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Research Article

Anthelmintic Activity of Three Indian Medicinal Plants

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ABSTRACT

The anthelmintic activity of the aqueous and methanolic extracts and the hexane, chloroform, n-butanol and water fractions of the leaf extracts of *Ocimum sanctum, Murraya koenigii* and *Mallotus phillipensis* was investigated *invitro* using egg hatch assay and larvicidal activity. The phytochemical analysis of the extracts was done using standard tests and the acute oral toxicity was assessed in rats at the dose rate of 2000mg/kg. The extracts were diluted at dose rated of 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml in distilled water and albedndazole at 0.5 and 1 mg/ml was used as the standard drug. The tests were done in triplicates with the ova exposed to the drug for 48 hours at 27°C where as larvae were exposed at room temperature. The number of dead ova/live larvae were counted after 48 hours to assess the effect on ova where as the time taken for cessation of motility of larvae was taken as the end point of larvicidal activity. The phytochemical analysis revealed the presence of tanins and flavonoids in all the extracts tested where as steroids were absent in all. The aqueous and methanolic extracts of *Murraya* and *Ocimum* and the methanolic extract of *Mallotus* showed significant ovicidal activity where as the minimum larvicidal concentration was not as pronounced as the ovicidal concentrations. The chloroform fraction of *Murraya* showed ovicidal activity at 0.78 mg/ml and larvicidal activity at 0.156 mg/ml showing that the anthelmintic activity is maximum with the chloroform fraction. None of the extracts showed any adverse reaction during the entire period of observation. Hence it could be concluded that all the three plants possess significant anthelmintic activity substantiating their use in folklore medicine in treatment of nonspecific diarrhea.

Keywords: Anthelmintic, *Ocimum sanctum, Murraya koenigii, Mallotus phillipensis*

INTRODUCTION

Prevalence of parasitic helminthes, especially gastrointestinal nematodes is recognized as a major constrain in the livestock industry due to the huge economic loss[1,2]. They affect the reproduction and production through mortality, weight loss, reduced milk yield and wool production[2]. Gastrointestinal helminthosis is controlled mainly by synthetic chemical anthelmintics, which have the disadvantages of being costly, risk of environmental pollution and development of resistant populations[3] . Anthelmintics derived from plants can be a solution to this world wide problem as they form safe and non-toxic agents with an altered site of action[3,4].

Ocimum sanctum, also called as holy basil (Tulsi) is an annual shrub and is known for its medicinal properties which include antibacterial, antifungal, hepato- protective, expectorant, anti-inflammatory and analgesic activity[5]. It contains eugenol, monoterpens and diterpenes which add to the medicinal properties[6].

*Mallotus Philippensis*is (Kamala) a small to medium-sized monoecious tree, up to 25 meters tall of the family Euphorbiaceae. The crude powder of Kamala obtained as a glandular pubescence from the exterior of fruits is found to have anthelmintic activity and active against thread worms, hook worms, round worms and earthworms. The drug was found to be 100% effective against tapeworms. The leaves are bitter, cooling, give appetite, causes flatulence and constipation[7].

Murraya Koenigii, commonly known as curry leaf tree, belongs to the family rutacea and is seen all over India, Srilanka and South East Asia. It is well known for its medicinal properties like antibacterial, antifungal, cytotoxic, antidiarrhoeal, anti-inflammatory[8]. Anthelmintic activity of *Murraya koenigii* extracts has been assessed in *Pheretima posthuma*[8]; but literature is scanty on the ovicidal and larvicidal activity against the nematodes. The present study has been undertaken to assess the ovicidal and larvicidal activity of different extracts of these plants against *Haemonchus contortus*.

MATERIALS AND METHODS

Plant Material

The leaves of *Ocimum sanctum, Mallotus phillipensis and Murraya koenigii* were collected from different parts of the district of Wayanad, identified and authenticated by a Botanist at MSSRF, Kalpetta. They were dried under shade, pulverized and extracted using methanol in soxhlet extraction apparatus. The aqueous extract was taken as a decoction. They were dried and stored under refrigeration till further use.

Fractionation of the extract

The methanolic extract was further fractionated in a

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Table 1: Phytochemical constituents of different leaf extracts.

Constituents	*Ocimum sanctum*						*Mallotus phillipensis*						*Murraya koengii*					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Phenolics	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+
Alkaloids	-	+	-	+	-	+	-	+	+	+	-	-	-	+	+	+	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenes	+	+	+	+	+	+	-	+	+	+	-	-	-	+	+	+	-	-
Saponins	-	+	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-
Flavanoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

1-Aqueous extract; 2- Methanolic extract; 3- Hexane fraction; 4- Chloroform fraction; 5- Butanol fraction; 6- Water fraction; (+) indicates presence and (–) indicates absence of the phytochemical in the extract.

separating funnel by taking solvents in order of increasing polarity viz, hexane, chloroform, n-butanol and water. They were also dried using rotary vacuum evaporator and stored under refrigeration till further use.

Phytochemical analysis

The extract as well as the fractions was analyzed qualitatively for various phytochemical constituents.

Identification of the larvae

The larvae were identified based on the morphometric studies and molecular methods[10].

Assessment of the anthelmintic activity

Egg hatch assay

Fresh ova were collected from faecal sample of goat infested with *Haemonchus contortus* and were concentrated by centrifugation. Eggs were washed with distilled water prior to the experiment. Aqueous and methanolic extracts as well as the hexane, chloroform, butanol and water fractions of the methanolic extracts of *Ocimum sanctum, Mallottus philippinensis and Murraya koenigii* were used for the study. Albendazole and Ivermectin were used as positive control whereas distilled water served as negative control. The extracts were diluted to concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 mg/ml in a total volume of 0.5 ml. In the experiment, about 50eggs/0.5 ml distilled water were counted and taken in marked 6- well tissue culture plates and were added with 0.5 ml of the extract as described earlier. The effective concentration of the drug in each petri plate was thus reduced to 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 mg/ml. Albendazole was also diluted using DMSO to provide concentration of 1 and 0. 5 mg/ml. The culture plates were incubated for 48 hrs at 27°C. The experiment was done in triplicates for each concentration. Hatched larvae (dead or alive) and unhatched eggs were counted under dissection microscope (magnification 40 X) [11-13]. The concentration that inhibited the hatch of 50% of the ova was considered as the minimum ovicidal concentration.

Assessment of the larvicidal activity

Five gram of dung from goats infested with *Haemonchus contortus* were incubated at room temperature with adequate humidity in dark for 10 days to get L3 larvae. The larvae were washed out into petriplates. The larvicidal activity was done as per the procedure of Rahman *et al.* with minor modifications. Approximately 100 motile larvae were collected in 100 µL water into which equal quantity of extract diluted in 10% DMSO were added. Aqueous and methanolic extracts as well as the hexane, chloroform, butanol and water fractions of the methanolic extracts of *Ocimum sanctum, Mallottus philippinensis and Murraya koengii* were used for the study. Albendazole and Ivermectin were used as positive control where as 10% DMSO served as negative control. The extracts were diluted to concentrations of 100, 50, 25, 12.5, 6.25, 3.125

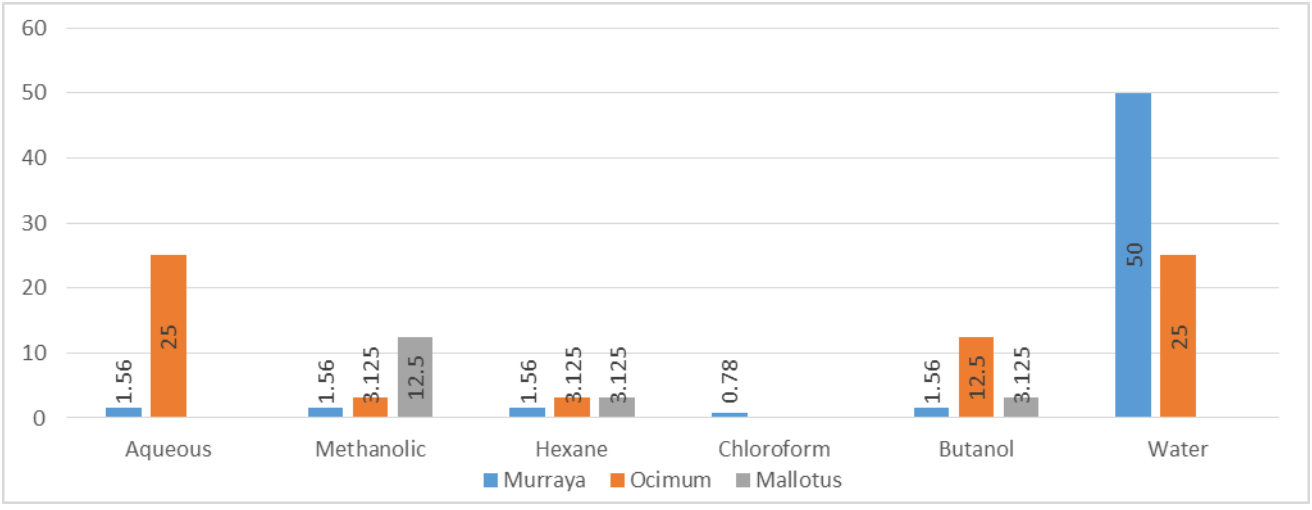

Fig 1: Minimum ovicidal concentration of different extracts against the ova of *Haemonchus contortus (mg/ml)*

Table 2: Minimum Larvicidal Concentration of different extracts on the L3 larvae of *Haemonchus contortus*

S no	Extract	*Ocimum sanctum*	*Mallotus philippinensis*	*Murraya koengii*
1	Aqueous	50	Nil	12.5
2	Methanolic	3.125	12.5	6.25
3	Hexane	25	3.125	3.125
4	Chloroform	nil	Nil	0.156
5	Butanol	Nil	3.125	3.125
6	Water	Nil	Nil	50

and 1.5625 mg/ml in a total volume of 0.2 ml. The loss of motility of the larvae was checked every 15 minutes and the % larvae found non-motile/ dead were calculated. The concentration that produced mortality in 50% of larvae within 4 hours was taken as the minimum larvicidal concentration.

RESULTS

Phytochemical Analysis

Phytochemical analysis of different extracts is summarized in table 1.

Tannins and flavonoids were present in all the extracts where as phenolic compounds were present in all the extracts except hexane fraction of all the three plants. Steroids were detected in none of the extracts where as glycosides were not detected in *O. sanctum.*

Assessment of the Anthelmintic Activity

All the extracts tested showed inhibition of hatching of *H. contortus* eggs in a dose dependent manner except for the aqueous extract of *M. phillipensis*, chloroform fractions of *O. sanctum* and *M. phillipensis* and water fraction of *M. phillipensis*. The chloroform fraction of *M. koeniggi* showed maximum potency with Minimum Ovicidal Concentration as low as 0.78mg/ml.

The morphology of the ova exposed to different treatments varied with the control in a dose dependent manner. The dead ova showed disintegration of the entire shell with breaking of the embryo inside in concentrations of 50 and 25 mg/ml whereas the shell was intact with dead embryos in drug concentrations of 12.5, 6.25, 3.125 and 1.56mg/ml. Albendazole and ivermectin showed disintegration of ova in all the doses selected for the study.

Assesment of larvicidal activity

The chloroform fraction of *Murraya koeniggi* extract showed maximum potency with a minimum larvicidal concentration of 0.156 mg/ml. The water fraction showed no activity whereas the aqueous extract of *Mallotus* also showed no effect. The control drugs, albendazole and ivermectin produced death of the larvae within 15 minutes of observation in both the concentrations.

The larvae on initial exposure showed wriggling movements, which slowed down on due course of time and finally the movements stopped and were found dead.

DISCUSSION

A large number of medicinal plants have been used for treatment of parasitic diseases in man and animals. Screening of anthelmintic activity is mainly through invitro tests including larval and adult paralysis/ death, egg hatch assays or motility and biochemical tests[1]. Invitro tests using the larvae of *Haemonchus contortus* is considered to be one of the best means of screening drugs for anthelmintic activity[15].

The aqueous and methanolic extracts of *Murraya* and *ocimum* and the methanolic extract of *Mallotus* showed significant ovicidal activity where as the minimum larvicidal concentration was not as pronounced as the ovicidal concentrations. The chloroform fraction of *Murraya* showed ovicidal activity at 0.78 mg/ml and larvicidal activity at 0.156 mg/ml showing that the anthelmintic activity is maximum with the chloroform fraction. Limited activity alone was shown by the aqueous extract and hence the choice of solvent shall be limited to methanol for anthelmintic activity. The control drug albendazole showed both ovicidal and larvicidal activities at the doses tested.

There are several reports on the invitro anthelmintic activity of *Murraya* and *Ocimum* against earth worms[9,16-19.] Earthworms, showing almost the similar structural and functional anatomy to helminths are taken as model for assessment of anthelmintic activity and hence the results can be extrapolated to the invitro studies with the adult worms. Studies in our laboratory has confirmed that presence of phenolics, tannins and flavonoids contributed to the anthelmintic activity of the extracts of *Mallotus phillipensis, Murraya koenigii* and *Ocimum sanctum* against *Ascaridia galli*[7, 20].

The presence of saponins and tannins in the leaf extracts of *Parkiabi globosa* inhibited the hatching of nematode eggs of ruminant parasites[13]. The biological effects of saponins are normally ascribed due to their interaction with the cell membranes, causing changes within the cell membranes, changes in the cell wall permeability and interaction with the collagen proteins from the cuticle of nematodes[21]. It was reported that the anthelmintic activity of tannins in *K. Sengalensis* because tannins have the capacity to bind to proteins and inactivate many mechanisms including the nutrient availability of the larvae[22]. This may impair vital process like feeding, reproduction of the parasite and disrupt the integrity of the cuticle[1].

The action on the cell membrane of the ova was very evident from the disruption and disintegration of the ova at higher doses of the drug treated groups. In the case of larvae, there was reduced motility from the initiation of the experiment itself which could be due to the effects of the extract on the energy metabolism of the parasite. Tannins can inhibit oxidative phosphorylation, thus decrease metabolism and availability of energy leading to death of the larvae[23].

From the study it could be concluded that the chloroform fraction of methanolic extract of *Murraya koeniggi* possess the best anthelmintic activity among the tested extracts. Further fractionation and testing can identify a potent anthelmintic molecule from the extract and provide a better solution to anthelmintic resistance.

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