

In Vitro Anthelmintic and Antimicrobial Activities of Methanolic Extracts of *Fumaria Indica*

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Abstract

Objective: The present study was undertaken to elucidate the anthelmintic and antimicrobial activities of *Fumaria indica*.

Methods: The methanolic extract of *Fumaria indica* was evaluated for in vitro anthelmintic efficacy against gastrointestinal nematodes of sheep (*Haemonchus contortus*) using adult motility assay. In vitro antimicrobial activities of various concentrations ranging from 100 to 500 mg/ml of alcoholic (methanol) extracts of *Fumaria indica* were analyzed on different clinical bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pseudomonas multocida* and *Klebsiella pneumonia*) and fungal strains (*Aspergillus flavus*, *Candida kruesie* and *Candida albicans*) using agar disk diffusion method and broth dilution method (MIC and MBC determination) for antimicrobial activity.

Results: Crude methanol extract of *Fumaria indica* resulted in mean percentage mortality of 94.44%, as observed after the worms were put in lukewarm PBS for 30 min after exposure to different treatments ($p < 0.01$). Highest mortality (95.00%) of worms was observed 8 hours post-exposure @ 50 mg/ml. There was 100% mortality of worms in Levamisole (used as a reference drug) within 4 hours post-exposure. In vitro antimicrobial activity results revealed that methanol extract of *F. indica* possess greater antibacterial activity than antifungal activity. MIC and MBC of methanolic extract showed that MIC values were 150 ml/ml against *E. coli* and 250 ml/ml.

Conclusions: It can be concluded that *Fumaria indica* has got a broad spectrum in vitro anthelmintic and antimicrobial activity and could be used as a potential alternative for treating various diseases.

Keywords: Anthelmintic activity; Antimicrobial activity; *Fumaria indica*; *Haemonchus contortus*; Methanolic extracts

Introduction

Nematode infections constitute serious health problems worldwide in humans and animals, causing hardship and stunted growth. These parasites cause frequent important economic losses due to the mortality in case of heavy infection. In addition, chronic infections cause lowering of the productivity, fertility, growth and milk and meat production in ruminants [1]. The resistance of nematodes to commercially available agents and the problems with their affordability, accessibility and the safety of anthelmintic substances present in foods derived from livestock which have recently been reported as considerably toxic to human health [2,3] have necessitated the need to search for safe and affordable anthelmintic agents from plants or natural products. Medicinal plants represent a rich source of antimicrobial and anthelmintic agents. They are used medicinally in different countries and are a source of many potent and powerful drugs [4]. Medicinal plants have served through the ages as a constant source of medicaments for the exposure of a variety of diseases. The history of herbal medicine is almost as old as human civilization. The

plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides [5,6]. A number of medicinal plants have been used to treat parasitic infections in man and animals [7-9].

Considering the vast potentiality of plants as sources of anthelmintic and antimicrobial drugs, the present study was undertaken to screen the in vitro and in vivo anthelmintic activity of *Fumaria indica* (local name Sheethar) commonly used in ethno-veterinary medicine in nomadic Gujars and Bakerwals of Kashmir Valley [10,11]. There is, however, no published scientific evidence for the anthelmintic and/or antimicrobial effects of *Fumaria indica*.

Materials and Methods

Plant material

Fumaria indica (Fumariaceae), an annual or perennial herb, locally called as Sheethar was collected from UpperWat, Gulmerg, Kashmir (34° 17' 04" N, 75° 13' 46" E Altitude 12068 ft) during May- August 2013. Gulmerg being hilly display an uneven topography having loamy sand soils with temperature ranging from 7-25°C in summers and -1 to -6°C in winters. The mature plants at peak of flowering were collected in polythene bags and were processed by standard technique

adopted by KASH (Kashmir University Herbarium). The labeled fresh specimens were discussed with local tribals regarding its uses/abuses. The data was reconfirmed by discussing/confirming the plants usage with other tribes/populations. Plant was identified and authenticated by plant taxonomist Prof. Irshad A. Nawchoo, Department of Botany, University of Kashmir, Srinagar, India. A voucher specimen (Voucher No. 1605) was deposited in KASH for further reference.

Preparation of plant extract

The collected plants were processed for shade drying at the environmental temperatures (25-30°C) in a well-ventilated room (Drying room at Department of Zoology, University of Kashmir, Srinagar). The dried plant parts (leaves, stem and flowers) were milled to a fine powder using an electric stainless steel blender and the plant powder (500 g) were extracted with methanol (1,000 ml, Qualigens) in a Soxhlet extractor at 60°C for 12 h. The extract was concentrated under reduced pressure of 22-26 mmHg at 40°C in a rotary evaporator (R-201, Shanghai Shenshen) then dried in a vacuum oven and stored at 4°C for further use. The yield for methanol was 7.76 g.

Evaluation of in vitro anthelmintic activity

Collection of worms

Collection of worms was done according to the method described by Jabbar et al. [12] and Lone et al. [13]. Briefly, mature live *H. contortus* female worms were collected from the abomasum of freshly slaughtered sheep then washed and finally suspended in a bottle containing lukewarm phosphate buffer saline (PBS) (pH 7.2) for further use in adult worm motility assays.

Adult motility assay (AMA)

In vitro anthelmintic activity of the plant materials was evaluated by exposing the adult *Haemonchus contortus* to methanolic extracts of *F. indica*. For each extract, five petri dishes were used, i.e., four for extract to be tested and one for 0.9% of PBS as control all the petri dishes were kept for incubation (at 37°C, 100% relative humidity in a 5%CO₂/air mixture. Adult live and motile *H. contortus* nematodes were collected from the gastrointestinal tract of sheep slaughtered at Srinagar slaughterhouse and immediately transferred to the petri dishes containing plant extracts and PBS. Observations were made on the motility/survival of nematodes at 0, 1, 2, 5 and 8 h post-exposure (PE). The number of worms found dead at 8 h PE to methanolic extracts of *F. indica* was compared to the control group and evaluated statistically through z test using SPSS (11.5) for Windows.

Antibacterial activity

Different concentrations of the prepared *F. indica* were tested by the disc diffusion method for the antibacterial activity against five strains of bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Pseudomonas multocida* and *Klebsiella pneumoniae*. The test microorganisms were seeded into respective medium by spread plate method with the 24-h cultures of bacteria grown in nutrient broth. After solidification, the filter paper discs (5 mm in diameter) impregnated with the extract was placed on test organism seeded plates. Streptomycin sulphate (200 µg/ml) was used as positive control, the antibacterial assay plates were incubated at 37°C for 24 h and the diameters of the inhibition zones were measured in millimeter. The micro dilution broth susceptibility assay was used for the evaluation of minimal inhibitory concentration (MIC), as recommended by NCCLS (1990) and Minimum bactericidal concentration (MBC). After incubation at 37°C for 24 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Using the values of MIC in micro broth dilution assay method.

Antifungal activity

The antifungal activity was tested by disc diffusion method [14] on Sabouraud's agar plates inoculated with 10-day-old fungal culture by point inoculation. Filter paper discs (5 mm in diameter) impregnated with different concentrations of the extract was placed on test organism seeded plates. Nystatin (200 µg/ml) was used as positive control. The activity was determined after 72 h of incubation at 30°C, and the diameters of the inhibition zones were measured in millimetre.

Results

In vitro anthelmintic activity

Effect of crude methanolic extracts of *F. indica* was dose and time dependent. Highest mortality (95.00%) of worms was observed 8 hours post-exposure @50 mg/ml (Table 1). The crude methanolic extract of *F. indica* resulted in mean percentage worm motility inhibition (%WMI) of 94.44%, as observed after the worms were put in lukewarm PBS for 30 min after exposure to different treatments. There was 100% mortality of worms in Levamisole (used as a reference drug) within 4 hours post-exposure. There was no mortality of worms kept in PBS till 6 hours post experiment.

Treatment	Conc. mg ml ⁻¹	Mean ± SEM of number of <i>Haemonchus contortus</i> worms showing motility up to 8 h exposure				
		0 h	1 h	2 h	5 h	6 h
Crude methanolic extract	50.00	20.00 ± 0.0	10.44 ± 0.6 (47.8)	8.00 ± 0.1 (60.0)	3.34 ± 0.2 (83.3)	1.00 ± 0.5 (95.0)
	25.00	20.00 ± 0.0	12.33 ± 0.7 (38.35)	10.67 ± 0.7 (46.65)	6.50 ± 0.4 (67.5)	3.50 ± 0.6 (82.5)
	12.50	20.00 ± 0.0	17.33 ± 2.2 (13.4)	13.33 ± 0.9 (33.5)	11.00 ± 0.6 (45.0)	8.00 ± 0.2 (60.0)

Levamisole (positive control)	0.50	20.00 ± 0.0	6.00 ± 0.4 (70.0)	2.00 ± 0.1 (90.0)	0.00 ± 0.0 (100.0)	0.00 ± 0.0 (100.0)
PBS (negative control)	0.99%	20.00 ± 0.0	20.00 ± 0.0	20.00 ± 0.0	18.50 ± 0.0	18.50 ± 0.0 (7.5)

SEM, standard error of mean; PBS, phosphate-buffered saline; CME, crude methanolic extract

Table 1: *In vitro* anthelmintic efficacy of crude methanolic extracts of *F. indica* on *Haemonchus contortus* of sheep

Antimicrobial Activity

In vitro antimicrobial activity results revealed that methanol extract of *F. indica* possess greater antibacterial activity than antifungal activity (Table 2). When tested by disk diffusion method it showed significant activity. Overall highest antibacterial activity was observed against *P. multocida* at a concentration of 500 mg/ml with an inhibition zone diameter of 22 ± 0.25 were as least inhibition of 3 ±

0.16 mm was also recorded for the *K. pneumoniae* bacterial strain at 200 mg/ml concentration. Antifungal activity of the extract was observed at 300 mg/ml and above with maximum inhibition zone diameter of 17 ± 0.66mm against *C. albicans* at 500 mg/ml and minimum inhibition zone diameter of 4 ± 0.09mm against *A. flavus* at 300 mg/ml.

Test organism	Zone of Inhibition in mm					Streptomycin sulphate (200 µg / ml)	Nystatin (200 µg / ml)
	Concentrations mg/ml						
	100	200	300	400	500		
<i>E. coli</i>	4 ± 0.09	10 ± 0.57	12 ± 0.33	18 ± 0.16	20 ± 1.20	22 ± 0.25	NC
<i>S. aureus</i>	7 ± 0.57	12 ± 1.0	16 ± 0.57	20 ± 0.11	22 ± 0.12	22 ± 0.57	NC
<i>P. multocida</i>	-	9 ± 0.06	10 ± 0.33	16 ± 0.57	22 ± 0.25	26 ± 0.14	NC
<i>P. aeruginosa</i>	-	-	9 ± 0.63	12 ± 0.66	18 ± 0.67	18 ± 0.20	NC
<i>K. pneumoniae</i>	-	3 ± 0.16	8 ± 0.09	13 ± 0.57	16 ± 0.57	18 ± 1.20	NC
<i>A. flavus</i>	-	-	4 ± 0.09	9 ± 0.33	15 ± 0.88	NC	22 ± 0.57
<i>C. albicans</i>	-	-	5 ± 0.011	11 ± 0.57	17 ± 0.66	NC	18 ± 0.16
<i>C. kruesie</i>	-	-	7 ± 0.11	10 ± 0.33	16 ± 0.57	NC	21 ± 0.33

Values are mean inhibition zone (mm) ± S.D of three replicates; NC: Not taken as positive control

Table 2: Antimicrobial activity of methanolic extract of *Fumaria indica*

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC of methanolic extract (Table 3) showed that MIC values were 150 ml/ml against *E. coli* and 250 ml/ml against *S. aureus*;

350ml/ml against *P. multocida*; 450ml/ml against *P. aeruginosa* and 500 ml/ml against *K. pneumonia*, in comparison to the positive control, Streptomycin sulphate.

Bacterial strains	MIC	MBC	Streptomycin sulphate µg/ml
<i>E. coli</i>	150 ± 25.1	450 ± 36.8	100 ± 30.55
<i>S. aureus</i>	250 ± 39.3	450 ± 40.0	100 ± 30.55
<i>P. multocida</i>	350 ± 20.8	500 ± 56.2	300 ± 35.11
<i>P. aeruginosa</i>	450 ± 40.0	100 ± 35.11	450 ± 40.0
<i>K. pneumoniae</i>	500 ± 56.2	150 ± 25.1	450 ± 26.12

Values are mean inhibition concentration \pm Standard Deviation of three replicates; MIC - minimum inhibitory concentration; MBC - minimum bactericidal concentration.

Table 3: The minimum inhibitory concentration and minimum bactericidal concentration of methanolic extracts of *Fumaria indica*

Discussion

In vitro anthelmintic activity

The present work was carried out in order to find a phytotherapeutic to help control gastrointestinal nematodes (*H. contortus*) in small ruminants and to provide an alternative use of a food processing byproduct. Hence development of new, safe, curative and economical drug has emerged as an active area of research. The anthelmintic activity of methanolic extract of *Fumaria indica* against *H. contortus* was evident from mortality of worms. A wide variation, however, was recorded in the anthelmintic effects among different concentrations as far as the intensity and dose-dependent effects were concerned [13]. The main route of acquisition of broad-spectrum anthelmintics by nematodes appears to be via trans-cuticular diffusion as proposed through oral ingestion [15]. We found that methanolic extract of *Fumaria indica* showed good *in vitro* anthelmintic activity and this could be due to the presence of a higher concentration of the alcohol-soluble active compounds in the extract [16]. Results obtained suggest that the extract exhibited significant *in vitro* anthelmintic activity against *H. contortus*, and has the potential to control gastrointestinal nematode parasites of sheep. Iqbal et al. [17] revealed significant *in vitro* anthelmintic activity of crude aqueous and methanol extracts of *Swertia chirata* against *H. contortus*. *In vitro* anthelmintic activity of methanolic extract of *Fumaria indica* which demonstrated a highest mortality (95.00%) of worms at 8 hours post-exposure @ 50 mg/ml is in agreement with [18-20] against ovine Gastrointestinal helminthes nematodes.

Antimicrobial activity

Presence of antimicrobial substances in plants is well established as they have provided a source of inspiration for novel drug compounds and have made significant contribution towards human and animal health. The antimicrobial activity of methanolic extract of *Fumaria indica* against the different clinical strains of bacteria and fungi supported the scientific validity of the plant being used traditionally as a medicine. The inhibition of a maximum of five bacterial strains by methanol extract may be attributed to the presence of soluble phenolic and polyphenolic compounds in the extract. The significant antimicrobial effects of this extract could be explained by disturbance of permeability barrier of bacterial membrane structures [21,22]. Indeed recent findings revealed that tea tree oil damages the cell membrane structure of *E. coli*, *S. aureus* and *C. albicans* [23]. The methanol extract also showed broad antimycotic activity against the tested fungal isolates at a final concentration of 500 mg/ml and the performance of this extract was similar to its antibacterial activity. The results are also in consonance with several other studies showing that the antimicrobial activity of plant extracts to be due to presence of saponins [24,25]. The MIC and MBC values supported the results obtained in the antibacterial screening, showing clearly that the methanol extract was most potent than ethanol extract as the MIC values were lower than the MBC values, similar to the results of Karou et al. [26]. George et al. explained that the observed differences to be due to the fact that while synthetic antibiotics are in a pure form, crude

plant extracts contains some impure substances that may be inert and do not have any antibacterial activities.

Conclusion

Based on the results of the present study, it can be concluded that *Fumaria indica* aerial parts (stem, leaves and flowers) tested in the form of crude methanolic extract showed significant *in vitro* anthelmintic activity at concentrations and doses tested against ovine nematodes as determined by motility inhibition of *H. contortus* of sheep and some clinical strains of bacteria and fungi. These findings suggest that *Fumaria indica* could form an alternative to commercially available synthetic anthelmintics; however, it is suggested that further research work may be carried out for phytochemical screening, its toxicity and to optimize the use of the plant, using appropriate technology as against the crude method used by the locals and nomads which is in vogue.

Conflict of Interest

The authors declare no conflicts of interest. Equipment brands, chemicals, and other trade names are mentioned here solely for the convenience of the reader and imply no endorsement by the authors.

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