

Antimicrobial activity of *Lannea coromandelica* bark extracts against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Background: In healthcare facilities, methicillin-resistant *Staphylococcus aureus* (MRSA) has long been a common pathogen. Indiscriminate and incomplete uses of antibiotics are creating MRSA more alarming day by day. This study aims to determine the antimicrobial activity of *Lannea coromandelica* (Jhika or Indian ash tree) bark extract against MRSA.

Methods: This experimental study was carried out in Department of Microbiology and Department of Pharmacology, Bangladesh University of Health Sciences, from January to July, 2021. In this study, a bark extract of *Lannea coromandelica* was prepared by macerating dried powder of the bark of the Jhika or Indian ash tree. Then bark extract was immersed in methanol, ethanol, and water for 48-72 hours, followed by solvent filtering and evaporation. MRSA were identified by biochemical test and then Kirby-Bauer disc diffusion method employed against MRSA isolates using commonly used antibiotics. Then the antibacterial activity of *Lannea coromandelica* extracts against MRSA was monitored. The microdilution method was used to assess the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of bark extracts. Finally, phytochemical screening was carried out only for methanolic extract.

Results: All MRSA isolates were completely resistant to ceftiofloxacin, oxacillin, gentamicin followed by tetracycline. Methanol, ethanol and aqueous extracts of *Lannea coromandelica* produce maximum zones of inhibition of 14 mm, 13 mm, and 12 mm, respectively, with MIC and MBC values ranging from 3.125 mg/ml to 12.5 mg/ml against MRSA. Phytochemical screening of methanolic extract determined the presence of tannin, saponin, flavonoid, phenol which may be the cause of the highest zone of inhibition against MRSA.

Conclusion: It can be concluded that methanol, ethanol and aqueous bark extract of *Lannea coromandelica* exhibited *in vitro* antibacterial activity against MRSA by disc diffusion method and detailed pharmacological screening should be carried out for the exploration of effective and natural drugs.

Key words: Methicillin-resistant *Staphylococcus aureus* (MRSA), *Lannea coromandelica*, bark extract, Gram-positive bacteria.

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens of hospital and community-associated infections and remains a global public health concern in both developed and developing countries.¹ In Bangladesh, the prevalence of MRSA (from hospital setting) varied between 15.38% to as high as 72%, however, the real picture is obscure due to the lack of nationwide data.^{2, 3} The treatment choices for MRSA are inadequate, which is making MRSA more multidrug-resistant (MDR) to commonly used antibiotics.^{4,5} Emergence and dissemination of MDR bacteria such as MRSA have become a significant public health threat as there are fewer or even sometimes no effective antimicrobial agents available to combat the infections caused by this pathogenic bacteria.^{6, 7}

Antimicrobial agents are essentially important in treating and reducing the global burden of infectious diseases.⁸ Plants produce a diverse range of bioactive molecules and studies showed that some of the modern drugs are analogs of these phytochemicals.⁹ Identification of the antimicrobial effect of medicinal plant extracts has become a new conduit for overcoming bacterial drug resistance and making them rich sources of different types of medicines. There have been positive outcomes for some plants (*Salvadorapersica*, *Ophiorrhiz-amungos*, *Nymphaeatetragona*, *Syzygiumaromaticum*, *Synedrellanodiflora*) in this direction.¹⁰⁻¹² The antimicrobial effect of plant extracts has been found against both Gram-positive and Gram-negative bacteria.¹³ The essential points of interest in utilizing plant constituents may be the best way to combat MDR and a source of alternatives affordable treatment and safer than synthetic alternatives.¹⁴

One of such plants is *Lannea coromandelica* (Jhika or Indian ash tree) which belongs to the family Anacardiaceae, a Bangladeshi medicinal plant, that has long been used in indigenous medicine.¹⁵ *Lannea coromandelica* has been documented for its anti-inflammatory, anti-hypertensive and wound healing properties.¹⁶⁻¹⁸ The plant also illustrated its beneficial effect on ulcerative stomatitis, dyspepsia, general debility, gout, cholera, diarrhea and dysentery, sore eyes, leprosy, sprains and bruises, elephantiasis, eruptions, snakebite, stomach ache and vaginal troubles.¹⁹⁻²⁴ It has shown antibacterial effects against *S. aureus*, *S. pyogenes* and fungi.^{25,26} However, the potential effect of these plant extracts against MRSA is yet to be resolved. In this study, we aimed to explore any antibacterial activity and minimal inhibitory

concentration (MIC), minimal bactericidal concentration (MBC) of different solvent extracts of *Lannea coromandelica* bark against MRSA.

METHODS

Collection, identification of plant and preparation of extracts

The healthy and fresh barks of *Lannea coromandelica* were collected from different zones of Dhaka, Bangladesh for this experimental study. It was identified by Bangladesh National Herbarium. The sample of the plant was submitted to the Bangladesh National Herbarium with the accession number DACB 65148. At first, barks were rinsed and air-dried, then were coarsely powdered in the pharmacology laboratory of Bangladesh University of Health Sciences (BUHS). Three types of solvents were selected for extraction: ethanol, methanol and water. Extraction was performed as described in previous studies with slight modification.^{18, 27} Thirty grams (30 g) of the dried powdered bark were soaked in 300 ml of distilled water at 80°C and cold-macerated in other organic solvents (ethanol, methanol) for 48-72h and then filtered. The filtrates were evaporated on a rotary evaporator to a semisolid mass and dried using a freeze drier. Filtrates were weighed and stored in sterile labeled containers and kept in the refrigerator at 4°C until required. The filtrate of water extract was then mixed with distilled water to prepare an aqueous extract solution and filtrates of organic extracts were mixed with dimethyl sulfoxide (DMSO) for the organic extract solutions.

Isolation and antibiogram of MRSA strains

Five MRSA isolates were obtained from Bangladesh Institute of Health Science (BIHS) General Hospital in Dhaka, Bangladesh and then strains were tested against various antibiotics like cefoxitin (30 µg/disc), oxacillin (30 µg/disc), vancomycin (30 µg/disc), ciprofloxacin (5 µg/disc), tetracycline (30 µg/disc), chloramphenicol (30 µg/disc), gentamicin (10 µg/disc), clindamycin (2 µg/disc) and cotrimoxazole (25 µg/disc).²⁸

Antibacterial activity of extracts

To evaluate the antibacterial activity of the extracts against the bacteria, dried and sterilized filter paper discs (6 mm diameter) were soaked with 20 µl of various concentrations of the extracts (50 mg/ml, 75mg/ml, 100 mg/ml).^{29,30} Discs were then placed on the Muller Hinton agar medium homogeneously seeded with the test microorganisms (10⁷ CFU/ml). Standard disc of

gentamicin was used as a positive control and discs impregnated with solvent were used as a negative control. Plates were then incubated at 37°C for 18-20 hours.

Determination of MIC of extracts

MIC of gentamicin and extracts were evaluated by the broth microdilution method in sterile 96-well polystyrene culture plates. 100µl of Mueller Hinton broth was dispensed into each well of the 96-well plate. A 100µl from the stock solution of test extracts (e.g. a concentration of 100 mg/ml) was added into the first row of the plate. Then, serial dilutions were performed to obtain concentration of extracts ranged from 50 to 0.09mg/ml. A negative control was prepared with plant extract and media, as well as a positive control was prepared with the inoculum and media. Then test plates were incubated at 37°C for 18 hours. The well with the lowest dilution with no recognizable growth by visual assessment was considered as MIC.¹³

Determination of MBC

Two-fold concentrated test product dilutions were plated to determine the MBC and enumerated to determine viable CFU/ml. After incubation, the concentration at which no visible growth was found was recorded as the MBC.¹³

Phytochemical screening of the plant extracts

Preliminary phytochemical screening of only methanolic extracts was performed following standard methods.³¹

RESULTS

Antibiogram of MRSA

All (5) MRSA strains were completely resistant to cefoxitin, oxacillin, gentamicin where isolates showed complete sensitivity to vancomycin. Strains also showed varied resistance against other antibiotics. Eighty two percent of MRSA were resistant to tetracycline followed by ciprofloxacin and chloramphenicol (Figure 1).

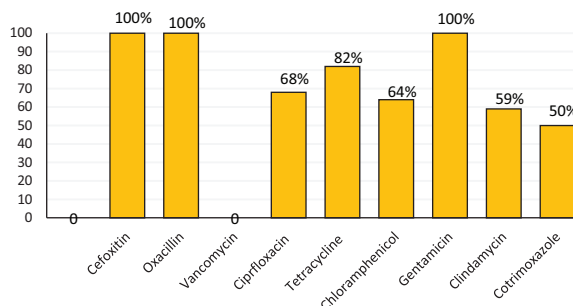


Figure 1 Resistance pattern of MRSA strain against different antibiotics

Antibacterial activity of plant extract

All types of extract (methanolic, ethanolic and aqueous) showed antibacterial activity against five MRSA isolates. Disk diffusion of the *Lannea coromandelica* extracts (methanolic, ethanolic and aqueous) against these MRSA strains showed an increasing zone of inhibition as the concentration of the extracts is increased (Table I). The highest zone of inhibition was 14 mm with 100 mg/ml methanolic extract. The MIC was 3.125 mg/ml and MBC was 6.25 mg/ml for maximum extracts (Table I).

Table I Zone of inhibition of *Lannea coromandelica* extracts (methanolic, ethanolic and aqueous) against MRSA strains and respective MIC and MBC values

Organism Id	Zone of inhibition (mm)									MIC (mg/ml)			MBC (mg/ml)		
	Concentration (mg/ml)									Zone of inhibition			Zone of inhibition		
	Methanol			Ethanol			Aqueous			Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous
	50	75	100	50	75	100	50	75	100						
S-1	10	11	12	9	11	12	9	11	12	3.125	3.125	3.125	6.25	6.25	6.25
S-2	11	13	14	10	11	13	9	10	12	3.125	6.25	6.25	6.25	12.5	12.5
S-3	9	11	13	10	11	13	11	11	11	6.25	3.125	6.25	12.5	6.25	12.5
S-4	8	10	11	7	9	10	9	10	11	6.25	3.125	3.125	12.5	6.25	6.25
S-5	10	11	13	8	10	12	8	9	12	3.125	6.25	3.125	6.25	12.5	6.25

Table II Phytochemical screening of methanolic solvent extracts of *Lannea coromandelica* bark

Tannin	Saponin	Alkaloid	Flavonoid	Phenol	Steroid	Terpenoid
+++	+++	-	+++	+++	-	-

Phytochemical screening result

Phytochemical screening of only methanolic solvent extracts of *Lannea coromandelica* bark showed the presence of tannin, saponin, flavonoid and phenol.

DISCUSSION

Plants are rich in a wide variety of phytochemicals like flavonoids, tannins, terpenoids, alkaloids, pigments, enzymes and minerals, which have disease preventive properties against plant pathogens as well as some antimicrobial activities.³¹⁻³³ In different investigations, low to moderate antibacterial activity of aqueous and ethanolic extract (bark) of *Lannea coromandelica* was observed against *S. aureus*, *Escherichia coli*, *S. pyogenes*, *Bacillus subtilis*.^{25,26} The zone of inhibition against *S. aureus* was reported 13 mm for ethanolic extract in a study by Das et al.²⁶ We found 14mm of the zone of inhibition of methanolic extract against MRSA. In a previous study²⁶, the MIC of the ethanolic extract against *S. aureus* was 12.5 mg/ml whereas we found the MIC and MBC both were as low as 3.125 mg/ml and 6.25 mg/ml for methanolic, ethanolic and aqueous extract.²⁶ For most of the MRSA strains, MIC and MBC values of the methanolic extract of *Lannea coromandelica* were lower than respective ethanolic and aqueous extract indicating that the methanolic extract contains more active constituents. All the MRSA in our study were completely resistant to gentamicin and oxacillin and showed varied resistance against commonly used antibiotics, the result coincides with the prevalence of MDR bacteria from different clinical and environmental specimens reported in Bangladesh.^{34,35}

On account of the highest activity of methanolic extract of *Lannea coromandelica* bark, we studied the preliminary phytochemical analysis of methanolic extract only. This analysis revealed the presence of tannins, saponin, flavonoids and phenols in our study. Earlier literature reported that the major active ingredients like flavonoids, phenolic compounds and some newer isolated compounds are responsible for the antimicrobial activity of *Lannea coromandelica*.³⁶ This study thus

indicates the presence of active chemicals in *Lannea coromandelica*, which can be very effective against MRSA. Further study delineating the active compounds would be of more supplementary.

It can be concluded that, *Lannea coromandelica* bark extract have immense potential effect against MRSA. Methanolic bark extract showed stronger activity against all the tested MRSA strain. It can be utilized to find bioactive natural compounds that could lead to the development of novel MRSA antibiotics that address unmet treatment requirements. Further pharmacological and toxicological studies are needed to evaluate a novel medication derived from *Lannea coromandelica* bark extract against MRSA.

Authors' contribution: RNF, AHP, SHK, MM, BR, SMZHA generated research idea. RNF, SSN, PKD, MM drafted manuscript. All authors read and approved final manuscript for submission.

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Conflicts of interests: Nothing to declare.

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