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RESEARCH ARTICLE

Anti-Helminthic Activity of *Leucas zeylanica Linn* Leaves

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ABSTRACT

The present study was examining the anti-helminthic activity of *Leucas zeylanica* leaves. For the present investigation, *L. zeylanica* leaves were collected in the month of January 2017 from Sai Nagar colony of the Karimnagar district. The leaves were dried and made into fine powder and subjected to acetone and ethanolic extraction by soxhlation, the phytochemical screening was done for extracts and the results showed that ethanolic and acetone extracts of the powdered leaves of *L. zeylanica* showed the presence of carbohydrates, alkaloids, phenols, tannins, flavonoids, steroids, and glycosides. Proteins and amino acids are absent. The extracts were studied for anti-helminthic activity against Indian earthworms *Pheretima posthuma*, acetone and ethanolic extracts showed the dose-dependent activity by paralysis followed by the death of earthworms. The observation of result shows that the anti-helmintic activity of ethanol extract is more potent compare to the acetone extract. The earthworms were more sensitive to the extracts of ethanol at 20 mg/ml concentrations as compared to the reference drug albendazole (10 mg/ml).

Keywords: Anti-helminthic, *Leucas zeylanica*, *Pheretima posthuma*, soxhlation.

INTRODUCTION

The term helminth has been derived from a Greek word meaning worm. It was originally meant to refer to only intestinal worms, but now includes tissue parasites as well as many free-living species. These are metazoa, an infection by a helminth is known as helminthiasis, soil-transmitted helminthiasis, helminth infection, or intestinal worm infection.

All helminths are multicellular eukaryotic invertebrates with tube-like or flattened bodies exhibiting bilateral symmetry. They are triploblastic (with endo-, memo-, and ecto-dermal tissues) but the flatworms are acoelomate (do not have body cavities) while the roundworms are pseudocoelomate (with body cavities not enclosed by mesoderm). In contrast, segmented annelids (such as earthworms) are coelomate (with body cavities enclosed by mesoderm).[1]

The number of different helminth species is vast: It is estimated to be around one million species. The nematodes are the most diverse of all the helminths with the highest number of species there may be as many as 300,000 species of parasites affecting vertebrates, and as many as 300 affecting humans alone.

The lifetime of adult worms varies tremendously from one species to another but is generally in the range of 1–8 years [Table 1]. This lifetime of several years is a result of their ability to manipulate the immune response of their hosts by secreting immunomodulatory products. Helminths can be either hermaphrodites (i.e., can have both sexes), such as tapeworms and the flukes (except the blood fluke which is not a hermaphrodite), or have their sexes differentiated, like the roundworms.[2]

The most common helminthiases are those caused by infection with intestinal helminths, ascariasis, trichuriasis, and hookworm, followed by schistosomiasis and lymphatic filariasis (LF).[3]

Adding to the global morbidity that results from human helminth infections are the observations that they have both direct and indirect effects on malaria and HIV/AIDS in developing countries. In Sub-Saharan Africa and elsewhere, helminthiases are frequently coendemic with malaria and HIV/AIDS. Indeed, it is not uncommon for an individual to be coinfected with the malaria-causing parasite and one or more parasitic worm or HIV and one or more parasitic worm. Such coinfections have additive effects, such as severe

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Table 1: Paralysis and death time for standard and test doses

Group	Treatment	Concentration (w/v) mg/ml	*P. posthuma*	
			Paralysis time (min)	Death time (min)
1	1% Tween 80 (control) (ml)	20	150±10	-
2	Albendazole	10	30	60
3	ESLE	10	120± 5	160±5
		20	70±10	120±10
4	ASLE	10	110±5	190±5
		20	95±10	150±10

anemia and synergistic effects, such as increased transmission of the malaria-causing parasite, HIV, and/or increased susceptibility to infection with these pathogens as well as cause an exacerbated progression of these two killer diseases.[4]

For reasons not well understood, compared with any other age group, school-aged children (including adolescents) and preschool children tend to harbor the greatest numbers of intestinal worms and schistosomes and as a result experience growth stunting and diminished physical fitness as well as impaired memory and cognition.[5]

Hookworm and schistosomiasis are also important diseases during pregnancy, causing neonatal prematurity, reduced neonatal birth weight, and increased maternal morbidity and mortality. Among some adult populations living in impoverished areas of developing countries, onchocerciasis is a leading cause of blindness and skin disease, while LF is a major cause of limb and genital deformities.

On-going scientific efforts to study hookworm are vital since it remains one of the most common chronic infections of humans, with an estimated 740 million cases in areas of rural poverty in the tropics and subtropics.[6]

Leucas zeylanica, belong to the family Lamiaceae commonly called as Ceylon slitwort,[7] synonyms include *Latin bancana* Miq, *Phlomis zeylanica* Linn, and *Spermacoce denticulate* Walp.[8,9] It is a small, earthy, nonwoody, annual erect plant or sometimes tufted, hispid and aromatic plant growing to a height of up to 120 cm, stipules absent. Stem is green in color. Leaves are oval in shape and green in color, which occur on opposite sides of stems and large in number. These are subsellile leaves which are liner lanceolate or elliptic-lanceolate which is 2.5–7.5 cm long. Roots are mainly taproot and fibrous. This is white or brown in color. Whorls of many flowers are bisexual, sessile, subsessile, usually in terminal curls is 1 to 2 cm in diameter, grouped together in an axillary, coralla is white in color and 2 cm long. Calyx is 5–7 mm long obliquely Turbient, with minute teeth, apex, acute, base acute, pinnately veined, and erect or spreading horizontally, It is reproduced by seed or pollinated by bees, moths, and flies.[10,11]

Plants exist in various habitats, a weed of sunny, dry localities, often on sandy soils, paddy dams, waste places, roadsides from the low land up to 1700 m altitude. Widely occurs throughout South East Asia.[12]

MATERIALS AND METHODS

Procurement of plant material

For the present investigation, *L. zeylanica* leaves were collected in the month of January 2017 from Sai Nagar colony of the Karimnagar district. The plant was identified and authenticated by BSI/DRC/16-17/Tech.05. The leaves were dried in the shade; it was powdered, passed through sieve No. 40 and stored in airtight bottles.

Selection of worms

An Indian adult earthworm was chosen for anti-helminthic activity as it has anatomical and physiological similarity with the intestinal roundworm parasites of human beings.

Administration of albendazole

Albendazole (10 mg/ml) was prepared using 1% v/v of Tween 80 as a suspending agent as administered as per the method of extract.

Preparation of 1% v/v of Tween 80

1% Tween 80 was prepared by taking 1 ml of Tween 80 in 100 ml of water or NaCl solution.

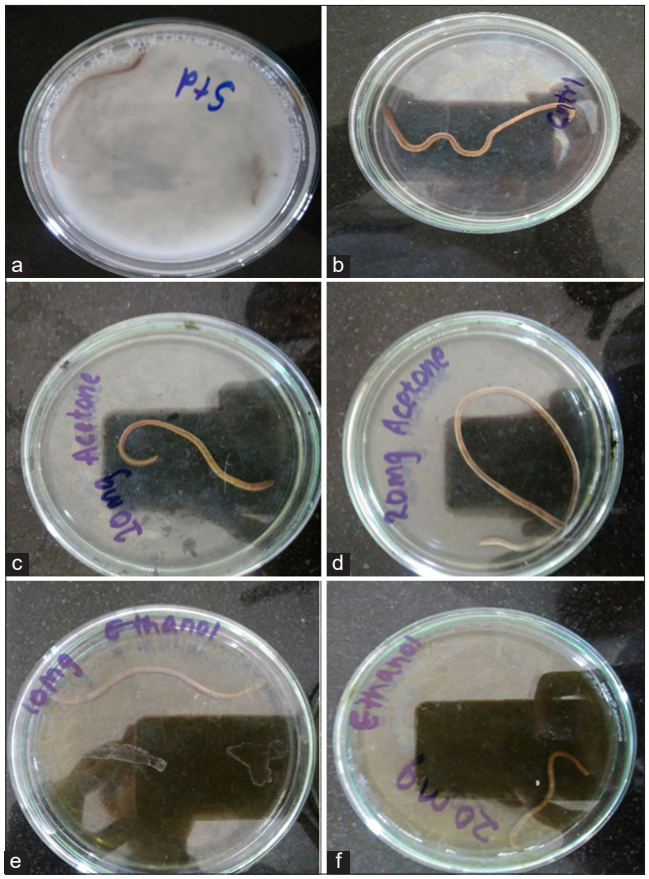

Figure 1: (a) STD: Albendazole, (b) control: 1% Tween 80, (c) 10 mg acetone, (d) 20 mg acetone, (e) 10 mg ethanol, (f) 20 mg ethanol

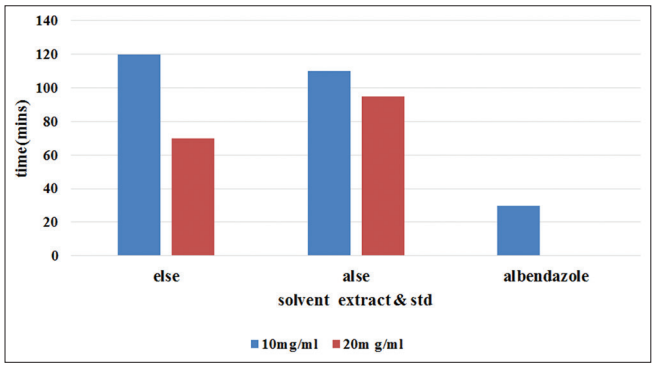

Figure 2: Comparative data of paralysis time at different concentrations against pheretima posthuma

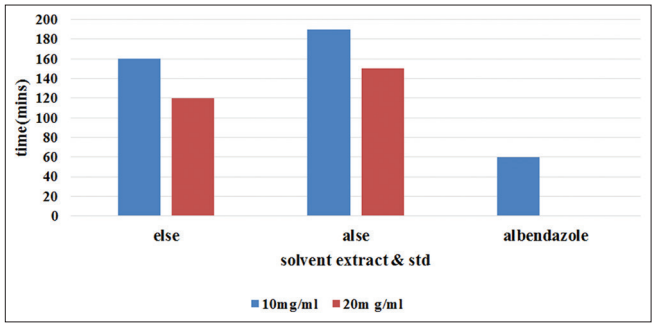

Figure 3: Comparative data of death time at different concentrations against pheratima posthuma

Preparation of extracts

Acetone and ethanolic extracts of *L. zeylanica* leaves were prepared by soxhlation methods at a suitable temperature. 50 g of the powder of leaves is prepared as a thimble and extracted with 300 ml of the solvent using soxhlation process was carried out for about 6 h for each solvent, and the extracts obtained were evaporated and dried in a desiccator.

Administration of extract

The suspension of acetone and ethanolic extract of *L. zeylanica* leaves of different concentrations (10, 20 mg/ml) were prepared using 1% v/v of Tween 80 as a suspending agent. A total of 20 ml for each concentration was prepared (200 mg in 20 ml for 10 mg concentration and 400 mg in 20 ml for 20 mg concentration). Albendazole was used as a standard. Groups of approximately equal size worms consisting of two earthworms individually in each group were releases into each 20 ml of the desired concentration of the drug and extract in the Petri dish.

Anti-helminthic evaluation

Experimental worms

Indian adult earthworms (*Pheretima posthuma*) were used to study anti-helminthic activity. The earthworms were collected from moist soil and washed with distilled water to remove all fecal matter. Earthworms 3–5 cm in length and 0.1–0.2 cm in width were used for the experiment.

Experimental design

The anti-helminthic activity was performed on adult Indian earthworm *P. posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *P. posthuma* was placed in Petri dish containing two different concentrations (10 and 20 mg/ml) of ethanolic and acetone extract of leaves of *L. zeylanica*. Each Petri dish was placed with 2 worms and observed for paralysis or death. Time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time of the death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were

compared with reference compound Albendazole (10 mg/ml) treated samples.[13]

RESULTS

From the results, it is observed that *L. zeylanica* shown potent anthelmintic activity while the *P. posthuma* has taken a long time for death (190 min–110 min) of worms. The earthworm selected for the anthelmintic activity was most sensitive to the different solvent extracts, namely ethanol and acetone.

Leaves extract of as *L. zeylanica*can be seen in Table 1. The anthelmintic activity result revealed dose-dependent paralysis is ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death at 10 and 20 mg/ml concentrations, paralysis, was observed, respectively, at 120 min and 70 min and death at 160 and 120 min in ethanol extracts. The acetone extracts of *L. zeylanica* also exhibited dose-dependent anthelmintic activities that caused paralysis at 110 and 95 min (at 10 and 20 mg/ml) and death at 190 and 150 min (at 10 and 20 mg/ml). The standard drug (albendazole) shows paralysis within 30 min and time of death 60 min in the two solvents extracts. The observation of result shows that the anti-helminthic activity of ethanol extract is more potent compared to the acetone extract. The earthworms were more sensitive to the extracts of ethanol at 20 mg/ml concentrations as compared to the reference drug albendazole (10 mg/ml). The results are furnished in Table 1, and graphs punished [Figures 1-3].

CONCLUSION

From the obtained results, it was concluded that acetone and ethanol soxhlation extracts of *L. zeyleanica* leave exhibited the dose-dependent anti-helminthic activity. Among them, ethanol (20 mg/ml extract causes paralysis in 70 min death in 120 min) extracts were more effective in causing the death of the worms as well as promoting paralysis compared to standard.

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