

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

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The whole plant of the methanolic extract from *Leucas cephalotes* was screened for *in vitro* antioxidant (using the DPPH method), *in vivo* analgesic (using hot plate test in mice) and anti-inflammatory (using rat paw edema test) activities. The methanolic extract of *Leucas cephalotes* (MELC) scavenged the DPPH radicals in a dose-dependent manner. The IC₅₀ value to scavenge DPPH radicals was found to be 421.3 µg/ml. A significant (p<0.0005) analgesic activity was observed at 60 min with 200 mg/kg, and 400 mg/kg 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 from *Leucas cephalotes* exerts significant analgesic and anti-inflammatory effects, which were comparable with standard drugs.

Uniterms: *Leucas cephalotes*/analgesic activity. *Leucas cephalotes*/antioxidant activity. *Leucas cephalotes*/anti-inflammatory activity. Diphenyl Picryl Hydrazyl assay.

O extrato metanólico total de *Leucas cephalotes* foi submetido à triagem para as atividades antioxidante *in vitro* (utilizando o método DPPH), analgésica (utilizando teste da placa quente, em camundongos) e antiinflamatória (utilizando teste de edema da pata de rato), nas doses de 200 e 400 mg/kg. O extrato metanólico de *Leucas cephalotes* (MELC) inativou radicais difenil picril hidrazila (DPPH) de forma dose-dependente. O IC₅₀ para essa atividade foi de 421,3 µg/mL. Observou-se atividade analgésica significativa (p<0,0005) a 60 minutos, com 200 mg/kg, e, com 400 mg/kg, observou-se atividade máxima. A resposta antiinflamatória máxima foi produzida, respectivamente, em 3 h e 2 h, com doses de 200 e 400 mg/kg. Estes resultados sugerem que o extrato metanólico de *Leucas cephalotes* apresenta efeitos analgésico e antiinflamatório significativos, comparáveis aos fármacos padrão.

Unitermos: *Leucas cephalotes*/atividade analgésica. *Leucas cephalotes*/atividade antioxidante. *Leucas cephalotes*/atividade antiinflamatória. Difenil Picril Hidrazila/análise.

INTRODUCTION

Leucas cephalotes (synonym: *L. Capitata*) is a herb of the family Labiatae and has been extensively used by rural people of Bihar, India (Kamat *et al.*, 1994). The plant is also known as “Dronapushpi in Sanskrit and Peddatumni in telugu by Indians” (Parrotta *et al.*, 2001). The whole plant was used to treat bronchitis, inflammation, asthma, dyspepsia, paralysis and skin diseases. The leaf

juice is sometimes 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 treating jaundice (Kamat *et al.*, 1994). The ethyl extract of whole plant of *Leucas cephalotes* has been reported for its protective effects on CCl₄-induced hepatotoxicity in mice and rats (Singh *et al.*, 1978). Nineteen compounds were isolated by chromatographic separation of 1-butanol-soluble fraction of the MeOH extract of *Leucas cephalotes* (Miyachi *et al.*, 2006). The structures of known compounds were identified as oleanolic acid, 7-oxysitosterol (Greca *et al.*, 1990; Pettit *et al.*, 1972), 7-oxostigmasterol (Katsui *et al.*, 1972), 7 alpha-hydroxysitosterol (Greca *et*

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al., 1990), 7 alpha-hydroxystigmasterol (Miyachi *et al.*, 2006), stigmasterol (Kolak *et al.*, 2005), 5-hydroxy-7, 4¹-dimeoxyflavone (Gonzalez *et al.*, 1989), gonzalitosin (Dominguez *et al.*, 1976), tricetin (Fujii *et al.*, 1995) and apigenin 7-0- β -D-(6-O-p-coumaroyl) glucopyranoside (Itokawa *et al.*, 1981). The aim of the present study was to evaluate the *In vitro* anti-oxidant, *In vivo* 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 Botany, Kakatiya University, Warangal, Andhra Pradesh, India.

Preparation of extract

The whole plant was cut into small pieces, shade dried and then ground into coarse powder (2kg). The powder was then subjected to exhaustive extraction by a maceration process using methanol as a solvent at room temperature for 7 days. The methanolic extract was concentrated by vacuum distillation to dryness; the yield obtained was 7.5% w/w with respect to dried plant material. The collected extract was stored in a desiccator. A suspension of the extract prepared in 2% gum acacia was used in experimental studies.

Drugs and chemicals

The drugs and chemicals used were carrageenan, ascorbic acid (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).

Animals

Albino mice (25-30g) and Wistar rats (175-250 g) of both sexes were used for assessing 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 consisting of six animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accor-

dance with guidelines of the Institutional Animal Ethics Committee, UCPSc, Kakatiya University, Warangal, India.

Phytochemical screening

Phytochemical properties of the extract were tested using the following chemicals and reagents (Trease and Evans, 1983): Alkaloids with Mayer and Dragendorff's reagents, saponins (frothing test), tannins (FeCl₃), glycosides (NaCl and Fehling's solution A and B), flavanoids (NaCl and HCl), anthraquinones (Borntrager's reaction), phenols (FeCl₃ and K₃Fe(CN)₆), carbohydrates (Molisch's test) and lipids (filter paper).

EXPERIMENTAL

Antioxidant activity

Diphenyl Picryl Hydrazyl (DPPH) method

The free radical scavenging activity of MELC was measured by DPPH using the method of Blies (Blies *et al.*, 1958). Ascorbic acid was used as a reference standard. The methanolic solution of DPPH (0.2mM) was added to different concentrations (100 to 800 μ g/ml in methanol) of MELC solution. After 30 min, absorbance was measured at 517 nm. The degree of discoloration 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.

Analgesic activity

Hot-Plate method

The hot plate test was used to measure analgesic activity by the method described by Eddy and Leimback (Eddy *et al.*, 1953) 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 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 damaging 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. Thirty minutes after administration of vehicle (2% gum acacia, p.o.) / methanolic extract (200 and 400 mg/kg, p.o.) / standard drug, animals were placed individually onto 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.

Anti-inflammatory activity

Carrageenan-induced oedema test

The normal paw volumes of all the rats were measured initially and then divided into four groups each comprising six animals, treated orally with the vehicle as controls (2% gum acacia, p.o.), with standard diclofenac sodium (20 mg/kg, p.o.), and with methanolic extract (200 and 400 mg/kg, p.o.), respectively. Carrageenan (0.1 ml of a 1% suspension in saline) was injected into the 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 a plethysmometer (Turner, 1965). A significant reduction in paw volume compared to vehicle-treated control animals was considered an inflammatory response.

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

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 statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis

Steroids, phenols, terpenoids, carbohydrates and glycosides were identified by preliminary phytochemical tests.

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 is shown in Figure 1. The IC_{50} value of the extract was

found to be 421.3 μ g/ml, which was significantly higher than that of standard antioxidant ascorbic acid (50 μ M). This indicates the lower *in vitro* free radical scavenging capacity (antioxidant activity) of MELC compared to ascorbic acid.

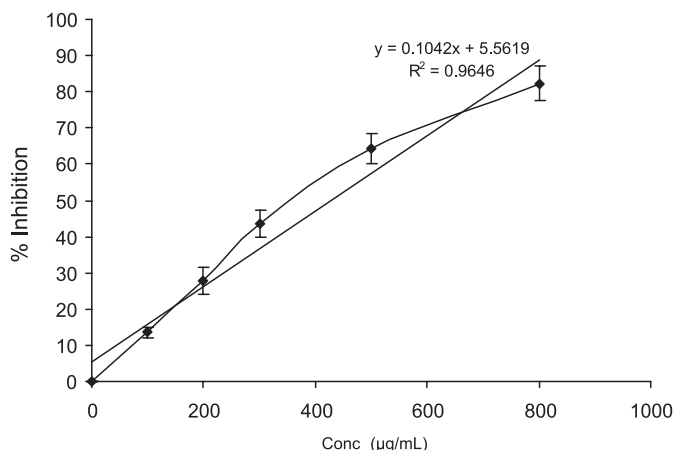


FIGURE 1 - *In vitro* Antioxidant activity of MELC using DPPH method.

Plants produce a variety of antioxidants against molecular damage from reactive oxygen species [ROS], produced by macrophages. Phenolic compounds are the major class of plant-derived antioxidants. Among the various phenolic compounds, flavonoids are perhaps the most important group (Kuo *et al.*, 1992). In the present study, the antioxidant activity of *L. cephalotes* might be due to the presence of flavonoids such as 5-hydroxy 7, 4'-dimeoxyflavone, pillion, gonzalitosin, and triclin, in the methanolic extract.

Analgesic and anti-inflammatory activity

The results of the hot-plate method for analgesic activity and of the rat paw edema method for anti-inflammatory activity, of methanolic extract of *L. cephalotes* are shown in Tables 1 and 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, exhibited the highest analgesic effect in response to the thermal stimulus at 120 min, where this was comparable to the effect of standard pentazocine.

After Carrageenan administration, paw edema in rats reached a peak value at 3 hr and the maximum anti-inflammatory response (inhibition of Carrageenan-induced paw volume) was produced at 3 hr and 2 hr with doses of 200 and 400 mg/kg, respectively.

In conclusion, the methanolic extract of *L. cephalotes* exhibited significant antioxidant, analgesic and anti inflam-

TABLE I - 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 expressed as mean ± SD; (n =6), a= p < 0.05, b= p < 0.005, c= P < 0.0005 Vs Control.

TABLE II - 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 expressed as mean ± SD; (n =6), a= p < 0.05, b=p < 0.001, c= p < 0.005 Vs Control.

matory activity. Further studies are needed for fractionation and purification of the compounds present in the methanolic extract of *L. cephalotes* to identify the chemicals responsible for the biological activities outlined above.

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