

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTHELMINTIC ACTIVITY
OF LEAVES OF *LEUCAS ZEYLANICA*

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ABSTRACT

In preliminary phytochemical analysis the extractives of leaves of petroleum ether, chloroform, ethanol (95%) and purified water showed positive reactions for alkaloids, tannins and carbohydrates. Aqueous and ethanol extracts of leaves of *Leucas zeylanica* Linn. (Moraceae) were evaluated separately for anthelmintic activity using Piperazine citrate as reference standard. The results indicated that ethanol extract was more potent than the aqueous extract.

INTRODUCTION

Leucas zeylanica leaves, Family – Lamiaceae, It is an annual herb with erect stem, 20-50 cm tall, many branches from the base. Stem is quadrangular, hairy leaves, simple, opposite, 3-9 cm long, upto 1-3cm wide. Lanceolate. Flowers white, bisexual, numerous and crowded in two or three whorls. Traditionally used for treating skin diseases, wounds, colic, flatulence, iches,^[1] etc.

The preliminary phytochemical examination^[2,3] of *Lecaus zeylanica* Linn leaves showed the presence of tannins and alkaloids. However, no systematic study on preliminary phytochemical screening and anthelmintic activity has been not reported in the literature. In this context the present study is focused to evaluate the anthelmintic activity of *Leucas zeylanica*.



Fig. 1: *Leucas zeylanica* Plant, Family- Lamiaceae.

MATERIAL AND METHODS

Leaves of *Leucas zeylanica* were collected from local region of Mangalore, Karnataka state, in winter season

and dried under shade. The Taxonomist Dr.P. Shivakumar, Department of Botany, Government Science College, Mangalore, and Karnataka identified

the plant. A voucher specimen SCP COGNOSY. 021 are preserved in our research laboratory for future reference.

The collected leaves of the plants were cleaned to remove the adhering soil and debris, dried for about 20 days under shadow. The dried plant material was reduced to powder and passed through Sieve number 10, and stored in air tight containers.

Preparation of the Extracts

Powdered leaves of *Leucas zeylanica* was taken separately into 5000 ml round bottom flask and extracted with following solvents in a ratio of 1:6 by successive extraction process.

- Petroleum ether (60-80°C)
- Chloroform
- Alcohol (95%)
- Chloroform water

Each time before extracting with next solvent the powdered material is dried. After refluxing with all the organic solvents, the marc was finally macerated with chloroform water for seven days to obtain the aqueous extract.

Each extract was concentrated to dryness in flash evaporator under reduced pressure and controlled temperature. The dried extracts were stored in airtight container in refrigerator below 10°C.

Phytochemical Analysis

In preliminary phytochemical analysis the extractives of leaves of petroleum ether, chloroform, ethanol (95%) and purified water showed positive reactions for alkaloids, tannins and carbohydrates.

The physical characteristics and percentage extractives of the extract are tabulated in the Table 1.

Table 1: Physical characters and gram % of various extracts of *Leucas zeylanica* Linn.

Sl. No.	Extract	%	Colour	Consistency	Odour
1	Petroleum ether	6 g	Reddish brown	Dry	None
2	Chloroform	6 g	Dark Green	Shining	Characteristic
3	Alcohol (95%)	18 g	Dark Green	Dry	Characteristic
4	Chloroform water	12 g	Reddish brown	Dry	None

Qualitative Tests for Phytoconstituents

All the extracts were then subjected to preliminary qualitative test to screen for presence of various

phytoconstituents. The test and the report are summarized in the Table.2

Table 2: Preliminary Phytochemical screening of various extracts of *Leucas zeylanica*.

Sl. No.	Chemical Tests	Pet. ether	Chloroform	Alcohol	Aqueous
I	Tests for Steroids				
	a) Salkaowski Test b) Lieberman Burchardt Test	----	----	----	----
II	Test for Triterpenes				
	a) Salkaowski Test	----	----	----	----
	b) LiebermanBurchardt	----	----	----	----
	c) Tschugajiu Test	----	----	----	----
	d) Brisekorn&Brinar Test	----	----	----	----
	e) Trichloro Acetic acid f) Kahlenberg Test	----	----	----	----
III	Test for Saponins				
	a) Foam Test b) Haemolysis Test	----	----	----	----
IV	Tests for Alkaloids				
	a) Mayer's Test	----	+	+	----
	b) Drageondroffs Test	----	+	+	----
	c) Hager's Test d) Wagner's Test	----	+	+	----
V	Tests for Carbohydrates				
	a) Molisch Test	----	----	----	----
	b) Fehlings Test	----	----	----	----
	c) Benedicts Test d) Barfoeds Test	----	----	----	----
VI	Tests for Flavonoids				
a) Ferric chloride Test	----	----	----	----	

	b) Shinoda Test c) Zinc-HCl Reduction d) Lead Acetate Test	----	----	----	----
VII	Test for Lactones a) Legal's Test b) Feigl's Test c) Baljet Test	----	----	----	----
VIII	Test for Tannins a) Ferric Chloride Test b) Gelatin Test	----	----	+	+
IX	Test for Proteins a) Ninhydrin reagent b) Xantho protein Test c) Biuret Test d) Million's Test	----	----	----	----
X	Test for Glycosides a) Baljet Test b) Legals Test c) Raymond Test d) Keller-Killanis Test e) Lugols Test f) Kedde Test	----	----	----	----

Anthelmintic activity

Preparation of the Extract

The collected leaves were shade dried, coarsely powdered and the powder was exhaustively extracted with chloroform and ethanol (95%) using Soxhlet apparatus.^[5,6] The solvent was then removed under reduced pressure using rotary flash evaporator. It was further concentrated and dried in the desiccator for further studies. The dried extracts were suspended in 1% Tween 80 in normal saline and used for anthelmintic activities.

Evaluation of Anthelmintic Activity

The anthelmintic activity was evaluated on adult Indian earthworms (*Pheretima posthuma* obtained from Horticulture Department). The method of Mathew *et al* and Dash *et al*,^[4-6] was followed for anthelmintic screening. Nine groups; each consisting of six

earthworms of approximately equal size (8±1cm) was released in to the 50 ml of desired formulation at room temperature.

Each group was treated with one of the following: vehicle (1% Tween 80 in normal saline), piperazine citrate (15 mg/ml) and extracts (10, 20 and 50 mg/ml) in normal saline containing 1% Tween 80. Observations were made for the time taken to paralysis and/or death of individual worms up to four hours of test period. The mean paralysis time and mean lethal time for each extract was recorded. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour. Worms were observed at regular intervals for paralysis and death in each concentration was recorded and the results are recorded in Table -3

Table 3: Anthelmintic activity of different extracts of the leaves of *Leucas zeylanica*.

Treatment	Concentration (mg/ml)	Time taken for paralysis (Minutes)	Time taken for death (Minutes)
Vehicle	----	----	----
Piperazine Citrate	15	16	----
Aqueous Extract	20	24	40
	40	20	37
	60	18	32
	80	16	19
	20	47	55
Ethanol Extract	40	28	40
	60	26	35
	80	16	20

RESULTS AND DISCUSSION

In preliminary phytochemical analysis the extractives of leaves of petroleum ether, chloroform, ethanol (95%) and

purified water showed positive reactions for alkaloids, tannins and carbohydrates.

Indigenous drug systems can be a source of variety of new drugs, which can provide to eliminate worms, but their claimed reputation has to be verified on scientific basis.

From the results shown in table, the predominant effect of Piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worms by peristalsis. Piperazine citrate act by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. It was observed that both extracts showed a remarkable dose dependent anthelmintic activity against *Pheritima posthuma*. Both the extracts showed paralysis of worms in a time nearer to that of Piperazine citrate, while showed death of worms in a less time compared to Piperazine citrate especially at higher concentration of 80 mg/ml. Aqueous extract took the least time to cause paralysis and death of the worms than the methanol extract. The observation of result show that the anthelmintic activity of ethanolic extract is more potent compared to aqueous extract. It is quite apparent from the studies that the aqueous extract possesses significant anthelmintic activity. It would be interesting to isolate the constituents responsible for the anthelmintic activity.

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