ANTHELMINTIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *PSEUDERANTHEMUM LATIFOLIUM*: AN IN-VITRO STUDY

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ABSTRACT

Helminthiasis also known as worm infestation is one of the most prevalent parasitic infestations both in developed and developing countries with about 1.45 million people infested with soil transmitted helminths. The emergence of resistance and toxicity to the conventional anthelmintic drugs and the increasing concern over the presence of drug residues in animal products has led to a renewal of interest in the use of plant-based drugs. Therefore, in the current study we aimed for evaluation of anthelmintic activity of ethanolic leaf extract of *Pseuderanthemum latifolium.* Leaves of *P. latifolium* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with ethanol. The *in-vitro* anthelmintic activity of ethanolic leaf extract of *P. latifolium* was determined. Results revealed that the mean time for paralysis (min) was found to be 19.54, 59.49, 48.69, and 29.65 in T_1, T_2, T_3, and T_4 respectively. The mean time for death (min) was found to be 23.65, 63.60, 52.80, and 33.76 in T_1, T_2, T_3, and T_4 respectively. The results inferred that ethanolic leaf extract of *P. latifolium* at 50 mg/mL shown anthelmintic activity in terms of time for paralysis and death at par with that of standard anthelmintic drug. In conclusion, ethanolic leaf extract of *P. latifolium* could be explored in development of natural anthelmintic formulations owing its anthelmintic potential.

Keywords: *P. latifolium*, Leaves, Anthelmintic potential, Tannins, Saponins, Alkaloids

INTRODUCTION

Helminthiasis also known as worm infestation is one of the most prevalent parasitic infestations both in developed and developing countries affecting around 1/3rd of the world's population. As per WHO, around 1.5 billion people are infested with soil-transmitted helminths worldwide.[1] India has the highest burden of soil-transmitted helminths among all the countries contributing to one-fourth of total global cases which is prevalent among

children of age group 1-14 years.[2] Overall prevalence of soil-transmitted helminths in India ranges from 13% to 66%.[3]

Parasites causing infections can be broadly classified into tapeworms, flukes, and roundworms. Helminths cause infection by faeco-oral route or by direct entry through the skin of the host and then lodging in the intestine.[4] Intestinal worms cause decreased absorption, blood loss, intestinal or lymphatic obstruction and secretion of toxins which leads to health hazards like undernourishment, anaemia, eosinophilia and pneumonia.[5] In extreme cases of intestinal infestation, the mass and volume of the worms may cause tear of the muscular layer leading to peritonitis, volvulus and gangrene of the intestine.[6]

Anthelmintic drugs either kill or expel the infesting worms. They include benzimidazole class like albendazole and mebendazole, macrocyclic lactone like ivermectin or pyrazinoisoquinoline derivative like praziquantel. Mebendazole and albendazole inhibits polymerization of β tubulin of the parasite. In addition, they probably block glucose uptake in the parasite and deplete its glycogen stores.[7,8]

Plants have traditionally played an important part in illness therapy. Every plant has unique phytochemicals that have a wide range of therapeutic properties. Plants are rich in medicinal ingredients; great emphasis has been paid to the creation of ethnomedicines because they include phenols, flavonoids, flavonols, alkaloids, tannins, proanthocyanidins, vitamins, terpenoids, and other phytochemicals responsible for various pharmacological effects.[9] These compounds show a variety of functions that can be used to create medications that are both more effective and less likely to cause adverse side effects than the existing state of abnormal health in humans or other animals.[10]

The emergence of resistance to all currently used anthelmintic drugs is a major concern and newer anthelmintics with novel mode of action is the need of the hour.[11] Growing concerns about helminths developing anthelmintic resistance led to the notion of evaluating

medicinal plants for anthelmintic action.[12] With this scenario, the present study was conducted with the main objective to evaluate anthelmintic activity of ethanolic leaf extract of *Pseuderanthemum latifolium*.

MATERIALS AND METHODS

Collection of *P. latifolium*

P. latifolium leaves (Figure 1) were collected from local market in Bengaluru urban district, Karnataka, India. The *P. latifolium* leaves were shade dried at room temperature for one week. The dried leaves of *P. latifolium* were crushed to fine powder with the help of electric grinder and stored in airtight containers for further analysis.

Figure 1. Showing leaves of *P. latifolium* plant

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *P. latifolium* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of ethanol. The extract was concentrated by distilling the solvents in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Qualitative analysis of ethanolic leaf extract of *P. latifolium* was carried out through phytochemical screening using standard procedures to detect phytoconstituents as described by Sofora,[13] Trease and Evans[14] and Harborne.[15]

Test for alkaloids

Approximately 0.2g of ethanolic leaf extract of *P. latifolium* was warmed with 2% H_2SO_4 (2.0mL) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for tannins and phenolic compounds

The ethanolic leaf extract of *P. latifolium* in small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins and phenolic compounds.

Test for carbohydrates

About 0.6g of ethanolic leaf extract of *P. latifolium* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of carbohydrates.

Test for saponins

About 0.2g of ethanolic leaf extract of *P. latifolium* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for flavonoids

0.2g of ethanolic leaf extract of *P. latifolium* was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for proteins

Two drops of 3% copper sulphate and few drops of 10% sodium hydroxide were added to 1 mL of ethanolic leaf extract of *P. latifolium.* Violet or red colour formation indicates the presence of proteins.

Evaluation of Anthelmintic Activity

A total of twenty-four adult earthworms *Pheretima posthuma* (5-8 cm in length) were collected from moist soil, and they were cleaned with normal saline to remove soil and faecal matter. The earthworms were divided into four groups (n=6) *viz.* Control (T_0), Standard (T_1), ethanolic leaf extract of *P. latifolium* (ELEPL), 10 mg/mL (T_2), ELEPL, 25 mg/mL (T_3), and ELEPL, 50 mg/mL (T_4). Standard drug (Albendazole) was dissolved in 2% gum acacia and 10 ml of the desired formulation were poured in separate petri dishes and were kept under room temperature. The earthworms were placed in the petri dishes containing the extract solutions or the standard drug.[16]

RESULTS

The major phytochemicals found in ethanolic leaf extract of *P. latifolium* were found to be alkaloids, carbohydrates, proteins, flavonoids, phenolic compounds, tannins, and saponins (Table 1).

Table 1. Qualitative analysis of ethanolic leaf extract of *P. latifolium*

Phytochemical Components	**Ethanolic Leaf Extract of *P. latifolium* (ELEPL)**
Alkaloids	+

Carbohydrates	+
Proteins	+
Flavonoids	+
Phenolic compounds	+
Tannins	+
Saponins	+

+: Present; ELEPL, Ethanolic leaf extract of *P. latifolium*

The results of *in-vitro* anthelmintic activities of standard and ethanolic leaf extract of *P. latifolium* was represented in Table 2. Results revealed that the mean time for paralysis (min) was found to be 19.54 ± 0.58, 59.49 ± 0.65, 48.69 ± 0.29, and 29.65 ± 0.47 in T_1, T_2, T_3, and T_4 respectively (Figure 2). The mean time for death was found to be 23.65 ± 0.16, 63.60 ± 0.61, 52.80 ± 0.46, and 33.76 ± 0.28 in T_1, T_2, T_3, and T_4 respectively (Figure 3). These findings implied that ethanolic leaf extract of *P. latifolium* at 50 mg/mL showed anthelmintic activity in terms of time for paralysis and death at par with that of standard anthelmintic drug (Albendazole).

Table 2. Effect of ethanolic leaf extract of *P. latifolium* on anthelmintic activity

Treatment Groups	**Time for Paralysis (min)**	**Time for Death (min)**
T_0 - Control	-	-
T_1- Standard	19.54 ± 0.58	23.65 ± 0.16
T_2 - ELEPL (10 mg/mL)	59.49 ± 0.65	63.60 ± 0.61
T_3 - ELEPL (25 mg/mL)	48.69 ± 0.29	52.80 ± 0.46
T_4 - ELEPL (50 mg/mL)	29.65 ± 0.47	33.76 ± 0.28

Values were expressed as mean ± SD; n=3; ELEPL, Ethanolic leaf extract of *P. latifolium*

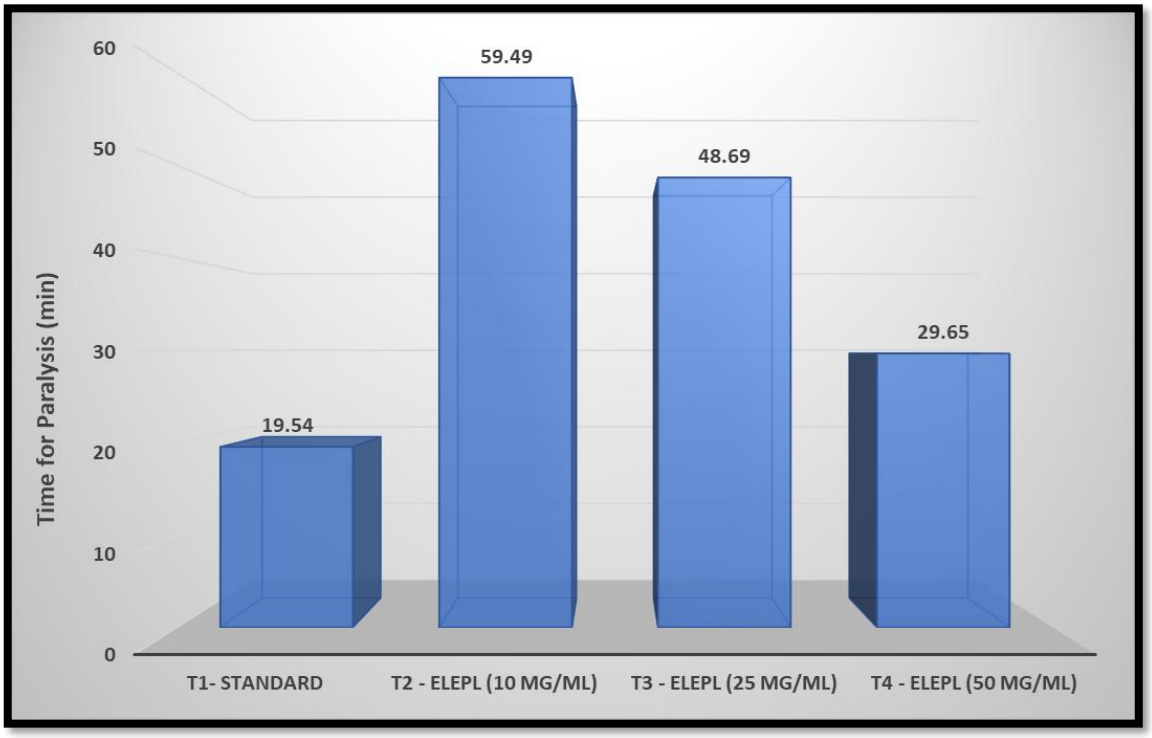

Figure 2. Effect of ethanolic leaf extract of *P. latifolium* on time for paralysis

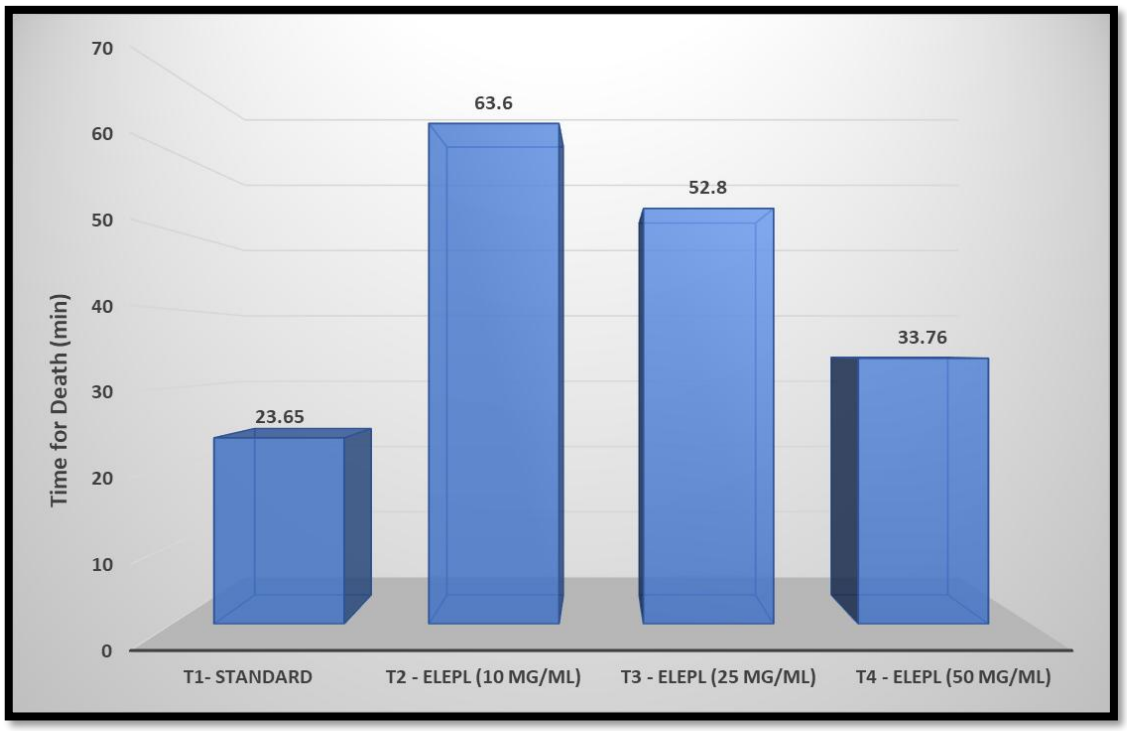

Figure 3. Effect of ethanolic leaf extract of *P. latifolium* on time for death

DISCUSSION

Helminthiasis is one of the most prevalent and preventable infestations worldwide. Globally about 1.45 million people are infested with soil transmitted helminths with the bulk of the burden shared by developing countries like India. The emergence of resistance and toxicity to the conventional anthelmintic drugs and the increasing concern over the presence of drug residues in animal products has led to a renewal of interest in the use of plant-based drugs. Evaluation of anthelmintic activity of new plant compounds by *in-vitro* methods using free living stages of parasitic nematodes have become popular.[17] Because of their low-cost effectiveness, simplicity and rapid turnover, *in-vitro* techniques are preferred over *in-vivo* methods.[18]

New medications with therapeutic benefits for humans may be derived from plants.[19] The Food and Drug Administration (FDA) has approved a large number of medications today, many of which have botanical origins. A wide range of substances found in plants used in traditional medicine can be utilized to treat infectious and chronic illnesses. In concurrence with literature findings in our study ethanolic leaf extract of *P. latifolium* at 50 mg/mL shown anthelmintic activity at par with that of standard anthelmintic drug such as Albendazole. Furthermore, time taken for paralysis and death of the worms by ethanolic leaf extract of *P. latifolium* are in concurrence with study reported by Shekhawat and Vijayvergia.[20]

The antihelmintic activity of ethanolic leaf extract of *P. latifolium* could be ascribed the secondary phytochemicals present in it mainly phenolic compounds, tannins, saponins, and alkaloids. Literature reports evidenced that tannins possibly can interfere with energy generation in helminths by uncoupling oxidative phosphorylation which is similar to some synthetic phenolic anthelmintics. Furthermore, literature reports revealed that tannins can also exert anthelmintic action by binding to free proteins in the gastro intestinal tract of host or glycoprotein on the cuticle of the parasite and causes death.[21]

In our study phytochemical investigation of the ethanolic leaf extract of *P. latifolium* also reveals presence of saponin which has membrane permeabilizing and pore forming property. This is similar to two conventional anthelmintic drugs such as praziquantel and toltrazuril. Thus, permeability of the cell membrane of the parasites is affected which forms vacuoles, resulting in disintegration of monogenean teguments.[22] In addition presence of alkaloids in ethanolic leaf extract of *P. latifolium* can block intake of acetylcholine from the host which expels out the worms by peristaltic movement of intestine.[23]

CONCLUSION

In conclusion, this preliminary pilot study evidenced anthelmintic potential of ethanolic leaf extract of *P. latifolium*. Hence, ethanolic leaf extract of *P. latifolium* could be explored in development of natural anthelmintic formulations. However, further studies are warranted to elucidate exact mechanism of action of anthelmintic potential of ethanolic leaf extract of *P. latifolium*.

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