

Antibacterial Activity of Sesamol against Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus*: An in Vitro Comparative Study

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ABSTRACT

Objective: To evaluate the in vitro antibacterial activity of sesamol against Methicillin-Sensitive *Staphylococcus aureus* (MSSA) and Methicillin-Resistant *Staphylococcus aureus* (MRSA), through the broth microdilution method.

Study Design and Setting: In vitro experimental study, conducted at the Microbiology Laboratory, Ziauddin University Hospital (North Nazimabad Campus), Multi-Disciplinary Research Lab, Ziauddin University Clifton Campus, Karachi, from 1st September 2025 to 28th December 2025.

Methodology: Sesamol (purity = 98%) was dissolved in 50% DMSO to obtain a 200 mg/mL stock solution. The antibacterial activity was evaluated using the Broth Microdilution Assay with several two-fold serial dilutions of sesamol against both drug-sensitive and resistant *Staphylococcus aureus* clinical isolates. The difference in MIC between the two groups was statistically processed by a Mann-Whitney U test.

Results: Sesamol showed concentration-dependent antibacterial inhibitory actions against all the MSSA and MRSA strains tested. The Minimum Inhibitory Concentration (MIC) of sesamol for MSSA and MRSA was 1.5 mg/mL and 2.0 mg/mL, respectively. The difference in MIC between the two groups was found to be statistically non-significant ($p=0.41$) between the two groups.

Conclusion: Sesamol showed concentration-dependent antibacterial activity, though the difference between MSSA and MRSA was not statistically significant ($p=0.41$). Its MIC against MSSA and MRSA indicates possible antimicrobial activity against these pathogens and warrants further investigation. These results suggest that sesamol can be a potential natural antibacterial agent that may proceed for further pre-clinical studies.

Keywords: Broth Microdilution, MIC, MRSA, MSSA, Sesamol, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the highly prevalent and clinically relevant gram-positive pathogens causing a broad range of superficial skin infections to severe life-threatening conditions such as bacteremia, endocarditis, pneumonia, osteomyelitis, and septic arthritis.¹ As part of the ESKAPE pathogen group (*Enterococcus faecium*,

Staphylococcus aureus, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*), it is considered one of the most challenging drug-resistant pathogens across the globe.² Particularly, the emergence of Methicillin-Resistant *S. aureus* (MRSA) has significantly complicated the treatment of staphylococcal infections, since these strains are resistant to multiple antibiotic classes, including beta-lactam antibiotics due to the *mecA* gene encoding the mutant penicillin-binding protein PBP2a.³

The global burden of MRSA is staggering, with 64% more deaths than its non-resistant counterparts.⁴ Further, in a 5-year longitudinal study from Macau (2017-2022), it was shown that MRSA prevalence rose from 30.1% to 45.7% - a rising tide that speaks volumes about poor infection control and inappropriate antibiotic use.⁵ In Pakistan also, *Staphylococcus aureus* remains a significant threat to public health owing to its high incidence rate and considerable involvement in cases of both community- and healthcare-acquired infections. A multi-center study from Karachi found that among 421 biochemically confirmed *S. aureus* strains, 89% were confirmed to be MRSA.⁶ Moreover, studies from major tertiary care hospitals in Karachi have

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reported MRSA colonization rates of 10.9-11.7% at the time of patient admission.⁷

Today, the main therapeutic arsenal against MRSA infections is limited to vancomycin, daptomycin, linezolid, and ceftaroline, which are associated with considerable drawbacks such as a parenteral route of administration, a significant adverse effect profile including nephrotoxicity, thrombocytopenia, high costs, and increasing resistance.⁸ Development of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) has further compromised the first-line therapies, demanding an urgent need for new antimicrobials with unique mechanisms of action.⁹ Therefore, the World Health Organization (WHO) has listed MRSA as a high-priority pathogen for new antibiotic development.³

In this grave situation, plants-derived phenolic compounds have attracted significant attention as adjunct or alternative antibacterial agents due to their broad-spectrum biological actions and relatively better safety profiles. Sesamol (3,4-methylenedioxyphenol), a phenolic lignan extracted from sesame seeds (*Sesamum indicum*), has been widely reported for its strong antioxidant activity mediated mainly by its free radical scavenging capabilities and influence on oxidative stress pathways, contributing to its cytoprotective effects. Besides, it has shown potent anti-inflammatory activity by targeting mediators and signaling cascades of inflammatory processes. It acts as an anticancer agent due to its proapoptotic effect and antiproliferative properties. Overall, the above-mentioned activities of sesamol make it a potential bioactive compound that warrants further investigations to develop possible clinical applications, including as an alternative antimicrobial drug in the current study.¹⁰ Recent studies have been conducted to unravel its antimicrobial activity, where sesamol has been demonstrated to have activity against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, with proposed mechanisms of action including disruption of the cell membrane of microorganisms, leakage of cellular contents, and interference with key cellular enzymes.¹¹

Although sesamol has demonstrated good antimicrobial efficacy against various pathogens, studies evaluating its antibacterial activity specifically in clinically established methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) using quantitative methods are limited. Most previous reports have focused on sesame oil as opposed to pure sesamol and have failed to distinguish between methicillin-resistant and methicillin-susceptible strains. In an attempt to bridge this gap, the current study aimed to assess the in vitro antibacterial effect of purified sesamol on clinical isolates of MSSA and MRSA using the Broth Microdilution Assay and to compare minimum inhibitory concentration (MIC) values between the two groups.

METHODOLOGY

This was an in-vitro experimental study carried out at the Clinical Microbiology Laboratory, Ziauddin University Hospital, North Nazimabad Campus, and Microbial Genomics Laboratories (MDRL1 and MDRL2), Ziauddin University, Clifton Campus, Karachi, from 1st September 2025 to 28th December 2025. This study was approved by the Ethical Review Committee (9860225SSPHA). M07, 11th Edition (2018) guidelines for antibacterial susceptibility testing were followed.¹²

Eight Culture and sensitivity proven MSSA and MRSA isolates were collected from the Microbiology Laboratory, Ziauddin University Hospital, North Nazimabad Campus by consecutive sampling. The MSSA isolates 1, 2 and 3 were obtained from blood, pus, urine samples respectively, with each isolate obtained from a different patient. While the MRSA-1 and 2 isolates were cultured from blood, MRSA-3 and 4 from pus and MRSA-5 from urine of the patients admitted in the Intensive Care Unit (ICU). Briefly, *Staphylococcus aureus* was identified by Gram staining (gram-positive cocci in clusters), growth on Mannitol Salt Agar (yellow colonies due to mannitol fermentation), coagulase test (positive by slide and tube method), and catalase test (bubbles in H₂O₂).¹³ The Oxacillin disc diffusion method, according to the CLSI guidelines (inhibition zone = 17 mm, MRSA) was used to confirm the presence of MRSA. Further confirmation of methicillin resistance was performed by growth on Chromogenic MRSA Agar (Oxoid, UK). MSSA was confirmed by sensitivity to Oxacillin (inhibition zone = 18 mm). Isolates showing mixed or contaminated growth were not included.¹⁴ Sesamol (purity =98%) was obtained from Macklin Biochemical Technology Co. and was dissolved in 50% Dimethyl Sulfoxide (DMSO) to make a stock solution of 200 mg/mL. Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) media were obtained from Oxoid, UK. Two-fold dilutions of sesamol were made in MHB to provide final concentrations of 0.5, 1.0, 1.5, 2.0, 4.0, and 6.0 mg/mL. All test wells had a final DMSO concentration of =1% (v/v) to rule out solvent-related antibacterial activity, which was confirmed by a DMSO control well.¹⁵

The antibacterial activity of sesamol was assessed by the standard Broth Microdilution method using M07, 11th Edition guidelines (2018).¹² Overnight cultures of MSSA and MRSA in MHB were diluted to the turbidity of 0.5 McFarland standard (1.5×10^8 CFU/mL). The inoculum was then diluted 1:150 in MHB to prepare the final working inoculum (approximately 5×10^5 CFU/mL per well). Along with bacterial inoculum and MHB, sesamol /DMSO were added to the tubes and were labelled as: (1) solvent control (50% DMSO + MHB + bacterial inoculum), (2) blank control (only sterilized MHB), (3) bacterial control (bacterial inoculum + MHB) (4) treatment control (various dilution of sesamol + MHB + bacterial inoculum) The final volume

in each well was 200 μ L.¹¹ All the tubes were incubated at 37°C for 18-24 hours at 120 rpm. After 24 hours, the lawning of each tube was done on agar plate with a sterile swab. The plates were again incubated at 37 °C for 24 h and observed for bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of sesamol that completely inhibited visible bacterial growth (absence of turbidity) in the broth after incubation. Subculturing from wells showing no visible growth was performed to confirm the MIC endpoint. Each clinical isolate was tested independently, and all sesamol concentrations in the broth microdilution assay were evaluated in three parallel wells (technical triplicates). MIC values were determined based on the consistent results obtained across these triplicate measurements.¹⁵ The mean $\pm$ standard deviation of the MIC values was calculated. The Mann–Whitney U test (non-parametric) was applied to compare the MIC between MSSA and MRSA. A p-value of <0.05 was taken as statistically significant. SPSS version 24.0 was used for statistical analyses.

RESULTS

Eight bacterial isolates (3 MSSA and 5 MRSA) were identified based on standard microbiological testing and CLSI-approved phenotypic tests. The isolates were identified as Gram-positive cocci, in clusters, coagulase positive, catalase positive, and fermented mannitol on MSA. MRSA isolates had an oxacillin inhibition zone of =17 mm and growth on chromogenic MRSA agar, while all MSSA isolates had an oxacillin zone of =18 mm and no growth on chromogenic MRSA agar.¹⁴ Sesamol showed dose-dependent antibacterial activity against all 8 tested MSSA and MRSA isolates, with largely comparable MIC values between the

two groups. The MIC of sesamol against all 3 MSSA isolates was 1.5 mg/mL (Figure 1f). The MIC for MRSA isolates was 2.0 mg/mL (Figure 2i). The broth blank control demonstrated no turbidity, indicating quality control (Figures 1c and 2b), while DMSO solvent showed turbidity and bacterial growth, confirming the absence of solvent-mediated antibacterial activity (Figures 1c and 2d). Bacterial growth control showed turbidity in all replicates, indicating viability and the integrity of the assay (Figures 1c and 2d).

The MIC value of sesamol against MSSA isolates was 1.5 mg/mL. Among MRSA isolates, four isolates demonstrated MIC values of 1.5 mg/mL, while the fifth isolate showed an MIC of 2.0 mg/mL. The mean MIC for MRSA isolates (1.6 ± 0.22 mg/mL) was slightly higher than that of MSSA isolates (1.5 ± 0.0 mg/mL), however comparative analysis using the Mann–Whitney U test revealed no statistically significant difference between MSSA and MRSA isolates ($p = 0.41$). Table 1 shows the MIC of all isolates. Table 2 presents the bacterial growth scores at each tested sesamol concentration across both organism groups. A clear concentration-dependent inhibitory effect was observed. In MSSA isolates, heavy growth (+++) was observed at 0.5 mg/mL, reduced growth (++) at 1.0 mg/mL, and complete inhibition (-) from 1.5 mg/mL onwards. In MRSA isolates, heavy growth (+++) persisted at 0.5 and 1.0 mg/mL, moderate growth (++) at 1.5 mg/mL, and complete inhibition (-) at 2.0 mg/mL and above (Figures 1d-1g and 2e-2j). Figures 1 and 2 display the photographic documentation of MHA subculture plates from MIC for MSSA and MRSA, respectively. Dense bacterial colonies are visible on plates sub cultured from concentrations below the MIC.

Table 1: Minimum Inhibitory Concentration (MIC) of Sesamol Against Individual MSSA and MRSA Isolates

Isolate ID	Isolate Type	MIC Replicate 1 (mg/mL)	MIC Replicate 2 (mg/mL)	MIC Replicate 3 (mg/mL)	Final MIC (mg/mL)
MSSA-1	MSSA	1.5	1.5	1.5	1.5
MSSA-2	MSSA	1.5	1.5	1.5	1.5
MSSA-3	MSSA	1.5	1.5	1.5	1.5
MRSA-1	MRSA	1.5	1.5	1.5	1.5
MRSA-2	MRSA	1.5	1.5	1.5	1.5
MRSA-3	MRSA	2.0	2.0	2.0	2.0
MRSA-4	MRSA	1.5	1.5	1.5	1.5
MRSA-5	MRSA	1.5	1.5	1.5	1.5
Mean $\pm$ SD	—	MSSA: 1.5 ± 0.0	MRSA: 1.6 ± 0.22		$p = 0.41$

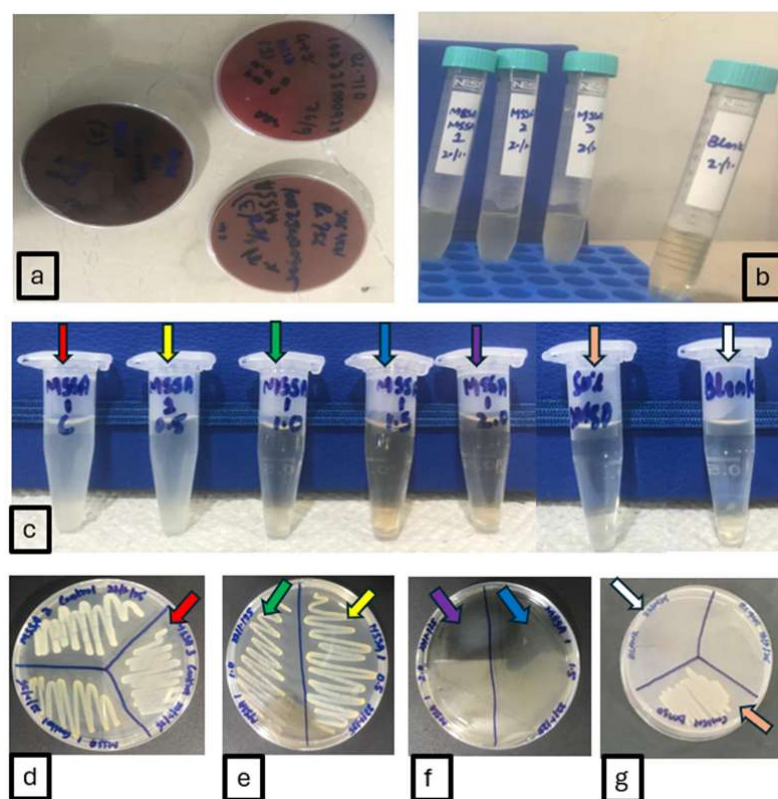
*Statistically non-significant (Mann–Whitney U test, $p > 0.05$). MSSA: Methicillin-Sensitive *S. aureus*; MRSA: Methicillin-Resistant *S. aureus*; MIC: Minimum Inhibitory Concentration; SD: Standard Deviation

Table 2: Bacterial Growth Score at Serial Sesamol Concentrations (Broth Microdilution, Triplicates)

Isolate Group	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL
MSSA (n=3)	+++	++	- (MIC)	-
MRSA (n=5)	+++	+++	+	- (MIC)

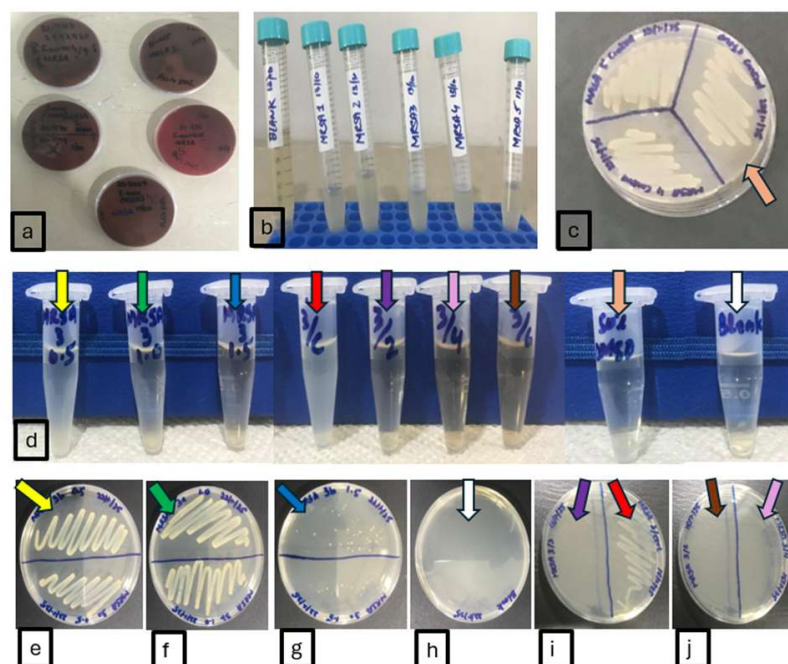
+++ : Heavy growth; ++ : Moderate growth; + : Sparse growth; - : No visible growth (inhibited). MIC = Minimum Inhibitory Concentration. Consistent results across all three replicates.

Figure 1: Steps of MIC determination against MSSA isolates.



(a) Culture and sensitivity proven clinical MSSA isolates (b) freshly inoculated bacterial samples in MHB (c) Working micro-dilutions of overnight grown MSSA in Eppendorfs with; 0.5 mg/ mL sesamol concentration (arrow yellow), 1.0 mg/ mL sesamol concentration (arrow green), 1.5 mg/ mL sesamol concentration (arrow blue), 2.0 mg/ mL sesamol concentration (arrow purple), sterile broth for quality assurance (arrow white), 50%DMSO control (arrow orange), MSSA control (arrow red), (d)lawning of MSSA after 24 hrs incubation for MIC determination; MSSA control (arrow red) (e) with 0.5mg/mL sesamol concentration (arrow yellow), 1.0mg/mL sesamol concentration (arrow green) (f) 1.5mg/mL sesamol concentration (arrow blue) and complete growth inhibition at 2.0mg/mL sesamol concentration (arrow purple) (g) sterile broth (arrow white), 50%DMSO control (arrow orange).

Figure 2: Steps of MIC determination against MRSA isolates.



(a) Culture and sensitivity proven clinical MRSA isolates (b) fresh inoculum containing overnight cultured bacterial samples in MHB (d) Working micro-dilutions of overnight grown MRSA in Eppendorfs; sterile broth for quality assurance (arrow white), 50%DMSO control (arrow orange), MRSA control (arrow red), with 0.5 mg/ mL sesamol concentration (arrow yellow), 1.0 mg/ mL sesamol concentration (arrow green), 1.5 mg/ mL sesamol concentration (arrow blue), 2.0 mg/ mL sesamol concentration (arrow purple), 4.0 mg/ mL sesamol concentration (arrow pink), 6.0 mg/ mL sesamol concentration (arrow brown) (e) lawning of MRSA after 24 hrs incubation for MIC determination; at 0.5mg/mL sesamol concentration (arrow yellow) (f) at 1.0mg/mL sesamol concentration (arrow green) (g) at 1.5mg/mL sesamol concentration (arrow blue) (h) sterile broth (arrow white) (i) MRSA control (arrow red), 2.0mg/mL sesamol concentration (arrow purple) (j) at 4.0mg/mL sesamol concentration (arrow pink), 6.0mg/mL sesamol concentration (arrow brown) (k) 50%DMSO control (arrow orange)

DISCUSSION

The present work aimed to investigate the in vitro antibacterial activity of sesamol, a hydroxylated phenolic derived from *Sesamum indicum*, against clinically isolated MSSA and MRSA samples via broth microdilution assay. Our results suggest that sesamol has a concentration-dependent inhibitory effect on both methicillin-sensitive and resistant strains, with MICs at 1.5 mg/mL and 2.0 mg/mL for MSSA and MRSA, respectively, supporting its antimicrobial efficacy.^{15,16} In contrast to MRSA isolates, MIC determination was performed on only three MSSA isolates because of the limited availability of MSSA clinical samples, attributable to the high prevalence of MRSA in the study setting. This predominance of MRSA highlights the growing burden of antimicrobial resistance and represents an important public health concern. Further, Mahendra et al in 2015 have previously demonstrated the antibacterial activity of sesamol against MSSA, which was found to be 2mg/ml. Despite the effectiveness of sesamol towards MSSA strains, it should be noted that no scientific research on pure sesamol against MRSA strains has yet been conducted, which was the rationale of our research. Interestingly, in our study, the statistical difference between the two groups was non-significant ($p=0.41$), confirming that sesamol has demonstrated antibacterial inhibitory activity against both MSSA and MRSA isolates. Therefore, this scientific comparative data offered a basis to consider sesamol as a possible antimicrobial compound effective against *Staphylococcus aureus* species. The sesamol antibacterial activity in the current study agrees well with recent studies that have demonstrated notable antibacterial activity against several bacterial species, including methicillin-sensitive *Staphylococcus aureus*. The reported minimum inhibitory concentration (MIC) of sesamol against MSSA was 2 mg/mL, while a similar MIC was observed for *Bacillus cereus*, which is also a gram-positive pathogen. Interestingly, in *Pseudomonas aeruginosa*, which is a gram-negative pathogen, sesamol exhibited somewhat parallel antibacterial inhibitory activity, at around >2mg/mL. These findings support the broad-spectrum antimicrobial potential of sesamol, indicating its anticipated antibacterial potential against both Gram-positive and Gram-negative pathogens.¹⁶ Additionally, the findings from the current study concur with those by Li et al. (2024), where the authors showed that purified sesamol displayed broad-spectrum antibacterial properties against some important pathogenic organisms, including foodborne bacteria such as *Listeria monocytogenes* and *Escherichia coli*, as well as *Staphylococcus aureus* with minimum inhibitory concentrations (MICs) recorded as 1.5 to 2.0 mg/mL through broth microdilution assays. The lower MIC levels demonstrate the high level of antibacterial ability inherent in purified sesamol, especially concerning gram-positive bacteria.¹⁵ Moreover, according to Yan et al. (2024), sesamol demonstrated an antibacterial

effect on two *Listeria monocytogenes* strains with a minimum inhibitory concentration (MIC) value of 2.0 mg/mL for both isolated strains, which aligns with the previously established range of MIC values of purified sesamol.¹⁷ These results are consistent with those of Li et al. (2024), where similar MIC values were obtained for *Listeria monocytogenes* isolates (1.5–2.0 mg/mL).¹⁵

The antibacterial action of sesamol is multi-layered and synergistic, interfering with many physiological processes of the pathogen. Its phenolic structure and the presence of a methylenedioxy moiety in the chemical structure of sesamol (3,4-methylenedioxyphenol), account for its antimicrobial action, which causes disintegration of the bacterial cell envelope, damage to the peptidoglycan layer, and the disorganization of the phospholipid bilayer present in the cytoplasmic membrane. Further, it has been reported that membrane disintegration results in the leakage of important bacterial intracellular constituents such as potassium ions, ATP, nucleic acids, and proteins. Therefore, loss of cellular balance occurs, leading to bacterial cell killing. Moreover, the increased membrane permeability can promote intracellular accumulation of sesamol, leading to damage of cytosolic structures. This mechanism has also been confirmed by Nair et al., describing that the biological activity of sesamol is related to the phenolic structure of the substance, which helps it interact with various essential microbial cell structures and disrupt their functions.¹⁰ In addition, it has been found that sesamol produces reactive oxygen species (ROS) in bacterial cells, which induces oxidative damage to lipids, proteins, and DNA.¹⁵ Moreover, sesamol has also been reported to have antibiofilm activities and inhibit quorum sensing in many microbes, which is one of the most common and robust mechanisms of drug resistance and bacterial survival in stressful conditions.¹⁸

All these mechanisms collectively produce deleterious effects in microorganisms. The slightly higher MIC seen for MRSA (2.0 mg/mL) versus MSSA (1.5 mg/mL) provides a clue to complex resistance mechanisms in MRSA strains. That is partly due to the presence of the *mecA* gene, which has a much stronger biofilm-forming potential.² In addition, MRSA strains have also overexpressed *MepA* and *NorA* efflux pumps of the Major Facilitator Superfamily (MFS), and Multidrug and Toxic Compound Extrusion (MATE) families, respectively,^{1,2} and an altered cell membrane phospholipid profile, among others, that together contribute to reducing the intracellular concentration and eventually efficacy of antimicrobial agents.¹⁹ Since the Sesamol MIC of 2.0 mg/mL against MRSA is statistically equivalent to MSSA, it is suggested that the resistance mechanism does not abolish the sesamol cell-damaging properties as observed with contemporary β -lactam antibiotics, in which bacterial resistance can be achieved mainly due to the mutation in PBP2a encoded by the *mecA* gene, resulting in decreased interaction of bacterial transpeptidase with the β -lactam ring,

thereby reducing their efficacy. Sesamol, on the other hand, acts via a membrane- integrating mechanism rather than through receptor-mediated processes, thus escaping this specific resistance tactic. The above hypothesis is consistent with data presented by Yang et al. (2024), according to whom membrane-targeting drugs such as isoxanthohumol-amine conjugates possess strong anti-MRSA properties owing to membrane disruption and loss of membrane potential with consequent leakage from the cells.²⁰ Overall, the comparable MIC values for both MSSA and MRSA in the current experiment indicate that sesamol has the potential to combat intractable pathogens that are refractory to conventional agents, either independently or as an adjuvant to conventional agents which is further supported by recent combination studies demonstrating a marked reduction in effective inhibitory concentrations when used alongside conventional or natural antimicrobial agents. Ji et al. reported that sesamol significantly enhanced the activity of colistin against *Pseudomonas aeruginosa*, where the MIC of colistin alone was substantially reduced in the presence of sesamol. In their study, colistin MIC values decreased 50-66% from the conventional inhibitory range of 8 µg/mL when combined with sesamol, reflecting a several-fold increase in bacterial susceptibility. This synergistic effect was attributed to sesamol-induced disruption of bacterial membrane integrity, increased permeability, and inhibition of quorum sensing, which collectively facilitated enhanced intracellular penetration of colistin and potentiated its bactericidal action.¹⁸ Similarly, Wu et al. demonstrated a strong synergistic interaction between sesamol and nisin, a naturally occurring lantibiotic against *Listeria monocytogenes*. In this study, the MIC of nisin was significantly reduced from 50–100 µg/mL to 6.25-25.00 µg/mL when combined with sesamol.²¹ The enhanced antibacterial effect in this study was attributed to greater membrane damage, intense leakage of intracellular components, and enhanced disruption of cellular morphology, indicative of synergism between the two compounds. Collectively, these findings support the concept that sesamol can function as an antimicrobial potentiator by lowering the effective MIC of established antimicrobial agents. Therefore, sesamol can be expedited as an adjuvant compound in combination therapy against multidrug-resistant *Staphylococcus aureus* infections.²¹

From a translational standpoint, sesamol represents many pharmacological benefits as an antibacterial compound. As mentioned earlier, it is a natural phenolic derivative of sesame, which is a commonly farmed and consumed food plant, and hence possesses food-grade and human safety attributes. Toxicological investigations in preclinical models have indicated that sesamol is GHS class 4 (LD50 300–2000 mg/kg body weight in rodents), and no toxic effects were reported at 300 mg/kg in-vivo studies^{5,19} Moreover, its physicochemical characteristics, such as superior aqueous solubility (38.8 mg/mL at 37°C) with moderate lipophilicity

(log P 1.29–1.37), facilitate good oral bioavailability and drug likeness, making it a possible pharmacologic scaffold for future drug development. Further investigations, including pharmacokinetic, pharmacodynamic, and cytotoxicity studies, are required to determine whether the in vitro antibacterial effects of sesamol can be attained safely in clinical settings.

Apart from this active phenolic compound, the whole herb of *Sesamum indicum* has demonstrated inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*, producing 8 mm of zone of inhibition against both organisms. Comparable activity was also observed with *Sesamum radiatum*, which also showed similar ZOI against both bacterial strains.²² Moreover, the extract of sesame seed also demonstrated antimicrobial activity across other pathogenic bacterial strains viz, *Bacillus subtilis*, *Pseudomonas species*, *Streptococcus pyogenes*, *Proteus vulgaris*, and *Klebsiella pneumoniae*. Interestingly, methanol-derived extract shows more promising antimicrobial activity than ethanolic extract, probably due to its superior efficacy for solubilizing lipid-soluble bioactive constituents, such as phenols and flavonoids, which are known to perturb bacterial cell walls and interfere with essential metabolic processes.²³ Yenn et al. (2023) in their study, demonstrated sesame oil also possess antibacterial properties and acts against both MSSA and MRSA isolates with the MICs of 3.1-12.5 mg/mL and zones of inhibition between 12.5-13.5 mm.²⁴ This is also in accordance with our study that shows that sesamol acts against both MSSA and MRSA isolates but in significantly smaller concentrations because of its purified form. These findings suggest that the whole genus of *Sesamum* and its derivatives, including sesamol, possess meaningful antimicrobial potential against both Gram-negative and Gram-positive bacteria.²⁴

The results of this study are of utmost importance, especially when the healthcare system of Pakistan is considered, where MRSA is highly prevalent, antibiotic stewardship is not satisfactory, and patient compliance towards expensive and parenteral antibiotics like ancomycin and daptomycin is limited.⁵ Although it requires relatively high concentrations to inhibit bacterial growth, than conventional anti-staphylococcal antibiotics, as a natural product with considerable activity against MRSA, a known safety and a cost-effective profile could be of considerable clinical relevance, either as a single agent or in combination with antibiotics. Future investigations include the exploration of the its potential synergistic effect with vancomycin and other anti-staphylococcal antibiotics, its anti-biofilm activity at sub-MIC concentrations, time-kill assays, and in vivo confirmation in murine infection models. In recent years, the design of novel hybrid compounds incorporating multiple pharmacologically active moieties has gained significant attention as an effective synthetic approach for producing agents with improved biological activity.²⁵ The development of hybrids containing two or more pharmacologically active groups has become an interesting avenue in medicinal

chemistry, as far as improving biological activity and overcoming limitations of individual drugs. As mentioned by Bachvarova et al., the recently developed hybrid compounds containing both sesamol and biologically active benzazole cores exemplify the rising trend in employing sesamol as an efficient pharmacophore for the design of multicomponent drug entities.²⁵ Taking into account the growing resistance against antibiotics, the use of sesamol-containing antibacterials seems to be a rational future direction in designing more effective drugs against *Staphylococcus aureus*. The formulation of the delivery systems (such as nano-emulsions, polymer-lipid nanoparticles) may optimally enhance the oral bioavailability of sesamol, further improving the antibacterial effect at the site of the infection.²⁶

Limitation: The present study has some limitations because of restricted time and financial resources, which warrant consideration. Firstly, since it was an exploratory study, the isolates were selected based on predefined inclusion criteria and their availability during the study period, with no formal sample size calculation. Secondly, the relatively small number of clinical isolates, limiting statistical power and validity of results, warrants further investigation with a large dataset. Third, MRSA identification was based on routine phenotypic microbiological methods, rather than molecular confirmation (e.g., detection of the *mecA* or *mecC* genes). Fourth, since this study was designed to evaluate and compare the antibacterial activity of sesamol between MSSA and MRSA, a standard antibacterial comparative agent, such as vancomycin or linezolid, having well-defined MIC ranges was not included. Fifth, the study aimed to evaluate the in vitro antibacterial inhibitory activity of sesamol and did not include cytotoxicity assessment, pharmacokinetic evaluation, or in vivo studies, prerequisite to determine the safety and therapeutic applicability of the obtained concentrations. Finally, since minimum bactericidal concentration (MBC) testing and time-kill kinetic studies were not performed, based on the current findings alone, sesamol cannot be classified as a bacteriostatic or bactericidal agent. Future detailed mechanistic studies addressing these limitations will provide a more comprehensive evaluation of the antimicrobial potential of sesamol.

CONCLUSION

In the present experimental study, sesamol exhibited concentration-dependent antibacterial activity for both MSSA and MRSA clinical isolates. The MIC for MSSA was found to be 1.5 mg/mL, and for MRSA was 2.0 mg/mL, which are statistically non-significant, rendering sesamol a potential antibacterial agent against drug-resistant *S. aureus*, thereby providing a logical basis to extend the investigation to pre-clinical studies, including detailed mechanistic, synergism with standard antibiotics, and in-vivo validation using animal models of infection.

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Authors Contribution:

Sabra Siraj: Conception and design, data collection, interpretation of data, and manuscript drafting.

Kauser Ismail: Supervised the study, critically revised the manuscript, and approve final version

Ambrina Khatoon: Conducted the molecular methodology and data analysis

Hafiz Syed Muhammad Osama Jafri: Assisted in manuscript writing and data compilation.

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