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Anthelminthic Activity of *Annona reticulata* Seed Extracts

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Authors' contributions

Authors may use the following wordings for this section: This work was carried out in collaboration between all authors. Author HJH designed and guided the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author DRYK carried out and managed the analyses of the study. Author ABV managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To evaluate the anthelminthic activity of *Annona reticulata* seeds extracts against *Pheretima posthuma.*
Study Design: Helminth infections have tormented humans and animals for thousands of years. The indiscriminate uses of the anthelminthic drugs have lead to the emergence of resistant helminths against many anthelminthic agents. Thus the need for the treatment of helminthic infections and the prevention of the emergence of resistant strains has led to the screening medicinal plants for their anthelminthic activity.
Place and Duration of Study: Department of Biotechnology, The Oxford College of Science, Bangalore, Karnataka, India, between January 2012 and March 2012.
Methodology: The main aim of the present exploratory study is to evaluate the anthelminthic activity of *A. reticulata* seeds extracts. The *in vitro* studies revealed that the anthelminthic effects of crude chloroform and ethanolic extracts of *A. reticulata* on *Pheretima posthuma* was evident from induction of paralysis and mortality. Piperazine citrate was used as the standard reference drug and normal saline as a control group.
Results: Among all the concentrations of chloroform and ethanolic extracts tested, 12.5 mg/ml showed significant ($p<0.01$) anthelminthic activity. The results indicated that the ethanolic extract of *A. reticulata* is a more potent anthelminthic agent against *Pheretima*

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posthuma when compared with chloroform extract but less potent when compared with the standard drug.
Conclusions: The results of this investigation justifies the use of the seed extracts of *A. reticulata* in traditional medical practice for the treatment of helminth infections.

Keywords: Annona reticulata; Annonaceae; Anthelminthic activity; Pheretima posthuma; ethanolic extract; chloroform extract.

1. INTRODUCTION

Helminthiasis is one of the most prevalent diseases and one of the most serious public health problems in the world. With the increased world travel and immigration from the developing countries, helminth infections are now prevalent throughout the world, affecting millions of humans and animals [1]. Parasitic diseases causing severe morbidities in many parts of the world include lymphatic filariasis, onchocerciasis, and schistosomiasis. To combat the chronic spread of helminth infections in humans and animals, many pharmaceutical industries have released many anthelminthic drugs, and the excessive use of these anthelminthics has lead to the emergence of resistant helminths against these synthetic anthelminthics and has became a severe problem worldwide [2]. Moreover, these drugs are unaffordable, inaccessible or inadequately available in most third world and/or underdeveloped countries.

Diseases caused by helminth infections in livestock's continue to be a major productivity constraint, especially in the tropical and subtropical countries [3]. Although plants act as nutrition for animals, they also appear to act as anti parasitic agents [4]. Investigation and exploration of biologically active principles from plant sources has always been of great interest for traditional medicinal practitioners and scientists, who are mainly working to unmask the novel useful drugs from plant source against infectious diseases. Nowadays, the use of medicinal plants is growing worldwide because of the increasing toxicity and side effects of the pharmaceutical drugs [5]. Evaluation of the bioactive principles of medicinal plants has indicated that many indigenous plants possess potent anthelminthic property, thus medicinal plants are getting attention of many scientists [6].

Annona reticulata Linn, commonly known as custard apple belongs to the family Annonaceae. This small tree species is found generally all through India. The fruit is variable in shape like heart-shaped or spherical. Study reveal that leaves are used as insecticides, anthelminthic, styptic and are also externally used as suppurant [7]. The bark contains the antidysentric and vermifuge and are used as astringent. Isoquinoline alkaloids are present in the various parts of the plant like root, bark, leaves and stem [8]. Acetogenins which are present in the leaves of *A. muricata* were found to be selectively cytotoxic to certain human tumors [9]. As per the literature survey the seed is used to treat various types of gastro intestinal problems. In the traditional medicine, the various parts of this plant are used for the treatment of fever, dysentery and heart diseases. *Annona reticulata* has been reported to contain aporphine, anonaine, norcorydine, coryeline and glaucine. It is also reported that the leaves of this plant also contains anonaine, benzyl tetrahydro-isoquinoline, borneol, β-caryphyllene, camphene, eugenol, farnesol, geraniol, 16-hetriacontanone, hexacontanol, isocorydine, limonine, linalool acetate menthone, methyl anthranilate, methylheptenone, methylsalicylate, octacosanol, pinene, rutin, stigmasterol, β-sitosterol, thymol and triacontanol. Alkaloids and proteins are reported to be absent in the leaves of this plant

[7,10]. Literature survey revealed that the anthelminthic activity of *A. reticulata* seed extract has not so far been scientifically reported. In our interest in this plant, we made an productive workout to assay the anthelminthic activity of the chloroform and ethanol extract of *A. reticulata* using *Pheretima posthuma* as an experimental helminth model.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

The standard drug piperazine citrate (K. Patel Chemo pharma Pvt.,Ltd, Mumbai). Ethanol was purchased from Hong, Yang Chemical Corporation, China. Chloroform (Merck, India).

2.2 Plant Resource

Annona reticulata seeds were collected by completely removing the above fruit flesh (Fig. 1). Fresh seeds were washed with distilled water thoroughly to remove traces of contaminants. These processed seeds were then shade dried for one month (Fig. 2). After complete drying the seed coat was broken and powdered mechanically and was subjected to cold extraction using chloroform as the solvent system for about 96 h. After every 24 h fresh chloroform was added and chloroform containing the crude extract was separated, followed by ethanol extraction sequentially in the similar fashion. Both extracts were filtered and concentrated in vacuo under reduced pressure and allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The yield of crude ethanol and chloroform extract for 1 kg of powdered seed material were 46 g and 53 g respectively.

Fig. 1. Shade dried *Annona reticulata* seeds

Fig. 2. Powdered *Annona reticulata* seeds

2.3 Test Organism

Indian adult earthworms (*Pheretima posthuma) were* collected from the Indo-American Hybrid Seeds, Bangalore. The earthworms were maintained under normal vermicomposting medium with adequate supply of nourishment and water, for about two weeks. Before the initiation of experiment the earthworms were washed with normal saline. Adult earthworms of approximately 4 cm in length and 0.2-0.3 cm in width were used for the experiment. This organism was selected as a model for anthelminthic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [11-12].

2.4 Extract Preparation for Experiment

The porously powdered *A. reticulata* seed material was used for extract preparation. After extraction, the crude extracts were stored in desiccators until further use. Test extracts and standard drug piperazine citrate were dissolved in 0.5% DMSO in normal saline (v/v) and were used for evaluation for anthelminthic activity.

2.5 Antihelmintic Activity

The experimental protocol for the anthelminthic activity was carried out as per the method reported by Dash et al. [13]. The anthelminthic activity of chloroform and ethanol extracts of *A. reticulata* was evaluated. The earth worms were divided into twelve groups with three earth worms in each group. Each earthworm was separately released into 20 ml of desired formulation in normal saline, Group I earthworm were released in 20 ml normal saline in a clean Petri plate. Group II, III, IV, V, VI earthworms were released in 2.5, 5.0, 7.5, 10.0 and 12.5 mg/ml of chloroform extract respectively. Similarly, group VII, VIII, IX, X, XI earthworms were released in 2.5, 5.0, 7.5, 10.0 and 12.5 mg/ml of ethanol extract respectively. Group XII earthworms were released in normal saline containing standard drug piperazine citrate (2.5 mg/ml). Earthworms were observed; and the time taken for paralysis and the time taken for death was monitored and documented in minutes. Paralysis time was analyzed based on the

behaviour of the earthworm with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body colour [14].

2.6 Statistical Analysis

The data of anthelminthic evaluations were expressed as mean ± S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at P< 0.01 was considered as statistically significant [13]. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

3. RESULTS AND DISCUSSION

In the present study, *A. reticulata* seeds were sequentially extracted using chloroform and ethanol as the solvent system. In continuation with our interest, this investigation on evaluating the anthelminthic property of *A. reticulata* was carried out.

Table 1. *In vitro* anthelminthic activity of chloroform and methanol extracts of *Annona reticulata* against *Pheretima posthuma*

Test samples	Concentration (mg/ml)	Paralysis Time (min)	Death Time (min)
Control (Normal Saline)	--	236.67 ± 5.04	246.33 ± 2.4
Chloroform extract of *Annona reticulata*	2.5	54.33 ± 3.76**	91.33 ± 2.03**
	5.0	52.67 ± 2.33**	96 ± 2.89**
	7.5	44.33 ± 2.03**	87.67 ± 2.33**
	10.0	43.33 ± 3.84**	84.67 ± 0.88**
	12.5	38.67 ± 2.03**	71.33 ± 0.88**
Ethanolic extract of *Annona reticulata*	2.5	85.33 ± 3.76**	108.67 ± 2.96 **
	5.0	75.33 ± 1.45**	97.67 ± 2.33**
	7.5	62.33 ± 2.6*	86.67 ± 2.33*
	10.0	59.33 ± 7.13**	74.33 ± 7.51**
	12.5	37.67 ± 2.91**	53.67 ± 4.1**
Piperazine citrate	2.5	19.33 ± 2.52**	36.67 ± 2.6**

*Values are the mean ± S.E.M. of three earthworms. Symbols represent statistical significance. * P < 0.05, ** P < 0.01, ns: not significant as compared with group.*

Chloroform extract at the concentration of 2.5 mg/ml showed the time of paralysis and death at 54 and 91 min respectively. For concentration of 5 mg/ml, the paralysis and the death time was found to be 52 and 96 min respectively. At the concentration of 7.5, 10 and 12.5 mg/ml, time for onset of paralysis were 44, 43 and 38 min respectively. Among the various concentrations tested, chloroform extract at 12.5 mg/ml showed efficient anthelminthic activity (Table 1). On the other hand ethanolic extract at the concentration of 2.5 mg/ml showed the time of paralysis and death at 85 and 108 min respectively. For concentrations at 5, 7.5, 10 and 12.5 mg/ml paralysis were shown at 75, 62, 59 and 37 min respectively and death occurred at 97, 86, 74 and 53 min respectively. Among all the concentrations ethanolic extract tested, 12.5 mg/ml produced significant results. Standard drug at 2.5 mg/ml showed paralysis at 19 min and death time was 36 min (Table 1, Fig. 3).

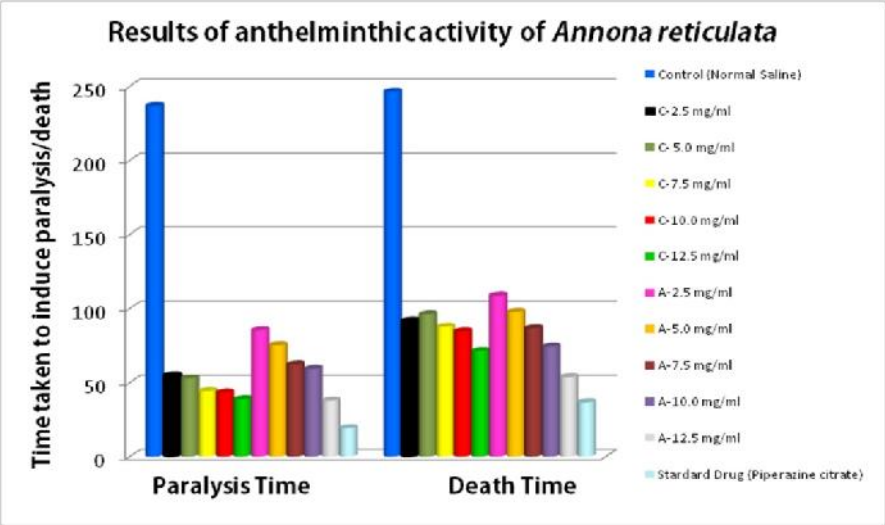

Fig. 3. Bar chart illustrating the comparative *in vitro* anthelminthic effect of different concentrations of chloroform and methanol extracts of *Annona reticulata.* (CE- Chloroform extract, ME- Ethanolic extract)

Many other investigators have reported the anthelminthic activity of different medicinal plant viz., *Manilkara zapota, Gynura angulosa, Calotropis procera* etc [15-17]. This investigation revealed that ethanolic extract of *A. reticulata* showed significant anthelminthic activity against *Pheretima posthuma* when compared chloroform extract. Ethanolic extract also proved to be more potent than the standard drug Piperazine citrate. This investigation reports a new potent anthelminthic agent that can be used as a potent drug for the treatment of helminth infections. The present study can act as a basis for further phytochemical evaluation of *A. reticulata* seeds to isolate potent anthelminthic compound.

4. CONCLUSION

In the present investigation we explored the anthelminthic property of *A. reticulata* seeds extract. However, ethanolic extract of *A. reticulata* seeds proved to be most potent when compared to chloroform extract. Further investigation need to be carried out to unmask its mode of action by *in vivo* studies using animal models.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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