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Full Length Research Article

Anti-microbial Evaluation of *Lagerstroemia speciosa* (L.) Pers (Lythraceae) Ethanolic extract of Leaf, Flower and Seed - In Vitro Studies

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Abstract

The aim of this study was to investigate the antibacterial activities of ethanolic extract of *Lagerstroemia speciosa*. The qualitative phytochemical analysis of ethanolic leaf, flower and seed extracts shows the presence of different types of compounds like terpenoids, tannins, deoxy sugars, saponins, phenolic compounds, and flavonoids, may contribute for the antimicrobial activity. The antibacterial study was carried out by Well diffusion method, Muller Hinton Media and Chloramphenicol (CLP) and Amoxicillin (AMX), standards were tested against different bacterial pathogens, Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*), and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) by using ethanolic extract. The result revealed that the ethanolic leaf extracts 50µl inhibits *Staphylococcus aureus* comparable to standard II. The ethanolic flower extracts inhibits the same in standard I. Higher inhibiting activity was shown in the ethanolic seed extracts towards standard I and II.

***Key words*:** Antibacterial, Amoxicillin, Chloramphenicol, Muller Hinton media, Medicinal plants, Pathogens

The active metabolites of medicinal plants represent rich source of antimicrobial agents. Plants are used as a traditional medicine in many countries and are the source of chemical compounds [1]. The secondary metabolites of plants were found to be source of various phytochemicals that could be directly used as intermediates for the production of new drugs [2]. Developing countries mainly depends on medicinal plants for the production of powerful drugs [3]. According to World Health Organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care needs [4]. Among the estimated 2,50,000-5,00,000 plant species, only a small percentage have been investigated phytochemicals and the fraction submitted to biological or pharmacological screening. Compound of natural or synthetic origin has been the source of innumerable therapeutic agents [5].

Amoxicillin (AMX) is one of the most common antibiotics prescribed for children. It is on the WHO's List of Essential Medicines. They are normally the only penicillin's added to feedstuffs at the maximum level of 500 mg kg^{-1} [6]. Chloramphenicol (CLP) is an effective antibiotic that has widely been used since the 1950s to treat food-producing animals. CLP is a wide range antibiotic which interferes with protein synthesis of many Gram-negative and Gram-positive bacteria [7], and has toxic effects on humans. These medicines may cause some serious side effects. The side effects of synthetic drugs are more when compare to natural medicines [8].

The medicinal plant *L. speciosa* belongs to the family Lythraceae and commonly called Queens Crape Myrtle. Leaves and flowers are considered hydragogue and drastic purgative. Bark is also considered stimulant and febrifuge. Roots are astringent and used as gargle. Seeds have narcotic properties [9]. The plant has been used as an anti-inflammatory agent in wound healing, anti-anxiety, anti-stress, anti-mutagenic, and spasmolytic agent and spasmolytic activities [10].

MATERIALS AND METHODS

Collection and authentication of plant samples

The leaves, flowers and seeds of *L. speciosa* were collected from PG Girls hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The *L. speciosa* were identified and authenticated at Botanical Survey of India, Coimbatore-03 (No: BSI/SRC/5/23/2020/Tech/51) and the voucher specimens was kept in Department of Zoology, Government Arts College, Coimbatore-18.

Plant extracts preparation

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The selected parts of the plant samples were collected and were washed thoroughly 2-3 times with clean running water and shade dried. The dried samples were then kept under the room temperature (27± 2 °C) for about 2 weeks till dried and pulverized, completely. The dried samples were finely powdered using an electric blender. The powdered plant material (100 g) was soaked in ethanol (1000 ml) in an airtight, wide mouthed bottle and kept for 4 days with periodic shaking. After that the extract was filtered using Whatman No. 1 filter paper and kept in plastic tray to dry at room temperature [11].

Phytochemical analysis

Qualitative phytochemical analysis of the leaf, flower and seed of *L. speciosa* Ethanolic extracts were carried out according to the methodology of Horborne [12], Trease and Evans [13]. The main group of chemical constituents was identified and listed in the (Table 1).

Microbial culture

A total four microorganisms were used to assess the antibacterial properties, it includes two gram-positive bacteria, (*Staphylococcus aureus, Enterococcus faecalis*), gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*). The microorganisms originally obtained from VJ Biotech, Biotechnology Company, Coimbatore, Tamil Nadu, India.

Media preparation

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated at 37 °C for 24 hours. A single colony was transferred to nutrient agar media slants were incubated at 37 °C for 24 hours. These stock cultures were kept at 4°C. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°C for 18-20 hours for bacterial culture.

Bacterial media (Muller Hinton media)

36g of Muller Hinton media (Hi-media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petri dishes. The solidified plates were poured with 5mm diameter cork bearer. The plates with wells were used for the antibacterial studies.

Well diffusion method

The antibacterial activity of crude extracts was determined by Well diffusion method [14]. MHA plates were prepared by pouring 20ml of molten media into sterile petri plates. After solidification of media, 20-25µl suspension of bacterial inoculums was swabbed uniformly. The sterile paper discs were dipped into required solvents then placed in agar plates. The tested cell suspension was spread on Muller Hinton Agar Plate. Wells were made in the agar plate using cork borer. The plant extracts were poured on to wells. Then the plates were incubated at 37°C for about 24 hours and control was also maintained. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the clear zone in cm.

Identification of active positive control

The chloramphenicol and amoxicillin are the commercially available standards used as a positive control. Antibacterial activity was confirmed based on the report and tested against 4 known pathogenic bacteria.

Antibacterial activity of the plant extract

The antimicrobial activity of *L. speciosa* ethanolic extract of leaf, flower and seed extracts of 25µg/ml and 50µg/ml were tested against different bacterial pathogens, Gram-positive bacteria (*S. aureus, E. faecalis*), and Gram- negative bacteria (*E. coli, P. aeruginosa*) by well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm corkborer. The ethanolic plant extracts was dissolved in sterile distilled water to form dilution such as 25µl and 50µl. Each concentration of the plant extract and 50µl of Chloramphenicol and Amoxicillin was tested against different bacterial pathogens. It was demonstrated by well diffusion assay [14]. The extracts were poured into the well using sterile syringe. The plates were incubated at 37 °C ± 2 °C for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in cm) including the well diameter. The readings were taken in three different fixed directions in all three replicates and the average values were tabulated.

RESULTS AND DISCUSSION

Out of three samples, the ethanolic flower extract was reported to be most active against all bacterial strains. The extract was found to be most effective against two bacterial strains viz. *E. coli* and *S. aureus* with minimum inhibited concentration of 50µg/ml.

Phytochemical analysis

The ethanol extract of leaf, flower and seed of *L. speciosa* revealed the following phytochemicals (Table 1).

Table 1. Phytochemical analysis of ethanolic leaf, flower and seed extracts of *Lagerstroemia speciosa*

Phytoconstituents	Leaf extract	Flower extract	Seed extract
Alkaloids	++	+++	+++
Flavonoids	++	+++	+
Saponins	+++	+++	-
Phenols	+++	+++	-
Tannins	+++	+++	+
Protein and amino acids	+++	+++	-
Reducing sugar	+	+++	+++
Steroids	++	++	+
Glycosides	+	++	++
Phytosterols	++	+++	+
Quinones	+	+++	-
Coumarins	++	++	-

'+' indicates the presence of phytoconstituents
'-' indicates the absence of phytoconstituents
'++' indicates the phytoconstituents present in a moderate level
'+++' indicates the phytoconstituents present abundantly

Antimicrobial activity

Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism [15] and many components of plants products

have been shown to be specially targeted against resistant pathogenic bacteria [16]. In vitro antimicrobial activities of ethanol leaf extracts of *L. speciosa* and standard antibiotic is show in (Table 2). The extracts of plants showed various antimicrobial activities against gram negative and positive microorganism, *E. coli*, *P. aeruginosa*, *S. aureus*, *and E. faecalis*, respectively. The leaf extract has the highest antimicrobial efficiency in *P. aeruginosa* (0.8cm/50µl), *S. aureus* (0.6cm/ 50µl) and *E. faecalis* (1.0cm/50µl) comparable to standard II. The (Table 3) shows the excellent antimicrobial activity towards the causative microbes. The ethanol flower extract of *L. speciosa* is the higher inhibition rate in *E. coli* (0.8cm/50µl), *S. aureus* (1.0cm/50µl), and *E. faecalis* (0.5cm/50µl). The antimicrobial activity of ethanol seed extract of *L. speciosa* did not show the inhibition activity in *E. coli*, *P. aeruginosa*, *E. faecalis* except in *S. aureus* in (Table 4). In ethanol seed extract of *Lagerstroemia speciosa*, *S. aureus* shows low inhibition rate 0.5cm/25µl and 0.8cm/50µl compare to standard II (1.0cm). Standard I and Standard II did not show any growth of all the test microorganisms, but inhibited activity against microorganism. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and antimicrobial activity of medicinal inflammatory properties of plants [8].

Table 2 Antibacterial activity of ethanolic leaf extract of *Lagerstroemia speciosa*

Organisms	Zone of inhibition* (in cm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Enterococcus faecalis*
25 µl	0.4 cm	0.3 cm	0.2 cm	0.4 cm
50 µl	0.7 cm	0.8 cm	0.6 cm	1.0 cm
Standard I (Chloramphenicol)	1.0 cm	0.4 cm	0.5 cm	1.2 cm
Standard II (Amoxicillin)	0.9 cm	0.4 cm	0.6 cm	1.0 cm

*Values are the means of three replications

Fig 1 Antibacterial activity of ethanolic leaf extract of *L. speciosa*

Table 3 Antibacterial activity of ethanolic flower extract of *L. speciosa*

Organisms	Zone of inhibition* (in cm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Enterococcus faecalis*
25 µl	0.4 cm	0.3 cm	0.6 cm	0.2 cm
50 µl	0.8 cm	0.7 cm	1.0 cm	0.5 cm
Standard I (Chloramphenicol)	1.2 cm	1.5 cm	1.0 cm	1.0 cm
Standard II (Amoxicillin)	0.8 cm	1.0 cm	1.2 cm	0.2 cm

*Values are the means of three replications

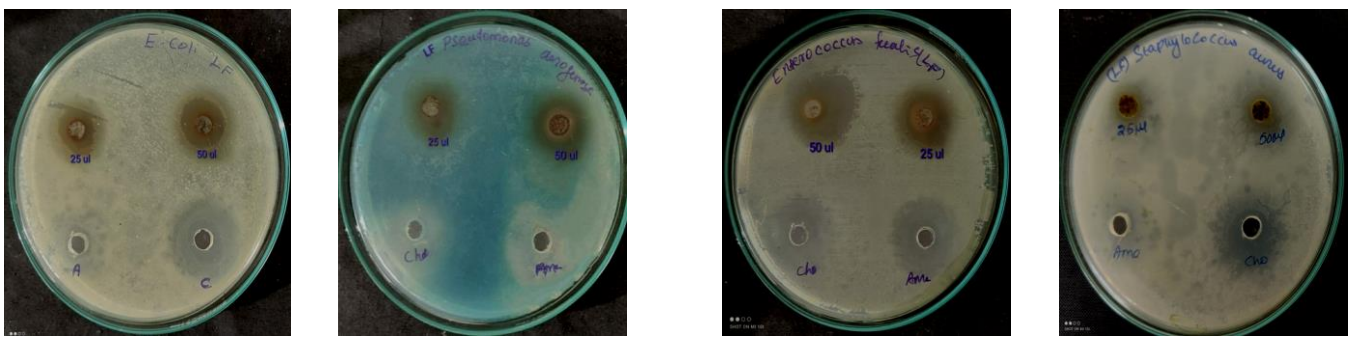

Fig 2 Antibacterial activity of ethanolic flower extract of *L. speciosa*

Table 4 Antibacterial activity of ethanolic seed extract of *L. speciosa*

Organisms	Zone of inhibition* (in cm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Enterococcus faecalis*
25 µl	0.5 cm	0.3 cm	0.5 cm	0.7 cm
50 µl	0.8 cm	0.6 cm	0.8 cm	1.3 cm
Standard I (Chloramphenicol)	1.2 cm	0.5 cm	1.0 cm	0.8 cm
Standard II (Amoxicillin)	1.0 cm	0.5 cm	0.3 cm	1.0 cm

*Values are the means of three replications

Fig 3 Antibacterial activity of ethanolic seed extract of *L. speciosa*

CONCLUSION

The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world population. The antibacterial activities of crude ethanolic extract of *Lagerstroemia speciosa* leaf and flower shows higher inhibitory activity of microbes compare to seed extract. It would suggest that the ethanol extract of *Lagerstroemia speciosa* plant extracts, especially leaf and flower can be used as anti-microbial agents in development of new drugs for the treatment of infectious disease.

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