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PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF THE FRUITS OF *LAGERSTROEMIA SPECIOSA* (L.) PERS

Sm Faysal Bellah [* 1], Km Rezwanul Islam [2], Md. Rezaul Karim [1], Md. Ashrafudoulla [1] and Mehedee Hasan[1]

Department of Pharmacy [1], Manarat International University, Dhaka - 1216, Bangladesh
Pharmacy Discipline [2], Life Science School, Khulna University, Khulna - 9208, Bangladesh.

ABSTRACT: The present study was investigated the phytochemical properties, analgesic and cytotoxic activities of the ethanolic extract of *Lagerstroemia speciosa* (L.) Pers. fruits (Family-Lythraceae). Phytochemical screening of the ethanol extract of fruits of *L. speciosa* showed the presence of Steroids, Tannins, and Saponins types of compounds. The pharmacological interest of these compounds encouraged to check *L. speciosa* Pers. for possible analgesic & cytotoxic activities. The crude extract at the dose of 500 mg/kg body weight exhibited moderate action against acetic acid-induced pain in mice significantly ($p<0.01$), where percentage of protection was found 55.21% while the standard drug diclofenac's percent of protection was found to be 84.37% at a dose of 25 mg/kg body weight. The extract showed potent cytotoxic activity to brine shrimp, and LC_{90} was found at the dose of 100 µg/ml as well as LC_{50} was 60 µg/ml. The obtained results must help use this plant in traditional medicine and its further investigation.

Keywords: *Lagerstroemia speciosa,* phytochemical test, Analgesic activity & Cytotoxic Activity

Correspondence to Author:
Sm Faysal Bellah

Lecturer,
Department of Pharmacy, School of Engineering, Science and Technology, Manarat International University, Dhaka - 1216, Bangladesh.

E-mail: faysal_Phku@yahoo.com

INTRODUCTION: Since, disease, decay, and death co-existed with the beginning of the 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 is obtained from plants either directly or indirectly [3].

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In the year of 1980, the 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 *Jarul*.

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 color [6]. Fruit-capsule of *L. speicosa* 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].

Its natural range includes Assam, Bengal, Chittagong, Western and Southern India, Sri Lanka, 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 many uses-laves are used in diabetes; Fruits are narcotic; Juice of the root is stimulant; Bark is astringent [10]. Several researchers have studied the phytochemical and various pharmacological activities from time to time with different species in different parts of the world of *L. speciosa* [11, 12, 13, 14, 15, 16, 17, 18, 19, 20]. The phytochemical and pharmacological studies of the fruit of *L. speciosa* ***is*** so far limited. Therefore, an attempt was made to evaluate phytochemical constituents and pharmacological effect of fruits derived from *L. speciosa* plant.

MATERIALS AND METHODS:

Plant Collection: For this present investigation, the fruits of *L. species* 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 into small pieces. The fruits were ground by a suitable grinder, and then the powder was stored into an airtight container until analysis commenced. Then kept the power in a cool, dark and dry place.

Ethanol Extraction: About 400 gm of powdered 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 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.

The 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: Artemia salina 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. The yellowish buff colored precipitate was not obtained indicate absence of alkaloid.

Dragendroff'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 Dragendroff's reagent was added. The orange-brown precipitate was not observed. 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. Yellow color was not found indicates the absence of glycosides.

Test for Steroids:

The 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.

Test 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**.**

Test for Flavonoids: Alcoholic extract of the plant material and concentrated hydrochloric acid were

shaken after taking according to the required amount. The 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 [21]. Experimental animals were divided into three groups such as group-I, group-II, and group-III and each group contained 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 a 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 a small amount of water to make a volume of about 2.5 ml. A 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. The 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 [22, 23]. In this study, 11 microorganisms were taken whereas positive and negative controls Standard Kanamycin (30µg/disc) and blank sterile filter paper disc was 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 37ºC for24 hours, the discs were then incubated on the plate aerobically. The diameter of the 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*. [24]. 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 napulii) can be used as a beneficial monitor for screening and fractionation in the discovery of new bioactive natural compounds [25].

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 25µg/µl.

Preparation of Sea Water: To make 1-litre solution, 20g pure NaCl and 18g table salt were weighed properly. Then it was dissolved 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 10µl/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 [26].

Counting of Nauplii: After 20 h, the test tubes were viewed. Then in each test tube, the amount of survived nauplii was counted for noting the result. From this, the calculation was performed in the percentage of the 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**.

TABLE 1: RESULTS OF DIFFERENT GROUP TEST IN ETHANOL EXTRACT OF *L. SPECIOSA*

Extract	Alkaloid	Glycoside	Steroid	Gums	Tannins	Flavonoids	Saponin
Ethanol extracts of *L. speciosa*	-	-	+	-	+	-	+

+ = Presence, - = Absence

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**.** The graphic presentation is shown in **Fig. 1** and **2**.

TABLE 2: TABULATION OF WRITHING

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

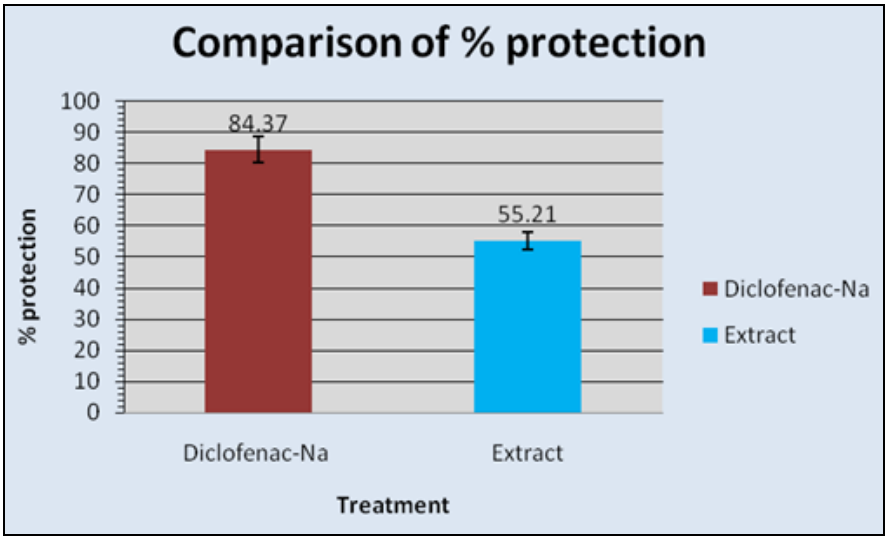

FIG. 1: PERCENT WRITHING INHIBITION OF METABOLIC EXTRACT OF FRUITS OF *LAGERSTROEMIASPECIOSA* ON ACETIC ACID INDUCED WRITHING IN MICE

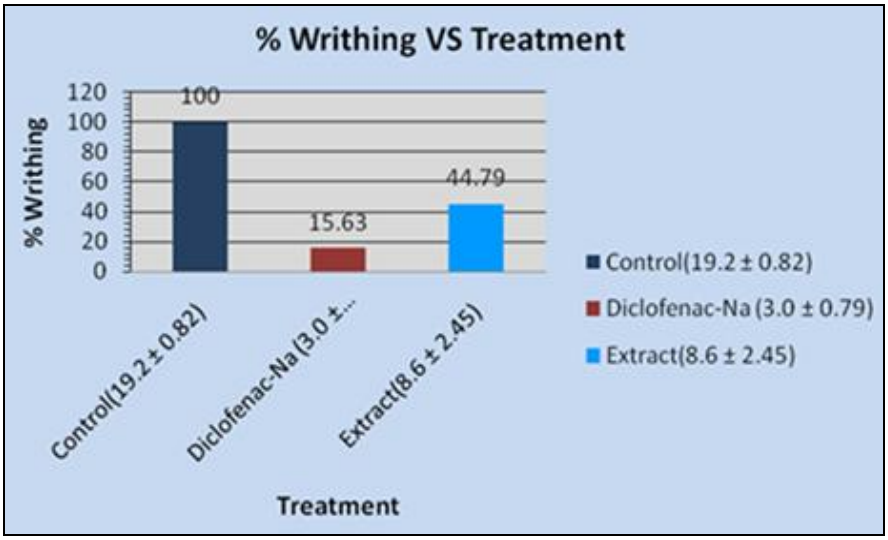

FIG. 2: COMPARISION OF ACETIC ACID INDUCED WRITHING IN CONTROL, POSITIVE CONTROL, AND TEST GROUP

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 **Table 4.**

Cytotoxic Activity Test: According to different concentrations, test sample showed different mortality rate and 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 approximately linear correlation between them. From the graph **Fig. 3** the concentrations at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation. The values were found to be 60µg/ml for the crude extract. The 90% mortality (LC_{90}) values were 100µg/ml respectively **Table 5**.

TABLE 3: STATISTICAL EVALUATION OF THE RESULTS

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

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

Bacterial strains	The diameter of the zone of inhibition in mm	
	Kanamycin (30µg/disc)	Ethanol extract (500µg/disc)
Staphylococcus epidermis	22	7
Salmonella typhi	19	0
Entero cocci	19	6
Proteus spp.	15	0
Escherichia coli	17	6
Spyo	17	0
Vibrio cholerae	16	0
Shigella boydii	15	0
Staphyllococcuss aprophyticus	21	6
Pleusomonus spp	16	0
Hafnia spp	22	0

TABLE 5: RESULT OF BRINE SHRIMP LETHALITY BIOASSAY OF ETHANOL EXTRACT OF FRUIT OF *LAGERSTROEMIA SPECIOS*

Test sample	Conc. (µg/ml)	Log Conc.	No. of shrimp	No. of alive shrimp	% mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Ethanol	10	1	10	9	10	60	
extract of	20	1.3	10	8	20		
L. speciosa	40	1.6	10	8	20		
	60	1.7	10	5	50		100
	80	1.9	10	2	80		
	100	2	10	1	90		

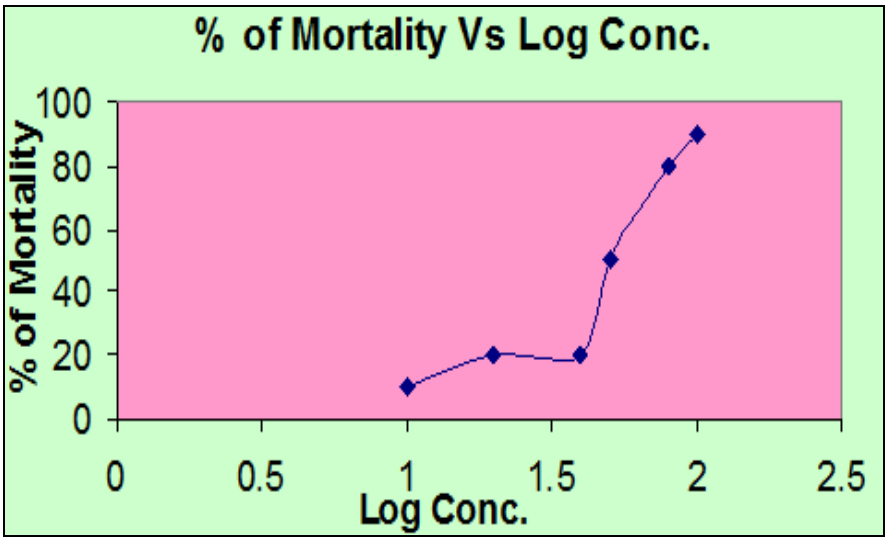

FIG. 3: CYTOTOXIC EFFECT OF EXTRACT OF FRUIT OF *LAGERSTROEMIA SPECIOSA*ON BRINE SHRIMP.

DISCUSSION: 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 [27, 28, 29, 30, 31, 32]. 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, anti-bacterial, and cytotoxic effect.

However, we do believe more advanced studies should conduct for further findings and for well-establishment of *L. Speciosa*as a vital plant with higher medicinal values. 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 [33]. 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.

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 were found to show potent cytotoxic effect by brine shrimp bioassay.

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COMPETING INTERESTS: The authors declare that they have no competing interests.

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