

**MANILKARA ZAPOTA SEED EMBRYO EXTRACT: A POTENT ANTHELMINTHIC AGENT****YASHWANTH KUMAR D. R.<sup>1</sup>, VURIVI HEMA<sup>1</sup>, MAYANK AGRAWAL<sup>1</sup>, PRAMODITHA SRUTHY C.<sup>1</sup>, VEDAMURTHY A. B.<sup>1</sup>, KRISHNA V.<sup>2</sup> AND JOY HOSKERI H.<sup>1,2,\*</sup>**

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**ABSTRACT**

Helminth infections have plagued humans since before the era of our earliest recorded history. They are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better known conditions. *Pherithema posthuma* a helminthes is commonly known as earth worm is used in the present study. In this connection the anthelmintic property of *Manilkara zapota* seed embryo extracts were evaluated against *Pherithema posthuma* as an experimental helminthes model. Piperazine citrate was used as the standard reference. Among the various concentrations of chloroform extract tested, 12.5mg/ml showed efficient anthelmintic activity and among all the concentrations of ethanolic extract tested, 12.5mg/ml showed significant results. This investigation revealed that ethanolic extract of *M. zapota* proved to possess significant anthelmintic activity against *Pheretima posthuma* when compared the chloroform extract and the standard drug. This investigation reports a new potent anthelmintic agent that can be used as a potential drug for the treatment of helminthes infection.

**Keywords:** *Manilkara zapota*; Sapotaceae; anthelmintic activity; *Pheretima posthuma*; Ethanolic extract; Chloroform extract.

**INTRODUCTION**

Approximately 3 million people are infected with helminthes world wide. Helminthes infections are commonly found in villages of developing countries and being recognized as a cause of much acute as well as chronic illness among the various human beings as well as cattles. More than half of the population of the world suffers from various types of infection and majority of cattles suffers from worm infections <sup>1</sup>. Parasitic helminthes live and feed on the living hosts by receiving nourishment and protection, while disrupting their host's nutrient absorption, causing weakness and disease in human and animals inflicting heavy production losses. Worms may also contribute to malnutrition by creating anorexia. Helminthes may also affect nutrition by inducing iron-deficiency anemia. This is most severe in heavy hookworm infections, as *N. Americanus* and *A. Duodenale* feed directly on the blood of their host. Once the links between helminth infection and various forms of malnutrition are established, there are a number of pathways by which parasite burden may affect cognition.

Treatment for helminthic infection is of utmost need. The high cost of modern synthetic anthelmintics have limited effect in control of these parasites. However, increasing problems of development of resistance in helminthes against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity <sup>2,3</sup>. Although the uses of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminth parasites and as a means of reducing the cost of controlling helminthic diseases <sup>4-7</sup>. Phytochemicals or plant based agents are known to provide a rich source of botanical anthelmintics <sup>8,9</sup>. Various medicinal plants have been reported on their application as anthelmintic drug in man and animals <sup>10,11</sup>. These plant based antihelminthics agents act by expelling parasitic helminthes worms from the body or by either stunning or killing them.

*Manilkara zapota* (Sapotaceae) commonly known as the sapodilla and is a long-lived, evergreen tree native to southern Mexico, Central America and the Caribbean, grown in huge quantities in India, Pakistan and Mexico. The fruit is a large ellipsoid berry, 4-8 cm in diameter, very much resembling a smooth-skinned potato and containing two to five seeds. The seeds are black and resemble beans, with a hook at one end that can catch in the throat if swallowed. The fruit has a high latex content and does not ripen until picked. The fruit has an exceptionally sweet, malty flavor. Many believe the flavor bears a striking resemblance to caramel or a pear candied with brown sugar. The unripe fruit is hard to touch and

contains high amounts of saponin, which has astringent properties similar to tannin, drying out the mouth. The sapodilla trees yield fruit twice a year, though flowering may continue year round. Literature survey revealed that there are few reports available on phytochemistry and pharmacological properties of this plant. However, anthelmintic activity of *Manilkara zapota* seed embryo extract has not yet been scientifically reported. In continuation with our interest in this plant, we made an attempt to assess the anthelmintic activity of the chloroform and ethanol extract of *Manilkara zapota* using *Pheretima posthuma* as an experimental helminthes model.

**MATERIALS AND METHODS****Drugs and chemicals**

The standard drug piperazine citrate (SD Fine Chemicals Ltd., Mumbai). Ethanol was purchased from Hong, Yang Chemical Corporation, China.

**Plant Resource**

*Manilkara zapota* seeds were collected by completely removing the above fruit flesh (Figure 1). Fresh seeds were washed with distilled water thoroughly to remove traces of contaminants. These processed seeds were then shade dried for one month. After complete drying the seed coat was broken and embryo was 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 the extracts were filtered and concentrated in vacuum 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 embryo material was 46 g and 53 g respectively.

**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 anthelmintic activity due to its

anatomical and physiological resemblance with the intestinal roundworm parasites of human beings<sup>12-13</sup>.



Figure 1: Shade dried *Manilkara zapota* seeds

#### Extract preparation for experiment

The porously powdered seed embryo material was used for extract preparation. After extraction, the crude extracts were stored in desiccator 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 anthelmintic activity.

#### Anthelmintic activity

The anthelmintic activity of chloroform and ethanol extracts of *Manilkara zapota* were evaluated as per the method reported by Dash et al., (2002). Twelve groups with three earthworms in each

group were used for the experiment. 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 50, 100, 150, 200 and 250 mg/20ml of chloroform extract respectively. Similarly, group VII, VIII, IX, X, XI earthworms were released in 50, 100, 150, 200 and 250 mg/20ml of ethanol extract respectively. Group XII earthworms were released in normal saline containing standard drug piperazine citrate (50 mg/20ml). 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 behavior of the earthworm with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body color<sup>14</sup>. The result of anthelmintic activity is depicted in Table 1.

#### Statistical analysis

The data of anthelmintic evaluations were expressed as mean  $\pm$  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. 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.

#### RESULTS AND DISCUSSION

In the present study, *Manilkara zapota* seed embryo was sequentially extracted using chloroform and ethanol as the solvent system. In continuation with our interest in helminth infections and biological properties of *Manilkara zapota* seed embryo, this investigation on evaluating the anthelmintic property of *Manilkara zapota* was carried out.

Table 1: *In vitro* anthelmintic activity of ethanol and chloroform extracts of *Manilkara zapota* against *Pheretima posthuma*.

Test samples	Concentration (mg/20ml)	Paralysis Time (min)	Death Time (min)
Control (Normal Saline)		93.67 $\pm$ 5.78	233.0 $\pm$ 3.79
Chloroform extract of <i>Manilkara zapota</i>	50	85.0 $\pm$ 2.65 ns	128.0 $\pm$ 2.31**
	100	36.33 $\pm$ 5.49**	55.67 $\pm$ 0.88**
	150	26.0 $\pm$ 2.08**	43.67 $\pm$ 5.7**
	200	10.67 $\pm$ 1.45**	28.33 $\pm$ 6.06**
	250	8.67 $\pm$ 1.45**	23.67 $\pm$ 3.84**
Ethanol extract of <i>Manilkara zapota</i>	50	5.0 $\pm$ 0.58**	9.33 $\pm$ 0.88**
	100	7.67 $\pm$ 1.45**	13.33 $\pm$ 0.33**
	150	4.33 $\pm$ 0.33**	12.0 $\pm$ 0.58**
	200	3.67 $\pm$ 0.33**	8.0 $\pm$ 0.58**
	250	3.33 $\pm$ 0.33**	7.33 $\pm$ 0.33**
Piperazine citrate	50	28.33 $\pm$ 1.45**	36.33 $\pm$ 1.45**

Values are the mean  $\pm$  S.E.M. of three earthworms. Symbols represent statistical significance.

\*  $P < 0.05$ , \*\*  $P < 0.01$ , ns: not significant as compared to control group.

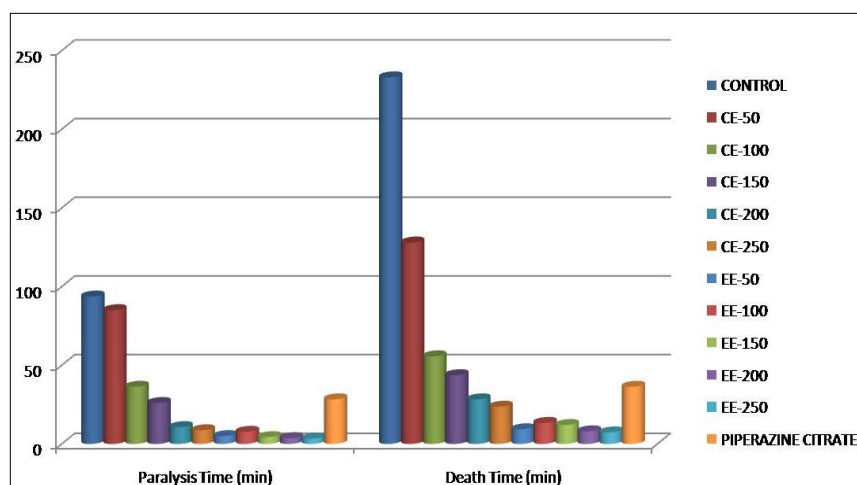


Figure 2: Bar chart illustrating the comparative *in vitro* anthelmintic effect of different concentrations of chloroform and ethanol extracts of *Manilkara zapota*.

Chloroform extract at the concentration of 2.5 mg/ml showed the time of paralysis and death at 85.0 and 128.0 min respectively. Chloroform extract at the concentration of 5 mg/ml induced the paralysis and the death by 36 and 55 min respectively. At the concentration of 7.5, 10.0 and 12.5 mg/ml, time taken to paralysis was 26.0, 10.0 and 8.0 min respectively and death time 43.0, 28.0 and 23.0 min respectively. Among the various concentrations tested, chloroform extract at 12.5 mg/ml (250 mg/20 ml) showed efficient anthelmintic 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 5.0 and 9.0 min respectively. For concentrations at 5.0, 7.5, 10.0 and 12.5 mg/ml paralysis was shown at 7.0, 4.0, 3.6 and 3.3 min respectively and death occurred at 13.0, 12.0, 8.0 and 7.0 min respectively. Among all the concentrations ethanolic extract tested, 12.5 mg/ml (250 mg/20ml) produced significant results. Standard drug at 2.5 mg/ml (50 mg/20ml) showed paralysis at 28.0 min and death time was 36.0 min (Table 1).

This investigation revealed that ethanolic extract of *Manilkara zapota* showed significant anthelmintic activity against *Pheretima posthuma* when compared chloroform extract and the standard drug Piperazine citrate. This investigation reports a new potent anthelmintic agent that can be used as a potent drug for the treatment of helminthes infection. The present study can act as the basis for further phytochemical evaluation of *Manilkara zapota* seed embryo to isolate potent anthelmintic compound.

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#### REFERENCES

1. Chaturvedi M, Dwivedi S, Dwivedi A, Barpete PK, Sachan R, Formulation and Evaluation of Polyherbal Anthelmintic Preparation, Ethnobot. Leaflet. 2009; 13: 329-331
2. Geert S, and Dorny P, Anthelmintic resistance in helminthes of animals of man in the tropics. Bulletin-des-Seances, Academic-Royale-des-Sciences 1995; 3: 401-423.
3. Coles GC, Nematode control practices and anthelmintic resistance on British sheep farms. Vet. Rec. 1997; 141: 91-3.
4. Kelly JD, Hall CA, Resistance of animal helminths to anthelmintics. Adv. Pharm. Ther. 1979; 16: 89-128.
5. Okon ED, Ogunsusi RA, Fabiyi JP, Survey and feasibility studies on fascioliasis and parasitic gastroenteritis of ruminants in Nigeria. Federal Livestock Department of Nigeria Report, Lagos; 1980; 1-55.
6. Taylor MA, Hunt KR, Anthelmintic drug resistance in the UK. Vet. Rec. 1989; 125: 143-147.
7. Coles GC, Roush RT, Slowing the spread of anthelmintic resistant nematodes of sheep and goats in the United Kingdom. Vet. Rec. 1992; 139: 505-510
8. Satyavati GV, Raina MK, Sharma M, Medicinal Plants of India. Vol. I. Indian Council of Med. Res., New Delhi, India. 1976; 201-06
9. Lewis WH and Elvin Lewis MPH, Medicinal Botany Plants Affecting Man's Health. John Wiley & Sons, New York; 1977.
10. Nadkarni AK, Indian Materia Medica, 3rd Ed. Popular Prakashan, Bombay, India; 1954.
11. Akhtar MS, Zafar I, Khan MN and Muhammad L, Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo-Pakistan subcontinent. Small Rumin. Res. 2000; 38: 99-107.
12. Thorn GW, Adams RD, Braunwald E, Isselbacher KJ and Petersdorf RG, Harrison's Principles of Internal Medicine. In: Mcgraw Hill Co., New York: 1977; 1088-1089.
13. Vigar Z, Atlas of Medical Parasitology. In: 2nd ed. P.G. Publishing House, Singapore: 1984; 216-217.
14. Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB, Evaluation of *Evolvulus alsinoids* Linn for anthelmintic and antimicrobial activities. J. Nat. Rem. 2002; 2: 182- 185.