Evaluation of Anti-Inflammatory Activity of *Lagerstroemia lanceolata* Wall Leaf Extract

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**Abstract:** The objective of the study was to evaluate the anti-inflammatory effect of *Lagerstroemia lanceolata* W. leaf methanolic extract along with quantitative determination of phenolic and flavonoid compounds. Quantitative determination of phenolic compounds present in *Lagerstroemia lanceolata* W. leaves was done by the modified Folin-Ciocalteau method. Acute oral toxicity of methanolic extract of *Lagerstroemia lanceolata* W. leaves was carried out. Anti-inflammatory effect was studied with the dose of 100, 200 and 400 mg/kg, p.o by carrageenan induced paw edema in rats. Quantitative analysis of *L. lanceolata* leaf extracts was performed in which the Total phenolic content was determined for ethyl acetate extract (3.472 mg/100 mg of dried extract) & methanolic extract (2.090 mg/100 mg of dried extract) which is compared against standard gallic acid. Total flavonoid content of methanolic extract is 1.475 mg/100 mg of dried extract which is compared against standard quercitin. Methanolic extract of *L. lanceolata* leaf showed a significant anti-inflammatory activity. The results of the experimental study confirmed that methanolic extracts of *Lagerstroemia lanceolata* W. has good quantity of phenolic and flavonoidal compounds present which may be responsible for its anti-inflammatory effect.

**Keywords:** *Lagerstroemia lanceolata*, Anti-inflammatory, total phenolic content & flavonoids.

**INTRODUCTION**

The Lythraceae is a small family of some 22 genera, which range in habit from herbs to shrubs and trees. They mainly occur in tropical regions, the representatives in temperate regions predominantly grow in damp to wet habitats [1]. *Lagerstroemia lanceolata* Wall is a plant belonging to this family. It is a moderate sized to large deciduous tree, sometimes attaining 30 m. in height and 2.4-3.0 m in girth, with a clean cylindrical bole of 12-15m. The tree grows well on hill slopes and in valleys preferring crystalline rock to laterite. It is usually found in mixed deciduous forests, but isolated specimens occur in evergreen forests. It attains its best development in regions of heavy rainfall, e.g. in Kanara, Malabar and Coorg regions of India. The phytoconstituents present in the plant are phenols, flavonoids, glycosides and terpenoids. Some of these phytoconstituents are useful in the treatment of inflammation.

Generally the phytochemicals present in plants possess strong antioxidant ability as well as anti-inflammatory action, which are also the basis of other bioactivities and health benefits [2-9]. The pathophysiological process involved in pain is a complex process which is mediated by a variety of signaling molecules produced by leucocytes, macrophages and mast cells along with activation of complement factors that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site [10-14]. Non-steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for the treatment of inflammation and pain, however, these drugs have too many side effects which has limited their use [15]. Therefore, there is a need to develop new and more substantial drugs with lesser side effects. The Western Ghat region of India is a rich source of flora and fauna which are used against various diseases in different systems of medicines [16].

The present study was planned to study the quantitative analysis of phenolic and flavonoidal compounds and to explore the anti-inflammatory potential of the methanolic extract obtained from the *Lagerstroemia lanceolata* W. leaves.
MATERIALS AND METHODS

Procurement and authentication of plant
The leaves of L. lanceolata W. was collected in the month of April from Maharashar Forest Department, Tansa Wildlife Sanctuary, Tansa WLS, Shahapur, Maharashtra. The plant was identified and authenticated by Mr. Saipun I Shaikh, MFS, Asst. Conservator of Forests and voucher specimen (550/ACF/TNWLS/TAXON/14-15) was deposited at the Department.

Chemicals
Carrageenan was purchased from Sigma-Aldrich, St. Louis MO, USA. Diclofenac was supplied as a gift sample from Emcure, Pune. All chemicals and reagents used were of analytical grade and procured from approved organization.

Preparation of extracts
The leaves were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place. 200g of dried plant material were exhaustively extracted with different solvents using maceration method. The extract was evaporated above their boiling points.

Determination of Total Phenolic Content
The concentration of total phenolics in the extract was determined according to previously reported methods [17, 18]. Briefly, a volume of 2 ml of various concentrations of extracts solutions was added to 1 ml of Folin-Ciocalteu (10%) and 1ml of 7.5% sodium carbonate was added. The mixture was vortexed for 15 seconds and allowed to stand for 15 minutes for colour development and the absorbance was recorded at 765 nm. Gallic acid was used as a standard. The concentration of total phenolic compounds in the extracts was determined as mg of gallic acid equivalent per 100 mg of extract.

Determination of Total Flavonoids Content
Total flavonoid concentration in the extracts was determined according to previously reported methods [19-23]. Briefly, 1 ml of 2% AlCl₃ solution in methanol was added to 1 ml of the extracts and allowed to stand for 15 minutes at room temperature, the absorbance was measured at 420 nm. Quercetin was used as a standard. Total flavonoid content was expressed as mg of quercetin equivalent per 100 mg of extract.

Acute toxicity study
Acute toxicity study was performed as per the OECD guideline No. 425 [24, 25]. Five female Swiss albino mice were used for study. The animals were fasted overnight providing only water. The test drug was administered orally at one dose level of 2000 mg/kg body weight. Animals were observed continuously for the first 4 h and then periodically up to 24 h for any toxic symptoms and mortality.

Anti-inflammatory activity (Carrageenan - induced hind paw edema in rats)
Acute inflammation was produced by injecting 0.1 ml of 1% lambda carageenan (Sigma Chemical Co., USA) in sterile normal saline into the sub plantar region of the rat left hind paw [26-29]. The rats were divided into five groups (n=6);
Group 1: Vehicle control (normal saline 0.1 ml/10g),
Group 2: Diclofenac (10 mg/kg),
Group 3: LLME (Lagerstroemia lanceolata Methanolic Extract) 100 mg/kg
Group 4: LLME 200 mg/kg
Group 5: LLME 400 mg/kg

Rats were pretreated orally with LLME and Diclofenac 1 hr before the carrageenan injection. The paw volume was measured at 0-5 h, at an hourly interval using plethysmometer. The mean changes in injected paw volume with respect to initial paw volume were calculated. Percentage inhibition of paw volume between treated and control group was calculated by the following formula.

\[ \text{% Inhibition} = \left(1 - \frac{V_T}{V_C}\right)\times 100 \]

Where, \( V_T \) and \( V_C \) are the increase in paw volume in treated and control groups, respectively.

RESULTS

Phenol and flavonoidal content
Phenolic compounds are known to possess the ability to reduce oxidative damage acting as antioxidants. They can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes [30].

Some studies associated the antioxidant capacity of phenolic compounds with an important role in stabilizing lipid peroxidation [31]. LLME exhibited a total phenolic content of 2.09 mg of GAE/100 mg of dried extract. Flavonoids and flavonols are an important sub-branch of the polyphenol family, synthesized by plants. In this study, total flavonoids were found to be 1.476 mg of quercetin/100 mg of dried extract.

Table-1 shows that ethyl acetate and methanol extract of L. lanceolata W. is richer in phenolic and flavonoidal content than the aqueous extract.
Table 1: Estimation of total phenolic and flavonoids content of *L. lanceolata* W

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracts</th>
<th>Total phenolic content (mg/100 mg of dried extract)</th>
<th>Total flavonoids content (mg/100 mg of dried extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethyl Acetate</td>
<td>3.472</td>
<td>1.200</td>
</tr>
<tr>
<td>2.</td>
<td>Methanol</td>
<td>2.090</td>
<td>1.475</td>
</tr>
<tr>
<td>3.</td>
<td>Aqueous</td>
<td>0.981</td>
<td>1.027</td>
</tr>
</tbody>
</table>

Acute Oral Toxicity Study

LLME was found safe up to 2000 mg/kg. Therefore the three doses 100 mg/kg, 200 mg/kg and 400 mg/kg were selected for the study.

Anti-inflammatory study (Carrageenan induced rat paw edema)

The paw volume was elevated in carrageenan control when compared to healthy control (*p* < 0.001). Treatment with the LLME at a dose of 200 mg/kg and 400 mg/kg exhibited a significant decrease (*p* < 0.001) in paw volume. LLME (200 and 400 mg/kg) showed significant decrease (*p* < 0.001) in paw volume at 4th hr. whereas LLME (100 mg/kg) did not show any significant decrease in paw volume at 4th hr. LLME (200 mg/kg) showed significant (*p* < 0.001) decrease in paw volume at 5th and 6th hr. LLME (400 mg/kg) also showed a significant decrease (*p* < 0.001) in paw volume at 5th and 6th hr. Diclofenac (10 mg/kg) exhibited a significant reduction in paw volume at 3rd, 4th, 5th and 24th h (*p* < 0.001) (Fig-1).

![Fig-1: Fig. Effect of LLME on the change in paw volume of paw volume in Carrageenan induced paw edema in female wistar rats](http://saspublisher.com/sajp/)

Values are expressed as mean ± SEM for six animals and analyzed by Two way ANOVA followed by Bonferroni post-hoc test. *p* < 0.05, **p** < 0.01, ***p*** < 0.001 when compared to carrageenan control and *p* < 0.05, **p** < 0.01, ***p*** < 0.001 when compared to healthy control.

Table 2 depicted the percentage inhibition of change in paw volume of LLME 100, 200 and 400 mg/kg as well as Diclofenac (10 mg/kg).

DISCUSSION

Phenolic compounds are a wide and heterogeneous group, ubiquitous in plant-based foods, and possess an amazing anti-inflammatory ability due to their inhibitory effect on pro-inflammatory mediators [27]. This has led researchers to propose dietary phenolic compounds as a potential natural alternative for the treatment of inflammation and related diseases, with minimal or null adverse side effects [32].

Prevention and cure of diseases using phytochemicals especially flavonoids are also well known. Variety of flavonoids which are found in nature possesses their own physical, chemical, and physiological properties. Medicinal usefulness of many flavonoids as antibacterial, hepatoprotective, anti-
inflammatory, anticancer, and antiviral agents is well established [13, 33-46].

Table-2: Effect of LLME on percent inhibition of paw volume in Carrageenan induced paw edema in female wistar rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dichlofenac (10 mg/kg)</th>
<th>LLME (100 mg/kg)</th>
<th>LLME (200 mg/kg)</th>
<th>LLME (400 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>46.26</td>
<td>5.91</td>
<td>13.58</td>
<td>25.59</td>
</tr>
<tr>
<td>2 hr</td>
<td>59.90</td>
<td>7.56</td>
<td>27.08</td>
<td>44.54</td>
</tr>
<tr>
<td>3 hr</td>
<td>71.26</td>
<td>18.18</td>
<td>26.05</td>
<td>44.54</td>
</tr>
<tr>
<td>4 hr</td>
<td>84.09</td>
<td>16.05</td>
<td>48.86</td>
<td>69.89</td>
</tr>
<tr>
<td>5 hr</td>
<td>85.86</td>
<td>20.00</td>
<td>63.33</td>
<td>76.98</td>
</tr>
<tr>
<td>6 hr</td>
<td>85.00</td>
<td>43.79</td>
<td>62.50</td>
<td>74.50</td>
</tr>
<tr>
<td>24 hr</td>
<td>83.79</td>
<td>47.39</td>
<td>58.62</td>
<td>61.03</td>
</tr>
</tbody>
</table>

There are different mediators which are released during inflammation process from tissues and migrating cells, and most strongly associated are the prostaglandins (PGs), leucotrienes (LTs), histamine, bradykinin, platelet-activating factor (PAF) and interleukin-1 [5-7, 9, 10, 12, 47, 48]. The carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatory activity of extracts [49]. Carrageenan is extracted from the red seaweed marine alga Chondrus crispu which is used in animal models of inflammation to induce swelling and pain [50]. The edema development in rat after the injection of carrageenan has been described as a biphasic event [51]. The edema induced by carrageenan is highly sensitive to NSAIDs and has been accepted as a useful indicator for identifying the new anti-inflammatory molecules. The results of the carrageenan induced paw inflammation revealed that LLME (200 & 400 mg/ kg) produced significant inhibition of paw edema as compared to carrageenan control group. The maximum percent inhibition was observed at 5th hr. 63.33%, and 76.98% for the dose of 200, and 400 mg/kg respectively. LLME contains glycosides, phenols, flavonoids, steroids moieties that may be responsible for the pharmacological activities. The methanolic extract of leaf of Lagerstroemia lanceolata contains different chemical constituents and possess potential anti-inflammatory activity.

Further studies can be performed for the isolation and identification of individual phenolic & flavonoidal compounds of the fractions.

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