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Original Article

Effect of various extracts of *Ocimum sanctum* and *Mallotus phillipensis* on *Setaria digitata*

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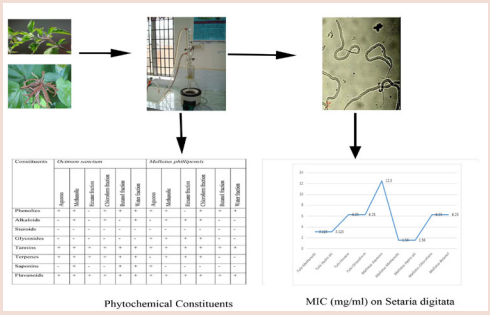
ABSTRACT

Introduction: The anthelmintic activity of various extracts of leaves of *Ocimum sanctum* and *Mallotus phillipensis* was assessed *in vitro* against *Setaria digitata*. **Materials and Methods:** The leaves of *Ocimum sanctum* and *Mallotus phillipensis* were collected and were extracted using methanol, dried and stored under refrigeration till further use. The aqueous extract was taken as a decoction. The methanolic extract was further fractionated by taking solvents of increasing polarity viz, hexane, chloroform, n-butanol and water. The extract as well as the fractions were analysed qualitatively for various phytochemical constituents. Fresh nematodes (*Setaria digitata*) were recovered manually from the peritoneum of infested buffalo, were washed and transferred to the extract containing petriplates (concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml) immediately and the motility/death of *Setaria digitata* was noted. **Results:** The presence of flavonoids and tannins were detected in all the extracts where was phenolics as absent in the hexane fraction. The methanolic extract of Tulsi and Kamla produced death of nematodes in concentrations of 3.125 mg/ml and the extract of tulsi was found to be more potent. Similar results were also observed in the case of hydro alcoholic extract whereas the aqueous extract showed no effect. The chloroform fraction of *Ocimum sanctum* and n-butanol and chloroform fractions of *Mallotus* were equally potent in inhibiting the motility and producing death of the worms. The control drug, albendazole produced death in 30 minutes in both the concentrations. **Conclusion:** It could be concluded that higher doses of the extract are as potent as albendazole.

Key words: Anthelmintic, Albendazole, *Mallotus phillipensis, Ociumum sanctum, Setaria digitata*.

SUMMARY

- The phytochemical analysis revealed the presence of tannins, flavonoids, terpenes and phenolic compounds in almost all extracts of *Ocimum sanctum* and *Mallotus phillipensis*.
- Methanolic and hydroalcoholic extracts of *Mallotus phillipensis* produced death of *Setaria* in concentrations of 1.56 mg/ml where as Tulsi extracts did it at 3.125 mg/ml.
- The extracts showed no toxicity on acute oral toxicity testing in rats.
- Presence of saponins and tannins may be the cause of the anthelmintic property of the extracts.



PICTORIAL ABSTRACT

Abbreviations used: MSSRF: MS Swaminathan Research Foundation, OECD: Organisation for Economic Cooperation and Development, MIC: Minimum Inhibitory Concentration.

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INTRODUCTION

Helminthosis is a major threat to ruminant populations leading to economic losses in areas where extensive grazing is practised. The major method of treatment of hemlinthosis is use of commercially available chemical anthelmintics. They are not economically viable and also possess the risk of anthelmintic resistance.[1] Resistance to the currently used anthelmintics has been well established and is being reported from all parts of the world even though all the three has different modes of action.[2] The signs of new class of drugs being developed with a different mechanism of action is also less.[3] The use of herbal preparations and plants as anthelmintics is prevalent in many parts of the world, mainly developing countries. The evidence of anthelmintic properties of plants is developed mainly through ethnoveterinary practices.[4] Anthelmintic activity of different plant extracts has been assessed in earth worm models[5-7] but literature is scarce on the adulticidal activity against the animal nematodes.[8-10]

Ocimum sanctum L. is a shrub grown all over India belonging to *Lamiacea*, possess a wide variety of pharmacological properties. It has proven analgesic, anti-inflammatory, antibacterial, antifungal, immunomodulatory, hepatoprotective, anticarcinogenic, antidiabetic and wound healing properties.[11] The volatile oil of tulsi contains mainly eugenol which attributes to the major pharmacological properties. *Mallotus Philippensis* is 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.[12] The present investigation was undertaken to assess the effect of different extracts of leaves of *O. sanctum* and *M. phillipensis* on *Setaria digitata*.

MATERIALS AND METHODS

Plant Material Collection and extraction

The leaves of *Ocimum sanctum* and *Mallotus phillipensis* were collected from different parts of Wayanad, Pookode, and were authenticated by

a Botanist at MSSRF, Kalpetta, dried under shade and pulverized. They were extracted using methanol in soxhlet extraction apparatus, dried using a rotary vacuum evaporator and stored under refrigeration till further use. The aqueous extract was taken as a decoction.

Fractionation of the extract

The methanolic extract was further fractionated in a separation funnel by taking solvents of increasing polarity viz, hexane, chloroform, n-butanol and water. They were also dried using the 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.[13]

Assessment of Nematodicidal activity

Collection of Nematode parasite

Fresh nematodes (*Setaria digitata*) were recovered manually from the peritoneum of infested buffalo slaughtered at the Malabar meat plant Sulthan Bathery, Wayanad in tyrodes solution. They were washed and transferred to the extract containing petriplates immediately.[14]

Identification of the parasite

The worms present in the peritoneum of buffaloes were collected in 10 per cent formalin solution and brought to the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Pookode, Wayanad for identification. The nematodes were dehydrated in ascending grades of alcohol and then cleared in creosote.[15]

Test drug preparation

Extracts were diluted in tyrodes solution at 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml concentrations in petriplates to get a total volume of 20 ml. albendazole @ 10 mg/ml and 1 mg/ml was used as positive control.

Test procedure

6 nematodes were placed in the petriplates containing the extract/drug and their motility/wriggling movements were noted every 15 minutes. The motility was Scored as described by[16] with minor modifications. Cessation of movements even on stimulation were considered as the death point. The experiment was done in triplicates.

Gross morphological examination

The treated worms as well as control worms were examined under light microscope for identifying gross morphological changes.[17]

Assessment of Acute Oral toxicity

The acute oral toxicity of the tested extracts were done in rats as per OECD guideline 420 in the limit dose of 2000 mg/kg body weight.

RESULTS

Phytochemical analysis

The results of the phytochemical analysis is presented in Table 1. Tannins and flavonoids were present in all the extracts where as terpenes were absent in the aqueous extract of *M. phillipensis* leaf. Phenolic compounds were absent in the hexane fraction of both the plants. Steroids were absent in all the extracts.

Adulticidal activity of different extracts against *Setaria digitata*

The effect of various extracts on the nematode is tabulated in Table 2. The methanolic extract of Tulsi and Kamla produced death of nematodes in concentrations of 3.125 mg/ml and 1.56 mg/ml respectively (Figure 1) and the extract of *Mallotus* was found to be more potent. Similar results were also observed in the case of hydro alcoholic extract where as the aqueous extract showed no effect. The chloroform fraction of *Ocimum sanctum* and n-butanol and chloroform fractions of *Mallotus* were equally potent in inhibiting the motility and producing death of the worms. The control drug, albendazole produced death in 30 minutes in both the concentrations. Hence it could be concluded that higher doses of the extract are as potent as albendazole.

Identification of the parasite

The worms were milky white in colour, tapering towards the hind end. The mouth was surrounded by cuticular ring. The tail of the male worms was with pre and post cloaca papillae and that of female with conical projections.

Gross Morphological Examination

Initially, before the treatment of extracts, the worms were highly motile and elongated. The gross morphological examination revealed shrinkage of the of the worms and they became slender and paralysed.

Acute Oral Toxicity

The acute oral toxicity test revealed no untoward clinical reactions or mortality in the entire time period of observation. There was no abnormal behavioural reactions.

Table 1: Phytochemical Analysis

Constituents	*Ocimum sanctum*						*Mallotus phillipensis*					
	Aqueous	Methanolic	Hexane fraction	Chloroform fraction	Butanol fraction	Water fraction	Aqueous	Methanolic	Hexane fraction	Chloroform fraction	Butanol fraction	Water fraction
Phenolics	+	+	-	+	+	+	+	+	-	+	+	+
Alkaloids	-	+	-	+	-	+	-	+	+	+	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	+	+	+	+	-	-
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Terpenes	+	+	+	+	+	+	-	+	+	+	-	-
Saponins	-	+	-	-	+	+	+	-	-	-	-	-
Flavanoids	+	+	+	+	+	+	+	+	+	+	+	+

Table 2: Effect of various extracts on the motility/ death of *Setaria digitata* (Duration; min)

	Concentration (mg/ml)	50	25	12.5	6.25	3.125	1.56
***Ocimum sanctum* leaf**	Methanolic	75	75	75	120	140	Nil
	Aqueous	Nil	Nil	Nil	Nil	Nil	Nil
	Hydroalcoholic	30	60	60	90	120	Nil
	Hexane	90	90	120	120	Nil	Nil
	Chloroform	30	60	60	90	Nil	Nil
	N-butanol	Nil	Nil	Nil	Nil	Nil	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil
***M. Phillipensis* leaf**	Aqueous	180	200	200	Nil	Nil	Nil
	Methanolic extract	30	30	60	60	90	90
	Hydroalcoholic	30	30	60	60	90	90
	Hexane	Nil	Nil	Nil	Nil	Nil	Nil
	Chloroform	30	90	90	120	Nil	Nil
	N-butanol	30	90	90	120	Nil	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil

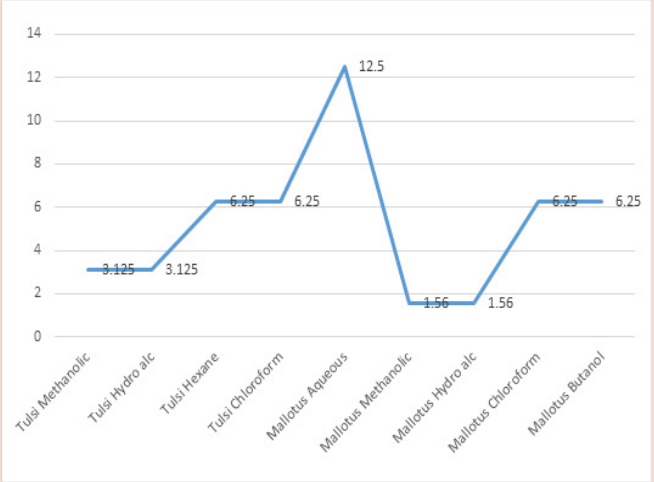

Figure 1: Minimum inhibitory concentration of the extracts on *Setaria digitata*

DISCUSSION

Screening of molecules for anthelmintic activity is mainly done using *in vitro* and *in vivo* techniques. *In vitro* techniques include (i) egg hatch assay for estimating the ovicidal activity, (ii) effect on the larvae assessed by larval motility assay, larval development assay and larval migration inhibition assay (iii) adulticidal activity assessed by the effect on motility, paralysis and death of the worms. Since the anthelmintic activity of a broad spectrum agents can be on any of the three stages viz, ova, larvae or adult or more than one of these, screening in all the three stages will provide the exact mechanism of action of the molecule. We have already reported from our laboratory, the effect of various extracts on the ova and larvae of nematode, *Haemonchus contortus*[12,18-20] In the present study, the effect of the extracts of tulsi and Kamla on the adult nematode, *Setaria digitata* was assessed.

From the results (Table 2), it is evident that the methanolic and hydroalcoholic extract of *Mallotus phillipensis* leaf showed better anthelmintic activity producing death at concentrations of 1.56 mg/ml by 1.5 hours where as the similar extracts of tulsi produced effect only upto 3.125 mg/ml, at 140 and 120 minutes respectively. The aqueous extract produced no effect which states that the phytochemicals present in the methanolic extract has got the potent anthelmintic property. On fractionation, none of the extracts showed similar potency as with the methanolic extract, which can be interpreted to be due to the combined effect of phytochemicals increasing the potency of the crude extract.

The phytochemical analysis (Table 1) revealed the presence of tannins, flavonoids, terpenes, phenolic compounds in almost all the extracts. Tannins will affect the energy metabolism of the parasites, may affect the integrity of the cuticle and also impair feeding and reproduction, mainly by their effect on proteins.[21,22] Saponins affect the cell wall integrity of the nematodes, interact with the collagen of the cuticle where by the cell will lose electrolytes and chemicals and thus the cuticular damage will be sufficient for the death of the parasite.[12]

Development and characterisation of the phytochemical with the evaluation of mode of action can be of significance in the quest for a novel drug that can avoid the anthelmintic resistance.

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CONFLICT OF INTEREST

The authors declare that there is no Conflict of interest.

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