Synergistic Antibacterial and Antibiofilm Activities of 3- and 6-Shogaols Against *Staphylococcus aureus*

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ABSTRACT

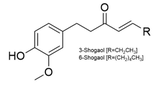
Background: *Staphylococcus aureus* poses a great clinical challenge due to its multidrug resistance and biofilm-forming ability. These traits greatly limit treatment efficacy and contribute to persistent infections. Hence, it is important to find new therapies to combat this bacterium. This study evaluated the antibacterial and antibiofilm activities of 3- and 6-shogaol against *Staphylococcus aureus*.

Method: 3- and 6-shogaol were synthesized via a modified literature method and characterized using FTIR and NMR spectroscopy. Minimum inhibitory concentrations against *Staphylococcus aureus* ATCC 43300 were determined, and combination effects with methicillin, oxacillin, amoxicillin, and gentamicin were assessed via checkerboard assay. Biofilm inhibition was evaluated against *Staphylococcus aureus* ATCC 35556 (strong biofilm producer) and ATCC 43300 (moderate biofilm producer) using the crystal violet assay.

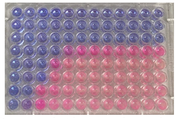
Results: The minimum inhibitory concentrations were 64 µg/mL for 3-shogaol and 1024 µg/mL for 6-shogaol, and 16, 64, 32 and 256 µg/mL for methicillin, oxacillin, amoxicillin, and gentamicin, respectively. Combination therapy revealed synergistic effects with all tested antibiotics, reducing the antibiotic concentration required for inhibition. Both shogaols significantly inhibited biofilm formation by ATCC 35556 ($p<0.05$), with 3-shogaol showing greater activity than 6-shogaol. No measurable biofilm inhibition was observed for ATCC 43300 due to its moderate biofilm production.

Conclusion: 3- and 6-shogaol exhibited potent antibacterial activity and significant biofilm inhibition against a strong biofilm-producing *Staphylococcus aureus* strain. Their synergistic interactions with antibiotics indicate potential to reduce antibiotic dosage requirements and slow the emergence of resistance. These results support the potential of shogaols as promising candidates for the treatment of *Staphylococcus aureus* infections.

METHODOLOGY



Synthesise of 3- and 6-shogaol using DIMCARB-mediated Mannich-type condensation

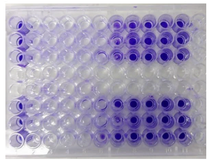


Synergistic study using checkerboard assay

Statistical analysis using ONE-WAY ANOVA & Dunnett's post hoc test

Characterisation of shogaols using NMR & FTIR

Biofilm Inhibition assay using crystal violet microtiter plate



CONCLUSION

In conclusion, the synergistic relationship between 3- and 6-shogaol and the antibiotics brings forth significant benefits towards the medical field as lower concentrations of antibiotics are required in the treatment of bacterial infections. Furthermore, there are fewer risks of the development of antibiotic resistance since it is a new drug combination. Additionally, both 3- and 6-shogaol also demonstrates strong biofilm inhibition even without the combination with antibiotics. In the future, the synergistic effects of 3- and 6-shogaol and antibiotics against biofilm formation can also be evaluated.

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INTRODUCTION

Antimicrobial resistance (AMR) is a major global health concern, projected to cause 10 million deaths annually by 2050. The widespread misuse of antibiotics has accelerated the rise of multidrug-resistant (MDR) pathogens, resulting in difficult-to-treat infections, prolonged illness, and higher mortality. *Staphylococcus aureus*, particularly methicillin-resistant *S.aureus* (MRSA), is a leading MDR pathogen responsible for both hospital- and community-acquired infections, ranging from mild skin conditions to life-threatening bacteraemia. Its resistance to β-lactams and ability to form biofilms severely limit treatment options and contribute to persistent infections.

Natural products remain a valuable source of new therapeutics. Ginger (*Zingiber officinale Roscoe*), known for its anti-inflammatory and antimicrobial properties, contains bioactive compounds such as gingerols, shogaols, and paradols. Among these, shogaols exhibit notable antimicrobial activity, highlighting their potential as candidates to combat resistant pathogens like MRSA.

RESULTS & DISCUSSION

Yields of 3- & 6-Shogaols

3-Shogaol

6-Shogaol

The yields obtained were 6.98% and 23.68% for 3- and 6-shogaol, respectively. The structures of 3- and 6-shogaol were confirmed with both 1H-NMR and 13C-NMR and FTIR.

Checkerboard Assay

3-Shogaol

Bacterium	MIC (µg/mL)					FICI			
	3S	GMX	AMX	MET	OXA	3S/GM	3S/AMX	3S/MET	3S/OXA
S.aureus ATCC 43300	64	256	32	16	64	0.1875–0.375*	0.375–0.5*	0.3125–0.5*	0.125–0.3125*

3S: 3-shogaol, GM: gentamicin, AMX: amoxicillin, MET: methicillin, OXA: Oxacillin

6-Shogaol

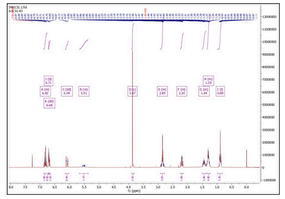
Bacterium	MIC (µg/mL)					FICI			
	6S	GMX	AMX	MET	OXA	6S/GM	6S/AMX	6S/MET	6S/OXA
S.aureus ATCC 43300	1024	256	32	16	64	0.313–0.5*	0.375–0.5*	0.5*	0.375–0.5*

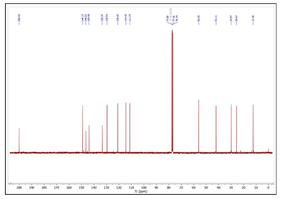
6S: 6-shogaol, GM: gentamicin, AMX: amoxicillin, MET: methicillin, OXA: Oxacillin

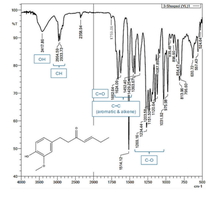
*FICI values of 0.5 and below indicate synergism

Characterisation of 3- & 6-Shogaols

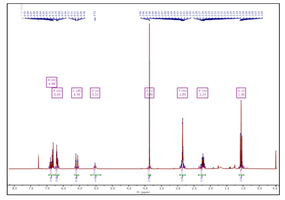
3-Shogaol

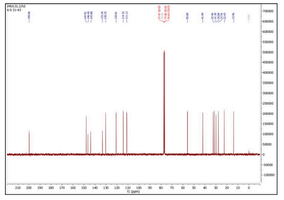


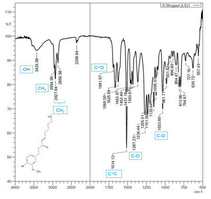




6-Shogaol



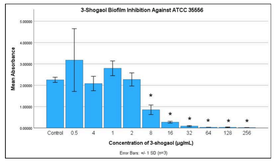


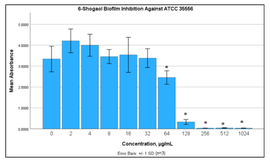


Biofilm Inhibition Assay

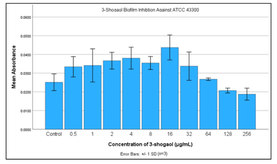
3-Shogaol 6-Shogaol

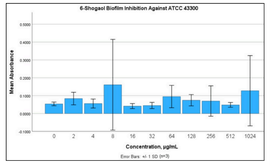
ATCC 35556





ATCC 43300





* $p < 0.05$ (statistically significant)