

Evaluation of the Anthelmintic Potential of *Pongamia pinnata* Root Hydroalcoholic Extract against *Tubifex tubifex*

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ABSTRACT

Background: *Pongamia pinnata* is a well-known plant traditionally employed in the treatment of various ailments. This study was conducted with the objective of evaluating the anthelmintic property of a hydroalcoholic extract of *Pongamia pinnata* root. **Materials and Methods:** *Tubifex tubifex*, commonly known as aquarium worms, were used as test worms for anthelmintic activity. The hydroalcoholic extract was prepared using soxhlation technique, and a comprehensive phytochemical analysis was subsequently conducted. The limit test, main test, and a comparative test were employed to evaluate the anthelmintic property of the root extract. The time taken for paralysis and death of *Tubifex tubifex* worms were recorded to determine the minimum effective concentration required to induce complete mortality within 4 hr, and to compare the effectiveness of the extract against the standard drug piperazine citrate. **Results:** Phytochemical screening of the extract indicated the presence of different phytoconstituents, such as alkaloids, flavonoids, glycosides and saponins. In the comparative test, the lowest concentration of the *Pongamia pinnata* root hydroalcoholic extract induced paralysis and death of all the worms in 167.83±3.85 min and 231.17±5.52 min, respectively. At the same concentration as the root extract, the standard drug piperazine citrate resulted in paralysis and death of all the worms in 89.33±2.76 min and 143.50±2.02 min, respectively. **Conclusion:** The hydroalcoholic extract of *Pongamia pinnata* root exhibits significant anthelmintic activity against *Tubifex tubifex*. Therefore, these findings support the rationale for using *Pongamia pinnata* root extract as a promising natural candidate for anthelmintic therapy.

Keywords: Anthelmintic, Death time, Paralysis time, Piperazine citrate, *Pongamia pinnata*, *Tubifex tubifex*.

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INTRODUCTION

Helminthic infections are a major health challenge, impacting not only human populations but also livestock and other economically valuable animals. These infections are especially common in areas where access to proper sanitation and healthcare is insufficient.^[1] They are caused by parasitic worms such as nematodes, cestodes, and trematodes, which have evolved to invade hosts, evade immune responses, and thrive in various environments. These infections can lead to nutritional deficiencies, stunted growth in children, and chronic anaemia in adults.^[2] In farming communities, the economic consequences are significant, as infected animals often exhibit reduced weight gain and overall diminished productivity,

thereby threatening food security and livelihoods. As a result, addressing helminthiasis requires comprehensive, multifaceted treatment and control strategies.

Anthelmintics are used to treat parasitic infections in humans and animals. They excrete parasites by killing or incapacitating them without significantly harming the host.^[3,4] Anthelmintics such as albendazole, mebendazole, ivermectin, piperazine etc. are used to combat infections with parasitic roundworms, pinworms, whipworms, hookworms, and tapeworms. One of the key challenges in modern helminth management is the growing drug resistance to conventional anthelmintics. Overuse of these drugs has reduced their effectiveness, pushing researchers to explore alternative therapies. Plant-derived compounds, with their diverse and synergistic bioactive components, offer a promising, sustainable solution rooted in traditional medicine, potentially decreasing reliance on synthetic drugs while addressing the helminthiasis public health burden.



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Pongamia pinnata is a fast-growing, medium-sized, glabrous, evergreen or temporarily deciduous shrub or tree. It typically grows between 15-25 M tall, with a straight or crooked bole that is at least 50-80 cm in diameter.^[5] The plant is also known as Indian beech, karum tree, pongam, and karanja. The plant contains various phytoconstituents such as flavonoids, alkaloids, glycosides, triterpenoids, tannins, saponins, and steroids. *Pongamia pinnata* is used in folk medicine to treat a variety of conditions, including piles, bleeding gums, ulcers, rheumatism, colds, coughs, diarrhoea etc. Various studies showed that plant *Pongamia pinnata* has a range of activity, including anti-inflammatory,^[6] anti-plasmodial,^[7] antioxidant,^[8] anti-diarrhoeal,^[9] anti-ulcer,^[10] anti-hyperglycaemic,^[11] antibacterial action^[12] etc. Thus, this study was conducted to scientifically evaluate the anthelmintic activity of *Pongamia pinnata* root extract.

MATERIALS AND METHODS

Worms used

Tubifex tubifex, commonly known as aquarium worms, were purchased from a nearby market. The average size of the worms were 1-1.5 cm. The worms were kept in a clay pot, and acclimated to the laboratory conditions before the commencement of the experiment.

Drugs and chemicals used

Ethanol (Loba Chemie Pvt. Ltd., Mumbai), piperazine citrate (Garima Healthcare Pvt. Ltd., Indore, Madhya Pradesh), and distilled water.

Preparation of root extract

The roots of the plant *Pongamia pinnata* were collected from Amta, West Bengal, India. The roots were first dried and ground into a powder, then subjected to an extraction process with 70% ethanol in a soxhlet apparatus for 48 hr. Next, the extract was collected and then evaporated under vacuum until it was completely dry. The dried extract was weighed and stored for experiment.

Preliminary phytochemical investigation

Hydroalcoholic extract of *Pongamia pinnata* root was evaluated for phytochemical investigation^[13] to check the presence of various phytoconstituents such as alkaloids, carbohydrates, saponins, flavonoids, glycosides, sterols, proteins etc.

Anthelmintic activity

The anthelmintic activity of the hydroalcoholic extract of *Pongamia pinnata* root was carried out through a method consisting of limit test, main test and comparative test.^[14,15] The minimum concentration required to cause paralysis and death in 100% of the worm population within 4 hr was determined. The complete paralysis and death time were noted. The onset

of pre-complete paralysis was considered to have begun when the worms ceased their normal movements. External forceful stimuli were applied to induce movement in a worm to confirm the complete paralysis time. The death time was confirmed when a worm completely ceased movement, its body colour turned pale, and it exhibited no response when immersed in warm water at 50°C. After that a comparative test was done to find out the effectiveness of the plant extract in comparison to the control and standard drug piperazine citrate.

In the limit test a concentration of 5000 mg/L of the *Pongamia pinnata* root hydroalcoholic extract was taken in six petri dishes, each containing 20 mL of distilled water. One worm was placed in each petri dish, and the test was conducted over a duration of 4 hr. All the worms died in this experiment, after which the main test was carried out.

For the main test, four different petri dishes were prepared, each containing 20 mL of distilled water, with 175 mg/L, 550 mg/L, 2000 mg/L, and 5000 mg/L of *Pongamia pinnata* root extract in each petri dish, respectively. For the control group 20 mL of distilled water was taken in a different petri dish. Six worms were placed in each petri dish, and the minimum concentration of the hydroalcoholic extract that caused complete mortality of the worms was determined. This study was conducted over a period of 4 hr, during which the paralysis time and death time were recorded. The minimum lethal concentration of the root extract, resulting in complete mortality of the worms, was determined through a series of dose divisions.

After the main test, a comparative test was conducted. The anthelmintic effect of *Pongamia pinnata* root extract was compared with standard drug piperazine citrate and untreated control group. The minimum lethal concentration of the root extract obtained from the main test and piperazine citrate (same concentration as the extract) were taken in separate petri dishes, each containing 20 mL of distilled water. For the control group 20 mL of distilled water was taken in a different petri dish. Then six *Tubifex tubifex* worms were placed in each petri dish, and their paralysis time and death time were recorded over a period of 4 hr.

Table 1: Result of preliminary phytochemical investigation of the hydroalcoholic extract of *Pongamia pinnata* root.

Sl. No.	Phytoconstituents	Result
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Proteins and amino acids	-
5	Flavonoids	+
6	Saponins	+
7	Steroids	+
8	Lipids or fats	+

Statistical analysis

The experimental result was shown as Mean±SEM for each treatment group. The significance of activity was assessed using one-way ANOVA, followed by Dunnett's test with $p < 0.01$ was considered statistically significant.

RESULTS

Preparation of root extract

Soxhlet extraction of *Pongamia pinnata* roots with 70% ethanol for 48 hr yielded 7.5% extract.

Preliminary phytochemical investigation

A preliminary phytochemical screening of the extract obtained from the *Pongamia pinnata* root was carried out and the presence of different phytoconstituents such as alkaloids, carbohydrates, glycosides, flavonoids, saponins and steroids was observed (Table 1).

Anthelmintic activity

In this study the anthelmintic potential of the hydroalcoholic extract of *Pongamia pinnata* root was evaluated using a sequential methodology comprising a limit test, a main test, and a comparative test. In the limit test all the worms died, after which the main test was performed. From the initial four doses used in the main test only 5000 mg/L and 2000 mg/L concentration of *Pongamia pinnata* root hydroalcoholic extract showed significant anthelmintic activity within 4 hr (Table 2). Then the minimum lethal concentration of the root extract was determined through various divisions of doses, and 1250 mg/L of the root extract was identified as the minimum concentration at which complete mortality of the worms occurred. The data revealed that the minimum concentration (1250 mg/L) of the root extract showed significant anthelmintic activity inducing paralysis and death of all the worms in 167.83±3.85 min and 231.17±5.52 min, respectively (Table 3). The same concentration (1250 mg/L) of the standard drug piperazine citrate caused paralysis and death of all the worms in 89.33±2.76 min and 143.50±2.02 min, respectively (Table 3).

Table 2: Effects of various doses of *Pongamia pinnata* Root Extract (PPRE) against *Tubifex tubifex*.

Treatment	Paralysis time (Minute)	Death time (Minute)
Control	-	-
PPRE 175 mg/L	-	-
PPRE 550 mg/L	-	-
PPRE 2000 mg/L	135.66±3.45**	197.33±2.54**
PPRE 5000 mg/L	98.83±1.20**	150.50±1.40**

All the values are shown as Mean±SEM, where $n=6$. ** $p < 0.01$ was considered statistically significant.

DISCUSSION

Parasitic helminths significantly impact both humans and animals, leading to severe hardship and hindered growth. Many helminth-related diseases are chronic and debilitating. The rise of anthelmintic resistance, coupled with the high cost of conventional drugs, has driven the exploration of medicinal plants as a potential alternative source of anthelmintic.

In this study, the anthelmintic activity of hydroalcoholic extract of *Pongamia pinnata* root was evaluated. The anthelmintic activity was performed on aquarium worms, *Tubifex tubifex*. These worms were chosen because their anatomical and physiological traits closely resemble those of the intestinal roundworm parasite.^[16] Additionally, their easy accessibility played a significant role in their selection.

Piperazine citrate was used as a standard anthelmintic drug. It induces muscle hyperpolarization of the worms through its GABA agonist action, which opens Chloride (Cl^-) channels. This process leads to muscle relaxation and reduces responsiveness to the contractile effects of acetylcholine, ultimately resulting in flaccid paralysis.^[17]

The study demonstrated that the hydroalcoholic extract of *Pongamia pinnata* root exhibits significant anthelmintic activity compared to the standard drug piperazine citrate and control group. Although the exact mechanism of action for the root extract remains unclear, its effectiveness is likely a result of its diverse chemical constituents. Phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, saponins and steroids, with several of these components contributing to the anthelmintic effect. For instance, alkaloids may exert their activity by interacting with acetylcholine receptors and hindering glucose uptake, leading to starvation of the helminths.^[18] Similarly, flavonoids appear to block both glucose uptake and auxin transport, and by interrupting phosphorylation reactions critical for energy production, they can cause the death of the parasites.^[19] Additionally, glycosides have shown potent activity against various helminths by disrupting the transportation of sodium and potassium ions within the parasite.^[20,21]

This comprehensive approach highlights the potential of *Pongamia pinnata* root extract as an alternative anthelmintic agent, suggesting further exploration into its active components could provide insights into new therapeutic strategies.

Table 3: Effects of different treatment groups against *Tubifex tubifex*.

Treatment	Paralysis time (Minute)	Death time (Minute)
Control	-	-
Piperazine citrate	89.33±2.76**	143.50±2.02**
PPRE	167.83±3.85**	231.17±5.52**

All the values are shown as Mean±SEM, $n=6$. ** $p < 0.01$ was considered statistically significant.

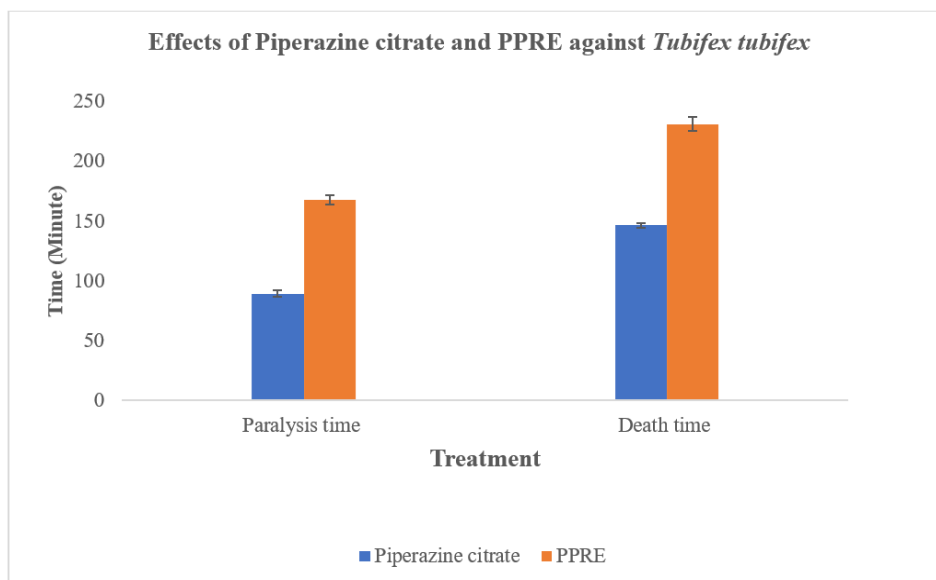


Figure 1: Effects of piperazine citrate and PPRE on the paralysis time and death time of *Tubifex tubifex*.

CONCLUSION

This study evaluated the anthelmintic potential of a hydroalcoholic extract of *Pongamia pinnata* root, with piperazine citrate serving as a standard anthelmintic drug. The extract showed significant anthelmintic activity by paralyzing and killing the *Tubifex tubifex* worms. Notably, the paralysis time and the death time of these worms when exposed to the *Pongamia pinnata* root extract was comparable to the effect produced by the piperazine citrate, indicating a significant level of effectiveness. This effect can probably be attributed to the phytoconstituents present, which include alkaloids, glycosides, and flavonoids. Overall, these findings indicate that *Pongamia pinnata* root extract could offer a promising alternative in anthelmintic therapy. However, further investigation is needed to isolate the actual phytoconstituents responsible for anthelmintic activity and to elucidate its mechanism of action. Overall, the study demonstrates the importance of exploring natural products as promising sources of anthelmintic agents, particularly in the context of growing drug resistance and the pressing need for alternative therapies.

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ABBREVIATIONS

ANOVA: Analysis of Variance; PPRE: *Pongamia pinnata* Root Extract.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

The author's contribution to this article is as follows: study conception and design: Shaon Dey, Kousik Saha, Urmistha Sarkar, Krishanu Pramanick; literature search: Shaon Dey, Sanjib Manna, Riju Koley; data collection: Shaon Dey, Sanjib Manna, Santanu Manna, Priya Roy, Riju Koley; analysis and interpretation of results: Shaon Dey, Kousik Saha, Urmistha Sarkar, Krishanu Pramanick; draft manuscript: Shaon Dey, Kousik Saha. All authors reviewed the results and approved the final version of the manuscript.

SUMMARY

Helminth infections remain a serious health and economic burden, and rising resistance to standard anthelmintics has spurred the search for plant-based alternatives. In this work, a hydroalcoholic extract of *Pongamia pinnata* root was prepared via soxhlet extraction and screened for phytochemicals before testing its anthelmintic efficacy against *Tubifex tubifex* worms. Using limit test, main test and comparative test, the extract was evaluated for its anthelmintic activity. Piperazine citrate was used as a standard anthelmintic drug. The root extract showed complete paralysis and death of all the worms at minimum concentration of 1250 mg/L. Phytochemical analysis revealed alkaloids, steroids, flavonoids and glycosides, pointing to multiple bioactive constituents that may underlie its potent anthelmintic action.

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