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Research Article

Effect of Various Extracts of *Ocimum sanctum* and *Vitex negundo* on *Gastrothylax crumenifer*

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ABSTRACT

The anthelmintic activity of methanolic, aqueous and hydro-alcoholic extracts of the leaves *Ocimum sanctum* and *Vitex negundo* and the hexane, chloroform and n-butanol fractions of methanolic extract were investigated on the trematode, *Gastrothylax crumenifer*. Adult motility assay was used in the study and the results were compared with the standard drug, oxyclosanide. The study was conducted at six different dilutions of extracts viz. 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml prepared in tyrodes solution. The Minimum Inhibitory Concentration of various extracts on *Gastrothylax crumenifer* was calculated by using the serial dilution technique. The phytochemical analysis of the extracts were done and the acute oral toxicity was assessed in rats. The methanolic extract of Tulsi as well as its chloroform fraction showed maximum potency with MIC of 1.56 mg/ml and the extracts of *Vitex negundo* were not as potent as Tulsi. On gross examination, the extract treated worms showed shrinkage, loss of motility and were dead in a dose dependent manner. Histopathology of the extract treated amphistomes showed damage to the syncytium and subsyncitium as well as parenchymal cells indicating the activity on the tegument. The extracts showed the presence of flavonoids, tannins and phenolics which may be the cause of anthelmintic activity,producing damage of tegument. None of the extracts showed any toxicity reactions in rats, hence *Ocimum sanctum* can be a lead for synthesis of a new trematodicidal drug.

Keywords: Anthelmintic, *Ocimum sanctum*, *Vitex negundo*, *Gastrothylax crumenifer*

INTRODUCTION

In a developing country like India, buffaloes deliver food self-sufficiency to poor rural farmers by providing milk, meat, skin, manure and traction. Due to the grazing nature of buffaloes in wet lands they are very vulnerable to various parasitic diseases. Armphistomosis is one of the economically considerable problem affecting these livestock industry by reducing the production [1]. These amphistomes impart serious pathogenesis especially the immature stages which are embedded in the mucosa and are plug feeders, drawing pieces of mucosa in to suckers causing necrosis and haemorrhages [2]. Chemotherapy is the widely accepted method to cure this infection, but high cost of the drugs and development of resistance are the problems to the poor farmers. Traditional plant based eco-friendly medicines offer an alternative to overcome some of these problems and are having high percentage of cure with a single therapeutic dose [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, hepatoprotective, expectorant, anti-inflammatory and analgesic activities [5] It contains eugenol, monoterpens and diterpenes which add to the medicinal properties [6]. *Vitex negundo* is a large shrub grown throughout India and has many medicinal properties which is made use of in the Indian systems of medicine. There are several reports on the anti-inflammatory, analgesic, antioxidant, antimicrobial and antifungal properties of the various extracts of the plant[7]. The present study investigates the adulticidal activity of *Ocimum sanctum* and *Vitex negundo* against *Gastrothylax crumenifer*.

MATERIALS AND METHODS

Plant Material

Collection and preparation of the extract

The leaves of *Ocimum sanctum and Vitex negundo* were collected from the campus of College of Veterinary & Animal Sciences, Pookode, 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. The aqueous extract was taken as a decoction. The hydroalcoholic extract was taken as a 1:1 combination of methanol and water in soxhlet extraction apparatus. All the extracts were stored under refrigeration after drying.

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 sored under refrigeration till further use.

Phytochemical Analysis

The extract as well as the fractions was analyzed qualitatively for various phytochemical constituents [8].

Assessment of Amphistomicidal activity

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Table 1: Phytochemical Analysis

Constituents	*Ocimum sanctum*						*Vitex negundo*					
	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 *Gastrothylax crumenifer* (Duration; min)

Ocimum sanctum leaf	Concentration (mg/ml)	50	25	12.5	6.25	3.125	1.56
	Methanolic extract	30	30	30	60	60	60
	Hydroalcoholic	90	90	90	150	150	Nil
	Hexane	Nil	Nil	Nil	Nil	Nil	Nil
	Chloroform	60	60	90	90	120	120
	N-butanol	120	120	120	150	150	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil
	Aqueous	Nil	Nil	Nil	Nil	Nil	Nil
Vitex negundo leaf	Methanolic extract	120	120	120	150	150	Nil
	Hydroalcoholic	Nil	Nil	Nil	Nil	Nil	Nil
	Hexane	Nil	Nil	Nil	Nil	Nil	Nil
	Chloroform	60	90	90	90	120	Nil
	N-butanol	Nil	Nil	Nil	Nil	Nil	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil
	Aqueous	Nil	Nil	Nil	Nil	Nil	Nil

Collection of Amphistomes

Fresh amphistomes were recovered manually from the rumen of buffalo slaughtered at the Malabar meat Plant, Sulthan Bathery, Wayanad and were collected in tyrodes solution. Care was taken not to damage the sucker. They were washed and transferred to the petriplates containing extracts.

Identification of the parasite

Some fresh amphistomes after washing were pressed between two slides, tied with twine and transferred to 10% formaline solution for 2-4 days. Then these amphistomes were put in carmine staining. After few days these were destained with 1% acid alcohol, dehydrated in ascending grades of alcohol and cleared in creosote, mounted in DPX for identification.

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. The negative control contained only tyrodes solution and oxyclosanide @ 10mg/ml and 1 mg/ml were kept as positive control.

Test procedure

Amphistomicidal activity was done as per [9] with minor modifications. Briefly, 6 amphistomes were placed in the extract containing petriplates and their motility/ wriggling movements were noted every fifteen minutes. Cessation of movements even on stimulation were considered as the death point. The experiments were done in triplicates and the average was taken.

Histopathology

The dead amphistomes were fixed in Bouins solution for 12 hrs and then transferred to 10% formalin for routine histopathological examination. The tissues were made into sections, stained using haematoxylin and eosin and then examined under oil immersion microscope for finding out the changes [9] .

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 of various extracts

All the extracts showed the presence of flavonoids and tannins whereas the hexane fraction of the methanolic extract of *Ocimum sanctum* showed absence of phenolics. The presence of flavonoids, tannins and phenolics could be implicated in the anthelmintic activity.

Adulticidal activity of different extracts against Amphistome

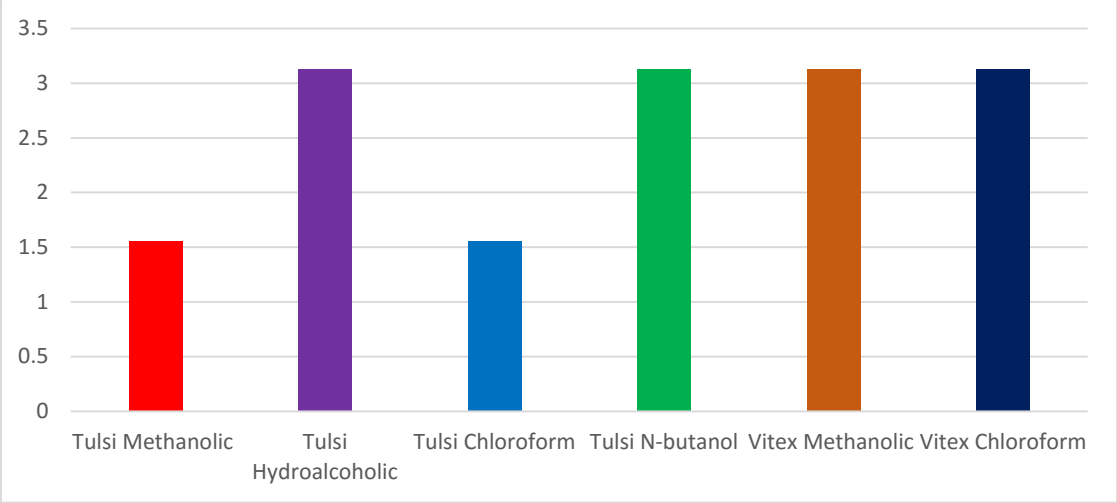

Figure. 1: Minimum Inhibitory Concentration of various extracts on *Gastrothylax crumenifer*

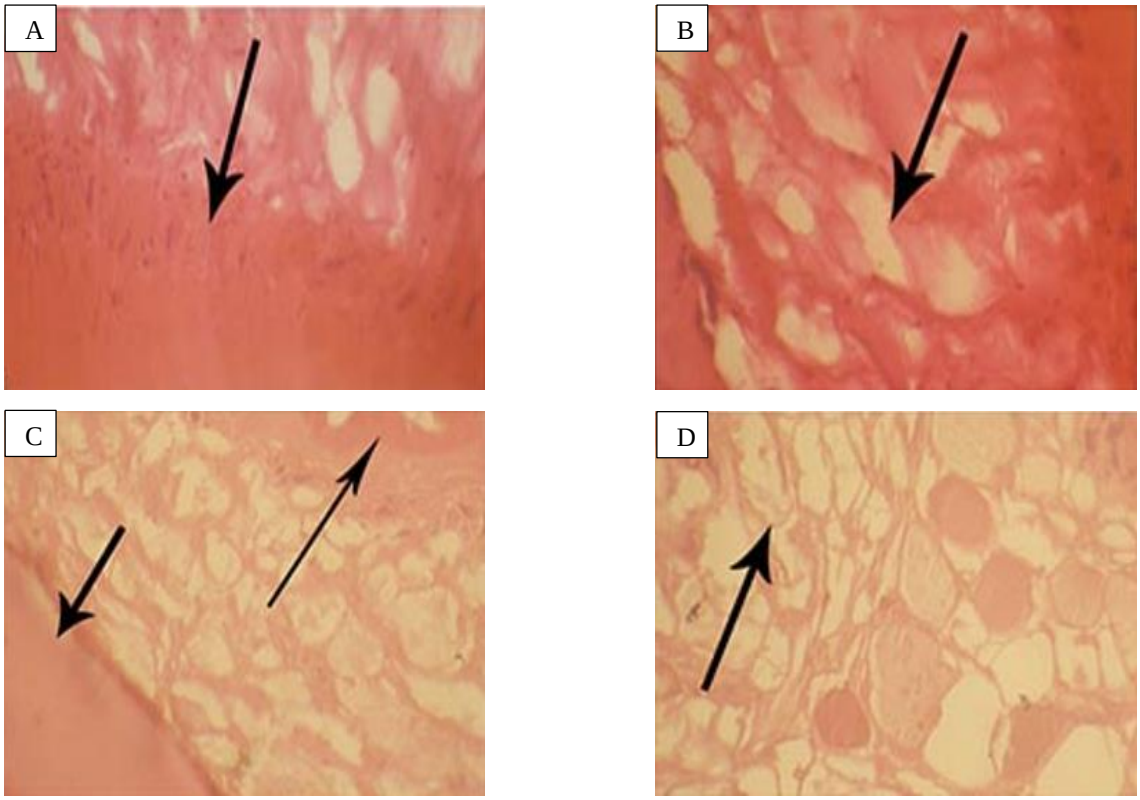

Figure. 2: Histopathology of amphistomes, A & B – Untreated Amphistomes, showing intact syncitium subsyncitial layer and parenchyma. C& D treated amphistomes showing of syncitium and rupture of parenchymatus cells.

The methanolic extract of Tulsi as well as its chloroform fraction showed maximum potency with MIC of 1.56 mg/ml. The extracts of *Vitex negundo* were not as potent as Tulsi. The positive control drug, oxyclosanide caused the death of all the worms with in 10 minutes of observation. Eventhough the activity of oxyclosanide and extracts could not be compared, the extracts could form a lead to the synthesis of a natural anthelmintic with less side effects.

Identification of the parasite

The worms showed an anteriorly opening ventral pouch extending the whole ventral surface, a posterior terminal sucker, intestinal caeca that extends upto the level of lobed horizontal testes and also showed the crossing of uterus from right to left. Thus it was confirmed that these amphistomes are *Gastrothylax crumenifer*.

Gross morphological examination

The treated worms became shrunken, paralysed and then finally died after 60 minutes in the case of methanolic extract of Tulsi and about 150 minutes in case of methanolic extract of *Vitex* in a dose dependent manner

Histopathology

The morphology of the normal and extract treated amphistomes were compared under light microscope and the extract treated worms showed detachment of the tegument and discontinuation of the syncytium (Fig 2).

The longitudinal and smooth circular muscles of the sub syncytial layer were found to be shrunken and disrupted in worms that were treated with higher dose of the extract where as only syncytial and subsyncitial changes were noticed in case of worms that were treated with lower doses. This showed a dose dependent activity on the cuticle as well as the tegument of amphistomes.

Acute oral toxicity

No mortality was detected in all groups of animals treated with the extract. Also no untoward clinical signs were noticed in any of the animals treated with the extract during the entire period of observation.

DISCUSSION

Anthelmintics are drugs that cause adverse effects on the helminths which include the effects on vital activities like feeding, neuromuscular transmission, ion exchange or on the tegument[11]. The normal phytochemicals present in the plant extracts like saponins, tannins, flavonoids and phenolic compounds act similar to the mechanisms exhibited by standard chemical anthelmintics like albendazole and fenbendazole. Saponins interact with the cell membranes, causing changes within the cell membranes, which cause changes in the cell wall[12]. Tannins have the capacity to bind to proteins impair vital process like feeding, reproduction of the parasite and disrupt the integrity of the cuticle[10]. The biochemical reactions between the condensed tannins and proline rich proteins on cuticle will interfere with the feeding and motility and other key metabolic processes like exsheathment and moulting of the parasites [13].

The effect of *Allium sativum* extracts against liver amphistomes was tested and it was found that complete paralysis of the worm occurred at 3000µg/ml[14]. The results of another study suggests that a dose rate of 1mg/ml produced moderate changes in the tegument and severe affect on muscle integrity. There were effects on the tegument which appeared blebbed and corrugated [11].

Histopathological examinations in the present study also suggests affection of the tegument and also the parenchymatous cells, which may be depicting an action similar to that of albendazole. The results were in accordance to many other similar works on amphistomes [9].

Studies in our laboratory has revealed the effect of various phytochemicals on nematodes, their ova and larvae[12, 16, 17,18,19]. The effect of these extracts on the trematodes and cestodes can be of vital significance because of the broad spectrum of activity of the drug. In era of emerging multidrug anthelmintic resistance, a drug that has evolved from natural produce that affects the system of all the parasitic helminths will be of immense therapeutic value.

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