Enhancing the antimicrobial sensitivity of *Staphylococcus aureus* with herbal extracts as an adjunctive treatment for reversing antibiotic resistance

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Research Article

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Abstract

Background and aim:

Staphylococcus aureus is a significant public health concern due to its ability to develop antibiotic resistance. Biofilm formation or the enhancement of bacterial cell membrane permeability contributes to antibiotic resistance ability. Herbal therapy presents a promising strategy to overcome antibiotic resistance challenges. This study aims to investigate the potential of herbal extracts to reverse antibiotic resistance in *S. aureus*.

Experimental Procedure:

In this study, both Wild-type and Kanamycin-adapted (Km-adapted) *S. aureus* strains were pre-treated with herbal extracts derived from *Zingiber zerumbet* (ZZ), *Eucalyptus globulus* (EG), *Andrographis paniculata* (AP), *Clerodendrum inerme*(Cl), *Combretum quadrangular* (CQ), and *Plectranthus amboinicus* (PA) at subinhibitory concentrations. The effects of these extracts on biofilm formation, bacterial cell surface hydrophobicity, cell permeability, and kanamycin sensitivity on pre-treated *S. aureus* were evaluated.

Results:

Our results demonstrated that *S. aureus* formed thick biofilms that were less sensitive to Km treatment, particularly in Km-adapted strains. However, extracts from ZZ, EG, and AP effectively reduced biofilm formation in both wild-type and Km-adapted strains and decreased bacterial cell surface hydrophobicity. Additionally, all herbal extracts increased the permeability of *S. aureus* cells, resulting in enhanced antibiotic sensitivity.

Conclusion:

Herbal therapy has the potential to reverse antibiotic resistance and reduce the necessary antibiotic dosage for treating S. aureus-related infections.

1. Introduction

Staphylococcus aureus is a round-shaped, Gram-positive bacterium that exhibits opportunistic pathogenicity in humans [1]. It is a significant contributor to nosocomial infections and can cause various infectious diseases such as pneumonia, skin infections, endocarditis, osteomyelitis, and sepsis which can be life-threatening [2–5]. The widespread use of antibiotics to treat *S. aureus* infections has led to the emergence of multiple strains of antibiotic-resistant bacteria, including those that are resistant to commonly used antibiotics such as methicillin, tetracycline, macrolide, chloramphenicol, and even current generation antibiotics [6]. Antibiotic resistance has resulted in difficulties in effectively treating *S. aureus* infections, and consequently, made it the second leading cause of mortality in over 200 countries and territories in 2019 [7].

The global concern about the slow development of new antibiotics compared to the rapid increase in antibiotic-resistant strains has led to the exploration of herbal medicine as a potential solution due to its multiple antibacterial effects and therapeutic benefits for antibiotic-resistant bacteria [8]. Even though plant-derived antimicrobial compounds are usually required at significantly higher doses than that of antibiotics to inhibit or kill bacteria, they usually do not cause rapid bacterial resistance [9]. A large number of studies have theorized and identified mechanisms of action driving the antimicrobial activities of plant-derived natural compounds, e.g. inhibiting the cell wall synthesis [10], protein expression [11], nucleic acid formation [12, 13], efflux pump expression [14], perturbing the cytoplasmic membrane [15], increasing membrane permeability [16], or attacking macromolecules in the cell and disrupting the metabolic network [17]. Additionally, interfering with bacterial biofilm formation presents a promising therapeutic approach against multidrug-resistant (MDR) pathogens since the biofilm formation is among the most effective and universal responses of MDR strains toward a wide range of antibiotics [18–20]. Biofilms are clusters of microorganisms that adhere to surfaces through hydrophobic interaction and produce an extracellular matrix that serves as a physical barrier to protect bacteria from environmental stresses, including antibiotics [21]. Eradication of biofilm-resident bacteria often requires antibiotic doses 100–1000 times higher than those for planktonic bacteria [22]. Several herbal extracts, such as limonene, citral, and carvacrol, have been demonstrated to reduce the biofilm formation of *S. aureus*, *Listeria monocytogenes*, and *Escherichia coli*, while enhancing antibiotic sensitivity [23–25].

Our previous study demonstrated that extracts from several herbal plants grown in Vietnam had antibacterial activity against *S. aureus* and its antibiotic-resistant variants, while not provoking the drug resistance after 30 passages [9]. This study aimed to explore the potential of herbal extracts as adjuvants to conventional antibiotics by investigating the possibility of increased antibiotic sensitivity in *S. aureus*, particularly for antibiotic-resistance strains. In addition, we investigated the possible mechanisms by which some herbal extracts sensitize *S. aureus* to antibiotics, particularly through biofilm formation and mediated pathways and cell permeability. The findings of this study may provide valuable insights into the development of alternative strategies to combat antibiotic resistance.

2. Material and methods

2.1. Microbial strains and growth conditions

S. aureus strain ATCC 25923 and its derived laboratory-evolved, Kanamycin-adapted (Km-adapted) strain were obtained from the NTT Hi-tech Institute laboratory (Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam) [9]. Since the Km-adapted strain was originated from the ATCC 25923, this initial strain is considered wild-type (WT) in this work. The strains stored in glycerol stocks were streaked onto tryptic soy agar (TSA, Himedia, India) at 37°C for 24 h for obtaining individual colonies. Before performing the antibiotic resistance assays, a single colony was inoculated into 5 mL of sterilized tryptic soy broth (TSB) and incubated at 37°C in a shaking incubator (130 rpm) for 24 h. In all the bacterial incubation experiments, 37°C was the default temperature, or specifically stated otherwise.

2.2. Preparation of herbal extracts

The herbal extracts used in this work were the leaves and stem, or rhizome prepared in diethyl ether and lyophilized as mentioned in our previous report [9]. The selected extracts are from *Clerodendrum inerme* (L.) Gaertn (glory bower), *Combretum quadrangulare* Kurz (sakae naa), *Plectranthus amboinicus* (Lour.) Spreng (Indian borage), *Andrographis paniculate* (Burm. F.) Nees. (green chiretta), *Zingiber zerumbet* (L.) Smith (shampoo ginger), and *Eucalyptus globulus* Labill (blue gum), abbreviated as CI, CQ, PA, AP, ZZ, and EG, respectively. The herbal extracts were acquired from NTT Hi-tech Institute laboratory (Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam). The concentrated extracts were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA) at 50 mg/mL concentration and kept at -20°C until experimentation.

2.3. Procedure for *S. aureus* biofilm formation

S. aureus harvested from TSB was resuspended at 1×10^7 CFU/mL, then 200 µL of the bacterial suspension was inoculated in 96-well plate at 37°C. Biofilm formation was measured after 8, 16, 24, 32, and 40 h of incubation. The medium containing only the Mueller-Hinton broth (MHB, Sigma-Aldirich, USA) was referred to as the control group. Biofilms formed by *S. aureus* were stained with crystal violet (1 mg/mL). Briefly, the supernatant containing the planktonic cells was extracted from each well, and the plates were rinsed with sterile distilled water to remove non-attached cells and left to air-dry condition for about 2 h. Subsequently, each well was filled with 300 µL/L of crystal violet solution (1 mg/mL) and incubated at room temperature (26°C) for 30 min. The crystal violet solution was removed, and the wells were further washed with distilled water. The crystal violet stain was solubilized in 33% acetic acid (Xilong Scientific, Guangdong, China), and the absorbance was measured at OD_{595} to quantify biofilm formation [26].

2.4. Determination of the minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC), of Kanamycin and herbal extracts on planktonic S. aureus

Microdilution method of 96-well plate and resazurin (Sigma-Aldrich, USA) colorimetric indicator was used for the determination of the MIC of plant extracts and Kanamycin (Km) on *S. aureus* [9]. Briefly, *S. aureus* and Km or herbal extract were all diluted in MHB (Sigma-Aldirich, USA). The bacteria were adjusted to a final concentration of approximately 1×10^7 colony-forming units (CFU) per mL. Then, 100 µL of bacterial suspension was added to the wells of 96-well plates containing two-fold serial Km or plant extract dilutions to a total volume of 200 µL. The wells containing only MHB were considered as negative controls. The plates were incubated at 37°C for 24 h, and then analyzed by the color shift of resazurin. The MIC values of Km or herbal extracts on *S. aureus* were determined by the lowest concentration of the compounds that made the mixture remained blue. All samples with concentrations of Km or herbal extracts that showed complete inhibition of visual bacterial growth were identified, and 50 µL of each culture was transferred onto the MHA plate, and then incubated for 24 h. The MBC was defined as the

complete absence of bacterial colonies on the agar surface in samples with the lowest Km or herbal extract concentration.

2.5. Determination of the minimum biofilm eradication concentration (MBEC) of Km on biofilm-resident *S. aureus*

The MBEC assay is a method used to determine the minimum concentration of an antimicrobial agent needed to eliminate bacterial colonies in biofilms [27]. Briefly, a 96-well plate was used to culture biofilm-forming *S. aureus* in MHB with or without herbal extracts and incubated at 37°C for 24 h. Planktonic cells were removed, and the wells were washed with phosphate-buffered saline (PBS) to eliminate loosely attached cells. The Km were then added into the wells at different concentrations, and the plate was incubated at 37°C for an additional 24 h. Afterward, the supernatant was removed, and the wells were rinsed twice with PBS. The biofilm cells were detached from the wells via pipetting and determined the viability using the drop-plate method on a TSA plate. The MBEC was defined as the lowest concentration of the antimicrobial agent that completely eradicated the biofilm colonies.

2.6. Effect of herbal extracts on biofilm formation of *S. aureus*

S. aureus harvested from TSB was resuspended at 1×10^7 CFU/mL, then 200 µL of the bacterial suspension strains were co-incubated with herbal extracts at the concentration of 1/2, 1/4, and 1/8 MIC in the wells of the 96-well plate for 24 h Biofilm formation of *S. aureus* strains was quantified *in vitro* by crystal violet staining [26]. The planktonic bacteria and biofilm-resident bacteria were collected and detected the sensitivity to Km by microdilution method. The results were expressed by MIC, MBC and MBEC values as described in previous methods.

2.7. Measurement of *S. aureus* cell surface hydrophobicity

The effect of herbal extracts on the hydrophobicity of *S. aureus* cell surfaces was evaluated by assessing cell adhesion to chloroform, as previously described [28]. Following exposure to sub-inhibitory concentrations of herbal extracts for 24 h, *S. aureus* cells were harvested through refrigerated centrifugation at 4000 × g for 5 minutes. The collected *S. aureus* pellet was subjected to two washes with distilled water and then resuspended in a 0.85% NaCl solution ($OD_{600} \approx 0.3$) to yield A1 value. One mL of *S. aureus* suspension was mixed with 1 mL of chloroform, followed by vortexing for 2 min and a 30-min incubation period. This resulted in the formation of a chloroform/water bilayer system. The OD_{600} value of the aqueous phase of the bilayer was measured as A2. The index of cell surface hydrophobicity (I) was calculated using the following equation:

$$I = (1 - A2/A1) \times 100\%$$

2.8. Measurement of *S. aureus* cell permeability with crystal violet

The alteration in membrane permeability was also evaluated by crystal violet (CV) assay [29]. *S. aureus* harvested from TSB was resuspended at 1 × 10^7 CFU/mL, then 200 µL of the bacterial suspension strains were co-incubated with or without herbal extracts at the concentration of 1/2 MIC in the wells of the 96-well plate for 24 h. Bacterial cell suspensions were harvested at 4,500 × *g* for 5 min at 4°C. The cells were resuspended in 0.5 mM potassium phosphate buffer solution (pH 7.4) containing 10 g/mL of crystal violet at 37°C for 10 min. The suspension was then centrifuged at 4000 × *g* for 15 min and the OD of the supernatant was measured at a wavelength of 590 nm. OD of the supernatant of the normal untreated cell was used as blank. The OD value of crystal violet solution, was considered as 100%. The percentage of crystal violet uptake was expressed as follows:

$$\text{Percentage uptake of crystal violet} = \frac{\text{ODvalueofCVsolution} - \text{ODvalueofsample}}{\text{ODvalueofCVsolution}} \times 100$$

2.9. Statistical Analysis

The experiment was conducted using a completely randomized design (CRD) with 3 replicates for each treatment. Results were calculated using the mean of triplicate readings and presented as the mean ± standard error of the mean data were analyzed using SAS 9.4 software (SAS, Inc., Cary, NC, USA). Statistical significance was assessed between groups, using T-test and Duncan's test. $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Biofilm development of *S. aureus*

The mass production of biofilm by both *S. aureus* strains was monitored periodically for 40 h using crystal violet staining. As shown in Fig. 1A, both *S. aureus* strains demonstrated strong biofilm formation, with the WT strain producing significantly more biofilm than the Km-adapted strain. Additionally, the cell proliferation of *S. aureus* was investigated by analyzing the growth curve (Fig. 1B). The growth curve revealed that the density of both *S. aureus* strains reached a peak at 24 h (Fig. 1B) after incubation, followed by a transition to the stationary phase and gradual decrease after 32 h of incubation (Fig. 1B). Thus, a 24 h incubation period for *S. aureus* was selected for subsequent experiments.

3.2. Biofilm formation promotes antibiotic tolerance in *S. aureus*

Differences were observed in the survival of planktonic bacteria and biofilm-associated bacteria when cultured with Km. The localization of *S. aureus* within the biofilm resulted in a 2-fold increase in the concentration of Km to eradication of both strains, compared to the planktonic bacteria (Table 1). The concentration of Km required to eradicate Km-adapted *S. aureus* in biofilm is up to 20,000 µg/mL, exhibiting an 800-fold higher than the WT strain (Table 1). This result indicated that biofilm formation might protect *S. aureus* from the antibiotic pressure.

Table 1
MICs, MBCs and MBECs of Km on *S. aureus*

	WT			Km-adapted		
	Planktonic		Biofilm	Planktonic		Biofilm
	MIC	MBC	MBEC	MIC	MBC	MBEC
Km (μg/mL)	6.25	12.5	25 #	2500 *	10000 *	20000 *#

WT: wild-type strain; Km-adapted: Kanamycin-adapted strain. * Indicates significant differences compared between the MIC, MBC, and MBEC values of kanamycin for Km-adapted and WT strains. # Indicates significant differences compared between the Km concentration required to eradicate biofilm-reside *S. aureus* (MBEC) and planktonic *S. aureus* (MBC).

3.3. Anti-biofilm effect of subinhibitory herbal extract concentrations

The potential activity of herbal extracts to act as biofilm inhibitors were first assessed by measuring the MIC of each extract against *S. aureus*. Interestingly, the MIC values of the herbal extracts were found to be similar on both strains of *S. aureus* (Table 2). Subsequently, sub-inhibitory concentrations for each herbal extract, namely 1/2 MIC, 1/4 MIC, and 1/8 MIC values, were used to determine the ability to inhibit *S. aureus* biofilm formation. Following a 24 h incubation in the presence of ZZ, EG, and AP, both *S. aureus* strains exhibited a dose-dependent decrease in biofilm production (Fig. 2D, E, F). In contrast, biofilm formation by *S. aureus* was not inhibited in the CQ, CI, or AP-treated groups (Fig. 2A, B, C).

Table 2
MIC values of herbal extracts on *S. aureus*

	MIC (μg/mL)					
	CQ	ZZ	CI	PA	EG	AP
WT	1250	250	625	2500	625	625
Km-adapted	1250	250	625	2500	625	625

3.4. Subinhibitory herbal extract concentrations enhance the effectiveness of Km to eradicate biofilm-resident *S. aureus*

The antimicrobial efficacy of Km against *S. aureus* in biofilms or planktonic cultures was significantly enhanced by pre-treating with sub-inhibitory concentrations of herbal extracts. The MIC and MBC values of Km for planktonic *S. aureus* strains were lowered in herbal extracts pre-treated groups, with the most substantial reduction observed when Km-adapted *S. aureus* was pre-treated with 312.5 μg/mL of AP or

125 µg/mL of ZZ (Table 3). The MBC value of Km for planktonic *S. aureus* decreased from 10,000 g/mL to 625 g/mL, resulting in a 16-fold decrease in survivability of the bacteria upon exposure to Km. The same pattern was observed in the biofilm-residing Km-adapted *S. aureus* strains, with the MBEC value of Km being reduced in all herbal extracts pre-treated groups as compared to the untreated group, despite biofilm-induced antibiotic resistance (Table 3).

Table 3
MICs and MBCs of Km on planktonic *S. aureus* and MBEC of Km on biofilm-resident *S. aureus* after 24 h co-incubation with different subinhibitory herbal extract concentrations.

		WT			Km-adapted		
		Planktonic		Biofilm	Planktonic		Biofilm
		MIC	MBC	MBEC	MIC	MBC	MBEC
	Untreated	6.25	12.50	25.00	2500.00	10000.00	20000.00
Subinhibitory herbal extract concentrations pretreatment	CQ (μg/mL)						
	312.50	3.13 *	6.25 *	12.50 *	625.00 *	5000.00 *	5000.00 *
	625.00	3.13 *	6.25 *	12.50 *	625.00 *	2500.00 *	5000.00 *
	ZZ (μg/mL)						
	62.50	1.56 *	6.25 *	12.50 *	625.00 *	2500.00 *	5000.00 *
	125.00	1.56 *	6.25 *	12.50 *	312.50 *	1250.00 *	2500.00 *
	CI (μg/mL)						
	156.25	6.25 ns	6.25 *	12.50 *	2500.00 *	2500.00 *	5000.00 *
	312.50	6.25 ns	6.25 *	12.50 *	2500.00 *	2500.00 *	5000.00 *
	PA (μg/mL)						
	625.00	6.25 ns	6.25 *	12.50 *	1250.00 *	2500.00 *	5000.00 *
	1250.00	6.25 ns	6.25 *	12.50 *	1250.00 *	1250.00 *	2500.00 *
	EG (μg/mL)						

WT: wild-type strain; Km-adapted: Kanamycin-adapted strain. * Indicates significant differences compared between the MICs, MBCs, and MBECs of Km on *S. aureus* in herbal extracts pretreatment groups and the untreated group, $p < 0.05$.

		WT			Km-adapted		
		Planktonic		Biofilm	Planktonic		Biofilm
	156.25	3.13 *	6.25 *	12.50 *	1250.00 *	5000.00 *	10000.00 *
	312.50	3.13 *	3.13 *	6.25 *	625.00 *	5000.00 *	10000.00 *
	AP (μg/mL)						
	156.25	1.56 *	6.25 *	12.50 *	625.00 *	1250.00 *	2500.00 *
	312.50	1.56 *	3.13 *	12.50 *	312.50 *	625.00 *	1250.00 *

WT: wild-type strain; Km-adapted: Kanamycin-adapted strain. * Indicates significant differences compared between the MICs, MBCs, and MBECs of Km on *S. aureus* in herbal extracts pretreatment groups and the untreated group, $p < 0.05$.

3.5. Effect of subinhibitory herbal extract concentrations on *S. aureus* cell surface hydrophobicity

The hydrophobicity of the bacterial cell surface is related to the biofilm formation ability. Therefore, a chloroform adhesion test was performed to see if the herbal extract affects the hydrophobicity [21]. The surface hydrophobicity of *S. aureus* cells was adjusted to the percentage affinity to chloroform as the hydrophobicity index. *S. aureus* cell hydrophobicity was significantly reduced in the presence of subinhibitory doses of ZZ, EG, and AP, associated with a decrease in biofilm, but not in the CQ, PA, or CI pretreatment group (Fig. 3).

3.6. Effect of subinhibitory herbal extract concentrations on *S. aureus* cell permeability

Bacterial cell permeability is closely linked to antibiotic resistance [30]. Therefore, *S. aureus* strains with or without extract pre-treatment were incubated at for 24 h and further examined the cell permeability. The results (Fig. 4) showed that the cell permeability of Km-adapted *S. aureus* was lower than the wild-type strain with less crystal violet uptake. The cell permeability of the Km-adapted *S. aureus* increased after treatment with herbal extracts compared to the untreated group, and the resistance to Km ability was lowered in the same pattern (Fig. 4).

4. Discussion

Treatment of *S. aureus* infections can be extremely complicated due to the elevated resistance of the organism to antibiotics [31]. Previously, we found that the continuous exposure to antibiotics after 30 passages developed the high antibiotic tolerance phenotype in *S. aureus*, in which the MIC/MBC values were increased by 400 and 800-fold compared with WT strains, respectively [9]. Moreover, *S. aureus* Km-adapted strain was also cross-resistant with other antibiotics such as chloramphenicol, streptomycin,... [9]. Therefore, new strategies are required to suppress *S. aureus* infection, including the development of herbal treatments could potentially reverse antibiotic resistance, thereby reducing the dose for antibiotic usage [8].

The elevated resistance of *S. aureus* to antibiotics is partly attributed to the formation of biofilms and changes in cell permeability [32]. Biofilms provide structural support and protection to bacterial cells from environmental stresses, including antibiotics and host immune defenses, and hydrophobic interactions are crucial for the initial attachment of bacteria to surfaces, facilitating biofilm formation [21]. In this study, the growth characteristics of *S. aureus* in biofilms were assessed. Compared to planktonic bacteria growing in liquid culture, *S. aureus* in biofilms displayed a much higher level of resistance to Km. Importantly, the Km-adapted *S. aureus* strain in biofilm demonstrated resistance to Km antibiotics with MBEC concentration up to 20,000 µg/mL, which is 800-fold higher than that of the WT strain. This finding aligns with the results of a prior study conducted by McLaughlin *et al*., in 2006, which demonstrated that biofilms protect *S. aureus* against antibiotic elimination [33]. Furthermore, the effects of herbal extracts on the hydrophobicity and biofilm formation of *S. aureus* were investigated. Our findings suggest that PA, EG, and AP extracts effectively reduced the surface hydrophobicity of *S. aureus*, associated with a reduction in biofilm formation. These extracts also decreased the MIC and MBC values of Km in both strains, indicating their potential to re-sensitize bacteria to previously treated antibiotics. Previous studies have indicated that various plant extracts, including limonene, citral, carvacrol, and methanol and ethyl acetate extracts of *P. amboinicus* and *A. paniculate* suppressed the biofilms formation of *S. aureus* through reduction the cell surface hydrophobicity, which interferes the interactions between bacterial cells and adhesion site, thereby preventing initial attachment and subsequent biofilm formation [34, 35]. These findings suggest that natural extracts such as PA, EG, and AP, have the potential to be utilized as an adjuvant to antibiotics, offering a promising strategy to combat *S. aureus* infections by reducing hydrophobicity, inhibiting biofilm formation, and enhancing the efficacy of antibiotic clearance.

Although CQ, PA, and CI extracts did not prevent the formation of biofilms in *S. aureus*, they increased the sensitivity of both the WT and Km-adapted *S. aureus* strains to Km, whether the cells were planktonic or biofilm-resident. The treatment group receiving 312.5 µg/mL of AP showed the most significant reduction, with a 16-fold reduction in the MBEC value in the Km-adapted *S. aureus* strain, from 20,000 µg/mL to 1,250 µg/mL, even in the presence of biofilm. This result is encouraging, given the slow pace of new antibiotic development, and pre-treatment with herbal extracts may offer a valuable approach to address antibiotic resistance. This observed effect could be attributed to the ability of these treatments to interfere with the permeability of the bacterial membrane. *S. aureus* has a cell membrane that is a phospholipid bilayer acting as a barrier to protect against harmful agents, including antibiotics [30].

Bacteria can modify their cell walls and membrane permeability to hinder antibiotic access, reducing antibiotic efficacy and increasing the chances of bacterial survival [36, 37]. We further investigated the membrane permeability and indicated that the Km-adapted *S. aureus* strain had lower permeability than the WT strain, with less CV absorption. However, treatment with herbal extracts increased the permeability of Km-adapted *S. aureus*, making the bacteria more susceptible to antibiotics. It was reported that certain compounds, such as *Chelerythrine* and *Rhodomyrtosone* B, can alter ion channels and membrane permeability, reducing antibiotic resistance [16, 38]. These findings provide scientific support for the use of herbal remedies in combination with antibiotics for the treatment of infectious diseases caused by *S. aureus*. Further investigation is required to explore the association between increased antibiotic sensitivity and *S. aureus* cell permeability induced by herbal extracts.

5. Conclusion

To summarize, the resistance of *S. aureus* to antibiotics is challenging to manage due to biofilm formation and altered cell permeability. The hydrophobic interactions between bacterial cells and surfaces are critical for biofilm formation, and natural extracts such as PA, EG, and AP have the potential to reduce this hydrophobicity, inhibiting biofilm formation, and re-sensitizing antibiotic-resistant *S. aureus*. Our findings suggest that these extracts can partly reverse the resistance of *S. aureus* strains to Km, regardless of their growth as planktonic or biofilm-residing cells, and permeabilize the *S. aureus* membrane, rendering them more sensitive to antibiotics. The use of these herbal extracts may offer a promising approach for treating *S. aureus* infections, but additional studies are needed to determine their safety, efficacy, and modes of action.

Abbreviations

MIC: Minimum inhibitory concentration

MBC: Minimum inhibitory concentration

MBEC: Minimum biofilm eradication concentration

Km: kanamycin

CQ: *Combretum quadrangulare* Kurz (sakae naa)

PA: *Plectranthus amboinicus* (Lour.) Spreng (Indian borage)

CI: *Clerodendrum inerme* (L.) Gaertn (glory bower)

ZZ: *Zingiber zerumbet* (L.) Smith (shampoo ginger)

EG: *Eucalyptus globulus* Labill (blue gum)

AP: *Andrographis paniculate* (Burm. F.) Nees. (green chiretta)

Declaration

Competing interests: The authors declare no competing interests.

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Figures

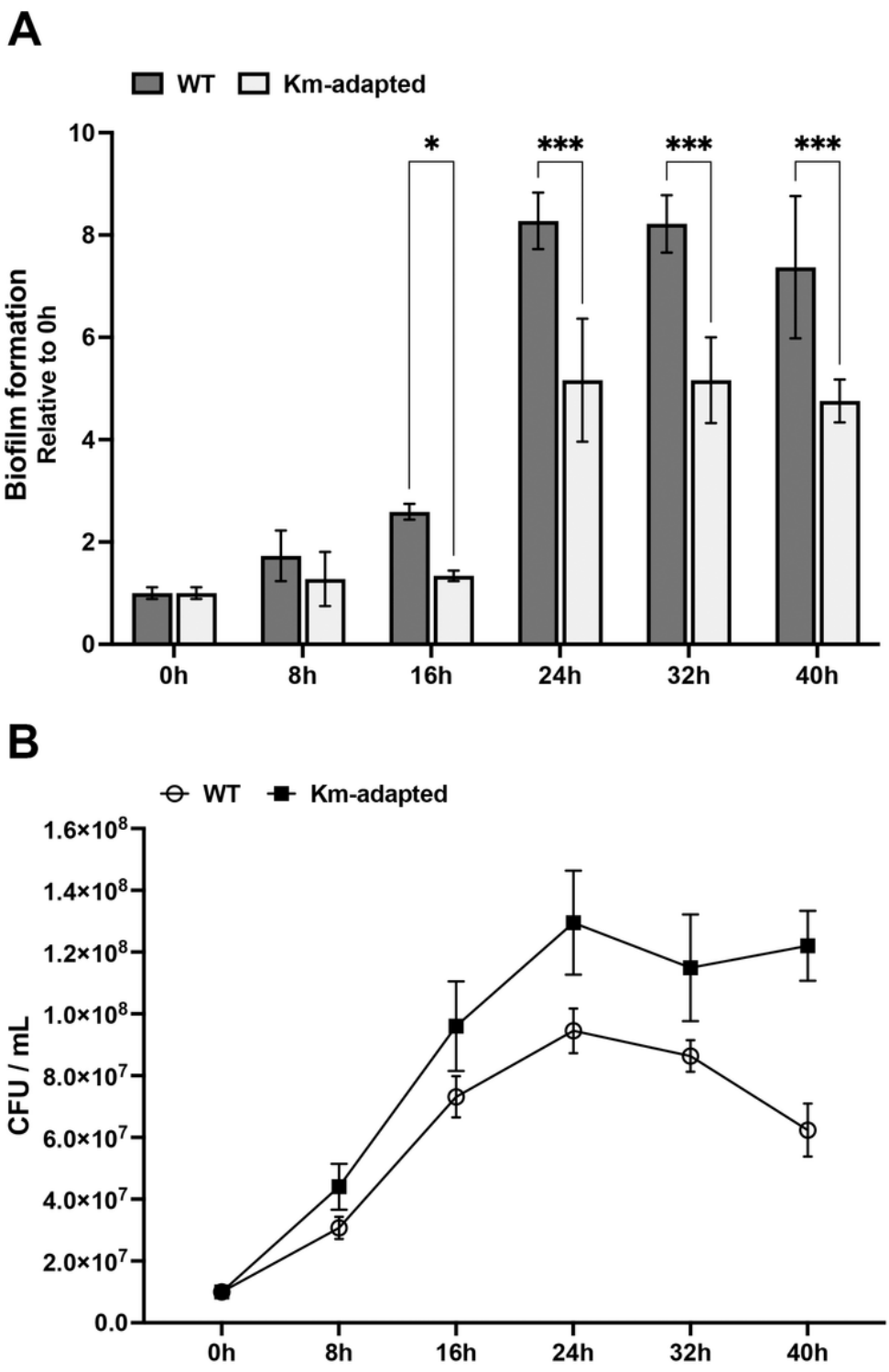

Figure 1

Biofilm mass production (A) and the growth curve (B) of *S. aureus* at different times interval. WT: wild-type strain; Km-adapted: Kanamycin-adapted strain. * Indicates significant differences compared between Km-adapted and WT groups, $p < 0.05$.

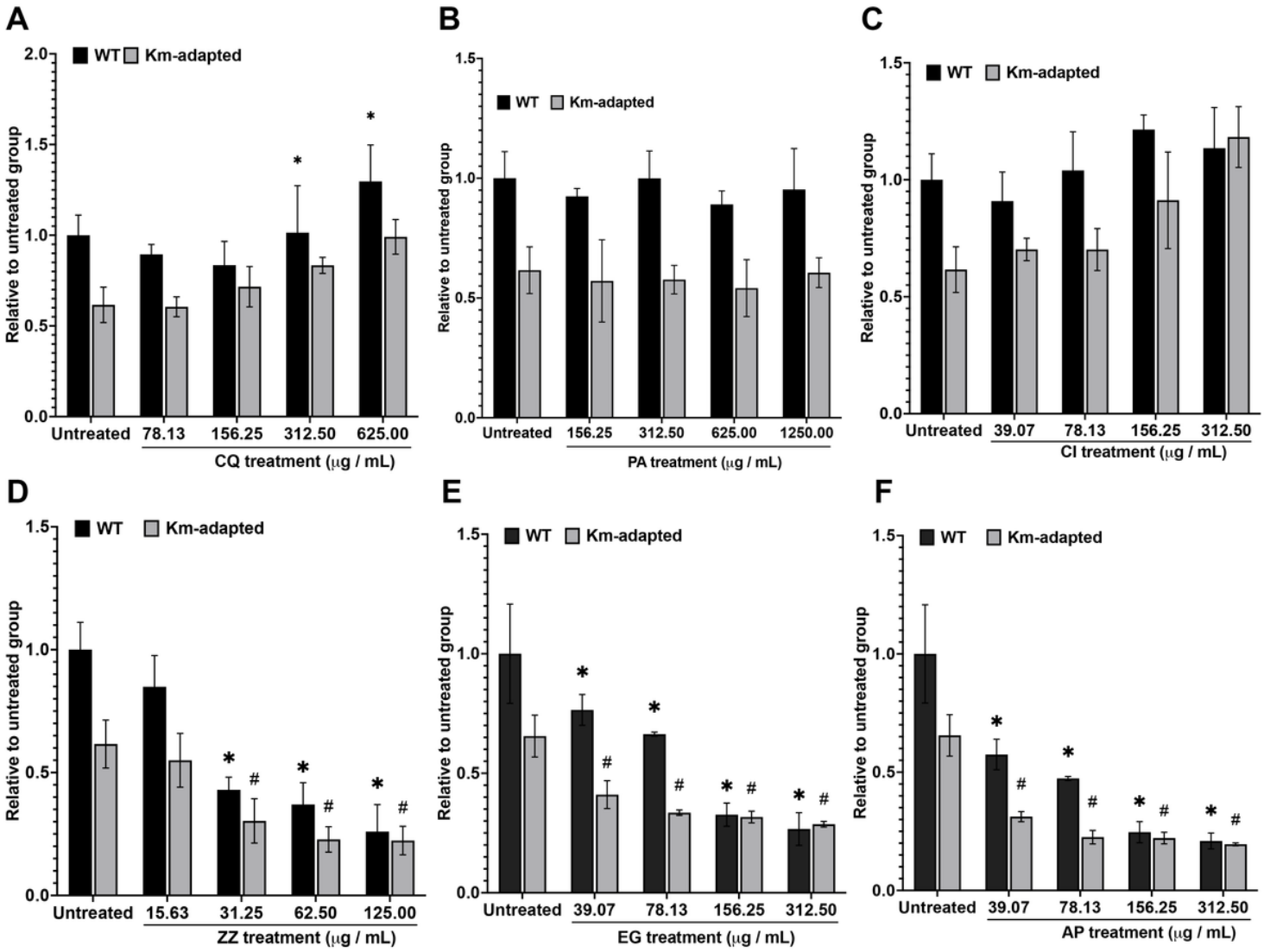

Figure 2

Effect of herbal extracts at different concentrations on the biofilm formation of S. aureus. WT: wild-type strain; Km-adapted: Kanamycin-adapted strain. * Indicates significant differences compared with the untreated group, $p < 0.05$.

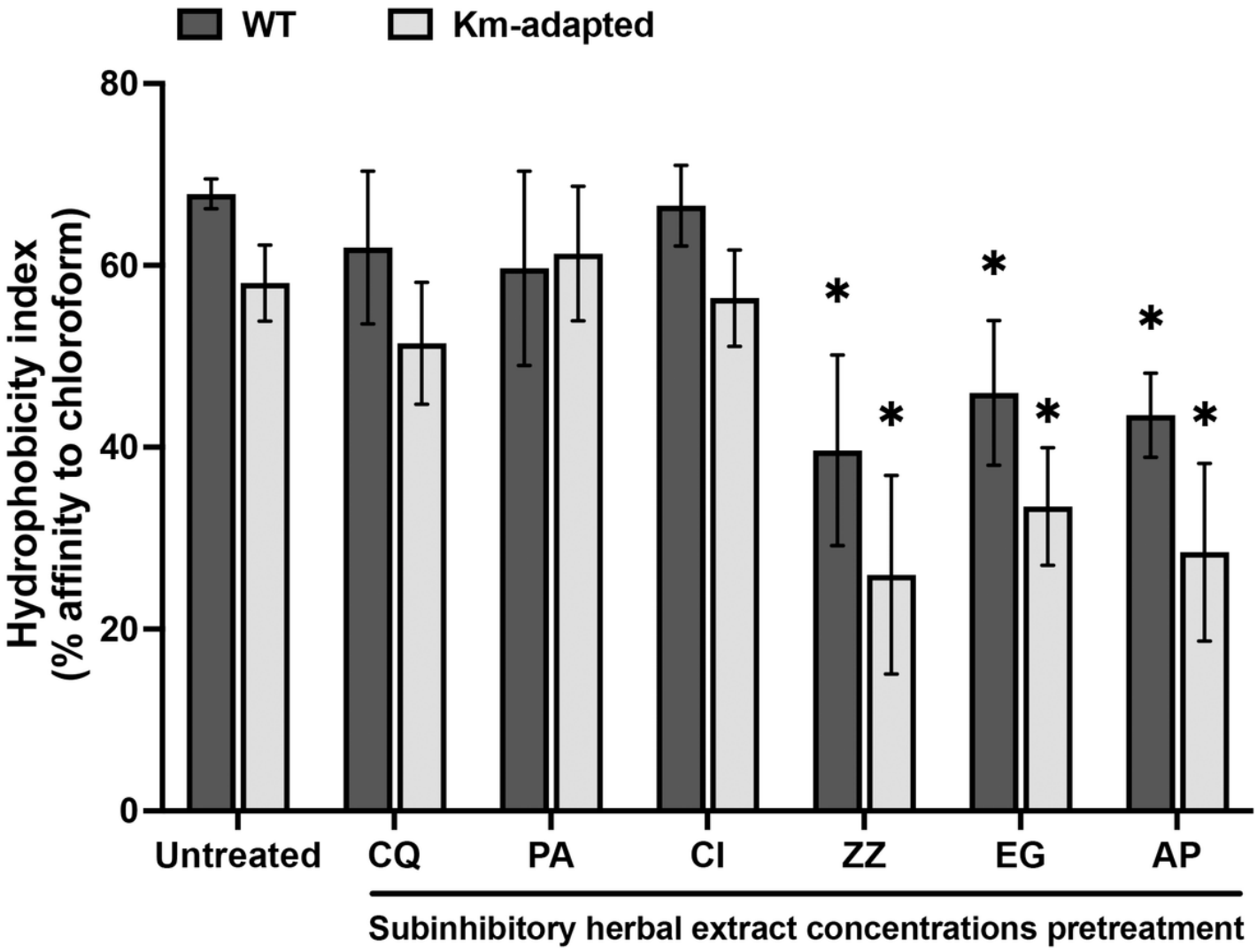

Figure 3

Effect of sub-inhibitory herbal extract concentrations on the cell surface hydrophobicity of *S. aureus*. * Indicates significant differences compared with the untreated group, $p < 0.05$.

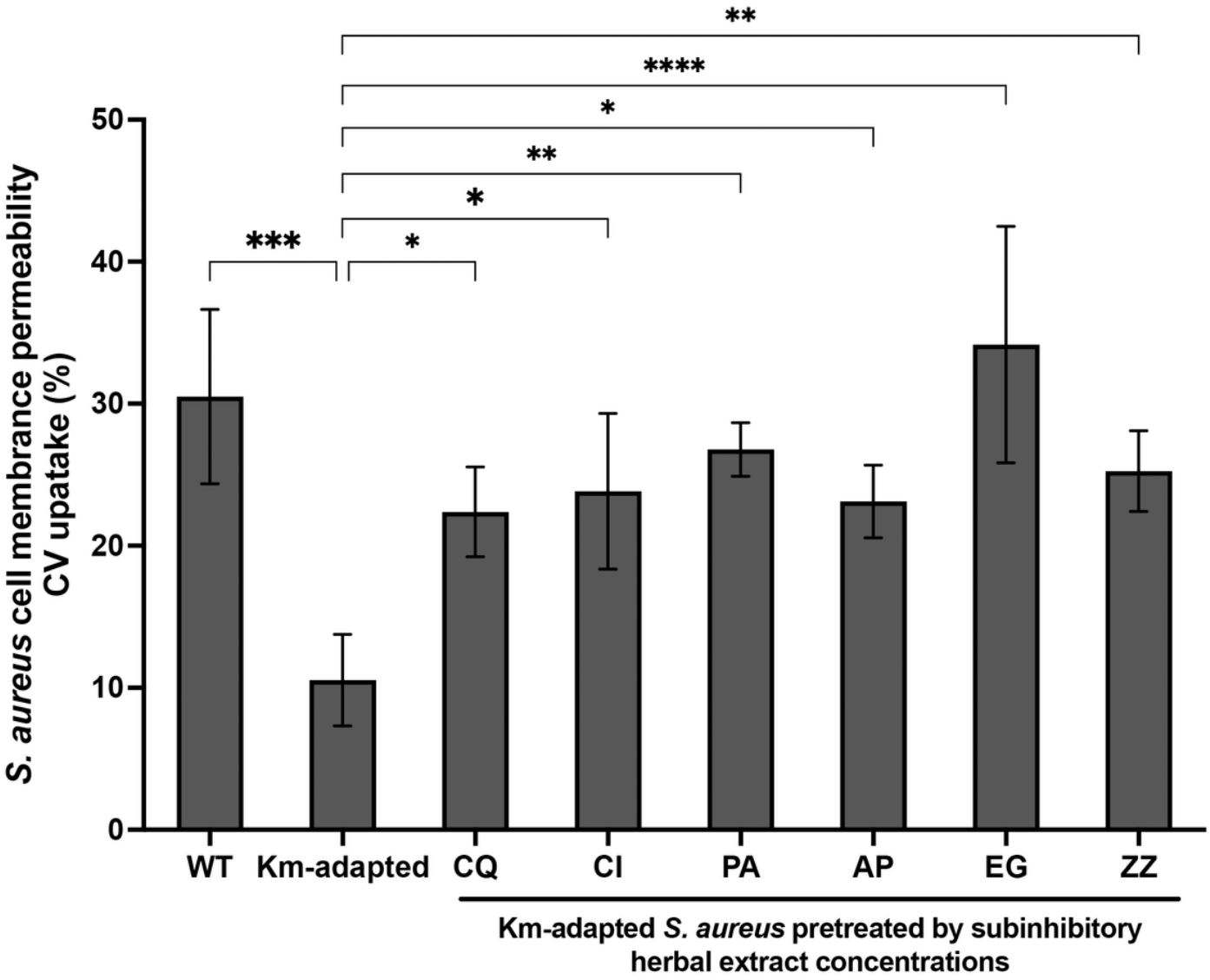

Figure 4

Effect of subinhibitory herbal extract concentrations change in S. aureus membrane permeability (assayed by crystal violet uptake) in the presence of different herbal extracts. WT: wild-type strain; Km-adapted: Kanamycin-adapted strain. * Indicates significant differences compared with the Km-adapted group without pretreated with herbal extracts, $p < 0.05$.

Supplementary Files

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- Graphicabstract.png