



# Research Journal of Pharmaceutical, Biological and Chemical Sciences

## Anthelmintic activity of *Annona reticulata* leaves.

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### ABSTRACT

Leaves of *Annona reticulata* L. (Annonaceae) were dried, powdered and extracted using ethanol and further fractionated using petroleum ether, chloroform, ethyl acetate, and ethanol. Anthelmintic activity of these various fractions was evaluated on adult Indian earthworms, *Pheretima posthuma*. Results showed that ethanol extract took less time to cause paralysis of the earthworms. In order to pinpoint the active constituent the ethanol fraction was further fractionated using column chromatography to yield fraction 1, 2, and 3 and again screened for activity. Results showed that the fraction 2 is most potent followed by fraction 3 and then fraction 1. It can be concluded that anthelmintic activity of the leaves of *A. reticulata* is due to compound present in fraction 2 of ethanol fraction of total ethanol extract.

**Keywords:** *Annona reticulata*, Anthelmintic activity, *Pheretima posthuma*.

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## INTRODUCTION

*Annona reticulata* L. (Annonaceae) is commonly called as 'Ramphal'. It is a small tree with glabrous branches. Leaves are membranous, oblong-lanceolate, acute or obtuse, cuneate or rounded at the base. Upper surface is glabrous and the lower is with a few scattered hairs. Flowers are two to four on lateral pedicels. Fruits are subglobose or somewhat heart shaped, roughish form outside, become yellow or yellowish red when ripe. Seeds are smooth and blackish [1].

The plant is indigenous to the West Indies but now naturalized in India and occurring in Bengal, Burma, and South India. Traditionally the bark is a powerful astringent and used as a tonic. Unripe and dried fruits are astringent and used to treat diarrhea. Leaves are used as an anthelmintic traditionally [2].

Anonaine, michelalbine, oxoushinsunine, and reticuline were isolated along with an unknown phenolic base from root bark [3, 4]. Dopamine, salsolinol, and coclaurine were isolated from leaves and stems (Rastogi & Mehrotra, 1993). The stem bark contains the diterpenes (-)-kaur-16-en-19-oic acid, 16- $\alpha$ -hydroxy(-)-kauran-19-oic acid, and methyl-17-hydroxy-16- $\beta$ -(-)-kauran-19-oate. From the seeds a series of N-fatty acyl tryptamine, in which acyl portion ranged from hexadecanoyl to hexacosanoyl have been characterized [5]. Various cytotoxic acetogenins as squamocin, cis-/trans-isomurisolenin, annoreticuin, annoreticuin-9-one, bullatacin, cis-/trans-bullatacinone, cis-/trans-murisalinone, solamin, annomonicin, rolliniastatin-1 and 2, squamone, and isoannonareticin, were isolated. Constituents of volatile oil viz.  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene, terpinen-4-ol, and germacrene D were identified, Plant is having in vitro antioxidant activity and cancer chemotherapeutic and chemopreventive potential.

The present research sought to validate the traditional anthelmintic use of the plant *A. reticulata*.

## MATERIALS AND METHODS

### Plant Material

Leaves of *A. reticulata* were collected in April 2006 from Ahmednagar district and were authenticated by Dr. Salunke, Head, Dept. of Botany, PVP College, Loni. A voucher specimen was deposited (No. - AR261).

### Drugs and Chemicals

The following drugs and chemicals were used. Drugs: Albendazole (BANDY, Mankind Pharma Ltd., New Delhi), Chemicals: Petroleum ether (60-80 °C) A.R. (PCL, Pune), Ethyl acetate A.R. (PCL, Pune), Ethanol A.R. (PCL, Pune), Dimethyl formamide (DMF) (PCL, Pune), Saline water (Claris Life sciences Ltd., Ahmedabad).

### Preparation of Extracts

Dried and coarsely powdered leaves (150 g, each) of *A. reticulata* were subjected for cold maceration using ethanol as a solvent. The extracts was concentrated by vacuum distillation and then dried in open air to yield 15.83 g of extracts. Further this total ethanol extract was made hydroalcoholic and fractionated in separating funnel using petroleum ether (60-80 °C), chloroform, ethyl acetate and ethanol. The fractions were concentrated by vacuum distillation and then dried open air to yield 3.39 g, 0.15 g, 0.13 g and 1.51 g fractions, respectively [6].

### Animals

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 all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [7, 8].



## Column chromatography

Ethanol fraction of total ethanol extract (4.69 g) of *A. reticulata* leaves was dissolved in ethanol and applied to silica gel column (2.9 X 44 cm), which was eluted by isocratic elution method by using methanol (400 ml) yielding 3 fractions. Fraction 1 (1.25 g); band of dark brown colour, fraction 2 (0.86 g); band of pale brown colour, fraction 3 (0.3 g); band of pale yellow colour. Fractions were collected according to its elution.

## EXPERIMENTAL

### Anthelmintic Activity [9]

All the extracts of *A. reticulata* were dissolved in minimum amount of DMF and then volume is adjusted to 10 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment.

In first phase, seven groups of six earthworms each were released into 10 ml of tested samples as follows; vehicles (5% DMF in normal saline), albendazole or total ethanolic extract or petroleum ether or chloroform or ethyl acetate or ethanolic fraction of total ethanolic extract of *A. reticulata* leaves (20 mg/ml, each) in vehicle. In the second set of experiment, five groups of six earthworms were released in to 10 ml of tested samples as follows; vehicle (5% DMF in normal saline), albendazole or fraction 1 or fraction 2 or fraction 3 of ethanolic fraction of total ethanolic extract of *A. reticulata* leaves (20 mg/ml, each) in vehicle. Observations were made for the time taken to paralysis. Paralysis was said to occur when the worms did not revive even in normal saline.

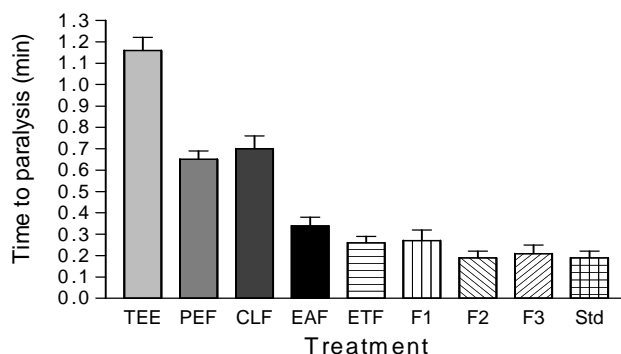
## RESULTS AND DISCUSSION

The data in fig 1 revealed that ethanol fraction of total ethanol extract of the plant *A. reticulata* showed significant anthelmintic activity compared with other fractions at 20 mg/ml concentrations. Thus it can be concluded that ethanol fraction is most potent and the active principle is located in that fraction.

In order to find out active constituents responsible for anthelmintic activity, ethanol fraction was further subjected to column chromatography yielding fraction 1, 2 and 3. Results showed that fraction 2 took least time to cause paralysis of the worms followed by fraction 3 and 1.

Thus it can be concluded that active constituents responsible for anthelmintic activity are present in the fraction 2 of ethanol fraction of total ethanol extract of leaves of *A. reticulata*.

Fig 1. Anthelmintic activity of *Annona reticulata* leaves.



Results are expressed as mean  $\pm$  SEM from six observations; control worms were alive up to 24 hrs of observation. TEE is total ethanolic extract, PEF is petroleum ether fraction; CLF is chloroform fraction; EAF is ethyl acetate fraction; ETF is ethanolic fraction; F1 is fraction 1, F2 is fraction 2 and F3 is fraction 3. All the fractions and standard drug were given at 20 mg/ml concentration.



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