

## Evaluation Of In Vitro Anti-Inflammatory Activity of the Methanolic Extract of *Litsea quinqueflora* (Dennst.) Suresh

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### **Abstract:**

**Objective:** The aim of this research work was to study the in vitro anti-inflammatory activity of the methanolic leaf extract of *Litsea quinqueflora* (Dennst.) Suresh.

**Methods:** Methanolic extract of the powdered leaves was subjected to preliminary phytochemical screening as per the standard protocols of Trease and Evans. In vitro anti-inflammatory activity was evaluated by HRBC membrane stabilization method with slight modifications.

**Results:** Preliminary phytochemical screening revealed the presence of flavonoids, coumarins, alkaloids, tannins, terpenoids, anthraquinones, phenols, reducing sugars and carbohydrates. In vitro anti-inflammatory activity was tested using various concentrations of the extract such as 400, 800, 1200, 1600 and 2000 µg/ml along with standard drug ibuprofen. As the concentration of the extract increased the anti-inflammatory property also increased and a maximum of 96.5% inhibition of red cell lyses was reported in 2000 µg/ml of the extract and the response was monophasic. Statistical analysis using one way ANOVA showed that the variations with respect to percentage of protection and drug concentration between and within groups were significant at 5% level ( $p < 0.05$ ).

**Conclusion:** On the basis of the present investigation it could be inferred that the methanolic extract of *Litsea quinqueflora* leaves possesses significant anti-inflammatory activity. Ongoing studies using cell lines could throw more light on the in vitro anti-inflammatory property and detailed phytochemical investigation could identify the exact compound responsible for it. The plant therefore could be considered as a natural source of membrane stabilizers and is capable of providing an alternative remedy for the treatment of inflammatory disorders.

**Keywords:** Phytochemical screening, *Litsea quinqueflora*, HRBC membrane stabilization, Anti-inflammatory activity.

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### I. Introduction

India is bestowed with a vast array of medicinal plants that are routinely used by most of the people as a remedy to various ailments. Many of the modern drugs have their origin either directly or indirectly from such plant sources. Medicinal and culinary herbs are rich sources of anti-inflammatory compounds such as flavonoids. Pharmaceutical drugs are built upon a single molecule while herbal remedies contain different active ingredients [1]. One of the wide spread complaint against modern medicines is its side effect that can be attributed to a single biochemical pathway that is triggered by the molecule of interest. On the contrary herbal medicines mediate multifaceted biochemical attack on inflammation due to the diversity and synergy of the anti-inflammatory compounds.

Inflammation is a protective response by our immune system against organisms which cause cell injury (e.g., microbes, toxins) and deals with the consequences of such injury. It may be acute or chronic, depending up on the nature of stimulus and the effectiveness of initial reaction in eliminating the stimulus or the damaged tissues. The main components of inflammation are a vascular reaction and a cellular response; both are activated by mediators that are derived from plasma proteins and various cells. The outcome of acute inflammation is either elimination of the noxious stimulus followed by decline of the reaction and repair of the damaged tissue, or persistent injury resulting in chronic inflammation[2].

Leukocytes, the key players of inflammatory response, can eliminate microbes and dead cells by phagocytosis, followed by their destruction in phagolysosomes. Destruction is caused by free radicals generated in activated leukocytes (neutrophils and monocytes) and lysosomal enzymes [3]. Enzymes and reactive oxygen species may be released into the extracellular environment where it acts as mediators of inflammation. Such mediators are mainly arachidonic acid metabolites, generated through Cyclooxygenase and Lipoxygenase pathways. Most of the anti-inflammatory drugs are targeted on these pathways [4].

In a different approach, rather than blocking a particular mediator or its pathway, preventing the release of inflammatory mediators could be considered as a better option. The possibility of this approach is revealed in this research by studying the ability of the plant extract to prevent the lysosomal membrane destruction. An

effective way to study this activity in vitro is to study the HRBC membrane stabilization activity of the plant extract. Lysosomal membrane and RBC membrane are similar in structure apart from the fact that luminal surface of the lysosomal membrane contains a glycoprotein coat which protects the membrane from digestion by lysosomal acid hydrolases[5]. This method has been used in most preliminary anti-inflammatory screening procedures [6].

Plants produce different bioactive compounds using secondary metabolic pathways in response to specific environmental stimuli such as herbivore-induced damage, pathogen attacks, or nutrient deprivation. These secondary metabolites can be unique to specific species or genera and perform a host of general, protective roles including anti-inflammatory and antioxidant activities [7]. The current study focuses on the evaluation of in vitro anti-inflammatory property and phytochemical nature of the leaf extracts of *Litsea quinquiflora* (Dennst.) Suresh.

*Litsea quinquiflora* (Dennst.) Suresh (Syn. *L. ligustrina*) belonging to the family Lauraceae is an important medicinal plant endemic to western ghats and is rare in Soth Sahyadri, Palakkadu hills, Wayanadu, Kottayam and Thiruvannathapuram districts of Kerala. It is used as a remedy for inflammatory disorders by local healers of Kottayam district, Kerala (unpublished oral data). This is for the first time we report the anti-inflammatory property of *Litsea quinquiflora*.

## II. Materials And Methods

### Plant Material

Leaves of *L. quinquiflora* were collected from Ponkunnam area, Kottayam District, Kerala, India and voucher specimen was deposited at the herbarium of Kerala Forest Research Institute, Peechi (**Herbarium No: KFRI 11102**), Thrissur, Kerala, after proper taxonomic identification. The leaves were washed in running tap water, shade dried in open air and powdered using electric blender.

### Chemicals and reagents

All chemicals used for the phytochemical analysis and membrane stabilization studies were of highest purity, analytical grade and purchased from Merck, USA.

### Preparation of plant extract

The dried and powdered leaf samples (20 g) were extracted with hot methanol for 24 hours using soxhlet apparatus at a temperature of 30 to 35°C. The extract was then concentrated in vacuum using rotary evaporator at 100 °C. The dried extract was preserved at -20°C in a deep freezer for subsequent experiments.

### Phytochemical Screening

The powdered leaves were subjected to various qualitative chemical tests for identification of its plant constituents by following the standard protocols [8].

### Anti-inflammatory activity

Anti-inflammatory activity of the leaf extract of *Litsea* was carried out as described by Oyedapo et al. [9]. The blood was collected from healthy human volunteers who had not taken any non-steroidal anti-inflammatory drugs for 2 weeks prior to the experiment. Blood samples were centrifuged at 3000 rpm for 10 min at room temperature. The supernatants (plasma and leucocytes) were carefully removed while the packed red blood cell was washed in fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation were repeated five times until clear supernatants were obtained. From this a 10% (v/v) human erythrocyte suspension was prepared using isosaline. Ibuprofen was used as standard drug. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer at pH 7.4 (25<sup>0</sup> C), 0.5 ml of 10% (v/v) HRBC suspension, 0.2-1.0 ml of drug/extract (concentrations ranging from 400µg -2000µg) and final reaction mixtures were made up to 4.5 ml with isosaline. Extra care must be given to avoid any water content. Drugs were omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 40°C for 45 min on a water bath, followed by centrifugation at 3000 rpm for 10 min at room temperature. The absorbance of the lysed haemoglobin was read at 560 nm using UV-Vis Spectrophotometer(Shimadzu, Japan). The percentage of membrane stability was estimated using the expression:

$$\text{Percentage of membrane stabilization} = 100 - \left[ \frac{\text{Drug Test} - \text{Drug Control}}{\text{Blood Control}} \right] \times 100$$

Where, the blood control represents 100% lysis or zero percentage stability. Each experiment was conducted in triplicates and the average percentage of stabilization at each concentration was calculated.

**Statistical analysis:** Results of the membrane stabilizing activity was statistically analysed using analysis of variance (ANOVA –Single factor) using the soft ware SPSS V.20 (IBM, USA).

### III. Results

#### Phytochemical screening

The results of various qualitative tests for the detection of chemical constituents of *L. quinqueflora* are shown in the table no.1. In the present investigation preliminary phytochemical screening revealed the presence of flavonoids, coumarins, alkaloids, tannins, terpenoids, anthraquinones, phenols, reducing sugars and carbohydrates while saponins, quinines and resins were absent.

**Table 1: Phytochemical screening of *L.quinqueflora* (Methanolic extract)**

Sl. No.	Components	Methanol Extract
1	Flavonoids	+
2	Coumarins	+
3	Alkaloids	+
4	Tannins	+
5	Steroids/Terpinoids	+
6	Saponins	-
7	Quinines	-
8	Anthraquinones	+
9	Phenols	+
10	Resins	-
11	Reducing Sugars	+
12	Proteins	-
13	Carbohydrates	+

#### Anti-inflammatory Activity

The results of anti-inflammatory activity showed a concentration dependant protection of cell membrane with a monophasic mode of action. The percentages of protection observed were 60, 73.2, 84.2, 92.15 and 96.5 for the respective concentrations of 400, 800, 1200, 1600, and 2000µg/ml (fig. 1). The standard drug, Ibuprofen showed 98.87 % and 101.12 % membrane stabilization at concentrations of 800µg/ml and 1600µg/ml respectively. Analysis of variance (ANOVA-Single factor) (table no. 2) of the results indicated that there were significant variations with respect to percentage of protection and drug concentration between and within groups and were significant at 5% level ( $p < 0.05$ ).

**Fig.1 :** Membrane stabilization activity of *L. quinqueflora* leaf extract (methanolic)

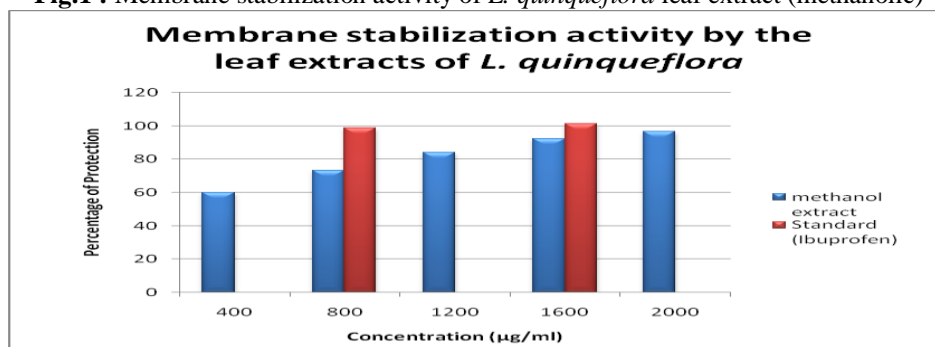


Table 2 - Anova: Single Factor				
Summary				
Groups	Count	Sum	Average	Variance
400	2	120	60	106.58
800	2	146.4	73.2	30.42
1200	2	168.4	84.2	0.72
1600	2	184.3	92.15	4.805
2000	2	193	96.5	2

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1752.864	4	438.216	15.16056	0.0053*	5.192168
Within Groups	144.525	5	28.905			
Total	1897.389	9				

\* Variation within and between groups is significant at 5% level (p<0.05).

#### IV. Discussion

Studies on the native or folk use of medicinal plants are gaining rapid importance in view of its potentiality as a newer drug source as evidenced by the centuries old traditional practical use. Plant extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. The medicinal value of these plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities which may protect against chronic diseases. For example steroids and saponins help in central nervous system functions [7], flavonoids against inflammation and pain [10], quercetin polyphenols act as anti-inflammatory, anti-proliferative and anti-atherosclerotic [11]). In the present investigation preliminary phytochemical screening has revealed the presence of flavonoids, coumarins, alkaloids, tannins, terpenoids, anthraquinones, phenols, reducing sugars and carbohydrates. Therefore the chance of getting similar biological properties from the methanolic extract of *Litsea* is high.

It has been reported that flavonoids and saponins exerted profound stabilizing effect on lysosomal membrane both in vitro and in vivo while tannins and saponins possess ability to bind cations there by stabilizing erythrocyte membranes and other macromolecules [12,13,14]. Anti-inflammatory property of Lauraceae trees [15] *Crocus sativus* cashmerianus [16], *Solanum aethiopicum* [17], herbal preparations containing combinations of plants [18] and *Centella asiatica*[19] have been reported earlier using HRBC membrane stabilization as a method. In vitro anti-inflammatory, anti-platelet and anti-arthritis activity of the methanolic leaf extract of *Anisomeles malabarica* L. was studied [20] and according to them HRBC membrane stabilization was 98.34% at 1000µg/ml of the extract. Similar results were obtained in the current experiment also. Thus the membrane stabilizing activity of the methanolic extract can be attributed to the presence of various metabolites identified from the screening tests.

The anti-inflammatory activity of *Cassia alata* leaf extract and its flavanoid glycosides were extensively studied [21]. They observed strong inhibitory effects on concavallin A induced histamine release, 5-lipoxygenase inhibition and inhibition of cyclooxygenase 1 and 2. Since the chemical constituents present in a plant are directly responsible for its therapeutic and other pharmacological properties, the constituents of the plant which are detected during this investigation should have some direct relationship with local medicinal uses. For preliminary in vitro anti-inflammatory screening HRBC and lysosomal membranes have similar structures. It is a well documented research method employed for studying the anti-inflammatory activity [9,22,23].

The in vitro anti-inflammatory activity reported in the present study can be attributed to the presence of chemical profile such as flavonoids, terpenes, and phenols that were capable of stabilizing red blood cell membranes against heat and hypotonic induced lyses. The results of the preliminary studies support the traditional use of this plant in some painful and inflammatory conditions. Further works are in progress to find out the exact class of compound which is contributing to the anti-inflammatory activity of the methanol extract of the leaves of *L. quinqueflora* and in vitro studies using established cell lines.

#### V. Conclusion

The current study provides evidence for the traditional use of *Litsea quinqueflora* against inflammatory disorders. The preliminary phytochemical screening revealed the presence of different class of compounds. The methanolic extract of the powdered leaves showed significant anti-inflammatory activity in a dose dependent manner, and thus, further research on the identification of bioactive components responsible for anti-inflammatory activity is highly recommended.

#### Conflict Of Interest

Declared None

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