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# Phytochemical Analysis and GC-MS Profiling of Ultrasound Assisted Extraction (UAE) Extract from *Lannea coromandelica* Leaves Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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**Abstract**

Methicillin-Resistant *Staphylococcus aureus* (MRSA) remains a major global health threat because of its resistance to multiple first-line antibiotics. This study evaluated the antibacterial activity and secondary metabolite profile of *Lannea coromandelica* leaf extracts obtained by Ultrasound-Assisted Extraction (UAE) using ethanol and methanol. Antibacterial activity was assessed against MRSA by the disc diffusion (Kirby–Bauer) method at extract concentrations of 50%, 75%, and 100%, with vancomycin as the positive control, while the chemical profile was characterized by Gas Chromatography-Mass Spectrometry (GC-MS). The methanol extract produced very strong inhibition at all concentrations (21.00±1.00, 20.67±1.15, and 21.00±1.00 mm), numerically similar to or slightly larger than the single vancomycin control disc (20.00 mm), whereas the ethanol extract produced strong inhibition (11.33–18.33 mm). Two-way ANOVA confirmed significant effects of solvent type ($p<0.001$), concentration ($p=0.001$), and their interaction ($p=0.007$) on inhibition-zone diameter. GC-MS identified 30 chromatographic peaks in each extract, dominated by Stigmast-5-en-3-ol/γ-sitosterol, phytol, and hexadecanoic acid, with neophytadiene and 1,13-tridecanediol diacetate detected exclusively in the methanol extract (tentative identifications, SI 82%). These findings indicate that the UAE methanol extract of *L. coromandelica* leaves exhibits in vitro antibacterial activity against MRSA, with inhibition zones numerically similar to the vancomycin control (equivalence was not tested), accompanied by a complex metabolite profile that may plausibly contribute to this activity.

**Keywords:** Antibacterial activity; GC-MS*; Lannea coromandelica;* MRSA; Ultrasonic assisted extraction.

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## INTRODUCTION

Indonesia is a tropical country that continues to face significant challenges associated with infectious diseases, a problem compounded by the uncontrolled use of antibiotics and the resulting rise of antimicrobial resistance. According to the Surveillance of Indonesian Network on Antimicrobial Resistance (SINAR), Methicillin-Resistant *Staphylococcus aureus* (MRSA) accounted for 37% of all *S. aureus* isolates in Indonesia (Perhimpunan Ahli Mikrobiologi Klinik Indonesia [PAMKI], 2025), and its prevalence has been reported to reach 43.4% among clinical isolates in several Indonesian hospitals, contributing to limited therapeutic options and increased morbidity and mortality (Nuryah et al., 2019). This situation makes the search for new, plant-derived antibacterial candidates an urgent priority.

*Lannea coromandelica* (Anacardiaceae), locally known as kayu jawa, is a medicinal plant rich in secondary metabolites such as flavonoids, saponins, tannins, and polyphenols (Ismail et al., 2016). Several studies have specifically tested its bark extracts against MRSA: methanol, ethanol, and aqueous bark extracts produced inhibition zones of 14 mm, 13 mm, and 12 mm, respectively (Ferdous et al., 2022), while a methanol bark extract showed activity

against *S. aureus* with zones of 7.3–10.4 mm at concentrations of 2.5–10% (Ahmad, 2016); the bark extract has also been formulated into an effective handsanitizer gel against *S. aureus* (Alyidrus et al., 2022). In contrast, studies on the leaves of *L. coromandelica* have so far been directed mainly at antibiotic-susceptible strains rather than MRSA: an ethanol leaf extract produced a maximum inhibition zone of 11.32 mm at 12.8% against skin-infection-causing bacteria (Kayla et al., 2023), and another ethanol leaf extract was evaluated against gastrointestinal-infection-causing bacteria using conventional maceration (Rabbana et al., 2023).

This comparison indicates a clear research gap: to date, no study has evaluated the direct antibacterial activity of *L. coromandelica* leaf extract against MRSA specifically, nor combined this with Ultrasound-Assisted Extraction (UAE) and GC-MS metabolite profiling to identify which compounds plausibly accompany the observed activity. Conventional extraction methods such as maceration are time-consuming and may promote degradation of thermolabile compounds, whereas UAE uses acoustic cavitation to mechanically disrupt plant cell walls and enhance solvent penetration, thereby improving extraction efficiency within a much shorter time while remaining consistent with green-extraction principles (Buanasari et al., 2019), and has also been shown to efficiently recover long-chain fatty acids and volatile terpenoids that contribute to disrupting the membrane integrity of resistant Gram-positive bacteria (Abbas et al., 2025) and to enhance the phenolic content and antibacterial activity of other leaf matrices such as *Moringa oleifera* (Tranquilino-rodríguez et al., 2025).

Chemical characterization of *L. coromandelica* leaves has so far been largely restricted to qualitative phytochemical screening, which only indicates the general classes of secondary metabolites present (Alyidrus et al., 2022). Gas Chromatography-Mass Spectrometry (GC-MS) offers a more comprehensive approach, capable of separating and identifying individual volatile and semi-volatile compounds based on their mass fragmentation pattern (Al-rubaye et al., 2017), and thereby provides a basis for relating specific compounds to observed biological activity.

Based on this background, the present study aimed to (1) evaluate the antibacterial activity of ethanol and methanol UAE leaf extracts of *L. coromandelica* against MRSA, and (2) characterize the chemical profile of both extracts using GC-MS, in order to examine which identified compounds plausibly accompany the antibacterial activity observed in this study.

## METHOD

### Study Design, Location, and Plant Material

This laboratory based experimental study was conducted at the Pharmacy Laboratory, Faculty of Medicine, University of Mataram, and the Microbiology Laboratory, Health Polytechnic of the Ministry of Health (Poltekkes Kemenkes) Mataram, Indonesia, from May to June 2026. The plant material consisted of young leaves (shoots) of *Lannea coromandelica* (Houtt.) Merr., collected from Mataram City, West Nusa Tenggara, Indonesia, and identified based on morphological characteristics. No herbarium voucher specimen was deposited for this study; this is noted as a limitation in the Study Limitations subsection.

### Sample Preparation

Fresh leaves were washed under running water, air-dried, rinsed briefly with acetone to remove surface dust and residual plant wax prior to drying, and then dried in a dehydrator at a controlled temperature of 40°C to preserve thermolabile compounds and standardize moisture content. Moisture content of the dried simplicia was determined using a moisture analyzer, following the maximum limit of 10% specified by the Indonesian Herbal Pharmacopoeia (Kementerian Kesehatan RI, 2017). The dried leaves were sorted, ground using a blender, and sieved through a 40-mesh sieve to obtain a fine powder.

### Ultrasound-Assisted Extraction (UAE)

Twenty grams of *L. coromandelica* leaf powder were extracted separately with 200 mL of methanol and 200 mL of ethanol (1:10, w/v) in an ultrasonic bath operating at 42 kHz for

20 minutes at room temperature. Each extract was filtered through a Büchner funnel, concentrated using a rotary evaporator, and further dried under a stream of $N_2$ gas. Extraction yield was calculated as Yield (%) = (weight of extract / weight of sample) × 100%. Dried extracts were stored at 4°C in sterile labeled containers and dissolved in dimethyl sulfoxide (DMSO) prior to the antibacterial and GC-MS assays.

**Phytochemical Screening**

Qualitative phytochemical screening was performed on both extracts following standard colorimetric procedures (Lona, 2018): alkaloids were tested with Mayer, Bouchardat/Wagner, and Dragendorff reagents, flavonoids by Mg/HCl reduction, saponins by the foam test, tannins with 1% $FeCl_3$ and steroids/triterpenoids with the Liebermann–Burchard reagent.

**Antibacterial Activity Assay**

Antibacterial activity was evaluated against MRSA using the disc diffusion (Kirby–Bauer) method. Nutrient Agar (NA) was used to reactivate the MRSA stock culture by streaking a single colony and incubating at 37°C for 24 hours, while Mueller-Hinton Agar (MHA) was used as the test medium. A bacterial suspension was standardized to 0.5 McFarland turbidity (≈$1.5\times10^8$ CFU/mL) and swabbed evenly onto MHA plates. Sterile paper discs (6 mm diameter) were impregnated with 20 µL of extract at concentrations of 50%, 75%, and 100% (dissolved in DMSO) and placed on the inoculated medium; vancomycin discs served as the positive control (a commercial antibiotic disc; one disc, single measurement, no replicates) and DMSO-only discs as the negative control. Plates were incubated at 37°C for 24 hours, and the diameter of the clear inhibition zone around each disc was measured with a caliper. Each extract treatment was tested in triplicate (n=3). Inhibition zones were descriptively categorized following the ranges commonly used in antibacterial screening of plant extracts (≤5 mm = weak, 5–10 mm = moderate, 10–20 mm = strong, ≥20 mm = very strong), consistent with the susceptibility-testing principles of CLSI M100-ED34 (2024) and the disc diffusion protocol of Hudzicki (2009).

**GC-MS Analysis**

The ethanol and methanol extracts were analyzed directly (without prior fractionation) using a Shimadzu GCMS-QP2010S instrument fitted with an Rtx-5MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). One microliter of sample was injected at an injector temperature of 200°C in split mode (ratio 1:50); helium was used as the carrier gas at a column pressure of 149.6 kPa (linear velocity 60.0 cm/s, flow rate 2.77 mL/min). The oven temperature program began at 40°C (held 5 minutes), increased at 30°C/minute to 260°C, and was held for 7 minutes, giving a total run time of 19.33 minutes. Compounds were identified by matching mass spectra against the WILEY7.LIB library based on molecular weight, fragmentation pattern, and chemical class. Compounds with a Similarity Index (SI) ≥85% were considered reliably identified; compounds with SI between 80–84% are reported as tentative identifications that would require confirmation against authentic reference standards**.**

**Data Analysis**

Data on moisture content, extraction yield, and qualitative phytochemical screening were analyzed descriptively and presented in tables. Inhibition-zone diameter data were first tested for normality (Shapiro-Wilk) and homogeneity of variance (Levene's test), then analyzed using two-way Analysis of Variance (ANOVA) with IBM SPSS Statistics 25 to examine the effects of solvent type, concentration, and their interaction. GC-MS chromatograms were interpreted based on retention time, relative peak area (% peak area), molecular formula, molecular weight, and SI. Major compounds (area >0.5% for the ethanol extract and >1% for the methanol extract) were considered together with the antibacterial results obtained in this study, and their potential biological activities were further examined through a literature review, to qualitatively evaluate their plausible contribution to the observed inhibition.

## RESULTS AND DISCUSSION

### Moisture Content and Extraction Yield

The moisture content of the dried *L. coromandelica* leaf powder was 9.10%, complying with the Indonesian Herbal Pharmacopoeia standard of a maximum of 10% (Kementerian Kesehatan RI, 2017), which helps minimize microbial contamination and enzymatic degradation during storage.

**Table 1.** Extraction Yield of *L. coromandelica* Leaf Extracts

| Extract | Yield (%) |
|---|---|
| Ethanol | 3.60 |
| Methanol | 6.43 |

UAE produced a higher extraction yield with methanol than with ethanol. This is primarily attributable to methanol's greater polarity: following the "like dissolves like" principle, methanol is more effective at dissolving polar phytochemicals from plant tissue, while the acoustic cavitation generated during UAE mechanically disrupts plant cell walls and facilitates solvent penetration into intracellular matrices, improving mass-transfer efficiency and shortening extraction time relative to conventional techniques (Buanasari et al., 2019).

### Qualitative Phytochemical Screening

**Table 2.** Qualitative Phytochemical Screening of *L. coromandelica* Leaf Extracts

| Phytochemical Class | Reagent/Test Method | Ethanol Extract | Methanol Extract |
|---|---|---|---|
| Alkaloids | Wagner/Dragendorff | + | ++ |
| Flavonoids | Mg + HCl + Amyl alcohol | + | ++ |
| Saponins | Foam test | + | + |
| Tannins | 1% $FeCl_3$ | + | ++ |
| Steroids | Liebermann–Burchard reagent | + | + |

*Note*: (–) not detected; (+) weak; (++) moderate reaction intensity

Both extracts tested positive for alkaloids, flavonoids, saponins, tannins, and steroids, with the methanol extract showing stronger reactions for alkaloids, flavonoids, and tannins, consistent with its higher extraction yield and methanol's greater efficiency at recovering polar secondary metabolites. This phytochemical profile is consistent with previous reports on *L. coromandelica* (Iqbal & Jariah, 2025). It should be noted that these qualitative "+/++" reactions indicate relative reaction intensity only and do not by themselves quantify metabolite abundance; the antibacterial and GC-MS results below provide the direct, quantitative evidence for this study's conclusions.

### Antibacterial Activity Against MRSA

The antibacterial activity of the *L. coromandelica* leaf extracts against MRSA was determined by measuring the diameter of the clear inhibition zone formed around each disc on the Mueller-Hinton Agar plates. Representative photographs of the resulting inhibition zones are shown in Figure 1, and the corresponding measurements are summarized in Table 3.

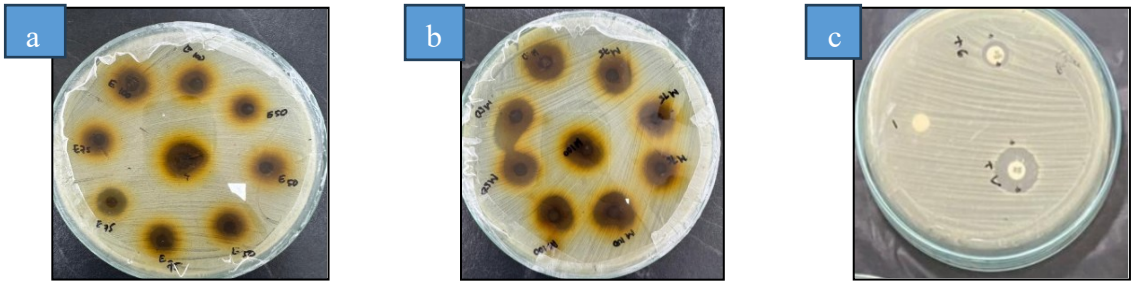


**Figure 1.** Antibacterial activity of *L. coromandelica* leaf extract

Note: Antibacterial activity of *L. coromandelica* leaf extract against MRSA by the disc diffusion method. (a) Ethanol extract at 50%, 75%, and 100% concentrations; (b) methanol

extract at 50%, 75%, and 100% concentrations; (c) positive control (vancomycin) and negative control (DMSO). Arrows indicate the inhibition zones that were measured.

**Table 3.** Inhibition Zone Diameter of *L. coromandelica* Leaf Extracts Against MRSA

| Extract | Concentration | Mean ± SD (mm) | Category |
|---|---|---|---|
| Ethanol | 50% | 11.33 ± 1.53 | Strong |
| Ethanol | 75% | 16.00 ± 1.00 | Strong |
| Ethanol | 100% | 18.33 ± 1.53 | Strong |
| Methanol | 50% | 21.00 ± 1.00 | Very strong |
| Methanol | 75% | 20.67 ± 1.15 | Very strong |
| Methanol | 100% | 21.00 ± 1.00 | Very strong |
| Vancomycin (positive control) | – | 20.00 | Very strong |
| DMSO (negative control) | – | 0.00 | No inhibition |

*Note*: n = 3 replicates per extract treatment; the vancomycin control was a single disc (one measurement, no SD) and was not statistically compared with the extracts.

The methanol extract produced inhibition zones classified as very strong at every tested concentration, numerically similar to or slightly larger than the vancomycin control disc (20.00 mm), while the ethanol extract produced strong inhibition that increased with concentration, remaining slightly below the vancomycin zone even at 100% (18.33 mm). This comparison with vancomycin is descriptive only and does not establish equivalent antibacterial efficacy: vancomycin was tested as a single disc without replicates or statistical testing, the extracts and the antibiotic differ in the amount and nature of the active material loaded per disc (a crude mixture versus a purified glycopeptide), and inhibition-zone diameter also depends on the diffusion rate of each agent through agar, so zone size alone is not a direct measure of relative potency. Equivalence would require MIC/MBC determination and a replicated, dose-matched comparison. No inhibition was observed for the DMSO negative control, confirming that the observed activity originated from the plant extract rather than the solvent. This pattern, with methanol outperforming ethanol against MRSA, is consistent with the bark-extract findings of Ferdous et al. (2022), where methanol (14 mm) also exceeded ethanol (13 mm) and water (12 mm) extracts of the same species. The comparatively larger zones obtained here for leaf extracts, relative to earlier leaf-extract studies against non-MRSA organisms (Kayla et al., 2023; Rabbana et al., 2023), likely reflect both the UAE method and the specific solvent-polarity profile used.

Mechanistically, methanol's lower molecular weight (32.04 g/mol, versus ethanol's 46.07 g/mol) and its combination of a polar hydroxyl group with a small nonpolar methyl group make it an efficient solvent for both polar and moderately nonpolar phytochemicals (Astarina et al., 2013), which is consistent with the stronger phytochemical reactions observed in Table 2 for the methanol extract. It is important to note, however, that this study evaluated crude extracts rather than isolated compounds; the relative contribution of individual metabolite classes to the observed inhibition cannot be established from these results alone.

**Statistical Analysis**

Prior to hypothesis testing, the inhibition-zone data satisfied the assumptions of normality (Shapiro-Wilk, $p = 0.356$) and homogeneity of variance (Levene's test, $p = 0.932$).

**Table 4**. Two-Way ANOVA of Inhibition Zone Diameter by Solvent Type and Concentration

| Source | df | SS | MS | F | p-value | Significance |
|---|---|---|---|---|---|---|
| Solvent type | 1 | 162.000 | 162.000 | 81.000 | <0.001 | Significant |
| Concentration | 2 | 48.111 | 24.056 | 12.028 | 0.001 | Significant |
| Solvent × Concentration | 2 | 31.000 | 15.500 | 7.750 | 0.007 | Significant |
| Error | 12 | 24.000 | 2.000 | – | – | – |
| Corrected total | 17 | 265.111 | – | – | – | – |

*Note*: Model $R^2 = 0.909$. Degrees of freedom (df): solvent type = 1, concentration = 2, solvent × concentration = 2, error = 12, corrected total = 17 (2 solvents × 3 concentrations × 3 replicates; N = 18).

Two-way ANOVA (N = 18; error df = 12) showed that solvent type [$F(1, 12) = 81.000$, $p < 0.001$], concentration [$F(2, 12) = 12.028$, $p = 0.001$], and their interaction [$F(2, 12) = 7.750$, $p = 0.007$] each had a statistically significant effect on inhibition-zone diameter, with the model explaining 90.9% of the observed variance. Examination of the simple effects indicated that concentration had a much stronger influence on the ethanol extract, whose zone diameter increased steadily from 11.33 mm (50%) to 18.33 mm (100%), whereas the methanol extract's activity was already close to its maximum at 50% and remained comparatively stable through 100%, suggesting that its antibacterial potential was largely saturated at the lowest concentration tested. These results provide direct, quantitative confirmation that the extracts possess genuine antibacterial activity against MRSA, rather than activity inferred solely from chemical composition.

## GC-MS Chemical Profile

GC-MS analysis of the ethanol and methanol extracts each produced 30 chromatographic peaks, reflecting a complex mixture of volatile and semi-volatile secondary metabolites, with SI values ranging from 82–97%. Following the ≥85% reliability threshold defined in the Method section, all compounds listed below reached this threshold except β-tocopherol (SI 84%, ethanol extract), neophytadiene (SI 82%, methanol extract), 1,13-tridecanediol diacetate (SI 82%, methanol extract), and lupeyl acetate in the methanol extract (SI 84%; the same compound had SI 89% in the ethanol extract), which are reported here as tentative identifications only. Tentative identifications are marked with † in Tables 5 and 6.

**Table 5.** Major Compounds Identified in the Ethanol Extract (area >0.5%)

| Compound | Molecular Formula | MW (g/mol) | Area (%) | SI (%) |
|---|---|---|---|---|
| 9-Octadecenal, (Z)- | C18H34O | 266 | 4.18 | 96 |
| Tetradecanal | C14H28O | 212 | 3.21 | 96 |
| 2-Heptadecanone | C17H34O | 254 | 1.59 | 96 |
| Hexadecanoic acid, methyl ester | C17H34O2 | 270 | 0.93 | 95 |
| Hexadecanoic acid (palmitic acid) | C16H32O2 | 256 | 8.61 | 95 |
| Phytol | C20H40O | 296 | 17.44 | 97 |
| Octadecanoic acid (stearic acid) | C18H36O2 | 284 | 4.08 | 94 |
| Bis(2-ethylhexyl) phthalate* | C24H38O4 | 390 | 2.44 | 97 |
| β-Tocopherol† | C28H48O2 | 416 | 0.67 | 84 |
| 1-Eicosanol | C20H42O | 298 | 2.35 | 95 |
| Alpha-tocopheryl-β-D-mannoside | C35H60O7 | 592 | 3.86 | 91 |
| Campesterol | C28H48O | 400 | 1.41 | 86 |
| Stigmast-5-en-3-ol (γ-sitosterol) | C29H50O | 414 | 21.74 | 90 |
| Lupeyl acetate | C32H52O2 | 468 | 1.97 | 89 |

*Note:* *Flagged as a likely exogenous/plasticizer contaminant (see discussion below); †tentative identification (SI 80-84%).

**Table 6.** Major Compounds Identified in the Methanol Extract (area >1%)

| Compound | Molecular Formula | MW (g/mol) | Area (%) | SI (%) |
|---|---|---|---|---|
| 9-Octadecenal, (Z)- | C18H34O | 266 | 5.76 | 96 |
| Tetradecanal | C14H28O | 212 | 3.99 | 96 |
| Neophytadiene† | C20H38 | 278 | 1.38 | 82 |
| 1,13-Tridecanediol diacetate† | C17H32O4 | 300 | 1.47 | 82 |
| 2-Heptadecanone | C17H34O | 254 | 1.50 | 92 |
| Hexadecanoic acid (palmitic acid) | C16H32O2 | 256 | 5.74 | 95 |
| Phytol | C20H40O | 296 | 11.39 | 97 |
| Diisooctyl phthalate* | C24H38O4 | 390 | 4.37 | 97 |
| 1-Eicosanol | C20H42O | 298 | 4.12 | 93 |
| Alpha-tocopheryl-β-D-mannoside | C35H60O7 | 592 | 4.30 | 89 |
| Stigmast-5-en-3-ol (γ-sitosterol) | C29H50O | 414 | 15.49 | 91 |
| Lupeyl acetate† | C32H52O2 | 468 | 2.33 | 84 |

*Note*: *Flagged as a likely exogenous/plasticizer contaminant (see discussion below); †tentative identification (SI 80–84%), reported here for completeness but not treated as confidently identified.

Stigmast-5-en-3-ol (γ-sitosterol), phytol, and hexadecanoic acid were the dominant compounds in both extracts, together accounting for 47.79% of the total peak area in the ethanol extract (21.74% + 17.44% + 8.61%) but only 32.62% in the methanol extract (15.49% + 11.39% + 5.74%). The two phthalate esters (bis(2-ethylhexyl) phthalate and diisooctyl phthalate) are widely recognized as plasticizers that readily migrate from plastic labware into extraction solvents (Ouchi et al., 2019). Given this, they are flagged here as probable exogenous contaminants rather than genuine plant metabolites, and are excluded from the bioactivity discussion below.

We note, for balance, that phthalate-class compounds have occasionally been reported as natural constituents of other plant extracts (Gultom et al., 2020); without dedicated solvent-blank analysis, however, this cannot be resolved for the present samples, and blank-corrected GC-MS analysis is recommended for future work.

**Chemical Profile and Its Plausible Relationship with the Observed Antibacterial Activity**

To avoid conflating chemical detection with proof of biological activity, the compounds discussed below are considered at three distinct levels of evidence: (i) compounds directly detected in this study by GC-MS, (ii) antibacterial or related bioactivity previously reported in the literature for those compounds (in isolation, not in this extract) and (iii) the antibacterial activity actually observed in this study's crude extracts (Table 3), which cannot be attributed to any single compound without further fractionation. It should also be emphasized that no correlation analysis (e.g., Pearson or Spearman correlation or multivariate analysis) was performed between compound abundance and inhibition-zone diameter; with only two extracts, such an analysis would have no meaningful statistical power. The relationships discussed below are therefore inferential and literature-based, not associations demonstrated statistically.

Stigmast-5-en-3-ol (γ-sitosterol) was the most abundant compound in both extracts and has been reported to interact with bacterial membrane lipids, altering membrane integrity and permeability, and to exert bacteriostatic effects against *S. aureus* by modulating protein-translation-related gene expression (Kurniawan et al., 2021; Ibrahim & Yaacob, 2017); campesterol, detected only in the ethanol extract, has similarly been reported to disrupt membrane stability in *S. aureus* (de Morais Oliveira-Tintino et al., 2025).

Phytol, the second most abundant compound in both extracts, has been reported to induce intracellular oxidative stress via reactive oxygen species and to increase bacterial membrane permeability (Almeida-Bezerra et al., 2024). Hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid) are long-chain saturated fatty acids repeatedly associated with antimicrobial activity against Gram-positive bacteria through destabilization of the phospholipid bilayer and disruption of membrane-associated enzymes (Casillas-Vargas et al., 2021).

Lupeyl acetate, present in both extracts (SI 89% in the ethanol extract, but only a tentative SI 84% identification in the methanol extract), is structurally related to lupeol, a pentacyclic triterpenoid reported to have both bactericidal and bacteriostatic effects against clinical MRSA isolates (Sohag et al., 2022). Neophytadiene and 1,13-tridecanediol diacetate, detected only in the methanol extract (as tentative SI 82% identifications), have been associated respectively with anti-inflammatory activity in macrophage models (Bhardwaj et al., 2020) and with membrane-permeabilizing ester structures; their exclusive presence in the methanol extract offers one plausible partial explanation for methanol's stronger observed activity, since a compound's biological contribution is not necessarily proportional to its relative abundance in a mixture (Casillas-Vargas et al., 2021).

Consistent with the phytochemical screening (Table 2), the steroid-positive reaction corresponds to the GC-MS detection of stigmast-5-en-3-ol and campesterol, both phytosterols sharing a cyclopentanoperhydrophenanthrene backbone (Piironen et al., 2000), while the saponin-positive reaction may be partly consistent with the triterpenoid skeleton of lupeyl

acetate, although this ester is not itself a saponin glycoside (Vincken et al., 2007) . Alkaloids, flavonoids, and tannins were detected in the phytochemical screening but did not appear in the GC-MS chromatograms; this is consistent with GC-MS's inherent bias toward volatile, thermally stable compounds, since polar, non-volatile, or thermolabile classes such as alkaloids and flavonoids typically require derivatization or complementary techniques such as LC-MS/MS or HPLC for detection, and some alkaloids are known to degrade at the high injector temperatures used in GC-MS (Frolova et al., 2026)

Taken together, the antibacterial activity documented in Table 3 is the direct experimental finding of this study, while the compound-level mechanisms discussed above remain literature-based inferences (not statistically demonstrated correlations) about which constituents plausibly contribute to that activity; because a crude, unfractionated extract was tested, these inferences should be confirmed through bioactivity-guided fractionation and testing of isolated compounds before any single metabolite is attributed a specific role.

**Study Limitations**

This study has several limitations that should be considered when interpreting the results. First, antibacterial activity was assessed only by the diffusion method; no minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) values were determined. In addition, vancomycin was tested as a single disc without replicates and was not statistically compared with the extracts, so the comparison with this antibiotic is descriptive and equivalence of antibacterial efficacy was not established. Second, only crude extracts were tested, without fractionation, isolation, or purification of individual compounds, so antibacterial activity cannot be attributed with certainty to any single metabolite. Moreover, the relationship between GC-MS compounds and antibacterial activity was interpreted qualitatively from the literature; no correlation analysis was performed, so these relationships remain inferential. Third, phytochemical screening was purely qualitative (colorimetric), without quantification of total flavonoid, phenolic, or tannin content, and GC-MS analysis was performed without a solvent blank run alongside the samples. Fourth, no in vivo or toxicity testing was performed, so the safety profile of the extracts has not been established. Finally, no herbarium voucher specimen was deposited for the plant material used in this study.

## CONCLUSION

Ultrasound-Assisted Extraction (UAE) of *Lannea coromandelica* leaves produced extraction yields of 6.43% (methanol) and 3.60% (ethanol), with both extracts testing positive for alkaloids, flavonoids, saponins, tannins, and steroids. Against MRSA, the methanol extract exhibited very strong antibacterial activity at all tested concentrations (20.67–21.00 mm), numerically similar to or slightly larger than the vancomycin control disc (20.00 mm; single measurement, equivalence not tested), while the ethanol extract exhibited strong, concentration-dependent activity (11.33–18.33 mm); two-way ANOVA confirmed that solvent type, concentration, and their interaction significantly affected inhibition-zone diameter ($p<0.05$ for all effects). GC-MS analysis identified 30 chromatographic peaks in each extract, dominated by γ-sitosterol, phytol, and palmitic acid, with neophytadiene and 1,13-tridecanediol diacetate detected only in the methanol extract (tentative identifications). These findings indicate that the UAE methanol extract of *L. coromandelica* leaves possesses in vitro antibacterial activity against MRSA, accompanied by a chemically complex bioactive profile that plausibly, though not yet conclusively, contributes to this activity.

## RECOMMENDATION

Further studies are recommended to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *L. coromandelica* leaf extracts against MRSA using the broth microdilution method. Bioactivity-guided fractionation, isolation, and purification of the major identified compounds (γ-sitosterol, phytol, and palmitic acid) are recommended to determine which specific metabolites are responsible for the

observed activity, ideally combined with in silico molecular docking studies. Complementary analysis using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) or High-Performance Liquid Chromatography (HPLC) is suggested to capture polar compound classes, such as alkaloids and flavonoids, that are not well represented in GC-MS chromatograms. In vivo acute and sub-chronic toxicity testing is also needed to establish a safety profile before any progression toward clinical application. Given that MRSA infections frequently manifest in skin and soft-tissue sites, the methanol extract's potential development into a standardized topical pharmaceutical preparation (such as a gel, ointment, or cream) also merits future investigation.

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**AUTHOR CONTRIBUTIONS STATEMENT**

| Name of Author | C | M | So | Va | Fo | I | R | D | O | E | Vi | Su | P | Fu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fitria Ernawati | ✓ | ✓ | | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | | |
| Bambang Fajar Suryadi | ✓ | ✓ | | ✓ | | | | | | ✓ | | ✓ | | |
| Emmy Yuanita | | | | | | | ✓ | | | ✓ | | ✓ | | ✓ |

**CONFLICT OF INTEREST STATEMENT**

The authors declare no conflict of interest.

**DATA AVAILABILITY**

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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