

# Anti-Inflammatory and Anti-Oxidant Activity of Lippia Alba Leaf

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## Abstract:

Inflammation is part of the body's defence mechanism which the immune system recognise and removes harmful and foreign stimuli and begins the healing process. The human body's oxidation process harm DNA, protein, lipids, and other components in addition to cell membranes. As oxygen is metabolized, it produces "free radicals," which are unstable chemicals that harm DNA and other cells by stealing electrons from other molecules. In this study, anti-inflammatory and Anti-oxidant activity of Lippia alba leaf extracts was investigated to overcome oxidation and inflammation. Lippia alba is a species of flowering plant in the Verbeanceae family having extensive properties like Anti-bacterial, Antiseptic, Anti-depressant, Anti-spasmodic, Anti-bronchitic etc.,. Phytochemical Analysis of extract LEE (T<sub>1</sub>) revealed the presence of phytosterol, alkaloids, flavonoid, phenolic compounds, carbohydrates, whereas the extract of LAE (T<sub>2</sub>) revealed the presence of alkaloids, flavoniods, carbohydrates, saponins. Anti-inflammatory effect was estimated by inducing carrageenan in four group of rats i.e., control, standard, T<sub>1</sub> and T<sub>2</sub>. Inflammation is caused by chemical mediators such as Histamine/Serotonin, Bradykinin, Prostaglandin, Lysosomes, Inhibition of carrageenan induced inflammation by leaf extracts could be due to inhibition of cyclooxygenase and prostaglandin synthesis. The LEE extract of Lippia alba leaf has shown significant anti-inflammatory activity. Anti-oxidant activity was estimated by mixing the sample with H<sub>2</sub>SO<sub>4</sub>, Ammonium molybdate, Na<sub>3</sub>PO<sub>4</sub> and incubated at 95°C for 10min and absorbance was measured at 695 nm. The LEE extract of Lippia alba leaf shown more anti-oxidant activity than LAE. This study has shown that LEE of Lippia alba has shown significant Anti-inflammatory and Anti-oxidant activity. These studies justify the traditional use of plants in some inflammatory and oxidant condition.

**Keywords:** Anti-inflammation, Anti-oxidant, Lippia alba, Bradykinin, Prostaglandin.

## INTRODUCTION:

Inflammatory response is the basic phenomenon of our body immunity, to fight against microbes and to accelerate healing mechanism. The local reaction to cellular damage, known as inflammation is characterized by capillary dilation, leukocytic infiltration, redness, heat, discomfort, swelling and occasionally loss of function as a result of pro-inflammatory chemicals. While inflammation is natural reaction to tissue damage, unchecked inflammation contribution to the early onset of chronic conditions such multiple sclerosis, asthma, rheumatoid arthritis, hepatitis, ulcerative colitis and Crohn's disease. A

drug or treatment that decrease inflammation or swelling is said to be anti-inflammatory or antiphlogistic agent. Approximately half of analgesics are anti-inflammatory medication, also referred to as anti-inflammatories. The human body's oxidation process harm DNA, protein, lipids, and other components in addition to cell membranes. As oxygen is metabolized, it produces "free radicals," which are unstable chemicals that harm DNA and other cells by stealing electrons from other molecules. Some free radicals are tolerated by the body and are necessary for proper bodily function. But over time, the harm brought on by an excess of free radicals could become irreversible and result in illnesses including liver and heart disease as well as some cancer such bowel, stomach, oesophageal and oral cancers. Antioxidants are defined as compounds that, when present in food, prevent, slow down or stop oxidation from occurring, and food quality from deteriorating, compounds known as anti-oxidants prevent oxidation. An anti-oxidant is a material that, in comparison to oxidisable substances, greatly retard or prevents the oxidation of that substance at low concentration.

## **METHODOLOGY:**

### **Plant collection and identification:**

The fresh leaves of *L. alba* were collected from nearby village Pothavarappadu, Vijayawada. These plants are authenticated in department of pharmacognosy of our college NRI collage of Pharmacy and identified the morphological characteristics of the plant. Leaves of plants were washed under tap water and kept under shade drying for 15 days to avoid the decomposition of thermolabile phytochemicals. After 15 days the dried leaves were grinded to powder with the help of blender and then used for further study.

### **Preparation of plant extract:**

Around 583.4gm of powdered sample of *L.alba* was extracted with 95% ethanol for 7 days with occasionally shaken in extraction bottle . After 7days , the mixture was filtered through whatsmann No.1 filter paper and was concentrated under reduced pressure at 50°C. The residue was extracted twice using same procedure and the filterates were weighed to get concentrated mass (34gm, 5.3%,w/w). An aliqueot of concentrated ethanolic crude extract ( LEE) was partitioned to get aqueous (LAE) fraction.

## **PHYTOCHEMICAL TESTS:**

### **Procedure for Phytosterols:**

#### ➤ **Liebermann Burchard's test:**

The ethanol extract was treated with few drops of acetic anhydride, boiled and cooled. On adding concentrated sulphuric acid, formation of a bluish green colour solution confirmed the presence phytosterols. When aqueous extract was treated with the same above procedure bluish green colour solution was not found and confirmed the absence of phytosterols.

### **Procedure for Alkaloids:**

#### ➤ **Dragondroff's reagent:**

To the aqueous extract potassium bismuth iodide solution was added. Reddish brown ppt was formed and confirmed that the presencce of alkaloids. When ethanol extract was treated with same above procedure reddish brown ppt was formed which confirmed the presence of alkaloids.

**Procedure for fixed oil:****➤ Spot Test:**

0.5 ml of solvent extract was taken and pressed in between the two filter paper. Formation of oil stains on the paper indicated the existence of fixed oil. When aqueous extract was treated with the same above procedure there was no formation of oil stains which indicates the absence of fixed oils. When ethanolic extract was treated with the same above procedure there was no formation of oil stains which indicates the absence of fixed oils.

**Procedure for flavonoids:**

➤ **Alkaline Test :** To the ethanolic extract few drops of solution of NaOH was added, yellow colour was observed upon addition of dilute HCl and confirmed the presence of flavonoids. When aqueous extract was treated with the same above procedure yellow colour solution was formed, which indicated the presence of flavonoids.

**Procedure for carbohydrates:**

➤ **Molish Test:** 1ml of aqueous extract was taken in a test tube. Few drops of molisch reagent was added. The test tube was shaken and 2 drops of conc. H<sub>2</sub>SO<sub>4</sub> added on walls of the test tube. Violet colour ring was observed at junction of 2 layers confirmed the presence of Carbohydrates. When ethanolic extract was treated with the same above procedure no violet ring was formed in between 2 layers, indicates the presence of Carbohydrates.

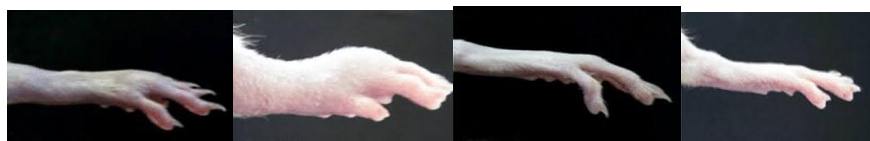
**Procedure for saponins:**

➤ **Foam test:** Small quantity of the aqueous extract was shaken with 2ml of water. Persistence of foam produced for 10min. Indicated the presence of saponins. When small quantity of the ethanolic extract was treated with the same above procedure of persistence foam was not formed, indicated the absence of saponins.

**ANTI-INFLAMMATORY ACTIVITY:****Carrageenan induced paw edema in rats:**

Adult Wistar albino rats of 130-160 gm were taken and they were housed in polypropylene cages under standard conditions. The animals were kept in conventional environment, which included a 12 hour dark cycle, a temperature of 27.0 ± 1.0°C, and a relative humidity of 55-65%. They were fed a typical diet. Using the carrageenan induced hind paw edema method, Anti-inflammatory activity was assessed. Rats of either sex were divided into four groups of six animals each. The first group was served as control and received only vehicle, second group was administered with standard drug Ibuprofen I.P 100mg/kg intra peritoneally. The animals of third and fourth group were treated with ethanol and aqueous extract LEE and LAE at a dose of 500mg/kg orally. After 30 min of above treatment 0.05ml of 1% w/v Carrageenan in saline was injected into sub plantar tissue of left hind paw of the animals.

The degree of paw edema of the entire group was measured at 0, 30, 60, 120, and 180 minutes by using Vernier caliper after administration of carrageenan. The anti-inflammatory effect was expressed as percent inhibition of edema. The result indicated that the major component responsible for anti-inflammatory may be present in LEE.



Standard

Control

LEE

LAE

**Anti-oxidant activity:**

**Determination of total antioxidant capacity:**

Total antioxidant capacity of Ethanol extract, Aqueous extract was evaluated by mixing samples (0.5 ml each) at 100µg/ml concentration with H<sub>2</sub>SO<sub>4</sub> (90.6M, 3ml), ammonium molybdates (1%) and Na<sub>3</sub>PO<sub>4</sub> (28Mm).The sample were incubated at 95°C for 10 min and absorbanc was measured at 695nm. It was described to measure the anti-oxidant activity of different extracts by using catechine as standard and the result showed ethanol extract (LEE) had significant anti-oxidant activity when compared to aqueous extract (LAE) at a concentration of 100µg/ml.

**RESULTS AND DISCUSSION:**

The Anti- inflammatory and Anti-Oxidant activity of the Lippia Alba leaf was found by using following methodologies. Anti-inflammatory activity of lippia alba leaf extract on wister albino rats are performed by carrageenan induced paw edema methods. Anti-oxidant activity of ethanolic and aqueous extract of lippia alba leaf was found by phosphomolybdenum method using catechin as standard drug. In carrageenan induced paw edema model four groups of animals were taken for the experiment and weighed. Marks are made on right hind limbs. Animals were divided into 4 groups each group comprising of 6 rats. Control group was injected with saline, standard was injected with Ibuprofen, T1(Test group-1) was injected with LEE & T2(Test group-2) was injected with LAE at a dose of 500mg/kg.

**Phytochemical studies:**

Phytochemical studies were performed with standard procedures on ethanolic and aqueous solvent extracts, which showed as below in Table no: 1.

**TABLE 1: Results of Phytochemical analysis**

Extract	Phytosterol	Alkaloids	Fixed oil	Flavonoid	Phenolic compounds	Carbohydrates	Saponin
LEE	+	+	-	+	+	+	-
LAE	-	+	-	+	-	+	+

+ =Present; - = Absent LEE and LAE represent Ethanol and Aqueous extract respectively.

**TABLE 2: Anti-inflammatory effect of Lippia alba Extracts on Wister Albino Rats**

S.no	Treatm et	Mean Paw Thickness (cm) <sub>±</sub> SEM (% Inhibition)				
		0 MIN	30 MIN	60 MIN	120 MIN	180 MIN
1.	Contro 1	4.77 <sub>±</sub> 0.026	5.33 <sub>±</sub> 0.035	5.54 <sub>±</sub> 0.039	5.9 <sub>±</sub> 0.043	6.22 <sub>±</sub> 0.02
2	Ibuprofe		4.53 <sub>±</sub> 0.04*(50	4.56 <sub>±</sub> 0.08*(54%)	4.63 <sub>±</sub> 0.07**(56	4.7 <sub>±</sub> 0.05**(66

	n	4.25±0.04	%)		%)	%)
3	LEE	4.33±0.06	4.78±0.04* (19.7%)	4.91±0.33** (30.5%)	4.96±0.06** (35.3%)	5.12±0.04** (40%)
4	LAE	4.51±0.05	4.81±0.06** (6.7%)	5.15±0.03** (7%)	5.15±0.1* (9.5%)	5.07±0.06** (11%)

Mean ± SEM, (n=6) “ \* ” indicates p <0.05 and “ \*\* ” p< 0.01.

**TABLE 3: Absorbance of Anti-Oxidant samples**

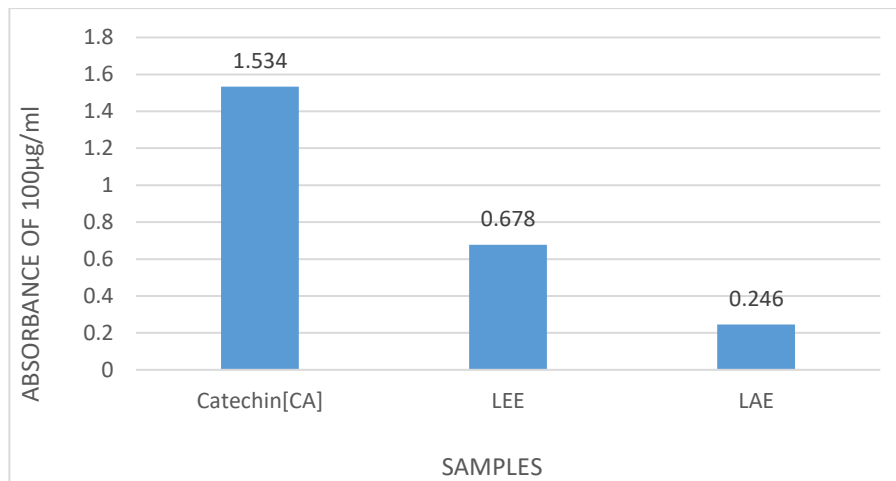
NAME OF THE SAMPLE	ABSORBANCE at 100µg/ml
Catechin[CA]	1.534
LEE	0.678
LAE	0.246

**Anti-inflammatory effect:**

The anti-inflammatory effect was expressed as percent inhibition of edema. The mean paw thickness of the standard ibuprofen group has shown 4.7±0.05\*\* with percentage of inhibition of about 66% at 180Min. The Mean paw thickness of the LEE group has shown more significant value 5.12±0.04\*\* with percentage of inhibition of about 40% at 180Min, whereas LAE group, has shown significant value of 5.07±0.06\*\* with percentage of inhibition of about 11%. It was observed that ethanolic extract at a dose of 500mg/kg showed the maximum anti-inflammatory activity amongst the other extract (aqueous) at 180Mins. The results indicated that the major component responsible for anti-inflammation may be present in ethanolic extract. Anti-inflammatory activity of ethanolic extract was may be due to the presence of flavonoids. Data was expresses as mean ± SEM and the statistical difference between the groups was analyzed by using Student’s t-test. The value of p<0.05 and p<0.01 was considered as statistically significant, which showed in Table: 2.

**Absorbance of Anti-oxidant sample:**

*Total Anti-oxidant* described to measure the anti-oxidant activity of different extractives by using catechin as standard and the results in shown figure-1. However, LEE and LAE extract of Lippia alba leaf are less than standard catechin. In total anti-oxidant activity assay LEE, LAE and standard showed absorbance as 0.678, 0.246 and 1.534 respectively. The results demonstrated that the LEE absorbance had significant anti-oxidant activity when compared to LAE. A higher value of absorbance indicates total anti-oxidant capacity, which showed in Table no: 3.



**Figure-1: Graphical representation of Anti-oxidant activity of Extract fraction.**

### Conclusion:

Replacement of synthetic anti-oxidants and anti-inflammatory drugs with natural may be beneficiary because of their less toxic nature, availability, extent of existence and less cost. In present study it was observed that ethanolic extract LEE at a dose of 500mg/kg body weight showed maximum anti-inflammatory activity than aqueous extract LAE, whereas total anti-oxidant capacity of ethanolic extract LEE of *Lippia alba* showed remarkable anti-oxidant activity when compared with that of aqueous extract LAE of *Lippia alba*. Hence, Ethanolic extract LEE of *Lippia alba* leaf is a potent source of natural anti-inflammation and anti-oxidant and help in traditional treatment of oxidative stress and inflammation.

### Reference:

- Rankin JA (2004) Biological mediators of acute inflammation. AACN Clin Issues 15: 3-17.
- Serhan CN, Ward PA, Gilroy DW (2010) Fundamentals of Inflammation. Yale J Biol Med 84: 64-65.
- Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420: 860-867.
- Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. Circulation 105: 1135-1143.
- Wyss-Coray T, Mucke L (2002) Inflammation in neurodegenerative disease—a double-edged sword. Neuron 35: 419-432.
- Australian Institute of Health and Welfare (2014) Australia's health 2014. Australia's health series no. 14, Canberra.
- Bacchi S, Palumbo P, Sponta A, Coppolino MF (2012) Clinical Pharmacology of Non-Steroidal Anti-Inflammatory Drugs: A Review. Antiinflamm Antiallergy agent Med Chem 11: 52-64.
- Dr. Steven T Leach (2016) A Brief overview of Nutrient Anti-Inflammatory Molecules and their In Vitro and In Vivo Activity.
- Charles A. Dinarello (2013) Anti-inflammatory Agents: Present and Future.
- NSAID Dr. Hashem Mansour.ppt download <https://slideplayer.com/amp/15915593/>.
- Nutrient reference values for Australia and New Zealand, National Health and Medical Research Council, Australian Government.
- Australian dietary guidelines, 2013, National Health and Medical Research Council, Australian Government.

13. <https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.slideserve.com%2Fnicole%2Ffree-radicals-and-antioxidants>.
14. Benny Simpson's Texas Native Shrubs. Texas A&M University. Retrieved 2010-01-2
15. P. Wilson, Bot. Porto Rico 6: 141. 1925; Rajendran & Daniel, Indian Verbenaceae 198. 2002.
16. Lantana alba Mill., Gard. Dict. ed. 8, 8. 1768.
17. Junaid R shaikh & M Patil, International Journal of Chemical studies 2020;8(2):603-608
18. Fred JA, Adisa A. Phytochemical and Pharmacognostic Studies of *Telosma africanum* (N.E.Br) Colville Leaf and Stem. Int J of Pharm Sci and Res 2012;3(6):1860-62.
19. Kokate CK. Practical Pharmacognosy. 3<sup>rd</sup> ed. New Delhi: Vallabh Prakashan; 1994.
20. Research J. Pharm. and Tech. 4(4): April 2011.
21. Ali et al./Bangladesh Pharmaceutical Journal 26(1): 7-14, 2023 (January).