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# Research on Synergestic Anthelmintic Activity of Methanolic Extract of the Leaves of Cyclea Peltata Lam and Roots of Cocos Nucifera Linn Against Adult Pheretima Posthuma Worms and it's Statistical Analysis

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**ABSTRACT:** The aim of the research is to develop a safe and effective anthelmintic from plants, due to the increasing toxic effect of synthetic drugs. The reason behind this research is the undesirable side effects of synthetic drugs available in the market like drug resistance. So there is a need for the exploration of more medicinal plants for the treatment of various worm infections.
Here the anthelmintic activity of combined methanolic extract of leaves of Cyclea peltata and roots of Cocos nuciferawere studied. The extract were prepared by maceration technique and evaluated the anthelmintic activity against adult Pheretima Posthuma worms via anthelmintic assay.
The reason for selecting these two plants for monitoring the anthelmintic activity because of the presence of tannins, which have been proved by previous phytochemical studies. Tannins have the capability to bind free proteins present in the cuticle, oral cavity etc, thus causing starvation of the larvae and thus possibly resultig in their death.
Combined Anthelmintic activity of the methanolic extract of the leaves of *Cyclea peltata* and roots of *Cocos nucifera* were 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 Statistical analysis also shows that the combined extracts increase the anthelmintic activity by each other, which can be monitored by analyzing the statistical significance and statistical non-significance, thus it proved to be synergistic. About three formulations were prepared from combined plant extract (25,50 and 100mg/5ml) and the time of paralysis and death were calculated, which establish significant synergistic anthelmintic activity against adult Pheretima Posthuma worms. Albendazole 25mg/5ml were used as a standard drug . Thus the plants can be used for the safe and effective management of helminthiasis.

**KEYWORDS:** Helminth, Anthelmintic assay, Synergistic, Cyclea peltata, Cocos nucifera

## 1. INTRODUCTION

Parasitic worms are called helminths. Helminthiasis plays a major role as the most widespread disease among men and animals. The gastrointestinal tracts and liver act as a living place for worms normally [1]. Parasitic worms are classified zoologically as Cestodes (Tapeworms), Trematodes (Flukes), and nematodes (Roundworms) [2]

### 1.1 Symptoms and diagnosis

The symptoms of helminthiases may vary, the most common symptoms include intestinal inflammation, abdominal pain, enlarged spleen and liver, anaemia, bowel obstruction. [3]

Diagnosis can be done by conventional and molecular methods [4]

1) Fecal egg examination
   Parasitic eggs in the feces of the host are identified microscopically.
2) Antigen test
   Hormones and enzymes produced by parasitic infections activate the immune system. This response is a biomarker, that can be quantified and qualified.
3) Serological test
   The serum of the host is examined for parasite-specific antibodies.
4) Nucleic acid-based diagnosis
   It can identify and isolate the DNAs of different parasitic species.
5) Urine examination
   Examining parasitic eggs on host urine microscopically [5], [6], [7] , [8]

### 1.2 Treatment /management

Anthelmintic drugs are used to remove the worms from the infected host or to destroy the worms that cause infections. Worms also can penetrate the tissues, thus the word anthelmintics is used to describe drugs that act against synthetic infections also.

There are many control programs launched to control lymphatic filariasis, onchocerciasis, schistosomiasis, cysticercoids, and soil-transmitted helminths (STH) [9]

A wide variety of synthetic drugs are available for the treatment of helminth infections like albendazole, mebendazole, praziquantel, piperazine, diethylcarbamazine, etc.

To manage helminthiases, proper hygiene maintenance is essential[10])

For the management of A. Lumbricoids albendazole, mebendazole, pyrantel pamoate, etc are used.[11] ,[12].

Intestinal obstruction requires iv support and anti-biotic treatment. Endoscopic and surgical therapy may be required if conservative therapy fails.

Praziquantel is the drug of choice for schistosomiasis[13]. Lymphatic filariasis can be treated by using mebendazole, flubendazole, diethylcarbamazine, etc[14], [15]

Even though all these synthetic drugs have so many adverse effects like toxicity they can produce side effects like nausea, vomiting, diarrhea, and they are not recommended for pregnant ladies and young children and also the problem of remaining drug residues on animal products[1], [16]

Neurocysticercosis is controlled by analgesics, anthelmintic therapy, and anti-convulsants [17], [18]

Gastro-intestinal helminths become more resistant to currently available synthetic anthelmintic drugs[19]. This needs the exploration of the herbal medicine system for the effective treatment of helminthiasis over man and animals and also to overcome all the resistance offered by the synthetic drugs.

Plants with medicinal properties were used from ancient times for the treatment and management of helminthiasis, even though without knowing their mechanism of action and compounds responsible for the activity. But now there is a huge advancement in the technologies and scope for research studies that help in the exploration of plants against various disease conditions including helminthiasis. Anthelmintic drugs obtained from plants are cheap and provide lesser side effects to the host organisms [20]

The plant constituent responsible for the antihelminthic activity is tannins especially condensed tannins [21]. Because tannins can bind the free proteins present in the cuticle, oral cavity, esophagus, thereby retarding nutrient availability and possibly results in larval starvation and death [3]

The reasons for which we select tanniferous plants for treating helminthic infections are ;

- Plants containing tannins increase the absorption and supply of digestible proteins, by forming non-biodegradable complexes with protein in the rumen, due to the direct anthelmintic activity of tannins towards animals.
- Plant chemical constituents like tannins, alkaloids, phenols are known to have significant anthelmintic activities.

  Phenolic compounds can interfere with the energy generation in helminths by uncoupling oxidative phosphorylation [22]

  *Cocos nucifera* Linn, a coconut tree is a member of an important family Arecaceae. Found abundantly in Malaysia, Indonesia, Philippines. The root system of coconut is fasciculated. [23] Used mainly for the treatment of vulnerable health problems [24]. The roots are found near the bottom surface of the coconut tree. Coconut root contains chemical constituents like flavonoids, steroids, glycosides, and the most important one tannins [25]

  In ancient scriptures of Ayurveda *Cyclea peltata* Lam is one of the important herbs. *Cyclea peltata* Lam belongs to the family Menispermaceae and grows throughout India and Sri Lanka. The leaves are simple, alternate, and heart-shaped, they have brownish cylindrical and tuberous roots. In Ayurveda, this herb is used for skin infections, fever, and urinary problems. The plant possesses chemical constituents like flavonoids, phenols, alkaloids, steroids, and mainly tannins [26], [27]

**Fig no. 1 Leaves and roots of *Cyclea peltata* and *Cocos nucifera***

## 2.0 MATERIALS AND METHODS

### 2.1 Source of the plant

The leaves and roots of *Cyclea peltata* Lam and roots of *Cocsnucifera* Linn were collected from Trivandrum district, Kerala, India during February 2021. Its botanical identity was confirmed by Dr. Sreeja. P, Assistant professor, Department of PG studies and research in botany Sir seyd college Thaliparamba, Kannur, Kerala. Plants parts are shade dried, size reduced, and are extracted by maceration technique by using methanol as a solvent.

### 2.2 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 Whatman filter paper grade 1. The extract was evaporated by using a distillation at a temperature of 60-70 ºc 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 [28].

### 2.3 Worm collection

Indian earthworm *Pheretima Posthuma* used for the study was procured from Kerala Agricultural University, Vellayani, Thiruvananthapuram. They were collected and washed with tap water for the removal of the adhering dirt. The average sizes of the worms were 8-14cm. They were selected to evaluate the activity because of its anatomical and physiological similarity with the intestinal roundworm parasite present in human beings.

### 2.4 Anti-helminthic Assay

Formulations of three different concentrations (25, 50, and 100 mg/5ml in distilled water) of the sample were prepared and six earthworms of approximately the same size were placed in it. Both the test solution and standard drug solution were freshly prepared and 'time for paralysis' was noted when no movement of any sort could be observed except when the worms were vigorously shaken. The 'time for death' of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°. A maximum time of 60 minutes was ascertained for the paralyzing as well as the death time of the worms. Albendazole (25mg/5ml) was used as a reference standard with distilled water as the vehicle control. [29]

## 3.0 RESULTS AND DISCUSSION

The anthelmintic activity of the combined extracts of leaves and roots of *Cyclea peltata* Lam and roots of *Cocos nucifera* Linn was carried out by the procedures mentioned in section 2.4. The results of the mentioned study were given in table no 1

**Table no. 1: Anthelmintic activity of the combined methanolic extract**

| 3 | Concentration of sample (mg/5ml) | Time of paralysis (in minutes) | Time of Death (in minutes) |
|---|---|---|---|
| Di | - | - | - |
| Control Drug (Albendazole) | 25 | 6±0.5 | 10±0.7 |
| MECN+MECYL combination | 25 (12.5+12.5) | 12 ± 1.2 | 19 ± 1.7 |
| | 50 (25+25) | 10 ± 1.4 | 15 ± 0.8 |
| | 100 (50+50) | 8 ± 1.1 | 12 ± 1.3 |

By evaluating the result can understand that all the three concentrations (25, 50, and 100mg/5ml) show paralysis and death of the adult Pheretima Posthuma worms. No one concentration produces anthelmintic activity greater than that of the standard drug albendazole, but when the concentrations increase there can see a decrease in the paralysis and death time of the worms. Due to this, we can foresee that using the higher concentrations may produce activities similar to or greater than that of standard drugs.

The figures for the estimated activity at corresponding concentrations were given

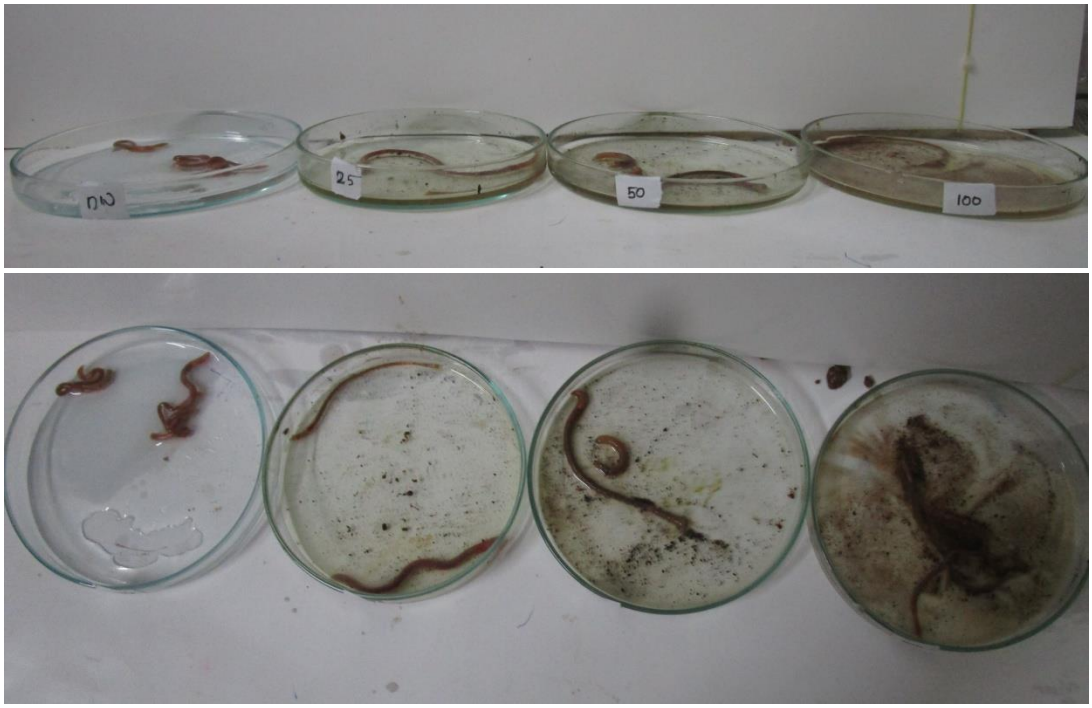


**Fig no.2: Anthelmintic activity produced by the combined extract.**

The individual anthelmintic activities of the methanolic extracts of leaves and roots of *Cyclea peltata* Lam and roots of *Cocos nucifera* Linn were given in table no 2 and table 3

**Table no. 2: Result of anthelmintic activity of Leaves and roots of *Cyclea peltata* Lam**

| | **Concentration of sample (mg/5ml)** | **Time of paralysis (in minutes)** | **Time of Death (in minutes)** |
|---|---|---|---|
| Di | - | - | - |
| Control Drug (Albendazole) | 25 | 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 |

**Table no. 3: Result of anthelmintic activity of roots of *Cocos nucifera* Linn**

| **Sample** | **Concentration of sample (mg/5ml)** | **Time of paralysis (in minutes)** | **Time of Death (in minutes)** |
|---|---|---|---|
| Di | - | - | - |
| MECN | 25 | 15 ± 1.5 | 22 ± 1.4 |
| | 50 | 12 ± 1.2 | 16 ± 2.0 |
| | 100 | 8 ± 0.5 | 10 ± 1.2 |

By comparing table 1 with table 2 and 3 we can understand that the time required for the paralysis and death of the worm by the combined extract is less than that of the individual extract, that means by comparing the two extracts there is an increase in the anthelmintic activity against adult Pheretima Posthuma worms. Ie, the

methanolic extracts of roots and leaves of *Cyclea peltata* Lam and roots of *Cocos nucifera* Linn synergistic activity so both extracts can be used together for the safe and effective management of helminthiasis.

## STATISTICAL ANALYSIS OF THE ANTHELMINTIC ACTIVITY OF COMBINED EXTRACTS

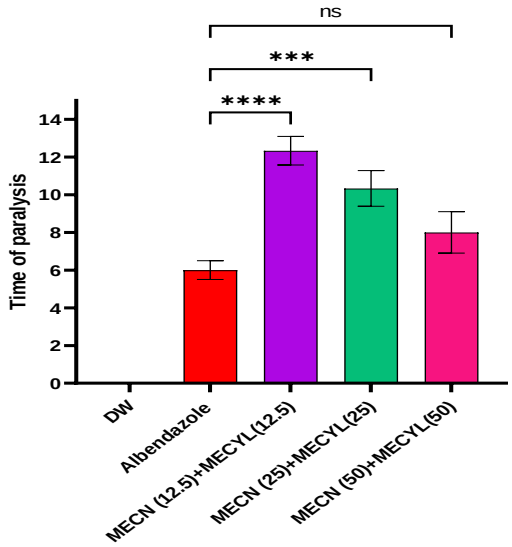


**Fig no. 3: Statistical analysis of the time of paralysis for the methanolic extract of *Cyclea peltata* Lam *and Cocos nucifera* Linn (*p-value;non-significant[ns],≤0.0001[****],0.0001[***].***

The graph shows the time of paralysis of the worms concerning their concentration. Here we can see that at lower concentrations (25mg/5ml) the anthelmintic activity of the plant extract is statistically significant with that of drug albendazole ie, the combined plant extract does not produce the activity greater than or similar to that of albendazole. But at a higher concentration (100mg/5ml), the activity becomes statistically nonsignificant, which means if we are using the higher concentration (above 100mg/5ml) the combined extract can produce activity greater than the control drug.

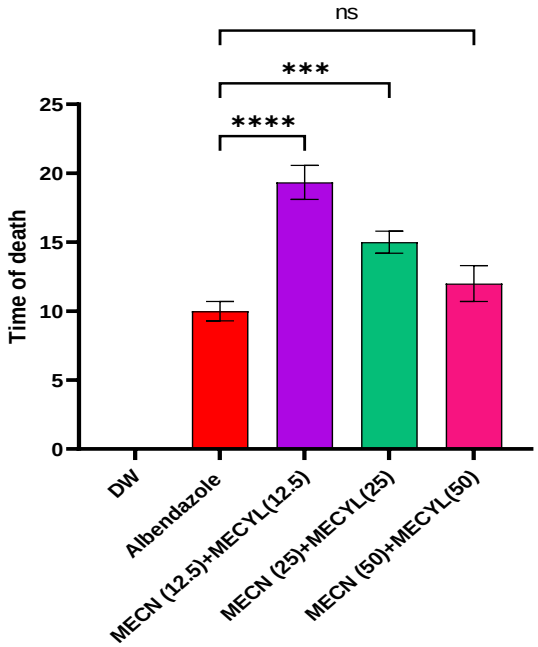


**Fig no. 4: Statistical analysis of the time of death for the methanolic extract of *Cyclea peltata Lam and Cocos nucifera* Linn*(p value;non-significant[ns],≤0.0001[****],0.0001[***].***

This graph shows the concentration of plant extract against the time of death of worms. This graph also shows at lower concentrations (up to 50mg/5ml) the concentration of combined extract and time of death becomes statistically significant. When the concentrations increase, it becomes statistically non-significant. This indicates that at higher concentrations it can produce greater anthelmintic activity than the albendazole against adult Pheretima Posthuma worms.

## CONCLUSION

By comparing the data obtained from both the individual and combined anthelmintic activity of methanolic extracts of roots and leaves of *Cyclea peltata* Lam and roots of *Cocos nucifera* Linn, there is a decrease in the time required for the paralysis and death of the worm when the concentration is increased. All three concentrations(25, 50, and 100mg/5ml) do not produce an activity similar to or greater than that of the standard drug Albendazole(25mg/5ml). But we can foresee that at higher concentrations (above 100mg/5ml) the extracts can produce anti-helminthic activity similar to or greater than that of standard drug.

When we are evaluating the data obtained from the anthelmintic activity of both individual and combined plant extracts, we can see that the time required for the paralysis and death of the worm in the combined extract is less than that of the individual extract. Such a great observation indicates that these two plant extracts have synergistic anthelmintic activities and can be used together for the safe and effective management of helminthiasis.

So we can conclude that methanolic extracts of both two plants have appreciable anthelmintic activities against adult Pheretima Posthuma worms and can be used effectively for the safe and effective management of helminthiasis with little side effects to man and animals and there is no resistance against helminths.

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## AUTHOR'S CONTRIBUTIONS

Snesha. S.R, the corresponding author actively participated in conducting the study, collection, and arrangement of data, its analysis, and writing of the article. Dr. Arthi. I, the corresponding author participated in the correction and arrangement of the article.

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