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Antibacterial Activity of *Metroxylon sagu* Rottb. Root Extracts and Fractions against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

***Introduction**: The increasing resistance of Methicillin-Resistant* Staphylococcus aureus *(MRSA) to multiple antibiotics has become a major challenge in infection management, underscoring the need to identify new antibacterial agents from natural sources. The roots of* Metroxylon sagu *Rottb. contain various secondary metabolites with potential biological activities.* ***Aims**: This study aimed to evaluate the antibacterial activity of the root extract and its fractions against MRSA and to determine their Minimum Inhibitory Concentration (MIC) values.* ***Methods**: Extraction was performed using Ultrasound-Assisted Extraction (UAE) with 96% ethanol, followed by liquid–liquid fractionation with n-hexane, ethyl acetate, and water. MIC values were determined using the microdilution method, and antibacterial activity was assessed using the disk diffusion method at concentrations of 1–10%.* ***Result**: The ethanol extract of sago roots (EEAR), the ethyl acetate fraction (FEAAR), and the aqueous fraction (FAAR) exhibited MIC values of 5 mg/mL, 1.25 mg/mL, and 2.5 mg/mL, respectively. The n-hexane fraction (Fn-HAR) showed no inhibitory activity. At a concentration of 10%, EEAR, FEAAR, and FAAR produced inhibition zones of 9.80 ± 0.07 mm, 10.84 ± 0.14 mm, and 10.06 ± 0.18 mm, respectively, while Fn-HAR produced none.* ***Conclusion**: The ethyl acetate fraction demonstrated the strongest antibacterial effect, supported by its lowest MIC value and largest inhibition zone, indicating that semipolar compounds may be responsible for the antibacterial activity. These findings suggest that* M. sagu *roots represent a promising natural source of antibacterial agents for inhibiting MRSA.*

***KEYWORDS**: Antibacterial, Metroxylon sagu root, MIC, disk diffusion, MRSA*

INTRODUCTION

Bacterial infections remain a major contributor to global morbidity and mortality, particularly in healthcare settings, and their clinical burden has been further intensified by the rapid emergence of antimicrobial resistance (AMR) worldwide. Specifically, methicillin-

resistant *Staphylococcus aureus* (MRSA) has emerged as a major pathogen responsible for both nosocomial and community-acquired infections, with a global prevalence estimated at approximately 14.69% in certain populations (Hasanpour *et al*., 2023).

The development of MRSA resistance is primarily attributed to the acquisition of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a), thereby reducing the binding affinity of β-lactam antibiotics and severely limiting available therapeutic options (Blázquez et al., 2014). This situation underscores the urgent need to identify alternative therapeutic agents that can effectively combat resistant bacterial strains (Touaitia *et al*., 2025). Furthermore, clinical and economic analyses have demonstrated that MRSA infections are associated with longer hospital stays and higher healthcare costs compared to infections caused by methicillin-susceptible *S. aureus* (MSSA) (Hirabayashi *et al*., 2024).

In the context of escalating bacterial resistance to conventional antibiotics and the declining efficacy of currently available antimicrobial therapies, the discovery of alternative antibacterial agents derived from natural sources has become increasingly urgent. Recent reports have estimated that antimicrobial resistance (AMR) contributes to millions of deaths annually and poses a substantial global healthcare burden, particularly in low- and middle-income countries (Murray *et al*., 2022). Exploring secondary metabolites from local plant species offers valuable opportunities to identify novel bioactive compounds with mechanisms of action distinct from those of existing antibiotics (Ho *et al*., 2024). Among diverse biological resources, endemic and traditional medicinal plants remain largely underexplored despite their long-standing ethnomedicinal use. One such endemic species with considerable pharmacological potential is the sago palm (*Metroxylon sagu* Rottb.), which commonly grows in tropical swamp regions such as Kalimantan. From an ethnopharmacological perspective, the Dayak community has traditionally utilized the roots of *M. sagu* to treat skin infections and wounds (Karmila *et al*., 2023). Therefore, this study aimed to evaluate the antibacterial activity of the extract and fractions of *Metroxylon sagu* root against MRSA as part of ongoing efforts to develop plant-derived antibacterial candidates against antibiotic-resistant pathogens.

Phytochemical screenings performed on different parts of *M. sagu* (leaves, pith, fruit, and roots) have consistently identified diverse classes of secondary metabolites, including flavonoids, tannins, saponins, phenolics, alkaloids, steroids, and glycosides, which are commonly associated

with antimicrobial and antioxidant activities in medicinal plants. These phytochemical profiles have been documented in recent surveys and reviews of *M. sagu* and its derivatives (Moshawih *et al*., 2025). Among the investigated plant parts, the roots were selected in the present study based on their traditional use by the indigenous Dayak community for the treatment of skin infections and wounds, indicating their potential as a source of antibacterial bioactive compounds. In addition, roots are known to accumulate defensive secondary metabolites that contribute to plant protection against soil-associated microbial challenges.

Several experimental studies have demonstrated the antibacterial activity of *M. sagu* extracts against common bacterial species such as *Staphylococcus aureus* and *Escherichia coli*, indicating an inherent antimicrobial potential in the crude extracts derived from its leaves and other plant tissues (Nurlila & Fua, 2023). However, reports specifically investigating the activity of *M. sagu* against methicillin-resistant *S. aureus* (MRSA), as well as systematic evaluations of root extracts and their chromatographic fractions, remain scarce. This research gap underpins the present study, which aims to evaluate the antibacterial activity of crude extracts and solvent-partitioned fractions of *M. sagu* roots against MRSA.

METHODS

Material

The instruments used in this study included an Ultrasound-Assisted Extraction (UAE) system, incubator (Memmert®), oven (Memmert®), autoclave (All American Autoclave®), microplate 96 well (Biologix®).

The materials used consisted of *Metroxylon sagu* Rottb. root simplicia processed into extracts with concentrations of 10%, 7.5%, 5%, 2.5%, and 1%. Additional materials included Mueller–Hinton Agar (MHA), Muller Hinton Broth (HiMedia®), Nutrient Agar (NA) (Oxoid®), 96% Ethanol (Brataco®), Ethyl Acetate (Brataco®), n-Hexane (Brataco®), DMSO (Emsure®), distilled water, NaCl, 0.5 McFarland standard (HiMedia®), and MRSA bacterial cultures. The minimum inhibitory concentration (MIC) assay was performed using the broth microdilution method in sterile 96-well microplates (Biologix®). Serial dilutions of the extracts and fractions were prepared in Mueller–Hinton Broth (MHB), followed by the addition of standardized bacterial suspensions equivalent to 0.5 McFarland turbidity standard. The microplates were incubated at 37°C for 24 h, and MIC values were determined as the lowest concentration showing no visible bacterial growth compared with the negative control.

Collection of Plant Material and Preparation of Simplisia

The roots of *M. sagu* (rumbia) were collected from Terantang Village, Alalak District, Barito Kuala, during a single harvest to ensure sample uniformity. The plant materials were carefully cleaned to remove soil and other impurities, followed by quick rinsing under running water. The roots were cut into small pieces of 5–10 mm and dried in a hot-air oven at a maximum temperature of 50 °C for 48 hours (2 × 24 h). Controlled drying at relatively low temperatures was intended to prevent the degradation of bioactive compounds. After drying, the samples were subjected to dry sorting, then pulverized into coarse powder. The powder was subsequently sieved through a 40-mesh sieve to obtain a homogeneous particle size for further extraction processes.

Preparation of Extracts

Extraction of the *M. sagu* root powder was performed using the Ultrasound-Assisted Extraction (UAE) method, which is known to enhance the recovery of bioactive compounds while minimizing solvent use and extraction time. A total of 500 g of powdered simplisia was macerated with 5000 mL of 96% ethanol at a ratio of 1:10 (b/v). The mixture was placed in an ultrasonic bath operated at 40 °C with a frequency of 47 kHz for 20–30 minutes to facilitate the release of active metabolites. The resulting extract solution was filtered to remove solid residues, and the filtrate was subsequently concentrated using a vacuum rotary evaporator or dehydrator at 40–50 °C until a thick crude extract was obtained (Demesa *et al.*, 2024).

Phytochemical Screening

Preliminary phytochemical screening was carried out to qualitatively determine the presence of major secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and terpenoids/steroids, following previously reported procedures with slight modifications (Hasani, *et al*., 2025). The presence of each metabolite group was confirmed through characteristic color reactions, precipitate formation, or foam stability tests (Dubale *et al*., 2023).

Liquid-Liquid Fractionation Procedure

The crude extract of *M. sagu* was dissolved in a minimal volume of semi-polar solvent and partitioned sequentially using immiscible solvent systems with increasing polarity to obtain n-hexane, ethyl acetate, and aqueous fractions. Following phase separation, each solvent layer was concentrated under reduced pressure at temperatures ≤50 °C to minimize thermal degradation. The obtained fractions were subsequently dried and subjected to antibacterial and phytochemical evaluations(Abubakar & Haque, 2020).

Sterilization of Instruments and Materials

All glassware and heat-resistant instruments, including test tubes, Petri dishes, Erlenmeyer flasks, and beakers, were sterilized using a hot-air oven at 160–170°C for 2 h following the dry heat sterilization method (Jain Aakanchhaand Jain, 2020). Culture media and heat-stable solutions, including Nutrient Agar (NA), Mueller–Hinton Broth (MHB) and Muller Hinton Broth (MHB) were sterilized by autoclaving at 121°C for 15 min under 15 psi pressure according to the moist heat sterilization method (Hasani *et al*., 2025). After sterilization, the NA and MHA media were cooled to 45–50°C before being poured into sterile Petri dishes, while MHB was allowed to cool to room temperature under aseptic conditions.

Antibacterial Activity and MIC Assay

All equipment and culture media were sterilized by autoclaving at 121 °C for 15 min prior to use. Sterile Mueller–Hinton Agar (MHA) was aseptically poured into sterile Petri dishes and allowed to solidify, while Nutrient Agar (NA) plates were prepared for the rejuvenation of methicillin-resistant *Staphylococcus aureus* (MRSA). The bacterial suspension was prepared in sterile 0.9% NaCl solution and adjusted to the 0.5 McFarland turbidity standard, corresponding to approximately 1.5×10^8 CFU/mL, to ensure uniform inoculum density during antibacterial susceptibility testing (CLSI, 2023).

Test samples were prepared at serial concentrations of 10%, 7.5%, 5%, 2.5%, and 1% using 1% DMSO as the solvent. A sterile cotton swab was used to evenly inoculate the bacterial suspension onto the surface of MHA plates. Sterile paper discs impregnated with the test samples were subsequently placed on the inoculated agar surface. Ciprofloxacin (5 µg/disc) and 1% DMSO served as the positive and negative controls, respectively. The plates were incubated at 37 °C for 18–24 h, after which the diameters of inhibition zones were measured (Callebaut *et al*., 2024). The minimum inhibitory concentration (MIC) assay was conducted using the broth microdilution method in sterile 96-well microplates. Serial dilutions of the extracts, fractions, and subfractions were prepared in Mueller–Hinton Broth (MHB), followed by the addition of standardized MRSA inoculum and ciprofloxacin as the reference control. The microplates were incubated at 37 °C for 24 h under sterile conditions. MIC values were defined as the lowest concentration exhibiting no visible bacterial growth compared with the negative control wells (Belley *et al*., 2025).

RESULTS AND DISCUSSION

Collection of Plant Material and Preparation of Simplisia

Fresh roots of *M. sagu* (red and light brown portions) were collected from Terantang Village, Semangat Karya, Alalak District, Barito Kuala Regency, South Kalimantan, Indonesia, during the morning to minimize post-harvest metabolic degradation and maintain sample freshness. A total of 6.8 kg of roots was thoroughly washed under running water, cut into 5–10 mm sections, and briefly sun-dried for 15–30 min under filtered sunlight prior to oven-drying at 50 °C for 46 h until a constant weight was achieved (Eapen *et al*., 2025). Controlled drying at moderate temperatures is widely recommended to reduce moisture content while minimizing thermal degradation of thermolabile secondary metabolites, particularly phenolic and flavonoid compounds (Al-Qudah *et al*., 2023). The dried roots were subsequently pulverized and sieved through a No. 40 mesh to obtain uniform simplicia powder (Batchelor *et al*., 2024), Particle size standardization is considered important for improving extraction efficiency by increasing solvent penetration and surface contact area during the extraction process. The overall drying and milling process yielded approximately 1,500 g of simplicia powder with a final recovery of 22.05%.

Preparation of Rumbia Root Extract

The roots of *M. sagu* were extracted using 96% ethanol through the Ultrasound-Assisted Extraction (UAE) method to enhance the recovery of bioactive constituents. This extraction process yielded 47.68 g of concentrated extract from 400 g of dried simplicia, corresponding to a percentage yield of 11.92%. The obtained yield indicates that the UAE technique was effective in facilitating solvent penetration and promoting the release of secondary metabolites from plant tissues. Previous studies have similarly reported that UAE improves the extraction efficiency of phenolic and flavonoid compounds compared with conventional extraction methods, such as maceration and reflux extraction (Hiranpradith *et al*., 2025). Furthermore, the extraction yield obtained in the present study falls within the commonly reported range for medicinal plant extracts, which generally varies between 8–12% depending on plant matrix characteristics and extraction conditions (González-Silva *et al*., 2022).

Phytochemical Screening

Preliminary phytochemical screening of the *M. sagu* root extract was performed to qualitatively identify the presence of major

Table 1. Phytochemical Screening Results of Rumbia Root Extract

Test	Reagent	Result	Observation
Alkaloids	Dragendorff's reagent	+	Formation of brownish-orange precipitate
	Mayer's reagent	+	Formation of white precipitate
	Wagner's reagent	+	Formation of reddish-brown precipitat
Flavonoids	2 N HCl, Mg powder, and amyl alcohol	+	Development of orange coloration
Quinones	1 N NaOH	+	Development of red coloration
Tannins	2% gelatin solution	+	Formation of white precipitate
	3% $FeCl_3$ solution	+	Development of bluish-green coloration
Saponins	2 N HCl	+	Formation of stable foam (±2 cm)
Steroids/Terpenoids	Acetic anhydride and concentrated H_2SO_4	+	Development of bluish-green coloration

classes of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, triterpenoids/steroids, and other phenolic compounds. The screening was conducted based on characteristic color changes and precipitate formation following treatment with specific phytochemical reagents, which served as positive indicators for each metabolite group. As summarized in Table 1, the extract exhibited positive reactions for several bioactive secondary metabolites potentially associated with antibacterial activity.

The phytochemical screening results presented in Table 1 demonstrated the presence of several major secondary metabolites in the *M. sagu* root extract, including alkaloids, flavonoids, quinones, tannins, saponins, and steroid/triterpenoid compounds.. This phytochemical profile is consistent with previous reports describing tropical medicinal plants as rich sources of bioactive secondary metabolites with potential antibacterial and antibiofilm activities (de Oliveira *et al*., 2024) Among the detected compounds, flavonoids, tannins, and quinones are particularly recognized for their important roles in inhibiting bacterial biofilm formation. In methicillin-resistant *Staphylococcus aureus* (MRSA), these compounds may interfere with multiple pathogenic mechanisms, including bacterial cell adhesion, extracellular polymeric substance (EPS) formation, and quorum sensing-mediated cell-to-cell communication. Such mechanisms are considered essential in reducing MRSA colonization and enhancing the susceptibility of bacterial cells to antibacterial agents (Moldovan *et al*., 2024).

Fractionation

The liquid–liquid fractionation (LLF) of the 96% ethanol extract of *M. sagu* root was performed to separate bioactive components based on polarity differences, resulting in three main fractions: n-hexane, ethyl acetate, and aqueous fractions. This separation aimed to group specific classes of metabolites, with non-polar compounds expected in the n-hexane fraction, semi-polar compounds in the ethyl acetate fraction, and highly polar compounds remaining in the aqueous fraction (Azzahra *et al*., 2023).

All obtained fractions were subsequently evaluated for antibacterial activity to assess the contribution of metabolite polarity to the bioactivity of *M. sagu* root extract. The fractionation process successfully separated compounds based on polarity differences, with non-polar constituents partitioning into the n-hexane fraction, semi-polar compounds into the ethyl acetate fraction, and polar compounds remaining in the aqueous fraction. This distribution pattern is consistent with previous reports on solvent polarity-based fractionation of tropical medicinal plants (Abubakar & Haque, 2020).

Antibacterial Activity Assay

The antibacterial activity of *M. sagu* root extract and its fractions was evaluated using the minimum inhibitory concentration (MIC) and disk diffusion assays against MRSA. The MIC assay was conducted to determine the lowest concentration capable of inhibiting visible bacterial growth, whereas the disk diffusion method was used to evaluate the inhibitory potential of each sample based on inhibition zone formation on agar media. These complementary approaches enabled comparative assessment of antibacterial potency among the ethanolic extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction.

Table 2. MIC of extract and fractions of *M. sagu* roots

Sample	MIC (mg/mL)
Ethanolic extract	5
n-hexane fraction	–
Ethyl acetate fraction	1.25
Aqueous fraction of	2.5

As summarized in Table 2, the ethyl acetate fraction exhibited the strongest antibacterial activity, with the lowest MIC value of 1.25 mg/mL, followed by the aqueous fraction (2.5 mg/mL), while the ethanolic extract and n-hexane fraction demonstrated MIC values of 5 mg/mL. The lower MIC value observed for the ethyl acetate fraction suggests that semi-polar compounds may contribute substantially to the antibacterial activity against MRSA.

The antibacterial activity was evaluated using the disc diffusion method. The extract and its fractions were prepared in serial concentrations of 10%, 7.5%, 5%, 2.5%, and 1%, with 1% DMSO used as the solvent and

Table 3. Antibacterial Activity of Extract and Fractions of *M. sagu* Roots

Sample	Inhibition zone diameter (mm)			Mean±SD
	1	2	3	
EEAR 10%	9,72	9,85	9,82	9,80±0,068
EEAR 7,5%	8,7	9	8,95	8,94±0,240
EEAR 5%	7,7	7,65	7,8	7,72±0,076
EEAR 2,5%	-	-	-	-
EEAR 1%	-	-	-	-
Fn-HAR 10%	-	-	-	-
Fn-HAR 7,5%	-	-	-	-
Fn-HAR 5%	-	-	-	-
Fn-HAR 2,5%	-	-	-	-
Fn-HAR 1%	-	-	-	-
FEAAR 10%	10,72	10,8	11	10,84±0,144
FEAAR 7,5%	8,25	9	8,6	8,62±0,375
FEAAR 5%	7,22	7,3	7,5	7,34±0,144
FEAAR 2,5%	-	-	-	-
FEAAR 1%	-	-	-	-
FAAR 10%	10,2	9,86	10,12	10,06±0,178
FAAR 7,5%	7,4	7	7,5	7,30±0,265
FAAR 5%	6,5	6,2	6,1	6,27±0,208
FAAR 2,5%	-	-	-	-
FAAR 1%	-	-	-	-
Kontrol (+)	26,82	25,45	26,38	26,22±0,699
Kontrol (-)	-	-	-	-

EEAR: ethanolic extract of *M. sagu* roots; Fn-HAR: *n*-Hexane fraction of *M. sagu* roots; FEAAR: ethyl acetate fraction of *M. sagu* roots; FAAR: aqueous fraction of *M. sagu* roots; Control (+): Ciprofloxacin disc (5 µg/disc); Control (-): 1% DMSO; (-): no inhibitory activity observed.

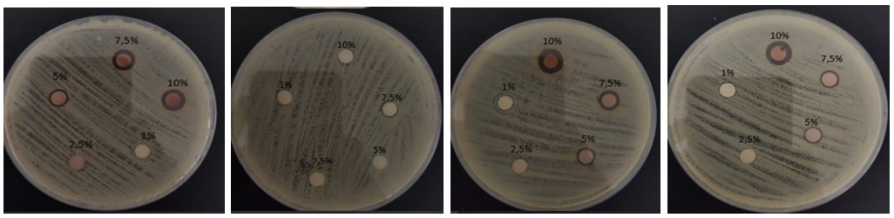

Figure 1. Antibacterial activity of *M. sagu* root extract and fractions against MRSA using the disk diffusion method: (a) ethanolic extract, (b) n-hexane fraction, (c) ethyl acetate fraction, and (d) aqueous fraction.

negative control, while ciprofloxacin discs (5 µg/disc) served as the positive control. The results of the assay are presented in the following Table 3.

Furthermore, the disk diffusion results presented in Table 3 demonstrated concentration-dependent inhibition against MRSA, with the ethyl acetate fraction producing the largest inhibition zone among the tested samples. Representative inhibition zones obtained from the antibacterial assay are illustrated in Figure 1.

The result revealed that the ethanolic

extract of *M. sagu* roots (EEAR) and its fractions exhibited measurable antibacterial activity at concentrations ≥5%. Among the tested samples, the ethyl acetate fraction at 10% concentration produced the strongest inhibitory effect, with an inhibition zone diameter of 10.84 ± 0.144 mm, followed by the aqueous fraction (10.06 ± 0.178 mm) and the ethanolic extract (9.80 ± 0.068 mm). The enhanced antibacterial activity observed in the ethyl acetate fraction may be attributed to the semi-polar characteristics of ethyl acetate, which favor the extraction of bioactive secondary metabolites such as flavonoids, tannins, and saponins (Br Sitepo Nadroh., 2020). These phytochemical constituents are widely recognized for their antibacterial mechanisms, including disruption of bacterial cell wall integrity, alteration of membrane permeability, inhibition of essential enzymatic activity, and interference with protein synthesis, which may collectively contribute to the superior antibacterial activity exhibited by the ethyl acetate fraction against MRSA. Similar antibacterial profiles have also been reported in ethyl acetate fractions of other medicinal plants rich in semi-polar flavonoid compounds, where LC–MS analysis confirmed the presence of bioactive flavonoids associated with strong antibacterial effects (Martha *et al*., 2024). his finding further supports the role of semi-polar phytochemicals as major contributors to antibacterial activity.

In contrast, the n-hexane fraction showed no activity at any tested concentration, likely because non-polar solvents primarily extract lipids, sterols, or hydrocarbons, which are relatively scarce in *M. sagu* roots and thus contribute minimally to antibacterial effects. The absence of activity in the n-hexane fraction further reinforces the notion that the bioactive metabolites responsible for bacterial inhibition in *M. sagu* are predominantly semi-polar to polar. Moreover, the substantially smaller inhibition zones produced by the extract and fractions compared with the positive control, ciprofloxacin (26.22 ± 0.699 mm), indicate that the antibacterial activity of *M. sagu* remains within the weak to moderate category (Moldovan *et al*., 2024). Although the plant-derived samples do not match the potency of synthetic antibiotics, their measurable inhibitory effects highlight the potential of *M. sagu* roots as a natural source of antibacterial agents that may be further purified or structurally optimized. These findings collectively suggest that the ethyl acetate fraction is the most promising candidate for future isolation and characterization of active constituents responsible for the observed antibacterial activity.

CONCLUSION

The present study demonstrates that the roots of *Metroxylon sagu* Rottb. possess promising antibacterial potential against MRSA. Among all tested samples, the ethyl acetate fraction exhibited the most pronounced activity, as reflected by its lowest MIC value and the largest inhibition zone, suggesting that the major bioactive constituents are likely semipolar compounds. These findings highlight the relevance of *M. sagu* roots as a potential source of antibacterial agents and provide a scientific basis for further phytochemical investigations, including the isolation, structural characterization, and mechanistic evaluation of the responsible active constituents. Future studies involving in vivo assessment and synergy testing with existing antibiotics are also warranted to validate its therapeutic applicability.

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