



Full Length Article

Anthelmintic Activity of *Fumaria parviflora* (Fumariaceae) against Gastrointestinal Nematodes of Sheep

I.R.M. AL-SHAIBANI¹, M.S. PHULAN[†] AND M. SHIEKH[‡]

Department of Veterinary Parasitology, Faculty of Agriculture and Veterinary Sciences, University of Thamar, Thamar, Yemen

[†]Department of Veterinary Parasitology, Sindh Agriculture University, Tando Jam, Pakistan

[‡]Department of Horticulture, Sindh Agriculture University, Tando Jam, Pakistan

¹Corresponding author's e-mail: dr_ibra67@yahoo.com

ABSTRACT

The anthelmintic activity of *Fumaria parviflora* was evaluated against the gastrointestinal nematodes of sheep through egg hatch and larval development tests *in vitro* and faecal egg counts reduction test *in vivo*. *In vitro* studies revealed that aqueous and ethanolic extracts at the concentration of 3.12, 6.3, 12.5, 25.0 and 50.0 mg/mL exhibited ovicidal and larvicidal effects ($P < 0.05$) against the eggs and larvae of gastrointestinal nematodes. The highest effective dose (ED₅₀) value of *F. parviflora* extract was recorded on the eggs of *Chabertia ovina* (14.45 mg/mL) with aqueous extract; whereas, the lower value was recorded on the eggs of *Haemonchus contortus* (9.12 mg/mL) with ethanolic extract. Similarly, the higher LC₅₀ value of *F. parviflora* extracts was recorded against the larvae of *Strongyloides papillosus* (16.60) and the lower value against the larvae of *H. contortus* (10.23 mg/mL) with aqueous and ethanolic extracts respectively. *In vivo* studies revealed that experimental animal groups treated with the doses of 200 mg/kg of either aqueous or ethanolic extracts of *F. parviflora* exhibited higher ($p < 0.05$) reduction rate on faecal egg counts (FEC) as compared to un-treated groups (negative control). The highest reduction rate on FEC of treated animal groups recorded was 77.6 and 70.05% with ethanolic and aqueous extracts, respectively at the dose of 200 mg/kg on the day 14 post treatment, whereas at the treatment doses of 50 and 100 mg/kg, the reduction rate ranged between 3.79 to 61.45% from day 3 to 14 post treatment. The current study showed that *F. parviflora* whole plant extracts possess anthelmintic activity, thus justifying their use in traditional veterinary practices.

Key Word: Anthelmintic activity; *Fumaria parviflora*; Gastrointestinal nematodes; Sheep

INTRODUCTION

Helminthiasis plays a crucial role in small ruminant's production leading to enormous economic losses. It causes loss of production through mortality, weight loss, reduced milk, meat and wool production (Ketziş et al., 2002; Githiori et al., 2003; Egual e et al., 2007). Helminth control in domestic animals is widely based on the use of anthelmintic drugs. However, the current efficacy of these drugs has been reduced, because of resistant nematode strains (Bartley et al., 2003; Jabbar et al., 2006; Artho et al., 2007). Furthermore, the high cost of these drugs, residual concern in food animals and environmental pollution have awaken interest in medicinal plants as an alternative source of anthelmintic drugs (Pessoa et al., 2002; Hordegen et al., 2003; Githiori, 2004; Bizimenyera et al., 2006). Plants have been used from ancient times to cure diseases of man and animals. This system of therapy is commonly referred as Unani, Folk, Eastern, or indigenous medicine in Indo-Pakistan sub-continent (Iqbal et al., 2006a & b). There are some reports on plants with anthelmintic properties from

Asia and Africa (Akhtar & Javed, 1985; Akhtar et al., 1999; Akhtar et al., 2000; Lateef et al., 2003; Alawa et al., 2003; Githiori, 2004; Maqbool et al., 2004; Iqbal et al., 2006a & b; Egual e et al., 2007) America (Pessoa et al., 2002) and Europe (Athanasiadou et al., 2001; Hordegen et al., 2003) that have shown that plants can be a good alternative for the treatment of helminthiasis.

Fumaria parviflora L. (Syn: *F. indica*) is an annual herb creeper mainly found in the Indo-Pakistan subcontinent belonging to the Fumariaceae family. This herb is used as an anthelmintic in the traditional veterinary practices in Pakistan. There are a couple of reports on the validation of *F. parviflora* as an anthelmintic using faecal egg count (FEC) reduction (Akhtar & Javed, 1985; Hordegen et al., 2003). This study was carried out to validate the anthelmintic activity of *F. parviflora* using a variety of *in vitro* and *in vivo* tests.

MATERIALS AND METHODS

Preparation of plant extracts. Whole plant materials of *F.*

parviflora were obtained from local market of Hyderabad, Pakistan. The plants were identified and authenticated by botanists in the Departments of Horticulture, Sindh Agriculture University (SAU), Tandojam. The Plant materials were dried in shade at ambient temperature, ground and milled to powder by electrical blender. The powdered materials were stored in dark tightly closed glass bottles until used.

The crude aqueous extract of *F. parviflora* was prepared according to the techniques described by Onyeyili *et al.* (2001) and Iqbal *et al.* (2006a). Briefly, the powdered plant materials were mixed with distilled water in a flask and boiled for 1.5 h. Following cooling to 40°C and residues were filtered using Whatman No. 1 filter paper. The extracts then were evaporated to dry by freeze dryer (ALPH 4-1, Martin Christ, Germany) and stored at 4°C until used.

Ethanolic extract was extracted according to the techniques described by Wang and Waller (2006). The powder material of *F. parviflora* was placed in a cellulose thimble and extracted with 90% ethanol in a Soxhlet's apparatus for 8-12 h. Solvents were removed at temperature below 50°C in an oven. The residue (extract) of respective plant material was stored at 4°C until used.

***In vitro* assay.** *In vitro* assays were conducted at the Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam. The techniques and recommendations of the World Association for Advancement of Veterinary Parasitology (WAAVP) for the detection of anthelmintic resistance in nematodes of veterinary importance (Hubert & Kerbouef, 1992; Coles *et al.*, 1992; Taylor *et al.*, 2002; Coles *et al.*, 2006) were modified for the eggs preparation, egg hatch and larval development assays as described below:

Recovery and preparation of eggs. Twelve sheep 6-10 months old of either sex of the local breeds were treated by single dose of commercial anthelmintic Levamisole (ICI Pakistan Ltd, Animal Health Division) at dose of 7.5 mg/mL body weight to free them from any naturally acquired helminth infections and kept indoor at experimental farm, SAU, Tandojam. Then experimental (artificial) infection was induced to sheep to obtain fresh mono-species eggs for egg hatch and larval development assays. Briefly, adult nematode females were collected from the abomasal and intestinal contents of sheep at necropsy from slaughter houses of Hyderabad. The adult worms were crushed gently to liberate eggs and then were cultured to obtain the third infective larvae (L₃). Two animals were infected orally each with *Haemonchus contortus*, *Trichostrongylus* spp., *Ostertagia circumcincta*, *Strongyloides papillosus*, *Oesophagostomum columbianum* and *C. ovina*. Eggs were collected after four weeks of post infection and the eggs per gram (EPG) were daily determined. The concentration of eggs was estimated by counting the number of eggs in aliquots of 50 µL by McMaster slide as techniques described by Urquhart *et al.* (1996). The infected animals served as source for fresh mono-species nematode eggs for *in vitro* tests.

Egg hatch test (EHT). The stock solutions of the crude extract *F. parviflora* initially were prepared by dissolving the crude extract in dimethylsulfoxide (DMSO) to improve its solubility in water. Aliquots of start solution (50 mg/mL) were taken for preparation final concentrations of 3.12, 6.3, 12.5, 25.0 and 50.0 mg/mL. In the assay, approximately 100 eggs in 200 µL of egg suspension were pipetted into each well of 96-well microtitre plate. In test wells, 200 µL of *F. parviflora* extract in concentrations of 3.12, 6.3, 12.5, 25.0 and 50.0 mg/mL was added. Levamisole (Nilverm 1.5% W/V; ICI Pakistan Ltd) at concentrations of 0.5, 0.5×10⁻¹, 0.5×10⁻², 0.5×10⁻³, 0.5×10⁻⁴ mg/mL was used as a positive control, while distilled water was utilized as negative control. Three replicates for each concentration of extract and control were performed. The plates were incubated under humidified condition at ambient temperature (27°C) for 48 h. A drop of Lugol's iodine solution was added to each well to stop further hatching and all the un-hatched eggs and L₁ larvae in each well were counted under an inverted microscope.

Larval development test (LDT). Eggs were obtained and estimated as described in egg hatch assay. One hundred eggs in 170 µL of egg suspension were put into each well of 96-well microtitre plate. A 20 µL of nutritive media (comprising of 1 g yeast in 90 mL of normal saline & 10 mL Earle's balanced salt) was added into each well. The plates were then incubated under humidified condition at ambient temperature for 48 h. Then 200 µL of *F. parviflora* extracts at same concentration as mentioned above and Levamisole control concentration were added to respective plates. There were three replicates for each concentration and control. The plates were further incubated for 5 days (total of 7 days). Further development was stopped by addition of one drop of Lugol's iodine solution. All L₁ and L₃ larvae in each well were counted under an inverted microscope.

***In vivo* experiments.** The field trials were conducted at sheep farms of small farmer at Tandojam area, Pakistan. Climatically, the study area is sub-tropical humid and receives average annual rainfall of about 129 mm. The average maximum temperature reached 40.8°C in May and minimum 7.9°C in January. The relative humidity is highest in the months of August (73%) and February (74%), whereas it's declined to the lowest level in the month of April (50%).

Experimental design. A total of fifty indigenous sheep, 8-12 months old of either sex, weighing ranging from 20 to 28 kg were used for evaluation of anthelmintic activity of *F. parviflora* extracts in field controlled trials. The animals were grazed on natural pasture during the day and housed in pens at night. Water was given *ad libitum*. The sheep had naturally acquired mixed infections with gastrointestinal nematodes. Infection was confirmed before the beginning of the experiments by collecting faecal samples from each animal by McMaster Flootation Technique (Urquhart *et al.*, 1996). Only those animals whose egg counts exceeded 1000

eggs per gram were selected. Prior to treatment, faecal samples were collected from each animal. The experimental animals (n = 50) were divided into five groups (n = 10) at random using age, weight, sex and level of helminths infection as the blocking factors and subjected to different treatments with single dose of plant extracts and/or commercial anthelmintic. The experimental doses of *F. parviflora* extracts were adjusted in the light of previous studies (Hordegen *et al.*, 2003; Maqbool *et al.*, 2004; Rao *et al.*, 2007). 1st, 2nd and 3rd groups received single doses of 50 mg/kg, 100 mg/kg and 200 mg/kg, respectively of *F. parviflora* extracts, while 4th served as a positive control group, which was given a single dose of levamisole 7.5 mg/kg and 5th group served as negative control group and received no treatment. There were two experiments one for aqueous extract and another for ethanolic extracts. Faecal samples were collected from each animal from 0 day pre-treatment and at 3rd, 7th and 14th day Post-treatment. Eggs per gram (EPG) were determined by modified McMaster technique (Urquhart *et al.*, 1996). The gastrointestinal nematode eggs were identified by faecal culture according to the techniques described by MAFF (1986) and Riche (1988). The anthelmintic efficacy of *F. parviflora* was evaluated or assessed by field controlled faecal egg count reduction test (Coles *et al.*, 1992; Taylor *et al.*, 2002; Coles *et al.*, 2006). The percent faecal egg count reduction was calculated using the following formula:

$$\% \text{ FECR} = a-b/a \times 100$$

Where, a = EPG pre-treatment and b = EPG post treatment.

Statistical analysis. The data from egg hatch (EH) and larval development (LD) assays were transformed to $\log_{10}(x + 1)$ and submitted to one-way analysis of variance. The means were compared by the Duncan test with 5% significant level using the SPSS 15.0 program. For EHT, effective dose (ED₅₀) was calculated as the concentration of drug or extract producing 50% inhibition of eggs hatching (Coles *et al.*, 1992; Bizimenyera *et al.*, 2006; Varady *et al.*, 2006; Eguale *et al.*, 2007); whereas, LC₅₀ was calculated as the concentration of drugs or extract inhibiting development of 50% of eggs into L₃ infective larvae (Athanasidou *et al.*, 2001; Konigova *et al.*, 2003; Maciel *et al.*, 2006). The ED₅₀ and LC₅₀ values were calculated graphically from linear regression with probit scale, $y = 5$, according to the techniques given by Fry (1993). The ED₅₀ and LC₅₀ values were back transformed and presented in milligram plant extract per milliliter.

The data obtained from faecal egg counts reduction test (FECRT) were normalized using ($\log + 1$) transformation before submitted to General Linear Model (GLM) Procedure. The difference between the means were considered significant at the $P < 0.05$.

RESULTS

The aqueous and ethanolic extracts of *F. parviflora*

exhibited ovicidal and larvicidal effects; whereas, egg hatching and larval development was not affected by the distilled water (negative control). The levamisole (positive control) at concentration of 0.5 mg/mL showed the highest ovicidal ($P < 0.05$) inhibition rate ranging from 90-99% (Nwosu *et al.*, 2008). The ethanolic extract was more potent (ED₅₀ = 9.12 mg/mL; $y = 2.73 + 2.37 \times R^2 = 1.00$) as ovicidal as well as larvicidal (LC₅₀ = 10.23 mg/mL, $y = 2.91 + 2.09 \times R^2 = 0.98$) against *H. contortus* compared with aqueous extract. The activity of plant extract was also superior against *H. contortus* compared with other nematodes (Fig. 1 & 2; Table I).

In vivo studies revealed that, no abnormal behavior was observed in animals treated either with aqueous or ethanolic extracts of *F. parviflora*. The average rectal temperature of all animals was within normal ranges (39-41°C). The nematode eggs recovered from the experimental animals were identified by faecal cultures as *H. contortus*, *Trichostrongylus* spp., *Ostertagia circumcincta*, *S. papillosus*, *Oesphagostomum columbianum*, *C. ovina* and *Trichuris ovis*. Results revealed that a gradual reduction in faecal egg counts (FEC) of experimental sheep treated with plant extracts were significant ($P < 0.5$) on day 14 post treatment from the 0 day pre-treatment (Fig. 3 & 4). At day 0, there was no difference between the FEC of treated sheep groups and un-treated groups (negative control). The FEC decreased ($P < 0.05$) from day 3 onward to day 14 post treatment. All doses of the plant extracts showed dose-dependent ($p < 0.05$) effect on reduction of FEC of experimental animals. The experimental animal groups treated with the doses of 200 mg/kg of either aqueous or ethanolic plant extracts exhibited higher ($p < 0.05$) reduction in FEC as compared to un-treated groups (negative control). The highest reduction rate in FEC was 77.6 and 70.05% at day 14 post treatment at the dose of 200 mg/kg for ethanolic and aqueous extracts respectively; whereas, the reduction rate was ranged between 3.79-61.45% from day 3-14 post treatment at doses 50-100 mg/kg of both aqueous and ethanolic extracts.

DISCUSSION

The main advantages of using *in vitro* tests/assays to screen the anti-parasitic properties of the plants and plant extracts are low costs and rapid turnover, which allow screening large number of plants. An additional advantage was that these tests measured the effect of anthelmintic activity directly on the processes of such hatching, development and motility of parasites without interference of internal physiological functions of the host on pharmacodynamic and pharmacokinetic of the drug (Assis *et al.*, 2003; Githiori *et al.*, 2006). As recorded in the present study, ovicidal and larvicidal effect of some plants have also been reported earlier against *H. contortus* eggs and larvae (Ketzis *et al.*, 2002; Assis *et al.*, 2003; Maciel *et al.*, 2006; Hordegen *et al.*, 2006; Bizimenyera *et al.*, 2006; Eguale *et*

al., 2007). Amarante *et al.* (1996) stated that resistant species of gastrointestinal nematodes to particular anthelmintic drug showed higher ED₅₀/LC₅₀ value than susceptible species. In the light of above definitions, current study revealed that eggs and larvae of *H. contortus* were more susceptible to ovicidal and larvicidal effect of *F. parviflora*; whereas, eggs of *C. habertia ovina* and larvae *S. papillosus* were relatively resistant. In spite of the advantages of *in vitro* tests on evaluation anthelmintic activity of plants, considerations should be borne in mind that potential bioactive substances used *in vitro* do not always correspond to *in vivo* bioavailability. Therefore, *in vitro* assays should always be accompanied by *in vivo* trials (as done in the present study) when used to validate anthelmintic activity of plant remedies.

In vivo studies are more relevant in control practices of gastrointestinal nematodes in farm animals and thus considered more reliable than *in vitro* studies in evaluation of plant extract properties, although costs of large scale screening of plant extract is probably inhibitory (Githiori *et al.*, 2006). Generally, the plant preparations may be whole plants, plants parts, extract or chemical isolated from plants. The *in vivo* studies normally have parasitized hosts being treated with known quantities of plant products and compared with un-treated control or with commercial standard anthelmintic. In trial or experiment, various measurements are usually recorded and these include expulsion of worms and counting parasites in faeces, regular worm counts in necropsied animals, or daily faecal egg excretion after consumption of plant products (Githiori *et al.*, 2005, 2006). Results of the current study revealed good anthelmintic activity (up to 77.6% reduction in FEC) of *F. parviflora* extracts. Though varying rate of efficacy, results are consistent with those of Akhtar and Javed (1985) and Hordegen *et al.* (2003), who have reported anthelmintic activity of the aqueous and ethanolic extracts of *F. parviflora* against the *H. contortus* and Trichostrongylid nematodes of sheep *in vivo*. The variation in efficacy of same plant in different studies may be due to different origin of the plant materials, different chemical constituents between individual plant due to genetic or environmental differences, development stages of plant at harvesting, drying process and storage technique (Hordegen *et al.*, 2003). Furthermore, Githiori (2004) reported that number of animals used in the trials during the evaluation of plant properties also can affect the results.

The mechanism action of *F. parviflora* is not yet fully understood, but the anthelmintic activity could be attributed to bioactive compounds of *F. parviflora* jointly or separately. The phytochemical analysis of *F. parviflora* revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and triterpenoids (Rao *et al.*, 2007). The main alkaloids of *F. parviflora* are protopine, fumarizine, papraine, papracine, papracinine, paprafumicine and papraraine (Gilani *et al.*, 1996; Bhatti, 1998; Heidari *et al.*, 2004; Rao *et al.*, 2007). Athanasiadou *et al.* (2001) suggested

Table I. The ED₅₀/LC₅₀ (mg/mL) of aqueous and ethanol extracts of *Fumaria parviflora* on egg hatching and larval development of gastrointestinal nematodes

Nematode species	Egg hatching		Larval development	
	Aqueous	Ethanol	Aqueous	Ethanolic
<i>H. contortus</i>	11.48	9.12	13.18	10.23
<i>O. circumcincta</i>	11.22	10.23	11.75	10.72
<i>Trichostrongylus Spp.</i>	10.72	10.72	13.18	12.30
<i>S. papillosus</i>	10.23	10.00	16.60	15.14
<i>Oe. Columbianum</i>	14.13	12.88	15.85	13.18
<i>Chabertia ovina</i>	14.45	12.88	15.14	12.59

Fig. 1. Linear relationship between eggs inhibition (%) of gastrointestinal nematodes and *F. parviflora* extract concentrations (mg/mL)

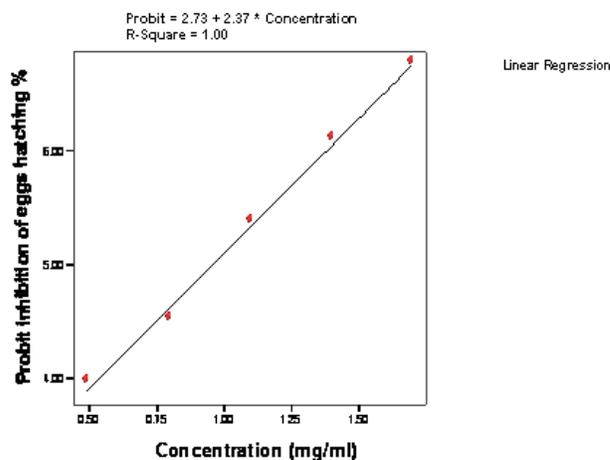
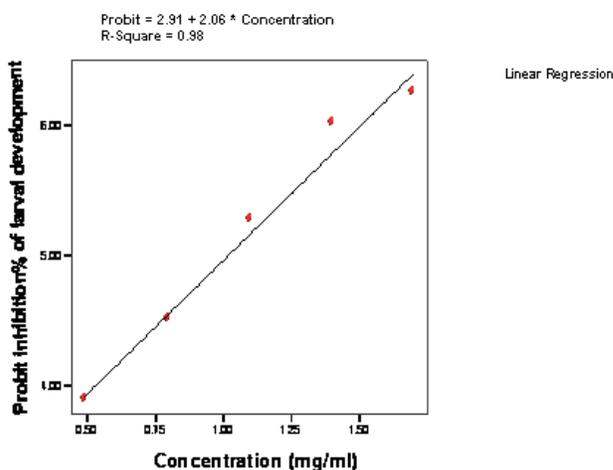


Fig. 2. Linear relationship between larval development inhibition (%) of gastrointestinal nematodes and *F. parviflora* extract concentrations (mg/mL)



that anthelmintic activity of plant extracts on larvae and adults of gastrointestinal nematodes could be attributed to tannins capacity to bind to proteins and could operate via several mechanisms. Condensed tannins may be bind to the cuticle of larvae, which is highly in glycoprotein and cause

their death. On adult worms, condensed tannins may create a hostile gut environment for the intestinal parasites, thus reducing their fecundity and consequently FEC in animals. On the other hand, Gilani *et al.* (1996), Cowan (1999) and Maqbool *et al.* (2004) suggested that the anthelmintic activity of *F. parviflora* may be due to the alkaloids of *F. parviflora*, which have ability to intercalate with DNA synthesis of parasites. In this study, the results also revealed that ethanolic extract were slightly more effective as compared to aqueous extract. Transcuticular diffusion is a common means of entry into helminth parasites for non-nutrient and non-electrolyte substances in nematodes. It has also been shown that this route is predominant for the uptake of major broad spectrum anthelmintics by different nematode, cestode and trematode parasites as opposed to oral ingestion (Egualé *et al.*, 2007). The possible explanation for better anthelmintic activity of ethanolic extract compared to aqueous extract on larvae and adults parasites could be due to easier transcuticular absorption of the ethanolic extract into body of parasites than the aqueous extracts. The commercial anthelmintic levamisole (positive control) exhibited the highest anthelmintic activity on reduction of FEC of animals infected naturally with gastrointestinal nematodes and the efficacy rate was observed as 100%. Levamisole acts as cholinergic agonist on neuromuscular junction in nematode parasites, which causes paralysis of the worm, leading to their death or expel them from the host (McKellar & Jackson, 2004).

In conclusion, *F. parviflora* has potential anthelmintic activity, thus justifying its use in traditional and ethno-veterinary medicine. However, it is suggested that further research work may be carried out on its toxicity and thus standardization of the dose.

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