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Phytochemical analysis and anthelmintic assessment of *Pongamia pinnata*: The Medicinal Tree

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Abstract

Herbal-based drugs are gaining popularity and are becoming an important part of the primary healthcare system. The current study aimed to assess the anthelmintic activity of *Pongamia pinnata* leaves against *Pheritima posthuma* and *Haemonchus contortus*. Five different concentrations (5, 15, 25, 50, and 75 mg/ml) of water-soluble, acetone, petroleum ether, and methanol extracts were evaluated on worms. It comprised determining the time of paralysis (P) and death (D) of the worms. The acetone extract showed more significant effects on paralyzing the worms than other investigated extracts. The impact of extracts on the paralysis and death of the *Pheritima posthuma*, as evidenced by the results, acetone > petroleum ether > methanol > aqueous. Similarly, among all the extracts obtained from *Pongamia pinnata*, acetone extract required the shortest time to cause death and paralysis of *Haemonchus contortus*, followed by petroleum ether < methanol < aqueous extracts. Albendazole was employed as a reference standard medicine, and saline water was used as the usual control.. The leaf extracts of *Pongamia pinnata* revealed a good remedy for worm infections and *in-vitro* trials proved its anthelmintic properties at higher doses.

Keywords: *Pheritimaposthuma*, *Haemonchuscontortus*, Albendazole, Anthelmintic property.

1. Introduction:

Since time immemorial nature has bestowed its benefits on mankind for betterment. It gives all we need for a healthy existence, including food, shelter, clothing, medicine, and spiritual support for disease prevention and treatment. Plants have been employed to produce therapeutic compounds since antiquity, and they continue to do so today. Few plants with the ability to be an excellent supplier of new bioactive compounds have been thoroughly investigated. According to estimates, just 5–15% of the 250,000 species of higher plants have been properly investigated [8] [6][33]. Plants and their by-products are essential components of the herbal medicine industry. Consequently, it has a strong focus.

Natural plants are the finest sources for healing a variety of diseases and conditions. Ancient books such as "The Rig Vedas" discuss the usage of plants to heal several human ailments. Medicinal plants have considerable pharmacological value [4][5][27]. In comparison to contemporary medicine, medications made from plants continue to be a crucial source of restorative agents because they are readily available, less expensive, and non-toxic [17]. It

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is inevitable to increase the preservation, effectiveness, and efficiency of both new and existing medicinal plants because they benefit humanity [25]. Potential therapeutic compounds derived from medicinal plants have a long history, such as reserpine and rescinnamine.

Since the dawn of time, numerous human and animal diseases have been treated using popular restorative agents from the kingdom of plants, which is considered to be a rich source of botanicals [31]. In ancient Indian, Chinese, and African cultures, the use of plants and plant parts for the prevention and treatment of various ailments was also acknowledged. Forages and plant parts have been used to treat worm diseases for a significant portion of human history, and natural products are still employed as herbal treatments in many parts of the world [2][13][18][26]. Plant- or herbal-based medications are receiving a lot of attention right now and are becoming an essential component of the primary healthcare system [19].

Pongamia Pinnata, a native Indian medicinal plant, has an extensive history of use in the Ayurvedic, Siddha, and Unani medical systems to treat a wide range of ailments and disorders in humans [28]. Ayurvedic medicine uses various parts of the plant to manage rheumatic joints, whooping cough, bronchitis, and diabetes-related dipsia. It is used locally to treat rheumatism, leucoderma, leprosy, foul-smelling gonorrheal lesions, and scrofulous enlargement [7]. Plants naturally create a variety of secondary chemicals that function as antioxidants. A phytochemical examination revealed that Pongamia plants contained flavonoids, saponins, glycosides, phenol, amino acids, diterpenes, and carbs. Crude extracts of *P. pinnata* are an excellent source of secondary plant-based constituents with strong antioxidant properties. The results of the study will be useful to pharmacologists, phytochemists, and the pharmaceutical industry. Many plant species, including, *Cymbopogancitratus*, *Chrysanthemum cinnararifolium*, *Allium sativa*, *Pongamiaglabra*, and others, have been claimed to have pest-controlling abilities [16].

Helminthic infestations are among the most widespread parasite infections of animals and people worldwide, and they are increasingly acknowledged as a severe veterinary and human health problem, both in developing and developed nations [23][24]. It is estimated that out of 3.5 billion people currently affected by intestinal parasites, approximately 2 billion are suffering from helminthiasis, out of which about 1.2 billion are infected with nematode parasites [34]. Different animals and humans suffering from helminthiasis excrete the worm eggs in their feces. These eggs contaminate the soil where open defecation is practiced or where there is inadequate sanitation. The eggs hatch in the moist soil, and eventually, the larvae or eggs reach the host either by ingestion of contaminated food (roundworm or whip worm) or by penetration through the skin (hookworm).

The primary and most frequent control method taken against helminth infections depends primarily on the use of a restricted number of commercial medications such as benzimidazoles (BZ), macrocyclic lactones, imidazothiazoles, and praziquantel (PZQ) [20][15]. Because of the absence of vaccines, there is currently no alternative to chemical control. Therefore, the present study was aimed at controlling helminthiasis using a crude extract of *Pongamia pinnata* as a biological control.

2. METHODS

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The *Pongamia pinnata* leaves were taken in September and October 2021 from the campus of the Govt. Vidarbha Institute of Science and Humanities in Amravati, India (Fig. 1). Standard floras [21][9] were used to help in identification. Associate Professor Dr. Manjusha R. Watt, of the Govt. Vidarbha Institute of Science and Humanities in Amravati, Maharashtra, identified and verified the plants. The Department of Botany's Herbarium houses the voucher specimen (78600).

2.1 Collection of the Plant material

Fig. 1. Site of Collection of Sample

The leaves were thoroughly washed and dried in the shade for two weeks. The dried leaves were crushed using a mixer grinder and sieved. Before extraction, the powder was stored at room temperature in airtight polythene bags.

2.2 Preparation of Extract

Crude extracts, viz., aqueous, methanol, acetone, and petroleum ether, from the powdered samples of *Pongamia pinnata* have been prepared in step with the same old methods with minor adjustments [11]. 25 g of crushed plant matter was macerated with 100 ml of methanol, acetone, petroleum ether, and water for 7 days before being filtered through a porous fabric and then filter paper. Following the extraction, the beverages were diluted to eliminate solvent waste products, and the procedure was repeated. The solvent from the whole extract was distilled, and the concentrate was evaporated in a water bath until dry and stored at 4°C until later use. On the day of the test, the crude extract was diluted in distilled water to make a stock solution and individual dilutions for anthelmintic activity analysis.

2.3 Phytochemistry

Phytochemical analysis was performed with the help of the following preferred techniques [10][30]. Various bioactive compounds were tested in the fresh as well as dry plant powder of the part used. The plants were thoroughly cleaned with water and then shade-dried. Dried plants and components were crushed and stored in polythene zip-lock bags at 30-32 degrees Celsius for analysis. All extracts were submitted to preliminary phytochemical screening to assess the presence or absence of various metabolites using the same old methods.

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Qualitative analysis was used to identify plant secondary metabolites including alkaloids, phenols, tannins, flavonoids, saponins, steroids, fixed oils, lignins, terpenoids, and glycosides.

PLATE – 1

Pongamia pinnata (L.) Pers

2.4 Experimental procedure

The anthelmintic activity was measured using the conventional technique of [1], with slight changes. The testing was carried out on adult Indian earthworms, *Pheritima posthuma*, and *Haemonchus contortus*, because they are anatomically and physiologically similar to the human intestinal roundworm parasites. Because of their ease of availability, earthworms are commonly utilized for the first in vitro assessment of anthelmintic substances. The bioassay evaluated several doses of each extract, including 5, 15, 25, 50, and 75 mg/mL. All of the extracts and standard medication solutions were newly produced before the trials began. The mean time for paralysis (in minutes) was recorded when no movement was seen except when the worm was aggressively shaken; the time for worm death (in minutes) was measured after ensuring that the worms did not move when shaken vigorously or immersed in warm water (50°C) Albendazole (15 mg/mL) used as a reference.

3. Statistical Analysis

The data were statistically examined using two-factor ANOVA and replications. The data were reported as mean values and standard deviations. Each essay was completed four times. The statistical analysis was done in Excel. To investigate the effects of various extracts and concentrations. The data gathered through experiments for various extracts is evaluated using the F test (ANOVA).

4. Results and Discussion

The phytochemical examination of the *Pongamia pinnata* aqueous extract (Table 1) revealed the presence of carbohydrates, steroids, glycosides, flavonoids, alkaloids, and tannins, whereas glycosides and alkaloids were present in the alcoholic extract, but all other phytochemicals tested were missing. It was also clear from the results that proteins and amino acids were absent from both the tested extracts. In a similar study, [32] reported more or less

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comparable findings while undertaking phytochemical screening on the same plant. The findings of our study were in line with the work of [3], who also reported the presence of similar phytochemicals and the absence of proteins and amino acids.

Table 1. Preliminary phytochemical screening of *Pongamia pinnata*

Test	Aqueous extract	Alcoholic extract
Test for carbohydrates Molisch test	+++	- - -
Test for reducing sugar Benedicts test Fehling test	+++ - - -	+++ +++
Test for monosaccharides Barfoeds test	+++	- - -
Test for hexose sugar Selwinoffs test	+++	- - -
Test for pentose sugar Test solution + HCL	+++	- - -
Test for proteins Biurets test	- - -	- - -
Test for amino acids Ninhydrin test	- - -	- - -
Test for steroids Salkowaski reaction	+++	- - -

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Test for glycosides (General test)		
Foam test	+++	+++
Cardiac glycosides (baljet test)	+++	+++
	+++	+++
Test for flavonoids		
Lead acetate test	+++	- - -
Alkali test	+++	- - -
Test for alkaloids		
Dragondorffs test	+++	+++
Test for tannins		
Ferric chloride test	+++	- - -

(Test was noted as "+ ++" represented as present while as "- - -" absent)

5. Anthelminthic Activity

The current study (Table 2) found that petroleum ether, methanol, aqueous, and acetone extracts of *Pongamia pinnata* had significant anthelminthic efficacy against *Pheretima posthuma* and *Haemonchus contortus* when compared to the standard medicine utilized in the study. Each extract of the plant at concentrations of 5, 15, 25, 50, and 75 (mg/ml) demonstrated anthelminthic activity differently, with *Pheretima posthuma* showing the shortest time of paralysis and death at 75 mg/ml concentration, as did *Haemonchus contortus.* Petroleum ether leaf extract of *Pongamia pinnata* at the maximum concentration (75 mg/ml) caused paralysis in 78 min and death at 90 min against *Pheretima posthuma*, but in the case of *Haemonchus contortus* paralysis and death were detected at 31.9 min and 40.4 min, respectively. The methanol extract showed paralysis in 185 min and death at 185.5 against *Pheretima posthuma* whereas paralysis and death were recorded at 20.7 min and 41.8 min against *Haemonchus contortus*, respectively. Similarly, aqueous extract at the highest concentrations showed paralysis in 278.35 min and death at 295 min against *Pheretima posthuma* whereas paralysis at 41.8 min and death at 49.5 min were reported against

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Haemonchus contortus, respectively. The acetone extract showed paralysis in 52.5 min and death at 59.5 against *Pheretima posthuma.* However, in the case of *Haemonchus contortus* paralysis and death were ascertained at 18.6 min and 25.2 min. The standard Albendazole drug showed the same at 290 min and 350 min as well as 11.95 min and 17.38 min against *Pheretima posthuma* and *Haemonchus contortus* respectively. As a result, among all the extracts produced from *Pongamia pinnata*, the acetone extract took the shortest time to cause paralysis and death of the earthworms, followed by petroleum ether, methanol, and aqueous extracts. Similarly, among the studied extracts, the acetone extract took the least time to produce paralysis and death of the roundworms, followed by petroleum ether, methanol, and aqueous extracts.

The current study's findings were consistent with previous publications on the anthelminthic effects of medicinal plant extracts. It has been found that an aqueous extract of *Pongamia pinnata* leaves had substantial anthelminthic action against *Pheretima posthuma*, with a paralysis time of 4.10 min and a death time of 9.22 min at a concentration of 50 mg/ml [29]. It was also discovered that the seed section of *Pongamia glabra* requires shorter paralysis and death time than the leaves of the same plant against *Pheretima posthuma*[21]. The current study's findings were also corroborated by the work of [14], who observed that fresh aqueous + acetone extract of *Pongamia pinnata* leaves at the highest concentrations (100 mg/ml) caused paralysis in 4.15 minutes and death in 25.75 minutes against *Pheretima posthuma*. Similarly, found that an ethanolic extract of *Pongamia pinnata* had strong anthelminthic action against *Pheretima posthuma*, with paralysis and death periods of 15.37 and 23.38 minutes, respectively [12].

Table 2. Anthelmintic activity of *Pongamia pinnata* at different concentrations against Earthworm and Roundworm.

Groups	Treatments	Conc.(mg/l)	*Pheretima posthuma* (Earthworm)		*Haemonchus contortus* (Roundworm)	
			P(min)	D(min)	P(min)	D(min)
I	**Normal Control**	-	-	--	-	--
II	**Standard Albendazole**	5	686 ± 1.41	763 ± 4.24	32.03±3.99	43.58±0.58
		15	623 ± 8.48	674 ± 9.89	23.46±1.35	32.19±2.03
		25	534.5±13.43	586±15.55	17.88±0.43	25.5±0.5
		50	388 ± 1.41	424.5±4.94	13.91±0.53	20.57±0.57
		75	290 ± 5.65	350 ± 9.89	11.95±0.55	17.38±0.43

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III	**Petroleum Ether**	5	253 ± 2.82	262 ± 1.41	51.8±6.53	79.19±4.05
		15	201 ± 4.94	207 ± 1.41	42±11.02	74.5±2.74
		25	197.5 ± 3.53	203 ± 5.65	41.8±8.92	64.4±4.39
		50	95.5 ± 4.94	107 ± 8.4	35.8±4.08	48±7.38
		75	78.0 ± 7.07	90 ± 1.41	31.9±5.62	40.4±3.15
IV	**Methanol**	5	280.5 ± 3.53	300 ± 2.82	74.4±5.02	93±1.76
		15	261 ± 8.48	272.5±6.36	71.2±5.54	92±2.57
		25	230 ± 7.07	236.5±9.19	43.4±7.30	65.4±4.39
		50	188.5 ± 4.94	195±8.48	32.5±6.65	52.3±4.23
		75	185 ± 5.65	185.5±2.12	20.7±4.08	41.8±4.81
V	**Aqueous**	5	328±11.31	386.5±9.19	77±4.10	84.4±3.36
		15	320.5±6.36	370±10.6	71±3.53	78.5±1.58
		25	313.5±4.94	356.5±4.94	46.4±1.29	56.6±1.85
		50	295.3±3.53	314±8.48	45.3±1.25	53.7±2.53
		75	278.35±3.04	295±4.24	41.8±1.68	49.5±2.64
VI	**Acetone**	5	117±12.72	132.5±14.84	33.8±4.20	55.5±4.35
		15	103.5±9.19	110±9.89	25.8±1.92	45.4±2.7
		25	82±9.89	85.5±2.12	24.4±4.03	42.6±4.5
		50	71±11.31	74±1.41	21±1.58	33.3±4.02
		75	52.5±6.36	59.5±6.36	18.6±2.88	25.2±2.38

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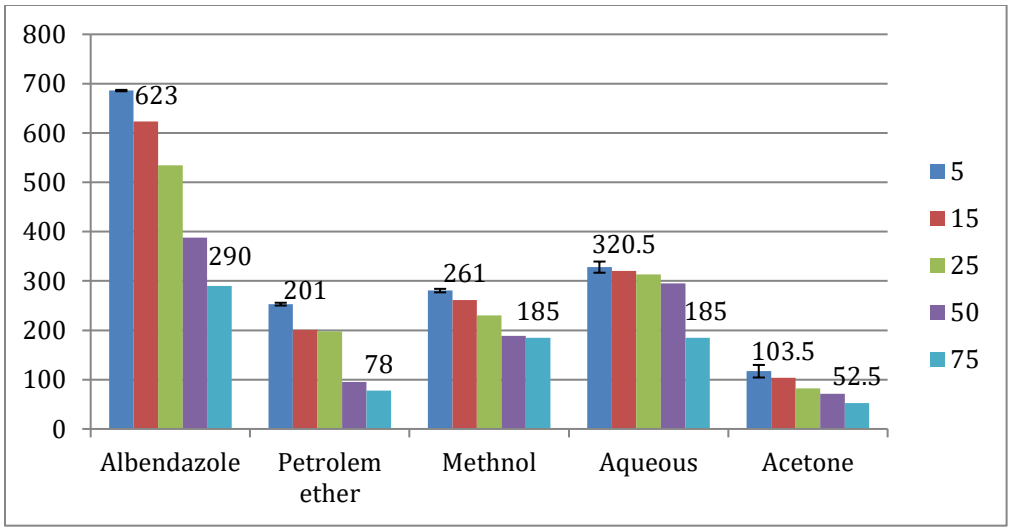


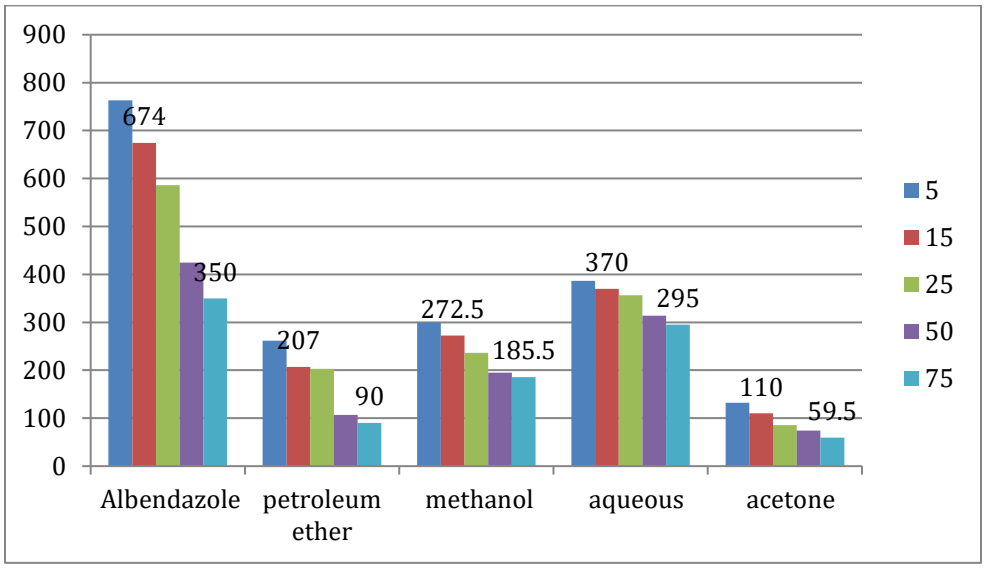
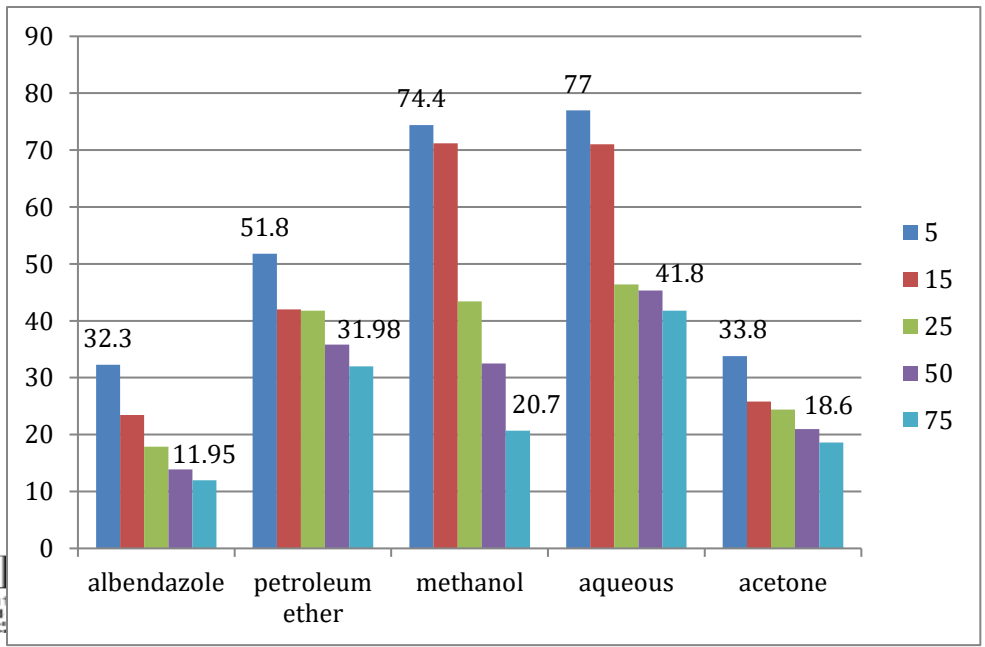


Fig.1a.2a Comparative in vitro anthelmintic action of various concentrations of *Pongamia pinnata* against exhibiting the paralysis and death time of *Pheretima posthuma*.



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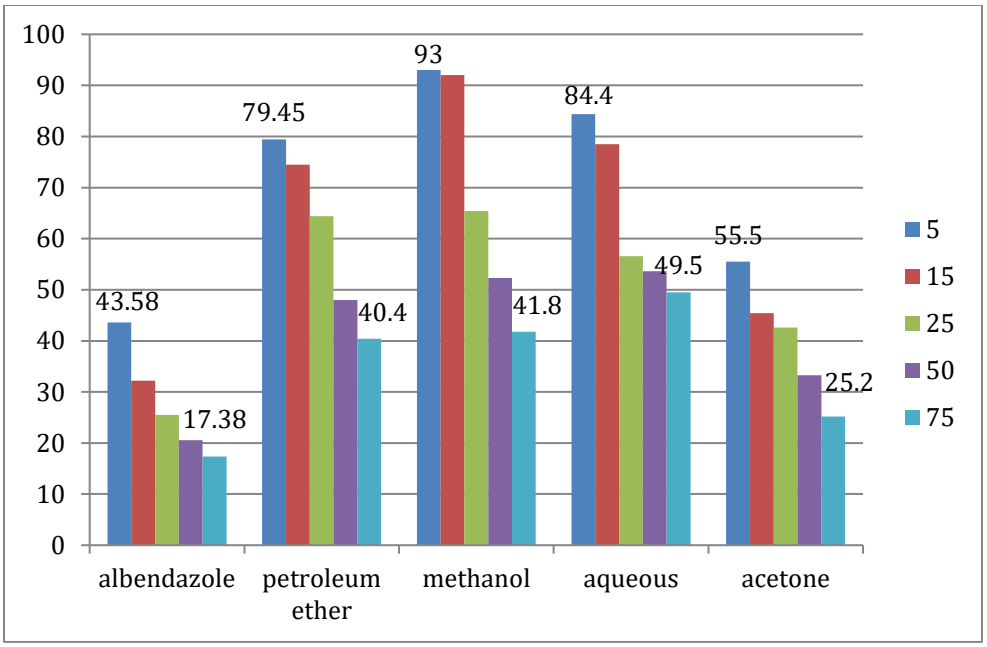

Fig.3a, 4a Comparative in vitro anthelmintic impact of various concentrations of *Pongamia pinnata* on *Haemonchus contortus* paralysis and death times.

6. Conclusion

From the foregoing results, it could be inferred that the traditional declaration of leaves of Pongamia pinnata as anthelmintic has been exhibited as the leaf extracts displayed pastime in opposition to the Indian earthworm (Pheretima posthuma) and Haemonchus contortus utilized inside the observation. Similarly, the use of *in-vivo* experiments is required to perform and establish the effectiveness and pharmacological reasons for using *Pongamia pinnata* as an anthelmintic drug. Furthermore, the drug might be investigated for the extraction and characterization of the active components responsible for anthelmintic activity, as well as for the establishment of movement mechanisms. The results of the present research hold a capacity promise for the future use of active standards from *Pongamia pinnata* as effective anthelmintics and may assist in designing incorporated answers for the management of parasites to be easy out of the host's body.

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