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Anthelmintic activity of *Pyrostegia venusta* using *Pheretima posthuma*

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ABSTRACT
Approximately 3 million people are infected with helminthes worldwide. Helminthes infections are commonly found in villages of developing countries and are being recognized as cause of much acute as well as chronic illness among the human beings as well as cattle's. Hence, the treatment for helmintic infection is of utmost need. The high cost of modern anthelmintics has limited the effective control of these parasites. However, increasing problems of development of resistance in helminthes against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. Literature survey revealed that there are only few reports available on phytochemical and pharmacological studies of this plant. In the present investigation we have made a sincere attempt to evaluate the anthelmintic property of chloroform and methanol extracts of *Pyrostegia venusta* using *Pheretima posthuma* as an experimental helminthes model. Piperazine citrate was used as the standard reference. Five different concentrations (2.5, 5.0, 7.5, 10.0 and 12.5 mg/ml) of chloroform and methanol extracts were used to determine their effect as time taken to paralysis and time to induce death in the worms. Among the various concentrations of chloroform extract tested, 12.5 mg/ml showed efficient anthelmintic activity with paralysis time (23 min) and death time (44 min). Among all the concentrations of methanolic extract tested, 12.5 mg/ml showed significant results with paralysis time (34 min) and death time (78 min) respectively. This investigation revealed that chloroform extract was more effective in its anthelmintic action against *Pheretima posthuma* when compared to methanolic extract. But both the extracts were less potent when compared with standard drug piperazine citrate.

Keywords: *Pyrostegia venusta*, Bignoniaceae, anthelmintic activity, *Pheretima posthuma,* chloroform extract, methanolic extract.

INTRODUCTION

Pyrostegia venusta, also known as flame vine or orange trumpet creeper, belongs to the family Bignoniaceae. It is a vigorous, fast-growing, evergreen woody vine that blooms in winter and spring with spectacular reddish-orange flowers. Flame vine branches profusely and climbs by clinging with its tendrils. *Pyrostegia venusta* is found in tropical and subtropical areas and is native to southern Brazil, northern Argentina and Paraguay. Flame vine tolerates acidic to alkaline soils. This is the popular ornamental vine found in Bangalore, Karnataka (India) for covering fences and walls (Fig. 1). Native Brazilians use the aerial parts of *P. venusta* for the treatment of cough and flu. Decoction of this plant was administered orally as a general tonic to treat diarrhoea, vitiligo, and jaundice. [1-2]

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Helminths are parasitic worms. They are the most common infectious agents of humans in developing countries and produce a global burden of disease that exceeds better-known conditions, including malaria and tuberculosis. [3] Helminthes infections are among the most common infections in man, affecting a large proportion of the world's population. There are two major phyla of helminthes *viz.,* nematodes and platyhelminths. The nematodes (also known as roundworms) include the intestinal worms and the filarial worms that cause lymphatic filariasis and onchocerciasis. Whereas the platyhelminths (also known as flatworms) include the flukes (also known as trematodes) such as the schistosomes and the tapeworms (also known as the cestodes) such as the pork tapeworm that causes cysticercosis. [4]

Many helminth infections occur in poverty-stricken and developing countries with warm, moist environments and poor sanitary conditions. [5] Helminths can live in humans and animals. These infections are usually transmitted through contaminated food or water, feces, and unwashed hands or contact with a contaminated object. Helminth infections

normally found in livestock can be transferred from animal to man through a process called zoonoses and can then cause increased prevalence among humans. Although few helminthes infections lead to death, most of them do cause severe physical impairment. Helminths live in the intestinal tract. Although helminth infections can affect anyone but children in developing nations are most at risk for helminth infections. [6] The World Health Organization reports a 35% infection rate for roundworm, which is a common parasitic worm. [7-8] The presence of these worms in the body challenges the immune system.

Fig. 1: *Pyrostegia venusta* vine.

Ideally an anthelmintic agent should have broad spectrum of action, high percentage of cure with a single therapeutic dose, free form toxicity to the host and should be cost effective. None of the synthetic drug available meets this requirement. [9] Development of resistance to most of the commercially available anthelmintics became a severe problem worldwide. Because of the increasing antihelmintic resistance and impact of conventional helminthes on the environment, it is important to look for alternative strategies against gastrointestinal nematodes. [10] Hence, the best alternative is plant based medicine. Plants are used as medicine against helminths since time immemorial. Most common drugs like piperazine salts have side effects. Moreover, these drugs are unaffordable, inaccessible or inadequately available to the resource-poor farmers of the developing countries. These factors paved the way for herbal remedies as alternative anthelmintics. Several research studies has been undertaken to evaluate plants for their proclaimed anthelmintic activity. [11] Evaluation of the activities of medicinal plants claimed for possessing the anthelmintic property is getting attention these days. Screening and proper evaluation of the claimed medicinal plants could offer possible alternatives that may be both sustainable and environmentally acceptable. Use of medicinal plants could be one of the major options to control these pathologies. *In-vitro* screenings mostly reported uses worm samples like Indian earthworm *Pheretima posthuma, Ascardia galli, Ascaris lumbricoids etc.* Adult *Pheretima posthuma* has been used in screening as it shows anatomical and physiological resemblance with the intestinal round worm parasite of human beings. [12] Because of easy availability they are used as suitable model organism for screening of anthelmintic drugs. [13] Literature survey revealed that there are no reports on antihelmintic activity of *Pyrostegia venusta* plant and considering the prevalence of helminthes infection and the need for an alternative treatment, hence this study was undertaken to evaluate the antihelmintic property of different extracts of *Pyrostegia venusta* plant against *Pheretima posthuma* to support its medicinal claims.

MATERIALS AND METHODS

Drugs and chemicals

The standard drug piperazine citrate (SD Fine Chemicals Ltd., Mumbai). Chloroform and Methanol (Merck, India).

Plant Resource

Stem and leaves of *Pyrostegia venusta* were collected from residential area of Bangalore City. Fresh plant material was washed thoroughly with distilled water to remove traces of contaminants. This processed plant material was then shade dried for one month. After complete drying the dried plant material was porously powdered mechanically and was subjected to cold extraction using chloroform as the solvent system for about 96 h. After every 24 h fresh chloroform was added and chloroform containing the crude extract was separated, followed by methanol extraction sequentially in the similar fashion. Both the extracts were filtered and concentrated in vacuum under reduced pressure and allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The yield of crude ethanol and chloroform extract for 1 kg of powdered seed material was 120.5 g and 142.3 g respectively.

Test organism

Indian adult earthworms (*Pheretima posthuma)* collected from the Indo-American Hybrid Seeds, Bangalore. The earthworms were maintained under normal vermicomposting medium with adequate supply of nourishment and water, for about two weeks. Before the initiation of experiment the earthworms were washed with normal saline. Adult earthworms of approximately 4 cm in length and 0.2-0.3 cm in width were used for the experiment. This organism was selected as a model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [13-14]

Extract preparation for experiment

The porously powdered *Pyrostegia venusta* plant material was used for extract preparation. After crude extraction, the crude extracts were stored in dessicator until further use. Test extracts and standard drug piperazine citrate were dissolved in 0.5% DMSO in normal saline (v/v) and were used for evaluation for *in vitro* anthelmintic activity.

Antihelmintic activity

The anthelmintic activity of chloroform and methanol extracts of *Pyrostegia venusta* was evaluated as per the method reported by Dash *et al.,* 2002. [15] Ten groups with three earthworms in each group was sorted and used to evaluate anthelmintic activity. Each earthworm was separately released into 20 ml of desired formulation in normal saline, Group I earthworm were released in 20 ml normal saline in a clean petri plate. Group II, III, IV and V earthworms were released in 100, 150, 200 and 250 mg/20ml of chloroform extract respectively. Similarly, group VI, VII, VIII and IX earthworms were released in 100, 150, 200 and 250 mg/20ml of methanol extract respectively. Group X earthworms were released in normal saline containing

standard drug piperazine citrate (50 mg/20ml). Earthworms were observed; and the time taken for paralysis and the time taken for death was monitored and documented in minutes. Paralysis time was analyzed based on the behavior of the earthworm with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body color. [15] The result of anthelmintic activity is depicted in Table 1.

Statistical analysis

The data of anthelmintic evaluations were expressed as mean ± S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at P< 0.01 was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

RESULTS AND DISCUSSION

In the present investigation, *Pyrostegia venusta* plant material was sequentially extracted using chloroform and methanol as the solvent system. In continuation with our interest in helminthes infections and biological properties of *Pyrostegia venusta* plant, we made an attempt to assess the anthelmintic property of *Pyrostegia venusta.*

Chloroform extract at the concentration of 5 mg/ml (100 mg/20 ml of normal saline) showed the time of paralysis and death at 33 and 45 min respectively. For concentration of 7.5 mg/ml (100 mg/20 ml of normal saline), the paralysis and the death time was found to be 30 and 55 min respectively. At the concentration of 10 and 12.5 mg/ml, time taken to paralysis was 35 and 23 min respectively and death time 43 and 42 min respectively. Among the various concentrations tested, chloroform extract at 12.5 mg/ml (250 mg/20 ml) showed significant anthelmintic activity (Table 1). On the other hand methanolic extract at the concentration of 5 mg/ml showed the time of paralysis and death at 65 and 88 min respectively. For concentrations at 7.5, 10 and 12.5 mg/ml paralysis was shown at 70, 66 and 34 min respectively and death occurred at 94, 91 and 78 min respectively. Among all the concentrations methanolic extract tested, 12.5 mg/ml (250 mg/20ml) produced significant results. Standard drug at 2.5 mg/ml (50 mg/20ml) showed paralysis at 29 min and death time was 32 min (Table 1).

Table 1: *In-vitro* anthelmintic activity of chloroform and methanol extracts of *Pyrostegia venusta* against *Pheretima posthuma*

Test samples	Concentration (mg/20ml)	Paralysis Time (min)	Death Time (min)
Control (Normal Saline)		104.33 ± 5.24	199.0 ± 6.56
Chloroform extract of *Pyrostegia venusta*	100	33.33 ± 2.91**	45.0 ± 8.66**
	150	30.33 ±8.51**	49.67 ± 15.39**
	200	35.33 ± 12.81**	43.67 ± 10.68**
	250	23.00 ± 5.69**	42.00 ± 10.54**
Methanol extract of *Pyrostegia venusta*	100	65.33 ± 5.61**	88.00 ± 11.06**
	150	70.33 ± 8.11*	94.00 ± 13.01*
	200	66.33 ± 6.98**	91.33 ± 6.84**
	250	34.00 ± 2.65**	78.00 ± 8.02**
Piperazine citrate		29.67 ± 2.33**	32.33 ± 1.88**

Values are the mean ± S.E.M. of three earthworms. Symbols represent statistical significance. * P < 0.05, ** P < 0.01, ns: not significant as compared to compared to control group.

This investigation revealed that chloroform extract of *Pyrostegia venusta* showed significant anthelmintic activity against *Pheretima posthuma* when compared methanol extract, but less potent than the standard drug Piperazine citrate (Fig. 2). This investigation reports a new potent anthelmintic agent that can be used as drug for the treatment of helminthes infection.

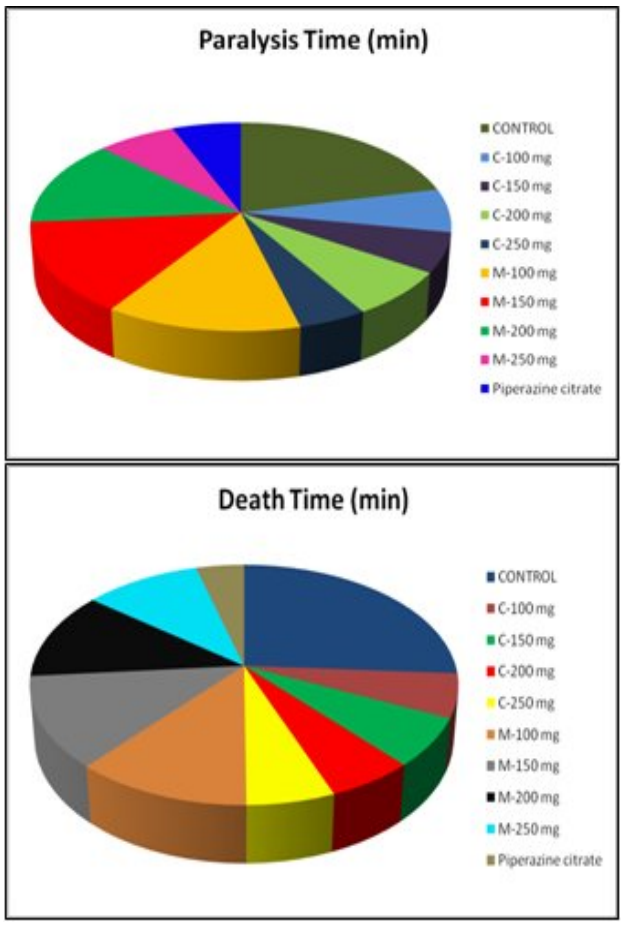

Fig. 2: Pie chart illustrating the comparative *in vitro* anthelmintic effect of different concentrations of chloroform and methanol extracts of *Pyrostegia venusta*

Synthetic anthelmintic drugs are usually associated with various side effects. More attention is attracted by the increasing problems of development of resistance in helminthes against synthetic anthelmintics. However, plants are the richest source for bioactive compounds. The best alternative over modern synthetic drugs is plant derived medicine. Many investigators have worker on the similar aspect and their reports support this investigation revealing that plants are potent anthelmintic agents. Swati *et al*., (2011) have reported the potency of *Catharanthus roseus* as anthelmintic plant. [16] Rajeshwar *et al*., (2011) have reported the anthelmintic property of *Tinospora cordifolia* [17] and anthelmintic and bactericidal activity of *Flaveria trinervia* by Joy *et al.*, (2011). [18]

Helminthiasis, the condition resulting from worm infestation, is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for wide spread nature of this disease in the developing countries including India. 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. [19] Anthelmintics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs; therefore, there is a foremost problem in treatment of helminthes diseases. [20] Moreover, these drugs are unaffordable because of their high cost. These factors paved the way for medicinal plants as alternative anthelmintics. In light to this issue our investigation presents *Pyrostegia venusta* as an new anthelmintic agents, this investigation acts as a basis for further phytochemical evaluation of *Pyrostegia venusta* seeds to isolate potent anthelmintic compound and carry out *in vivo* anthelmintic activity using animals.

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