



Anti-inflammatory Activity of Whole Plant Parts of *Leucas aspera* (Lamiaceae)

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ABSTRACT

In the present study, the anti-inflammatory potential of solvent extracts of whole plant parts of *Leucas aspera* (Lamiaceae) and isolation of anti-inflammatory compound from potent extracts was determined. Aqueous and methanolic solvent extracts of *Leucas aspera* were found to have significant anti-inflammatory activity at doses 50 and 100 mg/Kg during *in vitro* anti-inflammatory assay with standard Indomethacin 10 mg/kg. The anti-inflammatory activity of the methanol was evaluated by carrageenan-induced acute and formalin-induced chronic anti-inflammatory models in mice. The ethanolic extracts showed remarkable anti-inflammatory activity in both models at a dosage 100 mg/kg. Thus, results showed that extracts showed significant anti-inflammatory activity in dose-dependent manner. And also demonstrate that *Leucas aspera* has antinociceptive and anti-inflammatory activities in albino rats.

Key words: Anti-inflammatory, Lamiaceae, *Leucas aspera*

Today, the search for natural compounds from medicinal plants are rich in antimicrobial, antioxidant and anti-inflammatory properties is escalating due to their medicinal importance in controlling many related chronic disorders i.e. cancer, diabetes, arthritis, hypertension. Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of a large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti-inflammatory action (Marcocci *et al.* 2014). Inflammatory response is a series of well-coordinated dynamic mechanism consisting of specific vascular, humoral and cellular events that is characterized by the movement of fluids, plasma and inflammatory leukocytes (neutrophils, eosinophils, basophils and macrophages) to the site of inflammation (Conner *et al.* 1996). Inflammation and pain are the prime signs of acute and chronic conditions in various diseases. NSAIDs (nonsteroidal anti-inflammatory drugs) are commonly used to treat inflammatory conditions; however, their prolonged use has adverse side effects. Several plants of family Apocynaceae are known for the *in-vitro* and *in-vivo*

anti-inflammatory activity. Subraya and Gupta (2012) studied *in-vivo* anti-inflammatory activity of *Alstonia scholaris* stem bark methanol extract in rat paw edema models. The reported activity of the extract was found comparable to indomethacin (10 mg/kg) with paw edema inhibition of 64.86% and 67.29%, respectively, in carrageenan-induced rat paw edema models. Similarly, Jain *et al.* (2013) reported significant anti-inflammatory activity of different fractions of *Tabernaemontana divaricata* leaves extracts against croton oil-induced edema in male albino mice model. The activity of hexane and methanolic fractions was found comparatively better than indomethacin with IC50 less than 500 µg/cm².

Plants of genus *Leucas* (Lamiaceae) are traditional healers to cure many diseased conditions, which insinuated that this genus has immense potential for the discovery of new drugs. Generally, the plants of genus *Leucas* are shrubs, sub shrubs, annual herbs, or perennial herbs with woody root or stem base. The investigated parts of the *Leucas* species include roots, seeds, stem, leaves, and whole plant parts. The genus *Leucas* comprises about nearly 80 species. The highest species diversity has been found in East Africa and South Africa. In India, 42 species are available (Philipson and Wright 1996, Arunkuma 2009).

Leucas aspera commonly known as 'Thumbai' is one such medicinal plant which is being used traditionally as an antipyretic and insecticide. A part of the plant is also being

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used for many disorders like rheumatism and chronic skin eruptions. However, in this study we focused on anti-inflammatory activity of whole plant extract. The experiments were done by using albino rats according to animal ethics committee. A simple experiment and it could be complete within a weeks period and there is no harm for these rats on this study.

MATERIALS AND METHODS

Plant material: The important medicinal plants *L. aspera* was collected from higher altitudes of Easter Ghats, Lambasingi forest region, Visakhapatnam, Andhra Pradesh. The collected plant materials were grown in pots under suitable climatic condition and this material was used for *in-vitro* studies.

Preparation of extracts: The plant parts were collected and shade dried for about two weeks and ground into coarse powder. About 100g powder extracted with 500 ml of petroleum ether using Soxhlet apparatus. The same plant powders were also extracted with chloroform, acetone and methanol. The extracts were concentrated to dryness to yield crude residue. These residues were used for preliminary phytochemical analysis (Harborne 1973).

Determination of anti-inflammatory activity: Male albino rats (100–200 g) were used taking into account international principles and local regulations concerning the care and use of laboratory animals (Olfert *et al.* 1993). The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at $22 \pm 1^\circ\text{C}$ with a 12 h light/dark cycle. Albino rats were divided into four groups of six animals each for both inflammation models. Group I was kept as control group (vehicle). Group II and III were given methanol extract of *A. paniculatum* 100 mg/kg body weight and 200 mg/kg body weight, respectively. Group IV was given Indomethacin 10 mg/kg body weight.

Carrageenan-induced paw oedema: Paw swelling was induced by sub-plantar injection of 0.1 ml 1% sterile carrageenan in saline into the right hind paw. The solvent extracts of plant at dose of 150 and 200 mg/kg were administered orally 60 minutes before carrageenan injection.

Indomethacin (10 mg/kg) was used as reference drug. Control group received the vehicle only (10 ml/kg). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0, 1, 2, 3, and 4 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Formalin-induced paw oedema: Inflammation was produced by sub plantar injection of 20 μl of freshly prepared 2% formalin in the right hind paw of mice. The paw thickness was measured using vernier callipers 1h before and after formalin injection. The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0, 1, 2, 3, and 4 h after formalin injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined and the percent inhibition of edema was calculated in comparison to the control animals.

Statistical analysis: Two-way analysis of variance (ANOVA) was used for this study and the differences were deemed significant for a *P*-value below 0.05.

RESULTS AND DISCUSSION

The preliminary study on phyto chemical constituents of whole plant parts of *Leucas aspera* revealed the presence of many secondary metabolites like glycosides, flavonoids, tannins, carbohydrates, alkaloids, and phenols. The presence of these compounds can be the reason for medicinal properties and anti-oxidant property of *L. aspera*. The anti-inflammatory activity of methanol extract of *Leucas aspera* against carrageenan induced paw edema shows that the extracts have significant effect on inflammation and markedly reduced the swelling. The percentage reduction in the paw volume in the group of animals treated with *L. aspera* extract 150mg was 71.02%, 300 mg/kg was 81.13% and for the 500 mg/kg was 85.46% at 3 hours. It shows that the plant extract have significant ($P < 0.001$) anti-inflammatory effect and the results were compared with indomethacin 10mg/kg and show percentage paw volume reduction of 85.68%.

Table 1 Percentage of inhibition samples in difference concentrations

Concentration ($\mu\text{g/ml}$)	Petroleum Ether	Ethyl Acetate	Chloroform	Methanol
200	16.11	21.82	23.29	31.51*
100	10.29	15.98	14.09	22.19
50	4.02	8.22	8.27	15.13
25	0.92	4.61	7.59	9.31
12.5	0.50	1.71	2.01	5.11

Means followed by * are statistically significant at $p \leq 0.05$

The acute model of inflammation, upon challenge by phlogistic stimuli, ethanol extract of *Leucas aspera* whole plant showed significant ($P < 0.001$) anti-inflammatory activity. The edema and inflammation induced by carrageenan is shown to be mediated by histamine and 5-HT

during 1 hour, after which increased vascular permeability is maintained by the release of kinins up to 3 hours, the mediators appear to be prostaglandins, the release of which is closed associated with the migration of leucocytes into the inflamed site Rosa *et al.* (1971), Katara *et al.* (2012). Anti-

inflammatory activities of the ethanol extract of *Leucas aspera* in two models reduced the paw oedema significantly ($P<0.001$) (Table 1-2). The concentrations required to

inhibit the paw oedema in both type of inflammations was comparable to the standard reference drug diclofenac.

Table 2 Acute anti-inflammatory activity of methanolic extracts of *A. paniculatum* in carrageenin induced inflammation

Treatment groups	Initial paw thickness (cm)	Paw thickness after 5 hours (cm)	Increase in Paw thickness (cm)	Inhibition
Control	0.188±0.120	0.285±0.215	0.097±0.095	--
Indomethacin (25 mg/kg)	0.195±0.032	0.230±0.043	0.035±0.011	62
Dose I 50 mg/kg	0.202±0.045	0.224±0.076	0.022±0.031	73
Dose II 100 mg/kg	0.215±0.038	0.228±0.059	0.013±0.021	81

Means followed by * are statistically significant at $p\leq 0.05$

Table 3 Effect of chronic inflammatory activity of methanolic extracts of *A. paniculatum* in formalin induced inflammation

Treatment groups	Initial paw thickness (cm)	Paw thickness after 5 hours (cm)	Increase in Paw thickness (cm)	Inhibition
Control	0.198±0.020	0.270±0.025	0.072±0.005	--
Indomethacin (25 mg/kg)	0.195±0.032	0.230±0.078	0.035±0.046	54
Dose I 50 mg/kg	0.202±0.045	0.225±0.083	0.023±0.038	75
Dose II 100 mg/kg	0.228±0.038	0.245±0.059	0.017±0.023	83

Means followed by * are statistically significant at $p\leq 0.05$

Recent studies suggest that the inflammatory tissue damages are due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (Conner *et al.* 1996). In addition to this, nitric oxide is also implicated in inflammation, cancer, and other pathological condition (Farrel *et al.* 1992). (Table 3) represents the initial paw thickness and increase the paw thickness (cm) and inhibition. The development of carrageenan-induced oedema is biphasic; the first phase is attributed to the release of histamine, 5-HT, and kinins, while the second phase is related to the release of prostaglandins (Wheeler and Cowan 1991). The alkaloids, tannins, flavonoids and phenol compounds play a major role in preventing a number of

chronic diseases by a definite physiological action on the human body like anti-inflammatory, anti-thrombotic, anti-oxidant, hepatoprotective and anticarcinogenic activities. (Rumaisa *et al.* 2013).

It can be concluded that *L. aspera* has antinociceptive and anti-inflammatory activities in albino rats. This extract may also useful for the studies like anti inflammation in other animal models.

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LITERATURE CITED

- Arunkumar S and Muthuselvam M. 2009. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World Journal of Agricultural Sciences* **5**(5): 572-576.
- Conner E M and Grisham M B. 1996. Inflammation, free radicals, and antioxidants. *Nutrition* **12**(4): 274-277.
- Di Rosa M L, Giroud J P and Willoughby D A. 1971. Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *The Journal of Pathology* **104**(1): 15-29.
- Farrel A J, Blake D R, Palmer R M J and Moncada S. 1992. *Annals of Rheum Disease* **51**: 12-19.
- Harborne J B and Williams C A. 2000. Advances in flavonoid research since 1992. *Phytochemistry* **55**: 481-504.
- Jain S, Sharma P, Ghule S, Jain A and Jain N. 2013. In-vivo anti-inflammatory activity of *Tabernaemontana divaricata* leaf extract on male albino mice. *Chinese Journal of Natural Medicines* **11**(5): 472-476.
- Kataria S, Shrinivatava B, Kaur D and Sharma P. 2012. Antiinflammatory and antinociceptive activities of *Crotolaria burhia* Buch-Ham whole plant. *Indian Journal of Natural Products Research* **3**: 189-196.
- Marcocci L, Maguire J J, Droylefaix M T and Packer L. 1994. The nitric oxide-scavenging properties of Ginkgo biloba extract EGB 761. *Biochemical and Biophysical Research Communications* **201**(2): 748-755.
- Parke D V and Sapota A. 1996. Chemical toxicity and reactive oxygen species. *International Journal of Occupational Medicine and Environmental Health* **9**(4): 331.
- Phillipson J D and Wright C W. 1996. Plants with antiprotozoal activity, Tease and Evans, Pharmacognosy, 14th Edition, WB Saunders Company, London. pp 612.
- Rumaisa Y, Latha B, Soumya C K, Shafeena S and Sadhiya N. 2013. Phytochemical studies on *Leucas aspera*. *Journal of Chemical and Pharmaceutical Research* **5**(4): 222-228.
- Subraya C K and Gupta D. 2012. Antioxidant anti-inflammatory activity of *Alstonia scholaris* R. Br. stem bark extract. *Free Radicals and Antioxidants* **2**(2): 55-57.
- Wheeler-Aceto H and Cowan A. 1991. Neurogenic and tissue-mediated components of formalin-induced edema: evidence for supraspinal regulation. *Agents and Actions* **34**(1/2): 264-269.