

IN VITRO ANTHELMINTIC ACTIVITY OF *ABUTILON THEOPHRASTI* MEDIK. (MALVACEAE) AGAINST EGGS AND *L₃* LARVAE OF *HAEMONCHUS CONTORTUS*

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Received: 24 September 2018, Revised and Accepted: 09 January 2019

ABSTRACT

Objective: The present study was carried out to assess the in vitro anthelmintic activity of *Abutilon theophrasti* stem extracts.

Methods: Simple maceration was employed for extraction. Solvents such as methanol, water, and hexane were used. Egg hatch test (EHT) and larval motility test were employed to check the anthelmintic activity of crude extracts. Concentrations of 500, 250, 125, 62.5, and 31.25 mg/ml were made. Levamisole and distilled water served as control, respectively.

Results: All selected extracts displayed concentration-dependent inhibition except aqueous extracts. At higher concentration (500 mg/ml), stem extracts (methanol, aqueous, and ethanol) showed 74.39%, 72.5 and 70.03% of efficacy in EHT, respectively ($p \leq 0.05$). Meanwhile, inhibition of larval motility was seen higher with inhibition percentage of 79.79, 75.65, and 71.28 by methanolic, aqueous, and hexane extracts, respectively ($p \leq 0.05$).

Conclusion: The present study suggests active principles having anthelmintic efficacy in *Abutilon theophrasti* stem.

Keywords: *Haemonchus contortus*, Egg hatch test, Larval motility test.

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INTRODUCTION

Helminthiasis is one of the most important animal diseases worldwide, inflicting heavy production losses on grazing animals. Nematodes infect gastrointestinal tract adversely of small ruminants all over the world, especially in tropical and subtropical countries, and affect the overall productivity. The disease is, especially, prevalent in developing countries in association with poor management practices and inadequate control measures [1]. An integrated approach is required for the effective control of the helminths and careful management of grazing lands including control of stocking rates and appropriate rotation strategies; however, problems have emerged with the use of anthelmintics, and development of resistance in helminths is a problem of major concern. In addition, recognition of the antigenic complexity of parasites has slowed vaccine development. For these various reasons, interest in the screening of medicinal plants for their anthelmintic activity remains of great scientific interest despite extensive use of synthetic chemicals in modern chemical practices worldwide. Majority of the ethnopharmacological surveys and validation studies also indicate much wider and effective use of plants as anthelmintics compared to other diseases [2]. Many herbs belonging to the genus *Abutilon* are documented for their various medicinal benefits, but still they are not satisfactorily exploited. Our present study was done with the same aspiration to assess the anthelmintic activity of *Abutilon theophrasti*.

METHODS

Collection and authentication of plant material

A. theophrasti (Fig. 1) was collected from "Lower-Munda" District, Qazigund (J&k) with the latitude 33.56° and longitude 75.20°. The plant was identified and registered at Herbarium Center for Biodiversity and Taxonomy, "University of Kashmir," India. A voucher specimen (No.2113-KASH) (Fig. 1) was deposited in HCFBAT, University of Kashmir.

Preparation of extracts

The collected plant was washed with tap water and dried in shade. The dried plant samples were powdered and sieved to fine mesh and stored at room temperature before extraction. Methanol, distilled water, and hexane were used as solvents. Extraction was carried out by the maceration process. The extracts were concentrated to dryness using rotary evaporator.

Parasites

Eggs to perform egg hatch test (EHT) and for the larvae culture were obtained from fecal samples rectally from *Haemonchus contortus*-infected sheep. Nematode infection was confirmed by egg counting test (eggs per gram feces) [3].

EHT

About 20 g feces were collected rectally from the infected sheep (>2000 EPG). Feces were treated with water through a series of complied sieves (500, 150, 90, and 20 µm). Eggs which were retained at last sieve were obtained with a saturated saline solution by flotation. 100 µl containing 100 eggs approximately were distributed in microdilution plates. Different crude stem extracts (500, 250, 125, 62.5, and 31.25 mg/ml) were added to the plates. Levamisole (2 mg/ml) was taken as a positive control and distilled water was taken as negative control. Total time taken to complete the experiment was 24 h and the temperature was maintained at 27°C [4]. Eggs and first stage larvae (*L₁*) were counted as suggested by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P). The obtained results were expressed as percentage inhibition of egg hatches.

Larval motility test (LMT)

About 20 g of feces were taken and homogenized at a concentration of about 2000 EPG with the proportion of 1:2 v/v and incubated for 7 days at room temperature. The incubated material was moistened daily with

distilled water. L₃ larvae were collected by spontaneous migration with the aid of warm water (37°C). 50 µL suspension with 50 L₃ was placed in microdilution plates. Crude extracts (500, 250, 125, 62.5, and 31.25 mg/ml) were added to these L₃ containing microdilution plates and were incubated for next 24 h at 27°C. Motile and non-motile L₃ were counted by seeing keenly on the presence or absence of smooth sinusoidal movements. Levamisole (2 mg/ml) and distilled water were taken as positive and negative control, respectively [3]. Results were expressed as percentage inhibition of larval motility as a representation of three independent experiments performed in triplicate.

RESULTS

EHT

Percentage inhibition was concentration dependent. Methanolic stem extracts were more promising than aqueous and hexane extracts with inhibition percentages of 74.39, 71.91, 65.15, 62.41, and 47.38 on selected concentrations, respectively. Aqueous extracts displayed inhibition percentages of 72.5, 70.7, 63.94, 59.32, and 43.49. Hexane extracts showed inhibition percentages of 70.03, 64.84, 59.79, 53.14, and 48.16. Positive control levamisole (2 mg/ml) showed a inhibition of 98.6, while negative control (distilled water) showed a inhibition percentage of 1.67 (Fig. 2).

Data were statistically analyzed, and the level of significance was tested following the two-way analysis of variance.



Fig. 1: *Abutilon theophrasti*

LMT

An inhibition percentage of all stem extracts of *A. theophrasti* were seen concentration dependent. Methanolic stem extracts showed inhibition percentages of 79.79, 78, 72.31, 49.5, and 28.47 on selected concentration, respectively. Aqueous extracts showed percentage inhibitions of 75.65, 70.06, 68.45, 61.69, and 54.13. Hexane extracts showed inhibition percentages of 71.28, 70.98, 57.86, 33.1, and 19.59. Positive control levamisole (2 mg/ml) showed inhibition of 92.88, while negative control (distilled water) showed inhibition percentage of 2.34 (Fig. 3).

Data were statistically analyzed, and the level of significance was tested following the two-way analysis of variance.

DISCUSSION

All the treatments based on extracts of *A. theophrasti* stem exhibited anthelmintic activity ($p \leq 0.05$). Methanolic stem extracts were found to exert potential inhibition activity against eggs compared to aqueous and hexane extracts. Maximum inhibition was seen at the highest concentration (500 mg/ml) in all selected extracts. Levamisole served as reference standard drug displayed inhibition of 98.6%. Lowest inhibition was seen at a concentration of 31.25 mg/ml in all selected extracts; aqueous extracts were seen to show the lowest inhibition with inhibition percentage of 43.49%.

Phytochemical investigations revealed the presence of flavonoid glycosides, catechins, anthocyanidins, sterols, triterpenes, tannins, vitamins, sugars, rubber-like substances, etc. [5-10]. Tannins have been reported to complex with polysaccharides. Condensed tannins have been determined to bind cell walls of ruminant bacteria preventing growth and protease activity [11]. One of the molecular actions of the tannins is complex with proteins through so-called non-specific binding forces [12]. There are numerous reports indicating direct or indirect anthelmintic effects of condensed tannins, which are polyphenols [13,14]. Polyphenols have been attributed to the antihelminthic activity of white abrus [15]. Root and leaf extracts of *A. theophrasti* have been confirmed to possess antihelminthic activity [16], and hence, we find support to our study.

Variation in the anthelmintic activity of the extracts tested in the present study may be attributed to the disparity in the targets and qualitative and quantitative differences in the active phytochemicals of the stem. The targets to exert anthelmintic effects may differ in various parasite stages. Most of the screening *in vitro* tests are most easily applied to the

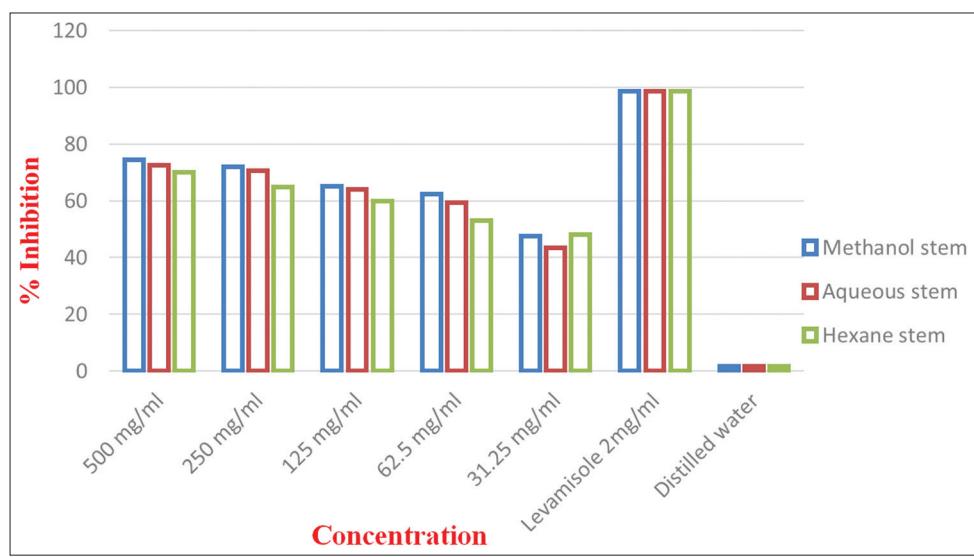


Fig. 2: Egg hatch test (stem)

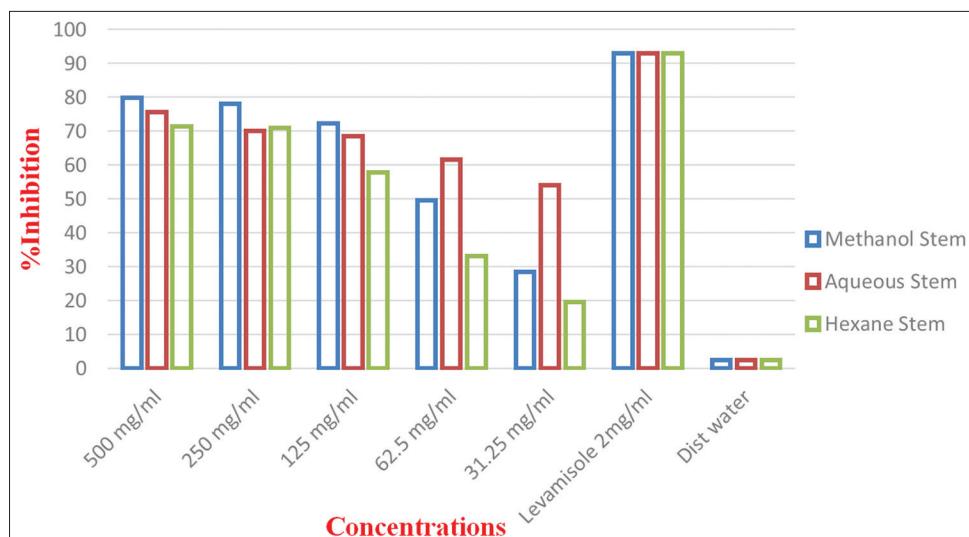


Fig. 3: Larval motility test (stem)

free-living stages of parasite species, i.e., eggs and larvae. The ultimate use of the anthelmintic will be directed at parasitic stages [17]. Certain guidelines have been designed for evaluating *in vitro* the anthelmintic efficacy of drugs that have been adopted by the WAAVP [18]. According to these guidelines, effective anthelmintic agents should inhibit worm egg hatching and larval motility by more than 90% and when inhibiting 80–90% should be considered moderately effective. Thus, our *in vitro* results obtained with *A. theophrasti* extract against *H. contortus* eggs and larvae, particularly in the higher dilutions, allow us to classify the tested extract as moderately effective. There is no clear evidence that synthetic anthelmintics leave no residues in meat that would pose potential public health hazards [19]. Henceforth, the identification of novel promising anthelmintic plant extracts such as *A. theophrasti* extract may contribute for the development of phytotherapeutic products that could be more cost-effective, safer, and more accessible and provide a lower risk of resistance than the conventional therapeutic arsenal currently employed.

CONCLUSION

The present study concludes with the finding that selected plant (*A. theophrasti*) is having potential anthelmintic activity against the eggs of *H. contortus*.

ACKNOWLEDGMENT

Authors are grateful to the Department of Zoology and Department of Biochemistry, Islamia College of Science and Commerce, Srinagar (Jammu and Kashmir), for providing the laboratory facilities to complete the research work.

AUTHORS' CONTRIBUTIONS

All the authors contributed equally to the conductance of the study, while as writing, and editing of the article was done by the first and corresponding author.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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