

IN VITRO SCREENING OF THE LEAVES OF *MUSA PARADISIACA* FOR ANTHELMINTIC ACTIVITY

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ABSTRACT

Helminthosis is one of the major problems of livestock production throughout the world, particularly in tropical and subtropical areas. Chemical control of helminths is in practice in most of the parts of the world. The problem of the development of resistance against the common chemical anthelmintics has renewed the interest in the study of medicinal plants for the development of novel anthelmintics. The present study has been planned to evaluate ovicidal efficacy of *Musa paradisiaca* leaves, used locally for worm control in sheep. For this purpose, egg hatch test (EHT) was conducted on nematodes ova to investigate the *in vitro* ovicidal effects of crude aqueous (CAE) and crude aqueous-methanol extracts (CAME) of the leaves of the plant. Lethal concentration 50 (LC₅₀) values of CAE and CAME of *Musa paradisiaca* leaves were 0.0207 and 0.4813, respectively. This study shows that *Musa paradisiaca* leaves possess *in vitro* anthelmintic activity. The study also suggests further large scale pharmacological and toxicological studies for their safer use in veterinary medicine.

Keywords: anthelmintic; gastrointestinal nematodes; *Musa paradisiaca*; sheep.

INTRODUCTION

Helminths due to their adverse effects are recognized as a major constraint to livestock production throughout the tropics and elsewhere (Githiori *et al.*, 2004). These adverse effects include retarded growth (Kochapakdee *et al.*, 1995), lowered productivity (Perry and Randolph, 1999), mortality (Sykes, 1994) and high economic losses (Iqbal *et al.*, 1993). The prevalence of helminthiasis in different species of animals has been reported ranging from 25.1 to 92% in Pakistan (Khan *et al.*, 1989).

Most of the parasite control programs are based upon a combination of chemotherapeutic control, grazing management, dietary management, biological control, vaccination and ethnoveterinary treatment (FAO, 2002). Chemotherapeutic control practices have evolved a number of problems including resistance of helminths to various groups of anthelmintic (Chartier *et al.*, 2001), chemical residues, toxicity problems, increased cost of treatment, non-adaptability of drugs and non-availability of the medicine in remote areas of Pakistan. Such problems diverted the researchers' attentions towards the development of alternate methods for the treatment of helminthiasis (Iqbal *et al.*, 2003). Due to the good efficacy and cost effectiveness herbal medicine have gained much importance in recent years. Plants provide a huge part of traditional veterinary practices and are a rich source of herbal anthelmintics of veterinary importance for centuries (Iqbal *et al.*, 2003; 2004)

Musa paradisiaca belongs to the family musaceae (banana family). The genus *Musa* is given to this plant in the honor of a Roman physician, Antonia Musa of first century. It is also called as plantain, banana, kadali, kela and Kel (Anjaria *et al.*, 2002). Plant is native to Southeast Asia and India including Pakistan and cultivated in tropical and subtropical regions. The fruit is picked when it is unripe and rich in starch, but when it ripens the starch turns into simple sugars (sucrose, glucose, and fructose). Fruit also has an ample quantity of vitamin A, B-6 and vitamin C. *Musa paradisiaca* is about 30 feet high and produces green or greenish-yellow seedless fruit, so fruit develops parthenocarpically (in the absence of seed development). The name "plantain" refers to *Musa paradisiaca* L., which requires cooking before it is eaten. An intoxicating drink can be prepared from the fruit. The leaves are cut into strips and woven into mats and bags (Anonymous, 2005)

The plant is used in inflammation, rheumatism, gripe, diabetes and hypertension. Unripe bananas are astringent, and used to treat diarrhea. The leaves are used for cough and bronchitis. The roots can arrest hemoptysis and possess strongly astringent and anthelmintic properties. Plantain juice is used as an antidote for snakebite. Other uses are asthma, burns, diabetes, dysentery, excessive menstrual flow, fever, gangrene, gout, headache, hemorrhage, inflammation, insomnia, intestinal parasites, sores, syphilis, tuberculosis, ulcers and warts (Morton, 1981). *Musa paradisiaca* roots have been reported to use as a tonic, antiscorbutic,

anthelmintic, antibilious, valuable alternative for the treatment of blood and venereal diseases. The stem of the plant is used as tonic and antiscorbutic while the ripe fruit is used as antiscorbutic, emollient, demulcent, mild astringent, nutrient, laxative and for the treatment of dysentery. Unripe fruit is used in combination with other drugs in diabetes. Juice of flower mixed with curd is used in dysentery and menorrhagea. Young leaves are also used as cool dressing for blisters and burns. Sap of stem is used in nervous affections like hysteria. It is also used in epilepsy, dysentery and diarrhea (Anjaria *et al.*, 2002). In ethnoveterinary medicine the leaves of *Musa paradisiaca* are used in the problems of hooves and injuries while young green fruit of banana is used in diarrhea (Lans *et al.*, 2006). So keeping in view the importance of the plant in ethnomedicine the present project was designed to study the anthelmintic effects of aqueous extracts and aqueous-methanol extracts of the leaves of the plant.

MATERIALS AND METHODS

1. Collection of Plant Material: Leaves of the plant were collected and identified from a botanist using preserved germplasm, Department of Botany, University of Agriculture Faisalabad. Voucher specimens were kept at the Herbarium, Ethnoveterinary Research and Development Center, Department of Veterinary Parasitology, University of Agriculture, Faisalabad. Leaves were dried under the shade and were ground to the powder in an electric mill and stored in cellophane bags at 4°C until use.

2. Preparation of crude aqueous extract: Crude aqueous extract (CAE) of powdered plants was prepared according to the standard methods (Onyeyili *et al.*, 2001). One hundred g of the powdered leaves were mixed with 500 mL of distilled water in a 1 L flask and boiled for 1.5 h. Brew was filtered using Whatman No.1 filter paper after cooling it to 40°C. The filtrate was concentrated in a rotary evaporator under vacuum and the extract was stored at 4°C until use.

3. Preparation of crude aqueous-methanol extract: Crude aqueous-methanol extract (CAME) of powdered plants was prepared according to the standard methods (Gilani *et al.*, 2004). Briefly, 1 kg of ground plant material was soaked in sufficient quantity of 70% aqueous-methanol by cold maceration at room temperature for three days after which the filtrate was collected through a piece of porous cloth and then filter paper and the plant material re-soaked twice. The combined filtrate was concentrated in a rotary evaporator at 40°C under reduced pressure to yield crude extract. This extract was stored at 4°C until use. The crude extract (as much as needed) was dissolved in distilled water on the day of the experiment to prepare stock solution and

different dilutions for the purpose of evaluating anthelmintic activity.

4. IN VITRO OVICIDAL ACTIVITY

4.1. Nematode egg recovery technique (Le Jambre, 1976): Approximately 50 gm of faeces obtained from the sheep, suspended in approximately 50 mL of water using electric mixer. This suspension was washed through a sieve with saturated NaCl solution. The mixture was poured into a shallow tray having the depth of 4 cm and a sheet of plastic cut to the shape of the tray was floated on top. As the specific gravity of the mixture is greater than some nematode eggs, so they float to the top and adhere to the plastic sheet. After about 15 min. the plastic sheet was removed and the eggs were washed off with a stream of water from a wash bottle into a beaker. Number of eggs was estimated by McMaster technique (Soulsby, 1982).

4.2. Egg suspension: The concentration of eggs was estimated in 50 µl samples and adjusted to 500 eggs mL⁻¹. The egg suspension was diluted with filtrate from the first step of egg extraction that had been centrifuged for 5 min at 100 × g to eliminate organic debris to provide bacteria for larval development. To avoid the proliferation of fungi 5 µg of amphotericin B was added per mL of suspension.

Test Procedure: The egg hatch assay was carried out using the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for determination of anthelmintic resistance (Coles *et al.*, 1992) with modifications that allowed the testing of the natural compounds (Alawa *et al.*, 2003). A suspension of 0.2 ml was distributed in a 24-well flat-bottomed microtitre plate containing approximately 100 fresh eggs and mixed with the same volume of plant extract having different concentrations (1, 2, 4 & 8 mgmL⁻¹). The control plates contained the diluent water and dimethyl sulfoxide (DMSO). The eggs were incubated in the mixture for 48 h at room temperature. After this time a Lugol's iodine solution was added to stop the eggs from hatching. All the eggs and first-stage larvae (L1) in each plate were counted. There were five replicates for each concentration and control. Data was expressed as percentage of unhatched eggs.

Statistical Analysis: Typical dose response curve (sigmoid) was transformed to linear function through probit transformation. The concentration of the extract required to inhibit 50% of eggs from hatching also called as lethal concentration 50 (LC₅₀), was calculated by the linear regression (for y = 0 on the probit scale).

RESULTS AND DISCUSSION

Aqueous extract as well as methanol extracts of *Musa paradisiaca* exhibited anthelmintic activity by inhibiting hatching of eggs of nematodes. The LC₅₀ of aqueous and aqueous-methanol extracts were calculated graphically by the regression equation as shown in the figs 1 and 2, respectively. The values of LC₅₀ of aqueous and aqueous-methanol extracts of *Musa paradisiaca* were 0.0207 and 0.4813 mgmL⁻¹, respectively. The regression values and correlation of regression of the aqueous extract were $y = -0.837x + 5.0174$ and $R^2 = 0.9954$ respectively while that of aqueous-methanol extract were $y = -0.6547x + 5.3151$ and $R^2 = 0.9052$, respectively. These data show that both aqueous and aqueous-methanol extracts possess dose-dependant anthelmintic activity. However, aqueous extract showed stronger anthelmintic activity against egg hatch than that of aqueous-methanol extract (figs 1 and 2).

These *in vitro* tests determine the effects of anthelmintic drugs on physiological processes like hatch, development, mortality and motility of the parasites (Varady and Corba, 1999). The *in vitro* assays provide cheaper, economical and rapid turn over in contrast of *in vivo* assays as for as anti-parasitic properties of plants and plant extracts are concerned (Githori *et al.*, 2006). Higher levels of anthelmintic activity of CAE of *Musa paradisiaca* revealed that active ingredient, responsible for the anthelmintic activity is relatively a polar compound. As for as ascertained, only one instance of anthelmintic activity of *Musa paradisiaca* against the eggs of gastrointestinal nematodes of ovine has been reported (Krychak-Furtado *et al.*, 2005). In this report, ethanolic extract and pure latex of *Musa paradisiacal* have been found possessing only low anthelmintic activity. In the present study, this plant has been tested for the first time for its anthelmintic activity against gastrointestinal helminths in Pakistan.

Other pharmacological uses of the plant have been reported. Reid (1961) used the plantain juice of the plant as an antidote for snakebite. The extract of *Musa paradisiaca* green fruits reduced hyperglycemia in normal and diabetic mice (Ojewole and Adewunmi, 2003), and protected the gastric mucosa from aspirin-induced erosion (Lewis *et al.*, 1999), and had direct vasodilation effect and nonspecific relaxing and inhibiting effect on aortic and portal smooth muscles (Orie, 1997). The plant has also been tested for the anti-ulcerogenic activity (Pannangpetch *et al.*, 2001). *Musa paradisiaca* contains tannins, eugenol, tyramine. Serotonin, levarterenol, norepinephrine and dopamine are available in the ripe fruit and peel. Other chemical constituents are alkaloids, steroidal lactones, and iron (Morton, 1981). The chemical constituent responsible for the anthelmintic activity of *Musa paradisiaca* has not yet been explored but this speculation is supported due to the

presence of phytochemicals like norepinephrine and alkaloids which have been reported to possess anthelmintic activity. Tannins, another constituent present in the plant also have anthelmintic activity (Molan *et al.*, 2000). Further research in this area may be helpful to jot down the exact mechanism of action of this plant.

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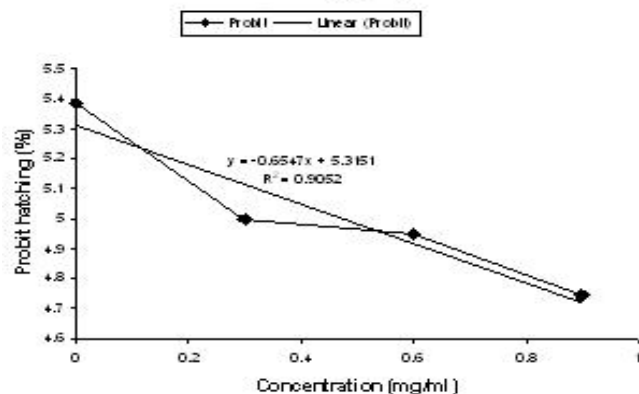


Fig. 1. Graph showing a linear regression between the egg hatching percentage on the probit scale of nematodes and crude aqueous extract (CAE) of *Musa paradisiaca*

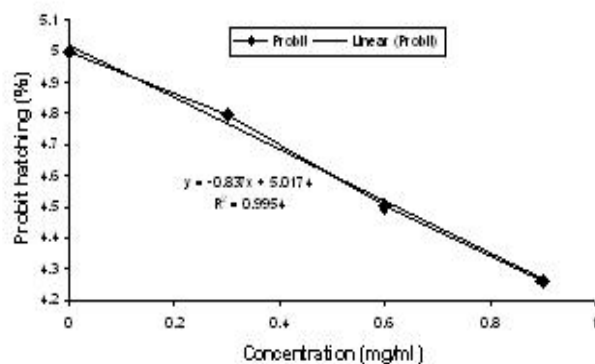


Fig. 2. Graph showing a linear regression between the egg hatching percentage on the probit scale of nematodes and crude aqueous-methanol extract (CAME) of *Musa paradisiacal*

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