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Research Article

In Vitro Anthelmintic activity of *Leucas cephalotes* leaf extract

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Abstract

Alcohol and aqueous extracts from the leaves of *Leucas cephalotes* were investigated for their anthelmintic activity against Pheretima posthuma and Ascardia galli. Three concentrations (25, 50 and 100 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Both the extracts exhibited significant anthelmintic activity at highest concentration of 100 mg/ml. Piperazine citrate in same concentration as that of extract was included as standard reference and distilled water as control. The anthelmintic activity of alcohol and aqueous extracts of *Leucas cephalotes* has therefore been demonstrated for the first time.

Keywords: Anthelmintic Activity, Ascardia Galli, *Leucas cephalotes*, Pheretima Posthuma.

Introduction

Leucas Cephalotes (Roth) Spreng. Syn.*Phlomis cephalotes* (Labiatae or Lamiaceae) rainy season weed mainly found in North India. It is commonly known as 'Kubo or Kubi' in traditional medicine of Gujarat. The genus *Leucas* includes about 100 Asiatic and African species. It is a valuable drug for snake bite. The plant is useful in bronchitis, inflammation, asthma, dyspepsia, paralysis and leucoma. The leaves are useful in fever and urinary discharge[1]. According to Ayurveda, the plant is mild stimulant, diaphoretic and used in fever and also used in liver disorder. Flowers mixed in honey are used as domestic remedy of cough and colds[2].

It is valuable homoeopathic drug and as such is used for the treatment of chronic malaria and asthma[3]. Dry leaves along with tobacco (1:3) are smoked to treat bleeding as well as itching piles[4]. The plant was evaluated for *in vitro* antifilarial activity[5] and antidiabetic activity[6],antioxidant activity,analgesic activity,anti-inflammatory activity[7].The plant was found to contain triterpenes,oleanolic acid, sterols and flavones[8].Other constituents such as lauric acid, tridecanoic acid, adipic acid, glutaric acid and labellenic acid (seed oil) was also reported[9-10]. Literature survey revealed that the plant extract has yet not been screened for its traditional claim of anthelmintic activity. The objective of the present study was evaluate anthelmintic activity of alcohol and aqueous extracts of *Leucas cephalotes*.

Material and Methods

Plant material

The Leaves of *Leucas Cephalotes* (Roth) Spreng.(Labiatae or Lamiaceae) was procured from local area of Dhule (Maharashtra). The plant and plant material were identified and authenticated in Department of Botany, S.S.V.P.S. society's Dr. P. R. Ghogrey Science College, Dhule and Voucher herbarium specimens was deposited in the Departmentof Pharmacognosy of our College. The plant material was dried in sunlight, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.

Preparation of extract[11-12]

Aqueous extract (Maceration method)

Powdered material of *Leucas cephalotes* leaves (200 gm) was kept for maceration with 1000 ml of distilled water for 12 hrs.

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The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath.

The extract was dried and used as a powder. The percentage yield of extract was found to be 3.56 percent.

Alcoholic extract (Continuous Soxhlet extraction method)

Powder was first defatted with pet. Ether and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract.

Drugs and chemicals

Piperazine citrate (Glaxo Smithkline) was used during the experimental protocol.

Experimental

Alcohol and aqueous extracts from the leaves of *Leucas cephalotes* were investigated for their anthelmintic activity against Pheretima posthuma and Ascardia galli. Various concentrations (10-100mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was included as standard reference and distilled water as control. The anthelmintic assay was carried as per the method of Ajaiyeoba[13] with minor modifications.

The assay was performed on adult Indian earthworm, Pheretima posthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings[14-17] Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro Indian adult earthworms (Pheretima posthuma) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Ascardia galli worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic drug was advocated earlier[18-25]. In the first set of experiment, six groups of six earthworms were released in to 50 ml of solutions of piperazine citrate, aqueous and alcoholic extracts of leaves of *Leucas cephalotes* (25, 50 and 100 mg/ml each) in distilled water. Piperazine citrate was used as reference standard while distilled water as control.

Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors. Same experiment was done for *Ascardia galli* worms only the difference was solutions were prepared in normal saline solutions.

Results and discussion

Preliminary phytochemical screening of alcoholic extract revealed the presence of anthraquinone glycosides,phenolic compounds and steroids while aqueous extract showed presence of glycosides and phenolic compounds. From the results shown in table no. 1, the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.

The alcoholic leaves extract of *Leucas cephalotes* demonstrated paralysis as well as death of worms in a less time as compared to piperazine citrate especially at higher concentration of 100 mg/ml. While water extract also shown significant activity. Phytochemical analysis of the crude extracts revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic

activity. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation . It is possible that phenolic content in the extracts of *Leucas cephalotes* produced similar effects.

Table 1: Anthelmintic activity of extracts of *Leucas cephalotes*

Extracts of LS	Concentration (mg/ml)	*Pheretima posthuma*		*Ascardia galli*	
AE		P	D	P	D
	25	89.03±0.4	120.21±0.6	54.15±0.76	74.5 ± 0.34
	50	72± 0.3	108.98±0.1	46.2 ± 0.21	60.2 ± 0.11
	100	64.73±0.8	95.63±0.1	27.5 ± 0.18	48.5 ± 0.48
EE	25	65± 0.14	72± 0.44	64.04 ± 0.9	79.5 ± 0.23
	50	43± 0.21	66± 0.11	49.7 ± 0.1	68.2 ± 0.1
	100	23± 0.9	33± 0.45	34.2±0.6	45.75±0.23
PC	25	1.5 ± 0.7	54.5±0.4	41.23±0.14	54.5 ± 0.4
	50	0.9 ± 0.12	30.2± 0.1	29.75 ± 0.5	30.2 ± 0.1
	100	0.5 ± 0.17	18.5± 0.8	20.05 ± 0.9	23.5 ± 0.8
Control	_	_		_	

Where, AE: Aqueous extract, EE: Alcoholic extract,PC: piperazine citrate, P: Time taken for Paralysis (min), D: Time taken for Death of worms (min)

Conclusion

From the above results, it is concluded that *Leucas cephalotes* used by tribals traditionally to treat intestinal worm infections, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

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