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Research Article

In-vitro Efficacy of Various Extracts of *Murraya koenigii* Leaf Against *Gastrothylax crumenifer*

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ABSTRACT

The anthelmintic effect of aqueous, hydroalcoholic and methanolic extracts of leaf of Murraya koenigii was evaluated invitro against rumen amphistomes, Gastrothylax crumenifer. The phytochemical analysis as well as acute oral toxicity of the extracts were also assessed in rats. The methanolic extract produced mortality against amphisotmes at dose rates of 3.125 mg/ml at 60 minutes, but the fractions were not as potent as the parent extract. No toxicity symptoms were noticed in the case of any of the extracts tested. There was gross and histopathological changes in the tegument and cuticle of the amphistomes tested indicating the effect on the cuticle. From the study it could be concluded that the leaf and flowers of Murraya koenigii contains phytochemicals that have a potential to become a broad spectrum anthelmintic.

Key words: *Murraya koenigii*, Anthelmintic, *Amphistomosis* , Acute oral toxicity

INTRODUCTION

Parasitic infestation, especially gastrointestinal helminthosis is a major constraint in the livestock industry which affect the health and production of livestock. The major helminthosis include nematode infections like stronglyosis, tape worm infections and trematode infections that include amphistomosis and fascioliosis. The helminths cause economic loss through lowered work efficiency, impaired fertility, lowered milk production and heavy treatment cost with reduced efficacy. Most of the effects may be subclinical without immediate notice to the owners, but with considerable economic loss[1-3]. The control of helminthosis include strict management practices and use of anthelmintic drugs which are pure chemicals. The three broad spectrum agents include the benzimidazoles, imidazothiazoles and macrocyclic lactones. Most of these drugs have reported resistance which is wide spread and no new anthelmintics with a different mechanism of action are appearing in the market[4]. Traditional medicines provide a great source for easily available and effective therapy for helminthosis and are used in developing countries for the prophylaxis of gastrointestinal helminthosis. The World health Organization also promotes research in herbal remedies for the primary health care, for its economic viability and safety[5]. Medicinal plants contain chemicals like alkaloids, tannins, flavonoids and phenolic compounds which are the major bioactive substances and they produce definite actions on the parasite during its life cycle or body[6].

Curry leaf (*Murraya koenigii)* is an aromatic tree, belongs to the family Rutacea, grows through out India and is used as a leafy vegetable. The leaves contain free aminoacids, glucocide, alkaloids, essential oils, terpenes etc[7,8]. The anthelmintic activity of the various extracts of *Murraya koenigii* were evaluated against the Indian earth worm, *Pheretima posthuma* with encouraging results[7-9]. Studies in our laboratory also has proved the activity against *Haemonchus contortus*[10]. But no reports are there, as far as our knowledge goes, on the effect of the extracts of *M. koenigii* on trematodes. Hence the study was undertaken to screen the effect of the plant extracts on the amphistome, *Gastrothylax crumenifer*.

MATERIALS AND METHODS

Plant Material

Collection and preparation of the extract

The leaves of *Murraya koenigii* were collected from a farm in Manathavady, Wayanad, identified and authenticated by a Botanist at MSSRF, Kalpetta, were dried under shade and pulverized. Thimbles were made out of the powdered leaves and were extracted using methanol in soxhlet extraction apparatus, dried in a rotary vacuum evaporator. The aqueous extract was taken as a decoction. The hydroalcoholic extract was taken as a 1:1 combination of methanol and water as described earlier in soxhlet extraction apparatus. All the extracts were stored under refrigeration after drying.

Phytochemical Analysis

The extract as well as the fractions was analyzed qualitatively for various phytochemical constituents[11].

Assessment of Amphistomicidal activity

Collection of Amphistomes

Fresh amphistomes were recovered manually from the rumen of buffaloes slaughtered at the Malabar meat Plant, Sulthan Bathery, Wayanad immediately after slaughter and were transferred into prewarmed (upto body temperature) tyrodes solution. Care was taken during the collection of the amphistomes to have the sucker intact.

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Table 1. Phytochemical analysis of the aqueous, methanolic and hydroalcoholic extracts of *Murraya koenigii*

Constituents	Extract		
	Aqueous	Methanolic	Hydro-alcoholic
Phenolics	+	+	+
Alkaloids	-	+	+
Steroids	-	-	-
Glycosides	+	+	+
Tannins	+	+	+
Terpenes	-	+	-
Saponins	+	-	-
Flavanoids	+	+	+

They were washed twice in the tyrodes solution to removed the coarse materials attached to it and transferred to the petriplates containing the extracts.

Identification of the parasite

A few of the collected 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 for 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 3.13 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[12] 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 end point of observation. The experiments were done in triplicates and the average value was taken.

Histopathology

The dead amphistomes were fixed in Bouins solution for 12 hours 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[12] .

Assessment of Acute Oral toxicity

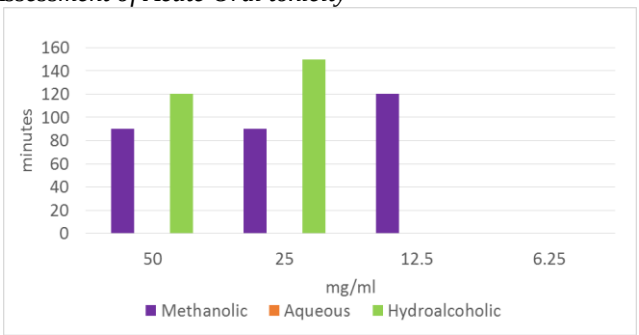

Figure 1. Effect of various extracts on the motility/ death of *Gastrothylax crumenifer* (Duration; min)

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

RESULTS

Phytochemical analysis of the extract

The methanolic and hydroalcoholic extract showed the presence of phenolics, alkaloids, glycosides, tannins and flavonoids where as steroids were absent in all the three extracts.

Adulticidal activity of different extracts against Amphistome

The methanolic and hydroalcholic extract produced mortality in the amphistomes where as no activity was noticed in the case of aqueous extracts. The methanolic extract killed the amphistomes at a doe rate of 12.5 mg/ml in 120 minutes where as the hydroalcoholic extract caused mortality only upto 25mg/ml in a duration of 150 minutes.

Histopathology

The histopathological sections of the methanolic extract treated amphistomes showed s shrinkage and condensation of the tegument and syncytium when compared to the normal control. The parenchymal cells were swollen and showed detachment (Fig 2).

The syncytium and tegument shows condensation and parenchymal cells are showing detachment.

Acute Oral toxicity

No toxicity symptoms were observed in any of the animals during the entire period of observation stating the absence of acute oral toxicity.

DISCUSSION

Gastrointestinal helminthosis is a major threat to livestock industry and the therapy includes use of pure chemicals. But continuous use of a limited range of drugs has resulted in the development of resistance among the helminths. Hence development of a novel molecule with a different mechanism of action is the need of the hour. The effect of various herbal extracts and molecules on the nematodes were demonstrated in our laboratory[13-16] and also from other laboratories[5,6,17,18], but reports on the activity on the flukes are limited[19-21].

Presence of the phytochemicals like tannins, spaonins, flavonoids and phenolic compounds are implicated in the anthelmintic activity of medicinal plants. Tannins are chemically polyphenolic compound sand many of the anthelminitcs are phenolic in nature which affect the

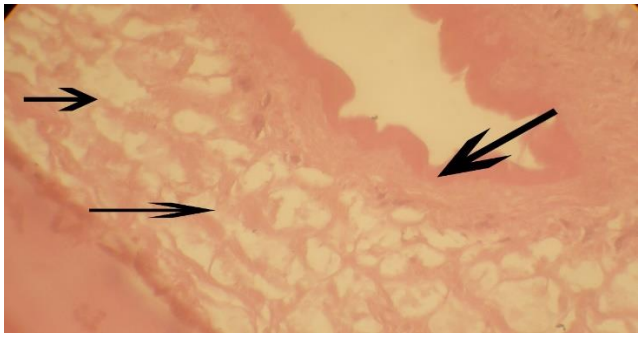

Fig. 2. Histopathology of methanolic extract treated amphistome (10X, H&E)

energy generation in the parasites by uncoupling the oxidative phosphorylation. Tannins may also bind to the glycoprotein in the cuticle of the parasite and cause death[22-24].

The histopathology of treated flukes showed changes in the syncytium, tegument and also on the parenchymatous cells which directly points to the action of the extract on the cuticle. The results are in accordance to various other studies[12,19-21]. In the light of the above study the methanolic extract on *Murraya koenigii* leaves has got broad spectrum anthelmintic property.

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