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Original Article

The Bioactive Compounds of Fruit of *Lagerstroemia speciosa L.* act as Potential Antimicrobial Agent

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ABSTRACT

Jarul (It is also known as Banaba) is a flowering plant that grows in warm climate like the Philippines, India and others. Jarul is widely used in the Philippines and as herbal medicine for diabetes. While in India, Jarul is also used in Ayurvedic medicine for the treatment of diabetes. The Jarul leaves and flowers contain corrosolic acid, a substance being studied for its insulin like effect of lowering the glucose in the body. Jarul is also being studied as a weight-loss supplement for its ability to delay or reduce the absorption of carbohydraes. Jarul is also rich in vitamins and minerals including zinc and magnesium. Jarul is also rich in dietary fibers. The scientific name the Jarul or Banaba is *Lagerstroemia speciosa L (*Lythraceae). It displayed that the leaves, bark, stem seeds of *Lagerstroemia speciosa L* was found to be contain several bioactive compounds such as terpenoids, steroids, saponins, flavonoids and glycosides and alkaloids etc. The objective of the present work is to evaluate the antimicrobial activity of fruit (without seeds) of *Lagerstroemia speciosa L.* Based on this, a new series of constituents had been planned to extract by Methanol (ML), Ethanol (EL), Chloroform (CF) from the fruits of *Lagerstroemia speciosa L.* The *in-vitro* antimicrobial activity of fruit (without seeds) of *Lagerstroemia speciosa L* were carried out by using agar diffusion method using bacterial cultures *Staphylococcus aureus (ATCC 9144), Bacillus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853,) Escherichia coli (ATCC 25922)* and fungal cultures *Aspergillus niger (ATCC 9029), Aspergillus flavus (ATCC 204304), Candida albicans (ATCC 10231).* By observing it was found that most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. The extracts were active against all the tested microorganism for anti bacterial activity with range of MIC values for *S.aureus* (MIC: 15-39 µg /ml), *E.coli* (MIC: 16-38 µg /ml) ,*P.aeruginosa* (MIC:15-39 µg /ml) and *B.subtilis* (14-39 µg /ml). The extracts were active against all the tested microorganism for anti fungal activity with the range of MIC values for *A.niger* (MIC :16-38 µg/ml), *A.flavus* (18-39 µg/ml) and *C.albicans* (16-38 µg/ml).

Keywords: *Lagerstroemia speciosa L*, Bioactive, Anti bacterial Activity, Anti fungal Activity, MIC etc.

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1. INTRODUCTION

The genus Lagerstroemia was first described by Carlos Linneaus. The name Lagerstroemia recognizes Magnus

von Lagerstroem, a Swedish naturalist who provided specimens from the East for Linnaeus. It is a small to medium-sized tree growing to 20 metres (66 ft) tall, with smooth, flaky bark. The leaves are deciduous, oval to elliptic, 8–15 cm (3.1–5.9 in) long and 3–7 cm (1.2–2.8 in) broad, with an acute apex. The flowers are produced in erect panicles 20–40 cm (7.9–15.7 in) long, each flower with six white to purple petals 2–3.5 cm (0.79–1.38 in) long. Folkloric uses of Banaba herbal medicine include the treatment for diarrhea, constipation, inflammation of kidneys, dysuria and other urinary dysfunctions. Banaba is a tropical flowering tree that grow up to 10 meters high. Banaba has large green oblong leaves that is about 3 inches in width and 7 inches in length. The flowers or Banaba are racemes and colored pink to lavender. Banaba bears nut-like fruits that are arranged in large clumps.It is grown in South East Asia, India and the Philippines. It is also widely cultivated as an ornamental plant in tropical and subtropical areas. [1, 2]

Banabá has a long history of folkloric medical applications that include blood pressure control, urinary dysfunctions (helps ease urination), cholesterol level control, treatment of diarrhea, facilitates bowel movement, diabetes and as an analgesic [3] The chemical compounds that have been isolated from the extract include corosolic acid, lager-stroemin, flosin B, and reginin A. The leaves of the Banabá and other parts are used widely in the Philippines, Taiwan, and Japan as a tea preparation. Banabá herb is one of the 69 herbal plants promoted by the Philippine Department of Health (DOH). [4]

Lagerstroemia speciosa have been previously reported to have hypoglycemic activity by reducing fasting blood glucose of strptozocin induced Diabetic rats. Apart from hypoglycemic activity [5, 6, 7] Banava leaves also possessed Antioxydant [8], Anti inflammatory [9], Antiobesity [10], Antifibrotic [11].

2. MATERIALS AND METHOD

Chemicals and drugs

The all chemicals used for the extraction and phytochemical screening were of LR and AR grade. Standard drugs Tetracyclin (Antibacterial) and Ketoconazole were purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from Institutional Store and were of AR grade. Bacterial cultures *Staphylococcus aureus (ATCC 9144), Bacilus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853,) Escherichia coli (ATCC 25922)* and fungal cultures *Aspergillus niger (ATCC 9029), Aspergillus flavus (ATCC 204304), Candida albicans (ATCC 10231)* were provided by the Biotechnology Lab of the CLBMCP, Chennai and maintained on Nutrient agar slant and fungal strains were maintained on Sabouraud dextrose broth at 4^0C.

Apparatus and chemicals required

Round bottom flask, water condenser, heating mantle, motor and pestle, methanol, ethanol, chloroform, dichloromethane, sodium chloride solution, magnesium sulfate etc.

Extraction

Weigh 50 g of fruits of *Lagerstroemia speciosa* (unripe and ripen can be mashed to prepare a paste) into a 500 ml round-bottomed flask. Add 200 ml of methanol and 240 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 250 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. [12] The same procedure has been followed for the preparation of EL and CF extracts.

Preliminary Phytochemical screening [13, 14, 15, 16, 17, 18, 19, 20]

Preliminary phytochemical screening of various extracts **(ML, EL and CF)** of fruits of *Lagerstroemia speciosa* had shown the presence of following bioactive compounds which were confirmed by their specific qualitative confirmatory chemical tests: Proteins and amino acids, Carbohydrates, Glycosides, Alkaloids, Terpenoids, Saponins, Phytosterols, Flavanoids, Gum and mucilage etc.

Evaluation of Antimicrobial Activity by paper disc diffusion method [21, 22, 23]

The sterilized (autoclaved at 120°C for 30 min) medium was inoculated (1mL/100mL of medium) with the suspension [10^5 cfu m/l (colony forming unit per milliliter)] of the microorganism (matched to McFarland barium sulphate standard) and poured in Petridish to give a depth of 3-4mm. The paper impregnated with the test compounds (50, 100,150 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1hr at RT and incubated at 37 °C for 24 hr for anti-bacterial and antifungal activities respectively. Tetracyclin (100 µg/disc) and Ketoconazoloe (100 µg/disc) was used as a standard.

Determination of MIC by agar streak dilution method [24]

MIC of the various extracts of fruit of *Lagerstroemia speciosa* were determined by agar streak dilution method. A stock solution of the extracts (100µg/ml) in Dimethylformamide was prepared and graded quantities of the test extract were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the compounds was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately10^5 cfu m/l and applied to plates with serially diluted compounds in Dimethylformamide to be tested and incubated at 37°C for 24hr. for bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate.

3. RESULTS AND DISCUSSION

The phytochemical screening of various extracts of fruit of *Lagerstroemia speciosa* were carried out by using standard procedure. The presence bioactive compounds in various extracts of fruit of *Lagerstroemia speciosa* were confirmed by their specific qualitative chemical confirmatory tests.

The zone of inhibition of various extracts of fruit of *Lagerstroemia speciosa* was compared with the standard drug tetracycline for the anti bacterial activity and Ketoconazole for the Anti fungal activity and Minimum Inhibitory Concentration (MIC) of various extracts of fruit of *Lagerstroemia speciosa* for bacteria and fungi were shown in **Table-1, 2, 3, and 4 .**

Table 1: The zone of Inhibition various Extracts of fruits of *Lagerstroemia speciosa* **(mm) for Anti bacterial activity**

Name of the extract	S. Aureus			E. Coli			P. Aeruginosa			B.subtilis		
	Concentration (µg/disc)											
	50	100	150	50	100	150	50	100	150	50	100	150
ML	16	30	37	18	33	39	16	30	36	19	26	35
EL	18	33	38	16	35	37	19	32	38	15	27	38
CF	19	26	31	19	25	35	18	25	33	17	22	36
Tetracyclin (100µg/ml)	27	48	57	28	49	58	29	46	55	27	40	56

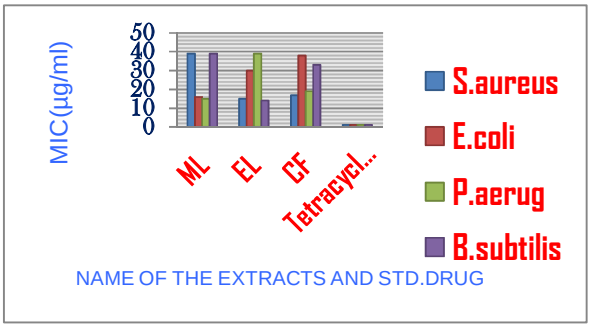

Fig 1: Graphical representation of Minimum Inhibitory Concentration of various Extracts of fruits of *Lagerstroemia speciosa*

Extract	Minimum Inhibitory Concentration (µg/ml)			
	S. aureus	E. coli	P. aeruginosa	B. subtilis
ML	39	16	15	39
EL	15	30	39	14
CF	17	38	19	33
Tetracyclin	1.2	1.2	1.2	1.2

Table 3: The zone of Inhibition of various Extracts of fruits of *Lagerstroemia speciosa* (Antifungal)

Name of the extracts	A. niger			A. flavus			C. albicans		
	Concentration (µg/disc)								
	50	100	150	50	100	150	50	100	150
ML	19	20	23	20	20	22	18	20	22
EL	18	19	21	19	19	21	17	19	21
CF	17	16	19	16	18	19	16	19	20
Ketoconazole (100µg/ml)	28	47	56	30	47	57	32	45	55

Table 4: Minimum Inhibitory Concentration of various Extracts of fruits of *Lagerstroemia speciosa* (Fungi)

Name of the extracts	Minimum Inhibitory Concentration(µg/ml)		
	A. niger	A. flavus	C.albicans
ML	17	39	32
EL	38	18	16
CF	16	31	38
Ketoconazole	6.1	6.1	6.1

Table 2: Minimum Inhibitory Concentration of various Extracts of fruits of *Lagerstroemia speciosa*

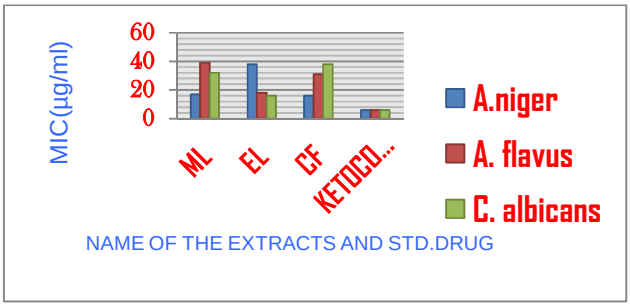

Fig 2: Graphical representation of Minimum Inhibitory Concentration of various Extracts of fruits of *Lagerstroemia speciosa*

The various extracts of fruits of ***Lagerstroemia speciosa*** possessed both anti bacterial and anti fungal activity. These activities were mainly due to the presence of bioactive compounds such as Proteins and amino acids, Glycosides, Alkaloids, Terpenoids, Saponins, Phytosterols, Flavanoids etc in the endocarp and exocarp of the fruit. The various extracts of fruits of ***Lagerstroemia speciosa*** were (50, 100 and 150 µg/ml) screened for antimicrobial activity by paper disc diffusion method. The experimental data had shown that most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. The MIC of the various extracts was screened by agar streak dilution method. The experimental data had shown that most of the extracts were active against all the tested microorganism for anti bacterial activity with range of MIC values for *S.aureus* (MIC: 15-39 µg /ml), *E.coli* (MIC: 16-38 µg /ml) ,*P.aeruginosa* (MIC:15-39 µg /ml) and *B.subtilis* (14-39 µg /ml) and the extracts were active against all the tested microorganism for anti fungal activity with the range of MIC values for *A.niger* (MIC :16-38 µg/ml), *A.flavus* (18-39 µg/ml) and *C.albicans* (16-38 µg/ml).

4. CONCLUSION

By observing it was found that most of the extracts (ML, EL and CF) exhibit moderate to good

antibacterial activity with a range of MIC between 15-39 µg/ml and antifungal activity with a range of MIC between 16-39 µg/ml. It was found that the extracts ML, EL and CF were exhibited good antibacterial and antifungal activity

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