Pharmacognostical, Phytochemical and Anthelmintic Evaluation of *Leucas indica* (L)

Ramani Ramalingam[1]*, Karra Hima Bindu[2], Boddupalli Bindu Madhavi[1], Anisetti Ravinder Nath[1], and Banji David[2]

[1]Faculty of Technology, Osmania University, Hyderabad. 500017, India.
[2]Nalanda College of pharmacy, Cherlapally Nalgonda. 508001, India.
* corresponding author : Phone: +919866297848 ; E-mail- ram_rpharmacist@yahoo.co.in

ABSTRACT

The present study deals with pharmacognostical, phytochemical and anthelmintic evaluation of entire plant of *Leucas indica*. This evaluation reveals the presence of many phytochemical constituents. Entire plant was extracted with petroleum ether, chloroform and methanol. Crude tannins were isolated from methanol extract and evaluated for anthelmintic activity. Chloroform methanol and crude tannin extracts showed very good activity. Paralysis and death time of crude tannins were very close to standard drug Albendazole.

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***Author for Correspondence:** ram_rpharmacist@yahoo.co.in

INTRODUCTION

*Leucas indica (*Linn) belonging to the family of Lamiaceae is commonly known as Tummi in Telugu. *Leucas indica* is distributed throughout India in common along road ways, waste lands, and river banks and on rocky hills. *Leucas indica* is an erect herb, branches are appressed and pubescent. Leaves are linear-lanceolate, entire undulate or distantly serrate. Flowers are white, shortly pedicelled in whorls, towards the end of the branches. Calyx tube slightly curved, 8- toothed, posterior tooth longer than the rest. Corolla is annulated within near the middle. Stamens are 4. Leaves are used as vermifuge, stomachic, sedative and sores.[1] Phenylethanoid glycosides were isolated from the aerial parts of *Leucas indica Linn* and they were found to contain antioxidant activity along with the inhibitory activity against xanthine oxidsase enzyme.[2] Methanolic extract of aerial parts of *Leucas indica* showed antipyretic activity.[3] Methanolic extract was found to show a potential reduction in spontaneous activity and cause a significant decrease in exploratory behavioural pattern by the head dip and Y-maze test. It also shows a significant reduction in muscle relaxant activity by rotarod, 30 inclined screen and traction tests. The extract shows a remarkable potentiation of pentobarbitone induced sleeping time in mice.[4] Methanolic extract of herb caused a significant reduction of blood glucose levels in streptozocin induced diabetes[5] and has antitussive activity [6] as well as wound healing activity.[7]

MATERIALS AND METHODS

Plant material

The whole plant of *Leucas Indica* was collected in February 2008 from Thirumala hills, Andhra Pradesh,India. *Leucas Indica* (L) R .Br. ex Vatke (Lamiaceae) was authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The entire plant was cleaned, dried at room temperature and stored properly in air tight container. The dried plant material was then subjected to size reduction to obtain coarse powder using grinder. This powdered material of no 16 mesh size was then used for further process.

Macroscopic and microscopic analysis

The macroscopy and microscopy of the powder were studied according to the method of Khandelwal and Kokate. For the microscopic studies, cross sections were

prepared and stained as per the procedure prescribed by Khandelwal and Kokate.[8–10] Micro powder analysis was done as per method prescribed by Vijayakumari. [11]

Physico- chemical analysis

Physico-chemical analysis like moisture content, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive and water soluble extractive values were performed according to the official methods prescribed by Indian Pharmacopeia[12], Khandelwal and Kokate.[8–10]

Preparation of different plant extracts

The powdered plant material (2 kg) was extracted with solvents of increased polarity such as, petroleum ether, chloroform and methanol for 24 h with each solvent by hot extraction using Soxhlet apparatus at 60 °C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a dessicator until used for further studies.

Isolation of crude tannins

1 g of methanolic extract of plant was agitated vigorously with 80% acetone at 55°C in a reactor fitted with propeller, stirrer and proper baffles to produce uniform mixing. The slurry after cooling was filtered and acetone was recovered. The left over slurry was suspended in distilled water (three times) and stirred to form a homogenous solution. It was filtered and dried.[13]

Percentage purity of isolated crude tannins

100 mg of isolated crude tannins were dissolved in 5 ml of water and filtered. From the filtrate 1 ml was pipetted out and diluted to 7.5 ml with water. To this 0.1 ml indigo sulphonic acid solution was added and titrated against 0.01N potassium permanganate solution until the colour changes to golden yellow. Percentage of total tannins was calculated as per standard procedure.[13]

Phytochemical study

Powder of entire plant material was subjected to fluorescence analysis. A portion of residue from each extract and crude tannins from methanol extract was subjected to phytochemical analysis in order to see the presence of steroids, alkaloids, tannins, proteins, glycosides, carbohydrates, phenols, flavonoids, volatile oils, saponins and starch.[8,10]

Thin layer chromatography study

All the extracts of plant were subjected to thin layer chromatographic study to determine the number of spots and corresponding R_f values by developing different solvent systems. TLC was performed using pre-coated silica gel TLC plates of E-Merck, Germany. The developed TLC plates were observed under daylight, UV light, iodine chamber and by spraying various detecting reagents.

Anthelmintic activity

Prepared extracts and isolated crude tannins were evaluated for anthelmintic activity separately. Adult Indian earth worms, *Pheretima posthuma* were chosen for the study due to their anatomical and physiological resemblance with the intestinal round worm parasite of human beings.[14] They were collected from in Nalgonda region and identified by Sri Prasad Traders, Nalgonda, and Andhra Pradesh, India.

The earthworms of nearly equal size (6 cm±1) were acclimatized to the laboratory condition before experimentation. The earth worms were divided into six groups of six earth worms in each. Albendazole was diluted with 5% DMF in Normal saline solution to obtain 10 mg, 25 mg and 50 mg served as standard and poured into petridishes. The extracts were dissolved 5% DMF in normal saline solution and diluted to prepare three concentrations such as 10 mg, 25 mg, and 50 mg and poured into Petri dishes. 5% DMF in normal saline solution was taken as control. Earth worms were placed in Petri dishes containing different concentrations of standard and extracts as well as crude tannins at room temperature. The mean paralysis time and mean death time for each sample was calculated (all the readings were taken in triplicate).The time taken for worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates and induce movement in earth worm if alive.[15]

RESUTS AND DISCUSSION

Macroscopic characters of powder – The colour of the powder is light reddish green, odourless and has bitter taste.

Microscopy characters

Transverse section of stem – The stem in cross – sectional view (Fig.1) consists of epidermis. Epidermis was the outer most layer composed of rectangular cells, which were closely arranged without intercellular spaces. Cortex was very small, with the presence of hypodermis. Hypodermis

was very thick found beneath the epidermis where the cell were sclerenchymatous and were arranged compactly without any intercellular spaces in between. Remaining part of the stem was occupied by the ground tissue called parenchymatous tissue with chloroplast. Vascular bundles were present in ground tissue where xylem vessels were separated by the multi seriate medullar rays. Lignified pericyclic fibres were present in form continuous circle. Pith was present in small central parenchymatous portion.

Transverse section of root - Transverse section of root was shown in Fig. 2. Cork appeared as yellowish brown present in many layers. It was followed by cortex which was not so distinct, but the endodermis consisted of rectangular cells which were arranged in single layer. It was followed by 3–4 layers of pericyclic fibres, xylem vessels and phloem cells. The remaining place in between the xylem and phloem is occupied by the parenchymatous cells and pith was present in the form of small central parenchymatous portion.

Powder characters – On microscopial examination the powder showed the following cells (Fig. 3, 4, 5 and 6). Cork cells were thick walled without intercellular spaces. Lignified fibers were thick walled uniform in thickness, long, vertical and present in single fragments. Epidermal cells were thick walled in nature. Trichomes were unicellular head and uniserate in nature. Lignified reticulated xylem vessels were present. The length and width of phloem fibre was found to be 81–124 µm and 30–60 µm respectively. The size of the calcium oxalate was found to be 5–25 µm (length) and 25 µm (width).

Micropowder analysis

Fluorescence analysis of powder – The powdered drug showed color change under visible light and ultraviolet light after treatment with different chemical reagents. This

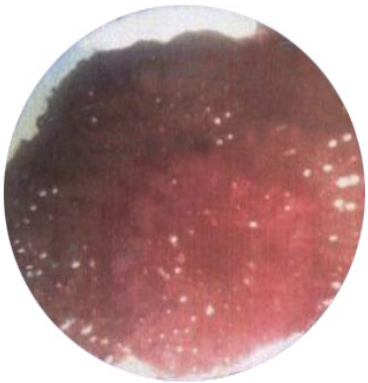

Figure 1. Transverse section of stem of *Leucas indica*

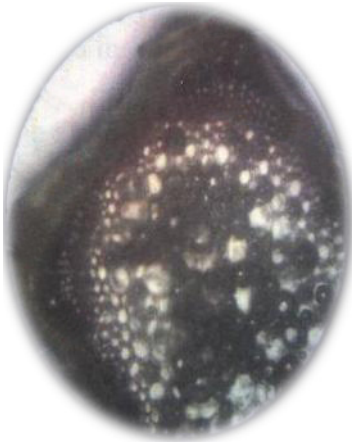

Figure 2. Transverse section of root of *Leucas indica*

Figure 3. Xylem fibres of *Leucas indica*

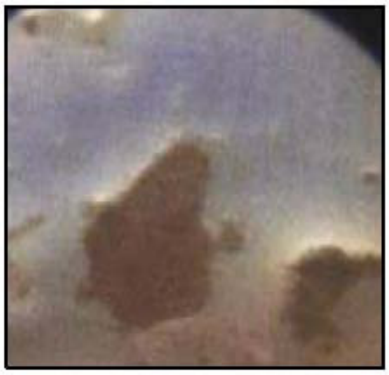

Figure 4. Cork cells of *Leucas indica*

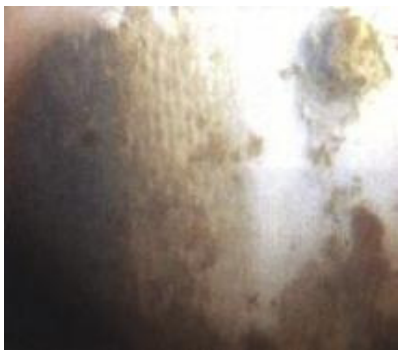

Figure 5. Epidermal cells of *Leucas indica*

Figure 6. Fibres of *Leucas indica*

fluorescence analysis revealed the presence of chemical constituents with fluorescent character. The results were shown in Table 1

Chemical analysis of powder – Treatment of powdered drug with different chemical reagents was revealed the presence of different chemical constituents (Table 2). In this analysis, on treatment with water it produced foam which indicates the presence of saponins. The foaming index was found to be 5%.

Physico-chemical analysis –Acid insoluble ash is used to know the percentage of dirt and sand. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The ash value, extractive value and loss on drying were shown in Table 3. Total ash values of a drug give information about inorganic compounds such as carbonates, phosphates, silica and silicates, which are naturally occurring in drug or deliberately added to it as a form of adulterant.

Extraction

Powder of the *Leucas indica* was extracted with petroleum ether, chloroform and methanol. % yield of petroleum ether, chloroform and methanol extracts were found to be 2.43 (green color mass), 2.87 (green color mass) and 4.53 (reddish brown color mass) respectively. % purity of tannins in chloroform and methanol extracts were found to be 0.089 ± 0.0577 and 0.16 ± 0.0034 respectively (n=3). Amount of tannin in methanolic extract is greater than chloroform extracts. Therefore, crude tannins were isolated from methanolic extract which yielded 0.0039%.

Table 2: Chemical analysis of entire plant of Leucas indica

Treatment	Observation
Powder as such	Light reddish green
Powder + 2% Ferric chloride	Dark green
Powder + 10% Sodium hydroxide	Yellow green
Powder + Sodium hydroxide +Water	Pale green
Powder + 5% Potassium hydroxide	Brownish green
Powder + Water, shake	Foam formation
Powder + Ethanol	Pale green
Powder + Sulphuric acid	Blackish brown
Powder + Hydrochloric acid	Greenish brown
Powder + Nitric acid	Brown

Table 3: Ash and extractive values of entire plant powder of Leucas indica

Parameters	*Leucas indica*
Total ash	5%
Acid insoluble ash	4%
Water soluble ash	6.8%
Alcohol soluble extractive	10.4%
Water soluble extractive	15%
Loss on drying	3.90%

The % purity of tannins in crude tannins was found to be 0.212 ± 0.0043 (n=3).

Phytochemical analysis

Preliminary Phytochemical screening revealed the presence of various phytochemicals given in Table 4. Petroleum ether extract showed the presence of only carbohydrates. Alkaloids, tannins, glycosides, phenols and volatile oil were found in chloroform extract. Alkaloids, tannins, glycosides, carbohydrates, phenols, flavonoids, and saponin were found in methanolic extract. Crude tannins isolated from methanol were subjected to phytochemical analysis showed positive result for tannins, phenols and flavonoids but negative for Alkaloids, glycosides, carbohydrates and saponins

Table 1: Fluorescence analysis of the entire plant of Leucas indica

Treatment	Visible light	Ultraviolet light
Powder as such	Dark green	Light reddish green
Powder + 5% Sulphuric acid	Reddish brown	Black
Powder + Ethanol	Light green	Dark green
powder + 1N sodium hydroxide	Reddish green	Pale green

Table 4: Phytochemical screening of the entire plant powder of Leucas indica

Constituents	Petroleum ether extract	Chloroform extract	Methanolic extract
Steroids	–	–	–
Alkaloids	–	+	+
Tannins	–	+	+
Proteins	–	–	–
Glycosides	–	+	+
Carbohydrates	+	–	+
Phenols	–	+	+
Flavonoids	–	–	+
Volatile oil	–	+	–
Saponins	–	–	+
Starch	–	–	–

"+" indicates the presence of constituents "–" indicates the absence of constituents

Table 5: TLC analysis of entire plant powder of Leucas indica

Extracts	Solvent system	R_f values
Petroleum ether	Benzene : Diethyl ether [6:1]	0.28, 0.55, 0.75, 0.86.
Chloroform	Toluene : Ethyl acetate: Methanol [7:2:1]	0.60, 0.78, 0.88.
Methanol	Toluene : Ethyl acetate [7:3]	0.41, 0.61, 0.21, 0.50, 0.82.

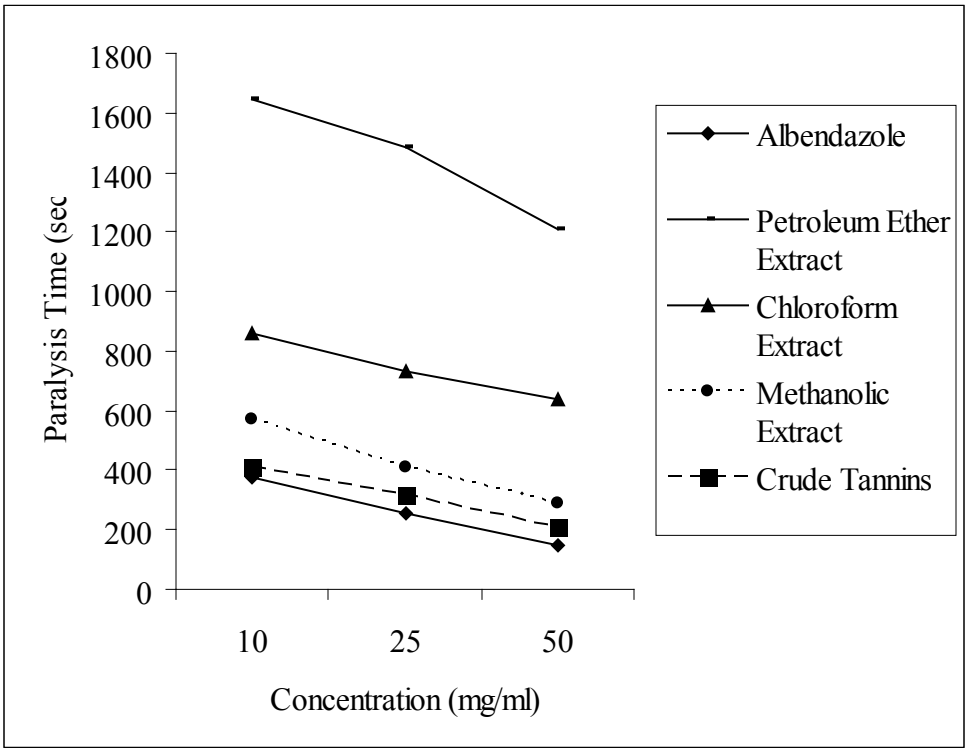

Figure 8. Death time for Anthelmintic activity of all extracts and crude tannins

indicating that crude tannins had some amount of phenol and flavonoids.

TLC analysis

Thin layer chromatography was performed for all the extracts and results were shown in Table 5. Crude tannins showed more than one spot on TLS plates. This may be due to presence of phenols and flavonoids along with tannins.

Evaluation of anthelmintic activity

Anthelmintic activity was evaluated for petroleum ether, chloroform, methanol extracts and isolated crude tannins. The results of paralysis and death time were shown in Table 6, Fig. 7 and 8. Among the extracts, methanolic extract showed very good anthelmintic activity. Methanolic extract had paralysed the earthworms within 9.3 min, 6.5 min and 4.5 min at the concentrations of 10 mg, 25 mg and 50 mg per ml respectively. Control was observed for more than 8 hrs and no paralysis and death were occurred. Crude tannins isolated from methanolic extract were evaluated for anthelmintic activity at various concentrations in mg per ml level. At all the concentrations, paralysis and death time of crude tannins were less than the all extracts. Paralysis and death times of crude tannins were very close to the paralysis and death times of Albendazole.

CONCLUSION

The present study on pharmacognostical evaluation on *Leucas indica* will provides useful information for its identification. Phytochemical analysis conform the presence of different phytochemicals in *Leucas indica*. The values of paralysis time and death time of methanolic extract as well as isolated crude tannins are very close to the values of albendazole. So the plant, *Leucas indica* possess anthelmintic activity. Future plan of work includes purification and characterization of isolated tannins from *Leucas indica*.

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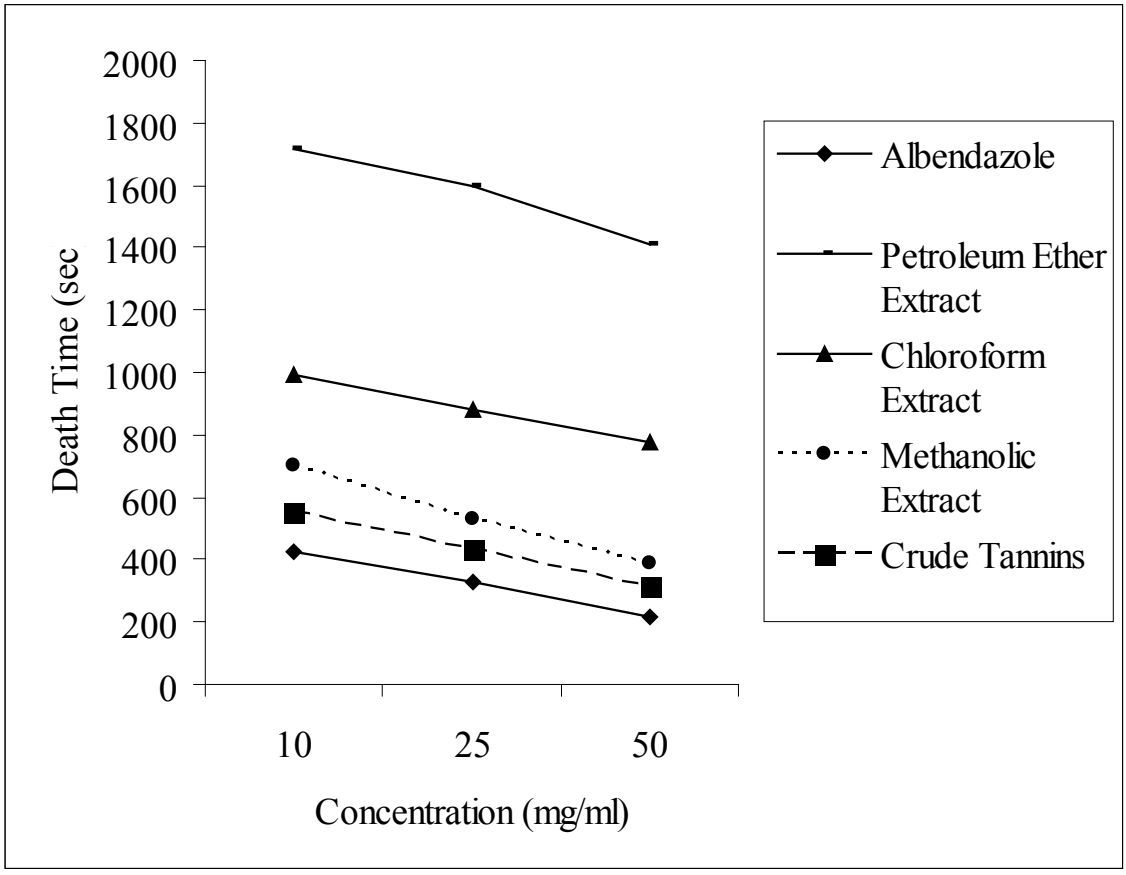

Figure 7. Paralysis time for Anthelmintic activity of all extracts and crude tannins

Table 6: Anthelmintic activity of entire plant powder of Leucas indica

Extract	Concentration (mg/ ml)	Time in minutes (Mean ± SEM where n=3)	
		For paralysis	For death
Control (5% Dimethyl formamide in Saline solution)		-----	-----
Standard Albendazole	10	6:16 ± 0.01	7:08 ± 0.034
	25	4:15 ± 0.011	5:30 ± 0.015
	50	2:28 ± 0.011	3:37 ± 0.0142
Petroleum ether	10	27:28 ± 0.020	28:36 ± 0.026
	25	24:42 ± 0.022	26:37 ± 0.013
	50	20:07 ± 0.06	23:34 ± 0.031
Chloroform	10	14:18 ± 0.07	16:35 ± 0.029
	25	12:15 ± 0.01	14:38 ± 0.017
	50	10:37 ± 0.046	12:55 ± 0.033
Methanol	10	9:31 ± 0.030	11:38 ± 0.027
	25	6:52 ± 0.048	8:53 ± 0.034
	50	4:50 ± 0.018	6:25 ± 0.02
Crude tannins	10	6:52 ± 0.017	9:11 ± 0.008
	25	5:13 ± 0.012	7:13 ± 0.020
	50	3:27 ± 0.020	5:10 ± 0.003

"----"indicates no paralysis and death occurred even after 8 hrs.

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