

E: ISSN 2278-4136
P: ISSN 2349-8234
JPP 2014; 3 (3): 29-32
Received: 06-07-2014
Accepted: 10-08-2014

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A comparative study on anthelmintic activity of various solvent extracts of *Clavaria rosea*

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Abstract

Helminths are the most common infections in man, affecting a large proportion of the world's population. The study includes, different solvent (Petroleum ether, chloroform, methanol and aqueous) extracts of the fruiting body of *Clavaria rosea* had been evaluated separately for anthelmintic activity on adult Indian earth worms, namely *Pheretima posthuma*. Three concentrations (25, 50 and 100 mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and the time for death of the worms. The results revealed that the petroleum ether and chloroform extract showed excellent anthelmintic activity at a concentration level of 50 and 100 mg/ml on time taken for paralysis (31 min, 34.16 min and 39.66 min, 40.66 min) and death (70.5 min, 98.5 min and 91.16 min, 114.83 min) of the worms. Activities on further comparison with the standard drug Piperazine citrate (10 mg/ml), the petroleum ether extract showed a significant effect as compared to other extracts.

Keywords: Anthelmintic activity, *Clavaria rosea*, Solvent extracts, *Pheretima posthuma*, Piperazine citrate

1. Introduction

Indian medicinal mushrooms are recognized to have great potential for preparing clinically useful drugs that, they can be used by allopathic physicians. In the present context, with the available talent in the country like pharmaceutical chemists, biotechnologist and interested allopathic physicians, made significant effort towards identification of an "active principle" of medicinal mushrooms to treat human and animal infections is a priority [1]. In this study anthelmintic activity of fruiting body extracts of *Clavaria rosea* was carried out.

Helminths infections, repeatedly entitled helminthiasis are among the most pervasive infection and a foremost degenerative disease, distressing a large proportion of the world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia [2]. The helminths parasites mainly subsist in the human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them [3].

Helminths infections are commonly found in community and being recognized as cause of much acute as well as cattle's. More than half of the population of the world suffers from worm infections [4].

Most diseases caused by helminths are of achronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Intestinal infections with worms can more easily treated than those the infections occur in other locations in the body; because the worms need to be killed by the drug and the drug need not be absorbed when given by oral route. Because of increasing anthelmintic resistance and impact of conventional anthelmintic on the environment, it is important to look for alternative strategies against gastrointestinal nematodes [5].

Chemical control of helminths coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminths [6, 7] against conventional anthelmintic is a foremost problem in treatment of helminths diseases [8, 9].

The World Health Organization estimates that a staggering two billion people harbour parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on the anthelmintic drug is sparse. According to the WHO, only a few drugs are used in treatment of helminths in humans. Anthelmintic from natural sources could play a key role in

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the treatment of these parasite infections^[10].

Helminths are the most common infections in man, affecting a large proportion of the world's population. Parasitic diseases may cause severe morbidities including lymphatic filariasis, onchocerciasis and schistosomiasis. Development of resistance to most of commercially available anthelmintic drugs became a severe problem worldwide^[11, 9].

In view of this, attempts have been made to study the anthelmintic activity of traditional medicinal mushrooms. Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal mushrooms for their anthelmintic activity.

2. Materials and Methods

2.1 Collection of Mushroom Material and Extraction

The *Clavaria rosea* were collected from Kotegudda, Koppa (T), Chickmagalur (D), Karnataka, during year 2013. The mushroom was picked from the soil surface and then they were cleaned. Identification was done by comparing their morphological, anatomical and physiological characteristics^[12]. The whole mushroom was shade-dried and coarsely powdered. The coarse powder was extracted with petroleum ether, chloroform, methanol and aqueous solvent using the Soxhlet apparatus. The extract was concentrated to dryness in vacuum.

2.2 Worm Collection

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter. Earthworms 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol.

2.3 Drugs and Chemicals

Piperazine citrates (GlaxoSmithkline), petroleum ether, chloroform and methanol (Sudha Chemicals Traders, India) was used during experimental protocol.

2.4 Anthelmintic Activity

The anthelmintic assay was carried out as per the method^[13] with minor modifications. The assay was performed on the adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance to the human intestinal roundworm parasite^[14, 15]. Due to their ready availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in-vitro*^[16-18]. The earthworms were divided into different groups, each group containing six worms. 50 ml formulations containing three different concentrations of petroleum ether, chloroform, methanol and aqueous extracts of *Clavaria rosea* (25, 50 and 100 mg/ml in distilled water) were prepared. The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The times of death of the worms were recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water (50 °C). Piperazine citrate (10 mg/ml) was used as reference standard while saline water served as a control.

3. Results and Discussion

From the results it is observed that *Clavaria rosea* shown potent anthelmintic activity while the *Pheretima posthuma* has taken long time for death (61.5 min-126.16 min) of worms.

The earthworm selected for the anthelmintic activity was most sensitive to the different solvent extracts viz., petroleum ether, chloroform, methanol and aqueous extract of *Clavaria rosea* as can be seen in Table-1. The anthelmintic activity result revealed dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death at 25, 50 and 100 mg/ml concentrations paralysis, was observed respectively at 51.83, 43.16 and 40.83 min and death at 131.5, 126.16 and 112.83 min in methanol extracts. The chloroform extracts of *Clavaria rosea* also exhibited dose dependent anthelmintic activities that caused paralysis at 43.16, 40.66 and 34.16 min (at 25, 50 and 100 mg/ml) and death at 123.66, 114.83 and 98.5 min. Same time petroleum ether extracts at 25, 50 and 100 mg/ml shows moderate paralysis 42.33, 39.66 and 31 min respectively and death time 100.16, 91.16 and 70.5 respectively (Graph-1 and 2).

The aqueous extracts of *C. rosea* also exhibited activities that caused paralysis at 25, 50 and 100 mg/ml was observed by respectively at 56.66, 52.5 and 45 mg/ml and death at 127, 122.5 and 114.5 mg/ml (Graph-1 and 2).

The standard drug (Piperazine citrate) shows paralysis within 25 min and time of death 61.5 min in all the four solvents extracts. The observation of result show that the anthelmintic activity of petroleum ether extract is more potent compare to the chloroform and methanol extract.

The earthworms were more sensitive to the extracts of *Clavaria rosea* at 50 and 100 mg/ml concentrations as compared to the reference drug Piperazine citrate (10 mg/ml). The petroleum ether and chloroform extracts were more effective in causing the death of the worms as well as promoting paralysis.

Most worm expellers like Piperazine citrate cause paralysis of the worms so that they are expelled in the faces. The petroleum ether extracts of the mushroom not only demonstrated this property but also killed the worms. The anthelmintic activity of the different solvent extracts of *Clavaria rosea* may be due to the presence of polyphenolic compounds^[19]. The wormicidal activity of the petroleum ether extract as described here in against earthworms suggests that it could be effective against parasitic infections of humans.

This may be due to the increased level of extraction of tannins in petroleum ether followed by chloroform and methanol extracts. The data presented and observations made thereof, lead to the conclusion that the different degree of helminthiasis of the different solvent extracts are due to the level of tannins present in compounds. Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators^[20-22].

Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation^[23] or, binds to the glycoprotein on the cuticle of parasite^[24] and cause death. Coming to the chemistry of nematode surface, it is a

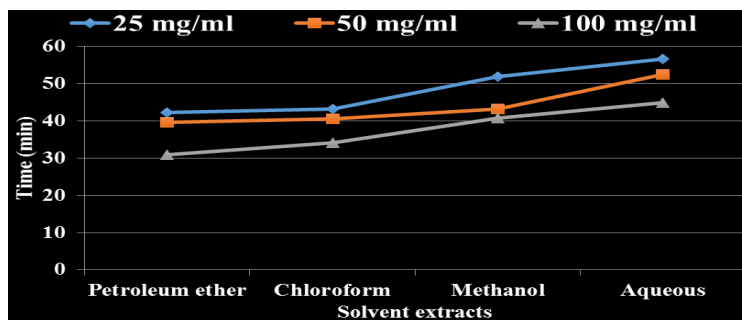
collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post-translational modification prior to assembly into higher order complexes (or) ECMS [25]. The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres. This results in the loss of flexibility in the collagen matrix and

gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannin of *Pheretima posthuma*, possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. Hence further investigation and proper isolation of the active principles might help in the findings of new lead compounds, which will be effective against various parasitic infections.

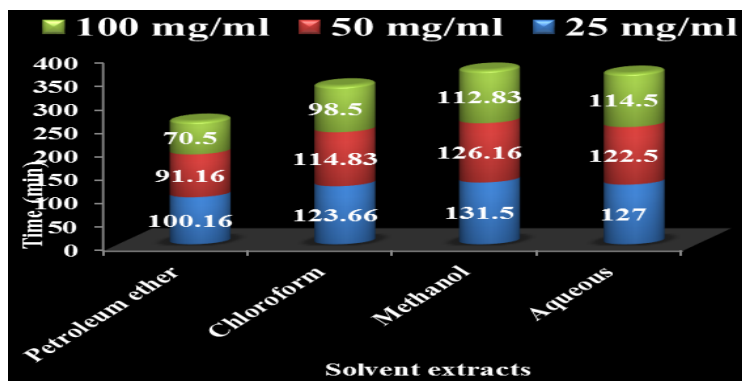
Table 1: The Anthelmintic activity of *Clavaria rosea* extracts

Group	Treatment	Concentrations (mg/ml)	<i>Pheretima posthuma</i>	
			Paralysis time (min)	Death time (min)
1	Control	-	-	-
2	Standard	10	25	61.5
3	Petroleum ether extract	25	42.33	100.16
		50	39.66	91.16
		100	31	70.5
4	Chloroform extract	25	43.16	123.66
		50	40.66	114.83
		100	34.16	98.5
5	Methanol extract	25	51.83	131.5
		50	43.16	126.16
		100	40.83	112.83
6	Aqueous extract	25	56.66	127
		50	52.5	122.5
		100	45	114.5

Note: Result expresses as mean ± SEM from six observations



Graph 1: Comparative data of paralysis time at different concentrations against *Pheretima posthuma*



Graph 2: Comparative data of death time at different concentrations against *Pheretima posthuma*

4. Conclusion

The wormicidal activity of various solvent extracts of *Clavaria rosea* has paralytic effect on Indian *Pheretima posthuma*, suggests that it is effective against parasitic infections of humans. The product is used as an anthelmintic agent. Further, in future it is necessary to identify and isolate the possible active bio-constituents responsible for the anthelmintic activity and study its pharmacological actions.

5. Acknowledgement

The authors are grateful to the Chairman, Dept. of Applied Botany, Kuvempu University, Shankaraghatta-577451, Shimoga (D), Karnataka, India for providing the facilities to perform this work.

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