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Research Article

Antihelminthic Activity in Aqueous Leaf Extract of *Moringa oleifera* Lam.

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ABSTRACT
The objective of the study is to evaluate the antihelminthic property of *Moringa oleifera* aqueous leaf extract by studying the paralysis and mortality time of *Tubiflex tubiflex* (blood worm). The aqueous extract at 200mg/ml exhibited paralysis time of 1 minute and death time of 2 minutes which was on par with synthetic drug Albendazole at 20mg/ml. Crude condensed tannin paralysed *T.tubiflex* at 60 seconds with mortality time of 9 minutes at 1mg/ml. *T.*tubiflex was selected for the study since they share a similar anatomical and physiological aspects with the intestinal round worm. The study indicated that *T.tubiflex* was inhibited effectively by condensed tannin in *Moringa oleifera* aqueous leaf extract and perhaps might be more effective on the round worms as well, since it is consumed as vegetable for its nutritive and medicinal values in tropical and subtropical regions.

Keywords: *Moringa oleifera*, *Tubiflex tubiflex*, antihelminthic activity, condensed tannin, Paralysis and Mortality time.

INTRODUCTION

Intestinal worm infestation is a global health problem. Although it is preventable, it seems to affect two billion people across the world, causing a high rate of morbidity and suffering[1]. Administration of albendazole (ALB) 400 mg or mebendazole (MBZ) 500 mg controls helminth infection[2]. According to WHO report by the year 2020, it is globally targeted to eliminate morbidity due to helminth[3] Using conventional antithelmintics has a major problem since, the gastro-intestinal helminthes have become resistant to currently available anthelmintic drugs . They cause side effect such as nausea, intestinal disturbance and giddiness[4]. An ideal antihelminthic drug must be cost effective, less side effects and broad spectrum of action. However the current scenario of synthetic drug do not meet the requirements. Hence search for plant based antihelminthic drug have gained momentum.

Moringa oleifera, Lam syn. *M. pterygosperma*, Gaertn (Family – Moringaceae), is native to India and the entire plant has been used to treat various ailments[5]. The leaves have enormous properties. They are antibacterial, antifungal, Antipyretic, wound healing, Antiasthmatic, Hepatoprotective, Antithyroid, Radioprotective, Antiulcer, Antihyperglycemic[6], Antitumor[7], Antiplasmodial, antioxidant activity[8], Prophylactic activity[9], analgesic, antifertility, anticonvulsant and antilipidermic[10]. *Moringa oleifera* is reported to contain a wide range of phytochemicals that are responsible for its activities. The antihelminthic activity due to condensed tannin in aqueous extract of *Moringa oleifera* has not been reported so far, hence an alternative strategies against gastrointestinal parasites has been studied.

MATERIALS AND METHODS

Preparation of extract

Moringa oleifera leaves were collected, washed thoroughly, shade dried and aqueous extracted. Different concentrations such as 10mg/ml, 20mg/ml, 50mg/ml, 100 mg/ml,150 mg/ml and 200mg/ml were prepared.

Antihelminthic assay

Tubifex tubifex (Bloodworm) was used in the study, since it resemble the intestinal round worm both anatomically and physiologically. The anthelmintic assay was carried as reported earlier[11] with minor modifications. The worms were collected, washed with normal saline. About 10 worms were subjected to each concentration of the aqueous extract.The paralysis and death time of the worms were recorded in terms of minutes.

Estimation of tannin

The total tannin in the *Moringa oleifera* was estimated by using Gallic acid as standard. To 1ml of aqueous extract 2.5 ml of 7% (w/v) Sodium carbonate and 0.7 ml of Folin phenol were added and incubated for 10 mins and the absorbance was read at 660nm.

Preparation of crude condensed tannin

Crude condensed tannin was prepared by weighing 20 g of *Moringa olerifera* leaves and extracted with 100 mL of ethanol and acetone (1;1), allowed to dry by placing it in the watch glass. To the diluted crude extract, a solution of

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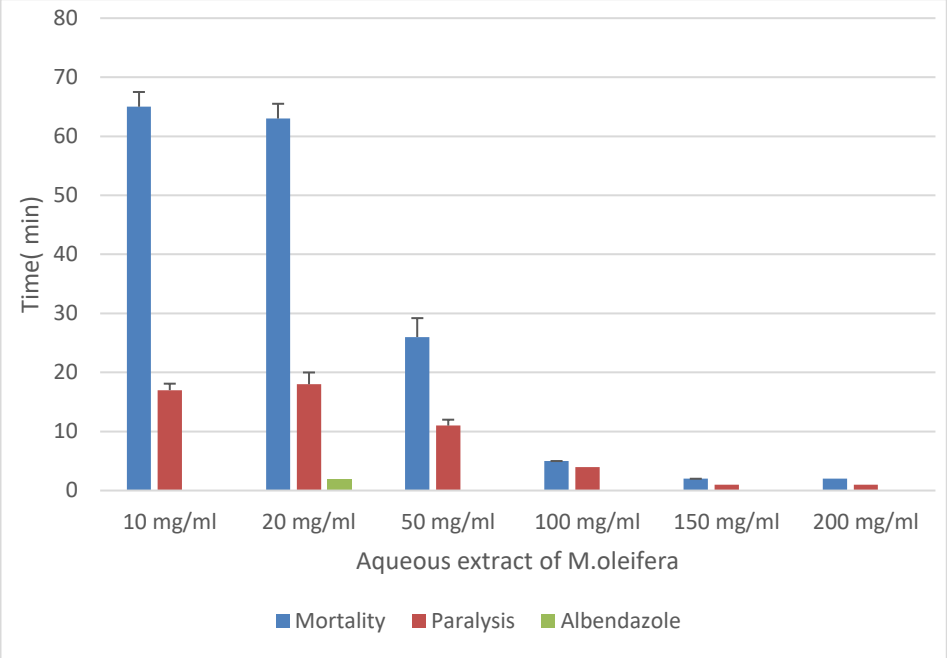

Figure 1: Paralysis and death time of *Tubiflex tubiflex*. treated with different concentrations of aqueous leaf extract of *Moringa oleifera.* Albendazole standard antihelminthic drug (20mg/ml) All values in triplicate, Mean ± SD.

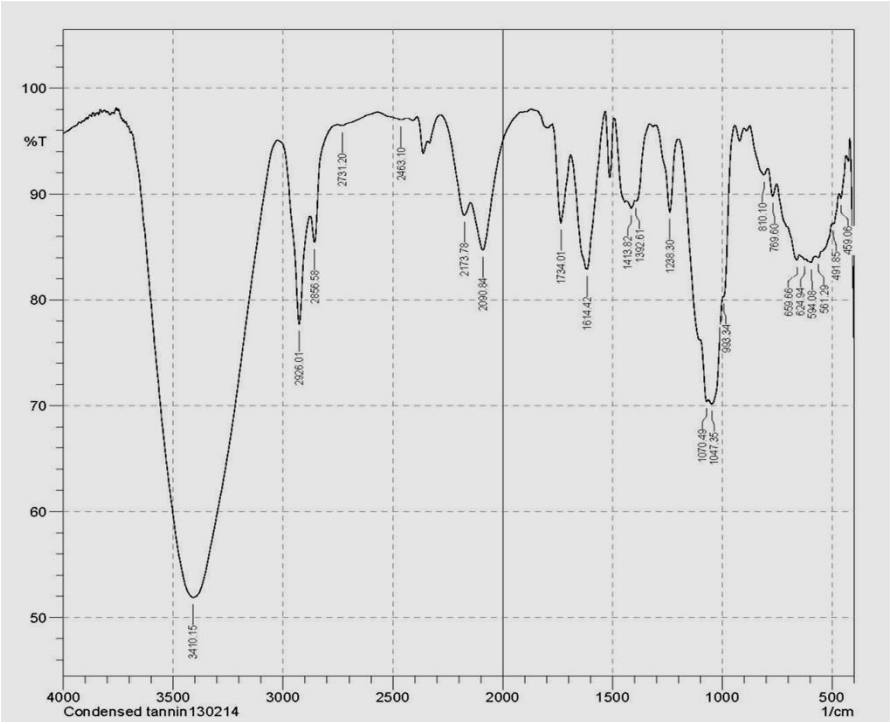

Figure 2: FT-IR Analysis of crude condensed tannin extracted from aqueous extracts *Moringa oleifera* leaves.

1% $FeCl_3$ was added to confirm the presence of condensed tannin.

FT-IR Analysis

The isolated crude tannin was subjected to FT-IR analysis. Potassium bromide was mixed with the sample in the ratio of 1:100 FTIR spectrum of samples was recorded on Shimazdu IR prestige – 21FTIR instrument with a diffuse reflectance mode (DRS-8000) attachment. The measurements were in the range of 650-4500 cm-1 at a resolution of 4cm-1. This range was used to study the fundamental vibrations and associated rational- vibrational structure.

Antihelminthic assay of condensed tannin

The crude residue containing tannin was suspended in water to obtain concentration of 100, 250,500, 750 and 1000 µg/ml. T.*tubiflex* was exposed to the crude extract and the paralysis and death time was recorded.

RESULTS AND DISCUSSION

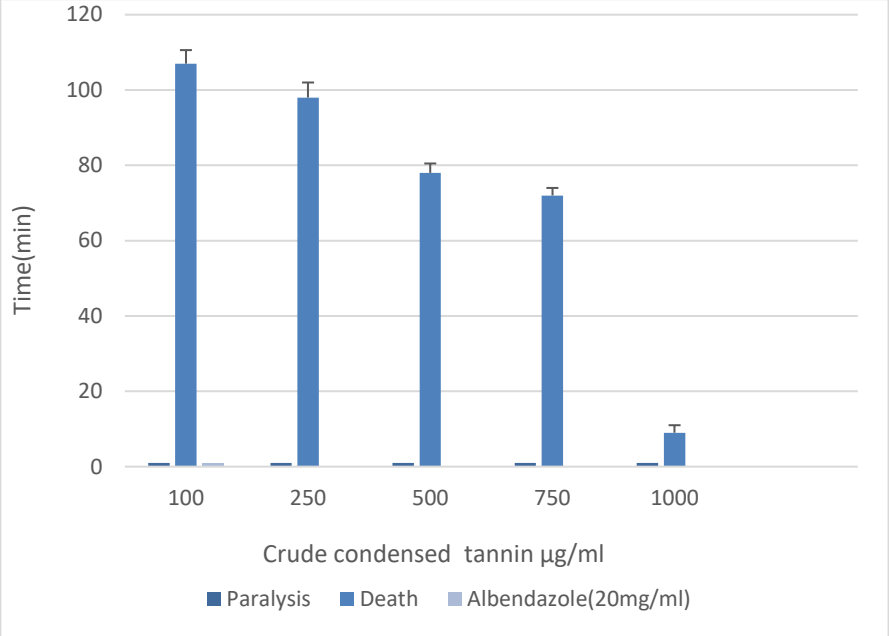

Figure 3: Paralysis and Mortality time of *Tubiflex tubiflex* treated with different concentrations of crude condensed tannin extracted from *M.oleifera*. All values in triplicate, Mean ± SD.

The paralyses and death time of *Tubifex tubifex* (blood worm) was found to be directly proportional to the concentration of the extract. The crude *Moringa oleifera* extract (200mg/ml) paralysed bloodworm in 60 seconds and caused death at 2 minutes (Fig-1) which was on par with pure albendazole at 20mg/ml.

The tannin content in *Moringa oleifera* leaves were found to be 530 µg/ml. The quantity of tannin estimated is directly proportional to the rate of total activity. The type of isolated crude tannin was confirmed to be condensed tannins based on the phytochemical test (since it resulted in brownish black colour on treating with ferric chloride). The FT-IR analysis revealed a very broad absorption band between 3,400 and 2,700 was due to the presence of hydroxyl groups. Three peaks occurring between 1392-1614 cm^{-1} were characteristic of aromatic compounds. The peaks in 600 to 900 cm-l and smaller peaks in the range of 1,000 and 1,200 cm^{-l} indicate substituted benzene rings (Fig-2). The extracted tannin was identified to be condensed tannin due to appearance of major peaks which are corresponding to condensed tannin reported earlier [12].

All concentration of the crude condensed tannin studied seemed to have similar effect on the rate of paralysis in *Tubiflex tubiflex*; it paralysed the blood worm at 60 seconds. The death time at higher concentration (1000 µg/ml) was 9 min; while at lower concentration (100 µg/ml) it was recorded to be 107 min(Fig-3).

Several factors may influence the anthehelminthic activity of a plant extract, since the active components contained in the plant may vary in relation to location, age, stage of development of the plant and the nature of collection [13]. *Moringa oleifera* contains several phenolic substances such as gallic acid, chlorogenic acid, ellagic acid, ferulic acid and vanillin from the aqueous extracts of leaves that are reported to impart certain physiological activities[14]. Tannins are polyphenolic substances that are reported to exhibit antihelminthic activity. Tannins are known to bind with free protein and reduce the availability of nutrient to the parasite, thereby subjecting it to starvation and death[15]. The mode action of these phenolic substances were shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[14] and most of the synthetic antihelminthics such as Oxyclozanide, Niclosamide, Nitroxynil, Bithionol etc. revealed a similar mechanism of action. Tannin or their metabolites have direct effect on the viability of the preparasitic stages of helminthes[16]. It was evident that lower concentration (0.1 mg/ml) of condensed tannin was ineffective in causing mortality of the *T.tubiflex* ; although they may be efficient against the actual intestinal parasites. From the study it is evident that the time of paralysis is important than the mortality of the worm, since paralysis can shut down vital activities of the worm and result in the depletion of energy resource which in turn would lead to the death of the parasite. Synthetic antihelminthic drug such as albendazole seems to have a similar mode of action. Hence it can be concluded that condensed tannin extracted from *Moringa oleifera* leaf aqueous extract is an effective antihelminthic substance.

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