

in vitro Anthelmintic activity of *Annona squamosa* (Annonaceae) Leaves

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Abstract: Pune region of Maharashtra is inhabited by several tribes and they are using several plants or plant-based preparations for the treatment of various ailments in their traditional system of medicine. During our course of studies on ethnomedicine of this region, the plant being used as anthelmintic is leaves of *Annona squamosa* (Annonaceae) This plant has a wide reputation among natives of being curative for intestinal-worm infections. Based on this, an attempt has been made to evaluate the anthelmintic potential of this plant.

Methanolic extract of *Annona squamosa* leaves was investigated for anthelmintic potential against earthworm (*Esenia fetida*). Various concentrations (25-100 mg/ml) of methanolic plant extract were tested in the bioassay and different parameters such as determination of paralysis and time of death of worms were recorded. It exhibits significant anthelmintic activity at highest concentrations of 100 mg/ml. Albendazole (20 mg/ml) was used as reference standard and distilled water as control.

Keywords: Anthelmintic activity, *Esenia fetida* , *Annona squamosa*.

INTRODUCTION

Helminth infections are among the most common infections in humans, affecting a large population of the world. Although the majority of infections due to worms are generally limited to tropical regions and pose a great threat to health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia¹. Parasites diseases causes severe morbidity affecting mainly population in endemic areas with major economic and social consequences². The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases³ ; hence there is an increasing demand towards natural anthelmintics.

Annona squamosa L. (Annonaceae) is a small, semi deciduous, much branched shrub or small tree about 3-8 m tall. Leaves are singly, pale green on both surfaces and mostly hairless while flowers are solitary in

nature, greenish yellow in color. It contains annoin, moupinamide, sachanoic acids, alkaloids, sqamocenin, reticulín and sqamocin⁴. Leaves are used in clarification of urine while roots are used in treatment of diarrhea⁵. Seeds are mostly used as abortifacient, insecticidal and used in destroying lice in hairs. Antimicrobial^{6,7}, hepatoprptective⁸, insecticidal⁹, antidiabetic^{10,11,12}, analgesia, anti-inflammatory¹³, anti-head lice^{14,15}, antimalarial¹⁶ and molluscicidal¹⁶ activities has been evaluated. It also shows fruits of *Annona* are Haematinic, cooling, sedative, expectorant, tonic, in making ice creams and milk beverages⁴.

Literature survey revealed that leaves extract has yet not been screened for anthelmintic activity. Hence, the present study was undertaken to evaluate the anthelmintic property of *Annona squamosa* (Annonaceae) leaves.

MATERIAL AND METHODS

Plant material

The *Annona sqamosa* (Annonaceae) leaves were collected from the Abhinav college of B Pharmacy campus, Narhegoan (MAHARSHTRA) in October 2010. The plant material were identified and authenticated in Botanical Survey of India, Koregaon park, Pune (Ref BSI/WRC/Tech/2009 Voucher no. KDHAS1 and Voucher herbarium specimens was deposited in the Department of Pharmacognosy of our College. The plant material was dried in sunlight, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.

Preparation of extract

Methanolic extract (Maceration method) of powdered material of *Annona sqamosa* (Annonaceae) leaves (200 gm) was kept for maceration with 1000 ml of distilled water for 12 hrs. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be 2.95 percent

Animals

Adult earthworms (*Esenia fetida*) were used to evaluate anthelmintic activity *in vitro*. *Esenia fetida* collected from moist soil of the botanical garden were washed with normal saline and used for the anthelmintic study. The earthworms (6-9 cm in length and 0.1-0.2 cm in width) were used. Earthworm were identified in Dept. of Zoology Padmashri Vikhe Patil Science College, Loni, Ahemadnagar and services of veterinary practioners were utilized to confirm the identity of worms

Drugs and chemicals

Albendazole (CIPLA) was used during the experimental protocol.

ANTHELMINTIC ACTIVITY

Anthelmintic bioassay

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.*, 2001¹⁷ with minor modifications. The assay was performed on adult Indian earthworm, *Esenia fetida* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings¹⁸. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*^{19,20,21}. Methanolic extract from the leaves of *Annona sqamosa* was investigated for their anthelmintic activity against *Esenia fetida*. Various concentrations (10-100 mg/ml) of each extract were tested in the bioassay, Albendazole was included as standard reference and distilled water as control. The assay was performed on *Esenia fetida*. The earthworms of 6-9 cm in length and 0.1-0.2 cm in width were used. In the experiment, six groups of six earthworms were released in to 50 ml of solutions of albendazole, methanolic extract of leaves of *Annona sqamosa* (25, 50 and 100 mg/ml each) in distilled water. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously nor when dipped in warm water (50 °C). Death was concluded when the worms lost their motility followed with fading away of their body colors²². All the results were shown in Table.1 and expressed as a mean \pm SEM of six worms in each group.

Table.1 Anthelmintic activity of *Annona sqamosa* leaves

Extract	Concentration (mg/ml)	Time required to paralyse. (min)	Time required to death of worm. (min)
Control	-	-	-
Methanolic extract	25	14.12 \pm 0.200	32.45 \pm 0.200
	50	6.56 \pm 0.060	19.27 \pm 0.270
	100	4.27 \pm 0.338	9.30 \pm 0.230
Albendazole	20	2.08 \pm 0.080	6.1 \pm 0.073

Each value represents mean \pm SEM (N=6).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of alcoholic extract revealed the presence of phenolic compounds, tannins, mucilage and alkaloids. From the results shown in table no. 1, the predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.

Results obtained indicates that the higher concentration of each plant extract produced paralytic effect much earlier and the time to death was shorter. The results of the current investigation indicate that among the extracts of *Annona squamosa*, the methanolic extract is the potent one and requires less time to the paralysis and death of the worms. Methanolic extract showed a concentration depended anthelmintic property (Table 1). Methanolic extract of *Annona squamosa* demonstrated paralysis as well as death of worms especially at higher concentration of 100 mg/ml while 50mg/ml concentration also shown significant activity. The preliminary phytochemical analysis of the extracts has shown the presence of phenolics²³ like tannins and saponins as well as alkaloids. Synthetic phenolic anthelmintics like niclosamide interfere with the energy generation in the helminth parasites by uncoupling the oxidative phosphorylation^{24,25}. Another possible mechanism of action is that they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and by this cause death. Tannins have also been shown to produce

anthelmintic activities. From the above results, it is concluded that *Annona squamosa* used by tribals traditionally to treat intestinal worm infections, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

CONCLUSION

The traditional use of leaves of *Annona squamosa* as an anthelmintic have been confirmed as the leaves extract displayed activity against the worms used in the study. Further studies need to isolate and reveal the active compound contained in the crude extracts of *Annona squamosa* and establish the mechanism of action are required.

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