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 cefoxitin, 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. 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. The treatment choices for MRSA are inadequate, which is making MRSA more multidrug-resistant (MDR) to commonly used antibiotics. 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.

Antimicrobial agents are essentially important in treating and reducing the global burden of infectious diseases. Plants produce a diverse range of bioactive molecules and studies showed that some of the modern drugs are analogs of these phytochemicals. 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*, *Ophiorrhizinamungos*, *Nymphatectragona*, *Syzygiumaromaticum*, *synedrellanodiflora*) in this direction. The antimicrobial effect of plant extracts has been found against both Gram-positive and Gram-negative bacteria. 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.

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. *Lannea coromandelica* has been documented for its anti-inflammatory, anti-hypertensive and wound healing properties. 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. It has shown antibacterial effects against *S. aureus*, *S. pyogenes* and fungi. 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 pharmacy 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. 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).

Antibacterial activity of extracts

To evaluate the antibacterial activity of the extracts against the bacteria, dried and sterilized filter paperdiscs (6 mm diameter) were soaked with 20 µl of various concentrations of the extracts (50 mg/ml, 75 mg/ml, 100 mg/ml). Discs were then placed on the Muller Hinton agar medium homogeneously seeded with the test microorganisms (10^7 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.\(^{31}\)

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**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).

**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).

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| 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) |
|           | Methanol | Ethanol | Aqueous | Methanol | Ethanol | Aqueous | Methanol | Ethanol | Aqueous |
| 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. 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*, and *Bacillus subtilis*. The zone of inhibition against *S. aureus* was reported 13 mm for ethanolic extract in a study by Das et al. We found 14mm of the zone of inhibition of methanolic extract against MRSA. In a previous study, 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. 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.

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, saponins, 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*. 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.

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**Conflicts of interests:** Nothing to declare.

**REFERENCES**

1. Yusuf MA, Islam KMS, Shamsuzzaman AKM, Ahmed I, Sattar AA. Burden of infection caused by methicillin-resistant *Staphylococcus aureus* in Bangladesh: A systematic review. Global Adv Res J Microbiol 2013; 2(11):213-23.
2. Hussain K, Rahman M, Nazir KHMNH, Rahman H, Khair A. Methicillin resistant *Staphylococcus aureus* (MRSA) in patients of community based medical College Hospital, Mymensingh, Bangladesh. Am J Biomed Life Sci 2016;4(3):26-9.
3. Parvez MA, Ferdous RN, Rahman MS, Islam S. Healthcare-associated (HA) and community-associated (CA) methicillin resistant *Staphylococcus aureus* (MRSA) in Bangladesh—Source, diagnosis and treatment. J Gen Eng Biotech 2018 Dec 1;16(2):473-8.
4. Gemmell CG, Edwards DI, Fraise AP, Gould FK, Ridgway GL, Warren RE et al. Guidelines for the prophylaxis and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in the UK. J AntimicrobChemother 2006; 57(4):589-608.

5. Doern CD. When Does 2 Plus 2 Equal 5? A review of antimicrobial synergy testing. J ClinMicrobiol 2014;52(12):4124-8.

6. Boucher HW, Talbot GH, Bradley JS. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009; 48(1):1–2

7. Giamarellou H. Multidrug-resistant Gram-negative bacteria: how to treat and for how long, Int J Antimicrob. Agents 2010; 36 (1):50–5.

8. Bhatia R, Narain JP , The growing challenge of antimicrobial resistance in the South-East Asia Region—are we losing the battle? Indian J Med Res 2010; 132(5): 482–86.

9. Abreu AC, McBain AJ, Simoes M. Plants as sources of new antimicrobials and resistance-modifying agents. Nat Prod Rep 2012; 29:1007-21.

10. Ahmed Z, Khan SS, Khan M, Tanveer A, Lone ZA. Synergistic effect of \textit{Salvadorapersica} extracts, tetracycline and penicillin against \textit{Staphylococcus aureus}. Afr J Basic Appl Sci 2010;2(1-2):25-9.

11. Lacmata ST, Kuete V, Dzoyem JP , Tankeo SB, Teke GN, Kuiate JR. Antibacterial activities of selected Cameroonian plants and their synergistic effects with antibiotics against bacteria expressing MDR phenotypes. Evid Based Complement Alternat Med 2012; 2012:623723.

12. Chowdhury SR, Akter S, Sharmin T, Mohammad FI. Antimicrobial Activity of five medicinal plants of Bangladesh. JPharmacPhytochem 2013 May 1;2(1): 164-70.

13. Farooqui A, Khan A, Borghetto I, Kazmi SU, Rubino S, Paglietti B. Synergistic Antimicrobial Activity of Camellia sinensis and Juglansregia against Multidrug Resistant Bacteria. PLoS ONE 2015;10(2):1-14.

14. Ojo SKS, Ejims-Enukwe O, Esumeh FI. Invitro antibacterial time-kill assay of \textit{Phyllanthusamarus} and \textit{Diodiascandens} crude extracts on staphylococci isolated from wounds and burns patients. Int J Pharm Sci Invent 2013;2(8):9-13.

15. Rahman M, Khatun A, Uddin SJ, Shilpi JA. Comparative effect of \textit{Lanneacoromandelica} (Houtt.) Merr. leaves and stem barks on acetic acid induced pain model in mice and chromagenic reagents: exploring the analgesic potential and phytochemical groups. Pharmacologyonline 2016 Apr 30;1(1):146-52.

16. Basuri TS, Patil C, Dhal NK. In vitro evaluation of antibacterial activity of crude ethanolic extract of \textit{Lanneacoromandelica}. J Pharm Res 2011;4:1246-7.

17. Sathish R, Mohd HA, Natarajan K, Lallitha KG. Evaluation of wound healing and antimicrobial activity of \textit{Lanneacoromandelica} (Houtt) Merrill. J Pharm Res 2010;3:1225-8.

18. Singh S, Singh GB. Hypotensive activity of \textit{Lanneacoromandelica} bark extract. Phytother Res 1996;10:429-30.

19. Jain SK, Tarefsler CR. Medicinal plants-lore of the sandsals-A revival of PO Bodding’s work. Econ Bot 1970;24:241-78.

20. Kirtikar KR, Basu BD. In: Mhaskar KS, E-Blatter, Caius JF, editor. Indian Medicinal Plants 2000; 3:933-6.

21. Hah GL, Yadav SS, Badri N. Medicinal plants from Dahanu forest division in Maharashtra state. J Econ Tax Bot 1983;4:141-51.

22. Shastri K, Chaturvedi G. CharakaSamhita: Vidyotini commentary. Varanasi: ChaukhambhaBharti Academy; 2004. P. 228- 229

23. Yadava T, Narayana R. SushrutaSamhita. 7th ed. Varanasi: ChaukhambhaOrientalia; Sutra sthana 2002; 38:14

24. Sharma S. AshitangaSangraha: Shashilekha Commentary. 2nd ed. Varanasi: Chaukhambha Sanskrit Office; Sutra sthana 2008; 16: 18-9.

25. Kaur R, Jaiswal ML, Jain V. Protective effect of \textit{Lanneacoromandelica}Houtt. Merrill. Against three common pathogens. J Ayurv Integrative Med 2013 Oct;4(4):224.

26. Das K. Phytochemical evaluation and comparative antibiocide efficacy of Aqueous, Ethanolic and equal mixture of aqueous and ethanolic (1:1) bark extract of \textit{Lanneacoromandelica} procured from Eastern region of India. International Letters of Natural Sciences 2014;21.

27. Ojo SKS, Ejims-Enukwe O, Esumeh FI. Invitro antibacterial time-kill assay of \textit{Phyllanthusamarus} and \textit{Diodiascandens} crude extracts on staphylococci isolated from wounds and burns patients. Int J Pharm Sci Invent 2013;2(8):9-13.

28. Performance standards for antimicrobial susceptibility testing; Twenty fourth informational supplement. Clinical and Laboratory Standards Institute. PA, USA; 2014.

29. Razmavar S, Abdulla MA, Ismail SB, Hassanardarvish P. Antibacterial activity of leaf extracts of \textit{Baeckea frutescens} against methicillin-resistant \textit{Staphylococcus aureus}. Bio Med Res Int 2014; 2014:5.

30. Bhandary SK, Kumari SN, Bhat VS, Sharmila KP, Bekal MP. Preliminary phytochemical screening of various
extracts of Punicagranatum peel, whole fruit and seeds. J Health Sci 2012;2(4): 35-8.

31 Bonjar GH, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J BiolSci 2004;4:405-12.

32 Monte J, Abreu AC, Borges A, Simões LC, Simões M. Antimicrobial activity of selected phytochemicals against Escherichia coli and Staphylococcus aureus and their biofilms. Pathogens 2014;3(2):473-98.

33 Manik MK, Wahid MA, Islam SM, Pal A, Ahmed KT. A comparative study of the antioxidant, antimicrobial and thrombolytic activity of the bark and leaves of Lannea coromandelica (Anacardiaceae). Int J Pharm Sci Res 2013 Jul 1;4(7):2609.

34 Hasan B, Sandegren L, Melhus A, Drohni M, Hernandez J, Waldenström J, et al. Antimicrobial drug–resistant Escherichia coli in wild birds and freerange poultry, Bangladesh. Emerg Infect Dis 2012;18(12):2055.

35 Adnan N, Sultana M, Islam OK, Nandi SP, Hossain MA. Characterization of Ciprofloxacin resistant Extended Spectrum [Beta]-Lactamase (ESBL) producing Escherichia spp. from clinical waste water in Bangladesh. Adv Biosci Biotechnol 2013;4(7B):15.

36 Toda M, Okubo S, Ohnishi R, Shimamura T. Antibacterial and bactericidal activity of Japanese green tea. Nippon Saikingaku Zasshi 1989; 44:669-72.