International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 4; January-March, 2015, pp. 213-216

http://www.krishisanskriti.org/ijbab.html

Antimicrobial Activity of Different Maturity of Lagerstroemia Indica L. on Pathogenic Bacteria

Chandra M

Department of Biosciences, Mangalagangothri, Mangalore University, Dakshina Kannada District, Karnataka-574199. India
E-mail: drchandram1@gmail.com

Abstract—*Different maturity (young, medium and coarse) of methanol extracts of Lagerstroemia indica leaves were subjected to analyse the antimicrobial activity against some pathogenic bacteria viz., Staphylococcus aureus, Escherichia coli and Shigella dysenteriae. The leaf methanol extracts at their different stages of maturity, exhibited therapeutic effect on the tested organisms. However, the coarse leaf extract had inhibitory affinity on the test organisms with halo ranging between 8mm-12mm in diameter, different from the medium leaf extract that ranged between 12 mm-18 mm. The young leaf extract of Lagerstroemia indica indeed showed highest inhibitory effect on test organisms with halo range between 10 mm-20 mm. Bacillus cereus and Shigella dysenteriae were most inhibited with this extract (20.6 mm) while Escherichia coli was the least inhibited (10.2 mm). MIC was effective by the young leaf extract at100-145 mg/mL, the medium leaf at 130-145 mg/mL and the coarse leaf extract at 130-350 mg/mL on the test organisms. The MBC of the young leaf extract was effective at 100-145 mg/mL, the medium leaf extract at 130-160 mg/mL and the coarse leaf extract at 145-300 mg/mL on the test organisms. The antibacterial potency of Lagsrstroemia indica is determined so that nutritional and medicinal properties could be exploited judiciously. The results confirm the effective use of this plant in medicine, food system and pharmacy.*

Key words: *Antimicrobial, Lagerstroemia indica, Pathogenic bacteria*

1. INTRODUCTION

Medicinal plants are the best source to obtain different drug, about 80% population of industrialized countries traditional medicines, which are derived from medicinal plants, so, the properties of plants must be investigated. In developing countries, more than 40% of the population are subjected to infection of the microorganism, besides this, infection is also due to spoilage of food materials and its pathogenecity [1]. The treatment and control of diseases by the use of available medicinal plants in a locality will continue to play significant role in medical health care implications in the developing countries of the world [2]. The antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [3]. Moreover, the increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggested that in order to find active compounds, a systematic study of medicinal plants is very important [4]. Antimicrobial agents, including food preservatives, inhibit food borne bacteria and preserve the food. Many naturally occurring extract from herb, medicinal plants are known to possess antimicrobial activities and can serve as antimicrobial agents against food spoilage [5]. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activities. It also facilitates pharmacology studies, leading to the synthesis of a more potent drug with reduced toxicity [6].

Lagerstroemia indica (Crepe Myrtle) belongs to the family *Lythraceae*. The stem bark is febrifuge, stimulant and styptic. The bark, flowers and leaves are considered to be hydrogogue and a drastic purgative. A paste of the flowers is applied externally to cuts and wounds. The root is astringent, detoxicant and diuretic. A decoction of the flowers is used in the treatment of colds.

2. MATERIALS AND METHODS

2.1. Preparation of plant sample

Apparently fresh and healthy young, matured and coarse leaves of *Lagerstroemia indica* was collected in Mangalore University Campus. Each portion of the leaves stages in growth were separated washed with running tap water and rinsed in distilled water. The leaves were dried at room temperature in the laboratory and homogenized. The obtained powders were extracted with sterile distilled water by soaking for 24 h and filtered. The filtrates were evaporated at 45^0C to dried pellets using rotary evaporator. The dried extracts were kept in sterile bottles prior use.

2.2. Tests for microorganisms

Test microorganisms used in this investigation were Bacillus cereus, staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Salmonella typhi and Shigella dysenteriae. Prior to use the isolates were inoculated into separate cotton-plugged test tube containing 10ml Muller Hinton broth which were inoculated at 37^0 C for 24 h.

2.3. Sensitivity Screening Method

The use of well-in-agar diffusion method was adopted. The bacterial isolates at concentrations of 10^7 cells/ ml in their log phase was pour plated with Muller Hinton agar. The plates were allowed to stand for 2 h for the test organisms to be fully embedded in the growth medium before a cork borer (No. 4) was flamed and used to bore wells. The different leaf extracts in their crude forms were filled in the wells and labelled appropriately. The plates were incubated at 37^0 C for 24 h. The sensitivity of the test organisms to the extracts was evaluated by measuring the inhibitory halo zones in millimetres (mm).

2.4. Phytochemical Screening

The phytochemical screening of methanol extract was done to identify the main groups of chemical constituents in methanol extracts *Lagerstroemia indica* and *Annona reticulata* by their color reaction (Evans, 1997).

2.5. Minimal Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the extracts was determined by broth dilution method. Decreased concentrations of the extract were prepared (400-100mg/mL). The extracts were weighed and reconstituted appropriately in sterile distilled water. In each test tube containing 8ml of sterile Muller Hinton broth, 1ml of the different extract concentration, and 1ml overnight broth culture of the test organism were introduced. The tubes were rolled between the palms for even mixed up and incubated at 37^0 C for 24 h. Turbid tubes after incubation indicates negative and the least extract concentrations where clarity in medium is visible to the naked eyes, determined the MIC of the extracts.

2.6. Minimal Bacterial Concentration

Minimal bacterial concentration (MBC) was determined by plating 1ml of the MIC positive tubes on nutrient agar to ascertain its bacteriostatic and bactericidal effect of the leaf extracts.

2.7. Conventional Antibiotic Disc Assay

The sensitivity disc (Gram –ve and Gram +ve) was used to assay the sensitivity pattern of the test organisms in comparison to the leaf extracts. The antibiotics and concentrations impregnated to the disc arms are Augmentin (AUG) 30µg, Amoxylin (AMX) 2µg, Ciprofloxaxin (CPX) 10µg, Gentamycin (GEN) 10µg, Nitrofuration (NIT) 200µg, Oflaxaxin (OFL) 54µg, Penfloacin (PFX) 5µg.

However, the same method used for the extract assay was also adopted for this test, except that the discs were picked with a sterile forceps and positioned at the centre of the seeded nutrient agar plates.

3. RESULTS AND DISCUSSION

The young leaf extracts of *Lagerstreomia indica* showed highest inhibitory potential than the mature and coarse leaf extracts. *B. cereus* and *S. dysenteriae* were each inhibited a zone of 20.6 mm. Despite the growth stages of the leaf inhibited all the test organisms with various degrees of inhibition, *B. cereus*, *S. aureus*, *S. typhi* and *S. dysenteriae* were the most inhibited.

However, it was observed that the inhibitory halo (10.2-18.5m) displayed by the matured leaf extract were close to the inhibitory sensitivity (10.2-20.6) displayed by the young leaf extract. The inhibitory potential observed with the coarse leaf extract (5-10) was not comparable to the therapeutic effect of young and matured leaf extracts on the test isolates (Table 1).

Table 1: Inhibitory assay of *Lagerstroemia indica* leaf extracts on the test organisms.

	Inhibitory assay of *L. Indica* extract (mm)		
Test organisms	**Young leaf**	**Matured leaf**	**Coarse leaf**
Bacillus cereus	20.0	17.0	7.0
Staphylococcus aureus	18.0	16.0	5.0
Pseudomonas aeruginosa	12.4	17.2	6.0
Escherichia coli	10.2	10.2	7.0
Proteus mirabilis	10.6	9.8	8.0
Salmonella typhi	18.3	9.4	8.0
Shigella dysenteriae	20.6	18.5	10.0

The phytochemicals quantitatively identified are tannins, saponins, flavonoids, alkaloids and phenol. The quantities of tannins, saponins, flavonoids, alkaloids and phenol in the young leaf extract were found to be 20.11±0.1, 3.2±0.1, 0.25±0.1, 1.53±1.2 and 0.34±0.0, respectively. For matured leaf extract, the same phytochemical constituents were, 1.6±0.1, 0.18±0.1, 19.36±0.1, 1.36±1.2 and 0.28±0.0 and for coarse leaf extract, 0.18±0.1, 0.10±0.1, 10.18±0.1, 1.03±1.2 and 0.20±0.0, respectively. The appreciable quantities of phytochemicals identified in the leaf extracts were observed. Their inhibitory potency on the tested isolates was as due to the crude form which contained the phytochemicals in large quantities, therefore directional to all kind of infections unlike purified antibiotics which are directional to the type of infection because the phytochemicals are separated into single or double entity for specificity in prevention and curing of diseases.

The MIC of the leaf extracts pointed out 100-145mg/mL, 130-145mg/mL and 200-350mg/mL respectively for the young,

matured and coarse leaf extracts as the actual therapy value. Comparing the bacterial load of the MIC positive tubes to the initial load (10^7cell/mL) of the test organisms, reduction in load was observed at 100mg/mL concentration. Though clarity were observed with the rated eyes in the positive MIC tubes, the MBC assay, revealed the concentrations at which the leaf extracts were bactericidal on the test organisms. The cidal and static activities of the leaf was in the trend of its antibacterial assay (Table 2).

Table 2: Extract concentration at which MIC and MBC are valuable on the test organisms

Test organisms	Extract concentrations (mg/mL)								
	Young leaf			Matured leaf			Coarse leaf		
	MIC	MBC	Action	MIC	MBC	Action	MIC	MBC	Action
Bacillus cereus	100	100	C	145	160	S	350	145	S
Staphylococcus aureus	100	115	S	130	160	S	300	130	S
Pseudomonas aeruginosa	100	145	S	140	130	S	300	130	S
Escherichia coli	100	100	C	130	130	C	300	300	C
Proteus mirabilis	145	145	C	130	130	C	300	300	C
Salmonella typhi	100	100	S	145	145	S	250	130	S
Shigella dysenteria	100	100	C	145	145	C	300	300	C

	Extract concentrations (mg/mL)

MIC = Minimal inhibitory concentration, MBC = Minimal bacterial concentration, S = Bacteriostatic, C =Bactericidal.

The employed commercial antibiotics (reference drugs), antibacterial activities in some cases showed higher inhibitory potency and in other cases showed lower inhibitory potency than the leaf extracts. NIT, AMX, CRO and CPX were the most potent on the test organisms. *S. aureus* was the most inhibited (10-28 mm) by the reference drugs followed by *S. dysenteriae* (13-26 mm) among the test bacterial isolates. All the test organisms were resistant to augmentin. However, *E. coli* was resistant to all the employed reference drugs. *S. typhi* was resistant to AUG, NIT, AMX, GRO, GEN and TET. TET showed the least therapeutic effect (2-5 mm) on the test bacterial isolates (Table 3).

It has been reported that gram negative bacterial are resistant to antibacterial agents and this was observed in the employed reference antibiotics. Hence, they are purified agents and the leaf extracts in this study acted better on both the Gram positive and negative considered test organism, their purification will evident a high therapeutic effect on certain diseases in which they are specifically manufactured for. The findings in this study, shows that the crude extract of the different growth stages of *L. indica* leaves acted significantly as antimicrobial agent. This was demonstrated by the various inhibitory halo measured from the in vitro bio-assay.

However, the active substances contained in the leaves made it possible for the inhibitory measure over the test organisms. Though, [9, 10] have reported the presence of some compounds such as saponin, glycosides, steroid, cardiac glycosides, tannins, volatile oils, phenols. The inhibitory effect against bacterial pathogens by *Terminalia catappa* with different maturity of leaf extracts and identified the phytochemicals with varying quality and quantities [11].

Since decreased inhibitory halo was observed in the stages of ageing in *L. Indica* leaf it implies that the active ingredients in the leaf decrease alongside advancement in age.

Table 3: Conventional antibiotic disc assay (mm)

Test organisms	AUG	NIT	AMX	CRO	COT	CPX	GEN	OFL	PEX	TET
Bacillus cereus	-	6	8	10	-	15	1	3	5	-
Staphylococcus aureus	-	29	20	15	-	28	10	12	15	2
Pseudomonas aeruginosa	-	11	20	15	1	10	2	5	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-
Proteus mirabilis	-	7	10	11	8	5	-	10	-	-
Salmonella typhi	-	-	-	-	2	1	-	2	3	-
Shigella dysenteri	-	15	18	26	26	22	-	13	-	5

AUG: Augmentin, NIT: Nitrofuration, AMX: Amoxicilin, CRO: Celtridzone, COT: Contrimoxazole, CPX: Ciprofloxacin, GEN: Gentamycin, OFL: Oflaxacin, PFX: Pefloxacin, TET: Tetracyclin.

4. CONCLUSION

The result of present investigation clearly indicate that the antimicrobial activity vary with the different maturity of plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.

REFERENCES

[1] M. Merino, C. Bersani, G. Como, "Impendence measurement to study the antimicrobial activity of essential oils from Laminaceae and Compositae", 2011, *Int. J. Food Microbiology*. 67:187-195.

[2] F. C. Akharaiyi, B. Boboye, "*J Nat Prod*", 2010, 3, 27-34.

[3] M. Lis-Balchin, S. G. Deans, "*J Appl Bacteriol* ", 1997, 82. 759-762.

[4] A. Nostro, M. P. Germano, V. D'Angelo, A. Marino, M. A. Cannatelli, "*Lett Appl Microbiol"*, 2000, 30, 379-384.

[5] C. F. Bogamboula, M. Uyttendale, J. Debevere, "Antimicrobial effects of spices and herbs on *Shigella sonnei* and *Shigella flexneri*", 2003, J Food Prot, 66: 668-673.

[6] A. Manna, M. E. Abalaka, 2000, *Spectrum J*, 7, 119-125.

[7] M. J. Ikenebome, P.O. Metitiri, Nigerian *Journal of Microbiology*, 8, 12-33.

[8] Yesser Diab, Khaled Atalla, Khaled Elbanna, "Antimicrobial screening of some Egyptian plants and active flavones from Lagerstroemia Indica L. leaves", 2012, Drug Discoveries and Therapeutics, 6(4):212-217.

[9] Bipul Biswas, Kimberly Rogers, Fredrick McLaughlin, Dwayne Daniels and Anand Yadav, "Antimicrobial activities of leaf extracts of Guava (*Psidium guajava* L.) on two Gram – Negative and Gram + positive bacteria", 2013, *Int J Microbiology*, Vol. 2(13), Article ID. 746165. 7 pages.

[10] F. C. Akharaiyi, R. M. Ilori and J. A. Adesida, "Antimicrobial effects of *Terminalia catappa* on some selected pathogenic bacteria", 2011, *Int J Pharm Biomed Res*, 2(2), 64-67.

[11] P. Neelavathi, Venkatalakshmi and Brindha, "Antibacterial activities of aqueous and ethanolic extracts of *Terminalia catappa* leaves and bark against some pathogenic bacteria", *Int J Pharm Pharmaceutical Sci.*,2013, Vol.3(1):140-120.