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Phytochemical Analysis and Invitro Ant-Inflammatory Activity of Leucus Aspera Leaves

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ABSTRCT : The systematic evaluation of the anti-inflammatory activity of indigenous plants used in wound healing will facilitate the development of efficient wound healing drugs. *Leucas aspera* is the medicinal plant commonly known as "Thumbai" is found all over India and reported to have anti-inflammatory, analgesic, antidiarrheal, antimicrobial, antioxidant, and insecticidal activities. *L. aspera* have been used in traditional medicine for treating wounds for fastening wound healing process. The present study was undertaken to evaluate the efficiency of anti-inflammatory activity of medicinal plant *Leucas aspera.* Hexane, ethyl acetate, acetone, and ethanol extracts of *L.aspera*, were subjected to of anti-inflammatory property of leaves by both *in vitro* and *in vivo* methods. *In vitro* method was estimated by human red blood cell membrane stabilisation (HRBC) method and *in vivo* method was estimated on the carrageenan induced paw oedima. These observations will stimulate further research in the clinical application of the plants under study. The study proves the anti-inflammatory efficacy of the plant and hold a good prospect for drug development against inflammatory diseases.

Keywords: *Leucas aspera,* Anti-inflammation, HRBC Carrageenan

1. INTRODUCTION

Many plants have been used for wound healing in traditional systems of medicine since ancient times. However prolonged inflammation may delay natural healing process, a good healing agent should possess anti-inflammatory activity for the proper wound management (Subramoniam et al., 2001, Annan and Houghton, 2008, Barreto et al., 2014). The systematic evaluation of the anti-inflammatory activity of indigenous plants will facilitate the development of efficient wound healing agents. *Leucas aspera* (Willd.) Linn. (Family: Lamiaceae) commonly known as 'Thumbai' is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperient, diaphoretic and insecticide. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites (Rai et al, 2005). *Leucas aspera* is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches (Figure 1). Preliminary chemical examination of *L. aspera* revealed presence of triterpenoids in entire plant. Among the 25 compounds identified from the leaf volatiles, u-farnesene (26.4%), x-thujene (12.6%) and menthol (11.3%) were the major constituents. Aerial parts are reported to contain nicotine, sterols, two new alkaloids (compound A m.p. 61-2°, α-sitosterol and β-sitosterol) (m.p. 183-4°), reducing sugars (galactose), glucoside (230-1°), diterpenes (leucasperones A and B, leucasperols A and B, isopimarane glycosides (leucasperosides A, B and C), together with other compounds like asperphenamate, maslinic acid, (-)-isololiolide, linifolioside, nectandrin B, meso-dihydroguaiaretic acid, macelignan, acacetin, apigenin 7-O-[6'-O-(p-coumaroyl)-3-D-glucoside], chrysoeriol, apigenin, erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxy phenyl)propan-1-ol, myristargenol B, and machilin C, (-)-chicanine, (7R,8R)- and (75,85)-licarin A (Mangathayaru et al., 2006; Sadhu et al., 2006).

Pharmacological studies revealed that *invitro* study of chloroform and ether extracts of *L. aspera* revealed its antifungal activity against *Trichophyton* and *Microsporum gypseum*. The minimum inhibitory concentration was found to be 5 mg/mL. *Leucas aspera* had both fungistatic and fungicidal actions (Thakur et al., 1987). The methanol extract of *L. aspera* flowers, its fractions, the alkaloidal residue, and the expressed flower juice showed good antibacterial activity for methanol extract and methanol fraction with maximum activity for the alkaloidal residue (Mangathayaru et al., 2005).The ethanolic extract was subjected to acetic acid induced writhing inhibition, 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay and brine shrimp lethality bioassay for screening of antinociceptive, antioxidant and cytotoxic activity, respectively. The ethanolic extract of *L. aspera* root produced significant inhibition in acetic acid induced writhing in mice at the doses of 250 and 500 mg/kg. The extract showed a significant free radical scavenging activity with an IC50 of 8 µg/ml. The extract showed significant lethality to brine shrimp (Rahman et al., 2007). In this study, different extracts of of *L. aspera* was selected to evaluate its anti-inflammatory activity.

2. METHODOLOGY

2.1 Chemicals and instruments

All chemicals used in the estimation were of analytical grade. Carrageenan was purchased from sigma chemicals. Reference standard diclofenac sodium was obtained as gift sample from CFTRI labs, Mysore. Shimadzu 1701 UV Visible spectrophotometer was used for the *in vitro* study.

2.2. Animals

Adult Wister albino rats (150 g -200 g) of either sex were used for the in vivo evaluation. They were housed under standard laboratory conditions and were fed with standard animal feed and water ad libitum. The experimental protocol was approved by institutional animal ethical committee.

2.3. Sample collection:

The *Leucas aspera* plants were collected in the local areas of Tumakuru. The plant leaves were separated and washed under the running tap water to remove the dust and sand particles and later rinsed with distilled water. Then they were shade dried for two to three days at room temperature and later dried leaves were grinded into fine powder (Figures 2 and 3).

Figure 1. *Leucas aspera* plant

Figure 2. Dry leaves

Figure 3. Powdered sample

2.4 Preparation of extract:

The fresh leaves were washed with tap water and were shade-dried at ambient temperature and then ground into powder using a grinder. The different extracts of leaves were prepared by Soxhlet extraction method. 100g of air-dried fine powder of each plant was extracted using Soxhlet extraction apparatus, successively with solvents of increasing polarity for 72 hours. Solvents used were hexane, ethyl acetate, acetone, and ethanol. All extracts were evaporated to dryness using rotary evaporator and the extraction yield for each extract was calculated.

2.5. Phytochemical Analysis:

The hexane, ethyl acetate, acetone, and ethanol extracts of *L. aspera* leaves were subjected to a qualitative phytochemical screening, using standard methods (Harbone, 1988)

2.6. Acute toxicity test:

Acute toxicity study was performed as per OECD guidelines 423 (Acute toxicity class method).

2.7. In vitro Anti-inflammatory activity

HRBC method was used for the estimation of antiinflammatory activity in vitro (Azeem et al., 2010). Blood was collected from healthy volunteers and approval of the Institutional ethics committee was obtained. Blood was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3 000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of antiinflammatory property. Different concentrations of extracts, reference sample and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of

HRBC suspension. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection= 100- (OD sample/ OD control) x 100

2.8. In vivo Anti-inflammatory activity

Paw oedema was induced on each rat by injecting 0.1 mL of carrageenan on physiological saline to the left hind paw (Gandhidasan et al., 1991). The extracts at different concentrations were administered orally 30 minutes prior to carrageenan administration. Paw volumes were measured at 60, 120, 180 and 240 minutes by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in extract treated groups was compared with control. Diclofenac sodium (5 mg/kg) was used as the standard.

2.9. Statistical Analysis

Statistical analysis was done using one-way analysis of variance followed by Dunnet's test. P values greater than 0.05 were considered as significant.

3. RESULTS

3.1 Preparation of Plant Extracts

Hexane, ethyl acetate, acetone and ethanol extracts of *L.aspera* leaves were prepared successively by Soxhlet extraction method and the yield calculated was reported in Table 1. Acetone extract of *L.aspera* leaves found to have more yield. The solvents, acetone, ethyl acetate and ethanol generally could extract more organic components from plant [6].

Table 1: Total yield of *L.aspera* leaves extracts (g per 100g dry plant).

Plant	Extract	Yield (g/100g)
Leucas aspera	Hexane	4.12
	Ethyl acetate	3.55
	Acetone	6.78
	Ethanol	1.33

3.2. Phytochemical analysis

Preliminary phytochemical screening of the hexane, ethyl acetate, acetone and ethanol extract of *L.aspera* leaves revealed the presence of various phytochemicals like phenols, flavanoid, alkaloids, saponins and terpenoids and the result was summarized in Table.2. The ethanol and ethyl acetate extracts of *L.aspera* leaves contain more phytochemicals than the other extracts. The qualitative phytochemical analysis of hexane, ethyl acetate, acetone and ethanol extracts of *L. aspera* supported by previous reports. [7,8,9]

Table 2: Qualitative phytochemical analysis.

Sl.No.	**Phytochemicals / Tests**	**Plant**			
		Leucas aspera			
		Hexane	**Ethyl acetate**	**Acetone**	**Ethanol**
1.	Carbohydrate				
	a. Molisch'sTest	-	-	+	+
	b. Barfoed's Test	-	-	+	+
	c. Benedict's Test	-	-	+	+
	d. Fehling's Test	-	-	+	+
2.	Alkaloid Test				
	a. Wagner's Test				
	b. Mayer's Test	-	-	+	+
		-	-	+	+

3.	Flavanoids(Alkaline reagent test)	-	+	-	+
4	Protein (Biuret test)	-	-	-	+
5	Phenols a. Lead acetate Test b. Ferric chloride test	 - +	 - +	 - +	 - +
6	Oils (Spot test)	+	+	-	-
7	Steroids(Salkowski's test)	+	-	-	+

Symbol (+) indicates presence and (-) indicates absence of phytochemicals.

3.3 Acute toxicity studies

The extracts of *L.aspera* leaves did not show any sign of toxicity up to 2000 mg/kg body weight and hence it was considered to be safe.

3.4 In vitro anti-inflammatory activity

Hexane, ethyl acetate, acetone and ethanol extract of *L.aspera* leaves at different concentrations (200, 300, 400 mg/mL) showed significant stabilization towards HRBC membranes. The percentage protection of ethyl acetate and ethanolic extracts at concentration 300 mg/mL was higher than that of concentrations. However, the percentage protection was found to be decreased at higher concentration. The results were tabulated in Table 3.

Table 3: In vitro anti-inflammatory activity of *L.aspera* leaves

Sl.No.	Extract type (mg/mL)	Percentage protection
1	Hexane-200	27.12
2	Hexane-300	25.58
3	Hexane-400	26.33
4	Ethyl acetate-200	29.53
5	Ethyl acetate-300	31.46
6	Ethyl acetate-400	28.85
7	Acetone-200	25.45
8	Acetone-300	24.22
9	Acetone-400	2567
10	Ethanol-200	27.45
11	Ethanol-300	32.22
12	Ethanol-400	26.67
13	Diclofenac sodium (5mg/mL)	35.34

3.5 In vivo anti-inflammatory activity

Hexane, ethyl acetate, acetone and ethanol extract of *L.aspera* leaves at different concentrations showed significant reduction in the paw volume of rats. The ethyl acetate and ethanolic extracts at concentration of 300mg/mL showed potent activity compared with the reference standard Diclofenac sodium. The results were tabulated in Table 4.

Table 4: In vivo anti-inflammatory activity of *L.aspera* leaves induced oedema

Drug	Dose (mg/ kg)	Carrageenan induced oedema (Volume in mL)			
		60 min	120 min	180 min	240 min
Control		0.40± 0.21	0.42± 0.93	0.47± 0.25	0.45± 0.44
Diclofenac	5	0.19± 0.33	0.17± 0.33	0.15± 0.27	0.12± 0.40
Hexane Extract	200	0.34± 0.28	0.32± 0.21	0.31± 0.20	0.28± 0.58
Hexane Extract	300	0.21± 0.54	0.20± 0.25	0.19± 0.31	0.17± 0.30
Hexane Extract	400	0.25± 0.31	0.23± 0.44	0.22± 0.22	0.20± 0.51

Ethyl acetate Extract	200	0.32± 0.26	0.30± 0.56	0.28± 0.67	0.26± 0.62
Ethyl acetate Extract	300	0.19± 0.61	0.18± 0.35	0.17± 0.34	0.16± 0.45
Ethyl acetate Extract	400	0.26± 0.55	0.23± 0.65	0.22± 0.45	0.20± 0.67
Acetone Extract	200	0.32± 0.76	0.30± 0.82	0.27± 0.67	0.24± 0.44
Acetone Extract	300	0.22± 0.82	0.20± 0.43	0.19± 0.32	0.17± 0.36
Acetone Extract	400	0.28± 0.17	0.25± 0.48	0.21± 0.38	0.20± 0.28
Ethanol Extract	200	0.30± 0.44	0.26± 0.47	0.22± 0.43	0.20± 0.24
Ethanol Extract	300	0.17± 0.67	0.15± 0.51	0.14± 0.54	0.12± 0.25
Ethanol Extract	400	0.24± 0.20	0.28± 0.69	0.21± 0.56	0.17± 0.28

4. DISCUSSION AND CONCLUSION

Inflammation is a common phenomenon, and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. The current study adds more scientific evidence for the traditional use *L.aspera*. Among the different plant extracts under study, the ethanol and ethyl acetate extracts of *L.aspera* exhibited significantly better anti-inflammatory activity than the other extracts and standard anti-inflammatory drug Diclofenac sodium. Here anti-inflammatory activity was performed based on the folk lore information using two methods. HRBC method was selected for the in vitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane (Shenoy et al., 2010)and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The result indicted that the leaf extract of *L.aspera* at various concentrations has significant anti-inflammatory property. Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect. The development of oedima in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, prostaglandin and the analogous (Georgewill et al., 2010 Winter et al., 1962 Georgewill and Georgewill, 2010). Different extract of *L.aspera* leaves showed significant anti-inflammatory activity. This significant antiinflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids (Rosa et al., 1971) present in the extract. The present result indicates the efficacy of *L.aspera* as an effective therapeutic agent in the treatment of acute inflammations. The result of present study validation the folk lore information on the anti-inflammatory property of the extract of *L.aspera* leaves Further research on the identification of bioactive components responsible for activity, will pursuit new phototherapeutics against inflammatory diseases.

REFERENCES

1. Annan K, Houghton PJ. Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of Ficus asperifolia Miq. and Gossypium arboreum L., wound-healing plants of Ghana. J Ethnopharmacol, 2008; 119(1): 141-4.
2. Azeem AK, Dilip C, Prasanth SS, Junise V, Hanan Shahima. Anti-inflammatory activity of the glandular extracts of Thunnus alalunga. Asia Pac J For Med 2010; 3(10): 412-420.
3. Barreto RS, Albuquerque-Júnior RL, Araújo AA, Almeida JR, Santos MR, Barreto AS, Quintans-Júnior LJ. A systematic review of the wound-healing effects of monoterpenes and iridoid derivatives. Molecules, 2014; 19(1): 846-62.
4. Gandhidasan R, Thamaraichelvan A, Baburaj. Antiinflamattory action of Lanea coromondelica by HRBC membarane stabilisation. Fitotherapia 1991; 62: 82-83
5. Georgewill OA, Georgewill UO, Nwankwoala RNP. Antiinflammatory effects of Morninga oleifera lam extract in rats. Asian Pac J Trop Med 2010; 3(2): 133-135.
6. Georgewill OA, Georgewill UO. Evaluation of the antiinflammatory activity of extract of Vernonia Amygdalina. Asian Pac J Trop Med 2010; 3(2): 150-151.
7. Harbone JB. Phytochemical Methods: A guide to modern technique of plant analysis. (3nd Edn.). Chapman and Hall, London, 1988; 1-138.
8. Mangathayaru K, Thirumuragan D, Patel PS, Pratap DVV, David DJ, Karthikeyan J. Isolation and identification of nicotine from Leucas aspera . Indian J Pharm Sci. 2006; 68,88–90.
9. Rahman MS, Sadhu SK, Hasan CM. Preliminary antinociceptive, antioxidant and cytotoxic activities of Leucas aspera root. Fitoterapia. 2007; 78:552-5.

10. Rai V, Agarwal M, Agnihotri AK, Khatoon S, Rawat AK, Mehrotra S. Pharmacognostical evaluation of Leucas aspera. Nat Prod Sci. 2005; 11,109–14.
11. Rosa MD, Giround JP, Willoghby DA. Studies of the acute inflammatory response induced in rats in different sites by Carrageenan and turpentine. J Pathol 1971; 104: 15-29
12. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. Diterpenes from Leucas aspera inhibiting prostaglandin-induced contractions. J Nat Prod. 2006; 69,988–94.
13. Shenoy S, Shwetha K, Prabhu K, Maradi R, Bairy KL, Shanbhag T. Evaluation of antiinflammatory activity of Tephrosia purpurea in rats. Asian Pac J Trop Med 2010; 3(3): 193-195.
14. Subramoniam A, Evans DA, Rajasekharan S, Nair GS. Effect of Hemigraphis colorata (Blume) HG Hallier leaf on wound healing and inflammation in mice. Ind J Pharmcol, 2001; 33(4): 283-5.
15. Thakur DK, Misra SK, Choudhuri PC. In vitro trials of plant extracts and chemicals for their antifungal activity. Indian J Animal Health. 1987;26:31–5
16. Winter CA, Risley EA, Nuss GW. Carrageenan induced oedema in hind paws of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Bio Med 1962; 111: 544-557.