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## SCREENING OF CRUDE EXTRACTS OF MEDICINAL PLANTS USED IN ERITREAN UNORTHODOX MEDICINE FOR ANTHELMINTIC ACTIVITY

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### ABSTRACT

The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. In view of this, an attempt has been made to study the anthelmintic activity to validate the Eritrean unorthodox medicines. In this study, aqueous extracts of *Acacia etbaica*, *Commiphora myrrha* and *Heliotropium cinerascens* were used and studied for paralysis and death of earthworm (*Pheretima posthuma*). The results were compared with that of standard drug. The aqueous extract of *Commiphora myrrha* showed better *in vitro* activity against the parasites than the standard (Mebendazole) in the concentration ranging from 50 to 100 mg/ml. Most of the extracts were found not only to paralyze (Vermifuge) but also having the cidal effect on the earthworms (Vermicidal). The present study reveals that the *Commiphora myrrha* was found to be most effective to execute the earthworm.

**Keywords:** Aqueous extract, anthelmintic activity, *Heliotropium cinerascens*, and *Acacia etbaica*.

### INTRODUCTION

For centuries, plants have provided mankind with useful, life saving drugs. Even as the world entered the 20<sup>th</sup> century about 100 years ago, 'modern' pharmaceutical manufacturers were primarily involved in extracting, developing and marketing the active constituents present in the medicinal plants<sup>1</sup>. 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</sup>. A number of medicinal plants have been used to treat parasitic infections in man and animals<sup>3,4</sup>. In the Developing countries, the greatest impact of parasitic diseases is in direct and potential productivity losses. Many researches show that

some plants not only affect the nutrition of animals, but also have antiparasitic effects. For example, plants that contain condensed alkaloids, tannins secondary metabolites, have these effects<sup>5,6</sup>. The world health organization estimates that a staggering two billion people harbor parasitic worm infection. Parasitic worm also infects liver stocks and crops; affecting food production with a resultant economic impact. Despite this prevalence of parasitic infection the research of anthelmintic drug is poor. As per WHO, only few drugs are frequently used in the treatment of helminthes in human beings. Anthelmintics from natural source may play a key role in treatment of these parasitic infections. In view of this an attempts has been made to study the anthelmintic activity of *Acacia etbaica*, *Commiphora myrrha* and *Heliotropium cinerascens*. Traditionally, these plants known to have activity against worms, and have been used in the treatment of dysentery caused by worms. In this study aqueous extracts of leaves were used and observed for paralysis and death of earthworm.

## **Materials and methods**

### ***Plant materials***

All the plant materials were collected from Adi-Nfas (Maekel, Asmara) in April 2010 and they were taxonomically identified by Dr. Ghebrehiwet Medhanie Ghebreamlak, plant taxonomist, Department of Biology, Eritrean Institute of Technology (EIT), Mai Nefhi, Eritrea.

### ***Earthworm collection and identification***

Eritrean adult earth worms, *Pheretima postuma* (Annelida) 5-6 cm in length, were collected from the

water logged soil of Adi Abeyto (Asmara) and were identified by S.D. Pandy, Professor, department of basic and behavioral sciences, Asmara college of health sciences, Eritrea.

### ***Drugs and chemicals***

The standard Drug is Mebendazole (Azel Pharmaceutical share Company LTD, Keren, Eritrea)

### ***Preparation of different extracts***

All the plant materials were shade dried and crushed so as they will pass through a sieve with a mesh size between 2mm and 250 micrometer. 50g of each sample was extracted with 300ml cold water for 24-48 hours on shaker. All the extracts obtained were concentrated to dryness in vacuum at 40°C and stored at 4°C in the refrigerator until further used.

### ***Evaluation of Anthelmintic activity***

The Anthelmintic assay was carried as per the method of Ajaiyeoba et al. with necessary modifications.<sup>7</sup> The assay was performed on adult Eritrean earthworm *Pheretima postuma*, due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being.<sup>8</sup>

Because of easy availability, earthworms have been used widely for initial evaluation of anthelmintic compounds in vitro.<sup>9</sup> Eleven groups of approximately equal size earthworms consisting of six earthworms in each group were used for the present study. Groups first served as control, received only phosphate buffered normal saline; Group second served as standard, received standard drug (Mebendazole 20mg/ml). The Rest nine Groups received 25mg/ml, 50mg/ml and

100mg/ml concentrations of each plant individually. Observations were made for the time taken to paralysis and death of individual worms. Paralysis was set to occur when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility when pinched followed with fading away of their body color.

### Statistical Analysis

The extract treated groups were treated with one way ANOVA followed by Dunnett's test and compared with vehicle treated group. The data is presented as Mean  $\pm$  SEM. The activities of the aqueous extracts were compared with the control. All the extracts showed significantly higher duration of paralysis and death<sup>10, 11, 12</sup>

### Results and discussion

All the aqueous extracts, from 3 plant species, were tested against anthelmintic activity. The paralysis and death time of the organism are presented in Table 1. For most plant parts, the aqueous extract of *Commiphora myrrha* were found to be more active on earthworms than other aqueous extracts of other plant species (Table 1). The aqueous extract of leaves of *Commiphora myrrha* was found to be the most potent extract against earthworms, with a paralyzing time of 3.5 minutes and a death time of 4.30 minutes at 100 mg/ml concentration. The effect of the leaf extract of the plant was compared with standard mebendazole (A standard anthelmintic drug) (Table 1).

**Table 1: Antihelminthic activity of different aqueous plant extracts**

Test substance	Concentration (mg/ml)	Time Taken for Paralysis(P) and death ( D) (In minutes)	
		<i>Pheritima posthuma</i>	
		P	D
<b><i>Acacia etbaica</i></b>			
Aqueous extract	25	18.32 $\pm$ 0.58*	36.3 $\pm$ 0.89*
Aqueous extract	50	11.66 $\pm$ 0.63*	29.23 $\pm$ 0.58*
Aqueous extract	100	8.33 $\pm$ 0.47**	27.15 $\pm$ 1.73*
Mebendazole	20	6.12 $\pm$ 0.55***	7.66 $\pm$ 0.33***
Vehicle	-	-	-
<b><i>Heliotropium cinerascens</i></b>			
Aqueous extract	25	-	-
Aqueous extract	50	-	-
Aqueous extract	100	-	-
Mebendazole	20	6.12 $\pm$ 0.55***	7.66 $\pm$ 0.33***
Vehicle	-	-	-
<b><i>Commiphora myrrha</i></b>			
Aqueous extract	25	24.3 $\pm$ 0.33*	49.7 $\pm$ 2.02*
Aqueous extract	50	5.77 $\pm$ 0.58***	7.7 $\pm$ 0.32***
Aqueous extract	100	3.5 $\pm$ 0.41***	4.3 $\pm$ 0.72***
Mebendazole	20	6.12 $\pm$ 0.55***	7.66 $\pm$ 0.33***
Vehicle	-	-	-

All values are Mean  $\pm$  SEM; n=6 in each group. Values are significantly different from reference standard (Mebendazole) \*p<0.05; \*\*p<0.01; \*\*\*p<0.001

It was found that the extract that was obtained with water were active than mebendazole at 50 mg/ml but less active at 25 mg/ml. On the other hand, the aqueous extracts of other plant species showed lesser anthelmintic activity than *Commiphora myrrha* and the standard drug. From the three plant extracts, *Acacia etbaica* showed moderate anthelmintic activity, with an 8.33 and 27.15 minutes for paralyzing and death time of earthworms respectively. *Heliotropium cinerascens* was not showing any effect on earthworms.

Among all the extracts tested, the crude aqueous extract of *Commiphora myrrha* was more active than other aqueous extracts of different plant species. The anthelmintic activities of crude extracts can be explained in part by their partial lipophilic nature, which renders the cells leaky and thereby results in cell death. The best plant extract against earthworms was the aqueous extract of *Commiphora myrrha* leaves.

*In Eritrea*, all the three plants those selected for the present study are used for various treatments by traditional healers or practitioners. They are used as decongestant when soaked in water for one night and consumed before breakfast on the empty stomach. It also combat any wound infections by washing the wounded area with the solution of this resin, more over the smoke of this resin inhibits uterus infection, also it could treat Asthma by drinking its solution continuously for about 14 days. Besides it is said to have anti-inflammatory action. This plant is a promising candidate as a source of

bioactive compounds for the treatment of trypanosomiasis. Literature surveys indicate the presence of flavonoids, hydroquinone, phenolic carboxylic acids<sup>13</sup> and triterpenes<sup>14</sup> in *heliotropium* species. The antitrypanosomal activity observed could be attributed to one of these classes of compounds or a combination of several.

### Conclusion

The two plants *Acacia etbaica* and *Commiphora myrrha* merit further consideration for the phytotherapy of trypanosomiasis as they showed good anthelmintic activities in dose dependent manner. Further bio-guided isolation of compounds from these two plants is, however, required to confirm which specific compounds are responsible for the observed anthelmintic activity. The observation that *heliotropium* extract were inactive in vitro does not mean that they do not have activity on other cell types. Unless the concentration of pure active principle(s) in crude extracts is stated, care should be taken by the general population of Eritrea in oral application of crude extracts, because they are known to be converted to toxic metabolites by liver cells and subsequently cause liver cirrhosis.

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