Phytochemical and Pharmacological Screening of the fruits of Lagerstroemia speciosa (L.) pers.

Sm Faysal Bellah1*, KM Rezwanul Islam2, Md. Rezaul Karim1, Md. Jahangir Hossain3, Md. Ashrafudoulla4 and Mehedee Hasan1

1Department of Pharmacy, Manarat International University, Dhaka-1216, Bangladesh
2Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh
3Quality Control Department, Beximco Pharmaceuticals Ltd. Tongi, Gazipur, Bangladesh

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ABSTRACT

The present piece of work was framed to investigate the phytochemical screening along with pharmacological activities (analgesic, cytotoxicity and antibacterial activity) of the ethanolic fruit extract of the plant Lagerstroemia speciosa (L.) from family of Lythraceae. Phytochemical tests confirmed Steroids, Tannins and Saponnins as its constituent compounds. Presence of these compounds further provoked to check for possible analgesic, cytotoxic and antibacterial activities of L. speciosa. The crude extract at the dose of 500 mg/kg body weight exhibited moderate analgesic action against acetic acid induced pain in Swiss albino mice, where percentage of protection was found 55.21% while the percentage of protection for standard drug diclofenac was 84.37% at a measure of 25 mg/kg body weight and the result was statistically significant (p<0.01). The extract showed moderate antibacterial activity against Staphylococcus epidermis, Enterococci, Escherichia coli and Staphylococcus saprophyticus. The extract also showed potent cytotoxic activity in brine shrimp where LC50 and LC90 was found at the dose of 60 µg/ml and 100 µg/ml respectively. The results of the study tend to suggest the traditional uses of L. speciosa and could be the basis of further investigations in future including isolation of novel compounds.

Key Words: Lagerstroemia speciosa, phytochemical test, analgesic activity, antibacterial activity & cytotoxic activity.

INTRODUCTION

Since, disease, decay and death co-existed with the beginning of human race, they always tried to be escaped and found plants, plants products as an effective therapeutic tools to treat disease and injuries [1]. So, undoubtedly nature blessed us providing a complete store house of remedies to cure all ailments of mankind [2]. An estimation says, almost 80% of the present day medicine are obtained from plants either directly or indirectly [3]. In the year of 1980, consumer paid 8 billion dollars in the United States for prescription drugs where the active ingredients are still derived from plants [4]. For this reason, plant screening has been given great impact and therefore, Hartwell established an extensive system of plant collection, screening and isolation at National Cancer Institute, USA [5]. L. speciosa (L.) Pers. is a species under Lythraceae family, locally known as Jaral. It’s a large deciduous tree with rounded crown reaching a height of 35-45 m and up to 4.2 m girth with a long cylindrical bole. The flowers are beautiful, pinkish in colour [6]. Fruit-capulse of L. speciosa ellipsoid or subglobose, oblong-ovoid, smooth, seated on the persistent somewhat woody, prominently ribbed enlarged calyx tube, 5-6 valved. Seeds 1-1.5 cm long along with wing [7]. It’s natural range include Assam, Bengal, Chittagong, Western and Southern India, SriLanka, West Bengal and some other parts of Bangladesh [8]. In its natural habitat the absolute maximum shade temperature varies from 32° to 43°C, the absolute minimum shade temperature various from 2° to 18°C. Normal rainfall varies from 1524 to 4572 mm per year [9]. Among of many uses-laves are used in diabetes; Fruits are narcotic; Juice of root is stimulant; Bark is astringent [10]. Several researchers have studied the phytochemical and various pharmacological activities from time to time with different species.
The phytochemical and pharmacological studies of fruit of *L. speciosa* so far limited. Therefore, an attempt was made to evaluate phytochemical constituents and pharmacological effect of fruits derived from *L. speciosa* plant. In past many other studies have been done by taking different parts of *Lagerstroemia speciosa* (L.) Pers, with taking methanolic and ethanolic extracts but we have studied that, those types of test we have studied here have not studied before in together by using ethanolic extracts of fruit of *L. speciosa* (L.) Pers. The present study is to find out the phytochemical test, analgesic activity and cytotoxic activity in together by using ethanolic extract of the fruit of *L. speciosa* (L.) Pers. We have focused our concentration only on the fruits of *L. speciosa* (L.) Pers. for finding our desired results by performing phytochemical test, analgesic activity and cytotoxic activity.

**MATERIALS AND METHODS**

**Plant collection:** For this present investigation the fruits of *L. speciosa* were collected from Khulna University campus. The plant was identified by Bangladesh National Herbarium (Acc. No. 31394)

**Preparation of crude drug**

**Drying and grinding:** The fruits were collected from plants or plant parts and after cutting they were sun-dried for one week to small pieces. The fruits were ground by a suitable grinder and then powder was stored into an airtight container until analysis commenced. Then kept the powder in a cool, dark and dry place.

**Ethanol extraction:** About 400 gm of powered material was taken glass container which was clean, flat bottomed and then soaked in 1700 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. After then the whole mixture was filtrated by a piece of clean, white cotton material and next it was filtered through whatman filter paper.

**Yield of *L. speciosa***: Fruit powder taken for extraction 400 gm. Yield extract = 9 gm

**Chemicals:** The chemicals used in this study were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO, USA). The chemicals which used were analytical grade.

**Test animals:** Young Swiss-albino mice were used for the experiment. Those were average weight 20-25 gm and aged 4-5 weeks. The mice were collected from the Animal Research Branch of ICDDR, B (International Centre for Diarrhoeal Disease and Research, Bangladesh) and formulated rodent food and water were used for their feeding.

**Test Organism:** Artemiasalina Leach (brine shrimp). The egg of the shrimp was collected from Katabon University Market.

**Phytochemical test**

**Tests for alkaloids:**

Mayer’s test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer’s reagent was added. Yellowish buff colored precipitate was not obtained indicate absence of alkaloid.

Dragendorff’s test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendorff’s reagent was added. Orange brown precipitate was not added. So, alkaloid is absent.

**Tests for Glycosides:**

Alcoholic extract of dried plant material was taken in 1ml of water which was small in amount. Then, a few drops of aqueous sodium hydroxide were added. A yellow color was not found indicates the absence of glycosides.

**Tests for Steroids:**

Sulphuric acid test: 1ml solution of chloroform extract was taken and then added 1ml Sulphuric acid. Chloroform layer. Acquired reddish brown color and acid layer showed green fluorescence. It indicates the presence of steroid.

**Tests for gums:**

5 ml extract solution was taken in a test tube. Then molish reagent and sulphuric acid were added. At the junction of two liquids there was not any red-violet ring which indicates the absence of gums.

**Tests for Tannins**

Ferric Chloride Test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added. The red color was found which indicates the presence of tannins.

**Tests for Flavonoids:**

Alcoholic extract of the plant material and concentrated hydrochloric acid were shaken after taking according the required amount. Immediate red color was not formed. Absence of flavonoids is ensured.
Test for Saponins:
1 ml solution of the extract was shaken in a graduated cylinder for 15 minutes after diluting to 20 ml. One Centimeter layer of foam. So, it indicates the presence of saponins.

Analgesic activity test:
Study of analgesic activity by acetic acid induced writhing method [20]. Experimental animals were divided into three groups such as group-I, group-II, and group-III and each group contains 5 mice. Each group received a particular treatment i.e. control, positive control and the test sample. After weighing each mouse properly the doses of the test samples was determined. To prepare suspension of the test samples at the dose 500 mg/kg per body weight, 125 mg were measured respectively. The extract was triturated by the addition of small amount of water to make a volume about 2.5 ml. Small amount of tween-80 was added to stabilize the suspension.

For the preparation of diclofenac sodium at the dose of 25 mg/kg-body weights, 12.5 mg of diclofenac sodium were taken and a suspension of 5 ml was made. Test sample, control (10 ml/kg body weight) and diclofenac sodium were used for oral route. About thirty minutes was needed for proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intraperitoneally to each of the animals of a group.

Antibacterial assay: The disc diffusion method was chosen for antibacterial assay [21], [22]. In this study, 11 microorganisms were taken where as positive and negative controls Standard Kanamycin (30μg/disc) and blank sterile filter paper disc were used. Nutrient agar medium was used to prepare fresh cultures for testing the sensitivity of the organisms. The sample discs, the control discs and the standard antibiotic discs were kept lightly on the previously marked zones in the agar plates which were previously inoculated with test bacteria. At 37°C for 24 hours the discs were then incubated on the plate aerobically. The diameter of zone of inhibition around each disc was measured and recorded at last of the incubation period.

Cytotoxic Activity Test: For the bioactive substances and natural product extracts brine shrimp lethality bioassay is a recently developed procedure. It indicates cytotoxicity and a wide range of pharmacological activities like anticancer, antiviral, pesticidal, etc. [23]. Bioactive compounds are almost always toxic in high doses. At a lower dose pharmacology is simply toxicology whereas toxicology is simply pharmacology at a higher dose. Thus, in-vivo lethality of an extract against a simple organism (brine shrimp nauplii) can be used as a beneficial monitor for screening and fractionation in the discovery of new bioactive natural compounds [24].

Preparation of stock solution: 250 mg of dried ethanol extract was taken in 10 ml volumetric flask and the volume was adjusted by DMSO. The concentration of this solution was 25 μg/μl.

Preparation of sea water: To make 1 litre solution, 20g pure NaCl and 18g table salt were weighed properly. Then it was dissolve in distilled water according to the rules.

Hatching of brine shrimp: In the divided tank shrimp eggs and were added and the shrimps were allowed for 20-22 hour for hatching and then also allowed to mature as nauplii (larvae). The hatched shrimps were recognized for bioassay.

Application of test solution and brine shrimp nauplii to the test tubes: 12 clean test tubes were taken, 6 of them were for the samples in six concentrations and other 6 for control test. Then 5ml of seawater was given to each of the test tubes. Then by using with the help of the micropipette specific volumes (2, 4, 8, 12, 16 and 20 μl) of samples were taken from the stock solutions to the test tubes to ensure desire sample concentrations of 10, 20, 40, 60, 80, and 100 μg/ml respectively. The concentration of DMSO in these test tubes did not exceed 10 μl/ml. For the control, same volumes of DMSO (as in the sample test tubes) were taken in the rest of the 6 test tubes. Finally 10 living shrimps were taken in each of the test tubes by using a Pasteur pipette [25].

Counting of nauplii: After 20 hours the test tubes were viewed. Then in each test tube the amount of survived nauplii was counted for noting the result. From this, calculation was performed in percentage of lethality of brine shrimp nauplii at each concentration for each sample.

RESULTS
Phytochemical test: Phytochemical studies showed that steroids, tannins, and saponins are present in the ethanolic extract which has shown in table 1.

Analgesic activity test: The ethanol extract of fruits of L. speciosa produced 55.88 % protection or writhing inhibition in mice at orally doses of 500 mg/kg body weights of mice which was analogous to the standard drug diclofenac sodium in where the inhibition was about 84.37 % at the dose of 25 mg/kg. Table 2 and 3 shows the effect of the
ethanolic extract of fruits of *L. speciosa* on acetic acid induced writhing in mice. It was found that the extracts cause a significant (P <0.01) inhibition on the writhing response. Graphic presentation shown in figure1 and figure 2.

**Antibacterial assay:** The zone of inhibition (mm) produced by the ethanol extract at the concentration of 500 μg/disc and Kanamycin at the concentration of 30μg/disc against different bacterial strains, are represented in the table 4.

**Cytotoxic Activity Test:** According to different concentrations, test sample showed different mortality rateand the mortality rate of brine shrimp was increased according to the concentration of the sample .The percent mortality versus log concentration plot on the graph paper created an approximate linear correlation between them. From the graph (figure 3) the concentrations at which 50% mortality (LC50) of brine shrimp nauplii occurred were obtained by extrapolation. The values were found to be 60 μg/ml for the crude extract. The 90% mortality (LC90) values were 100 μg/ml respectively (Table 5).

**DISCUSSION**

We highly focused on its fruit and got some influential findings such as steroid, tannins, saponins like specific compounds in the fruit of *L. Speciosa* and very much effective analgesic, antibacterial and cytotoxic effect. In this study, phytochemical test has shown the presence of Steroid, Tannins and Saponin whereas Alkaloid, Glycoside, Gums and Flavonoids are absent .Analgesic activity also shown that 55.88 % withing inhibition 500 mg/kg body weight in compare with diclofenac 84.37 % at the dose 25 mg/kg. In antibacterial test, ethanolic extract (500 μg /dise) showed zone of inhibition against *Staphylococcus epidermis, Enterococci, Escherichia coli* and *Staphylococcus saprophyticus* diameter of zone were respectively 7, 6, 6 and 6. In case of cytotoxic study, 50% mortality occurred at the concentration 60 μg/ml whereas 90% mortality found at 100 μg /ml of concentration. Although, research from various regions of the world found out significant phytochemical and pharmacological effect of different parts (leaf, barks, and flowers) of *L. Speciosa* like anti-diabetic, anti-oxidant, anti-inflammatory activities and other chemical compounds like alkaloids, glycosides [26],[27],[28],[29],[30],[31]. The cytotoxic activity was determined by using ethanol extract of fruits of *L. speciosa* (L.) Pers. and brine shrimp lethality bioassay. For the bioactive compounds it is very helpful in the bioassay and brine shrimp lethality bioassay introduce cytotoxicity as well as pharmacological activities such as antimicrobial, pesticidal, antitumor etc [32]. The extract had shown potent activity in opposition to the brine shrimp nauplii and the positive response had found in this assay which indicates that the extract may contain antibacterial or pesticidal compounds.

The possibility of the presence of phytochemical constituents like tannins, proteins, alkaloids, flavonoids and saponins may increase or decrease according to the condition of areas such as dry and shady areas [33].

**CONCLUSION**

The experimental findings from the study showed that the ethanolic extract has organic compounds which can show extensively pharmacologic activity. From the above observation it can be suggested that the ethanolic extract of fruits of *L. speciosa* had shown analgesic activity. The ethanolic extract of *L. speciosa* showed antibacterial activity. The crude extracts was found to show potent cytotoxic effect by brine shrimp bioassay.

**Competing interests:** The authors declare that they have no competing interests.

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**Table 1:** Results of different group test in Ethanolextract of *L. speciosa*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloid</th>
<th>Glycoside</th>
<th>Steroid</th>
<th>Gums</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Saponin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extracts of</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>L. speciosa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = Presence        - = Absence
Table 2: Tabulation of writhing

<table>
<thead>
<tr>
<th>Substance Administered (dose)</th>
<th>Group</th>
<th>Numbering of mice</th>
<th>Body Weight (gm)</th>
<th>Dose (ml)</th>
<th>Total writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group 0.1 % tween-80 in distilled water</td>
<td>I</td>
<td>1</td>
<td>26</td>
<td>0.26</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>25</td>
<td>0.25</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>25</td>
<td>0.25</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>26</td>
<td>0.26</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>28</td>
<td>0.28</td>
<td>18</td>
</tr>
<tr>
<td>Standard group Diclofenac sodium (25mg/kg)</td>
<td>II</td>
<td>1</td>
<td>23</td>
<td>0.23</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>25</td>
<td>0.25</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>22</td>
<td>0.22</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>22</td>
<td>0.22</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>21</td>
<td>0.21</td>
<td>4</td>
</tr>
<tr>
<td>Test group Ethanol Extract (500 mg/kg)</td>
<td>III</td>
<td>1</td>
<td>24</td>
<td>0.24</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>22</td>
<td>0.22</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>25</td>
<td>0.25</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>22</td>
<td>0.22</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>25</td>
<td>0.25</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3: Statistical evaluation of the results

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Total Writhing</th>
<th>Mean Writhing</th>
<th>Standard deviation (SD)</th>
<th>Standard error (SE)</th>
<th>% Writhing</th>
<th>% Protection</th>
<th>T-test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control n=05</td>
<td>96</td>
<td>19.2</td>
<td>1.64</td>
<td>0.82</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac sodium n=05</td>
<td>15</td>
<td>3</td>
<td>1.58</td>
<td>0.79</td>
<td>15.63</td>
<td>84.37</td>
<td>14.21 (P&lt;.001)</td>
</tr>
<tr>
<td>Extract (500mg/kg) n=05</td>
<td>43</td>
<td>8.6</td>
<td>4.9</td>
<td>2.45</td>
<td>44.79</td>
<td>55.21</td>
<td>4.14 (P&lt;.01)</td>
</tr>
</tbody>
</table>

n = Number of mice; Significance: Control Vs Diclofenac sodium: Significant (P< 0.001), Control Vs Extract (500 mg/kg): Significant (P< 0.01)

Table 4: In vitro anti-microbial activity of Ethanol extract

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Diameter of zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kanamycin (30μg/disc)</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>22</td>
</tr>
<tr>
<td>Enterococci</td>
<td>19</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>17</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>21</td>
</tr>
</tbody>
</table>

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Table 5: Result of Brine shrimp lethality bioassay of ethanol extract of fruit of *Lagerstroemia speciosa*

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Conc. (µg/ml)</th>
<th>Log Conc.</th>
<th>No. of shrimp</th>
<th>No. of alive shrimp</th>
<th>% mortality</th>
<th>LC₅₀ (µg/ml)</th>
<th>LC₉₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of <em>L. speciosa</em></td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.3</td>
<td>10</td>
<td>8</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1.6</td>
<td>10</td>
<td>8</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.7</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.9</td>
<td>10</td>
<td>2</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Percent writhing inhibition of metabolic extract of fruits of *Lagerstroemia speciosa* on acetic acid induced writhing in mice.

Fig. 2: Comparison of acetic acid induced writhing in control, positive control and test group.
Fig 3: Cytotoxic effect of extract of fruit of Lagerstroemia speciosa on brine shrimp.

REFERENCES