Research Article

Antibacterial Impact of *Syzygium aromaticum* Extract on Clinical and Sub-Clinical Methicillin-Resistant *Staphylococcus aureus*

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Abstract | Clove (*Syzygium aromaticum*) is famous for its' antibacterial properties. The current study aimed to investigate the antibacterial properties of clove extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) strains (n=9), which were previously isolated and confirmed from clinical and subclinical cases. The objective was also to determine the optimal concentration of clove extract required to inhibit MRSA growth. The extract was tested at various concentrations in a liquid growth medium using the agar well diffusion method. The ethanolic extract demonstrated antibacterial activity, with inhibition zone diameters ranging from 14 mm to 43 mm. No inhibition was observed at concentrations of 6.25 mg/mL and 12.5 mg/mL, whereas significant activity was noted at 50.25 mg/mL. All MRSA strains showed resistance to nine conventional antibiotics. The minimum inhibitory concentration (MIC) values ranged from 12.5 to 25 mg/mL, with a MIC of 25 mg/mL indicating notable susceptibility. The minimum bactericidal concentration (MBC) values ranged from 25 to 50 mg/mL. Overall, the findings suggest that ethanolic extracts of *S. aromaticum* exhibit promising antibacterial activity against *S. aureus*, including MRSA strains.

Keywords | *Syzygium aromaticum*, *Staphylococcus aureus,* MRSA, MIC, Broth serial dilution

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INTRODUCTION

According to data from the World Health Organization (WHO), approximately 80% of individuals in low-income countries rely on medicinal plants to meet their primary healthcare needs. This widespread dependence reflects both cultural traditions and limited access to conventional medicine. Simultaneously, the global healthcare landscape is undergoing significant shifts, driven by growing concerns over the adverse effects of pharmaceutical drugs, questions about their long-term efficacy and safety, and the escalating cost of medical treatments. Chronic diseases that remain unresponsive to conventional therapies further intensify these concerns (Mancusi *et al.*, 2024). One of the most pressing challenges is the rise in antimicrobial resistance, which hampers the effectiveness of standard treatments for infectious diseases such as pneumonia, typhoid, and tuberculosis. Antibiotic resist-

ance stems from complex biological mechanisms, enabling microbes to develop resistance across entire classes of antibiotics due to shared resistance pathways. This phenomenon threatens the successful treatment of once-manageable bacterial infections and poses serious risks to human and animal health worldwide, affecting both medical and veterinary practices (Zafar *et al*., 2024).

Medicinal herbs offer significant therapeutic advantages and, in many cases, exhibit greater efficacy in treating various ailments than conventional antibiotics, while posing fewer adverse effects. Approximately 80% of individuals continue to rely on traditional therapies, utilizing botanicals such as cloves, ginger, garlic, turmeric, and other herbs for their health-promoting properties (Muhammad *et al*., 2024). Among these, clove (*Syzygium aromaticum*), a fragrant flower bud cultivated primarily in tropical and subtropical regions, has been extensively used in both traditional medicine and the fragrance industry. Studies report that clove essential oil (CEO) comprises approximately 15–20% of the plant's total weight and contains a high concentration of phenolic compounds, including eugenol, α-humulene, and β-caryophyllene. These bioactive components exhibit broad biological activities, including antibacterial, antifungal, insecticidal, and antioxidant effects (Ahmad and Ibrahim, 2024; Haro-González *et al*., 2021).

Cloves are versatile in their applications, being utilized in forms such as capsules, infusions, tinctures, extracts, powders, and essential oils. Despite sometimes being undervalued, their rich history in traditional medicine highlights their pharmacological potential (Ullah *et al*., 2023). Experimental studies have confirmed the inhibitory effect of clove essential oil on *Staphylococcus aureus*, suggesting that the oil interacts with bacterial cell walls and membranes, leading to cellular disruption, leakage of intracellular contents, and ultimately, bacterial death. The oil's ability to penetrate the cytoplasmic membrane interferes with vital processes such as DNA replication and protein synthesis, thereby halting bacterial growth (Xu *et al*., 2016).

One of the most concerning pathogens in modern medicine is Methicillin-resistant *Staphylococcus aureus* (MRSA), which has developed multiple defense mechanisms, including biofilm formation, to evade antibiotics and the host immune response. MRSA is responsible for a spectrum of infections, ranging from superficial skin conditions to life-threatening diseases such as endocarditis and osteoarticular infections. These infections contribute significantly to global morbidity and mortality. The ability of MRSA to produce biofilms is a key factor in its persistence and resistance to antibiotic treatment (Kaushik *et al*., 2024). The present study was therefore designed to investigate the antibacterial effects of clove extract against MRSA.

MATERIALS AND METHOD

Ethical Approval

Methicillin-resistant *Staphylococcus aureus* (MRSA) stock isolates were obtained and identified from previously collected clinical and subclinical cases. As no new samples were collected directly from human or animal subjects and all materials were acquired from existing sources, ethical approval was not required in accordance with the institutional guidelines of our university.

Methanolic Extraction of Clove

Clove samples were procured from a local marketplace in Najaf, Iraq. The extraction process was conducted in the Department of Physiology and Pharmacology at the Faculty of Veterinary Medicine. A total of 50 grams of dried clove buds were ground using a dry blender. The ground material was then soaked in 500 milliliters of 75% ethanol and stirred continuously with a magnetic stirrer for 24 hours at 45°C. Following extraction, the mixture was filtered using Whatman No. 2 filter paper to remove solid residues. The resulting filtrate was concentrated using a rotary evaporator at 150 rpm and 50°C for 60 to 90 minutes to obtain a semi-solid extract. The final clove extract was stored at -4°C until further use (Abood *et al*., 2021).

Isolation and Identification of MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were obtained from blood samples collected at the Faculty of Veterinary Medicine, University of Kufa. A total of nine blood samples were tested and confirmed as MRSA based on microbiological and biochemical criteria. Initial identification of MRSA colonies was based on characteristic morphological and cultural features, including the presence or absence of hemolysis on blood agar and the inability to grow on MacConkey agar. Biochemical confirmation involved catalase and coagulase tests, along with identification using the VITEK automated system. The biochemical profile also included the assessment of mannitol fermentation under anaerobic conditions to support the identification process.

Determination of MIC and MBC

A stock solution of methanolic clove extract was prepared and used to create a series of serial dilutions. Four concentrations were tested: 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. A total of nine bacterial isolates, previously identified as MRSA, were used in the assay. Each diluted extract was mixed with 1 mL of an actively growing bacterial culture containing approximately 10^7 colony-forming units per milliliter (CFU/mL). The mixing and preparation were conducted under sterile conditions in a Gusto Laminar Flow Cabinet II (Gusto, USA). Following preparation, the mixtures were incubated at 37°C for

24 hours. Post-incubation, bacterial cultures were plated on nutrient agar for colony enumeration. Colony counts were performed after a further 24-hour incubation period to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the clove extract. Interpretations were made as follows: the presence of a single colony indicated "sensitivity," more than ten colonies indicated "resistance," and an intermediate number of colonies was classified as "intermediate sensitivity" (Dadazadeh and Nourafcan, 2021).

Testing Antibiotic Sensitivity

Antibiotic susceptibility testing of MRSA isolates was carried out using the Kirby-Bauer disk diffusion method, as described by Bauer et al. (1966). The selection of antibiotic disks was based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). Bacterial suspension turbidity was adjusted to match the 0.5 McFarland standard, corresponding to approximately 10^7 colony-forming units per milliliter (CFU/mL), to ensure standardized inoculum density. Mueller-Hinton agar (HIMEDIA, India) was used as the culture medium. The following antibiotic disks were applied to the agar surface: chloramphenicol (C-30), amoxicillin (Amc-30), cefotaxime (CTX), ampicillin (Am), ceftriaxone (Cro), tetracycline (TE), gentamicin (CN), oxacillin (Ox), and amoxicillin (Ax-25). After placement of the antibiotic disks, the plates were incubated at 37°C for 18–24 hours. The diameter of the inhibition zones surrounding each disk was measured and interpreted according to the clinical and laboratory standards outlined by Patel (2015).

Table 1: Antibiotic profile of the studied MRSA bacteria.

Sample codes	Antibiotic disc with zone of inhibition (mm)								
	AM 25µg	OX 5µg	C 10µg	AMV 30µg	CN 10µg	AMX µg	CTX 30µg	CRO 30µg	TET 10µg
S1 p	R	R	R	R	R	R	R	R	R
S2	R	R	R	R	R	R	R	R	R
S3	R	R	R	R	R	R	R	R	R
S4	R	R	R	R	R	R	R	R	R
S5	R	R	R	R	R	R	S	S	R
S6	R	R	R	R	R	I	R	R	R
S7	R	R	R	R	R	R	I	S	R
S8	R	R	R	R	R	R	R	R	R
S9	R	R	R	R	R	R	R	R	R

AM: ampicillin; **OX:** oxacillin; **C:** chloramphenicol; **AMV:** Amoxicillin-clavulanic acid; **CN:** gentamicin; **AMX:** amoxicillin; **CTX:** cefotaxime; **CRO:** ceftriaxone; **TET:** tetracycline; **p:** pathogen; **R:** resistant; **S:** sensitive; **I:** intermediate.

Assay of Antibacterial Activities

Following the guidelines set by the National Committee for Clinical Laboratory Standards (Patel, 2015), the agar well diffusion method was employed to evaluate the antibacterial activity of both aqueous and solvent-based clove extracts. A standardized inoculum of 10^6 colony-forming units per milliliter (CFU/mL) for each bacterial strain under investigation was prepared. The bacterial suspensions were uniformly spread onto nutrient agar plates using a sterile swab dipped in the respective cultures. After inoculation, 8-mm diameter wells were created in the agar medium. Each well was filled with 100 µL of the prepared plant extract and left at room temperature for two hours to allow diffusion. Control wells containing an equivalent volume of sterile distilled water served as negative controls. The plates were then incubated at 37°C for 24 hours. Following incubation, zones of inhibition around each well were measured in millimeters to assess antibacterial activity. Each extract was tested in triplicate against each bacterial isolate to ensure reliability and reproducibility of the results.

RESULTS AND DISCUSSIONS

According to the World Health Organization (WHO), infectious diseases are a leading cause of global morbidity and mortality, accounting for nearly 50% of all deaths in tropical regions (World Health Organization, 2022). Medicinal plants have long played a pivotal role in traditional healthcare systems worldwide, both for the prevention and treatment of various illnesses (Picking, 2024).

The inhibitory effect of *S. aromaticum* (clove) extracts on MRSA is demonstrated in Table 1. The results of our study show that the ethanolic extracts of *S. aromaticum* exhibited a significant antibacterial effect against MRSA isolates. As shown in Table 3, the inhibition zone diameters observed at a concentration of 50 mg/mL ranged from 14 mm to 43 mm, indicating strong antibacterial activity and dose-dependent efficacy.

At lower concentrations (12.5 and 6.25 mg/mL), the clove extract showed no inhibitory effect on the isolated MRSA strains. All tested MRSA isolates exhibited resistance to the nine antibiotics assessed, with the exception of sample 5, which demonstrated sensitivity to cefotaxime and ceftriaxone. Additionally, sample 7 showed sensitivity to ceftriaxone and intermediate sensitivity to cefotaxime, as detailed in Table 2.

The findings of our study align with previous research, which has demonstrated that ethanolic plant extracts exhibit strong antibacterial activity against *S. aureus*. Specifically, Mostafa *et al.* (2018) reported that ethanol extracts of clove showed significant antimicrobial effects against *S. aureus*. Ethanol-based extracts of spices are known to contain a wide range of bioactive compounds, including esters

of weak acids, fatty acids, phenolic compounds, terpenes, and various derivatives. These chemical constituents have the ability to target multiple sites within bacterial cells, thereby enhancing their antimicrobial efficacy, as supported by the findings of Mostafa *et al.* (2018).

Table 2: Antimicrobial effect of different concentrations of clove extract on MRSA.

Sample code	Syzygium aromaticum extract concentrations			
	50 mg/ml	25 mg/ml	12.5mg/ml	6.25 mg/ml
S1 p	S	R	R	R
S2	S	I	R	R
S3	S	S	R	R
S4	S	R	R	R
S5	I	R	R	R
S6	S	I	R	R
S7	S	R	R	R
S8	S	S	R	R
S9	S	S	R	R

p: pathogen; **R:** resistant; **S:** sensitive; **I:** intermediate.

Syzygium aromaticum (clove) demonstrated notable antimicrobial activity against MRSA isolates. As shown in Table 2, the MIC values for the tested microorganisms ranged from 12.5 to 25 mg/mL. *S. aureus* was identified as the most susceptible strain, with a MIC of 25 mg/mL. The minimum bactericidal concentration (MBC) values varied between 25 mg/mL and 50 mg/mL, indicating concentration-dependent bactericidal activity.

Table 3: Diameter of the inhibition zones (in mm) produced by clove extract against MRSA isolates at different concentrations.

Sample ID	Syzygium aromaticum extract concentrations			
	50 mg/ml	25 mg/ml	12.5mg/ml	6.25 mg/ml
S1 p	35mm	20mm	R	R
S2	40mm	25mm	R	R
S3	42mm	30mm	R	R
S4	43mm	25mm	R	R
S5	30mm	R	R	R
S6	39mm	30mm	R	R
S7	41mm	14mm	R	R
S8	43mm	40mm	R	R
S9	37mm	38mm	R	R

R: resistant; **p:** pathogen.

These results are consistent with the MIC and MBC values reported by Chikowe *et al.* (2013) and Uddin *et al.* (2022). Furthermore, the methanolic extract of clove showed superior antimicrobial efficacy across all tested isolates, corroborating the findings of previous studies (Silva *et al.*, 2023). The effectiveness of clove extract increased with concentration, likely due to enhanced diffusion of active compounds through the bacterial cell membrane. This process leads to membrane disruption and ultimately cell lysis, as observed in earlier studies (Thangiah *et al.*, 2013; Pasaribu *et al.*, 2021).

CONCLUSIONS AND RECOMMENDATIONS

Susceptibility testing plays a crucial role in evaluating the antibacterial potential of plant-derived substances. The effectiveness of antimicrobial agents, including plant extracts, is influenced by both their concentration and the duration of exposure. In this study, *S. aromaticum* (clove) demonstrated significant antibacterial activity against all tested MRSA isolates. The MIC values ranged from 12.5 to 25 mg/mL, while the MBC values varied from 25 to 50 mg/mL. These findings highlight the potential of clove extract as a natural antibacterial agent, particularly in the context of rising antibiotic resistance. It is advisable to perform additional research involving laboratory animals to further elucidate the toxicological effects of *S. aromaticum*.

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NOVELTY STATEMENTS

The findings suggest that ethanolic extracts of S.aromaticum exhibit promising antibacterial activity against *S. aureus*, including MRSA strains. This is important in alternative medicine.

AUTHOR'S CONTRIBUTIONS

All listed authors have made significant intellectual and academic contributions to this study. Each author has reviewed and approved the final manuscript for publication.

Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Abood ZA, Al-Juraisy YH, Al-Halbosiy MM (2021). Antioxidant and Antibacterial Effects of Methanolic and Oil Extracts of Clove (*Syzygium aromaticum*) Buds. Biochem. Cell. Arch., 21(2).

Ahmad RM, Ibrahim OMS (2024). Anti-inflammatory

activity of clove (*Syzygium aromaticum*): oil extract against chronic inflammation in rat. Adv. Anim. Vet. Sci., 12(1): 77-84. https://doi.org/10.17582/journal.aavs/2024/12.1.77.84

Bauer, A.W., (1966) Antibiotic susceptibility testing by a standardized single isk method. American Journal of Clinical Pathology, 45, 493-496.

Chikowe G, Mpala L, Cock IE (2013). Antibacterial activity of selected australian syzygium species, Pharmacogn. Commun., 3(4).

Clinical and Laboratory Standards Institute (CLSI) (2018) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15.

Dadazadeh A, Nourafcan H (2021). Determination of clove (Eugenia caryophyllata), essential oil and comparison of its antibacterial properties with some conventional antibiotics. J. Food Sci. Technol., (Iran): 18(119): 47-59. https://doi.org/10.52547/fsct.18.119.47

Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H (2021). Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction chemical composition food applications, and essential bioactivity for human health. Molecules, 26(21): 6387. https://doi.org/10.3390/molecules26216387

Kaushik A, Kest H, Sood M, Steussy BW, Thieman C, Gupta S (2024). Biofilm Producing Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infections in Humans: Clinical Implications and Management. Pathogens, 13(1): 76. https://doi.org/10.3390/pathogens13010076

Mancusi A, Egidio M, Marrone R, Scotti L, Paludi D, Dini I, Proroga YTR (2024). The In Vitro Antibacterial Activity of Argirium SUNc, against Most Common Pathogenic, and Spoilage Food Bacteria. Antibiotics, 13(1): 109. https://doi.org/10.3390/antibiotics13010109

Manesh A, Varghese GM (2021). Rising antimicrobial resistance: an evolving epidemic in a pandemic. Lancet Microbe, 2(9): e419-e420. https://doi.org/10.1016/S2666-5247(21)00173-7

Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakri MM (2018). Antimicrobial activity, of some plant extracts against bacterial strains, causing food poisoning diseases. Saudi J. Biol. Sci., 25(2): 361-366. https://doi.org/10.1016/j.sjbs.2017.02.004

Muhammad N, Nisa I, Gilani SF, Jamal B, Kamal R, Afzal M, Khattak B (2024). Medicinal Plants-fabricated AgNPs, and Their Role as an Antibacterial, Agent against Drug-resistant Uro-pathogens. Nano Biomed. Eng., 16(1). https://doi.org/10.26599/NBE.2024.9290045

Pasaribu T, Sinurat AP, Wina E, Cahyaningsih T (2021). Evaluation of the phytochemical content, antimicrobial and antioxidant activity of Cocos nucifera liquid smoke, Garcinia, mangostana pericarp *Syzygium aromaticum* leaf, and Phyllanthus niruri L. extracts. Vet. World, 14(11): 3048 https://doi.org/10.14202/vetworld.2021.3048-3055.

Patel JB (2015). Performance standards, for antimicrobial susceptibility testing, twenty-fifth informational supplement, 35 (3): M100-S25

World Health Organization (2022). Global Health Estimates 2020, Deaths by Cause, Age, Sex, by Country and by region, 2000–2019. WHO. https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates

Picking D (2024). The global regulatory framework for medicinal plants. In Pharmacognosy, 769-782. https://doi.org/10.1016/B978-0-443-18657-8.00023-2

Puangpronpitag D, Niamsa N, Sittiwet C (2009). Anti-microbial properties of clove (Eugenia caryophyllum Bullock and Harrison) aqueous extract against food-borne pathogen bacteria. IJP-Int. J. Pharmacol., 5(4): 281-284. https://doi.org/10.3923/ijp.2009.281.284

Silva BN, Bonilla-Luque OM, Possas A, Ezzaky Y, Elmoslih A, Teixeira JA, Gonzales-Barron U (2023). Meta-analysis of in vitro antimicrobial capacity of extracts and essential oils of *Syzygium aromaticum*, Citrus L. and Origanum L.: contrasting the results of different antimicrobial susceptibility methods. Foods, 12(6): 1265. https://doi.org/10.3390/foods12061265

Thangiah AS, Obey J, Mutuku NC (2013). Phytochemical analysis of Vernonia adoensis leaves and roots used as a traditional medicinal plant in Kenya. Int. J. Pharm. Biol., 3(3):46–52.

Uddin AN, Hossain F, Reza AA, Nasrin MS, Alam AK (2022). Traditional uses, pharmacological activities, and phytochemical constituents of the genus *Syzygium*: A review. Food Sci. Nutr., 10(6): 1789-1819. https://doi.org/10.1002/fsn3.2797

Ullah MA, Hassan A, Hamza A (2023). Role of Clove, in Human Medical History. SAR J. Anat. Physiol., 4(2): 10-19. https://doi.org/10.36346/sarjap.2023.v04i02.001

Xu JG, Liu T, Hu QP, Cao XM (2016). Chemical composition, antibacterial properties, and mechanism of action of essential oil, from clove buds against *Staphylococcus aureus*. Molecules, 21(9): 1194. https://doi.org/10.3390/molecules21091194

Zafar H, Kamboh AA, Zain-ul-Abideen TM, Ahmad S, Zafar U (2024). Antimicrobial resistance profiling of E. coli isolated from various sources of broiler farms in district quetta, Balochistan. J. Anim. Health Prod., 12(1): 24-30. https://doi.org/10.17582/journal.jahp/2024/12.1.24.30