

# In vitro Evaluation of the Anti Helminthic Activity of Selected Eritrean Traditional Medicinal Plants



Yohana Habte G Amlak<sup>1</sup>, Yohana Ghirme Weldu<sup>1</sup>, Yodit Cheay G Silasie<sup>1</sup>, Ariam Yemane Fisehaye<sup>1</sup>, Jeevan Jyoti kaushik<sup>2</sup>, Dawit Eman<sup>2</sup> and Hagos Andom Tuumzghi\*<sup>2</sup>

<sup>1</sup>MOH, central region, Asmara, Eritrea

<sup>2</sup>Asmara College of Health Sciences, Asmara, Eritrea

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\*Corresponding author: Hagos Andom Tuumzghi, MOH, central region, Asmara, Eritrea, PO Box: 8566

## Abstract

The aim of the study was to determine the anti helminthic activity of two medicinal plants (Taget esminuta and Moringa Olifera) in Eritrea. Fractionated extracts of the plants were prepared using different solvents and their anthelmintic activity was tested on earthworms (Pheretima postuma). The histopathological studies of the worms was conducted to compare the histological damage on the test animals in comparison with the standard drug. Methodology the anthelmintic activity was carried out on freshly collected adult earthworms (Pheretima postuma). The study was carried as per method of Ajaiyeoba et al. [1]. The earthworms of similar length and width were subjected to different concentrations of each plant extract. At the same time, positive and negative controls were carried out. Time taken for paralysis and death of individual worms were recorded.

**Results:** The highest yield percentage (22%) was obtained with aqueous ethanol and chloroform solvents from Moringa Olifera leaves and with aqueous-ethanol in case of T.minuta leaves. Chloroform extract of Tagetes diminuta showed the most remarkable activity in paralyzing and killing the earthworms whereas aqueous-ethanolic extract of Moringa Olifera leaves was the most potent extract than the other extracts. From the histopathological studies it was observed that the leaf extract of Moringa Olifera as well as that of T.minuta have worm icidal effect. The fruit extract of Moringa Olifera has better damaging effect than that of the leaf extract of this plant.

**Conclusion:** From going observation on the effect of the extracts of two selected plants lead us to reach the conclusion that both the leaf and fruit extract of M.olifera as well as the leaf extract of TMinuta have worm icidal effect. To conclude, in future studies, there is need for thorough phytochemical, clinical and possible studies on molecular mechanism of action of these plants.

**Keywords:** In vitro Evaluation; Medicinal Plants; Anti Helminthic; Eritrea; Moringa Olifera; Phyto Chemical; Helminthes; Anaemia; Eosinophilia; Filter Paper; Autoclave Tape

## Introduction

Human kind has been exposed to infection by microorganism since before the dawn of recorded history [1]. Diseases caused by intestinal parasites (helminthic) pose a great threat to health and contribute to the prevalence of malnutrition, anaemia and eosinophilia [2]. All cultures have folk medicine traditions that include the use of plants and plant products [3]. Traditional medicinal plants are linked to traditional culture with traditional healers and herbalists acting as important stakeholders [3]. The plants are known to be a rich source of botanical and the helminthics. It is estimated that two thirds of the world population still rely on traditional medical remedies, mainly plants, because of limited availability and affordability of pharmaceutical medicines [4]. Today more than 70% of the people in Africa refer to traditional healers concerning health issues [4]. The world health organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the health care program of developing countries because of the great potential they hold in combating various diseases [4].

Thus, limitations of reliance on chemotherapy are the threat of parasites developing resistance to drug treatment, the cost of drugs for people in developing countries and for some helminthes, lack of efficacy of current available drugs [5]. Therefore, novel and complementary helminthes control options are urgently needed. Taget esminuta is a strongly scented annual herb with stiff erect stems up to 2m tall. Leaves stalked, opposite, light green, 7-15cm long, pinnately dissected into 4-6 pairs of pinnate. Secretions from roots are effective against nematodes, worms, insects, fungi and perennial weeds [6]. It has antimicrobial, antiparasitic, sedative, fungicide and hypertensive properties [6]. Moringa Olifera is often referred to as the "miracletree" because of its uniquely diverse array of nutritional, medicinal, and purifying properties, it is a small or medium sized tree, fast growing deciduous or sharp tree that reaches 12m height when mature [6]. It's known to have anti-diabetic, anti-hypertensive, anti-inflammatory, anti-microbial, anti-viral, anti-parasitic, anti-tumor and anti-aging activities [6].

## Methods and Materials Used

### Collection and Identification of Earth worms

The assay was performed on adult earthworm (Pheretima postuma) owing to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Easy availability of earth worms and free-living lifestyle prompts their extensive use for preliminary in-vitro evaluation of anthelmintic compounds. Earthworms were collected from the water-logged soil besides Expo ground and were identified by an anatomist. All earthworms were of approximately equal size (15cm long).

### collection and identification of selected plants

Two plants (Tagetes minuta and Moringa Olifera) which are locally used by traditional healers were collected in the month of March 2015 from different locations like Elabered and Asmara and were used for the present experimental study. The leaves of Tagetes minuta and leaves and fruits of Moringa olifera were used for the present study. The selected plants were authenticated by Botanist. The collected plants were deposited in the department of Clinical Laboratory Sciences, School of Allied Health Profession, ACHS, Asmara.

### Standards

The anthelmintic activity was carried out on freshly collected adult earthworms (Pheretima postuma). The anti-parasitic activity was carried out as per method of Ajaiyeoba et al. [7]. The earthworms were similar in length and width. They were washed with normal saline to remove soil, dust and mud from them and were put in petri-dish. Each Petri dish had three worms of approximately equal size (Figure 1). Three different concentrations of 10mg/ml, 50mg/ml and 100mg/ml of each plant extract were used. The solutions were introduced into the respective petri-dishes. At the same time, positive (Mebendazole, 20mg/ml) and negative control (normal saline) were carried out.



Figure 1: Treatment with 70% ethanol.

### Chemicals

The two plants were subjected to cold extraction using aqueous ethanol, Petroleum ether, Chloroform; Methanol and percentage yield was obtained.

### Extraction method

The plant materials were washed under running tap water to remove mud and dust. The samples were air-dried for 7 days and

were well pulverized into a fine powder with a mixer grinder. The powder was stored in air tight aseptic containers ( $28^{\circ}\text{C}\pm 2$ ) for subsequent use. Weighed material (50g each) was placed in conical flask containing the different solvents and then placed on an electronic haker for 48 hours at the speed of 100rpm. The fluids were then filtered using Whatman No.1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in the fridge ( $4^{\circ}\text{C}$ ) prior to use.

### Quality Control

All the equipments used for bioassay were autoclaved/heat sterilized. The petri-plates and beakers prepared were autoclaved at  $121^{\circ}\text{C}$  for 30 minutes along with Bowie-Dick autoclave tape. During the analytical process Mebendazole as positive control and normal saline as negative control was used.

### Histo-Pathological Studies

Histo-pathological slides were made from the treated earthworms with plant extracts. Sections from the earthworms in portions before and after the clitellum were selected. Fixations of these portions were made by 10% formalin for 24 hours. These fixated pieces were put into the processing machine (alcohol, xylene and Wax). They were further directed to embedding and moulding in an embedding medium causing them to solidify. The embedded portions were refrigerated, and the cooled portions were cut by microtome to 5 micron thickness and were transferred to water ( $150^{\circ}\text{C}$ ). Slides were made and transferred to oven with xylene coat. The prepared slides were examined by a pathologist. Finally, different pictures of the slides were taken and comparison with the control was made.

### Statistical Analysis

The precision of this test methodology was determined by statistical analysis of all results on paralysis (P) and/or death (D) time. Repeatability was considered as the difference between successive results obtained by the same technician under constant conditions on identical worms, in the correct and standard procedure of the test methodology. Reproducibility was considered as the difference between two independent results obtained by different technicians working in different laboratories on identical test worms in the correct and standard procedure of the test methodology.

### Results and Discussion

The highest yield percentage (22%) was obtained with aqueous ethanol and chloroform solvents from Moringa Olifera leaves. While the lowest yield percentage (7%) was obtained from the fruits of Moringa olifera by using methanol. Similarly highest yield percentage (18%) was obtained with aqueous ethanol in case of Tagetes minuta leaves and the lowest yield (8%) was obtained with chloroform and petroleum ether (Table 1). The aqueous ethanol extracts of Tagetes minuta showed paralysis and death of the earthworms at  $17\pm 2.9$  min and  $35\pm 0.9$  min respectively at highest concentration of 100mg/ml and  $32\pm 1$  min and  $210\pm 3.6$  min respectively upon treatment with 10mg/ml. Results are found to be dose dependent with increasing concentration.

**Table 1:** The percentage yield of the selected plants.

Selected plants	Part of plant used	Solvent used	Wt. of the material	Wt. of the Extracts	Yield Value
Tagetes diminuta	Leaves	Aqueous-ethanolic	50g	9g	18%
	Leaves	Petroleum ether	50g	4g	8%
	Leaves	Chloroform	50g	4g	8%
	Leaves	Methanol	50g	8g	16%
Moringaolifera	Leaves	Aqueous-ethanolic	50g	11g	22%
	Leaves	Petroleum ether	50g	4g	8%
	Leaves	Chloroform	50g	11g	22%
	Leaves	Methanol	50g	4g	8%
Moringaolifera	Fruits	Aqueous-ethanolic	50g	5g	10%
	Fruits	Methanol	50g	3.5g	7%

**Table 2:** Anti Helimetic activity of Moringaolifera and Tagetes diminuta leaves.

Group	Treatment of extracts	Concentration (mg/ml)	Time of paralysis Moringao lif era leaves(min)	Time of death of Moringao lif era leaves(min)	Time of paralysis of Tagetes diminuta leaves (min)	Time of death of Tagetes diminuta Leaves (min)
1	Control Saline	-	-	-	-	-
2	Mebendazole	20	37±2.1min	60±2min	37±2.2min	60±2min
3	Aqueous Ethanol	10	11±1.2min	35±1.25min	32±1min	210±3.6min
		50	6±0.6min	20±2.8min	27±3.8min	45±2.6min
		100	57±0.025sec	13±1.25min	17±2.78min	35±0.86min
4	Petroleum Ether	10	12±2min	50±4.24min	8±2min	90±4min
		50	9±1.1min	38±1.6min	7±1min	60±1.6min
		100	6±1min	20±3min	5±1min	50±2.8min
5	Chloroform	10	20±1min	58±0.7min	4±1.6min	13±2.1min
		50	18±1.8min	50±0.5min	2±1.27min	7±3.12min
		100	16±2min	38±1min	55±0.6sec	4±0.9min
6	Methanol	10	9±1min	53±2min	13±2.5min	43±2.26min
		50	6±2min	41±1.7min	9±2.3min	19±3.2min
		100	4±0.8min	28±2min	7±1.5min	12±2.7min

Data is an average of two experiments consisting three earthworms in one plate.

All the values represent Mean±S.D; n=3 in each group.

The death time of earthworm when treated with petroleum ether extract was 50±2.78min, 60±1.58min and 90±4min respectively using 100mg/ml, 50mg/ml, 10mg/ml concentrations, which was observed to have the longest death time as the concentration of plant extract was less than the two higher concentrations. Chloroform extract of Tagetes diminuta showed the most remarkable activity and paralyzed the earthworms within 55±0.6sec using 100mg/ml of plant concentration, so it can be considered as most potent extract of the plant. The fourth solvent used for the extraction of Tagetes diminuta was methanol. It paralyzed the earthworms at 13±2.5min, 9±2.3min and 7±1.5min using the 10mg/ml, 50mg/ml and 100mg/ml concentration while the death time was recorded 43±2.26min, 19±3.2min, 12±2.7min for the three concentrations (10mg/ml, 50mg/ml and 100mg/ml) (Table 2).

Aqueous ethanolic (70% ethanol) extract of Moringaolifera leaves showed a variable effect when three different concentrations

(10mg/ml, 50mg/ml, 100mg/ml) were used. The highest concentration (100mg/ml) resulted in death of earth worms at 13±1.2min, whereas at the lowest concentration (10mg/ml) death was observed at 35±1.25min (Table 2). In the case of Petroleum ether, paralysis of the earth worms was observed in 12±2min for the lowest concentration and 6±1min for the highest concentration. Death time with petroleum ether extract was recorded at 50±4.24min, 38±1.6min, and 20±3min at 10mg/ml, 50mg/ml and 100mg/ml concentration respectively. Chloroform extract also showed good and the lminthic activity. Results clearly indicated that with increased concentration, the effect also increased. Among all the solvents used, Methanol showed the third better activity as the paralysis and death time at the highest concentration was observed at 4±0.8min and 28±2min respectively (Table 2).

Aqueous-ethanol and methanol were used for extraction of Moringaolifera fruits. Aqueous-ethanol extract with Concentration of 10mg/ml, 50mg/ml and 100mg/ml showed a paralysis time in 4±1.8min, 2±1.2min and 55±0.4sec respectively, whereas the death

time were recorded at  $8 \pm 2.1$  min with the highest concentration and  $45 \pm 2.7$  min with the lowest concentration. For the methanol extract, paralysis and death time of the earthworms were recorded  $50 \pm 0.5$  sec and  $4 \pm 2.2$  min at the highest concentrations respectively. Both the leaf and fruit extract of *M. olifera* have homicidal effect. Also the fruit extract of *M. olifera* has still better damaging effect than that of leaf extract of this plant. The effect of extracts of *Moringa Olifera* leaves on the paralysis of the worm, according to the results (Table 3) may be indicated as Aqueous ethanol >Methanol >Petroleum Ether>Chloroform.

**Table 3:** Anthelmintic activity of *Moringa olifera* fruits.

Groups	Treatment of extracts (min)	Concentration (mg/ml)	Time of paralysis (min)	Time of death (min)
1.	Control saline	-	-	-
2.	Mebendazole	20	$37 \pm 2.1$ min	$60 \pm 2$ min
3.	Aqueous ethanol	10	$4 \pm 1.8$ min	$45 \pm 2.7$ min
		50	$2 \pm 1.2$ min	$40 \pm 1$ min
		100	$55 \pm 0.8$ sec	$8 \pm 2.1$ min
4.	Methanol	10	$3 \pm 2.3$ min	$20 \pm 4.4$ min
		50	$2 \pm 0.8$ min	$12 \pm 4.35$ min
		100	$50 \pm 0.5$ sec	$4 \pm 2.2$ min

Data is an average of two experiments consisting three earthworms in one plate.

All the values represent Mean  $\pm$  S.D; n=3 in each group.

The combination of the *M. olifera* (fruit and leaves) and *T. Minuta* (leaves) showed paralysis at  $15 \pm 3$  min with the lowest concentration of Aqueous ethanolic extract and at  $3 \pm 2$  min with the highest concentration, where as death time was recorded at  $40 \pm 3.6$  min and  $12 \pm 3$  min respectively. The 50 mg/ml concentrations paralyzed the earthworms within  $8 \pm 1$  min and killed them in  $20 \pm 4.2$  min. The plants' extractives showed the lminthic activity and were dose-dependent that increase in concentration of plant showed increased destruction on earthworms. In the combination, the antihelmintic activity of aqueous ethanolic extracts of *M. olifera* (fruit and leaves) was reduced when tested in combination with *T. Minuta* although a clear dose-dependent effect indicating increased activity with increased concentration has been found (Figure 2).



**Figure 2:** Control.

Similar kind of study was done by Vidyadhar et al. in 2010 on various extracts of the whole plant of *Enicostemma littorale*. Various concentrations (25, 50, 100, 200 mg/ml) of all extracts were tested and results were expressed in terms of time for paralysis and time

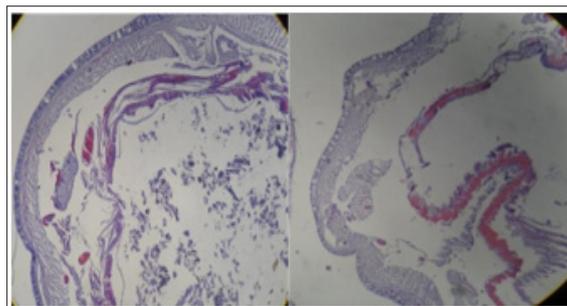
for death of worms. Albendazole was used as a reference standard and saline as a control group. The ethanol extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of ethyl acetate, water, chloroform, hexane extracts. In particular the ethanol extract exhibited an increased paralytic as well as helminthic effect overall bendazole at the given experimental concentrations [8].

In another study the root part of *Capparis decidua* Edge was evaluated for the anthelmintic activity of the plant at 10, 50 and 100 mg/ml using ethanol as the main extraction solvent against Indian earthworm *Pheritima posthuma*. The activity was found dose dependent compared with the standard drug used at 10 mg/ml activity increased with increase concentration of plant [9]. A study conducted on the anti-helmintic activity of the drupe extracts of *Melia azadirachta* L. (Meliaceae) growing in Argentina against a tape worm and an earthworm, showed to be better than the standard piperazine phosphate, which is used in the treatment of Cestoda infections as it showed mean paralysis and death time value at 32 min and 68 min using 70% Ethanol at high (80 mg/ml) and low (20 mg/ml) concentration [10].

In the present study when the fruits of *Moringa Olifera* were extracted in aqueous ethanol (70%) and methanol, both showed excellent anthelmintic activity. The earthworms were paralyzed in  $55 \pm 0.8$  sec and  $50 \pm 0.5$  sec in aqueous ethanol (70%) and methanol extracts respectively at 100 mg/ml while death was caused at  $8 \pm 2.1$  min with aqueous ethanol extract and  $4 \pm 2.2$  min with methanol extract at same concentration. Several studies showed that extracts from plants grown to serve as human food for example: coconut, onion and garlic, annaas, chicory have high Anti Helminthic potential against intestinal nematodes, cestodes, and trematodes [11].

### Histo pathological studies

**Effect of treatment with *Tagetes diminuta* extract:** Histo pathological observations revealed that treatment of earthworms with leaf extract of *T. minuta* at 100 mg/ml (Chloroform extract) were effective. The epidermis and the overlying cuticle were damaged; epidermal cells appeared to be bulged. There was reduction in the height of circular muscle layer (CML) and longitudinal muscle layer (LML) in comparison to the control (saline). The coelomic septa and gut epithelium appeared to be distorted. The vasculature of the body and the VNC were found to be intact. The histopathological damages due to these extracted plants were compared to that of control animal picture (Figure 3).



**Figure 3:** HS of *T. Minuta* leaves.

### Effect of treatment with *Moringa Olifera* extracts

Histopathological studies revealed that animals treated with leaf extracts of *M.olifera* (100 mg/ml of 70%-ethanol) showed damaging effect on their body wall as well as visceral organs. The epiderm is of the body wall was more or less completely destroyed. Epidermal cells were variously damaged, although cuticle, the upper most lining of the body wall was less affected. The musculature of the body wall has also showed reduction. The CML and LML appeared to be reduced in their height in comparison to the selayer of normal control animals. The visceral organs such as coelomicsepta (CS) and gutepi the lium were completely dismantled. However, the dorsal and ventral blood vessels were found to be less affected. The ventralnervcord (VNC) on the other hand was intact (Figure 4). It is worth to mention here that fruit extract of same plant was more effective than its leaf extract (Figure 5)

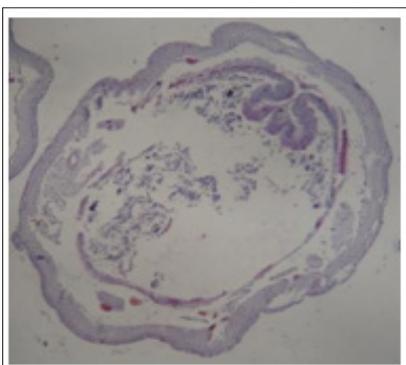


Figure 4: HS of *M.olifera* leaves.

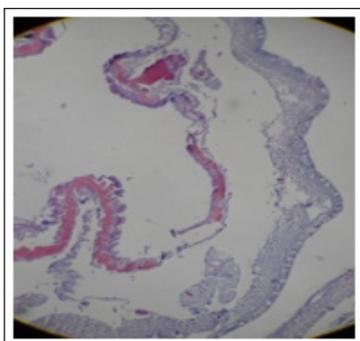


Figure 5: HS of *M.olifera* fruits.

### Effect of treatment with combined extracts

The histopathological studies pertaining to effect of combined extracts of *M.olifera* and *T. Minuta* on the experimental worm revealed that there was no remarkable damage on the body wall. However, the epidermal cells seemed to be partly damaged. The cell size was relatively enlarged and some of the cells were completely damaged. The height of CML and LML was also reduced as it was observed in animals treated with leaf extracts of both plants. The visceral organs such as coelomic septa and gut were also damaged to a large extent. Vasculature and nervous tissues, especially VNC do not seem to be much affected (Figure 6). Animals treated with reference drug did not show any remarkable damaging effect on the tissues.

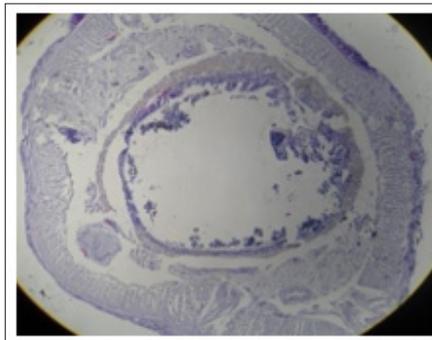


Figure 6: HS of combined extracts

### Conclusion

The present study was an attempt to conduct in vitro antihelmintic activity of selected Eritrean medicinal plants to substantiate the folk claim. This study reveals new scientific information about the leaf extracts of *Taget esminuta* and leaf and fruits extractives of *Moringa Olifera* rain regard to a the lminthic activity. The plants' extractives showed a the lminthic activity and were dose-dependent that increase in concentration of plant showed increased activity on earthworms.

Fore going observation on the effect of the extracts of the two selected plants lead us to reach the conclusion that both the leaf and fruit extract of *M.olifera* as well as the leaf extract of *T.Minuta* have wormicidal effect. Also the fruit extract of *M.olifera* has still better damaging effect than that of leaf extract of this plant. Present observation suggests that these herbal materials should be regularly used to get protected from the ill effects of the worms because these herbs have worm icidal effects. The exact mechanism of action of these plants is not known. However, it is apparent that these extracts cause damage to tissues of worms. Since these plant extracts have quick paralytic effect, it is advisable to make regular use of such herbs. Because once the worm is paralyzed, there is likely hood of worms to be easily flushed out from the body.

To conclude, in future studies, there is need for thorough phyto chemical, clinical and possible studies on molecular mechanism of action. At the same time efforts should be made to standardize the plant extracts with good antihelmintic activity and formulate best alternative herbal preparations to replace or complement the synthetic drugs which are currently in use (Table1)

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Hagos Andom Tuumzghi. Biomed J Sci & Tech Res



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