



**PHYTOCHEMICAL INVESTIGATION AND IN-VITRO ANTHELMINTIC ACTIVITY
OF *PONGAMIA PINNATA***

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ABSTRACT

Many herbal extractions individual or combination have been recommended in various medical values for the cure of different diseases. The present research work deals with phytochemical investigation and anthelmintic activity of *Pongamia pinnata* bark. Standardization of crude drug extracted from plants play an important role in identifying the quality and purity of drugs. Phytochemical constituents are extracted by petroleum ether, acetone, chloroform and ethanol from *Pongamia pinnata* belongs to the family *Fabaceae*. This research gave information that ethanolic extracts of *Pongamia pinnata* bark had the highest number of phytochemicals compared to other solvent extracts. Hence, ethanolic extracts of *Pongamia pinnata* holds the greatest potential to treat various human diseases and has profound medical applicability. In-vitro anthelmintic activity was determined by taking adult Indian earthworms, *Pheretima posthuma* having anatomical and physiological resemblance with intestinal roundworms. These earthworms were washed in normal saline solution before they were used. To observe anthelmintic activity, all the investigations were carried out by ethanolic extract with different concentrations of 10, 25, 50mg/ml, significant activity like time of paralysis and time of death were reported. At highest concentrations of 50mg/ml, a significant anthelmintic activity was observed and compared with piperazine citrate as standard reference with difference concentrations 10, 25, 50mg/ml and distilled water as control. Herbal drugs and synthetic drugs were equally effective in helminthic infestations but ethanolic extract of *Pongamia pinnata* bark exhibits potentiality and have maximum anthelmintic activity.

KEYWORDS: *Pongamia pinnata* bark, Phytochemical investigation, Piperazine citrate, *Pheretima posthuma*.

INTRODUCTION

In developing countries, major people are depends on traditional practitioners and using medicinal plant products in order to get their health in good conditions.^[1,2] Many herbal extractions individual or combination have been recommended in various medical values for the cure of different diseases. The usage of medicinal products and supplements has increased exceedingly over the past years, not less than 75% of world population are depends on medicinal plant products for their primary health. *Pongamia pinnata* belongs to family *Fabaceae*, native to eastern and tropical Asia also found in Southeast Asia, Australia, and Pacific islands, distributed in the greater part of India. In the traditional systems of medicines, the *Pongamia pinnata* plant was used for anti inflammatory, anti plasmodial, anti hyperglycaemics, anti diarrhoeal, anti ulcer, anti hyperammonic and antioxidant.^[3-6] In Ayurvedic medicines, *Pongamia pinnata* plant root and bark having anthelmintic activity and useful in abdominal enlargement, biliousness, eye, skin itching, piles, ulcers, wounds, the sprouts, digestive and laxative,

piles and wounds. Various phytochemical constituents with medicinal properties are used to cure various health illnesses have been revealed every day by researchers.^[7-13] Helminthiasis is one of the major and common diseases that widespread to human beings, animals due to poor management. To treat parasitic manifestations in human and animals, a large number of medicinal plants are being used. Parasitic infections are common in the tropical countries that infect humans. Bioactive plant metabolites are cheap, cost, effective and easily affordable drugs against parasitic infections. On adult Indian earthworms, *Pheretima posthuma*, the anthelmintic activity was observed due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. *Pheretima posthuma* were used for evaluation of in vitro anthelmintic activity because of their easy availability, the present research work deals with evaluation of phytochemical screening and of anthelmintic activity of *Pongamia pinnata* bark.

MATERIAL AND METHODS

Collection of the Plant Material

Pongamia pinnata bark was collected from the college garden, Mother Teresa Pharmacy College, Sathupally, Khammam, Telangana.

Preparation of *Pongamia pinnata* bark powder

Pongamia pinnata plant barks are collected and naturally dried to prevent it from direct sunlight impact to minimize undesirable chemical reactions of plant metabolites. Drying of plant product is crucial to prevent the formation of artifacts as a result of microbial fermentation and subsequent degradation of the plant metabolites. Hence for the present research, barks are dried in shade and then powdered in a mechanical grinder. The bark powder was passing through sieve number 44 were taken for extraction and stored in an airtight container for further studies.

Preliminary Phytochemical Screening Preparation of Plant Extract

The finely grounded bark powder was placed in a filter paper bag and placed in soxhlet apparatus. The solvent is heated to get evaporation, vapour moves into a distillation arm, and floods into the chamber housing the thimble of bark powder. The condensed solvent dipped into the filter paper bag containing the bark powder. The compound dissolved in the solvent, when the chamber containing the leave powder slowly fills with condensed solvent. When the Soxhlet chamber was filled, the chamber is emptied by the siphon. The solvent is returned to the round bottom flask. The solvent movement does not transport any powdered material to the still round bottom flask. This cycle was repeated for many times. Different solvents like petroleum ether, acetone, chloroform and ethanol are used for extraction of *Pongamia pinnata*.

Phytochemical Investigation

The phytochemical investigation was performed regarding the presence of different compounds possessing therapeutic effects. The different solvent extracts (petroleum ether, acetone, chloroform and ethanol) of *Pongamia pinnata* bark powder was used for screening the presence of carbohydrate, glycosides, alkaloids, flavonoids, steroids, tannins, saponins, phenolic compounds, protein, and triterpenoids according to the standard procedures.^[14]

Anthelmintic activity of *Pongamia pinnata* bark

Preparation of earthworms

Pheretima posthuma (Indian earthworm), were used to observe the anthelmintic activity of *Pongamia pinnata*, they are collected from the water logged areas of soil at Sathupally. The earth worms *Pheretima posthuma* are one of the most crucial soil invertebrate that promotes soil fertility. Earthworm feeding and burrowing activities helps to breakdown of organic matter, improve aeration, release nutrients, aggregation of soil. The average sizes

of *Pheretima posthuma* were 8-11 cm with weight 0.9-2.30g were used for all experimental work. Earthworms were washed with normal saline to remove all the fecal matter around their body. The earthworms are anatomically and physiologically resemble with the intestinal roundworm parasites, hence they are used for the study of anthelmintic activity.^[15,16]

Experimental method

Earth worms were segregated into different groups, in each group five earth worms are taken. The bark extract concentrations and the standard drug concentrations were freshly prepared before conducting the experiments. The bark extract was dissolved in minimum quantity of 2% v/v Tween 80 and the volume was made upto 10ml with normal saline for the preparation of various concentrations like 10, 25 and 50mg/ml. Earthworms were washed with normal saline solution before they were placing into 10ml respective formulation, vehicle (2% v/v Tween 80 in normal saline), bark extract (10, 25 and 50 mg/ml) and Piperazine Citrate as standard drug (10, 25 and 50mg/ml) were prepared to observe anthelmintic activity. 10ml formulation containing three different concentrations of ethanolic extract (10, 25 and 50 mg/ml) were prepared and poured in different petriplates, earthworms were kept in the different solutions. Observations were recorded as the time taken to paralysis and death of individual worms.

RESULTS AND DISCUSSION

Phytochemical investigation of plant extracts were essential to detect bioactive principles which is a new source of industrially and therapeutically valuable compounds that may lead to the discovery of new drugs. In the present research study, the presence of phytochemicals were screened with the petroleum ether, chloroform, acetone and ethanol extracts of the *Pongamia pinnata* bark and the results are shown in Table 1. Crude plant extracts and medicines are manufactured based on the principles of natural compounds even by pharmaceutical companies, may lead to large scale exposure of humans to natural products. Presence or absence of essential bioactive compounds in an extracts were identified by color reactions with specific chemicals, this procedure is simple for preliminary pre-requisite before going to phytochemical investigation. Hence, in the present work, the crude extracts obtained by petroleum ether, chloroform, acetone and ethanol solvents were screened for the presence of phytochemicals. The ethanol extract shows the presence of carbohydrate, glycosides, alkaloids, flavonoids, steroids, tannins, saponins, phenolic compounds, protein, and triterpenoids have health benefits such as anthelmintic activity, eye, skin itching, piles, ulcers, wounds, the sprouts, digestive and laxative, piles and wounds.

Table 1: Preliminary phytochemical screening of *Pongamia pinnata* bark.

Test	Petroleum ether	Chloroform	Acetone	Ethanol
Alkaloids	+	+	+	+
Steroids	+	+	+	+
Tannins	-	-	-	+
Saponins	+	-	-	+
Phenols	+	-	-	+
Flavonoids	+	+	+	+
Triterpenoids	-	+	+	+
Carbohydrates	-	-	-	-
Glycosides	-	+	+	+
Proteins	-	-	-	-

+ indicates the presence of the phytochemical;
- indicates the absence of the phytochemical

The present research work finding highlights that ethanolic extracts of *Pongamia pinnata* bark had the highest number of phytochemicals compared with other solvent extracts. Hence, ethanolic extracts of *Pongamia pinnata* bark holds the great potential to treat various human diseases and has profound medical applicability. Due to presence of secondary metabolites, the plant barks extract exhibits anthelmintic activity to bind free

proteins in the gastrointestinal tract of host animal and glycoprotein on the cuticle of the parasite. The results of anthelmintic activity on earthworms *pheretima posthuma* was shown in Table-2. Different concentrations (10, 25 and 50mg/ml) prepared for evaluation of paralysis and death of earthworms and it was compared with the same concentration for Piperazine citrate.

Table 2: Anthelmintic activity of Methanolic extract of *Pongamia pinnata* bark.

Groups	Dose in Concentration (mg/ml)	Time of paralysis (min)	Time of death (min)
Control	-	-	-
Ethanolic Extract	10	45.19±3.52	56.17 ± 2.53
	25	27.27± 4.11	42.16±2.14
	50	13.37 ± 3.22	22.13 ± 3.32
Standard drug	10	17.32 ± 1.51	21.24 ± 2.31
	25	11.11±1.42	16.26 ± 1.23
	50	8.46 ± 0.44	11.36 ± 0.36

The ethanolic extracts of *Pongamia pinnata* bark and standard drug solution not only illustrate paralysis, but also causes death of worms especially at higher concentration of 50 mg/ml, in very less time was shown in Fig 1,2.

**Figure-1: Anthelmintic activity of ethanolic extract.****Figure-2: Anthelmintic activity of standard drug.**

In addition, Tannins or their metabolites have an undeviating effect on the possibility of the pre-parasitic stages of helminthes and other phytochemical constituents might be responsible for an anthelmintic activity include flavonoids and terpenoids. This present research work shows the presence of different phytochemical constituents like carbohydrate, glycosides, alkaloids, flavonoids, steroids, tannins, saponins, phenolic compounds, protein, and triterpenoids with biological activity that can be valuable therapeutic index. The plant bark extracts can be used for further

isolation of compounds for their anthelmintic activity.

CONCLUSION

The present research concludes that *Pongamia pinnata* bark contains potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising. The presence of phytoconstituents, such as phenolic compounds and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing a number of diseases through free radical scavenging activity. Since the plant *Pongamia pinnata* bark has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identify bioactive compounds. The present results will form the basis for collection of new plant species for further investigation in the potential discovery of new bioactive compounds. Further studies are required for in-vitro studies to establish effectiveness and pharmacological rationale for the use of bark extracts as anthelmintic drug. The present research concluded that the plant *Pongamia pinnata* bark extract has significant anthelmintic activity.

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