e- ISSN: 2394-5532
p- ISSN: 2394-823X
General Impact Factor (GIF): 0.875
International Journal of Applied And Pure Science and Agriculture
www.ijapsa.com

Effect of Mallotus Phillipensis Flower Extracts against the Third Stage Larvae of Haemonchus contortus

Deepa, C.K[1]., Darsana, U[2]., Sujith, S[3] Priya, M. N.[4] and Juliet S[5]

[1,4]Assistant Professor, Department of Veterinary Parasitology, College of Veterinary & Animal Sciences, Pookode, kerala-673576
[2]Research Assistant, Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Pookode, Kerala-673576.
[3,5]Assistant Professor, Department of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Pookode, kerala-673576.

Abstract

The anthelmintic activity of methanolic, aqueous and hydroalcoholic extracts of flower of Mallotus phillipensis was assessed invitro using larvicidal activity on L3 stage larvae of Haemonchus contortus. Phytochemical analysis was done using standard techniques and the acute oral toxicity of the extracts were studied in rats as per OECD guidelines 420. Freshly collected faeces from infected goats were cultured using modified Veglia's method and the larvae were collected after 12 days for performing larvicidal activity. Extracts were diluted using distilled water to provide final concentrations of 50, 25, 12.5 and 6.25 mg/ml and Albendazole and ivermectin served as positive control whereas distilled water served as negative control. Phytochemical analysis revealed the presence of phenolics, tannins and saponins in all extracts and the effect of the extracts could be due to these components. The methanolic extract caused death of larvae even at 6.25 mg/ml whereas the aqueous extract showed activity only at 50 mg/ml. The larvae showed initial increase in wriggling movements and then showed contractions and death. None of the extracts showed any toxicity reaction during the entire 14 days of observation. From the study it could be found that the methanolic extract of flower of Mallotus phillipensis possess potent anthelmintic property and the isolation of molecule from the same can lead to the synthesis of a novel anthelmintic.

Keywords: Anthelmintic, mallotus phillipensis, Larvicidal activity

I. INTRODUCTION

Mallotus philippensis (Lam.) Muell. Arg (Euphorbiaceae) are shrubs or small trees which grow on mountain slopes or valleys, limestone hills or river valleys and forests at an altitude of 300–1600 m in Asia and Australia. Different parts of the plant have been used in traditional medicine. Kamala, a red powder consisting of glandular hairs from plant capsule has been used as anthelmintic and cathartic in traditional medicine [1,2] and an orange dye for silk [3]. Kamala is commonly administered in its curd form for the elimination of intestinal worms and also for skin irritation, ringworm, and freckles [4]. The fruit of the plant is purgative for animals [5].

The therapy for helminthosis include use of chemicals and rotation of the available drugs. Most of the present day anthelmintics viz, benzimidazoles, macrocyclic lactones and imidazothiazoles show development of resistance worldwide, which may be for a single class or multi drug resistant and hence an alternate has to be found out. Herbal agents form a better solution as they are non toxic and economically viable and so research on the anthelmintic activity of medicinal plants has got a great drive [6]. Plants contain various phytochemicals that can inhibit or kill parasites and the identification of such

phytochemicals can be an answer for the long lasting problem of anthelmintic resistance. Hence, the present study investigates the larvicidal activity of the extracts of leaves of *Mallotus phillipensis* on the third stage larvae of *Haemonchus contortus.*

II. MATERIALS AND METHODS

2. 1. Plant material

2.1.1 Plant Material

The flowers of *Mallotus phillipensis* was collected from different parts campus of college of Veterinary & Animal Sciences Pookode identified and authenticated by a Botanist at MSSRF, Kalpetta. The collected leaves were cleaned, dried under shade and pulverized in a pulveriser without over heating. The crude powder was made into thimbles and extracted using methanol in soxhlet extraction apparatus, dried using a vacuum evaporator and stored under refrigeration till further use. The aqueous extract was taken as a decoction.

2.1.2Phytochemical Analysis

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

2.1.3Identification of the larvae

The larvae were identified based on the morphometric studies as well as molecular methods described. [7]

2.2.Assessment of the Anthelmintic activity

2.2.1 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. 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 *V. negundo* 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 50, 25, 12.5, 6.25, 3.125, 1.1.5625 and 0.78 mg/ml in a total volume of 0.2 ml. The loss of motility of the larvae were checked every 15 minutes and the % larvae found non-motile/ dead were calculated[8].

2.3.Assessment of the acute oral toxicity

The acute oral toxicity of all the extracts tested were done in rats at the dose of 2000mg/kg as per OECD guidelines 420.

III. RESULTS

3.1 Percentage yield of the extract

The aqueous extract yielded 18% , methanolic extract provided an yield of 14% where as the hydroalcoholic extract yielded 21%.

3.2 Phytochemical analysis

The results of the phytochemical analysis of the different extracts are shown in table 1.

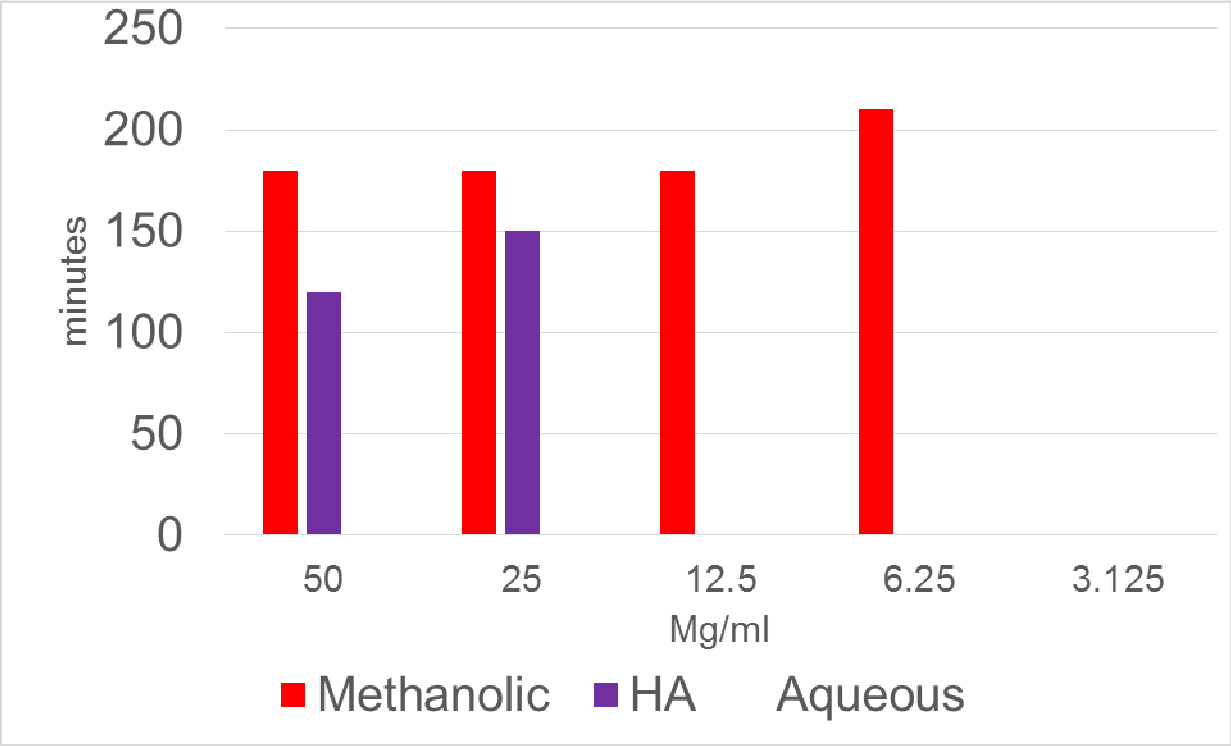
Table 1. Phytochemical constituents of extracts of *M. phillipensis*

Extract	Phenolics	Alakloids	Steroids	Glycosides	Tannins	Terpenes	Saponins	Flavanoids
Aqueous	+	-	+	+	+	+	+	+
Methanolic	+	+	+	-	+	+	-	+
Hydroalcoholic	+	+	+	+	+	+	+	+

Phenolics, steroids, tannins, terpenes and flavonoids were present in all the extracts where as alkaloids were absent in aqueous extract and glycosides and saponins were absent in methanolic extract.

3.3. Larvicidal activity

Fig 1. **.** Effect of the various extracts of flower of *Mallotus phillipensis* on the L3 larvae of Haemonchus contortus



The methanolic and hydroalcoholic extracts inhibited the activity of the larvae and caused their mortality. Even though the hydroalcholic extract at the dose of 50mg/ml caused complete mortality of the larvae in 120 minutes, the activity decreased with dose and no activity was found from the dose rate of 12.5 mg/ml. The methanolic extract inhibited the movement of the larvae even at 6.25mg/ml causing their death and hence was found to be more potent than the hydroalcoholic extract.

3.4 Acute oral toxicity

The rats kept for the assessment of acute oral toxicity did not show any visible or clinical signs of toxicity or discomfort, showing that the extract was nontoxic at the dose rate of 2000 mg/kg.

IV. DISCUSSION

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 [9] *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 [10].

In the present study, it is seen that the methanolic extract possess anthelmintic activity at large dose range where as the hydroalcoholic extract possess good activity at very high doses only. The

activity of the extracts can be due to the presence of flavonoids, terpenes, phenolic compounds, tannins etc. These phytochemicals have been proven to act on various metabolic pathways of the larvae disrupting their homeostasis.

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 affect the energy utilization mechanism of the larvae by inhibiting the oxidative phosphorylation and they starve for energy required for survival[11]. The presence of flavonoids in the plant extracts affect the moulting as well as the survival of various larvae and potentiates the activity of various other drugs, chemicals etc.[12, 13, 14]. saponins are normally ascribed due interact with the cell membranes, causing changes in cell membranes, cell wall permeability and they also interact with the collagen proteins from the cuticle of nematodes [15].

From the study it could be concluded that the flower of *Mallotus phillipensis* contains phytochemicals, especially phenolics and tannins which can be isolated and used for the development of a novel anthelmintic.

BIBLIOGRAPHY

[1]. Satyavati G. V, Gupta K. A and Tandon N .1987. *Medicinal Plants of India Volume 2. New Delhi: Indian Council of Medical Research*: 201-206.

[2]. Gupta A K, Chauhan J. S. 1984. Constituents from the stem of *Bauhinia variegate*. *Nat Acad Sci Lett* .,**7**:15-16.

[3]. Lounasmaa M, Widen C. J, Tuuf C. M and Huhtikangas A. 1975. On the phloroglucinol derivatives of *Mallotus philippinensis*. *Planta Med.,* **28:**16-31.

[4]. Usmanghani K, Saeed A and Alam M . T. 1997. Indusynic Medicine., Karachi: *Research Institute of Indusyunic Medicine***:** 285-287.

[5]. Zabihullah O, Rasheed A and Akhter N. 2006. Ethnobotanical survey in Kot Manzaray Baba valley Pakistan**.** *J Plant Sci*., **12**: 115-121.

[6]. Iqbal Z, Lateef M, Ashraf M and Jabbar, A. 2004. Anthelmintic activity of Artemisia brevifoliain sheep. *J. Ethnopharmacol*., **93:** 265–268

[7]. Coles, G. .C.; Jackson, F.; Pomroy, W.E.; Prichard, R.K.; von Samson-Himmelstjerna, G.; Silvestre, A.; Taylor, M.A.; and Vercruysse, J. 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* **136** : 167–185

[8]. Rahman, W.A., Lee, R. and Sulaiman, S.F. 2011. Invitro Anthelmintic activity of Neem Plant *(Azadirachta indica)* extracts against third stage *Haemonchus contortus* larvae from goats. *Global veterinaria* **7(1):**22-26.

[9]. Githiori J.B Athanasiadou, S and Thamsborg, S.M 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet. Parasitol.*,**139:** 308–320

[10]. Priya, M.N.; Sreeshitha, S.G.; Sreedevi, R.; Sujith, S.; Deepa, C.K.; Suja R.S. and Juliet, S. 2014. Anthelmintic activity of different extracts of *Mallotus phillipensis invitro. Life Sci. Int. Res J*. **1:** 152-155

[11]. Athanasiadou S, I.; Kyriazakis, F.; Jackson and Coop, R.L. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. *Vet Parasitol.* **99:** 205-219.

[12]. Hrčkova, G and Velebný, S. 2010. Flavonoid silymarin potentiates antihelmintic effect of praziquantel via down-regulation of oxidative stress and fibrogenesis in the liver. *Proceedings of the World Medical Conference* 250-257.

[13]. Azando, E.V.B; Hounzangbe-Adote, M.S.; Olounlade, P.A.; Brunet, S and fabre, N. 2011. Involvement of tannins and flavonoids in the invitro effects of *Newbouldia laevis* and *zanthoxylum zanthoxyloides* extracs on the exsheathment of third stage infective larvae og gastrointestinal nematodes. *Vet. Parasitol*.**180**: 292-297.

[14]. Williams, A.R., Ropiak, H.M., Fryganas, C., Desrues, O., Muller-Harvey, I and Thamsborg, S.M. 2014. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of *Oesophagostomum dentatum*. *Parasites vectors* **7**: 518-530.

[15]. Hernandez-Villegas, M.M., Borges-Argaez, P., Rodriguez-Vivas, R.I., Torres-Acosta, J.F.J., Merndez-Gonzalez, M. and Cacers-Farfan, M. 2011. Ovicidal and larvicidal activity of the crude extracts from Phytolacca icosandra against Haemonchus contortus. Vet. Parasitol. 179: 100-106.

