Evaluation of *in vitro* anthelmintic activities of *Brassica nigra*, *Ocimum basilicum* and *Rumex abyssinicus*

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**ABSTRACT**

Helminthes infections are now being recognized as the cause of many acute as well as chronic illnesses in human beings and in cattle, particularly in tropical and subtropical areas. The development of anthelmintic resistance and the high cost of conventional anthelmintic drugs led to evaluation of herbal remedies as alternative anthelmintic for the control of these parasites. In the current study, *in vitro* experiments were conducted to determine the possible anthelmintic effects of crude aqueous and methanolic extracts of *Brassica nigra* (seeds), *Ocimum basilicum* (whole plant) and *Rumex abyssinicus* (roots) in a dose of 20 and 40mg/ml against Eritrean adult earthworm *Pheretima posthuma* to justify the traditional claim using mebendazole as a reference standard. From the results, the paralysis and death time of worms in all extracts was found to be dose dependent and the potent anthelmintic activity was observed in aqueous extracts in comparison to methanolic extracts. Among the three plants *Ocimum basilicum* showed a potent anthelmintic activity with least paralysis time and death time, whereas *Rumex abyssinicus* extracts showed an intermediate activity and *Brassica nigra* has taken long time for death of worms. All the values were found to be statistically significant with P value of 0.00 using one way ANOVA. Based on the findings it may be concluded that *Brassica nigra*, *Ocimum basilicum* and *Rumex abyssinicus* possess varying degree of anthelmintic activity and also justifies its folklore claims for potential anthelmintic property.

**Key words:** Anthelmintic activity, *Brassica nigra*, *Ocimum basilicum*, *Rumex abyssinicus*.

**INTRODUCTION**

Helminthes are recognized as a major constraint to livestock production throughout the tropics and elsewhere and are considered important in causing enormous economic losses through morbidity and mortality in livestock. As most of the developing countries of the world lie in tropical and subtropical region, warm and humid climatic conditions in the tropic/subtropics provide favorable environment for development of worm eggs to infective larvae almost throughout the year.[1]

Among the parasitic diseases, gastro-intestinal (g/I) nematodes such as *Haemonchus contortus*, *Trichostrongylus spp.*, *Cooperia spp.*, *Oesophagostomum columbianum*, *Trichuris spp.* and *Strongyloides papillosus* are most common cause. This group of gastrointestinal nematodes is associated with anaemia and gastroenteritis resulting loss of body weight, stunted growth, diarrhoea etc. that greatly hamper the normal growth and production of goats. Thus parasite problem is unquestionably being a major limiting factor in the improvement of livestock production.[2] *Haemonchus contortus* is a highly pathogenic helminthes of small ruminants in all age groups, and is one of the top 10 constraints of sheep and goat production in East Africa.[3]

Helminthes infections are also among the most common infections in human, affecting a large proportion of the world’s population in developing countries and produce a global burden of disease and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia which more often physically impair their hosts than kill them.[4] Control of gastrointestinal Helminthes infections in the livestock relies mainly on the use of anthelmintic in combination with farm management.[5]

Various problems have been evolved with chemotherapeutic control practices such as parasites are developing resistance
to several families of chemical anthelmintics,[6] chemical residues and toxicity problems,[7] un-economical and non-availability of drugs in remote areas. Furthermore, it has been recently recognized that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health.[8]

For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific significance despite extensive use of synthetic chemicals in modern clinical practices all over the world. In early times of the eldest human societies (Babylonia, Egypt, Greece, and S. Klimpel Rome), but also stretching from the early medieval centuries until the nineteenth century, plant diets were used to control parasites inside the house, in farm animals, as well as in humans based more on belief than on knowledge.[9] The plant kingdom is known to provide a rich source of botanical anthelmintics, antibacterials and insecticides.[10] A number of medicinal plants have been used to treat parasitic infections in man and animals.[11] However, their scientific evaluation as compared to commercial anthelmintics is limited. These factors paved the way for herbal remedies as alternative anthelmintic.

World Health Organization estimates that 80% of people in developing countries rely on traditional medicines.[12]

In Africa and other developing countries, most people depend on traditional herbal medicine for health needs, and these herbal remedies are usually safer than active compounds isolated from plants. Plant-derived anthelmintic products have the advantage of being more biodegradable and environmentally friendly.[13] A larger number of plants are naturally available in the Eritrea, which possess narrow or broad spectrum anthelmintic activities. Therefore the phytochemical analyses of naturally available plants and control anthelmintic trials along with contemporary knowledge of parasite control strategies may offer new opportunities for effective and economical control of parasitic diseases.

Based on information obtained from ethno-medicinal survey and on information obtained from herdsmen and pastoralists, the plants “Brassica nigra”, “Ocimum basilicum” and “Rumex abyssinicus” were selected. A review of the literature also revealed that the anthelmintic activity of these plants has not been subjected to scientific evaluation for its anthelmintic activity. Hence, the present study was carried out on aqueous and methanolic extracts of these three medicinal plants against Eritrean adult earthworm “Pheretima posthuma” to justify its folklore claim also aimed to make documentation and validation of these plants in a scientific approach in Eritrea. Recognizing the value of this indigenous knowledge empowers livestock owners to attempt to solve their animal health problems in a cost effective way.

**MATERIALS AND METHODS**

**Plant material**

The 3 medicinal plant materials i.e., Seeds of *Brassica nigra* (Brassicaceae), English name: Black mustard and Vernacular name: Adri, Whole plant of *Ocimum basilicum* (Lamiaceae), English name: Sweet basil and Vernacular name: Seseg and Roots of *Rumex abyssinicus* (Polygonaceae), English name: Spanish rhubarb and Vernacular name: Mokmoko were procured from the local market/field (Medeber), Asmara, Eritrea in April 2010. The collected plant materials were authenticated by the Taxonomist Dr. Gebrehiwet Medihanie, Department of Biology, Eritrean institute of technology (EIT), Mai Nefhi, Eritrea and a voucher specimen was numbered and kept in our research laboratory for further reference.

**Extract preparation**

All collected plant materials were thoroughly cleaned with water to remove adulterants and dried under shade at a well ventilated place for five days at 35-40°C until it became grindable. The dried materials were ground with the help of electric grinder and preserved separately for further extraction with respective solvents.

**Aqueous extracts preparation**

Powdered materials of *Brassica nigra* (60 g), *Ocimum basilicum* (20 g) and *Rumex abyssinicus* (50 g) were extracted separately in soxhlet apparatus using distilled water at 50°C. then the extract was filtered using What man No 1 filter paper, the clear filtrate obtained was concentrated by a rotary evaporator at a temperature not exceeding 40°C under reduced pressure, lyophilized and the resulting powder was stored at 4°C until use, and dissolved in solution of 0.1% Tween 80 in a normal saline on the day of the experiment to prepare stock solution and different test dilutions for the purpose of evaluating anthelmintic activity. The percentage of extraction was calculated by using the following formula: percentage of extraction = weight of the extract (gram)/ weight of the plant material (gram) × 100.

**Methanol extracts preparation**

Powdered materials of *Brassica nigra* (50 g), *Ocimum basilicum* (20 g), and *Rumex abyssinicus* (60 g) were used for extraction. Each plant materials were separately soaked in sufficient amount of methanol by cold maceration at room temperature for a total of 3 days. After that the filtrates were collected through a What man No 1 filter paper under vacuum pressure and the plant materials re-soaked twice. The combined filtrates of each plant material was concentrated in a rotary evaporator at 40°C under reduced pressure to yield a thick and dark coloured crude extract, lyophilized and the resulting powder was stored at 4°C until use and...
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**Preliminary phytochemical screening**

Standard methods were used for preliminary phytochemical screening of methanolic and aqueous extracts to know the nature of phyto constituents present in it.

**Evaluation of anthelmintic activity**

**Drugs**

The aqueous and methanolic extracts of *Brassica nigra*, *Ocimum basilicum* & *Rumex abyssinicus* were tested in two doses i.e., 20 mg/ml & 40 mg/ml in each group. Tween 80 (0.1%) in normal saline was used as control. The standard drug Mebendazole was obtained from Azel Pharmaceuticals, Keren as a gift sample for comparative study with aqueous and methanolic extracts. All other chemicals and solvents used were of analytical grade and obtained locally.

**Experimental animal model**

Healthy adult Eritrean earthworms, *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings were used in the present study. Because of easy availability, earthworms have been widely used for the initial evaluation of anthelmintic compounds *in vitro*.

**Worm collection and authentication**

Eritrean adult earthworms (*Pheretima posthuma*; Annelid, Megascolecidae) collected from moist soil of “Maitemenay” around “Enda kirbit” of Asmara (Eritrea) and washed with normal saline to remove all extraneous matters. All earthworms were of approximately 4-8 cm in length and 0.1-0.2 cm in width was used for all the experimental protocol. These Earth worms were identified and authenticated by Mrs. Ghinjia, Parasitologist, Head, School of Allied Health Professions, Asmara College of Health Sciences, Asmara, Eritrea.

**Grouping of worms**

Four groups of approximately equal size earthworms consisting of three earthworms in each group were used for the present study. **Group first** serve as control, receive only Tween 80 (0.1%) in normal saline, **Group second** serve as test-1, receive Methanolic extracts, **Group third** serve as test-2, receive aqueous extract and **Group four** serve as standard, receive standard drug Mebendazole of different concentration.

**Anthelmintic assay**

Screening of plants for their anthelmintic activity has multiple objectives. These include:

1. Validation of the claims of the farmers using different plants for anthelmintic purposes using standard parasitological procedures
2. Exploring the possibilities of discovering new plants with anthelmintic properties.

The anthelmintic activity was evaluated as per the method of Dash et al with slight modification. The extracts were suspended in Tween 80 (0.1%) in normal saline. All the drugs and extracts were prepared freshly before starting the experiment. Twelve groups of six earthworms each were released into 10 ml of desired formulation as follows; vehicle (Tween 80 (0.1%) in normal saline, Mebendazole (20 and 40 mg/ml), methanol and aqueous extract (20 and 40 mg/ml, each) in Tween 80 (0.1%) in normal saline.

Observation was made for the time taken to paralysis and death of individual worms up to 4 hrs of the test period. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Death was concluded when the worms lost their motility followed with fading away of their body colours. All the results were expressed as a Mean ± SEM of six animals in each group.

**Statistical analysis**

All the data’s were subjected to one way ANOVAs analysis for calculating P value using the SPSS software Version 18. Results with \( p < 0.05 \) were considered to be statistically significant.

**RESULTS**

In the present work, aqueous and methanolic extracts of selected plants used in traditional medicine in Eritrea were tested for their anthelmintic activity. The percentage yield of aqueous extracts of *Brassica nigra*, *Ocimum basilicum* & *Rumex abyssinicus* were found to be 1.4, 4.5, 5.54% w/w respectively with reference to the dried plant material. Similarly the percentage yield of Methanolic extracts of *Brassica nigra*, *Ocimum basilicum* & *Rumex abyssinicus* were found to be 6.52, 10.8, 10.73% w/w respectively with reference to the dried plant material. Preliminary phytochemical screenings on these extracts revealed the presence of flavonoids, alkaloids, saponins, carbohydrates and tannins in the extracts.

From Table 1 and Table 2, it is very clear that methanol and aqueous extracts showed better anthelmintic activity.

When compared with standard drug at the same concentration, vehicle worms were alive up to above 12 hrs of observation. From the observations made, the paralysis and death of worms in different doses were found to be dose dependent. Among extracts, potent anthelmintic activity was observed in case of aqueous extract than the methanolic extracts.

From the results, it was observed that *Ocimum basilicum* showed a potent anthelmintic activity from all the aqueous and methanolic extracts. Aqueous and methanolic *Rumex abyssinicus* extracts shows an intermediate activity. Whereas extracts of *Brassica nigra* took longer time to bring death of worms. Even if this was the case these effects are even comparable with the paralysis and death time of Mebendazole.

**DISCUSSION**

This study revealed that crude extracts of *O. basilicum, B. nigra* and *R. abyssinicus* exhibited strong in vitro anthelmintic activity against the model organism. Based on the preliminary phytochemical screening crude extracts of *Brassica nigra, Ocimum basilicum* and *Rumex abyssinicus* revealed presence of tannins as one of the chemical constituent, which are known for their antimicrobial activity and also have their application as anthelmintic.[18] Chemically tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death.[19,20] Presence of Tannins may be responsible for anthelmintic activities of plant extracts.

Mebendazole, a benzimidazole drug that acts by interfering with carbohydrate metabolism and inhibiting polymerization of microtubules. The function of the anthelmintic drugs like Mebendazole is to cause paralysis of worms. So, that they are expelled in the faeces of man and animals.[21] The extracts not only demonstrated paralysis of worms, also caused death of the worms. Hence our study has shown that, all the extracts have significantly determined anthelmintic activity. Statistical analysis reveals that significant in vitro anthelmintic activity (*P* = 0.00) was observed between the concentrations and time of exposure of all the extracts. The present investigation indicated that all the plant extracts has antihelminthic effects against Eritrean adult earth worm in vitro at the various concentrations tested.

**CONCLUSION**

The experimental evidence obtained in the laboratory model could provide a rationale for the folklore claims of *Brassica nigra, Ocimum basilicum & Rumex abyssinicus* for its potential anthelmintic property. These analyses have revealed for the

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Doses in mg/ml</th>
<th>Time taken for paralysis (P) and death (D) in minutes</th>
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<tbody>
<tr>
<td></td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>Brassica nigra</td>
<td>40</td>
<td>146.33 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>156.67 ± 0.719</td>
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<tr>
<td>Ocimum basilicum</td>
<td>40</td>
<td>22.67 ± 0.27</td>
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<td></td>
<td>20</td>
<td>96.67 ± 0.103</td>
</tr>
<tr>
<td>Rumex abyssinicus</td>
<td>40</td>
<td>29.67 ± 0.98</td>
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<tr>
<td></td>
<td>20</td>
<td>58 ± 1.25</td>
</tr>
<tr>
<td>Mebendazole (Standard)</td>
<td>40</td>
<td>73.3 ± 0.72</td>
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<td></td>
<td>20</td>
<td>101.33 ± 0.72</td>
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The value paralysis time (P) and death time (D) refer the mean ± SEM

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<tr>
<td></td>
<td>P</td>
<td>D</td>
</tr>
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<td>Brassica nigra</td>
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<td>17 ± 0.00</td>
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<tr>
<td></td>
<td>20</td>
<td>23.66 ± 0.271</td>
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<td>Ocimum basilicum</td>
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<td></td>
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<td>8 ± 0.47</td>
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<tr>
<td>Rumex abyssinicus</td>
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<td>12 ± 0.00</td>
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<td>20</td>
<td>36.67 ± 0.54</td>
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<tr>
<td>Mebendazole (Standard)</td>
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The value paralysis time (P) and death time (D) refer the mean ± SEM
first time that the components present in the solvent extracts of *O. basilicum*, *B. nigra* and *R. abyssinicus* have anthelmintic activity. These results strongly indicate that plant extracts derived from *O. basilicum*, *B. nigra* and *R. abyssinicus* contain promising bioactive compounds that might be useful in the control of helminth infections by interrupting the worms’ life cycle and preventing their growth. Since the motility of worms is also affected, it may be possible that these plant extracts also act on worm muscles and paralyse them. Remarkably, none of these selected plants have been previously tested. Our data reinforces the existing knowledge and the regular use of plants by herdsmen and pastoralists for the treatment of worm infections. So, it may be suggested that those 3 plants with strong wormicidal activity could provide alternatives in the control of helminthic infections. The high anthelmintic activity observed make these plants provide alternatives in the control of helminthic infections.

In future studies, we will isolate active compounds and investigate their mode of action. Additionally we are looking into safety aspects to determine the feasibility of developing effective and safe drug preparations for the treatment of helminth infections.

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