ANTHELMINTIC ACTIVITY OF DIFFERENT EXTRACTS OF *MALLOTUS PHILIPPENSIS INVITRO*

PRIYA, M. N, SREESHITHA, S. G, SREEDEVI, R, SUJITH, S, DEEPA, C. K, SUJA, R. S, JULIET, S.

Abstract: The an thematic activity of different extracts of *Mallotus Philippensis* was assessed *invitro* using earth worm, *Ascaridia galli* and larvae of strongyle worms. Aqueous and methanolic extracts were used for the study and the activity was compared with that of standard drug Ivermectin. *Ascaridia galli* were collected from slaughter house specimens, earth worms (*Pheretima posthuma)* were grown in our laboratory under specified conditions and the larvae were cultured from the fecal sample of goats. The aqueous extract @ 200 mg/ ml killed earth worms with in192.16 ± 1.87min, *A. galli* in 24.16 ± 1.06 and the extract @ 25 mg/ml killed the larvae in 4 hours. The results suggest a potential role of *Mallotus Philippensis* as an anthelmintic against intestinal nematodes of poultry and ruminants.

Key words: Anthelmintic activity, *Ascaridia galli, Mallotus philippensis*, *Pheretima posthuma*

Introduction: The prevalence of gastrointestinal nematodes is of primary concern in livestock and poultry industry, especially in developing countries. Nematodes that feed on blood of the host are responsible for specific clinical and subclinical symptoms and pose great loss to ruminant industry [1]. Due to the great economic losses by this parasitism, it is necessary to have adequate methods of control. Conventionally controls of gastrointestinal parasites are widely based on the use of anthelmintic drugs [2]-[3]. However, the extensive use of these drugs reduced their efficacy because of the development of resistance in parasites [4]-[5]. Since high cost is involved in the development of newer drugs, there has been increasing search for medicinal plants as an alternative source of anthelmintic drugs due to their less toxic, biodegradable and environment friendly nature [6].

Mallotus Philippensis (Kamala tree) 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 [7]. The drug was found to be 100% effective against tapeworms. The leaves are bitter, cooling, give appetite, causes flatulence and constipation [8]. The present study investigated the anthelmintic activity of aqueous and methanolic extracts of *Mallotus Philippensis* using the earth worm model, *Ascaridia galli* and larvae of strongyle worms.

***Materials and Methods:*Plant materials** Leaves of *Mallotus Philippensis* were collected from different localities of the district of Wayanad (Kerala) during December 2012 to April 2013 and identified at MS Swaminathan Research Foundation, Kalpetta.

Extraction of plant material For the preparation of methanolic extract, the air dried and powdered plant materials (100g) were extracted using methanol in a soxhlet and evaporated to dryness using rotary vacuum evaporator and stored under refrigeration. 250 g of each plant material were mixed with 5 times water and a decoction was prepared by boiling to produce the aqueous extract. The extract was dried using rotary vacuum evaporator and stored under refrigeration.

Preparation of the extract: 200 and 100 mg/ ml of the methanolic and aqueous extracts were prepared in normal saline and were used to test the adulticidal activity whereas 25, 12.5 and 6.25 mg/ml were used to test the larvicidal activity.

Phytochemical analysis: Qualitative phytochemical analysis was done using color reactions and on TLC plate [9].

Evaluation of the *invitro* anthelmintic activity: Adulticidal activity *Ascaridia galli:* *Invitro* anthelmintic activity of the plant materials were evaluated as per the method [10] with necessary modifications. The adult live and actively motile *Ascaridia galli* worms were collected from the GI tract of chicken slaughtered in the local slaughter houses and transferred to petri dishes containing the plant extracts and normal saline. For each extract, seven petri dishes were used; six for the extracts to be tested and one for control (normal saline). Each petridish contained 2 worms and experiment was done in triplicate. Observations were made every 15 minutes till the fifth hour of the experiment and the number of worms paralyzed/ dead were counted and compared with the normal.

Earth worm model: Indian earth worms, *Pheretima posthuma* which resemble the intestinal round worm parasites were used to assess the anthelmintic activity. The procedure was repeated as in the case of *Ascaridia galli.*

Larvicidal activity: Culturing of Third Stage Larvae Fresh fecal samples were collected from the

rectum of goats maintained in the Sheep and goat farm of College of Veterinary and Animal Sciences, Pookode and incubated at room temperature in vermiculties for 10 days. The third stage infective larvae were recovered and twelve larvae in 3 replicates were used for assessing the larvicidal activity [11].

Assessment of the Larvicidal activity: The larvicidal activity was determined in 96 well microtitre plate in 3 replicates for each concentration. Twelve strongyle larvae of similar size were placed in each well and the extracts were then poured into the well. Mortality of the larvae was recorded every hour till the 7th hour of experiment. Normal saline was kept as control. The complete loss of motility/ death was the end point of the experiment [11]. The results were statistically evaluated through ANOVA and Tukeys test using SPSS 21 for windows.

Results:

Table 1: Phytochemical constituents of the methanolic and aqueous extracts of *Mallotus Philippensis*

Phytochemical constituent	Alcoholic extract	Aqueous extract
Steroid	-	-
Alkaloid	+	-
Tannins	+	+
Flavonoids	+	+
Glycosides	+	+
Phenolics	+	+
Diterpenes	+	-
Triterpenes	+	+
Saponins	-	+

Tannins, flavonoids, glycosides, and phenolics were present in all the extracts and none of the extracts showed the presence of steroids.

Table 2: Adulticidal activity of aqueous and methanolic extracts of *Mallotus Philippensis* on *Ascaridia galli* and *Pheretima posthuma*

Treatment	Dose (mg/ ml)	*Ascaridia galli*		*Pheretima posthuma*	
		Time taken for		Time taken for	
		Paralysis (min)	Death (min)	Paralysis (min)	Death (min)
Aqueous	200	94.5±0.69^{b}	192.16 ± 1.87^{c}	10.33±0.76^{e}	24.16±1.06^{g}
	100	180.5± 2.07^{c}	193.33±1.30^{c}	23.66±0.96^{g}	32.5±1.14^{h}
Alcoholic	200	141.33±1.44^{bc}	180.33±1.59^{c}	15.16±1.06^{f}	30.00±1.05^{h}
	100	170.83± 1.84	231.16±1.67^{d}	21.0±0.78^{g}	30.67±0.77^{h}
Ivermectin	0.1	20.66 ± 0.69^{a}	48.00±1.05^{ab}	9.33±1.21^{e}	14.88±0.54^{f}
	0.05	85.67±0.99^{b}	122.66±1.46^{bc}	12.33±0.87^{e}	16.67±0.68^{f}
Normal saline	……	……..	……...	……...	……

Table 2 shows the effect of exposure of *Ascaridia galli* and *Pheretima posthuma* to the aqueous and alcoholic extracts of *Mallotus Philippensis*.

The aqueous extract displayed significant anthelmintic activity in a dose dependent manner giving shortest time for paralysis and death with 200 mg/ml concentration for both *Ascaridia galli and Pheretima posthuma*. The alcoholic extract took more time for causing paralysis and death and ivermectin did the same at 21 min and 8 minutes and 48 and 15 minutes respectively for both *Ascaridia and Pheretima posthuma.*

Table 3 shows the rate of mortality of larvae after treatment with different concentrations of *Mallotus Philippensis* and Ivermectin with in a period of 7 hrs. The larvae treated with the aqueous and alcoholic extract @ 25mg/ml showed substantial mortality by third hour itself whereas in lower doses mortality occurred by fourth hour of treatment

Table 3: Mortality rate (%) of larvae treated with different concentrations of *Mallotus Philippensis*

Extract	Concentration (mg/ ml)	Time (in hrs) taken for mortality of larvae							
		0	1	2	3	4	5	6	7
Aqueous	25	0	0	30	66	100	100	100	100
	12.5	0	0	0	0	91	100	100	100
	6.25	0	0	0	0	75	100	100	100
Alcoholic	25	0	0	0	50	58.3	91	100	100
	12.5	0	0	0	50	50	91	100	100
	6.25	0	0	0	0	41	58.3	83.3	83.3
Ivermectin	0.08	0	100	100	100	100	100	100	100
Normal Saline	…….	0	0	0	0	0	16	33.3	33.3

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Discussion: Anthelmintics are drugs that are used to destroy the infesting helminthes in human beings and animals without causing any harm to the host. Phenolic compounds, tannins, flavonoids, saponins and alkaloids have been implicated in the pharmacological activities of medicinal plants including the anthelmintic activity [12].The presence of active phytochemicals like phenolics, tannins and flavonoids contribute to the anthelmintic activity of the extracts of *M. phillippensis*. Polyphenolics, flavonoids, glycoflavones, tannins etc affect the metabolism as well as cuticle of the nematodes [13]-[14]-[15] Various reports are available on the effect of phytochemicals on inhibition of nematode larval development and egg hatching. The crude methanolic extract of *Ziziphus mummularia* bark and *Acacia nioltica* fruit demonstrated good anthelmintic activity against nematodes of sheep [16]. Papaya latex was shown to have anthelmintic activity *invitro* and *invivo* [17]-[18]. The aqueous extracts of neem leaf, stem and root barks reduced the hatching of eggs and survival of larvae of strongylid nematodes of goats there by showing potent anthelmintic property [19].
The novel phytochemicals with anthelmintic property with new mechanism of action will be highly efficacious especially in the scenario of severe resistance to the currently available anthelmintics (Tariq *et al.,* 2009)[18].The findings of the study will contribute to the development of an effective natural anthelmintic.

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Assistant Professor, Department of Veterinary Parasitology,
e-mail: mnpriyar@yahoo.com
Research Assistant, Department of Pharmacology & Toxicology,
e-mail: drsreeshitha@gmail.com
RResearch Assistant, Department of Pharmacology & Toxicology,
e- mail: sreedeviramachandran2007@gmail.com
Assistant Professor, Department of Pharmacology & Toxicology,
e-mail: sujith@kvasu.ac.in
Assistant Professor, Department of Veterinary Parasitology,
e-mail: deepack@kvasu.ac.in
Assistant Professor, Department of Pharmacology & Toxicology,
e-mail: sujavet@gmail.com,
Assistant Professor and head, Department of Pharmacology & Toxicology,
e-mail: sanis@kvasu.ac.in
College of Veterinary & Animal Sciences, Pookode, Wayanad, Kerala-673576.