Value: The total ash obtained was boiled for 5 min with 25 ml of 2N HCl, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in tarred crucible cooled and the residue obtained was weighed. Finally the percentage of acid insoluble ash was calculated with reference to the air-dried drug (**Table 2**).

Determination of Water-Soluble Ash Value: The total ash obtained was boiled with 25 ml of water for few minutes. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 mins at temperature not exceeding 450°C. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug (**Table 2**).

TABLE 2: ASH VALUE OF *PONGAMIA PINNATA* LINN. LEAVES

Name of plant	Ash Value		
	Total Ash	Acid insoluble ash	Water soluble ash
***Pongamia pinnata* Linn.**	7.93 %	2.49 %	8.36 %

Determination of Alcohol Soluble Extractive Value: 5 gms of air dried, coarsely powdered leaves of *Pongamia pinnata* powdered was macerated with 100 ml of ethanol (70%) in a Soxhlet's apparatus for 10 days, shaking frequently during the first 6 hrs and was allowed to stand for 18 hrs Then, it was filtered rapidly and precautions were taken against loss of alcohol.

25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentages of alcohol soluble extracts were calculated with reference to the air-dried drug. The procedure followed as above using chloroform water instead of alcohol.

TABLE 3: EXTRACTIVE VALUE OF PONGAMIA PINNATA LINN. LEAVES

Name of plant	Extractive values (Percentage w/w)		
	Ethanol soluble extractive value	Water soluble extractive value	Petroleum ether extractive value
***Pongamia pinnata* Linn.**	12 %	23 %	43 %

Determination of Moisture Content : Accurately weighed 5 gms of powdered leaves *Pongamia pinnata* Linn. was taken in a china dish. It was kept for 30 mins in a hot air oven at 105 - 110°C. The percentage of moisture content was then calculated with reference to the air-dried drug at different times.

Preliminary Phytochemical Screening: The powdered leaves were subjected to systematic phytochemical screening by successively extracting them in different solvents and testing for the presence of chemical constituents.

Qualitative chemical examination of extracts:

Detection of Alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloid reagents.

Meyer's Test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of brown or reddish brown precipitate indicated the presence of alkaloids.

Detection of Flavonoids:

Lead Acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Shinoda Test: The extracts were treated with few fragments of magnesium metal separately followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta colour indicated the presence of flavonoids.

Detection of Proteins and Amino Acids:

Millons Test: The tracts were treated with 2 ml of Millons reagent. The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.

Biuret Test: The extract were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet colour indicated the presence of proteins.

Ninhydrin Test: To the extracts 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicated presence of amino acid.

Detection of Glycosides:

Modified Borntrager's Test: The extracts were treated with ferric chloride solution and heated on a boiling water bath for about 5 mins. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. The formation of rose pink or cherry red colour in the ammonical layer indicated the presence of anthranol glycoside.

Legal's Test: The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red colour indicated the presence of cardiac glycosides.

Baljet Test: The extract of drug was treated with sodium picrate and the formation of a yellowish orange colour confirmed the presence of cardiac glycosides.

Liebermann-Burchard's Test: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The form of brown or pink coloured rings at the junction confirmed the presence of steroidal or triterpenoids, saponins and glycosides respectively.

Keller Killani Test: 0.5 g of dried extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solutions. This was then under laid with 1 ml of concentrated H_2SO_4. A brown ring obtained at the junction of two liquids indicates the presence of a deoxysugars.

Detection of Saponins:

Froth's Test: The extracts (alcoholic and aqueous) were diluted with 20 ml of distilled water separately and further shaken for 15 min in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.

Detection of Phytosterols:

Liebermann-Burchard's Test: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride boiled and cooled concentrated sulphuric acid was added through the sides of the test tube. The formation of brown coloured ring at the junction of two liquids confirmed the presence of steroids.

Detection of Phenolic Compounds and Tannins:

Ferric Chloride Test: The extract was treated with few drops of neutral ferric chloride solution (5%). The formation of bluish black colour indicated the presence of phenolic compounds.

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicated the presence of tannins.

Lead Acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Alkaline Reagent Test: The extract was treated with few drops of sodium hydroxide separately. Formation of intense yellow colour, which turned colourless on addition of few drops of dilute acid, indicated the presence of flavonoids.

Vanillin in Hydrochloric Acid Test: The extracts were treated with few drops of vanillin hydrochloride reagent. The formation of pinkish red colour indicated the presence of tannins.

TABLE 4: PHYTOCHEMICAL TESTS OF ETHANOLIC EXTRACT OF *PONGAMIA PINNATA* LINN.

TESTS	ETHANOLIC EXTRACT
Alkaloids	
a) Mayer's test	+ve
b) Wagner's test	+ve
c) Hagner's test	+ve
Carbohydrates	
a) Molisch test	+ve
b) Fehling test	+ve
c) Benedict test	+ve
Flavonoids	
a) Shinoda test	+ve
b) Lead acetate solution	+ve
Protein	
a) Biuret test	-ve
b) Millions test	-ve
Steroids	
a) Salkowski's test	-ve

Amino acid		
a)	Ninhydrin test	+ve
b)	Tyrosine test	+ve
Glycosides		
a)	Keller Killani test	+ve
b)	Borntrager test	+ve
c)	Legal test	+ve
Tannins/Phenolic Compounds		
a)	5% $FeCl_3$ test	+ve
b)	Iodine test	+ve
Terpenoids		
a)	Liebermann Burchard's Reaction	+ve
b)	Salkowski's test	+ve

("+" means present, "-" means absent)

Thin layer chromatography:

Preparation of plates: Silica gel G was used as the adsorbent. Slurry of it was prepared with distilled water in a glass pestle mortar. The slurry was poured on the clean and dry glass plates and spread on the plate as a uniform coating using a glass rod. These plates were then placed on a levelled surface in the horizontal position and allowed to air dry for 20-25 minutes.

Activation of plates: When the plates were dried they were placed in an oven, maintained at 1100 C for 30 minutes. The prepared plates were stored in a closed desiccated cabinet and removed only when required for use.

Preparation of Samples: About 100 mg of test material was dissolved in 10 ml of the respective solvent and was used for the TLC studies.

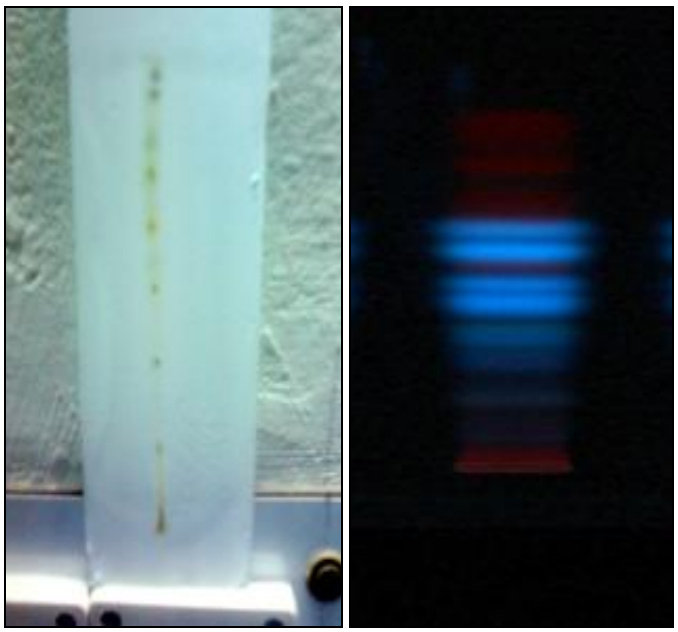
Application of Spots: The spots were applied on the activated plate at a distance of 2 cm from one end of the plate and 3 cm from each other with the help of a fine capillary tube or diameter less than 1 mm. The solvent was removed from spot by air-drying. The position of the origin was marked.

Development of Chromatograms: Chromatograms were developed by one way ascending TLC. The plate carrying spots was placed squarely in the developing chamber and the lid was replaced as quickly as possible to minimize disturbance of the solvent saturated atmosphere. The developing solvent was allowed to travel up the plate until it reached the desired level (10 to 15 cm). The plate was then removed from the chamber; solvent front was marked and dried in air at room temperature [7].

Detection of Spots: The number and position of the various constituents present in the mixtures was determined by spraying the plate with the 1% vanillin in sulphuric acid and the plate was heated at 110^{o}C for 10 minutes and the spots were marked. R_f value was calculated for well [8, 9.]

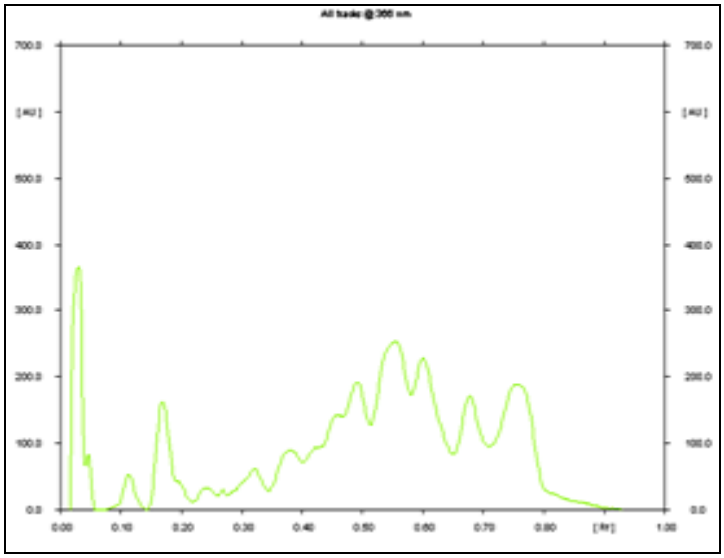
Observation: The ethanolic extract of *Pongamia pinnata* leaves showed the best result in the solvent system Toluene: Ethyl Acetate (70:30) and gave 8 spots. Their R_f values were 0.05, 0.09, 0.15, 0.21, 0.61, 0.72, 0.75 and 0.79.

High Performance Thin Layer Chromatography (HPTLC): High performance thin layer chromatography was carried out by National Botanical Research Institute, Lucknow (U.P.). It also gives seven numbers of peaks. The analysis results are shown in **Fig. 2 and 3**:



(A) **(B)**

FIGURE 2: (A) TLC CHROMATOGRAM OF ETHANOLIC EXTRACT OF *PONGAMIA PINNATA* LINN. (B) HPTLC CHROMATOGRAM OF ETHANOLIC EXTRACT OF *PONGAMIA PINNATA* LINN. ON THE UNDER 366 nm



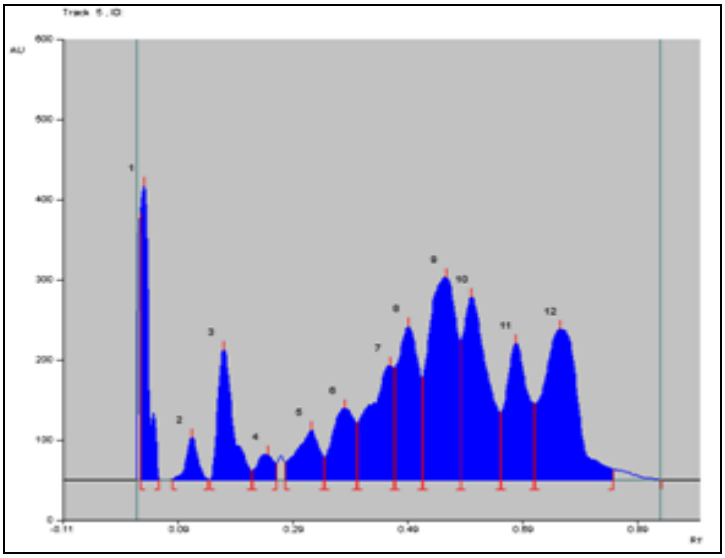


FIG. 3: HPTLC PEAKS OF ETHANOLIC EXTRACT OF *PONGAMIA PINNATA LINN.*

Sample- 2 mg/ml; **Application-** Linomat 5 Applicator (Camag); **Volume applied**-2 µl; **Solvent System-** Toluene : Ethyl Acetate (70 : 30); **TLC plate Development-** Pre-saturated Camag Twin Trough Chamber

TABLE 5: HPTLC PEAKS OF ETHANOLIC EXTRACT OF *PONGAMIA PINNATA* LINN. R_F VALUE

Peak	Start Position	Start Height	Max. Position	Max. Height	Max %	End Position	End Height	Area	Area %	Assigned Substance
1.	0.02 Rf	332.4 AU	0.03 Rf	366.6 AU	18.89%	0.06 Rf	1.7 AU	4175.3 AU	7.19%	Unknown*
2.	0.08 Rf	1.6 AU	0.11 Rf	52.7 AU	2.72%	o.14Rf	0.2 AU	877.5 AU	1.51%	Unknown*
3.	0.14 Rf	0.1 AU	0.17 Rf	162.7 AU	8.38%	0.22 Rf	11.2 AU	3523.6 AU	6.07%	Unknown*
4.	0.22 Rf	11.5 AU	0.24 Rf	32.2 AU	1.66%	0.26 Rf	21.1 AU	764.9 AU	1.32%	Unknown*
5.	0.28 Rf	22.4 AU	0.32 Rf	62.3 AU	3.21%	0.34 Rf	28.3 AU	2049.7 AU	3.53%	Unknown*
6.	0.34 Rf	28.4 AU	0.38 Rf	89.4 AU	4.61%	0.40 Rf	71.2 AU	2851.8 AU	4.91%	Unknown*
7.	0.40 Rf	71.5 AU	0.46 Rf	142.7 AU	7.36%	0.47 Rf	40.0 AU	5030.7 AU	8.67%	Unknown*
8.	0.47 Rf	140.2 AU	0.49 Rf	191.5 AU	9.87%	0.51 Rf	28.0 AU	5699.1 AU	9.82%	Unknown*
9.	0.52 Rf	128.8 AU	0.56 Rf	253.1 AU	13.04%	0.58 Rf	72.9 AU	10140.7AU	17.47%	Unknown*
10.	0.58 Rf	173.0 AU	0.60 Rf	228.2 AU	11.76%	0.65 Rf	94.0 AU	8106.2 AU	13.96%	Unknown*
11.	0.65 Rf	84.1 AU	0.68 Rf	170.8 AU	8.80%	0.71 Rf	94.8 AU	5407.8 AU	9.31%	Unknown*
12.	0.71 Rf	95.0 AU	0.75 Rf	188.5 AU	9.71%	0.85 Rf	12.8 AU	9430.7 AU	16.24%	Unknown*

RESULT AND DISCUSSION: Phytochemical evaluation of ethanolic extract of *Pongamia pinnata* Linn. showed the presence of flavonoids, alkaloids, carbohydrate, proteins, terpenoids and glycoside compounds. Best result of Thin Layer Chromatography in the solvent system Toluene: Ethyl acetate (70:30) was found in which 8 spots were present with R_f values were 0.05, 0.09, 0.15, 0.21, 0.61, 0.72, 0.75 and 0.79.

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