IN VITRO ANTHELMINTIC ACTIVITY OF CRUDE EXTRACTS AND ALKALOID FRACTIONS FROM *STRYCHNOS POTATORUM* L .f.

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Summary

The solvent successive crude extracts *viz*. petroleum ether, chloroform, ethanol and water from the root, stem bark, seed, and also seven indole type alkaloid fractions separated by column chromatogram from the seed of *Strychnos potatorum* L.f. (Loganiaceae) were screened for anthelmintic activity against a nematode pathogen, *Heterorhabditis indicus* Poinar *in vitro* at the concentration 1.0 mg/ml. The findings have revealed that all organic plant crude extracts and more particularly the ethanol extracts were showed significant activity (75 - 100%) in terms of observed paralysis and/or mortality of the parasite tested at room temperature (27± 2^{o} C) after 2h. Further, PB - IV fraction (diaboline), the most abundant indole alkaloid has shown considerable paralytic and/or mortality activity (≥ 50%) which is comparable to that of albendazole drug, included as positive control.

Key words: *Strychnos potatorum*, Anthelmintic activity, Loganiaceae, diaboline, alkaloid fractions.

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Introduction

Parasitic nematodes are among the most common infectious diseases of young children and grazing live stock, especially in small ruminants in the tropics and sub-tropics [1]. Reported cases of anisakidosis i.e., worm infections in man have increased recently in many countries. The most effective therapy is larval extirpation by endoscopy when the parasite is located in the digestive tract and is accessible by fibroscopy. Otherwise, the treatment is surgical, by resection of the affected region [2]. Because of the rapid escalation of anthelminitc resistance worldwide, other approaches to nematode control in developing countries are becoming urgent [3]. This has stimulated research into alternative medications, such as medicinal plants for anthelmintics that are both sustainable and environmentally acceptable.

Strychnos potatorum L.f. (Family:Loganiaceae) (English: Clearing nut; Sanskrit: Kataka/ Ambuprasada) is a medium size deciduous tree occurs both in Indian subcontinent and north-eastern part of Africa. It is one of the important medicinal plants for treating various ailments including helminthiasis both in traditional medicinal systems (Ayurveda, Unani and Siddha) and folk medicine since time immemorial [4]. An attempt therefore has been made here to provide scientific basis in this regard. Keeping this objective, the crude extracts and alkaloid fractions of seed from *S. potatorum* have been screened for their anthelmintic activity.

Materials and Methods

Collection of Plant material: The root, stem bark and seed material of *Strychnos potatorum* were collected from Karpakpalli Reserve forest, Bidar District, Karnataka, India during December 2002 and identified [5]. A voucher specimen of the same was deposited in the Herbarium, Gulbarga University, Gulbarga (HGUG-214).

Extraction of crude drugs and alkaloid fractions: Five hundred grams of the powdered plant material *viz.,* root, stem bark and seed were subjected to the Soxhlet successive extraction method (60-80^{o}C) using about 2.5 liters of petroleum ether, chloroform, ethanol (95% v/v) and distilled water in the order of increasing polarity of solvent for a period of 18 h. However, the aqueous extracts of seed were obtained by cold extraction method owing to its jelly nature. The extracts obtained were concentrated to dryness *in vacuo* at 40^{o}C and stored at 4^{o}C in the refrigerator until further use. Further, seven prominent indole alkaloid fractions ranging from PB-I to PB-VII were purified using column chromatogram coupled with preparative thin layer chromatography as described earlier [6].

Chemicals and drugs: i) 0.9% Phosphate Saline Buffer**:** 8 g sodium chloride; 0.34 g potassium dihydrogen phosphate and 1.21 g dipotassium hydrogen phosphate were dissolved in 1000 ml sterilized distilled water and adjusted pH to 7.3.

ii) Preparation of drug solutions: All the extracts were dissolved in 10% dimethyl sulfoxide (DMSO) and further mixed with phosphate saline buffer (PSB) so as to get 1 mg/ml final concentrations. Similarly, albendazole (Pfizer Ltd. Mumbai) and mebendazole were included as positive controls.

The pathogen: The nematode used for study is *Heterorhabditis indicus* Poinar, an entamopathogen pure culture maintained in the Parasitological Laboratory, Department of Zoology, Gulbarga University, Gulbarga.

***In vitro* Anthelmintic assay:** The *in vitro* anthelmintic activity was carried out as described by McGaw et al. [7]. 20 ml of the extract containing 0.9% phosphate saline buffer (1 mg/ml) was taken into an equal size Petri plate. Then, 50 – 60 *H. indicus* infectious juveniles were inoculated to each plate. Similarly, a set of plates containing 1 ml of Tween – 80 PSB inoculated with parasites was included as negative control. These plates were incubated in darkness at 27 ± 2^{o}C for 2 h. These plates were observed under Olympus binocular compound microscope (10X) for the non-motility or paralysis condition of parasites i.e., mortality or death of parasites. The experiments were realized in triplicates and the observations were recorded.

Results and Discussion

The petroleum ether, chloroform, ethanol and water extracts of the root, stem bark and seed of *S. potatorum* and also the alkaloid fractions of seed were screened for *in vitro* anthelmintic activity against *Heterorhabditis indicus* and the results obtained are recorded in the table 1.

Table 1. *In vitro* anthelmintic activity of *S. potatorum* extracts and alkaloid fractions

Drugs Used	Name of the drug (1 mg/ml)	Paralysis or mortality of *H. indicus* after 2 hours		
		Root	Stem Bark	Seed
Crude Extracts	Petroleum ether	++	++	++
	Chloroform	++	++	++
	Ethanol	+++	+++	+++
	Water	-	-	-
Alkaloids (Seed)	PB – I	+		
	PB – II	+		
	PB – III	+		
	PB – IV	++		
	PB – V	+		
	PB – VI	+		
	PB – VII	+		
Positive Control (Standards)	Albendazole	++		
	Mebendazole	++		
Negative control	Tween 80	-		

Note :

'–' = Not active (≤ 25%); '+' = Less active (≥ 25%)
'++' = Active (≥ 50%); '+++' = Highly active (75 – 100%)

All the crude extracts excluding the aqueous extracts of root, stem bark and seed of *S. potatorum* have shown moderate to significant anthelmintic activity at the tested concentration of 1 mg/ml. Of these, the ethanol extracts have displayed the most significant activity compared to the petroleum ether and chloroform. These extracts completely arrested the motility of parasite or led to the paralysis and / or death of the total parasites within an incubation period of 2 h. Whereas, the petroleum ether and chloroform extracts have shown moderate activity. The activity of extracts may be attributed to the presence of active principles like triterpenes, phenols and saponins [8].

Among the alkaloid fractions of seed tested, PB – IV, fraction (diaboline) has shown moderate anthelmintic activity by arresting the motility or death of parasite to an extent of ≥ 50%. Where as, the remaining fractions have displayed feeble activity (≥ 25%). On the other, albendazole and mebendazole, the standard drugs (positive control) showed moderate activity (≥ 50%) against *H. indicus*. This finding is as evidenced the use of this plant as anthelmintic in the traditional systems of medicine and also in the folklore practices. However, further there is a need to find out the active principles involved and also to test its efficacy *in vivo* model as well to consolidate these findings.

The significant anthelmintic activity of the ethanolic extract of root peel of *Flemingia vestita* against various intestinal nematodes was reported [9, 10]. Similar observations were made for the methanolic extract of *Butea monosperma* seed against *Caenorhabditis elegans* at the concentration of 1200 μg/ml [11]. Further, tribulosin (a steroidal saponin) and β-sitosterol-d-glucoside, compounds of *Tribulus terrastris* were observed as the active principles for displaying anthelmintic activity [12].

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