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Research Article

ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *CYCLEA PELTATA* LAM AND ITS STATISTICAL ANALYSIS

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ABSTRACT

The aim of this research is to develop a safe and effective anthelmintic from plants, due to the resistance and toxic effect of synthetic anthelmintics on animals and humans. There are several side effects for synthetic drugs including drug resistance, toxicity, and drug residual in animal products. So there is a need for the exploration of medicinal plants for the treatment of different types of worm infections.

In this research a medicinally and traditionally important plant is used, leaves of *Cyclea peltata* Lam. The methanolic extract of the leaves were prepared by maceration technique and are monitored for the anti-helminthic activity against adult Pheretima posthuma worms. The reason for selecting this plant is due to the presence of tannins, which have been proved by previous phytochemical studies. Tannins have the capability to bind free proteins in the cuticle, oral cavity of helminths, thus causing the death. Albendazole (25mg/5ml) is used as a controlled drug by using the adult motility assay method. Three concentrations were prepared (25, 50, and 100mg/5ml). The time required for the immobility and death of the worms were determined. This study deals with the statistical analysis by one way ANOVA followed by Tukey's multiple comparison test at 95% confidence interval using graph pad prism software version 9.0.0 reveals the result that the methanolic extract of the Leaves of *Cyclea peltata* Lam causes the immobility and death of the vermicular in a concentration-dependent manner as compared to the standard drug Albendazole.

INTRODUCTION

Parasitic worms are otherwise known as helminths. The worm infections, commonly known as helminthiasis which cause morbidity to their host. Such an infection in man and animals causes stunted growth and a considerable threat to their health. The person suffering from helminthiasis may experience several symptoms including fatigue, enlarged liver, and spleen, abdominal pain, diarrhea, malnutrition, etc. [1]

Anthelmintics are drugs that destroy the parasitic worms or remove them from the host. The drugs that kill worms are commonly known as vermicides, drugs that affect the parasitic activity of worms and expel them from the gastrointestinal tract are known as vermifuges.[2]

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Currently using synthetic anthelmintics have several minus marks including toxicity, resistance, and presence of drug residues in animal products. These negative concerns regarding the current therapy lead to the need for the exploration of plants for the treatment of various worm infections. The reason behind this study is to develop a safe and effective anthelmintic which have to be overcome the unwanted effects of synthetic drugs.

The reason for selecting this plant is due to the presence of tannins which have been proved by previous phytochemical studies. Tannins can bind with the free proteins present in the oral cavity, cuticle of helminths etc thereby causing their starvation and death.

Parasitic worms are classified according to the zoological features in to four, cestodes, nematodes, trematodes, and filarial worms.

Cestodes commonly referred to as tapeworms, causes the infection cestodiasis.

Nematodes are otherwise known as flatworms which cause enterobiasis. Trematodes known as flukes causes schistosomiasis.

Another type of worm is the filarial worm which causes filariasis. [3,4]

The most common infection of the human beings are helminthic infections, in which human helminths are carried through the water, food, air which causes disease states, secrets toxins, and consumes the essential nutrients from the host bodies.[5,6,7]

Worm infections of the man and animals are mainly structured by three classes of new psycho active substances like benzimidazoles and the nicotinic acetylcholine agonists which are licensed for the treatment with humans. In developing countries of the world, intestinal worm infections are identified to be the most important imminence to economic success, in order to overcome the resistance offered by synthetic drugs, there is a need to explore the medicinal plants to develop a more efficient herbal anthelmintic with minimum side effect and resistance. [8,9,10]

Parasitic helminths cause considerable harm full effect in animal husbandry globally and considerable productivity losses to farmers [11,12]. The management of these nematodes has been based on the application of a limited number of vermicides. Neverthless the defiance too many of these vermicide is now widespread, and so on, there is a need to find new drugs to ensure sustained and effectual therapy and control in the future. [13,14]

Plant based preparations have relevant therapeutic activity and can be used for both animal and human treatments. Imported plant and their products have major dewormers activity which have been grown in the Scandinavian environment. [15,16]

The beneficial effect of condensed tannins against internal parasites includes;

- They expand the bestow and absorption of digestible proteins, by the capability of tannins to form non-biodegradable complexes with proteins in the abdomen.
- Under acidic pH condition these complex dissociate to liberate more proteins for metabolism in the small intestine of ruminants, this improves host resistance to nematode parasitic infections.
- Tannins have a direct vermicidal activity on the worm population. [19]

The phytonutrients from plants provide a distinctive good time in the search for new, effective and safe vermifuges. Many of these natural medicines may be acting on pathways in worms that differ from targets of currently used parasiticidal drugs that can cause mortality of nematodes that are resistant to one or more vermifuges. [20,21,22]

Medicinal plants produce some chemical constituents which possess numerous biological activities and help the plants to resist pests and diseases (Vaarst et al., 1996). Some previous studies showed that chemical constituents like alkaloids, tannins, flavonoids, phenols, etc possess appreciable anthelmintic activities. [23,24]

Figure 1: Leaves of *Cyclea peltata*

Selection of the Plant

Tannins contribute the anthelmintic activity by uncoupling the oxidative phosphorylation in the helminth thereby interfering with the energy generation in the helminth [25]. Tannins also exert anthelmintic activity by binding free proteins present in the esophagus, cuticle, oral cavity, etc and reduce nutrient availability thereby causing larval starvation and death.[26]

The beneficial effect of condensed tannins against internal parasites includes;

- They enlarge the provision and absorption of digestible proteins, by the capability of tannins to form non-biodegradable complex with proteins in the stomach.
- Under low pH condition of abomasums these dissociate to liberate more proteins for metamorphosis in the small intestine of ruminants, this ameliorates host resistance to nematode parasitic infections.
- Tannins have a direct vermifuge effect on the worm population. [9]

Here, the anthelmintic activity of leaves of *Cyclea peltata* Lam have been studied, the previous phytochemical studies revealed that the methanolic extract of leaves of *Cyclea peltata* Lam was shown the presence of tannins.

Leaf of *Cyclea peltata* Lam is commonly known as velvetleaf, a slender twining shrub that belongs to the family Menispermaceae [27]. In Ayurveda classics, Cyclea is mentioned as Raja Patha and it exists as a wonderful ingredient in several formulations of medical practice[28,29,30]. It contains chemical constituents like tannins, alkaloids, terpenoids, saponins etc. which have been proved by previous phyto-chemical studies.

MATERIALS AND METHODS

Source of the plant

The leaves of *Cyclea peltata* Lam were collected from Trivandrum district, Kerala, India during February 2021, its botanical identity was confirmed by Dr. P.Sreeja, Assistant professor, Department of P.G Studies and Research in Botany, Sir Seyd college, Thaliparamba, Kannur, Kerala.

Plant parts are shade dried, size reduced, and are extracted by maceration technique by using methanol as a solvent.

Extract preparation

The extract for the evaluation of anthelmintic activity was prepared by the cold maceration process.

The leaves were pulverized and size reduced by using a mixer grinder. The pulverized leaves were passed through sieve number 20. About 350 grams of the powdered leaves were placed in a 1000ml beaker and a sufficient amount of methanol with a solvent level of a few centimeters above the drug level was added. The solvent drug mixture is stirred well. The mouth of the beaker is covered with an aluminum foil kept for seven days and it is occasionally stirred. After seven days it is filtered through a cotton plug and funnel followed by whatmann filter paper grade 1. [31]

The extract was evaporated by using a distillator at a temperature of 60-70°C in order to evaporate the solvent and to obtain a residue of the extract and the obtained residue was weighed, after it is placed in a desiccator for 3 days. Then it is placed in a vial and kept in a refrigerator, until it is required to use. [32]

Worm collection

The Indian earthworm Pheretima Posthuma used for the study was procured from Kerala agricultural university, Vellayani, Trivandrum. They were assembled and washed with fresh water for the removal of cohering filth. The average size of the worm was 8-14 cm. The Pheretima Posthuma was selected to evaluate the activity in view of the fact that its anatomical and physiological similarity with the intestinal roundworm parasite present in human beings.

Anti-helminthic Assay

The anti-helminthic activity of the methanolic extract of the Leaves of *Cyclea peltata* Lam was evaluated by using the Indian earthworm Pheretima Posthuma having 8-14cm length. Because of its physiological and anatomical similarity with intestinal roundworm parasite of human beings.

Formulations of three different concentrations (5, 10, and 15mg/5ml in distilled water) of the sample were prepared and six earthworms of approximately the same size were placed in it. The test solution and standard drug solution were freshly prepared and 'time for paralysis' was noted when no movement of any kind could be observed excluding when the worms were vigorously shaken. The 'time for death' of worms was recorded after shaking with warm water kept at 50°C A maximum period of 60 minutes was work out for the immobilization as well as the death time of the worms. Albendazole (25mg/5ml) was used as a criterion with distilled water as the vehicle control. [33]

RESULTS AND DISCUSSION

The anthelmintic study of the methanolic extract of the leaves of *Cyclea peltata* Lam were carried out by the methods described in section 2.0. The results shows that all three concentrations (25, 50, and 100mg/5ml) show paralysis and death of adult Pheretima Posthuma worm. The standard drug albendazole (25mg/5ml) shows paralysis at 6±0.5 and death of the worm at 10±0.7 minutes respectively.

25mg/5ml of methanolic extract of *Cyclea peltata* Lam were shows paralysis at 17±1.4 and death at 24±1.9 minutes. The paralysis and death time for 50 and 100mg/5ml is 15±1.5, 10±0.1, 20±2.0, 13±2.2 minutes respectively (Table no: 1)

Table 1: Result of anthelmintic activity of Leaves of *Cyclea peltata* Lam

	Concentration of sample (mg/5ml)	Time of paralysis (in minutes)	Time of Death (in minutes)
Di	-	-	-
Control Drug (Albendazole)	25 mg/5ml	6±0.5	10±0.7
MECYL	25	17 ± 1.4	24± 1.9
	50	15 ± 1.5	20 ± 2.0
	100	10 ± 0.1	13 ± 2.2

Di- Distilled water

MECYL - Methanolic extract of *Cyclea peltata* Lam

The figures for the death of the worms in corresponding concentrations were given (see figure; 1, 2, 3, 4, 5)

Figure 2 A) Standard drug B) Control drug

C) 25 mg/5ml D) 50mg/5ml E) 100 mg/5ml

The results of antihelminthic activity of the methanolic extracts of leaves of *Cyclea peltata* Lam were shown in table 1. The result shows that all three concentrations cause paralysis and death of the worm. The higher concentration (100mg/5ml) causes paralysis at a time of 10±0.1 minute and death at 13 ± 2.2 minute, compared to the standard drug. Albendazole which takes 6±0.5 and 10±0.5 minutes at a concentration of 25mg/5ml. The statistical studies show that at lower concentrations the paralysis time of the standard drug and the methanolic extract is statistically significant but at higher concentrations, it becomes statistically non-significant. The death time required for the standard drug and plant extract is statistically significant at lower concentrations but at higher concentrations, they become statistically non-significant.

The statistical analysis of determination for the methanolic extract of *Cyclea peltata* Lam was carried out by one-way ANOVA followed by Tukey's multiple comparison test at 95% confidence interval using graph pad prism software version 9.0.0.

The graph shows (see figure 6) that the paralysis effect produced by the standard drug albendazole and methanolic extract of *cyclea peltata* Lam at a concentration of 25 and 50mg/5ml is statistically significant. This means they are not producing the good paralysis effect as that of the control drug albendazole. But when the concentration increases i.e., at 100mg/5ml they become statistically known significant. So we can understand that at higher concentrations (e.g. at 150mg/5ml) the methanolic extract of the *Cyclea peltata* Lam can produce paralysis effect same or greater than that of standard drug albendazole against adult Pheretima Posthuma worm.

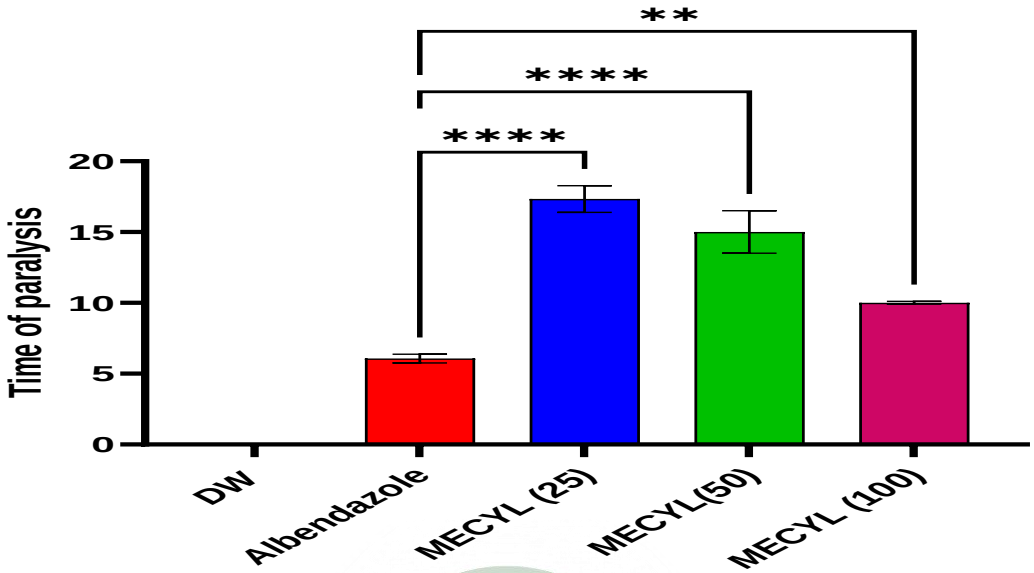

Figure 3: Statistical analysis of the time of paralysis for the methanolic extract of leaves of *Cyclea peltata* Lam (p-value; ≤0.0001 [****], 0.002 [**]

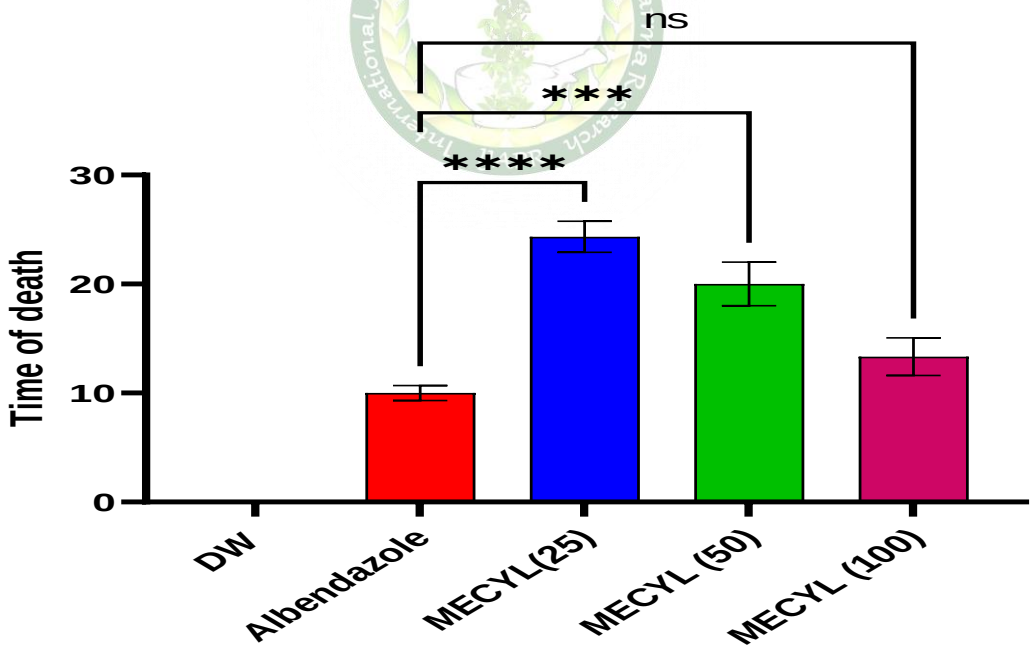

Figure 4: Statistical analysis of the time of death for the methanolic extract of *Cyclea peltata* Lam (p value; non-significant [ns], ≤0.0001 [****], 0.0001 [***]

From this graph, we can see that the death time required for the standard drug albendazole and the methanolic extract of the *Cyclea peltata* Lam at concentrations 25 and 50mg/5ml were statistically significant (see fig 7). That means at 25 and 50mg/5ml the plant extract can't produce the same anthelmintic effect as that of a standard drug. But at 100mg/5ml i.e., at higher concentration, the anthelmintic effect of the standard drug and methanolic plant extract are statistically non-significant. This means at a higher concentration methanolic extract of *Cyclea peltata* Lam can produce the anthelmintic effect similar to or greater than the standard drug albendazole.

CONCLUSION

Based on the results obtained from the present study, it shows that paralysis time required for the standard drug and the methanolic extract of leaves of *Cyclea peltata* Lam were statistically significant, ie, they are not producing the paralysis effect similar to or greater than that of standard drug, but at higher concentrations (100mg/5ml) the statistical significance decreases and they may produce paralysis of the worm similar to or greater than that of standard drug. At lower concentrations (25,50mg/5ml) the death time required for the methanolic extract of leaves of *Cyclea peltata* Lam is statistically significant with standard drug albendazole indicating that at lower concentration the plant extract has no significant anthelmintic activity as that of standard drug. But at higher concentrations (100mg/5ml) the death time of standard drug and plant extract becomes statistically non-significant. i.e., at higher concentration the methanolic extract of leaves *Cyclea peltata* Lam has similar or greater anthelmintic activity as that of standard drug albendazole. So we can conclude that methanolic extract of leaves of *Cyclea peltata* Lam has significant anthelmintic activity against adult Pheretima Posthuma worms compared to the standard drug albendazole.

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