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**PRECLINICAL EVALUATION OF *MORINGA OLEIFERA* FLOWER'S  
HYDROALCOHOLIC EXTRACT FOR ITS ANTHELMINTIC  
ACTIVITY ON *TUBIFEX TUBIFEX***

**MANNA R<sup>\*1</sup>, CHANDA A<sup>1</sup>, SAHA P<sup>1</sup>, BISWAS D<sup>1</sup>, ROY P<sup>1</sup>, MAJUMDAR M<sup>1</sup>, ROY  
S<sup>1</sup> AND CHATTOPADHYAY S<sup>2</sup>**

**1:** Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy,  
Chakdaha, Nadia- 741222, West Bengal, India

**2:** Department of Pharmaceutical Chemistry, Netaji Subhas Chandra Bose Institute of  
Pharmacy, Chakdaha, Nadia- 741222, West Bengal, India

**\*Corresponding Author: Ms. Ruchismita Manna: E Mail: [mannaruchismita@gmail.com](mailto:mannaruchismita@gmail.com)**

Received 16<sup>th</sup> Jan. 2023; Revised 7<sup>th</sup> April 2023; Accepted 3<sup>rd</sup> July 2023; Available online 1<sup>st</sup> Feb. 2024

<https://doi.org/10.31032/IJBPAS/2024/13.2.7803>

**ABSTRACT**

Helminthic infections are among the most human illness affecting among half of the world's population. In developing nations, they represent a serious threat to public health and increase the risk of pneumonia, eosinophilia, anaemia, and malnutrition.

Herbs are the source of different phytoconstituents that are responsible to treat different disease, helminthic infection is one of them. In our traditional medicine system, different herbs are used to treat the helminthic infection, *Moringa oleifera* is one of them.

The present study aimed to evaluate the anthelmintic activity of *Moringa oleifera* flowers on *Tubifex tubifex* to find out the minimum lethal dose 100 and compare its anthelmintic activity with albendazole as well as a herbal standard drug within 4 hours. The experiment was conducted using freshly collected *Tubifex tubifex*, which were exposed to different concentrations of *Moringa oleifera* flower extract, albendazole, and the herbal standard drug. The anthelmintic activity was determined based on the time taken for paralysis and death of the worms within 4 hours.

The results showed that the *Moringa oleifera* flower extract had a dose-dependent anthelmintic activity on *Tubifex tubifex*, with a significant reduction in the time taken for paralysis and death of the worms compared to the control group. Furthermore, the anthelmintic activity of *Moringa oleifera* flowers was

found to be comparable to that of albendazole, a known synthetic anthelmintic drug, and the herbal standard drug.

Therefore, the present study demonstrates the potential of *Moringa oleifera* flowers as an effective natural anthelmintic agent.

**Keywords:** *Moringa oleifera*, hydroalcoholic extract, phytoconstituents, albendazole, herbal standard, anthelmintic activity

## INTRODUCTION

Plants are used for food, fuel, fodder, and many other things, therefore they play a crucial role in society [1]. Man has relied on plants for medicine from the earliest times. It is clear from historical evidence that civilization's fascination with plants predates humanity itself. The plant kingdom is a vast repository of organic compounds, many of which have been used medicinally and could be used as a starting point for the future development of novel agents that are effective against a variety of pathological disorders [2]. The basic healthcare provided by herbal plants is significant to society. The most important obstacle to the acceptance of herbal medicines is the lack of research and quality control. Attempting to standardise the plant material that will be used as medicine thus becomes extremely important<sup>1</sup>. More infections and diseases appear to affect humans than any other animal species. Nature has given us a complete approach of cures for all human ills and diseases that are related to them [3]. A significant fraction of the majority suffers from helminthic infections, which are among the most widespread illnesses in

humans [4]. Helminthiasis is thought to affect around half of the majority, and the prevalence is rising daily. Because of poor hygienic conditions, hunger, and dense living conditions, it is endemic in many areas. Today, there are strong anthelmintics on the market, and various medications can be used to treat the condition. Nearly 80% of the world's population still believes in the usage of traditional medicine for their health, which mostly involves plant extract. Even if allopathic medication is necessary, there are medicinal plants that can be utilised instead of allopathic drugs. Numerous herbal preparations are employed as anthelmintic [5]. There are some allopathic medicines is available but contain lots of side effects. This time, pharmaceutical technology demonstrates a greater interest in herbal medicines, both domestically and internationally, as people hold the belief that they are less toxic than synthetic medications. Herbal medicines are being used as primary health care needs by nearly 80-90 percent of people [6]. The pricey modern anthelmintics, however, has limited their ability to effectively control

parasites and also breeds resistance, which leads to a decrease in their use. Few plants are traditionally used as anthelmintics, such as *Ocimum sanctum* L., *Mimusops elengi* L., *Melia azedarach* L., *Punica granatum* L., and *Carica papaya* L. [3].

The most widespread species grown throughout the world's tropical regions is *Moringa oleifera* Lam. (family Moringiaceae) [7]. It is commonly used as anthelmintic. Drugs known as anthelmintics either kill (vermicides) or expel (vermifuge) invading helminths [8]. Anthelmintic medications are used to get rid of or lessen the number of helminthic parasites in the body's tissues or the intestines. Although these parasites share many biochemical and physiological processes with their human hosts, there are slight variations that are starting to be discovered by pharmacologic research [9]. There are 12–14 species in the Moringiaceae family, all of which are members of the *Moringa* genus. All of the species originated in north India, from which they have spread to all warm climates worldwide [10]. According to reports, the plant contains a variety of amino acids, fatty acids, vitamins, and nutrients. Leaf, flower, fruit, and bark have all been recorded to be utilised as herbal remedies. Many ayurveda doctors utilise the plant *Moringa oleifera* as a medication to treat chronic rheumatism and asthma [11].

The whole plant of *Moringa oleifera*, which is also known as “drumstick” has been used in Indian folk medicine as anthelmintic agent traditionally to eradicate or reduce the number of helminthic infections in intestinal tract. But there was no scientific proof about this when literature review was studied about the plant. While studying the literature review about the plant we found out several phytochemical constituents which are responsible for anthelmintic activity. There are several researches that confirmed about the anthelmintic activity of various other parts of *Moringa oleifera* but there were not any such researches about the flower as far we studied. Literature review of the phytoconstituents of the flower confirmed about the presence of alkaloids, flavonoids, saponins, tannins, glycosides that are responsible for anthelmintic activity. Therefore, we chose to perform this experiment and evaluated the anthelmintic activity of *Moringa oleifera* flower's hydroalcoholic extract on *Tubifex tubifex*, as well as tried to scientifically establish or prove the folklore practice.

## MATERIALS AND METHODS

### Plant materials:

The fresh flowers of *Moringa oleifera* were collected during the month of December-January from the local market and local area of Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, Nadia.

**Preparation of extract: [12, 13]**

Under the shed, the flowers of *Moringa oleifera* were gathered, gently cleaned with distilled water, and dried and manually crushed the flowers. 100 gm of crushed leaves were percolated for two days with 70% hydro ethanolic mixture. Vacuum desiccators were used to dry the extract. The extracts were sealed in aluminium foil and kept at 4°C.

**Phytochemical screening: [14]**

For the purpose of identifying the various active ingredients, the crude extracts underwent qualitative analysis. Standard procedures were performed to conduct phytochemical tests.

**Animals:**

Aquatic worm *Tubifex tubifex* (Annelida) were collected from the market, the average size of worm being (0.5 – 6 cm) long. They were washed and cleaned with tap water for the removal of the adhering dirt. Then the worms were kept in a beaker under running water.

Due to its morphological and physiological similarities to human intestinal roundworm parasites, the testing was carried out on aquatic worms (*Tubifex tubifex*). These worms are readily available in the market and are a good model for testing anthelmintic medications.

**Determination of anthelmintic activity: [15, 16]**

*Tubifex tubifex* (Annelida), aquarium worms were bought from the local market of Chakdaha. The size of the worms varied from 1cm. to 1.5 cm. The worms were kept in laboratory conditions before testing.

To find out the anthelmintic potency of hydro alcoholic extract of *Moringa oleifera* flowers, Limit test and Main test has been performed. The dose required to cause Paralysis and lethality in 100% of the population within 4 hours was determined. The complete paralysis as well as lethal time were also noted. When the worms lost their normal motions or movements, the time was taken as pre-complete paralysis stage. External forceful stimuli were applied to induce movement in an aquatic worm to conform the complete paralysis time. The lethal time or death time was confirmed when the aquatic worms (*Tubifex tubifex*) lost their complete motions, body colour became pale, as well as they showed no movement when dipped in warm water (50°C), that time was recorded.

After that a comparative study was also performed to find out the potency of our test drug as compare to control and marketed standard drug as well as marketed herbal standard drug.

**Limit test:**

The test dose 2000mg/kg was added to a petri dish containing 20ml of distilled water for one animal. If the animal died, the primary test/main test was performed.

If the animal survived, a total of four additional animals placed in same dose one after the other, making five animals in all that were assessed. If three animals died during the limit test, the primary/main test was carried out. If three or more animals survived till 4 hours then the LD100 was greater than 2000 mg/kg. If an animal unexpectedly died late in the trial and there were other survivors, then additional dosing was stopped and observed rest of the animals to find out, if any animal/s died within the same observation period (up to 4 hours).

According to research requirements, a limit test of 5000 mg/kg body weight was conducted on one animal for 4 hours, and the main test was then carried out. Two additional animals were dosed if the animal lived. The test was stopped if both individuals survived.

#### **Main test:**

To perform the main test, 4 petri dishes were taken containing 20 ml of distilled water, then as per OECD guidelines 175mg/kg, 550mg/kg, 2000mg/kg, 5000mg/kg doses were added or mixed within each petri dish respectively. Then in each petri dish number of 6 *Tubifex tubifex* were placed to find out the minimum LD100 dose within the 4 hours and corresponding paralysis time and death time were also noted to evaluate the anthelmintic activity. If the research needed,

then further dilution or division of dose was prepared and applied.

#### **Comparative Study:**

After finding out the minimum dose, the comparative study was performed between our minimum LD100 dose of hydroalcoholic extract of *Moringa oleifera* flowers, untreated control, standard as well as a herbal standard drug at the same dose as compared to the test substance. Distilled water was taken as untreated control, and the LD100 dose of the hydroalcoholic extract of *Moringa oleifera* flowers was taken as test and marketed Albendazole ('NO WORM' from Alkem Laboratories Ltd) was taken as standard group-I, as well as a herbal standard drug ('KRIMI MUDGAR RAS' from Dabur India Ltd.) was also taken as standard group-II at the same dose of LD 100 of *Moringa oleifera* for the comparative study.

As like main test 3 petri dishes were taken for each group containing 20ml of distilled water and the test substance and standard drugs were mixed or added within their respective petri dish. Then in each petri dish number of 6 *Tubifex tubifex* were placed for 4 hours, and their paralysis time as well as death time were noted down to perform the comparative study.

#### **Statistical Analysis: [17]**

The outcomes are shown as mean  $\pm$  S.E.M. By using an ANOVA followed by a Dunnett's test, the mean and SEM were

statistically analysed,  $P < 0.001$  vs control was regarded as a significant value.

## RESULT AND DISCUSSION

### Extract preparation:

The *Moringa oleifera* flowers were extracted by cold percolation method for 72 hours, using 70% hydroalcoholic solution and 4.48 % yield was procured.

### Phytochemical investigation:

Through the different phytochemical investigation, the hydroalcoholic extract of *Moringa oleifera* flowers showed the presence of different following phytochemicals (Table 1).

### Anthelmintic activity:

The research work was designed to find out the minimum lethal dose 100% within 4 hours of *M. oleifera* and find out the potency of our test product (hydroalcoholic

extract of *M. oleifera* flowers) as compared to control along with marketed standard drug Albendazole ('NO WORM' from Alkem lab.) and marketed herbal standard ('KRIMI MUDGAR RAS' from Dabur India Ltd.) (Table 2) (Figure 1) (Figure 2). Through the Limit test and Main test *M.oleifera* 2000mg/kg dose has shown the maximum paralysis time and lethality in 100% of the population within 4 hours, to confirm the dose further division of doses were carried out (1800 mg/kg, 1600 mg/kg, 1400 mg/kg, 1200 mg/kg, 1000 mg/kg), but within 4 hours these doses did not show any result. Therefore the *M.oleifera* 2000mg/kg dose was demonstrated as the minimum lethal dose 100% within 4 hours (Table 3) (Figure 3).

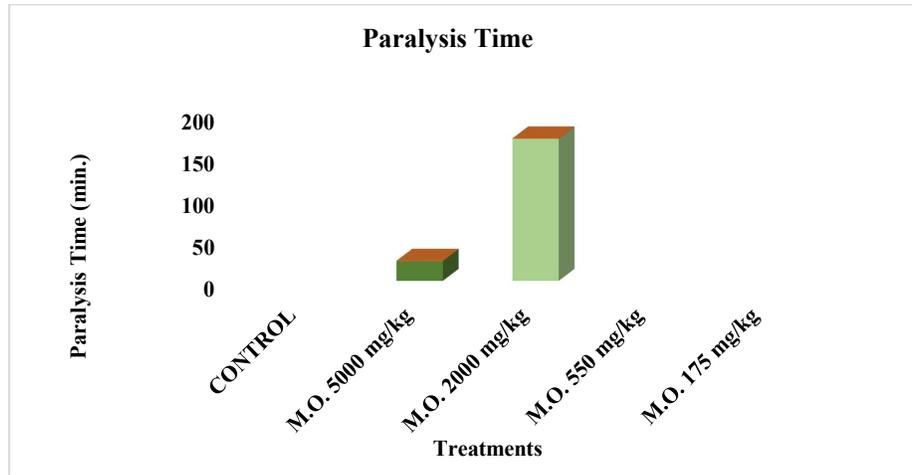
Table 1: Phytochemical investigation results of hydroalcoholic extract of *Moringa oleifera*

Phytoconstituent	Test	Observation	Result
Carbohydrate	Molish's test	Purple Colour	Present
Alkaloid	Dragendroff test	Reddish Brown Precipitate	Present
Flavonoid	Ferric chloride test	Blackish Red Colour	Present
Saponin	Foam test	Formation of Stable Foam	Present
Tannin	Lead Acetate test	White Precipitate	Present
Polyphenol	Ferric Chloride test	Red Colour	Present
Glycoside	Kellar Killiani test	Blue Colour at Junction	Present

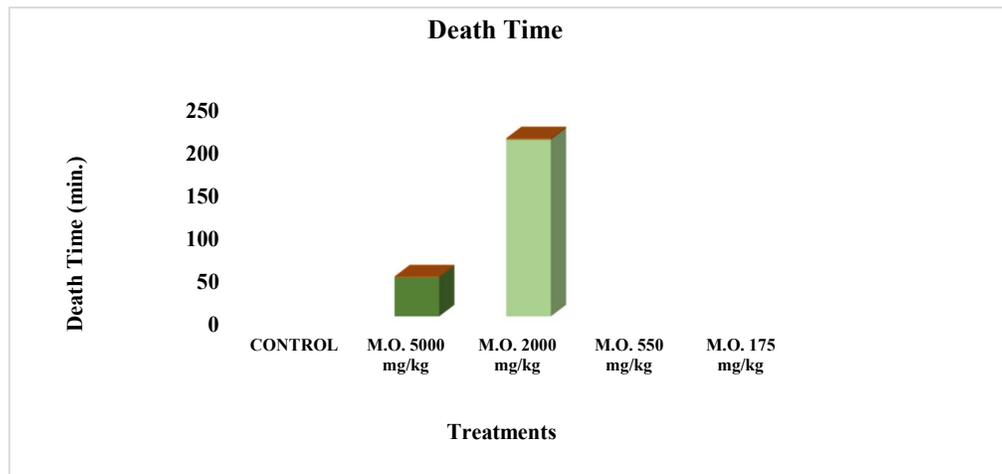
Table 2: Effect of *Moringa oleifera* various doses as an anthelmintic activity

Treatments	Paralysis Time	Death Time
Control	-	-
<i>M.oleifera</i> 5000 mg/kg	23.48±0.42***	44.89±1.37***
<i>M.oleifera</i> 2000 mg/kg	169.12±0.51***	204.64±1.75***
<i>M.oleifera</i> 550 mg/kg	-	-
<i>M.oleifera</i> 175 mg/kg	-	-

All values are mean±SEM, n=6, \*\*\*p<0.001 vs control



All values are mean±SEM, n=6  
 Figure 1: Effect of *M.oleifera* various doses on paralysis time on *Tubifex tubifex*

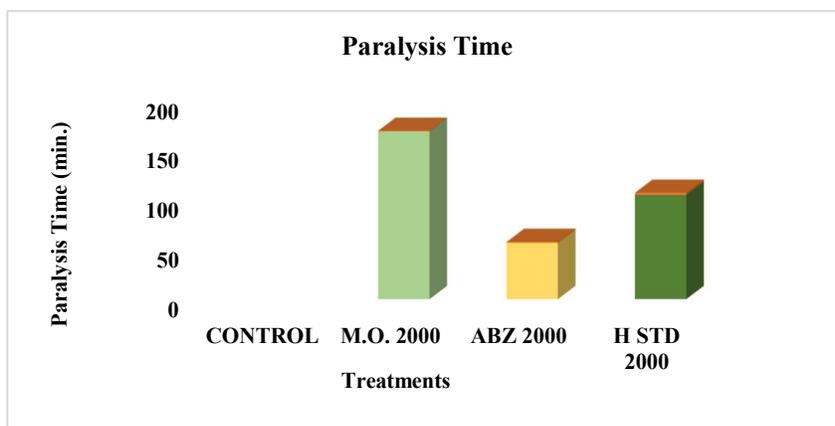


All values are mean±SEM, n=6  
 Figure 2: Effect of *M.oleifera* various doses on death time on *Tubifex tubifex*

Table 3: Result of comparative study on paralysis time and death time

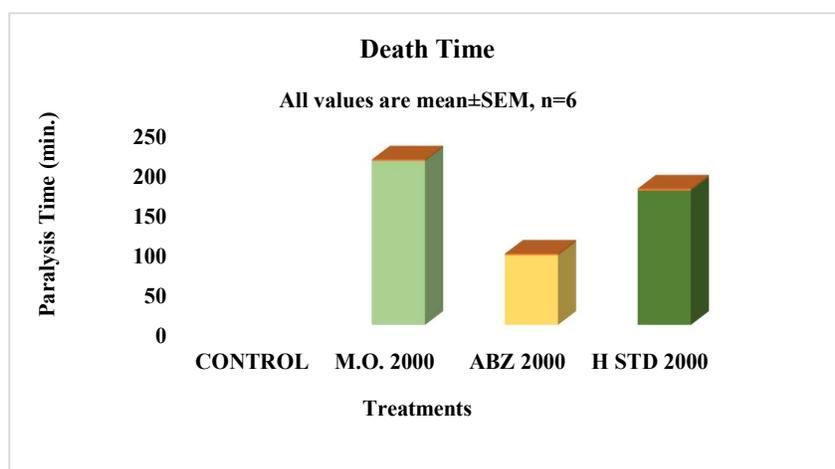
Treatments	Paralysis Time	Death Time
Control	-	-
<i>M.oleifera</i> 2000 mg/kg	169.12±0.51***	204.64±1.75***
Albendazole 2000 mg/kg	56.74±0.90***	87.01±1.83***
Krimi Mudgar Ras 2000 mg/kg	105.19±2.00***	167.72±2.39***

All values are mean±SEM, n=6, \*\*\*p<0.001 vs control



All values are mean±SEM, n=6

Figure 3: Graphical representation of comparative study on paralysis time



All values are mean±SEM, n=6

Figure 4: Graphical representation of comparative study on death time

To find out the potency of *M.oleifera*, a comparative study was performed between *M.oleifera* as test group, normal distilled water as control, marketed standard Albendazole ('NO WORM' from Alkem Laboratories Ltd) as standard group-I, and marketed herbal standard ('KRIMI MUDGAR RAS' from Dabur India Ltd.) as standard group-II. The logic behind the selection of an herbal standard to compare the anthelmintic activity of our test drug is that our *M.oleifera* is a herbal drug so to

better understand the potency of our drug we need to compare the efficacy of our drug with a marketed standard as well as a marketed standard herbal drug also.

In the comparative study the *M.oleifera* 2000 mg/kg dose had been shown potent anthelmintic activity as compared to control as well as provided a comparable result as compare to Albendazole and herbal standard drug. The marketed standard Albendazole and the marketed herbal standard proved its dynamic known anthelmintic activity again.

Plants are a huge store house of various phytochemicals. There are some phytochemicals that are responsible for anthelmintic activity. Through the phytochemical investigation *M.oleifera* showed the presence of Alkaloid, Flavonoid, Tannin, Polyphenol.

Alkaloid attacks the central nervous system causing paralysis in the motility of the worms and ultimately causing death [18]. Tannins and polyphenols bind to the cuticle protein of the worm, causing breakdown of the protein and ultimately death [19]. Additionally Flavonoid is one of the other important phytoconstituents that is responsible for anthelmintic activity. It is a strong antioxidant that causes scavenging of the free radical and inhibits lipid peroxidation [20].

Therefore these phytochemicals played an important role in constructing the *M.oleifera* as an anthelmintic agent.

The aquatic worm model used in this investigation is a well-known method for determining anthelmintic properties of plant extracts, and the findings from this method would be helpful in the development of novel anthelmintic drugs. In order to evaluate the efficacy and safety of *M.oleifera* extract in animal models and human subjects, additional research is needed to isolate and define the active substances responsible for the anthelmintic properties of the extract.

## CONCLUSION

*Moringa oleifera* flowers have been used traditionally as an anthelmintic agent which did not have any scientific proof to it. The research was conducted to gain scientific knowledge about the traditional use. The hydroalcoholic extract of the flower was assessed against the marketed Albendazole drug and its effects were studied and compared.

In the research the hydroalcoholic extract of flower of *Moringa oleifera* shown valuable anthelmintic activity. The findings suggest that a natural anthelmintic could be made from the hydroalcoholic extract of *Moringa oleifera* flowers. Before considering the extract for therapeutic use, more research is required. Overall, this work emphasises the value of looking into natural compounds as prospective sources of anthelmintic drugs, particularly in light of rising medication resistance and the demand for alternative remedies.

## ACKNOWLEDGEMENT:

We like to express our special thanks to the principal, Dr. Arnab Samanta, of our institute Netaji Subhas Chandra Bose Institute of Pharmacy. We are also thankful to all the lab attenders, our family members, and our friends for their support.

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