Invitro Antihelmintic Activities of Four Medicinal Plants against *Haemonchus contortus*

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***Abstract- In vitro* experiments were conducted to find out the anthelmintic effects of crude aqueous extracts of the leaves of *Cordia africana, Achyranthes aspera, Croton macrostachyus and Schinus molle* against adults of live *H. contortus* and the efficacy of these crude aqueous extracts of leaves were determined based on the mortality rate of the adult parasite. The plants tested for the efficacy at 4mg/ml of the aqueous extracts of *Cordia africana, Achyrantus aspera, Schinus molle* and *Croton macrostachyus* was 95%, 90%, 80% and 70% respectively against the adult stage of *Haemonchus contortus.* The efficacy of Albendazole against the adult parasite, on the other hand, was dose dependent and all the adult worms were dead at a concentration of 0.5mg/ml within 24 hours. The overall findings of the current study indicated that most of the plants have potential anthelmintic effect and further *in vitro* and *in vivo* evaluation should proceed using these plants in the future.**

***Index Terms*— Antihelmintics, Haemonchus contortus, in vitro experiment, Plant extracts**

I. Introduction

Parasitic infections remain a major constraint to livestock production globally (Tembely *et al.*, 1994; Waller, 1997). Among these, gastrointestinal parasites are one of the factors that contribute to low productivity of livestock in developing countries (Waller and Thamsborg, 2004). *Helminthosis* play a crucial role in small ruminant production leading to enormous economic losses particularly in areas where extensive grazing is practiced .It causes loss of production directly and indirectly. The direct loss is manifested through mortality, loss of blood and plasma protein by blood sucking behavior of the parasites and leakages into GIT, depression of mineral level and diarrhea, all contributing to weight loss, reduced milk and wool production (Soulsby, 1986). The indirect economic impact is manifested by increased cost of control strategies like antihelmintics, labor, drenching equipments and other parasite-related penalties such as delay in achieving target weights, increased feed requirements to achieve target weight and reduced quality of carcass and predisposition to other diseases (Kassai, 1999).

Haemonchus contortus, the causative agent of *Haemonchosis*, is also known as red stomach worm, wire worm or barbers pole worm. Commercial antihelmintics have been used for some decades throughout the world to combat *Hemonchosis*, and other worm infections for a long time, but resistance of the parasites against these chemicals is growing. The threats of antihelmintics resistance, risk of residue, availability and high cost, especially to farmers of low income in developing countries, have led to the notion that sustainable helminthes control cannot be achieved with commercial antihelmintics alone. Other alternative options like; biological control, vaccine, and traditional medicinal plants are being examined in different corners of the world (Bain, 1999; Chandrawathani *et al.*, 2003; Githiori, 2004; Waller and Thamsborg, 2004).

Since time immemorial, plants have been indispensable sources in both preventive and curative traditional medicine preparations for human beings and livestock (Derry *et al.*, 1999). According to the World Health Organization (WHO), medicinal plants form the bases of traditional or indigenous healthcare systems used by the majority of the population of most developing nations. Indeed, it is reported that more than 3.5 billion people rely on plants for the treatment of both human and livestock diseases. In south Asian countries alone 500 million people are reported to seek health security from the leaves, roots and barks of trees. This global utilization of medicinal plants has considerably increased in the last two decades (Medhin Zewdu *et al*., 2001).

Evaluation of the activities of medicinal plants claimed for anthelmintic property is getting attention these days (Gathuma *et al.*, 2004; Githiori, 2004). Rich literature is available on ethno veterinary use of medicinal plants as anthelmintics. However, there is only a brief description about the plants used and the conditions they are used during treatment. Most of these reports did not provide detailed information on the part of the plants used and method of preparation. Often no validation of the effect against the disease conditions is provided (Waller and Thamsborg, 2004). This study was, therefore, aimed to

assess the antihelmintic activity of leaves of *Cordia africana*, *Schinus molle, Achyrantus aspera* and C*roton macrostachyus invitro* using adults of live *H. contortus.*

II. MATERIALS AND METHODS

Study Area

The study was conducted in Addis Ababa, the capital city of Ethiopia. Geographically Addis Ababa is located at 9°2' N, 38°42' E with an elevation of about 2500 m.a.s.l. the area receives a mean annual rainfall of 1,800 mm in bimodal distribution. The long rainy season last from March to May. The average minimum and maximum temperature are 10.7° C and 23.6° C, respectively (NMS, 2005).

Plant Collection

Plant materials evaluated in the current study had been identified from various sources to serve as antihelmintic agents by traditional healers or farmers in different parts of Ethiopia. Those plants that believed to have anthelmintic activity but not scientifically evaluated for the reported activity were selected and the required parts of the plant species were collected from their natural habitats and transported to Aklilu Lemma Institute of Pathobiology (ALIPB) of the Addis Ababa University. All the plants were identified by a plant taxonomist and voucher specimens of each species were deposited at the ALIPB, Endod and Other Medicinal Plants Unit. The collected plant parts were air-dried at room temperature ground and stored until extraction starts. Names (scientific and local names) of the plant species used in this study, parts used, and areas of collection are shown in Table 1.

Extraction Method

Aqueous extraction of the plants was conducted in ALIPB. After collection, the leaves were dried by air. The dry leaves then crushed and 50 g of the dry powder from each extract was socked in 50 ml of distilled water and put in graduated flasks and shaken for 24 hours by electric shaker. For each plant species about six flasks that contains 50g of the powder and 50ml of distilled water were used. Then the suspension was filtered using mesh and the filtrate was filtered again using filter paper. After that the filtrate kept in deep freezer for 24 hours. After 24 hours it was lyophilized using lyophilizer. The lyophilized dry powder was then collected, weighed and kept in a dry place to avoid absorption of water until being used for the test.

Collection of Parasites

Adult parasites of *H. Contortus* were collected from the abomasum of sheep obtained from Addis Ababa Abattoir's Enterprise. The abomasum from 40 sheep were collected immediately after slaughtering and transported to ALIPB laboratory. In laboratory abomasum were washed by running water and the parasites were kept in Phosphate Buffer Saline (PBS) until the *in vitro* evaluation starts.

In vitro Evaluation of Plant Extracts on Adult Worms

The test was performed in 5cm diameter glass Petri dish. A total of about 420 adult parasites of *H. contortus* were used in the study. Four concentrations were employed for each plant extract. Twenty adult worms were placed in each Petri dish filled with 4, 2, 1, and 0.5mg/ml of the extract of the plant material and those parasites with PBS alone were served as a negative control group. Albendazole dissolved in Dimethyl Sulphoxide (DMSO) at the concentration of 0.5, 0.25, 0.125 and 0.0625 mg/ml was used as a positive control. After 24 hours, the extract was washed away and the parasites were re-suspended in PBS for 30 minutes for possible recovery of the parasite motility. Finally, the number of motile (alive) and immotile (dead) worms were counted under dissecting microscope and recorded for each concentration. A mortality index was calculated as the total number of dead worms divided by the total number of worms per Petri dish.

III. RESULTS

In vitro Effects of Plant Extracts on Adult Parasites

All the extracts showed inhibitory effect on the survival of *H. contortus* in a dose dependent manner. C*ordia africana, Achyrantus aspera, Schinus molle*, and C*roton macrostachyus* produced mortality of adult *H. contortus* to the level of 95%, 90%, 80%, and 70% respectively, at a concentration of 4mg/ml. 65%, 60%, 60%, and 50% mortality rate at a concentration of 2mg/ml, 40%, 45%, 35%, and 25% at a concentration of 1mg/ml, and 30%, 25%, 25%, and 15% at a concentration of 0.5mg/ml. (fig-1)

In vitro Effects of Albendazole on Adult Parasites

The *in vitro* evaluation was also conducted using Albendazole which was used as a positive control. The concentrations of the Albendazole were not the same as the concentrations of the plant extracts. Four concentrations were used, 0.5, 0.25, 0.125 and 0.0625 mg/ml. Albendazole brought about 100% parasite mortality at a concentration of 0.5mg/ml within 24 hours. When the concentration reduces to 0.25 the mortality becomes 30%. At a concentration of 0.125, 15% mortality effect was produced and at a concentration of 0.0625, and 5% mortality within 24 hours was obtained. The efficacy of the Albendazole was also dose dependent like the plant extracts.

IV. DISCUSSION

Several *in vivo* and *in vitro* techniques have been developed to detect antihelmintic resistance in nematodes (Craven *et al.*, 1999). However, *in vivo* tests are not the best model to screen plants extracts with antihelmintics activity, since these tests are time-consuming, expensive and present low precision and reproducibility due to inter-animal variation and pharmacodynamics of the drugs in the host (Lacey *et al*, 1990). The *in vitro* tests using free living stages of parasitic nematodes offer a means of evaluating the antihelmintic activity of new plant compounds, as already reported by various authors (Asase *et al*., 2005).

In traditional medicine, *Cordia africana* was used in treating a variety of GIT infections and the different parts of the plant have been used as antibacterial treatment (Manandhar, 2002). This finding is confirmed by the current study that the leaves of *Cordia africana* showed relatively higher mortality of the parasite. It showed 95% adult mortality of *H. contortus* at a concentration of 4mg/ml. The roots, leaves and the juice extracted from the root of *Achyrantus aspera* are used traditionally against ecto and endo-parasites (Markus and Ernst, 2005) and the present study also showed that the aqueous extract from the leaves of this plant exhibited 90% of adult mortality of *H. contortus* at a concentration of 4mg/ml. The leaves of *Schinus molle* is used to treat infections caused

by different GIT parasites (Machadoa *et al*., 2007). This is evident from the current study which showed 80% mortality of adult parasites of *H. contortus* at a concentration of 4mg/ml. In Tigray, the crushed leaves and seed of *Croton macrostachyus* are drunk in water for tapeworm, the fruit is eaten and a root-decoction drunk for venereal disease, and the seed eaten to induce abortion (Burkill, 1985), in this study aqueous extract of *Croton macrostachyus* exhibited 70% of adult mortality of *H. contortus* at a concentration of 4mg/ml.

Based on the ability of adult parasite mortality, the most potent extracts in increasing order were *Croton macrostachyus*, *Schinus molle, Achyrantus aspera* and *Cordia Africana*, which showed 95%, 90%, 80% and 70% respectively at a concentration of 4mg/ml.

Most of the plant extracts in the current study showed good inhibitory effect on the survival of *H. contortus* at concentration of 4mg/ml as compared to other plant extracts that studied previously. For example, aqueous extract of the leaves of *Carisa spinarum* and *Azadrichta indica*, stem bark of *Acacia tortolis* and fruits of *Phytolacca dodecandra* produced mortality of adult *H. contortus* to the level of 96.8, 93.9%, 53.3% and 68.1% respectively (Mohammed *et al*., 2013)

In present study, all the four extracts showed efficacy (70-95%) for adult mortality of *H. contortus* at a concentration of 4mg/ml. 50-65% of adult mortality of *H. contortus* was obtained from the extracts at a concentration of 2mg/ml. At a concentration of 1mg/ml of the plant extracts 25-40% of adult mortality of *H. contortus* was obtained. The extracts showed 15-30% mortality of adult parasites of *H. contortus* at a concentration of 0.5mg/ml. Poor efficacy were seen at low concentrations (0.5mg/ml and 1mg/ml) while better efficacy were seen at a concentration of 2mg/ml and the highest efficacy were obtained at higher concentration (4mg/ml). On the other hand Albendazole showed 100% efficacy at low concentration (0.5mg/ml).

V. CONCLUSION

In this study, extracts of all the study plants (*Acyarantus aspera, Cordia africana*, *Croton macrostachyus*, and *Schinus molle*) have shown promising *in vitro* anthelmintic activity against adult parasites of *H. contortus.* Extracts from *Acharantus aspera, Cordia Africana* and *Schinus molle* have shown relatively good effect on the adult mortality, while extracts of *Croton macrostachyus* demonstrate lesser effect than others.

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CONFLICT OF INTERESTS

The author(s) declare that they have no competing interests.

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Figure 1: Percentage mortality of adult parasites of H. contortus after 24 hours exposure to plant extracts at different concentrations (mg/ml).

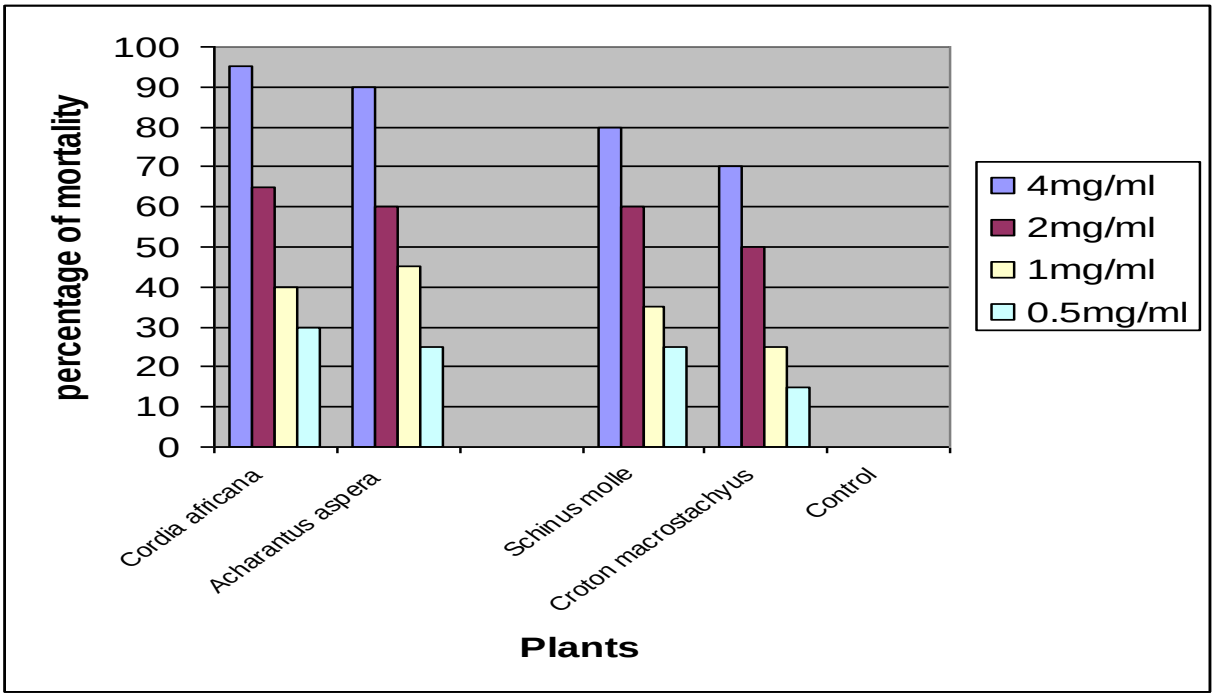

Table 1: Species, local name, area of collection and plant parts used in the study.

Species Name (Family)	Local Name	Area of Collection	Part used
Cordia africana lam	wanza	Arsi	Leaves
Achyranthes aspera	Dargu arbaa	Aklilu Lemma Institute of Pathobiology compound and from Alert hospital	Leaves
Croton macrostachyus	Bissana	Butajira	Leaves
Schinus molle	Tikur berbere	Sheko	Leaves