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Evaluation of Anthelmintic Activity of *Scleroderma bermudense* of Different Solvent Extracts

Ashok B Chittaragi* and Jayashree K Kodiyalmath

Research Scholars, Dept. of P. G. Studies and Research in Applied Botany, Mycology Lab, Bio-Science Complex, Jnana Sahyadri, Kuvempu University, Shankaraghatta-577451, Shimoga, Karnataka, India.

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Abstract

Objective: Development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal mushrooms as an alternative source of anthelmintics. The aim of the present study was to investigate the anthelmintic activity of the fruiting bodies of *Scleroderma bermudense* extract using adult earthworm, *Pheritima posthuma*.

Methods: The fruiting body extracts of *Scleroderma bermudense* at different concentrations of 25mg/ml, 50mg/ml and 100mg/ml were tested, which involve determination of paralysis time and death time.

Results: It was found that the extract exhibited significant dose dependent anthelmintic activity. Piperazine citrate at various concentrations of 10mg/ml as standard reference.

Conclusion: The fruiting body extracts of *Scleroderma bermudense* possess anthelminthic property. Further investigations on pharmacological activity need to be carried out to unmask its mode of action.

Keywords: *Scleroderma bermudense*; Macrofungi, Anthelmintic activity; Mushroom extracts, *Pheritima posthuma*, Western Ghats.

Contents

***Corresponding author**
Ashok B Chittaragi
Dept. of P. G. Studies and Research in Applied Botany, Mycology Lab, Bio-Science Complex, Kuvempu University, Karnataka, India
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1. Introduction

Helminthic infections continue to be major health hazard of people, especially those living in tropical developing countries. Current estimates suggest that over half of the world population is infected with intestinal helminths, such as Ascaris, hookworms, Trichuris, Enterobius, Strongyloides, and tapeworms, and that most of these infected people live in remote rural areas in the developing countries [1]. People living in poverty in developing countries often suffer from helminthic infections, which more often physically impair their hosts then kill them. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climates [2]. Besides, the continued usage of current

anthelmintic drugs is also posing a major problem of drug resistance in several parasite species as well as unwanted adverse effect such as abdominal discomfort, nausea vomiting, diarrhea, drowsiness vertigo,rashes are common and they are also contraindicated for certain groups of patients like pregnant and lactating woman [3]. Mushrooms are similar to plants which are having a great potential for the production of useful bioactive metabolites such as phenolic compounds, polyketides, terpenes, steroids, lectins, lactones, alkaloids and metal chelating agents. Polyphenols play an important role in human as preventative against several diseases, and hence they are a prolific resource for drugs [4].

Helminthiasis are infection with parasitic worms, affects over two billion people worldwide. Human beings can spread these pathogens to previously uninvolved population through travel, migration, and military operations. Worms pathogenic for human beings are Metazoa, classified into roundworms (nematodes) and two types of flatworms, flukes (trematodes) and tapeworms (cestodes). These biologically diverse eukaryotes vary with respect to life cycle, bodily structure, development, physiology, localization within the host, and susceptibility to chemotherapy. Immature forms invade human beings via the skin or gastrointestinal tract and evolve into well-differentiated adult worms that have characteristic tissue distributions. With few exceptions, such as *Strongiloides* and *Echino coccus*, these organisms cannot complete their life cycles that is they replicate themselves, within the human host. Therefore, the extent of exposure to these parasites dictates the severity of infection, and reduction in the number of adult organisms by chemotherapy is sustained unless reinfection occurs. The prevalence of parasitic helminthes typically displays a negative binomial distribution within an infected population such that relatively few persons carry heavy parasite burdens. Without treatment, those individuals are most likely to become ill and to perpetuate infection within their community [5].

Anthelmintic are drugs that either kill (vermicide) or expel (vermifuse) infesting helminthes. Helminthiasis is prevalent globally (One third of world€s population harbours them), but is more common in developing countries with poorer personal and environmental hygiene. Multiple infestations in the same individual are not infrequent. In the human body, gastrointestinal tract is the abode of many helminthes, but some also live in tissues or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasisis rarely fatal, but is a major cause of ill health [6]. There have been few studies on primary and secondary phytochemical analysis, physic-chemical properties, antifungal and antibacterial activities of *S. bermudense,* there is insubstantial information available on this wild fungi. Therefore, this study focuses on the anthelmintic activities of *S. bermudense*.

2. Materials and methods

Mushroom material

Various extracts of the experimental fruit body was prepared according to the methodology [7]. Fruit bodies of a wild macrofungi *Scleroderma bermudense* was collected from forest regions of Sringeri (T), Chikmagalur (D), Karnataka. They were harvested fresh during rainy season in the month of August to October-2013 and the mushroom species was identified as *Scleroderma bermudense* with the help of faculty of Department of Applied Botany, Kuvempu University, Shankaraghatta-577451,Shimoga (D),Karnataka.

Preparation of extract

The powdered material was subjected to Soxhlet extraction with various solvents ranging from non-polar to polar. The solvents used were petroleum ether, chloroform and methanol. Each time before extraction with next solvents the marc was air-dried. All the extracts were concentrated by rotary flash evaporator. Three different solvent extracts was selected for anthelmintic activity on the basis of biochemical screening and literature survey.

Animals

Indian adult earthworms (*Pheritima posthuma*) were used to study anthelmintic activity. The earthworms were procured from the Department of Horticulture Shimoga, Karnataka collected from moist soil and washed with normal saline to remove all faecal matter earthworms were identified in the Department of Applied Zoology Kuvempu University Shankaraghatta-577541, Shimoga district of Karnataka.

Anthelmintic activity

The anthelmintic assay was carried as per the method [8] with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [9-12]. Because of easy availability, earthworms have been used widely for the initial *in-vitro* evaluation of anthelmintic compounds [13]. Three sets (petroleum ether, chloroform and methanol extract treatment) in five groups were taken each containing six earthworms of approximately equal size (6.0 ± 1.0cm). Piperazine citrate (10mg/ml) was taken as standard drug. The fruiting body extracts of *Scleroderma bermudense* different concentrations were prepared by dissolving in minimum quantity of DMSO and making up to the final volume with normal saline to obtain 25mg/ml, 50mg/ml and 100mg/ml concentrations. One of the groups is taken as control group, which was treated with normal saline. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after

as pertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50•C. All the test solution and standard drug solution were prepared freshly before starting the experiments [14].

3. Results and Discussion

The different concentrations of various solvents viz., petroleum ether, chloroform and methanol extracts of *Scleroderma bermudense* were evaluated for anthelmintic activity using adult Indian earthworm model. The extract exhibited a dose dependent inhibition of spontaneous motility (paralysis).The chloroform extract of the *Scleroderma bermudense* showed significant anthelminthic activity compared to the other extracts. From the above study, it was observed that the chloroform extract showed dose dependent anthelmintic activity as compared to a standard drug Piperazine citrate (Table-1).

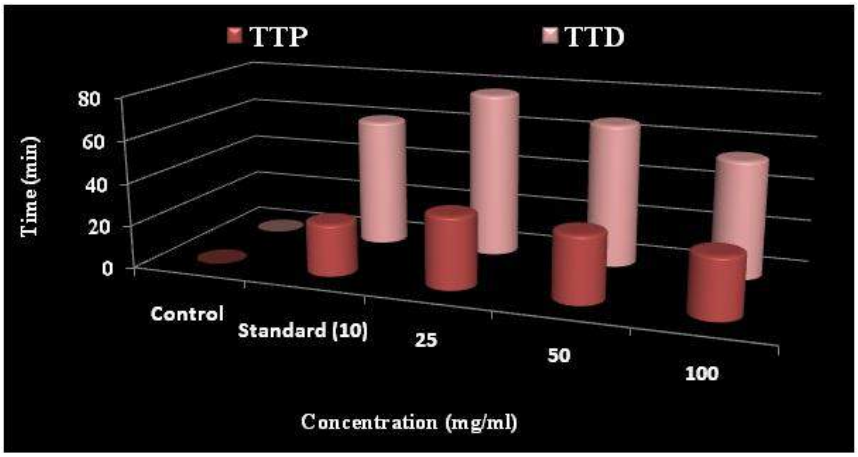
The mean paralyzing time of *Pheritima posthuma* with the concentrations 25, 50 and 100mg/ml of petroleum ether extract were found to be 33.66, 31.16 and 28 min respectively (Graph-1), whereas in chloroform extracts 31.66, 30.16 and 28.83 minutes (Graph-2). In methanol extract shows 35.16, 30.5 and 29.83 minutes respectively (Graph-3). The standard Piperazine citrate (10mg/ml) was found to be 24.5 minutes. The mean death time of *Pheritima posthuma* with the concentrations 25, 50 and 100mg/ml of petroleum ether extract were found to be 77.16, 66.83 and 54.83 minutes. Whereas, in chloroform 77.83, 70.5 and 52.66 minutes. In methanol extract shows 84.66, 74.83 and 58 minutes respectively. Death time of standard Piperazine citrate (10mg/ml) was found to be 60.5 minutes (Graph-1, 2 and 3).

Helminthic infections are among the most common infections in man, affecting a large proportion of the world€s population. Parasites have been of concern to the medical field for centuries and the helminthes still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. Consequently, the discovery and development of new chemical substances for helminthic control is greatly needed and has promoted studies of traditionally used anthelmintic mushrooms, which are generally considered to be very important sources of bioactive substances [15]. In this study, the anthelmintic activity of various solvent extracts of *Scleroderma bermudense* may be due to the presence of constituents.

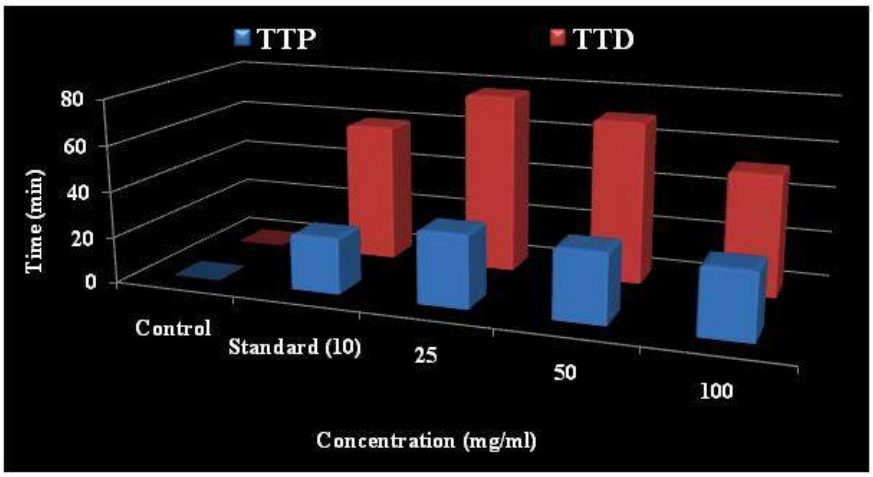
Table 1: Anthelmintic activity of *Scleroderma bermudense* of different solvents extracts

Concentration (mg/ml)	Solvent extracts					
	Petroleum ether		Chloroform		Methanol	
	TTP in minutes	TTD in minutes	TTP in minutes	TTD in minutes	TTP in minutes	TTD in minutes
Control	-	-	-	-	-	-
Standard (10)	24.5	60.5	24.5	60.5	24.5	60.5
25	33.66	77.16	31.66	77.83	35.16	84.66
50	31.16	66.83	30.16	70.5	30.5	74.83
100	28	54.83	28.83	52.66	29.83	58

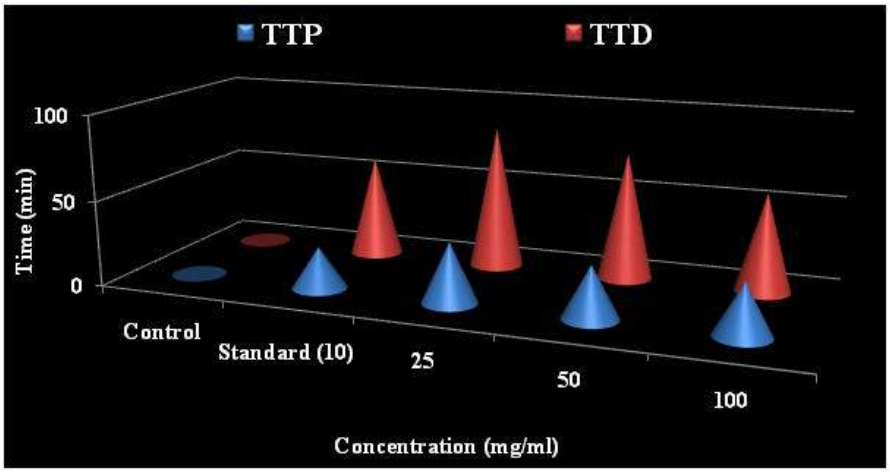
Note: TTP = Time taken for Paralysis, TTD = Time taken for Death.



Graph 1: Comparative data of paralysis time and death time in Petroleum ether extract against *Pheretima posthuma*



Graph 2: Comparative data of paralysis time and death time in Chloroform extract against *Pheretima posthuma*



Graph 3: Comparative data of paralysis time and death time in Methanol extract against *Pheretima posthuma*

4. Conclusion

The results of the present study clearly indicated that the various solvents extract of *Scleroderma bermudense* did produce anthelmintic activity against Indian earthworm *Pheretima posthuma.* The mushrooms possesses significant anthelmintic activity at 100mg/ml concentration measured by time taken for paralyse / death of the earthworms. The current investigation leads to conclusion that the fruiting body of *S.bermudense* has potent anthelmintic activity when compared with the conventionally used drug. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminthes. Further studies using *in-vivo* models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rational for the use of *S. bermudense* as an anthelmintic drug.

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