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PHYTOCHEMICAL, PHARMACOGNOSTICAL AND QUANTITATIVE ESTIMATION OF *PONGAMIA PINNATA* LEAVES EXTRACT-A PRELIMINARY STUDY TO IDENTIFIED PHYTOCONSTITUENTS

S. K. Sarje[1*], Shagufta Farooqui[1], Shinde Punam[1], Sontakke Shital[1] and Narote Mayur[1]

[1]Department of Pharmacology, Nanded Pharmacy College, Nanded, Maharashtra.

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***Corresponding Author**
S. K. Sarje
Department of Pharmacology, Nanded Pharmacy College, Nanded, Maharashtra.

ABSTRACT

Objective: The objective of this study was to carry out phytochemical and pharmacognostic and quantitative evaluation of leaves of *Pongamia pinnata* L. (Fabacea). **Method:** The present study provides pharmacognostic, phytochemical and quantitative details of the leaves of *P. pinnata*. **Results:** The macroscopic study showed that the leaf was ovate or elliptic with smooth margins, short petiole, alternate imparipinnate, hairless, acuminate at apex, rounded to cuneate at base and slightly thickened. Microscopic study revealed collateral, closed vascular bundles, trichomes, paracytic stomata, xylem vessels and prismatic calcium oxalate crystals. Qualitative Phytochemical screening showed the presence of alkaloids, glycosides, carbohydrates, steroids and flavonoids and phenolic compounds in both the extracts. Total Poly phenol content & total flavonoid content was determined by Folin Ciocaltue & Aluminium trichloride method respectively by using UV-Visible spectrophotometer. DPPH scavenging assay were performed to evaluate the antioxidant activity which was found maximum at 125 µg/ml concentration for both the extracts. **Conclusions:** The results of this study can serve as valuable source of information for identification of this plant for future investigation and applications.

INTRODUCTION

Plants have been the foundation of traditional medicine system throughout the world and continue to nurture mankind with new remedies. The research pertaining to medicinal plants is rapidly increasing at national and international levels.[1] Further investigation of traditional systems of medicine with emphasis on safety, efficacy and quality will help to rationalize the

use of natural products in healthcare.[2] In the Indian systems of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and an understanding of the medicinal components and their effects. Pharmacognosy is the study of medicines derived from plants and it is the preliminary step in the standardisation of crude drugs. Authentication and standardisation are prerequisite steps for herbal drugs and their formulations in traditional systems of medicine.[3]

Phytochemical are compounds that occur naturally in plants. They contribute to the color, flavor and smell of plants. Phytochemical are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids.[4] Phytochemical can act as agents to prevent undesirable side effects of the main active principle.[5] The capacity of flavonoids to act as antioxidant depends upon their molecular structure.[6] The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities.[7]

Pongamia pinnata L. belongs to family Fabacea. It is native to India and widely distributed along Southeast Asia to the West Pacific and northern Australia.[8] It is also called *Deris indica, Pongamia glabra* and *Milletia pinnata*. The plant has been documented in Ayurveda, an alternative medicine in India. All parts of this plant are used in the treatment of abscess, bronchitis, diarrhea, itches, piles, skin diseases, tumors, painful rheumatic joints, ulcers, whooping cough quench dipsia in diabetes, blood purifier and as an antiseptic to treat wounds and cuts.[9] Some of the reported activities of this plant include antioxidant, antimicrobial, anti-inflammatory, antiulcer, antihyperglycemic amongst others.[10-12]

MATERIALS AND METHODS

Plant Collection and Extraction

On the basis of literature survey leaves of ***Pongamia Pinnata pierre*** **Linn (Fabaceae)** was collected from the Local region of Nanded. The Morphological study identification is carried out and further processed for authentication. The authentication was done by Dr. S. S. Bodke, HOD, Dept. of Botany, Yeshwant Mahavidyalaya, Nanded. Authentication of plant *Pongamia pinnata* was done specimen. No.NPC/Herberium/2018-2019. H- 06 is allotted to the plant.

Collection, authentication, Identification, processing and storage has been done according to standard procedure for the plant material. According to the literature survey & nature of phytochemicals present in drug, the extraction method was selected.

Fig no.1: Leaves and dry powder of *Pongamia pinnata.*

Preparation of plant extract

Pongamia Pinnata Leaves were shade dried, leaned and pulverized by hands made to obtain coarse powder of mesh size #40. Coarse powder (1000 g) of *Pongamia Pinnata Leaves* was exhaustively defatted using petroleum ether (60-80 ºC) (PP-PE) and extracted successively with Ethyl Acetate (PP-EA) and ethanol (PP-ET) using Soxhlet apparatus. All the extracts were collected, filtered through whatman filter paper, concentrated and stored in tight desiccator and percentage yield was calculated.

PHARMACOGNOSTIC EVALUATION OF PLANT MATERIAL

Macroscopic characteristics

Visual observation provides the simplest and quickest means to recognize identity and possibly also the quality of the plant material. Various macroscopic characters of fresh leaves including shape, venation, margin, presence or absence of petiole were recorded.

Microscopic characteristics

The microscopic evaluation was performed by taking free hand sections of fresh leaves and staining with safranine to confirm lignifications. Various identifying characters such as trichomes and cell composition were recorded and then pictomicrography studies were undertaken. Powder microscopy of the dried leaf powder was studied under microscope. The characteristic structures and cell components were observed and their photographs were taken.

Development of TLC Fingerprints Profile of The Extracts

All the extracts of selected plant material were subjected to TLC studies using various solvent systems to determine the presence of various phytoconstituents. The R_f values of observed compounds were noted for all extracts. The characteristic fingerprint of the various chemical constituents in each extract under UV light and after derivatization with suitable reagents was recorded.

Preliminary phytochemical screening revealed the presence of carbohydrate, proteins flavonoids, alkaloids, fixed oils, &steroids. Compounds of varying polarity in the extracts well separated using various solvent systems on TLC. R_f value of the separated compounds were recorded. (The Indian Pharmacopoeia, 2007).

PHYTOCHEMICAL EVALUATION

Determination of Total Poly-phenolic Content

The total phenolic content of *Pongamia pinnata* leaves extracts was determined by using Folin- Ciocalteu method. A gallic acid calibration curve was designed by preparing the aliquot of (20, 40, 60, 80, and 100µg/ml) in distilled water from standard 1 ml solution of gallic acid was added to 10 ml volumetric flask in methanol i.e.100 µg/ml. Reagent blank using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 5 ml of 10% Na2CO3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 765 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic acid Equivalents (mgGAE/g). All the experiments were performed in triplicate.[13]

Determination of Total flavonoid content

The total flavonoid content of *Pongamia Pinnata* extract was determined by the aluminium chloride colorimetric method. Crude extract (1 mg/ml) were made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% $NaNO_2$ solution; 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation and the mixture was allowed to stand for 6 min. Then, 2 ml of 1M NaOH solution were added and the final volume of the mixture was brought to 10 ml with distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight.[14]

***In vitro* Anti-Oxidant Activity**

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. The antioxidant activity of *Pongamia pinnata* was determined by DPPH free radical scavenging assay method.[15]

Preparation of test solution: *Pongamia pinnata* extracts (Ethyl acetate and Ethanolic) 50mg was separately dissolved in 50ml of methanol from which different concentration of 25µg/ml, 50µg/ ml, 75µg/ml, 100µg/ml, 125µg/ml were prepared.[16]

Preparation of Standard solution: Standard i.e. rutin ascorbic acid, gallic acid(1mg) was dissolved in 1ml of methanol from which different concentration of, 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml125µg/ml were prepared.

METHODS

2, 2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) Activity

Principle

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 2, 2-diphenyl-1-picrylhydrazyl.

Procedure

Different concentrations (25, 50, 75,100,125µg/ml) of both the extracts were prepared with methanol and 1ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 30 min. After 30 min, the absorbance of the mixtures was measured at 517 nm. 1ml of DPPH and 1 ml of methanol was taken as control.[17-18]

The percentage of inhibition can be calculated using the formula:

$$(\%)\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$: Absorbance of control.

A_{test}: Absorbance of test.

RESULTS

Macroscopy of leaf

The macroscopic study involved visual examination of the morphological characters of the leaves. The fresh leaves of *P. pinnata* were glossy dark green above and dull green with prominent veins beneath when mature. The size of leaves varied from 6- 16.5 cm in length and 4-8.3 cm in breadth. The leaf was ovate or elliptic with smooth margins, short petiole, alternate imparipinnate, hairless, acuminate at apex, rounded to cuneate at base and slightly thickened.

Microscopy of Leaf

The transverse section of *Pongamia. pinnata* leaf showed the presence of single layered epidermis, consisting of tubular cells. The epidermis was covered with a single layer of cuticle. Epidermis was followed by 3-4 layers of collenchymatous hypodermis. The vascular bundle was surrounded by 4-6 layers of cortex. Cortex consisted of oval shaped parenchymatous cells. Pericyle can be observed in the form of sclerenchymatous sheath. Vascular bundles were collateral, closed and arranged in discontinuous ring. Xylem was lignified, phloem was non-lignified. In the central portion, compacted parenchymatous pith was present. Prismatic crystals of calcium oxalate, pointed trichomes and paracytic stomata were found. Thus the salient diagnostic features of leaf were collateral, closed vascular bundle, paracytic stomata, xylem vessels and prismatic calcium oxalate crystals**.**

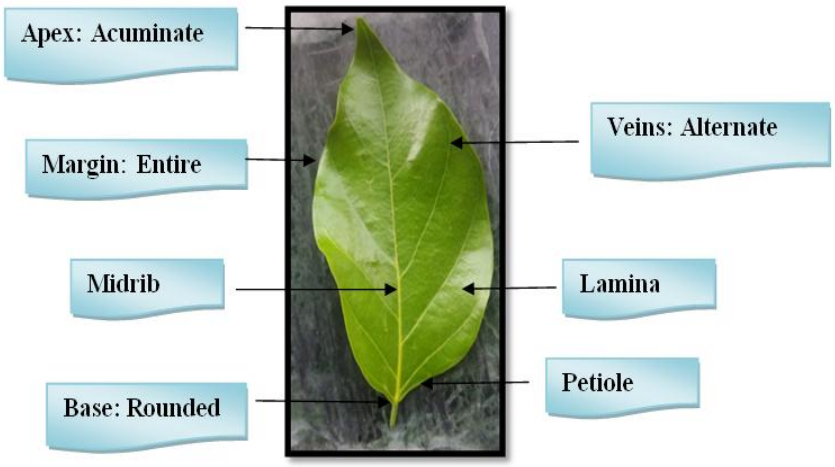


Fig. 2: Macroscopy of leaf.

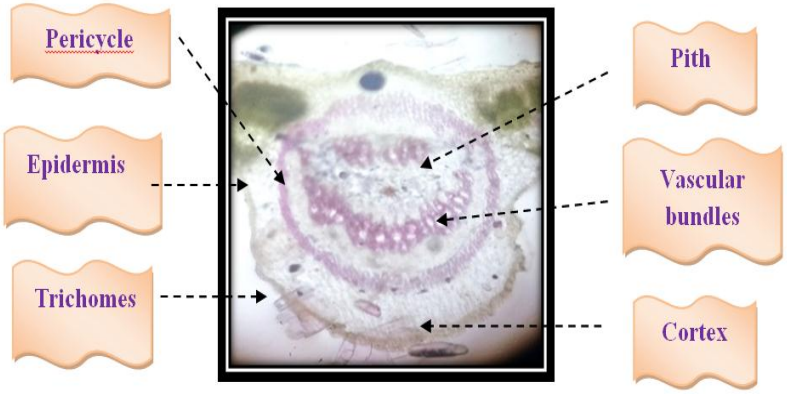


Fig no.3: Transverse section of *Pongamia Pinnata* leaves.

Phytochemical screening of *Pongamia pinnata* extract showed positive test constituents like alkaloids, carbohydrate, flavonoids, phenols, protein, and tannin (table no.1). Preliminary phytochemical evaluation of ethyl acetate and Ethanolic extracts was carried out for the determination of presence of phytoconstituents along with TLC fingerprinting both the extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, phenolic, flavonoids, and saponins. The spots at R_f values (ethyl acetate extract) 0.07, 0.22, 0.40, 0.44, 0.66, 0.75, 0.79, 0.93 and (Ethanolic extract) 0.24, 0.42, 0.56, 0.66, 0.86 represents the presence of kemferol, rutin, Flavanone, 6-hydroxy flavones, Flavone in the extracts. 0.09, 0.15, 0.28, 0.38, 0.44, 0.46, 0.68, 0.76, 0.77, 0.92. (table no.2).

As depicted in observation table-3 reveals that Petroleum ether, Ethyl acetate and Ethanol have Phenolic content as 17.87 (mg GAE/g DW), 46.87 (mg GAE/g DW), 48.48 (mg GAE/g DW) respectively. Ethanolic extract shows more phenolic content than Petroleum ether and Ethyl acetate as per comparative evaluation of phenolic content of extracts.

As depicted in observation table- 4 reveals that Petroleum ether, Ethyl acetate and Ethanol have Flavonoid content as 42.92(mg QE/g DW), 62.92 (mg QE/g DW), 69.51 (mg QE/g DW) respectively. Ethanol extract shows more Flavonoid content than Petroleum ether and Ethyl acetate as per comparative evaluation of Flavonoid content of extracts.

In DPPH Scavenging assay method, the % inhibition of Ethyl acetate and Ethanolic leaf extracts *Pongamia pinnata* at 517 nm has been recorded at different concentration of 25µg/ml, 50µg/ml and 75 µg/ml 100µg/ml 125g/ml respectively. The results were compared with Ascorbic acid as a reference standard and both extracts showed very significant % inhibition close to reference standard. Among both extracts, Ethanolic extract exhibited highest DPPH radical scavenging activity with 89% at 125µg/ml concentration (which is nearly close to the value of Ascorbic acid i.e.). In overall comparison, Ethanolic leaf extracts of *Pongamia pinnata* shows highest % inhibition activity (89% at 125µg/ml concentration) followed by Ethyl acetate extract (87.7 at 150µg/ml concentration).

Table 1: Phytochemical evaluation *Pongamia pinnata* of leaves extracts.

Sr no.	Chemical test	Pet ether	Ethyl acetate	Ethanol
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Proteins	+	+	+
4	Glycosides	+	+	+
5	Saponins	-	+	+
6	Amino acids	-	-	+
7	Steroids	-	+	+
8	Phenolic compounds	+	+	+
9	Flavonoids	-	+	+

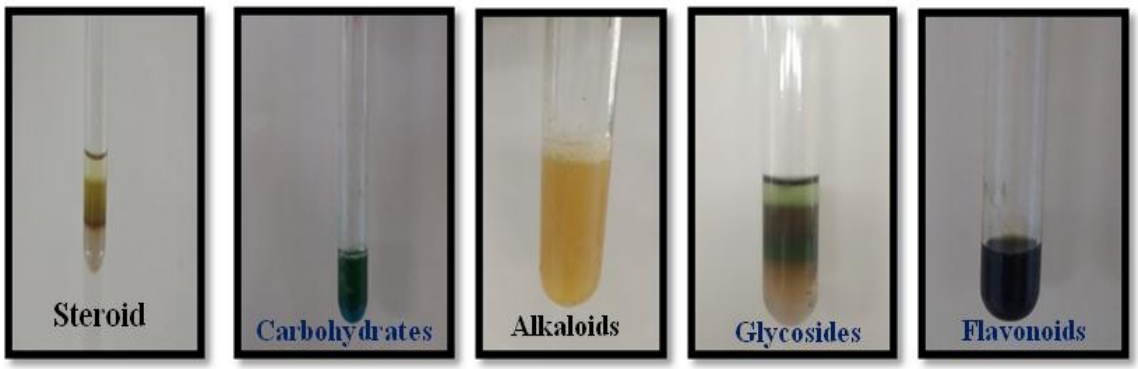
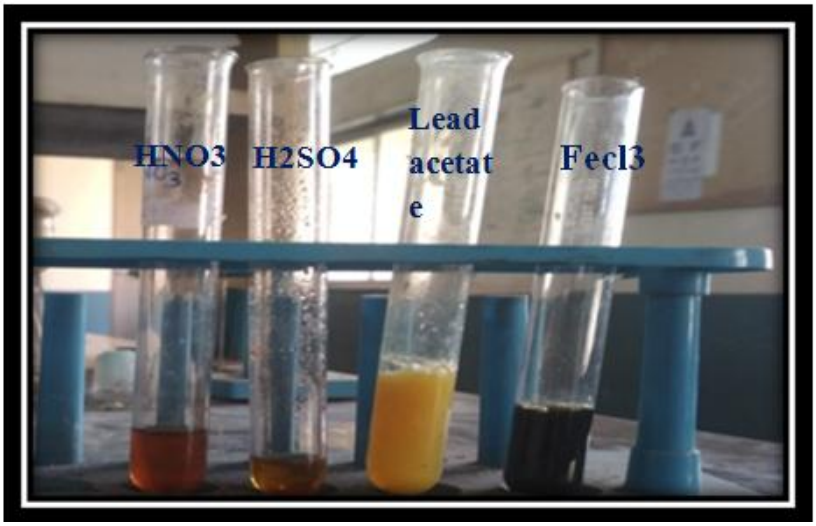


Fig no.4: Chemical tests of plant extracts.



Figno.5: Phenolic compounds.

Table 2: TLC Analysis of *Pongamia pinnata* leaves Extracts.

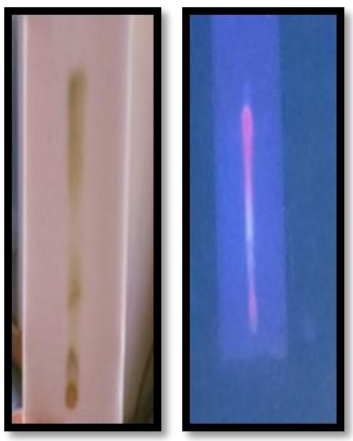
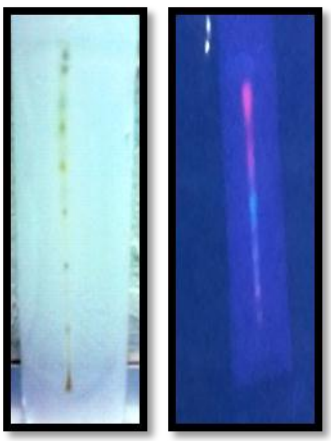
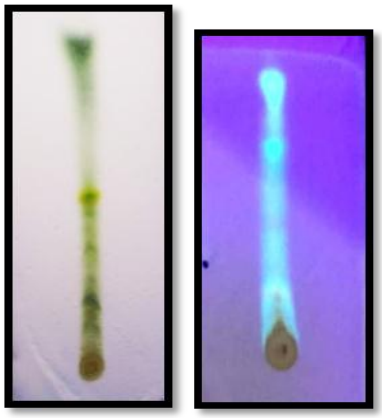
	Pet ether	**Ethyl acetate**	**Ethanol**
Solvent systems	Benzene:4 Chloroform:1 Ethanol:1 	Benzene: 7 chloroform:2 ethanol:1 	Benzene:6 chloroform:1 Ethanol:2 formic acid:1 
R_f Values	0.04,0.52,0.72,0.82	0.07,0.22,0.40,0.44 0.66,0.75,0.79,0.93	0.09,0.15,0.28,0.38,0.44, 0.46,0.68,0.76 0.77,0.92

Table 3: Total phenolic content of *Pongamia pinnata* leaves extracts.

Sr. no	**Extracts**	**Concentration (µg/ml)**	**Absorbance**	**TPC mg/g of GAE**
1	Pet ether	100	0.059	17.87±0.20
2	Ethyl acetate	100	0.154	46.87±0.37
3	Ethanolic	100	0.160	48.48±0.24

(N=3) Note: GAE/g DW denotes Gallic Acid Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, Ethyl acetate and Ethanol have Phenolic content as 17.87 (mg GAE/g DW), 46.87 (mg GAE/g DW), 48.48 (mg GAE/g DW) respectively. Ethanolic extract shows more phenolic content than Petroleum ether and Ethyl acetate as per comparative evaluation of phenolic content of extracts.

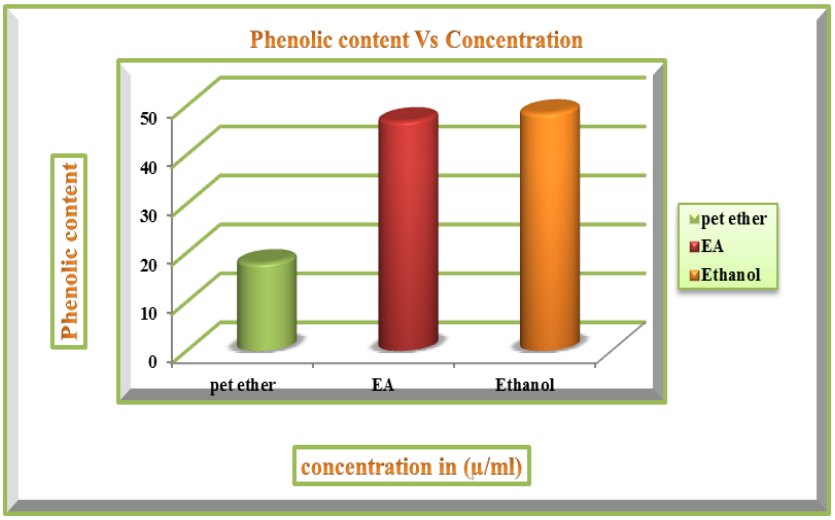


Chart no.1: Total Phenolic Content of *Pongamia pinnata* leaf extracts.

Table 4: Results of Total Flavonoid content.

Sr. no	Extracts	Concentration (µg/ml)	Absorbance	TFC mg/g of Rutin
1	Pet. ether	100	0.176	42.92±0.10
2	EA. Extract	100	0.2850	62.92±0.24
3	Eth. Extract	100	0.2580	69.51±0.22

(N=3) Note: RU/g DW denotes Rutin Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, Ethyl acetate and Ethanol have Flavonoid content as 42.92(mg QE/g DW), 62.92 (mg QE/g DW), 69.51 (mg QE/g DW) respectively. Ethanol extract shows more Flavonoid content than Petroleum ether and Ethyl acetate as per comparative evaluation of Flavonoid content of extracts.

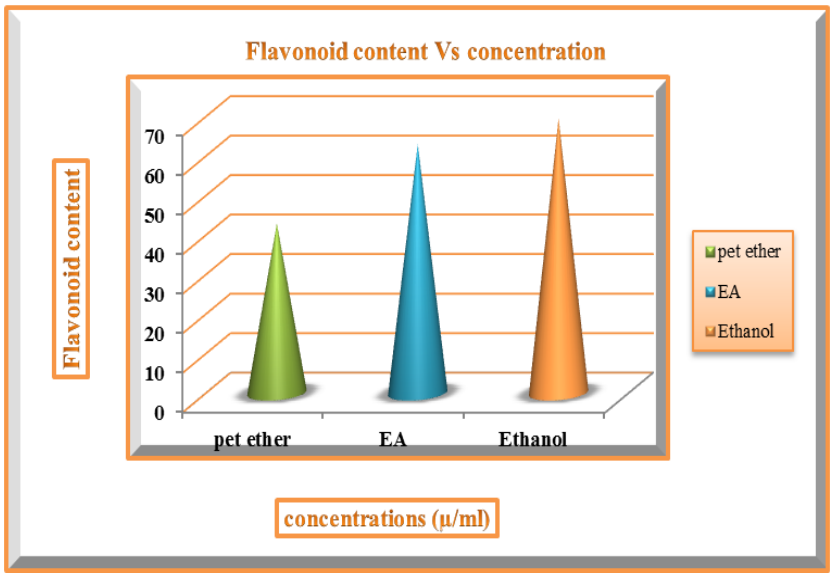
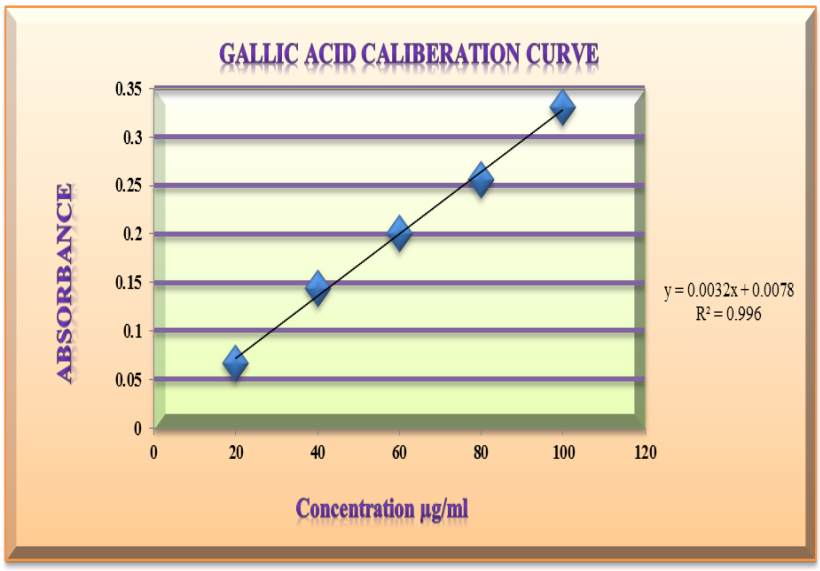
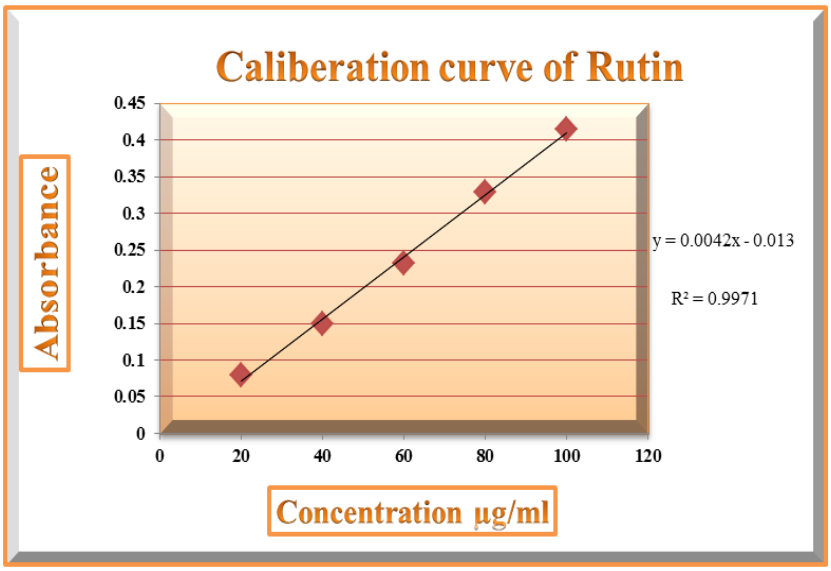


Chart 2: Total Flavonoid Content of *Pongamia pinnata* leaf extracts.



Graph no.1: Calibration Curve of Gallic acid.



Graph no. 2: Calibration Curve of Rutin.

Table 5: DPPH (2, 2-Dipheny1, 1-Picrylhydrazyl) radical scavenging activity of standard.

Sr no.	Conc	Absorbance of rutin	%inhibition of Rutin	Absorbance of ascorbic acid	%inhibition of ascorbic acid	Absorbance of gallic acid	%inhibition of gallic acid
1	25	0.285	41.487	0.182	62	0.287	41
2	50	0.236	51.54	0.129	73	0.224	54
3	75	0.124	74.53	0.088	81	0.116	76.18
4	100	0.102	79.21	0.059	87	0.093	80.9
5	125	0.032	93.42	0.024	95	0.043	90.3

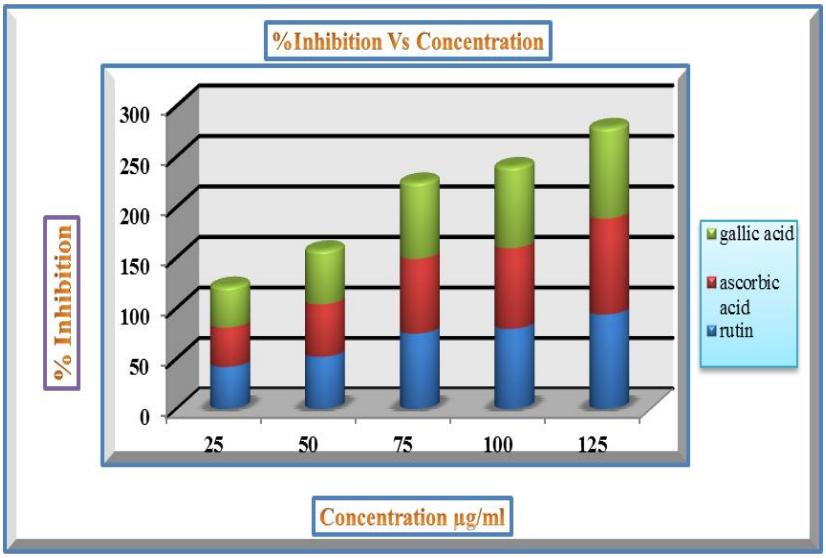
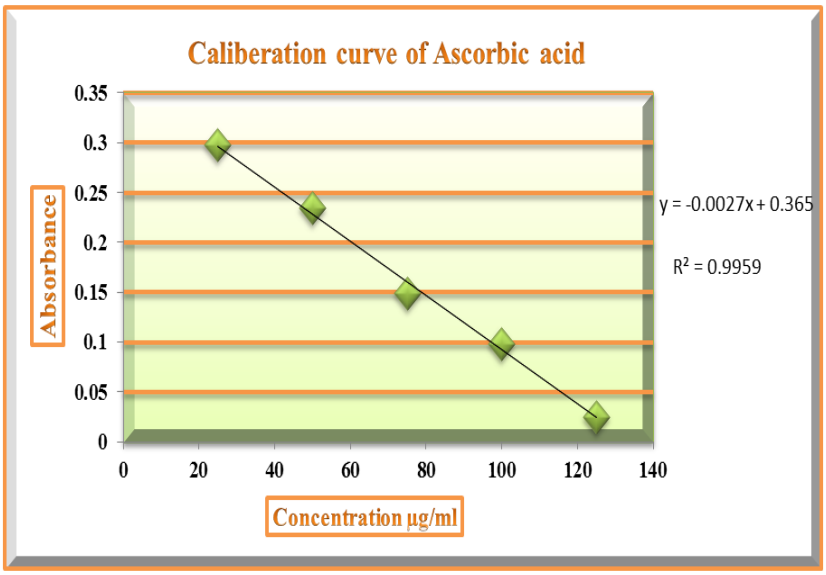


Chart No.3: Effect of % inhibition of standard drugs.



Graph no.3: Calibration curve of Ascorbic acid.

Table 6: Comparative DPPH Scavenging assay method of *Pongamia pinnata* (Ethyl acetate and Ethanolic) leaf extracts.

Sr. no.	Concentration (µg/ml)	Ascorbic acid (%inhibition)	Ethyl acetate extract (%inhibition)	Ethanolic extract (%inhibition)
1	25	62±0.316	59.7±0.14	60.77±0.2
2	50	73±0.54	69.3±0.27	71.45±0.15
3	75	81±0.31	78.02±0.26	80.69±0.16
4	100	87±0.316	84.98±0.67	86.23±0.19
5	125	95±0.54	87.67±1.4	89.11±0.21

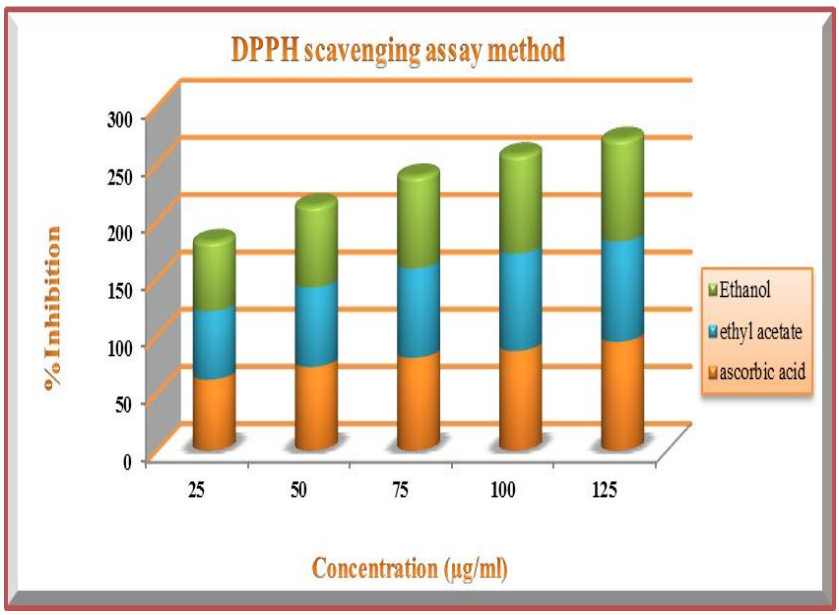


Chart no.4: DPPH scavenging activity of *Pongamia pinnata* leaf extracts.

DISCUSSION

Preliminary phytochemical evaluation of ethyl acetate and Ethanolic extracts was carried out for the determination of presence of phytoconstituents along with TLC fingerprinting. Both the extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, phenolic, flavonoids, and saponins. Ethanolic extract shows more phenolic and flavonoid content than Petroleum ether and Ethyl acetate extracts.

Antioxidant property of *Pongamia pinnata* leaves extracts was carried out by using DPPH radical scavenging assay technique. This provides evidence that Ethanolic extract of *Pongamia pinnata* leaves has potent antioxidant activity and it can be used as an antioxidant agent.

CONCLUSION

Different extracts of *Pongamia pinnata* leaves showd presence of various phytoconstituents such as alkaloids, glycoside, flavonids, tannis and phenolic compounds.

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