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Invitro ovicidal activity of *Allophyllus cobbe* leaf extracts against *Haemonchus contortus*

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Abstract

The anthelmintic activity of methanolic, aqueous and hydroalcoholic extracts of Allophyllus cobbe was assessed invitro using egg hatch assay. Fresh ova of Haemonchus contortus from infected goat were concentrated using faecal flotation method and were washed with normal saline to remove the debris. 100µL of the washing containing approximately 100 ova were transferred to a 6 well plate and equal volume of extracts were added at dilutions of 50, 25, 12.5 and 6.25 mg/ml. Albendazole and ivermectin at dose rates of 1 and 10 mg/ml served as positive control where as normal saline acted as negative control. They were incubated at 28ºC for 48 hours and the number of dead, live or hatched larvae and ova were counted under the microscope to assess the percentage of hatching. The phytochemical analysis of the extracts were done using standard protocols as well as the acute oral toxicity was assessed at the dose rate of 2000mg/kg in rats. The alcoholic extract at dose rates of 50, 25 and 12.5 mg/ml completely inhibited the hatching of ova where as the extract in the dose of 6.25 mg/ml produced only 50% inhibition. The ova were found disintegrated in the treatment groups with 50 and 25 mg/ml and also with the control drugs. None of the extracts showed any toxic reactions during the entire period of observations. From the study it could be concluded that the methanolic extract of Allophyllus cobbe showed potent anthelmintic property and the further isolation and characterization of the extract could prove a lead for the synthesis of a novel anthelmintic molecule.

Keywords: Anthelmintic, Allophyllus cobbe, Egg hatch assay, Oral toxicity

I. INTRODUCTION

Prevalence of parasitic helminthes, especially gastrointestinal nematodes are 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[1]. 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]. Most of the present day anthelmintics viz, benzimidazoles, macrocyclic lactones and imidazothiazoles show development of resistance world wide, which may be for a single class or multi drug resistance. [4,5]. 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[6,3]

Allophyllus cobbe which belongs to the family sapindacea has got strong ethnopharmacological activities and are being used in the treatment of bones, fractures, wounds etc and has been shown to have potent antibacterial property [7]. The present investigation was carried to investigate the effect of various extracts of *A. cobbe* leaves against the ova of *Haemonchus contortus*

II. MATERIALS AND METHODS

2. 1. Plant material

2.1.1.Plant Material

The leaves of *Allophyllus cobbe* was collected from different parts of the District of Wayanad, identified and authenticated by a Botanist at MSSRF, Kalpetta, were dried under shade and pulverized. They were 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.

2.1.2. Phytochemical Analysis

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

2.1.3. Identification of the larvae

The larvae were identified based on the morphometric studies as well as molecular methods described. [5]

2.1.4. Assessment of the Anthelmintic activity

Egg Hatch Assay

Fresh ova were collected from 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 *A. cobbe* were used for the study. Albendazole and Ivermectin were used as positive control where as 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 (Tarson) and were added with 0.5 ml of the extract ad described earlier. The effective concentration of the drug in each well 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) [8,9,10]

2.1.5. 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 yeilded 17% , methanolic extract providedan yield of 12% where as the hydroalcoholic extract yielded 19%.

3.2 Phytochemical analysis

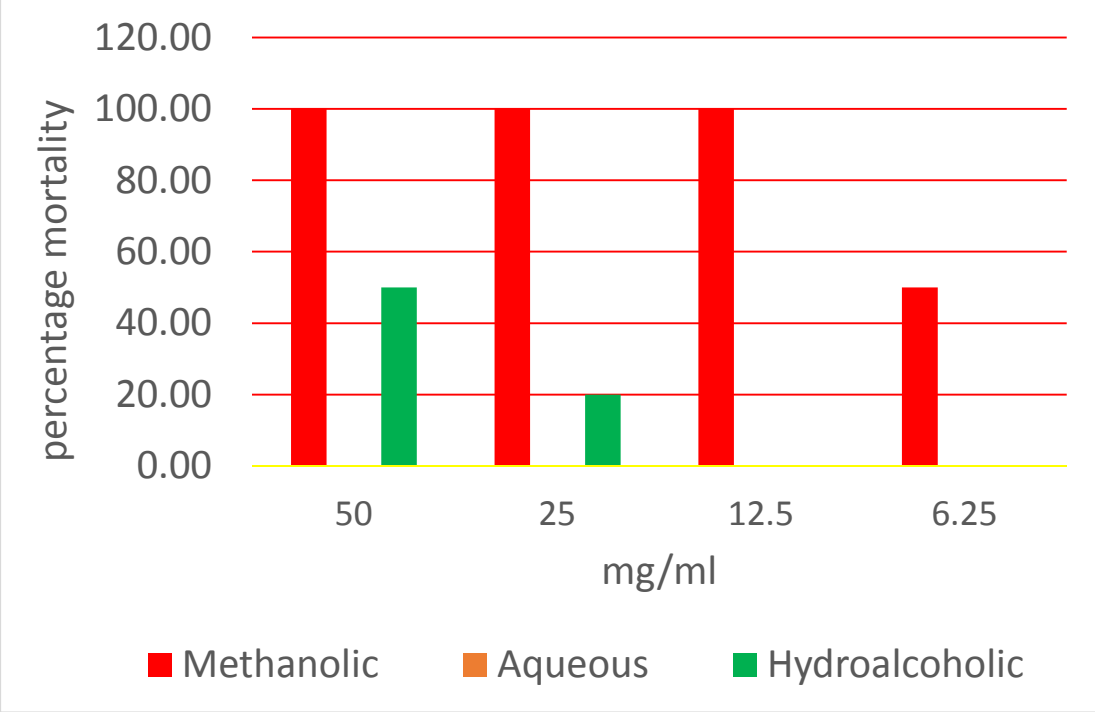
The results of the phytochemical analysis of the different extracts are shown in table 1.

Constituent	Phenolics	Alkaloids	Steroids	Glycosides	Tannins	Terpenes	Saponins	Flavonoids
Aqueous	+	-	+	-	+	+	+	+
Methanolic	+	-	-	+	+	+	+	+
Hydroalcoholic	+	-	-	-	+	+	+	+

All the three extracts tested were positive for the presence of phenolics, tannins, terpenes, saponins and flavonoids. The anthelmintic activity present in the extracts may be due to the presence of these compounds.

3.3 Egg hatch Assay

Fig 1. **. Percentage mortality of *H. contortus* ova on exposure to various extracts of *A. cobbe*.**



From the figure, it is evident that the aqueous extract has got no potential activity against the ova where as the methanolic extract has got very potent activity with 100% mortality of the ova even at a concentration of 12.5 mg/ml. The hydroalcoholic extract produced only 50% mortality even at higher

doses which indicate that the concentration of the various phytochemicals in the methanolic extract is much high when compared to the other extracts.

3.4. Acute oral toxicity

No symptoms of any toxicity was noticed in any of the extracts during the entire period of observation.

IV. DISCUSSION

Since time immemorial, plants formed the part of therapy against helminth infections of both humans and animals. Later on, chemicals were used for the therapy which included phenothiazines, benzimidazole, macrocyclic lactones and levamisole. But, the rapid emergence of resistance among the helminthes pose a great risk to the livestock industry. Screening of anthelmintic activity is mainly through invitro tests including larval and adult paralysis/ death, egg hatch assays or motility and biochemical tests [2] Invitro tests using the larvae of *Haemonchus contortus* is considered to be one of the best means of screening drugs for anthelmintic activity [10]

The aqueous, hydroalcoholic, methanolic extracts of *Allophyllus cobbe* was subjected to the egg hatch assay. Eventhough the phytochemical constituents were almost similar in all the three extracts, the methanolic extract was seen to be the most potent one with ovicidal activity even at 6.25 mg/ml where as the hydroalcoholic extract did not produce the same effect. The ova at higher concentration were found to be disintegrated where in very low doses, the ova were seen embryonated and dead or they hatched and the larvae were found dead. This showed a dose dependent effect. The control drug albendazole showed both ovicidal activity at the doses tested and almost all the ova were found disintegrated. .

Studies in our laboratory has confirmed that presence of phenolics, tannins and flavonoids contributed to the anthelmintic activity of the extracts of *Mallotus phillipensis, Azadirachta indica*, *Murraya koenigii* and *Ocimum sanctum* against *Ascaridia galli* [11,12]. The presence of saponins and tannins in the leaf extracts of *Parkia biglobosa* 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 [14]. The anthelmintic activity of tannins in *K. sengalensis* resides in the tannins as they have the capacity to bind to proteins and inactivate many mechanisms including the nutrient availability of the larvae [15]. This may impair vital process like feeding, reproduction of the parasite and disrupt the integrity of the cuticle [2]. 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 [16] From the study it could be concluded that *A. cobbe* possess good anthelmintic activity. 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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