



Antioxidant, analgesic and anti-inflammatory activities of *Leucas Cephalotes* (Roxb.ex Roth) Spreng

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ABSTRACT

The whole plant of the methanolic extract from *Leucas Cephalotes* was screened for *in vitro* antioxidant (using DPPH method), analgesic (using hot plate test in mice) and anti-inflammatory (using rat paw edema test) activity at the doses of 200 and 400 mg/kg. The methanolic extract of *Leucas Cephalotes* (MELC) has scavenged the DPPH radicals in a dose dependent manner. The IC₅₀ value to scavenge DPPH radicals was found to be 427.3 µg/ml. A significant (p<0.0005) analgesic activity was observed at 60 min with 200 mg/kg and 400 mg/kg has exhibited maximum activity. The maximum anti-inflammatory response was produced at 3 hr and 2 hr with doses of 200 and 400 mg/kg respectively. These results suggest that the methanolic extract *Leucas Cephalotes* has exhibited significant analgesic and anti-inflammatory effects, which were comparable with standard drugs.

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KEYWORDS

Leucas Cephalotes;
Antioxidant;
Analgesic activity;
Anti-inflammatory activity;
DPPH.

INTRODUCTION

Leucas Cephalotes (synonym: *L. Capitata*) is an herb of the family Labiatae, have been extensively used by rural people of Bihar, India^[1]. The plant is also known as "Dronapushpi in sanskrit and Peddatumni in telugu by Indians"^[2]. It is distributed in India, Nepal, Pakistan and Afghanistan. Medicinally it is used as diaphoretic, anti inflammatory and obstinate urinary troubles in the ayurvedic system of medicine^[3]. The whole plant was used to treat bronchitis, inflammation, asthma, dyspepsia, paralysis and skin diseases. The leaf juice, some times mixed with honey to treat coughs and colds among

the Santhalis in southern Bihar and by rural inhabitants of Gujarat in India, where it is also used for the treating jaundice^[1]. This study has been undertaken to evaluate the *In vitro* anti-oxidant, analgesic and anti inflammatory activities of the methanolic whole plant extract of *Leucas Cephalotes* (MELC).

MATERIALS AND METHODS

Plant material

The whole plant of *L. Cephalotes* was collected from Kakatiya University, Warangal. The plant material was authenticated by Prof. V. S. Raju, Dept of

Full Paper

Botany, Kakatiya University, Warangal, Andhra Pradesh, India.

Preparation of extract

The fresh plants were shed dry and coarse powdered store in a airtight container till further use. Then the powder was subjected to exhaustive extraction by maceration process using methanol as a solvent at room temperature. The methanolic extract was concentrated and it is stored in a vacuum desiccator. The suspension of the extract prepared in 2% gum acacia was used in the entire experimental studies.

Drugs and chemicals

The drugs and chemicals used were carrageenan (SD fine chemicals Limited, Mumbai), gum acacia and diclofenac sodium (Dr. Reddy's Labs, Hyderabad), pentazocine (Pure Pharma Ltd., Mumbai), methanol (Merck, Mumbai), DPPH (Sigma, USA).

Phytochemical screening

The methanolic extract was screened for the presence of various phyto-constituents like steroids, alkaloids, terpenoids, glycosides, flavonoids, phenols and carbohydrates^[4].

In vitro antioxidant activity using Diphenyl Picryl Hydrazyl (DPPH) method

The free radical scavenging activity of MELC was measured by DPPH using the method of Blis^[5]. Ascorbic acid was used as a reference standard. The methanolic solution of DPPH (0.2mM) was added to different concentrations (100 to 800 mg/ml) of MELC solution. After 30 min, absorbance was measured at 517 nm. The degree of discolouration indicates the scavenging potential of the extract. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The IC₅₀ (Inhibitory concentration) is the concentration of sample required to scavenge 50% of DPPH free radicals.

Animals

Albino mice (25-30g) and Wister rats (175-250 g) of either sex were used for the assessing for the biological activity. The animals were maintained under standard husbandry conditions and had free access to food and water *ad libitum*. The animals were allowed to

acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee.

Evaluation of Analgesic activity of MELC using Hot- Plate method

The hot plate test was used to measure analgesic activity by the method described by Eddy and Leimback^[6] with minor modifications. In this experiment, the hot plate was maintained at $55 \pm 0.5^\circ\text{C}$. All animals were selected 24 hr prior to experimentation and the animals were selected on the basis of their normal reaction time i.e., pain response to the hot plate to the minimum and maximum of 2-15 sec respectively. In order to avoid the damage to the paws of the animals, the time standing on the plate was limited to 25 sec. Pentazocine 10 mg/kg was administered intraperitoneally as a reference standard. 30 min after administration of vehicle (2% gum acacia, p.o.) / methanolic extract (200 and 400 mg/kg, p.o.) / standard drug animals were placed individually on to the hot plate and the time from placing the animal on the hot plate to jumping of the animal from the hot plate was recorded as the reaction time or latency of the pain response.

Evaluation of Anti-inflammatory activity of MELC using Carrageenan induced paw edema method

The normal paw volumes of all the rats were measured initially and were divided into four groups each consists of six animals treated orally with the vehicle as control (2% gum acacia, p.o.), standard diclofenac sodium (20 mg/kg, p.o.) and methanolic extract (200 and 400 mg/kg, p.o.) respectively. Carrageenan (0.1 ml of a 1% suspension in saline) was injected sub plantar region of the right hind paw of each rat. The vehicle, drug and extract were administered 30 min prior to the injection of Carrageenan. The paw volumes of all the rats were recorded at 1, 2, 3 and 4 hr after Carrageenan treatment by using plethysmometer^[7]. A significant reduction in the paw volume compared to vehicle treated control animals was considered a inflammatory response.

$$\% \text{ Inhibition} = \frac{[(V_T - V_0) \text{ control} - (V_T - V_0) \text{ treated groups}]}{(V_T - V_0) \text{ control}} * 100$$

V_0 = Paw volume of the rat before administration of Carrageenan

V_T = Paw volume of the rat after administration of Carrageenan at different time intervals

Statistical analysis

All the results were expressed as mean \pm SD and analyzed by one way ANOVA followed by Dunnet's test and $P < 0.05$ was considered as a statistical significant.

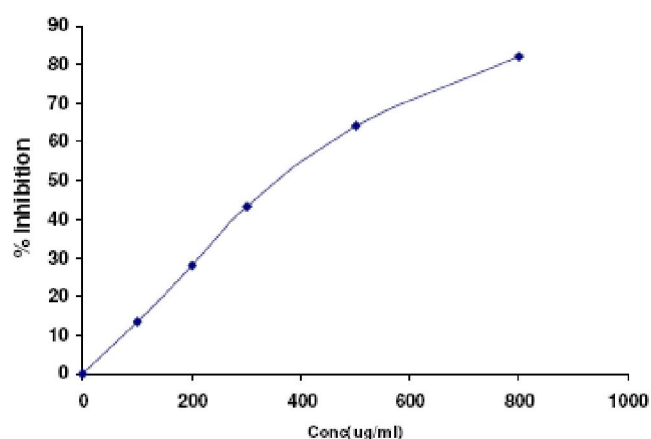
RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary phytochemical screening of the methanolic extract of *L. Cephalotes* reveals the presence of steroids, phenols, terpenoids, carbohydrates and glycosides. Regarding the chemical constituents of the methanolic extract of *Leucas Cephalotes*, sterols, β -sitosterol and its glucosides has been reported^[8]. Yukunori *et al.*,^[9] also reported that the methanolic extract of whole plant of *Leucas Cephalotes* consists of labdane, norlabdane, abietane-type diterpenes named leucadins and two protostane-type triterpenes named leucastrins; five sterols and eight flavones. The structures of known compounds were identified as oleanolic acid^[10], 7-oxysitosterol^[11,12], 7-oxostigmasterol^[13], 7 alpha-hydroxysitosterol^[11], 7alpha-hydroxystigmasterol^[14], stigmasterol^[15], 5-hydroxy-7, 4¹-dimeathoxyflavone^[16], pillion^[17], gonzalitosin^[18], tricinin^[19], apigenin 7-0- β -D-(6-O-p-coumaroyl) glucopyranoside^[20] and anisofolin A^[21].

Antioxidant activity

In the present study, DPPH radical was used as a substrate to evaluate the free radical scavenging activity of MELC extract. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate antioxidant activity of MELC extract. The DPPH scavenging property of MELC was showed in Figure 1. The IC₅₀ value of the extract was found to be as 427.3 μ g/ml.



Plants produce a variety of antioxidants against molecular damage from reactive oxygen species [ROS], produced by arterial wall macrophages and phenolics compounds are the major class of plant derived antioxidants. Among the various phenolic compounds, the flavonoids are perhaps the most important group^[22]. In the present study, the antioxidant activity of *L. Cephalotes* might be due to the presence of flavonoids like 5-hydroxy 7, 4¹-dimeathoxyflavone, pillion, gonzalitosin, tricinin the methanolic extract.

Analgesic and anti-inflammatory activity

The results of hot plate method for analgesic and rat paw edema method for anti inflammatory activity of methanolic extract of *L. Cephalotes* were reported in TABLE 1 and TABLE 2. A significant ($p < 0.0005$) analgesic effect was observed at 60 min with 200 mg/kg of *L. Cephalotes*. Higher doses i.e 400 mg/kg of methanolic extract had exhibited highest analgesic effect to the thermal stimulus at 120 min which is comparable to the effect of standard pentazocine.

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer and vascular disease^[23-25]. Carrageenan has been widely used as a noxious agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity^[26]. The development of edema induced by carrageenan corresponds to the events in the acute phase of inflammation, mediated by histamine, bradykinin and prostaglandins produced under an effect of cyclo-oxygenase^[27]. After Carrageenan administration paw edema in rats reached to peak value at 3 hr and the maximum anti inflammatory response

Full Paper

was produced at 3 hr and 2 hr with doses of 200 and 400 mg/kg respectively. These analgesic and anti-inflammatory activities of MELC may be due to the presence of above mentioned flavones, terpenes, glycosides

and steriols.

In conclusion, the methanolic extract of *L. Cephalotes* have exhibited a significant antioxidant, analgesic and anti inflammatory activity.

TABLE 1 : Effect of methanolic extract from *L. Cephalotes* on the hot plate test in mice

S. No	Group	Dose (mg/kg)	Reaction time after administration of control/ standard/ extract in sec			
			0 min	60 min	120 min	240 min
1.	Control		2.17 ± 0.75	2.33 ± 0.52	2.17 ± 0.41	1.67 ± 0.52
2.	Pentazocine	10	2.83 ± 0.75	6.83 ± 0.75 ^c	6.33 ± 1.63 ^b	2.33 ± 0.52 ^a
3.	<i>L. Cephalotes</i>	200	2.67 ± 0.82	7.33 ± 1.03 ^c	8.00 ± 1.10 ^c	2.00 ± 0.63
4.	<i>L. Cephalotes</i>	400	2.83 ± 0.75	8.17 ± 1.17 ^c	9.17 ± 0.75 ^c	2.17 ± 0.75

Values are in mean ± SD; (n =6), a = p < 0.05, b = p < 0.005, c = P < 0.0005 Vs Control.

TABLE 2 : Effect of methanolic extract from *L. Cephalotes* on the paw edema test in rats

S. No	Group	Dose (mg/kg)	Paw edema volume after			
			1 hr	2 hr	3 hr	4 hr
1.	Control		0.18 ± 0.02	0.20 ± 0.03	0.22 ± 0.03	0.18 ± 0.02
2.	Diclofenac Sodium	20	0.14 ± 0.02 ^a	0.13 ± 0.02 ^c	0.12 ± 0.02 ^c	0.12 ± 0.02 ^c
3.	<i>L. Cephalotes</i>	200	0.15 ± 0.01 ^a	0.14 ± 0.01 ^b	0.13 ± 0.02 ^c	0.14 ± 0.02 ^a
4.	<i>L. Cephalotes</i>	400	0.14 ± 0.01 ^a	0.12 ± 0.01 ^c	0.12 ± 0.02 ^c	0.13 ± 0.03 ^a

Values are in mean ± SD; (n =6), a= p < 0.05, b=p<0.001, c= p < 0.005 Vs Control.

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Full Paper

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