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Available online at: www.jpardonline.com**Anthelmintic activity of ethanolic extract of *Annona reticulata* bark**

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ABSTRACT:

Background: *Annona reticulata*, a member of the *Annonaceae* family, is used for a variety of medicinal purposes, including analgesic, anti-inflammatory, antipyretic, wound healing, and cytotoxic actions. **Aim:** The aim of this study was to determine the anti-helminthic activity of an ethanolic extract of *Annona reticulata* bark on adult Indian earthworms. **Method:** There are 7 groups containing six earthworms in each petri dish. The normal control, standard, and test were treated with normal saline, three different concentrations of albendazole (10, 20, and 40 mg/ml), and ethanolic extract of *A. reticulata* bark (10, 20, and 40 mg/ml) in each petri dish respectively. The activity of assessment was performed based on the time taken for complete paralysis and death was recorded in min. **Results:** The results showed that ethanolic extract of *A. reticulata* bark (40 mg/ml) shows significant ($p < 0.05$) anti-helminthic activity in comparison with the standard drug, albendazole (40 mg/ml) by reducing the time taken for paralysis and death of helminths. **Conclusion:** It could be concluded that the bark extract of *A. reticulata* exhibited a potent anthelmintic activity at a concentration of 40 mg/ml.

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INTRODUCTION:

There are various worms that affect both humans and animals. Anthelmintics are a class of anti-parasitic medications that paralyze or kill parasitic worms and other internal parasites without inflicting significant harm to the host ^[1]. Helminthes affect around 24 % of the global population and are mostly transmitted via the soil. Countries like America, China, Sub-Saharan Africa, and East Asia recorded the greatest number of cases ^[2]. It is one of the greatest neglected tropical diseases. Helminthiasis may cause stunted growth, elephantiasis, vitamin deficiencies, anaemia, blindness, protein-calorie undernutrition. The worms ingest on host tissues and

Keywords: *Annona reticulata* Bark, *Annonaceae*, Albendazole, Anti-helminthic, Paralysis, Cytotoxic effects.

blood and cause loss of iron and protein ^[3]. There are diverse helminths infections, soil-transmitted helminths are the most common followed by *Schistosomas*, filarial worms, lymphatic filariasis, onchocerciasis, ascariasis, trichuriasis, platyhelminthes, and enterobius ^[4]. An evaluation of the anthelmintic activity has been attempted on *Eisenia fetida* of almost equal sizes using the bark extract of *Annona reticulata* ^[5]. *A. reticulata* (Fig 1) belonging to the family *Annonaceae* is best known for its fruit which is the custard apple.



Fig 1. Plant - *Annona reticulata*.

A. reticulata is native to the Caribbean and Central America. It is cultivated in Southeast Asia, Taiwan, India, Bangladesh, Pakistan, Australia, and Africa ^[6]. The main phytoconstituents of *A. reticulata* bark are Tannins, Alkaloids, and Phenolic compounds. The chemical present in the bark is monotetrahydrofuron acetogenins, reticulatacin, alkaloids such as liriodenine, copaene, patcholane, bullatacin ^[7]. They possess several medicinal properties like anthelmintic, analgesic, anti-inflammatory, anti-pyretic, wound healing, and cytotoxic effects ^[8]. The bark of *A. reticulata* has not yet been proved for anti-helminthic activity. So, the present study has planned to study the ethanolic extract of *Annona reticulata* bark for anthelmintic activity in helminths.

MATERIALS AND METHODS:

Chemicals and reagents:

The standard drug, Albendazole was procured from Cadila Pharma, Mumbai. The ethanol and acacia were procured by Karnataka Fine Chemicals, Karnataka. The chemicals and reagents used in this study were of analytical grade and procured from an authorized dealer.

Bark extract:

The dried bark of *A. reticulata* was obtained from the approved market (Amruth Keasari) at Bengaluru. The

bark in dried form was ground into powder and the powder was extracted with 70 % ethanol using Soxhlet apparatus (Borosil, India). The extract (200 mg) of *A. reticulata* bark was weighed and the suspension was prepared using 1 % acacia. The different concentrations of *A. reticulata* bark extract were prepared at concentrations of 10, 20, and 40 mg/ml for the study.

Preparation of albendazole standard:

The standard Albendazole with 1 % w/v of acacia was used to make albendazole suspensions at strengths of 10, 20, and 40 mg/ml.

Animals:

Pheretima posthuma (Indian adult earthworms) of about 4 to 6 cm were collected from the local area of Amruth Keasari, washed, and kept in distilled water for the study.

Evaluation of Anthelmintic activity:

The study included *Pheretima posthuma* (Indian adult earthworms) of about identical size (4 to 6 cm). Prior to the investigation, the worms were acclimatized to the laboratory conditions. In each petri dish, earthworms were separated into seven groups, each with six earthworms. The normal saline was used as a control. Albendazole suspension of different concentrations of 10, 20, and 40 mg/ml was poured into Petri dishes of the standard group. Ethanolic extract of *A. reticulata* bark suspension of different concentrations of 10, 20, and 40 mg/ml was poured into Petri dishes of the test group. The time it took for total paralysis and death to occur was measured in min. Each earthworm was applied superficial inducements to assess its movement and to determine if it were alive ^[9].

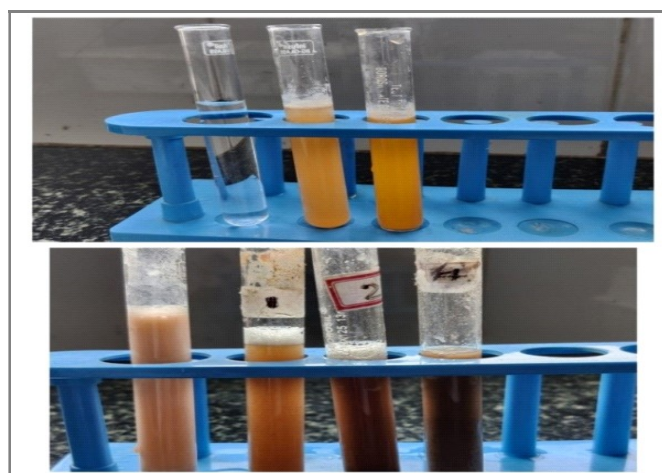


Fig 2. Test tubes containing control, various concentrations of Albendazole and *Annona reticulata* bark extract.

RESULTS AND DISCUSSIONS:

The results of the effect of *A. reticulata* bark extract on helminths were represented in Table 1 and Fig 2 to 6. Among the various concentrations of standard

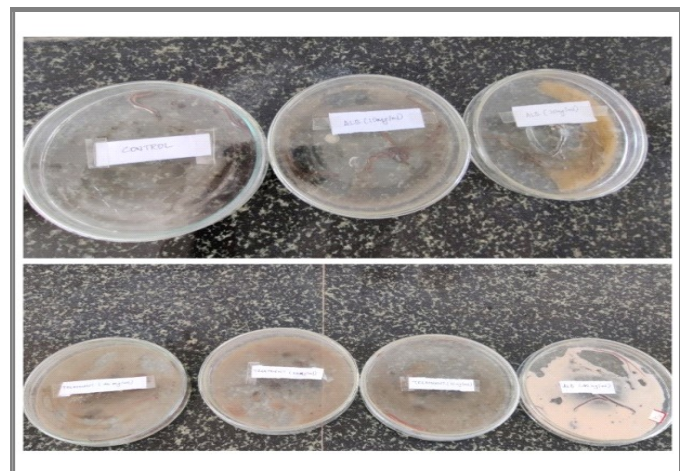


Fig 3. Petri dish containing worms of approximately the same size in various concentrations of albendazole and *Annona reticulata* bark extract.



Fig 4. Petri dish containing various concentrations of Albendazole and *Annona reticulata* bark extract at time 0.

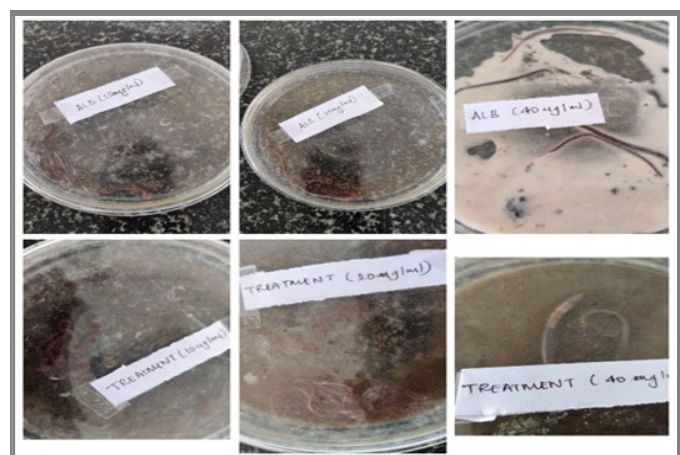


Fig 5. Petri dish containing various concentrations of Albendazole and *Annona reticulata* bark extract at 500 min.

albendazole and ethanolic extract of *A. reticulata*, the concentration of 40 mg/ml of *A. reticulata* showed a notable anthelmintic activity.

The results thus obtained depict that the bark extract of *A. reticulata* is a more significant ($P < 0.5$) anthelmintic agent against the *Pheretima posthuma* when compared to the standard drug. The time taken for paralysis was 245 min and the time taken for death was 314 min by the ethanolic extract of 40 mg/ml of *A. reticulata* whereas albendazole shows the time taken for paralysis is 259 min and time taken for death is 366 min respectively.

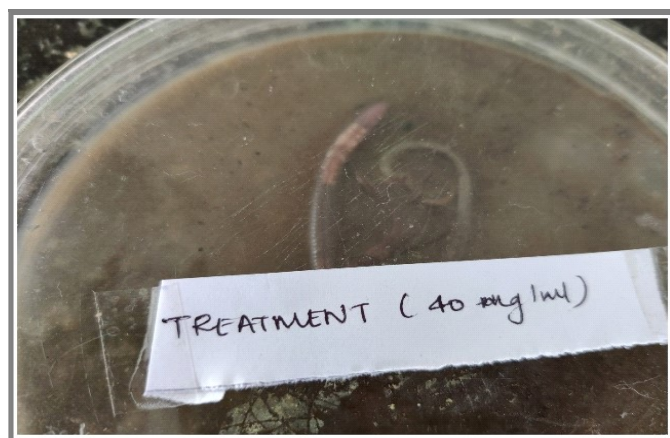


Fig 6. Petri dish containing Treatment group (*Annona reticulata* bark extract – 40 mg/ml).

The control group containing normal saline when exposed to composting worms showed no change in movement and remained active. Albendazole treated group with the concentration of 40 mg/ml when exposed to composting worms displayed paralysis and death in 257 and 366 min respectively. When 20 mg/ml concentration of the albendazole and extract were exposed to composting worms, the time required to obtain total paralysis and death was at 262 and 446 min respectively. The 10 mg/ml concentration displayed a prominent delay to obtain paralysis and death when exposed to albendazole extract and the time required was at 292 and 476 min respectively.

A. reticulata bark involves a number of phytochemicals with a variety of pharmacological properties. Alkaloids, Steroids, Tannins, Lignin, Phenolic compound, Lipids, and Oils were discovered in the bark [10]. The evidence reveals that the alkaloidal content shows the anthelmintic activity in the bark extract. The paralysis of the worms is primarily caused by the alkaloid content [11]. Tannins chemically are polyphenolic compounds that possess anthelmintic activity. Some of the Tannins

Table 1. Effect of *Annona reticulata* bark extract on helminths.

Sl. No.	Treatment group	Concentration of drug	Time taken for paralysis (min)	Time taken for death (min)
1	Control (Normal saline)	0.9 % w/v	-	-
2	Albendazole	10 mg/ml	292±4.56	476±4.20
3	Albendazole	20 mg/ml	262±5.67	446±3.68
4	Albendazole	40 mg/ml	259±5.50	366±3.44
5	<i>Annona reticulata</i> bark extract	10 mg/ml	330±4.89	458±5.23
6	<i>Annona reticulata</i> bark extract	20 mg/ml	318±4.28	426±5.17
7	<i>Annona reticulata</i> bark extract	40 mg/ml	245±5.20*	314±4.60*

All the values are expressed in Mean±SEM (n=6). The statistical analysis was carried out using one-way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett multiple comparison test. * $P<0.5$, to standard ALB (Albendazole) group.

are responsible for uncoupling the oxidative phosphorylation process and interfering in the energy production process of worms [12]. Tannins also bind to the free protein present in the GIT of the host species or also bind to glycoprotein present in the cuticle of the parasite ultimately causing the death of the organism [13]. The bark extract of *A. reticulata* contains aporphine alkaloids with a variable spectrum of biological activity [14]. The aporphine mainly binds to the alpha 1-adenoreceptor associating with the sleep pattern and ultimately resulting in flaccid paralysis. The methylenedioxy ring present is mainly responsible for the cytotoxic role of aporphine. They inhibit the calcium channels and also impact sodium ion channels [15,16].

Thus, when exposed to Indian adult earthworms, *Annona reticulata* bark extract showed a dose-dependent activity in paralysis and death of the organism and displayed a significant anthelmintic activity at a dosage of 40 mg/ml. When compared to albendazole the ethanolic extract of *Annona reticulata* bark extract possessed a potent anthelmintic activity due to the presence of various phytochemicals in the bark of *Annona reticulata*. The synergistic effect of multiple phytochemicals presents in the bark extract of *A. reticulata* is responsible for obtaining paralysis and mortality against Indian adult earthworms.

CONCLUSION:

The bark extract of *Annona reticulata* was tested against *Pheretima posthuma* at various dosages (10, 20, and 40 mg/kg), and it was found that the bark extract has significant anthelmintic activity at a dose of 40 mg/ml. When compared to the conventional treatment albendazole (40 mg/kg), the worms displayed paralysis followed by death in the shortest period of time at this dose. Thus, the *A. reticulata* bark extract caused

mortality in a short period of time, indicating its potent anthelmintic activity.

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