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# IN VITRO ANTHELMINTIC ACTIVITY OF LEAF EXTRACT OF AVERRHOA CARAMBOLA AGAINST PHERETIMA POSTHUMA

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#### **Summary**

The aim of the present study was to evaluate the anthelmintic potential of *Averrhoa carambola* using *Pheretima posthuma* as the test worm. Various concentrations (10-100mg/ml) of the drug were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Albendazole (10-100mg/ml) was included as standard reference and distilled water as control. The result of the present study indicated that *Averrhoa carambola* significantly demonstrated paralysis, and also causes death of worms especially at higher concentration of 100mg/ml, as compared to standard reference Albendazole. In conclusion the anthelmintic activity of *Averrhoa carambola* has been confirmed.

Key Words: Averrhoa carambola, Pheretima posthuma, Albendazole.

#### Introduction

Soil-transmitted helminth (STH) infections are among the most prevalent of chronic human infections worldwide<sup>1</sup>. Helminthes are generally restricted to tropical regions and cause serious problems to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia<sup>2</sup>. After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani<sup>3</sup>. The WHO has estimated that 80% of the world's population already relies on traditional medicine for their primary healthcare needs, therefore traditional medical plants being an important component of complementary and alternative medicine may be useful for drug discovery and development<sup>4</sup>.

*Averrhoa carambola*, traditionally known as '<u>karmakh</u>', and commonly known as <u>star</u> <u>fruit</u> because of its peculiar shape, belongs to the plant family *Oxalidaceae*, has been widely used in Ayurveda, preparations of its fruit and leaves are used to pacify impaired kapha, pitta, skin diseases, pruritis, worm infestations, diarrhea, vomiting, hemorrhoids, intermittent fever, over-perspiration and general debility. It is also used in traditional medicines in countries like China, Phillipines, Brazil for various ailments<sup>3</sup>.

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Recent studies show that *Averrhoa carambola* is a very rich source of natural antioxitants<sup>5</sup>, studies have also proven hypoglycaemic effects<sup>6</sup>, antimicrobial propery<sup>7</sup>of this plant.

The plant is believed to have originated in Ceylon and is commonly grown in the provinces of Fukien, Kuangtung and Kuangsi in southern China, in Taiwan and India. It is rather popular in the Phillipines and Queensland, Australia and is widely used as an ornamental plant and in pickles and jams<sup>8</sup>.

*Averrhoa carambola* is a small, evergreen, multistemmed tree, 3-5m high or, rarely, 10m high. At the base, the trunk reaches a diameter of 15cm, the bark is light brown, smooth or finely fissured. Leaves are 15-25cm long alternate, pinnate, disposed more or less in a horizontal plane, shortly petiolate with 7-9 pendant leaflets; leaves have the peculiarity of being sensitive to touch in the same way as certain Mimosa species<sup>[8, 9]</sup>.

Preliminary phytochemical analysis indicated presence of saponins, tannins, alkaloids and flavonoids<sup>3</sup> hinting the presence of anthelmintic activity, but as there are no scientific evidences to support the same, the aim of this study was to test the anthelmintic activity of the leaf extract of *Averrhoa carambola* on adult Indian earth worms (*Pheretima posthuma*).

# Materials and Methods

# Plant material

*Averrhoa carambola* leaves were collected by A.N. Shah from Pune region in December 2010 and identified by Pharmacognosy department of MAEER's Maharashtra Institute of Pharmacy, Pune.

## Worm collection

Healthy adult Indian earthworm *Pheretima posthuma* about 5-7 cm were purchased from Enora Pvt. Ltd, Pune, washed and kept in purified water.

## **Preparation extract**

The leaves of *Averrhoa carambola* were dried and ground to coarse powder and macerated for 72 hours. The aqueous extract obtained was filtered and concentrated by evaporating to get extract.

# **Preparation of test sample**

Samples for in-vitro study were prepared by dissolving 2.5gms of extract in 25ml of distilled water to obtain stock solution of 100mg/ml .From this stock solution different working dilutions were prepared to get a concentration range of 10, 50 and 100mg/ml.

# Anthelmintic Assay

The anthelmintic assay was carried out as per Ajaiyeobaet al<sup>9</sup>, with minor modifications. The assay was performed on Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with intestinal roundworm parasite of human beings<sup>[10-12]</sup>. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in-vitro<sup>[13-15]</sup>. 50 ml formulations containing three different concentrations each of extract (10, 50, 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when worms were shaken vigorously. Time of death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously or when dipped in water (50<sup>0</sup>C). Albendazole was used as reference standard (10, 50, 100 mg/ml) while distilled water as the control.

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# **Results and Discussion**

Preliminary phytochemical analysis indicated presence of saponins, tannins, alkaloids and flavonoids in aqueous extract of *Averrhoa carambola*. The leaves of *Averrhoa carambola* displayed a significant anthelmintic activity in dose depended manner giving shortest time of paralysis (P) and death (D) with100mg/ml concentration as shown in Table 1. The anthelmintic activity of aqueous extract was comparable with that of standard drug at 100mg/ml concentration, plant extract showed paralysis in 10 minutes and death in 16 minutes while Albendazole exhibited similar effects at 10 and 21 minutes respectively. As the plant extracts are found to contain tannins, which are polyphenolic compounds, there is a possibility that their effects are like synthetic phenolic anthelmintics e.g.- niclosamide, oxyclozanide, bithionol etc. which are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation<sup>16</sup>. Another possible anthelmintic activity of tannins is that they can bind to free proteins in gastrointestinal tract of host animal<sup>17</sup> or glycoprotein on the cuticle of parasite<sup>18</sup> and may cause death.

Sample	Concentration (mg/ml)	Time taken for Paralysis (P) and Death (D) of <i>Pheretima posthuma</i> in minutes	
		Р	D
Albendazole	10	31.16±2.48	72.16±8.47
Albendazole	50	14.16±1.16	47.5±1.22
Albendazole	100	10.5±1.04	21.33±1.63
Plant extract	10	87±13.57	179.5±24.22
Plant extract	50	26.83±3.71	35±2.82
Plant extract	100	10.83±1.72	16.16±1.85

#### Table No. 1: Anthelmintic activity of Averrhoa carambola

All values represent Mean  $\pm$  SEM; n=6 in each group. Control worms were alive up to 24 hours.

## Conclusion

*Averrhoa carambola* showed anthelmintic activity. However dose and form in which they can be used requires standardization. Future scope involves isolation of phytoconstituents responsible for activity.

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