



In-Vitro Anthelmintic Potential Of Aqueous And Ethanolic Leaves And Roots Extracts Of *Annona Muricata* Against Earthworm

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Article History	Abstract
Received: Revised: Accepted:	Helminths or Parasitic worms of humans may cause chronic and sometimes deadly diseases considered as Neglected Tropical Diseases that infect around two billion people worldwide. Many of synthetic drugs available, for the treatment of various worm infection like alendazole, mebendazole and Ivermectin with more of adverse effects so the need of herbal formulations are essential for teating worm infections. Plants have been used as anthelmintic from ancient times. In our study we selected <i>Annona muricata</i> is well known traditional plant which is used for the treatment of many worm infection without adverse effect. Phytochemical screening was conducted using standard qualitative methods, it reveals that they were rich in secondary metabolite compounds such as alkaloids, saponins, tannins, flavonoids. The <i>in-vitro</i> anthelmintic activity was determined using earthworm. In this ethanolic extract of 200 mg/ml concentration shows high anthelmintic activity when compared to other concentration of <i>Annona Muricata</i> leaves extract.
CC License CC-BY-NC-SA 4.0	Keywords: Helminths, Helminthiasis, anthelmintic, <i>Annona Muricata</i>

INTRODUCTION

Diseases known as parasitic infections are brought on by creatures that feed on other living things. They may result in neurological symptoms, rashes on the skin, fever, exhaustion, and digestive issues. Insect bites, undercooked meat, contaminated food, water, or surfaces can all spread them. Treatment for parasitic infections involves the use of antiparasitic drugs.^[1]

The three primary parasite species that infect people are as follows:

- Protozoa
- Helminths

- Ectoparasites ^[1]

1.1. HELMINTHIASIS

The invertebrates known as helminths are distinguished by their elongated, flat, or spherical bodies. A portion of the body gets infected by parasitic worms like pinworms, roundworms, or tapeworms in helminthiasis, a macro parasitic disease that affects both people and animals.^[1]

Parasites are entry into human body through

- Ingestion,
- Arthropod stings, and
- Penetration of undamaged skin or mucous membranes^[2]

1.2. CLASSIFICATION OF HELMINTHS

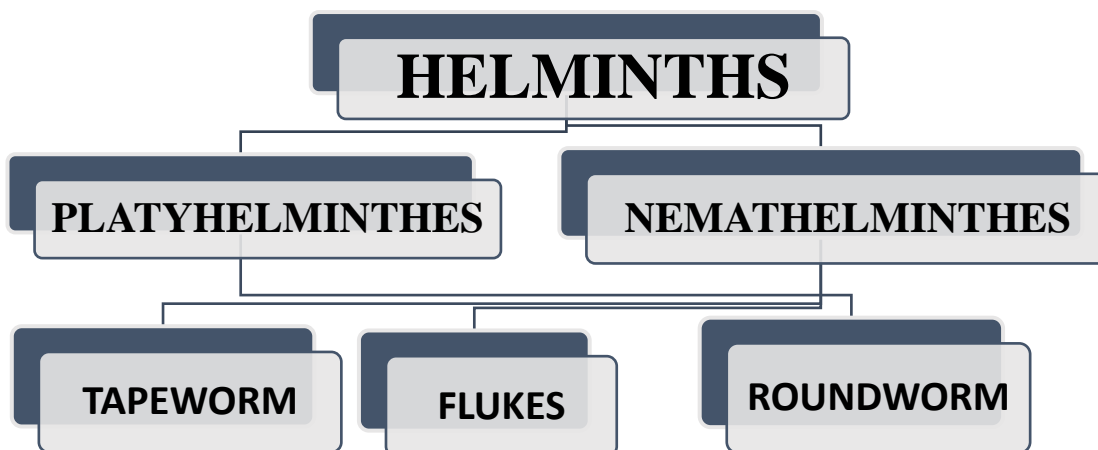


Fig No: 1 CLASSIFICATION OF HELMINTHS

1.3. DISEASE CAUSED BY HELMINTHS

- Enterobiasis
- Acariasis
- Taeniasis
- Cysticercosis
- Schistosomiasis ^[2]

1.4. ANTHELMINTIC

- Anthelmintics are medications that eradicate or drive out helminth infestations.
- *Pheretima posthuma* has been employed as a test worm in the majority of screenings for anthelmintics.^[2]

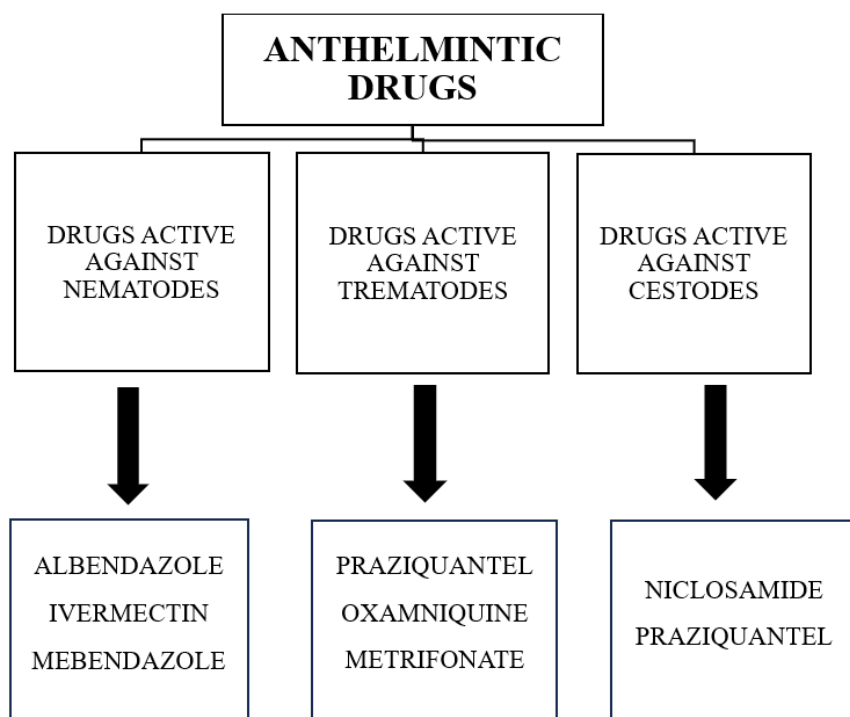


Fig No:2 ANTHELMINTIC DRUGS

1.5. HERBS USED AS ANTHELMINTIC

- Ocimum Sanctum Linn*
- Carica papaya*
- Nigella sativa*
- Annona Muricata*^[2]

1.6. ANNONA MURICATA

Annona muricata Linn is a member of the Annonaceae family. It has been the subject of extensive research in recent decades, because of its potential for therapeutic use. The plant leaf is known as "the cancer killer" and is, as its name implies, also employed in traditional medicine to cure cancer.^[3]

The fruit of *Annona muricata* is reported to taste both sweet and sour, hence the popular name "soursop." The soursop tree is a small, fast-growing perennial with branches.^[3]

1.6.1. USES OF ANNONA MURICATA

Like other *Annona* species, such as *Annona squamosa* and *Annona reticulata*, all parts of the *Annona muricata* tree are widely utilized as traditional medicines to treat a variety of human maladies and diseases, particularly cancer and parasitic infections.^[4]

Annona muricata is also known for its anti-inflammatory, hypoglycemic, sedative, smooth muscle relaxant, hypotensive, and antispasmodic properties found in its leaves, bark, and roots. Apart from their traditional therapeutic applications, fruits are extensively utilized in the creation of drinks, candies, frozen desserts, shakes, and syrups. It can also be used to treat diabetes, fever, respiratory, and skin conditions, as well as analgesia.^[5]

MATERIALS & METHODS AND

2.1. Collection and Identification of Plant Material

Fresh leaves of *Annona muricata Linn* (Annonaceae) were collected from a household garden in Coimbatore, Tamil Nadu. The taxonomic identities of the plant is confirmed by Botanical survey of india. Fresh leaf material was washed under running tap water, air dried in shade and then homogenized to fine powder and stored in sterile air tight bottles for the experimental work.

2.2. Extraction

The extraction was done by cold maceration using ethanol and distilled water as solvents. 25 gm of the dried powder of *Annona Muricata* leaves was weighed into two separate round bottom flasks and 250 ml of the

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solvents were added to each flask containing the powder, the mixture was allowed to stand for 5 days at room with agitation at regular intervals. The extracts were filtered separately through a filter paper. The extracts was concentrated to remove the solvents.

$$\% \text{ yield} = \frac{\text{Weight of the crude extract}}{\text{Weight of the sample}}$$

2.3. PRELIMINARY PHYTOCHEMICAL SCREENING

The crude ethanolic and aqueous extract was assessed for secondary metabolites such as alkaloids, Tannins, Glycosides, Steroids, Terpenoids, Flavonoids, Coumarins, and Phenols using standard methods.

1) TEST FOR ALKALOIDS

Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrate were treated with dragendroff's reagent. Formation of red colour precipitate indicate the presence of alkaloid.

2) TEST FOR FLAVANOIDS

Dilute ammonia was added to a portion of an aqueous filtrate of the extract. Then, concentrated sulphuric acid was added. A yellow colouration indicated the presence of flavonoids.

3) TEST FOR SAPONINS

Extract was added to 5ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

4) TEST FOR TANNINS

The extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration. It indicate the presence of tannins.

5) TEST FOR TRITERPENOIDS

The extract was dissolved in 1ml of chloroform. 1ml of acetic anhydride was added, followed by the addition of 2ml of concentrated sulphuric acid. Formation of reddish violet colour indicates the presence of triterpenoids.

2.4. IN-VITRO ANTHELMINTIC ACTIVITY:

Anthelmintic activity of ethanolic and aqueous leaf extract of *Annona Muricata*

2.4.1. Experimental worms

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for the experiment due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human being.



Earth Worm

2.4.2. Administration of extract

The suspension of Ethanolic and aqueous leaf extract of *Annona muricata* concentration (100mg/ml, 200mg/ml) were prepared by using Normal saline as a vehicle and final volume was made up to 100ml for respective concentration. Groups of approximately equal size worms consisting of four earthworms individually in each group were released into in each 10 ml of desired concentration of extracts in the petridish and compared to the standard drug (Albendazole).

RESULTS & DISCUSSION

3.1. PERCENTAGE YIELD OF LEAVES 7 ROOTS EXTRACTS

Annona muricata leaves and roots powder was extracted by using the cold maceration method. The aqueous and ethanol was used as a solvent in the ratio of 1:10. Maceration is carried out for 5 days. After the filtration the filtrate was evaporated to remove the solvent.

S.NO	WEIGHT OF SAMPLE (gm)	SOLVENT	IMMERSION TIME	WEIGHT OF CRUDE EXTRACT(gm)	PERCENTAGE YIELD
1)	25	AQUEOUS	5 DAYS	1.2	4.8 %
2)	25	ETHANOL	5 DAYS	1.9	7.6 %

TABLE NO: 1

The table 1 shows that the result of extracted *Annona muricata* leaves by the cold maceration method.

3.2. PHYTOCHEMICAL SCREENING

S.NO	COMPOUND	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
1)	ALKALOIDS	+ve	-ve
2)	FLAVANOIDS	+ve	+ve
3)	SAPONINS	+ve	+ve
4)	TANNINS	+ve	+ve
5)	TRI-TERPENOIDS	+ve	+ve

TABLE NO:2

Table 2 shows that the phytochemical screening of ethanolic and aqueous extracts of *Annona Muricata* leaves.

3.3. IN-VITRO ANTHELMINTIC ACTIVITY:

To evaluate the anthelmintic activity of aqueous and ethanolic leaves extracts of *Annona muricata* and which was found to dose dependants. The data were expressed in mean \pm SEM (Statistical analysis) values and compared with control or standard drug. The experimental results of anthelmintic activity in earthworm were

given in table which reveal that the leaves extracts shown the paralysis and death condition in different concentration of extracts and it was compared with the standard drug albendazole.

OBSERVATION:

Observations were made for noted the time taken for the paralysis and death of during the investigation. The paralysis time was noted when there was no movement of any part of the body. Death time was noted followed by paralysis condition. Death time was noted when no movement of body parts after being vigorous shaking and also fading of body colour of the worms.

S.NO	EXTRACT	CONCENTRATION (mg/ml)	PARALYSIS TIME (min)	DEATH TIME (min)
1.	AQUEOUS EXTRACT	100 mg/ml	+ 155	+ 268
		200 mg/ml	+ 99	+ 172
2.	ETHANOLIC EXTRACT	100 mg/ml	+ 122	+ 214
		200 mg/ml	+ 88	+ 104
3.	STANDARD DRUG (ALBENDAZOLE)	100 mg/ml	+ 69	+ 98



ETHANOLIC EXTRACT (100mg/ml)



AQUEOUS EXTRACT (100mg/ml)



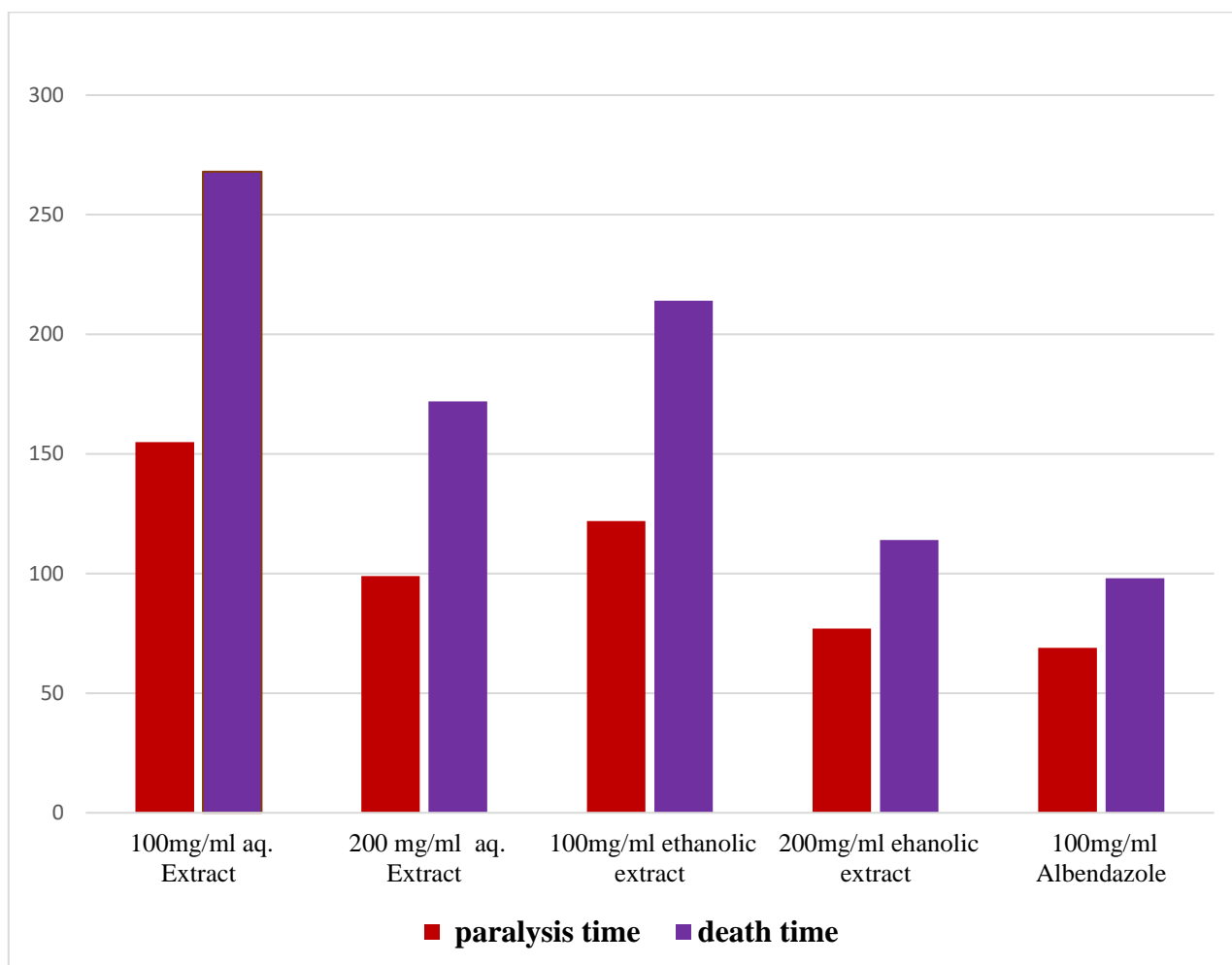
ETHANOLIC EXTRACT (200mg/ml)



AQUEOUS EXTRACT (200mg/ml)



STANDARD DRUG (ALBENDAZOLE-100mg/ml)



ANTHELMINTIC ACTIVITY OF ETHANOLIC LEAVES & ROOTS EXTRACTS OF *ANNONA MURICATA* AGAINST EARTHWORM

3.4. EXTRACTION OF PLANT MATERIAL

- The dry powder of *Annona muricata* leaf was extracted with distilled water and ethanol by cold maceration method. The percentage yield of aqueous and ethanolic leaf extracts was found to be 4.8 % and 7.6% respectively

3.5. PHYTOCHEMICAL SCREENING METHOD

- In our finding this study revealed that the presence of secondary metabolites in the both leaf extracts. The ethanolic leaves extract of *Annona muricata* shows the presence of alkaloid, flavonoids, tannins, terpenoids, saponins. Also, aqueous leaves extracts of *Annona muricata* shows the presence of flavonoids, saponins, tannins, terpenoids and absence of alkaloid. The secondary metabolites were present in both extracts, but with the noticeable difference in relative abundance ranging from low, average, high.

3.6. *IN-VITRO* ANTHELMINTIC ACTIVITY:

- Commonly used anthelmintic drug are made from the synthetic medication which have a number of side effects. so, the drug derived from the medicinal plant (Herbs) may have less side effects or no side effects.
- The anthelmintic potential of *Annona muricata* leaf extract is assessed against earthworm by tracking the time for paralysing and death.
- This investigation is possible to assess if *Annona muricata* leaves may have the source of anthelmintic medication.
- The aqueous and ethanolic leaf extracts of *Annona muricata* was administrated to the earthworm at the different concentration (100mg/ml, 200mg/ml). These extracts show the paralysis and death in both concentrations. This effect is compared with the effect produced by the standard drug (Albendazole) against earthworm.

CONCLUSION:

The World Health Organisation has estimated more than 80% of the population in developing countries depends primarily on herbal medicine for their healthcare needs in recent years. For the last few years *Annona muricata* has been studied due to their application in the treatment of parasitological disease. In the extraction process we get more yield in ethanolic leaf and Root extract than that aqueous leaf and Root extract. In this ethanolic extracts of 200 mg/ml concentration elicit high anthelmintic activity when compared to other concentration of *Annona Muricata* extracts. Further studies are required to find out and to establish the effectiveness and to establish pharmacological motivation by using *in-vivo* model for the leaves along with roots as an anthelmintic drug. And also required to isolate active constituents from the extracts and establish the mechanism of action.

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