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in vitro Evaluation of Few Indigenously Known Herbal Plants for their Anthelmintic Property

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The messy use of anthelmintics leads to the establishment of parasitic resistance. Thus present study was designed to determine anthelmintic activity of some indigenously known angiospermic plants. Four plants were selected according to their local availability in Hisar (Haryana, India). Extracts in four different solvents were prepared from each plant and were evaluated qualitatively for the presence of phytochemical constituents. *In vitro* anthelmintic activity of each plant extracts were evaluated against *Haemonchus contortus* by larval motility assay method. Mortality of L3 larvae were recorded from a concentration range of 1 mg/mL to 400 mg/mL. Mean mortality was found to be highest for chloroform methanol extract (ranged from 55.81 to 82.56 % at the highest concentration level). Efficacy of albandazole (anthelmintic drug), a positive control was 65.21 % at 400 mg/mL which is comparable to efficacy of few plant extracts. Thus, it may be concluded that plant parts used in the present study may work as effective substitute of commonly used anthelmintic drugs.

Keywords: Gastrointestinal, Physico-chemical properties, *in vitro* model, Albizia, Anthelmintic, Embelia, Nyctanthes, Solanum.

INTRODUCTION

Helminth infections are a major threat to livestock productivity and cause enormous economic losses [1]. Among different nematodes of animal origin *Haemonchus contortus* (a nematode) is a pathogenic parasite of ruminants and causes acute disease and high mortality [2]. Nematodes have developed resistance against several families of antiparasitic drugs [3,4]. In addition to anthelmintic resistance, inadequate availability and high cost of commercial anthelmintics are other important constraints of helminth control in developing countries. Plant kingdom is known to have phytochemicals with anthelmintic, antibacterial and insecticidal properties. A number of plant parts have been documented in ancient literature for their use as anthelmintics [5]. However, active chemical ingredients responsible for these activities still need investigations. Screening, structure elucidation and testing the efficacy of active ingredients from particular part of medicinal plant may boost the sustainability and acceptance of the same as an active anthelmintic for livestock. Keeping this in view, certain parts of indigenously known herbal plants were studied for their chemical constituents and their *in vitro* efficacy was tested against *Haemonchus contortus,* a common gastrointestinal parasite present in ruminants.

EXPERIMENTAL

Four angiospermic plants *viz.*, *Embelia tsjeriam cottam* (Vidang, Bibadang), *Nyctanthes arbor-tristis* (Harshingar), *Albizia lebbeck* (Siris) and *Solanum xanthocarpum* (Kantkari) known in ancient literature and easily available in India were taken for the study. Fruits of *Embelia tsjeriam cottam* and *Solanum xanthocarpum* and bark of *Nyctanthes arbor-tristis* and *Albizia lebbeck* were collected locally. Authentication of plants was done by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India.

Preparation of plant extract: Shade dried and powdered authenticated plant samples were subjected to extraction successively with petroleum ether, chloroform-methanol (4:1) and methanol using Soxhlet apparatus. Aqueous extracts were prepared by hot extraction method. Extracts so prepared were concentrated to near dryness on rotary vacuum evaporator at 40-50 °C. Different concentration levels of extracts were suspended in Tween-80 for determining their biological activity against *Haemonchus contortus*.

Phytochemical screening: Qualitative chemical tests were performed for determination of phytochemical constituents present in the plant extracts. Tests were performed for the

presence of alkaloids, carbohydrates, proteins, glycosides, saponins, steroids, tannins and flavonoids using the method of Neelima *et al.* [6] and Tiwari *et al.* [7].

Anthelmintic activity: Fresh faecal samples from grazing animals were collected from the field. Representative samples were evaluated for the presence of eggs by floatation method. Positive samples were cultured to obtain their infective larval stage (L3). Faeces were mixed with charcoal so that it was neither too wet nor too dry and cultured at 27 °C for 7-10 days. Collection of larvae was done by Berman's technique. Larvae load was counted on simple microscope at 10x level. Equal volume of each extracts was added to 100 µL solution containing 10-15 larvae. Number of motile and non-motile larvae were counted under microscope after a time interval of 2 h of incubation.

RESULTS AND DISCUSSION

Screening of plant extracts for the presence of various phytoconstituents: Phytochemical screening of various extracts *viz*. petroleum ether, chloroform- methanol, methanol and water extracts were performed and is presented in Table-1. The presence of carbohydrates, alkaloids, tannin, glycosides and steroids in *Embelia tsjeriam cottam* was observed. However, chloroform-methanol extract of *Embelia tsjeriam cottam* contained only alkaloids while methanolic extract contains a maximum number of three components *i.e.* alkaloids, proteins and tannins. In aqueous extract only steroids were present. In *Nyctanthes arbor-tristis*, carbohydrate, steroids, tannins and flavonoids were present. Flavonoids were present only in the chloroform-methanol extract, while methanolic part showed the presence of tannins and flavonoids both. In aqueous extracts of *Nyctanthes arbor-tristis* carbohydrates and steroids were present. In *Albizia lebbeck*, different extracts contained the entire range of compound analyzed except saponin. Glycosides and steroids were present in chloroform-methanol extract, while methanol contained alkaloids, protein, tannins, flavonoids and glycosides. Aqueous extract had only carbohydrates. *Solanum xanthocarpum* was having alkaloids in its chloroform-methanol extract, while in methanolic extract maximum numbers of constituents were present *viz*. alkaloids, carbohydrates, proteins, steroids, tannins and flavonoids. In aqueous extract only carbohydrates and flavonoids were present.

Efficacy of extracts against L3 larvae of *Haemonchus contortus*: Efficacy of different solvent extracts against *H. contortus* was studied at various concentration levels (1-400 mg/mL). The per cent mortality in third stage larvae is presented in Table-2. Per cent mortality was found to be highest for chloroform-methanol extract amongst all the plant parts studied. In chloroform-methanol extracts mortality varies from 55.81 to 82.56 % at 400 mg/mL concentration level for different plant part's extracts. The variation between anthelmintic activities in four solvent extracts of a plant part might be due to difference in concentration of active components responsible for the anthelmintic activity. On comparing the results of plant part extracts with common anthemintic drugs like albendazole, it was observed that most of the plant part extracts at higher concentration were superior in their anthelmintic properties over the activity of albendazole (65.21 % mortality at 400 mg/mL).

The results obtained for *Embelia tsjeriam cottam* showed the presence of alkaloids, flavonoids, proteins and gums in ethanolic extracts of leaf, while roots had only alkaloids and gums [8]. In present study, methanolic extract showed the presence of alkaloids, proteins and tannins, although the work was done in fruits of *Embelia tsjeriam cottam*. Results of *Nyctanthes arbor-tristis* confirmed the presence of tannins and flavonoids in ethanolic extract while aqueous extract contain carbohydrates, proteins and amino acids in addition to glycosides, tannins and flavonoids [9]. *Albizia lebbeck* showed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, steroids in ethanolic extracts [10]. Presence of alkaloids, carbohydrates and steroids using petroleum ether, alcohol and acetone solvent extracts has been reported in fruits of *Solanum xanthocarpum* while tannins were present only in petroleum ether solvent [11].

A study with neem plant extracts reported mortality of about 40 % after a time interval of 24 h at concentration level of 4 mg/mL [12]. In another study, 60 % mortality was reported after 2 h using methanolic extracts of *Euphorbia helioscopia*

TABLE-1
PHYTOCONSTITUENT ANALYSIS OF DIFFERENT PLANT EXTRACTS

Plant	Extract	Alkaloids	Carbohydrates	Proteins	Glycosides	Saponin	Steroids	Tannins	Flavinoids
Embelia tsjeriam cottam	Petroleum ether	–	–	–	+	–	–	–	–
	Chloroform-methanol	+	–	–	–	–	–	–	–
	Methanol	+	–	+	–	–	–	+	–
	Aqueous	–	+	–	–	–	+	–	–
Nyctanthes arbor-tristis	Petroleum ether	–	–	–	–	–	–	–	–
	Chloroform-methanol	–	–	–	–	–	–	–	+
	Methanol	–	–	–	–	–	–	+	+
	Aqueous	–	+	–	–	–	+	–	–
Albizia lebbeck	Petroleum ether	–	–	–	–	–	–	–	–
	Chloroform-methanol	–	–	–	+	–	+	–	–
	Methanol	+	–	+	+	–	–	+	+
	Aqueous	–	+	–	–	–	–	–	–
Solanum xanthocarpum	Petroleum ether	–	–	–	–	–	–	–	–
	Chloroform-methanol	+	–	–	–	–	–	–	–
	Methanol	+	+	+	–	–	+	+	+
	Aqueous	–	+	–	–	–	–	–	+

TABLE-2
PER CENT MORTALITY (HARMONIC MEAN) OF *Haemonchus* CONTORTUS LARVAE (L3) AFTER 2 H OF INCUBATION

Extract		1 mg/mL (%Mean±SD)	10 mg/mL (%Mean±SD)	100 mg/mL (%Mean±SD)	200 mg/mL (%Mean±SD)	400 mg/mL (%Mean±SD)
Embelia tsjeriam cottam	Petroleum ether	8.33±3.18 aBC	11.11±1.25 abBC	15.38±3.78 bBC	21.05±1.11 cCD	40.54±2.20 dF
	Chloroform-methanol	9.53±5.15 aBC	14.29±2.09 aC	21.05±1.11 bC	38.10±5.57 cFG	82.57±0.76 dJ
	Methanol	11.11±6.35 aC	13.33±5.09 abC	16.67±6.36 bC	30.00±3.04 cE	37.74±2.14 dF
	Aqueous	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	6.45±1.07 bA
Nyctanthes arbor-tristis	Petroleum ether	6.25±0.40 aBC	11.77±2.16 abBC	16.67±2.87bC	23.53±1.39 cD	33.33±0.00 dEF
	Chloroform-methanol	6.90±0.72 aBC	14.29±2.09 bC	16.67±6.36 bC	36.92±0.57 cFG	67.24±8.26 dHI
	Methanol	7.14±4.12 aBC	6.67±0.45 aB	10.00±1.01 aB	17.39±8.24 bC	52.94±3.13 cG
	Aqueous	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	5.56±0.63 bB	16.67±1.40 cC
Albizia lebbeck	Petroleum ether	8.33±4.81 aBC	14.29±8.25 bC	21.05±6.02 cC	27.27±2.50 dDE	37.33±2.50 EF
	Chloroform-methanol	8.89±2.33 aBC	12.50±3.37 aC	30.77±2.38 bD	46.67±3.13 cG	55.81±1.30 dG
	Methanol	7.69±4.44 aBC	11.11±6.41 aBC	21.05±6.02 bC	41.38±1.43 cFG	40.26±5.23 cF
	Aqueous	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	10.53±1.71 bAB
Solanum xanthocarpum	Petroleum ether	7.14±4.12 aBC	10.00±5.77 aBC	20.00±0.00 bBC	17.39±4.00 bC	32.00±6.71 cE
	Chloroform-methanol	9.09±5.25 aBC	11.76±0.70 abBC	15.38±3.78 bB	43.64±0.79 cG	71.82±0.40 dH
	Methanol	5.56±3.21 aB	12.50±1.44 bC	15.38±3.78 bcB	19.05±0.91 cCD	25.30±2.47 dD
	Aqueous	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	8.33±0.70 bB	13.80±0.48 bBC
Albendazole		9.67±4.21aB	15.38±2.87bC	41.38± 1.39cE	55.81±1.40 dH	65.21±8.08 eH
Negative control		0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA	0.00±0.00 aA

Different superscript in a row (a,b,c) and column (A,B,C) are different significantly ($P < 0.05$).

at a concentration of 50 mg/mL [13]. Mortality of larvae in present study was found to be on higher side, although the concentration level was different from the above mentioned studies. In crude aqueous methanolic extract of *Albizia lebbeck* leaves 100 % mortality was reported at dose rate of 50 and 100 mg/mL after 4 h post treatment [14]. Similarly other concentrations also showed time and dose dependent response. *Albizia lebbeck* in our study showed 55.81 % mortality of worms however these results were obtained after 2 h time interval. Maphosa and coworkers [15] reported that aqueous extract of *A. ferox, E. elephantia* and *L. leonurus* showed 100 % inhibition of egg hatch and larval development of *H. contortus*. Results were comparable to the findings of this study (82.57 %). In another study of root extracts of *Elephantorrhiza elephantina* the effect of ethyl acetate and aquous fractions was much faster even at very low concentration of 0.312 mg/mL [16] but in this study we did not use the extract of ethyl acetate and even the response of aquous extract was lowest among different extracts. Highest activity of aquous extract was observed in *nyctanthes* at its highest level of concentration. On the basis of present study it may be concluded that extract of *Embelia tsjeriam cottam*, *Nyctanthes arbor-tristis*, *Albizia lebbeck* and *Solanum xanthocarpum* showed good anthelmintic activity and thus these plant parts may be an alternate for commonly used costly anthelmintic drugs for deworming of ruminants. In *Embelia tsjeriam cottam* and *Solanum xanthocarpum* alkaloids may be responsible for the anthelmintic activity while in *Albizia lebbeck* and *Nyctanthes arbor-tristis* activity may be due to the presence of glycosides and flavonoids respectively.

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