Evaluation of *in vitro* anthelminthic activity of *Leucas aspera* extracts

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ABSTRACT

Helminths infections are also among the most common infections in human, affecting a large proportion of the world's population in developing countries and produce a global burden of disease. *Pherithema posthuma* a helminthes is commonly known as earth-worms, *Leucas aspera* herb is distributed throughout India. The anthelminthic property of *Leucas aspera* was evaluated using *Pherithema posthuma* as an experimental model. Piperazine citrate was used as the standard reference. Earthworm belonging to control group showed paralysis time as 64.33 min and death time as 200 min. Among the various concentrations of aqueous extract tested, concenration at 250 mg/ml showed efficient anthelminthic activity and among all the concentrations ethanol extract tested, concentration at 250 mg/ml gave significant results. This investigation revealed that ethanol extract of *Leucas aspera* showed significant anthelminthic activity against *Pheretima posthuma* when compared aqueous extract. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *Leucas aspera* as anthelminthic plant.

Key words: Lamiaceae; *Leucas aspera*; Anthelminthic activity; *Pheretima posthuma*; Ethanol extract; Aqueous extract.

INTRODUCTION

Parasitic helminthes are worm-like organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and disease in human and animals inflicting heavy production losses. Helminths infections are also among the most common infections in human, affecting a large proportion of the world's population in developing countries and produce a global burden of disease and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia which more often physically impair their hosts than kill them.[1] Anthelminthics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them.[2] Various problems have been evolved with chemotherapeutic control practices such as parasites are developing resistance to several families of chemical anthelminthics,[3] chemical residues and toxicity problems,[4] un-economical and nonavailability of drugs in remote areas. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health.[5] For these various reasons, interest in the screening of medicinal plants for their anthelminthic activity remains of great scientific significance despite extensive use of synthetic chemicals in modern clinical practices all over the world.[6]

Helminthes infections are commonly found in community and being recognized as cause of much acute as well as chronic illness among the various human beings as well as cattle's. More than half of the population of the world suffers from various types of infection and majority of cattle's suffers from worm infections.[7] However, the high cost of modern anthelminthics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelminthics[8] has led to development of resistance and hence a reduction in the usefulness of available anthelminthics.[9] Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of

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DOI: 10.5530/pj.2011.24.15

helminth parasites, and as a means of reducing the cost of controlling helminthic diseases.[10-13]

Leucas aspera (Willd.) Linn. (Family: Lamiaceae), a herb commonly known as 'Thumbai is distributed throughout India from the Himalayas down to Ceylon.[14] The plant is used as an insecticide and indicated in traditional medicine for coughs, colds, painful swellings, and chronic skin eruptions.[15] Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites.[14,16] Compounds isolated from the plant include, long-chain aliphatic compounds, a triterpene-leucolactone, sterols- sitosterol, campesterol, stigmasterol and a novel phenolic compound.[17-20] However, anthelminthic activity of *Leucas aspera* whole plant extract is not scientifically and reported. To justify the traditional claims of *Leucas aspera,* we made an efficient attempt to assess the anthelminthic activity of *Leucas aspera.*

MATERIALS AND METHODS

Drugs and chemicals

The standard drug piperazine citrate (SD Fine Chemicals Ltd., Mumbai). Ethanol was purchased from Hong, Yang Chemical Corporation, China.

Plant Resource

Leucas aspera plant material was collected from agricultural fields of Tinsukia, Assam, India. The plant was authenticated by Prof. V. Krishna, Kuvempu University. Fresh plant material was washed thoroughly in tap water to remove traces of soil and other contaminants. It is then shade dried. Further, the whole plant was chopped finely and was shade dried, powdered mechanically. The powdered plant material was transported in a vacuum sealed container to Bangalore for further experimental studies. This plant material was subjected to cold extraction using ethanol as the solvent system for about 96 h, after every 24 h fresh ethanol was added and ethanol containing the extract was separated, followed by double distilled water (with 5% ethanol, to avoid microorganism contamination) for 96 h. 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 ethanol crude and aqueous extract for 1 kg of powdered plant material was 36 g and 45 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 model for anthelminthic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.[21,22]

Extract preparation for experiment

The porously powdered plant material was used for extract preparation. After extraction, the crude extract was stored in dessicator until further use. Ethanol extract and standard drug piperazine citrate were dissolved in 0.5% DMSO in normal saline (v/v). Whereas, the crude aqueous extract was directly dissolved in normal saline and used for evaluation for anthelminthic activity.

Antihelminthic activity

The anthelminthic activity of whole plant extracts of *Leucas aspera* was evaluated as per the method reported by Dash et al.[23] Twelve groups of animals with three earthworms in each groups, each earthworm were separate released into 20 ml of desired formulation in normal saline, Group 1 earthworm were released in 20 ml normal saline in a clean petri plate. Group II, III, IV, V, VI earthworms were released in 20 ml normal saline containing 50, 100, 150, 200 and 250 mg/ml of ethanol extract respectively. Similarly, group VII, VIII, IX, X, XI earthworms were released in 20 ml normal saline containing 50, 100, 150, 200 and 250 mg/ml of aqueous extract respectively. Group XII earthworms were released in 20 ml normal saline containing standard drug piperazine citrate (50 mg/ml). Earthworms were observed; 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 lose of motility with faded body color.[24] The result of anthelminthic activity is depicted in Table 1.

Statistical analysis

The data of anthelminthic 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

Leucas aspera is a well known medicinal plant and is widely used in folk medicine/ ayurvedic system of medicine. In

Table 1: *In vitro* anthelminthic activity of ethanol and aqueous extracts of *Leucas aspera* against *Pheretima posthuma*

Test samples	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (Normal Saline)		64.33 ± 0.88	200.00 ± 2.60
Ethanol extract of *Vinca rosea*	50	20.00 ± 0.58**	26.33 ± 0.88**
	100	16.00 ± 0.58**	23.00 ± 0.58**
	150	10.00 ± 0.33**	14.00 ± 0.58**
	200	6.33 ± 0.33**	11.00 ± 0.58**
	250	4.33 ± 0.88**	9.33 ± 0.33**
Aqueous extract of *Vinca rosea*	50	117.00 ± 1.15**	168.33 ± 4.06**
	100	100.67 ± 1.45**	154.00 ± 8.14**
	150	62.00 ± 1.53^{NS}	123.33 ± 2.60**
	200	56.00 ± 0.58**	115.00 ± 2.65**
	250	50.33 ± 0.88**	96.67 ± 2.33**
Piperazine citrate	50	31.33 ± 1.86**	40.67 ± 0.88**

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.

the present study solvents namely ethanol and water were used sequentially for crude extraction of *Leucas aspera* whole plant. To justify the ethnomedical claims of *Leucas aspera* we made an efficient attempt in evaluating the anthelminthic property of *Leucas aspera.*

Earthworm belonging to control group showed paralysis time as 64.33 min and death time as 200 min. Aqueous extract at the concentration of 50 mg/ml showed the time of paralysis and death at 117 and 168 min respectively. For concentration of 100 mg/ml, the paralysis and the death time was found to be 100.67 and 154 min respectively. At the concentration of 150, 200 and 250 mg/ml, time taken to paralysis was 62, 56 and 50.33 min respectively and death time 123.33, 115 and 96.67 min respectively. Among the various concentrations tested, aqueous extract at 250 mg/ml showed efficient anthelminthic activity (Table 1). On the other hand ethanol extract at the concentration of 50 mg/ml showed the time of paralysis and death at 20 and 26.33 min respectively. For concentrations at 100, 150, 200 and 250 mg/ml paralysis was shown at 16, 10.67, 6.33 and 4.33 min respectively and death occurred at 23, 14, 11 and 9.33 min respectively. Among all the concentrations ethanol extract tested, concentration at 250 mg/ml gave significant results. Standard drug at 50 mg/ml showed paralysis at 31.33 min and death time was 40.67 min (Table 1). This investigation revealed that ethanol extract of *Leucas aspera* showed significant anthelminthic activity against *Pheretima posthuma* when compared aqueous extract. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *Leucas aspera* as anthelminthic plant.

ACKNOWLEDGEMENT

The authors are grateful to Department of Biotechnology, The Oxford College of Science, Bangalore, for providing the facilities to carry out the entire experiment.

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