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Antibacterial Mechanism of a Novel Peptide Dendrocin-ZM1 Against *Staphylococcus aureus* by Increasing Membrane Permeability

Sima Sadat Seyedjavadi[1] · Mehdi Razzaghi-Abyaneh[1] · Parmida Bagheri[2] · Mehdi Goudarzi[3]

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Abstract

Staphylococcus aureus is a prevalent pathogen responsible for healthcare and community-acquired infections, which lead to elevated rates of fatality and morbidity. With the emergence of bacterial strains that are resistant to multiple drugs, antimicrobial peptides are increasingly recognized as a promising alternative to antibiotics due to their distinctive mode of action and other notable attributes. In our previous investigation, we isolated and characterized an antibacterial peptide from *Zataria multiflora Boiss*, known as Dendrocin-ZM1, which exhibits potent antibacterial properties against a diverse range of bacterial strains.

In this study, we aimed to investigate the anti-staphylococcal mode of action of Dendrocin-ZM1. We examined the interactions of the peptide with the cytoplasmic membrane using DiSC (3)-5, NPN, and PI dyes. Additionally, we analyzed the release of ATP, DNA/RNA from cells exposed to the peptide. Our findings revealed that Dendrocin-ZM1 can eliminate 99.9% of bacteria within 60 min. Furthermore, this peptide demonstrated good stability in the presence of physiological concentrations of salts. Through bactericidal mechanism analysis, we discovered that Dendrocin-ZM1 has the ability to disrupt the integrity of the bacterial membrane, increase its permeability, and cause leakage of cellular content, resulting in membrane damage. Observation of the treated bacteria using transmission electron microscopy (TEM) provided evidence of damage to the cell membrane and subsequent cell lysis. Based on these results, we conclude that Dendrocin-ZM1 shows promising prospects as a new antimicrobial agent.

Keywords Antibiotic Resistance · *Staphylococcus aureus* · Antimicrobial Peptide · Dendrocin-ZM1 · Mode of Action · Membrane Destruction

Introduction

Staphylococcus aureus, commonly referred to as *S. aureus*, is a gram-positive bacterium that frequently colonizes the human nose and skin as a commensal microorganism (Sakr et al. 2018; Tong et al. 2015). About 30% of the populations carry *S. aureus* and 20% of which are categorized as persistent carriers (Accorsi et al. 2020). Significantly, it has been revealed that the colonization of *S. aureus* can lead to a potential risk for subsequent infection (Accorsi et al. 2020; Sakr et al. 2018; Tong et al. 2015). In recent decades, there has been a significant increase in the incidence of *S. aureus* infections, which has been associated with the emergence of antibiotic-resistant strains (Guo et al. 2020). Due to the increasing number of multidrug-resistant (MDR) strains, this has become a major public health concern, only a limited number of antibiotics such as vancomycin, currently effective in the treatment of *S. aureus* infections. Evidence indicates that the emergence of vancomycin-resistant *S. aureus* strains (VRSA) is increasing, necessitating the prompt discovery of effective and durable antibiotic alternatives (Wu et al. 2021; Assis et al. 2017).

Antimicrobial peptides (AMPs) are a class of molecules found ubiquitously across all domains of life and are

✉ Mehdi Razzaghi-Abyaneh
mrab442@yahoo.com

✉ Mehdi Goudarzi
m.goudarzi@sbmu.ac.ir; gudarzim@yahoo.com

[1] Department of Mycology, Pasteur Institute of Iran, Tehran 1316943551, Iran

[2] Department of Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

[3] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

well-known for their efficacy against a broad spectrum of microorganisms, including fungi, viruses, Gram-positive, and Gram-negative bacteria. Despite variations in their amino acid sequences, they are typically characterized by their small size, cationic charge, and amphipathic characteristics. Additionally, AMPs have been identified as promising alternatives to conventional antibiotics (Lei et al. 2019).

In recent years, AMPs have been reported to exhibit a wide range of antibacterial activities with rapid bactericidal properties, making them promising candidates for the development of innovative antibacterial compounds (Luo and Song 2021). Compared to conventional antibiotics that target specific biosynthetic processes essential to cell wall or protein synthesis, a considerable fraction of AMPs function by physically disrupting the bacterial membrane, leading to immediate membrane permeabilization and effective elimination of multidrug-resistant bacterial strains (Lei et al. 2019; Luo and Song 2021). However, the precise mode of action of AMPs remains to be fully elucidated, limiting their potential applications. In most cases, the presence of a cationic charge in AMPs facilitates their interaction with the cellular membrane of the targeted microorganism, inducing membrane permeabilization or pore formation (Luo and Song 2021). However, certain AMPs have the ability to interact with intracellular targets (Lei et al. 2019; Luo and Song 2021). Numerous studies have documented the inhibitory effects of AMPs on various intracellular processes, including DNA replication, RNA and protein synthesis, cell wall formation, and enzyme activity, ultimately leading to cellular demise (Luo and Song 2021). Evidence indicates that the mechanism of membrane damage and intracellular action of AMPs often complement each other in exerting antibacterial activity (Erdem Büyükkiraz and Kesmen 2022; Lei et al. 2019).

In our previous study, we successfully isolated a novel antimicrobial peptide, Dendrocin-ZM1 (TTLRLNTLAYKVAWLVNVKAFWAAGRALKKVGR), consisting of 33 amino acids, from *Zataria multiflora Boiss*. Dendrocin-ZM1 exhibited significant antimicrobial efficacy against both Gram-negative and Gram-positive bacterial strains, with a minimum inhibitory concentration (MIC) of 8 µg/mL against *S. aureus* ATCC 25,923 (Seyedjavadi et al. 2022).

The present study aims to analyze the impact of Dendrocin-ZM1 on the cellular membrane of *S. aureus* using transmission electron microscopy and flow cytometry. Additionally, the study examines the interaction between Dendrocin-ZM1 and bacterial nucleic acid.

Materials and Methods

Chemicals, Reagents and Ethical Approval

Mueller-Hinton broth (MHB), Muller-Hinton Agar (MHA) and Tryptic Soy Broth (TSB) were acquired from Merck (Darmstadt, Germany). Potassium chloride, zinc chloride, ammonium chloride, magnesium chloride, and sodium chloride were obtained from Kermel (China). Ethanol (99%), glutaraldehyde (50% in H2O), 3,3'-dipropylthiadicarbocyanine iodide (diSC3-5), N-phenyl-1-naphthylamine (NPN), and propidium iodide (PI) were purchased from Sigma-Aldrich (China). This study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences in Tehran, Iran (IR.SBMU.MSP.REC.1398.802).

Synthesis of Dendrocin-ZM1

Dendrocin-ZM1 (TTLRLNTLAYKVAWLVNVKAFWAAGRALKKVGR) (≥95% purity) was synthesized using the 9-fluorenylmethoxycarbonyl solid-phase peptide synthesis (Fmoc-SPPS) method (Gongora-Benitez et al. 2013). The confirmation of the synthetic peptide's purity was accomplished through the utilization of RP-HPLC, which demonstrated a purity level exceeding 85%. Additionally, the molecular weight of the peptide was determined using electrospray ionization mass spectrometry (ESI-MS).

Antibacterial Susceptibility Testing

The broth microdilution technique, as outlined by the Clinical and Laboratory Standards Institute (CLSI) (2023), was used to determine the minimum inhibitory concentration (MIC) of peptide (CLSI). Briefly, bacterial cells were diluted to obtain final concentrations of 5×10^5 CFU/mL in MHB, followed by incubation with different concentrations of the peptide (from 1 to 128 µg/mL) in a 96-well plate for 24 h at 37 °C. Positive and negative controls were included, using broth containing bacterial cells and broth with no additives, respectively. The MIC was set as the lowest concentration of peptides that reduced bacterial growth by ≥90% while the MBC was set as the lowest concentration of peptides that reduced bacterial growth by >99.99% after enumeration of viable bacteria by plate counts compared to bacteria grown without either AMP. The experimental procedure was conducted in triplicate.

Salt Stability

To detect the influence of salt on the bactericidal activity of Dendrocin-ZM1, the peptide was incubated while exposed to different salts at certain physiological concentrations

(150 mM NaCl, 4.5 mM KCl, 8 mM $ZnCl_2$, 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 4 mM $FeCl_3$), and the MIC of Dendrocin-ZM1 was determined on *S. aureus* ATCC 25,923 (Dong et al. 2014). All experiments were conducted three separate times independently.

Time Killing Kinetics

The time killing kinetics of Dendrocin-ZM1 on *S. aureus* ATCC 25,923 was determined using a colony count-based method (Wang et al. 2019). *S. aureus* cells in the logarithmic phase were collected by centrifugation (1000 × g for 5 min) and washed twice with PBS. The resulting solution was then diluted to a concentration of 2×10^5 CFU/mL. Subsequently, *S. aureus* was treated with different concentrations of Dendrocin-ZM1 (4, 8, and 16 µg/mL). The solutions were incubated at 37 °C with shaking at 120 rpm. At certain time points (15, 30, 45, 60, 90, and 120 min), a volume of 50 µL of the bacterial solution was extracted, diluted, and plated on MHA. Bacterial colonies were counted after a 24 h incubation period at 37 °C. A control experiment was conducted on bacterial suspensions in the absence of the peptide, under the same conditions. The experiment was conducted in triplicate, with each trial being carried out independently.

Cytoplasmic Membrane Disruption Assay

Propidium Iodide Uptake Assay

To confirm that Dendrocin-ZM1 can permeabilize the bacterial membranes of *S. aureus* ATCC 25,923, flow cytometry was used to measure the incorporation of propidium iodide (PI) into cells with damaged cell membranes (Joshi et al. 2010). *S. aureus* cells in the logarithmic phase were centrifuged (1000 × g for 5 min) and diluted to an $OD_{600} = 0.05$. Dendrocin-ZM1 (16 µg/mL) was added to the suspension and incubated at 37 °C for different times (5, 15, 30 min). Subsequently, propidium iodide (50 µg/mL) was added, and the final solution was incubated for 15 min, protected from light exposure. The number of PI-stained bacterial cells was evaluated using a fluorescence-activated cell sorting (FACS) Calibur flow cytometer (BD Biosciences, San Jose, CA, United States) at excitation and emission wavelengths of 580 and 620 nm, respectively. Sterile water and triton X-100 (1%) were used as the negative control and positive control, respectively. The results are presented from three independent experiments.

NPN Uptake

The N-phenylnaphthylamine (NPN) uptake assay was conducted to investigate the outer membrane (OM) permeability of Dendrocin-ZM1. *S. aureus* cells in the logarithmic phase were diluted to an $OD_{600} = 0.05$. An NPN solution was added to a final concentration of 10 µM. The solutions were thoroughly homogenized and incubated for 20 min. Different concentrations of Dendrocin-ZM1 (4, 8, and 16 µg/mL) were added to the wells. Triton X-100 (1%) and sterile water were used as the positive control and negative control, respectively (Wang et al. 2020). A microplate reader was used to evaluate the fluorescence intensity and measure the uptake of NPN at excitation and emission wavelengths of 350 and 420 nm, respectively.

DiSC3-5 Assay

The fluorescent dye DiSC3-5 was used to detect the depolarization activity of Dendrocin-ZM1 against the *S. aureus* ATCC 25,923 cellular membrane, as previously described (Rasul et al. 2010). *S. aureus* cells in the logarithmic phase were centrifuged and re-dissolved in sterile PBS, adjusted to an $OD_{600} = 0.05$. A DiSC3-5 solution was added to achieve a final concentration of 1 µM. The cells were incubated for 20 min, protected from light exposure. Different concentrations of the peptide were added. Triton X-100 (1%) and sterile water were used as the positive control and negative control, respectively. Subsequently, a microplate reader was used to evaluate the fluorescence intensity at excitation and emission wavelengths of 622 and 670 nm, respectively.

Leakage of Intracellular Contents

ATP Release

The effect of Dendrocin-ZM1 on the leakage of ATP was measured using a previously described technique with slight modifications (Rasul et al. 2010). *S. aureus* cells in the logarithmic phase were incubated with Dendrocin-ZM1 at different concentrations at 37 °C for 1 h. Samples were collected at 2 min intervals, followed by centrifugation (9000 × g for 5 min at 4 °C). The supernatant was then extracted and stored on ice. Subsequently, released ATP was identified using an ATP bioluminescence kit (Invitrogen, Eugene, OR, United States) according to the manufacturer's recommendations.

Loss of DNA/RNA

The leakage of DNA/RNA was assessed using a previously described method (Carson et al. 2002). *S. aureus* cells in the logarithmic phase were incubated with Dendrocin-ZM1 at different concentrations at 37ºC. The bacterial suspension was collected at 5 min intervals, centrifuged at 9000 × g for 5 min, and the supernatant was filtered using 0.22 µm pores

(Merck, Darmstadt, Germany). The OD_{260} nm of the filtrates was evaluated using a UV-visible spectrophotometer.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was used to determine the structural alterations of *S. aureus* ATCC 25,923 after exposure to Dendrocin-ZM1 (Joshi et al. 2010). A suspension of bacteria (1×10^6 CFU/mL) was incubated with a peptide (16 µg/mL) for 30 min, followed by centrifugation for 30 min. The cells were washed three times for 5 min. The pellet was then fixed using a 2.5% (v/v) glutaraldehyde solution for 3 h at 4ºC. After washing the pellet with PBS, the samples were post-fixed in a solution of 1% osmium tetroxide in PBS for 70 min. Subsequently, the specimens underwent two rounds of PBS washing, followed by gradual dehydration in acetone solutions and embedding in Epon 812. The specimens were then sectioned with an ultramicrotome and stained with uranyl acetate and lead citrate. Finally, the specimens were observed using a Zeiss EM-900 TEM apparatus operating at 80 kV. Bacterial growth in PBS without the presence of the peptide was performed according to the same protocol as the control.

Statistical Analysis

Each experiment was performed in triplicate. Statistical analyses were conducted using GraphPad Prism software (version 8.0, La Jolla, CA, USA). The outcomes are presented as the mean value accompanied by the standard deviation.

Result

Antimicrobial Activity of Dendrocin-ZM1

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Dendrocin-ZM1 against *S. aureus* ATCC 25,923 were found to be 8 µg/mL and 16 µg/mL, respectively (Seyedjavadi et al. 2022).

Time Killing Kinetics

To determine the relationship between exposure time to Dendrocin-ZM1 and bacterial survival, the killing kinetics of the peptide on *S. aureus* ATCC 25,923 were investigated. As shown in Fig. 1, at a concentration of 16 µg/mL (2 × MIC), Dendrocin-ZM1 effectively killed *S. aureus* ATCC 25,923 within 60 min of exposure, demonstrating good bactericidal activity. The antimicrobial activity of Dendrocin-ZM1 was found to be dose and time dependent.

Salt Stability

The activity of antimicrobial peptides (AMPs) can be affected by saline environments. Therefore, in this study, the sensitivity of Dendrocin-ZM1 to salt was confirmed by adding different salts at physiological concentrations. The results of the MIC values of Dendrocin-ZM1 while being exposed to salts are presented in Table 1. The MIC of Dendrocin-ZM1 against *S. aureus* ATCC 25,923 did not change in the presence of Na^+, K^+, Fe^{3+}, and Mg^{2+} at physiological concentrations. However, the antimicrobial activity of Dendrocin-ZM1 was decreased in the presence of Ca^{2+}, resulting in an increase in MIC value from 8 to 16 µg/mL. Overall, Dendrocin-ZM1 exhibited high tolerance to physiological salts.

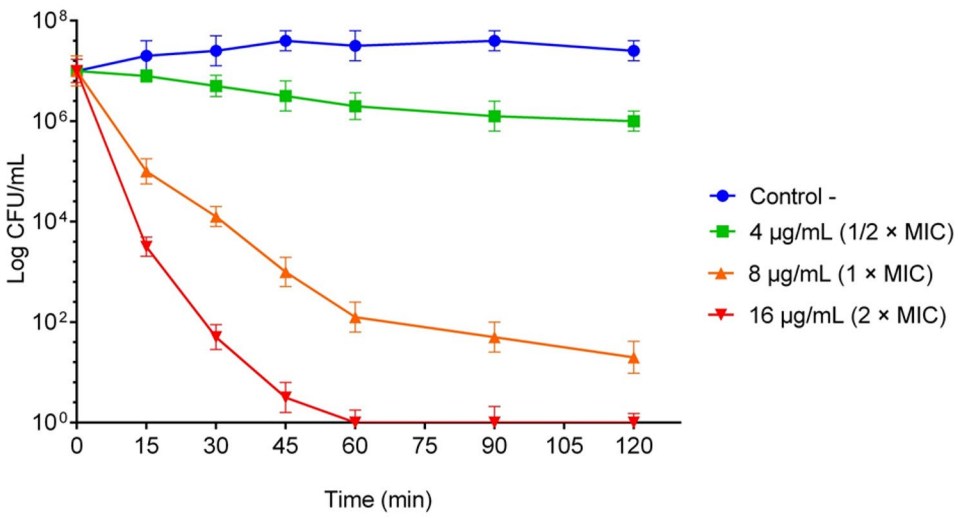

Fig. 1 Bacterial killing kinetics. Time kill kinetics of Dendrocin-ZM1 against *S. aureus* ATCC 25,923. Bacterial cells (2×10^5 CFU/mL) suspensions were treated with different concentrations of Dendrocin-ZM1 (4–16 µg/mL). Data presented as means (± SD) of three independent repeats in triplicate cells

Table 1 MIC values of Dendrocin-ZM1 against *S. aureus* 25,923 in the presence of physiological salts

Peptides	MIC (μg/mL)	NaCl (150 mM)	KCl (4.5 mM)	$MgCl_2$ (2 mM)	$CaCl_2$ (1 mM)	$ZnCl_2$ (8 mM)	$FeCl_3$ (4 mM)
Dendrocin-ZM1	8	8	8	8	16	4	16

The data were derived from a representative value of three independent experimental trials

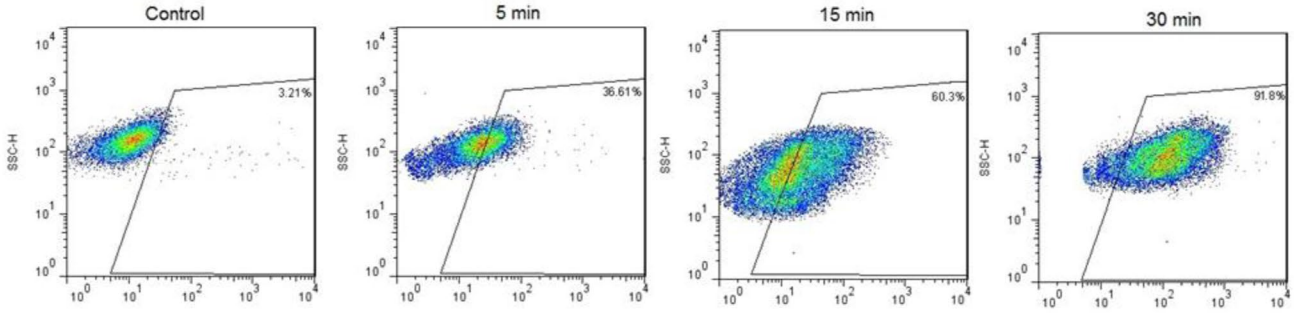

Fig. 2 Effect of Dendrocin-ZM1 on the cell membrane permeability of *S. aureus* ATCC 25,923 measured by PI uptake. Flow cytometric analysis of membrane permeabilization assay by PI uptake. Approximately 1×10^6 CFU /mL cells were incubated with Dendrocin-ZM1 (16 μg/mL) for 5, 15 and 30 min

Cytoplasmic Membrane Disruption Assay

To investigate the killing mechanism of Dendrocin-ZM1 against *S. aureus* ATCC 25,923, its effect on cell membrane disruption and permeabilization was examined. Flow cytometry and Propidium Iodide, a DNA intercalating dye, were used to determine the permeabilization effect of Dendrocin-ZM1 on the cytoplasmic membrane. This dye is selectively absorbed by cells with damaged membranes, attaches to nucleic acids, and emits a fluorescent signal.

As shown in Fig. 2, treatment with Dendrocin-ZM1 at a concentration of 16 μg/mL caused prompt permeabilization of the bacterial membrane. Within 5 min, there was a noticeable increase in PI fluorescence for *S. aureus* ATCC 25,923 compared to the control group that did not receive the peptide treatment. These results indicate that Dendrocin-ZM1 disrupts the membrane and permeabilizes the outer membrane of *S. aureus* ATCC 25,923.

NPN, a hydrophobic fluorescent reagent, was used to investigate bacterial cell membrane destruction. NPN uptake increases with damage to the bacterial membrane. As shown in Fig. 3a, Dendrocin-ZM1 increased NPN uptake within 5 min, resulting in significantly higher fluorescence intensity compared to the control. These results suggest that Dendrocin-ZM1 destroys the bacterial membrane.

Bacterial cytoplasmic membrane depolarization caused by Dendrocin-ZM1 was confirmed using DisC3-5, a cationic fluorescent probe. Fluorescence from DiSC3-5 is detectable upon depolarization of the cell membrane. As shown in Fig. 3b, the depolarization induced by various concentrations of Dendrocin-ZM1 was observed for a duration of 60 min. The fluorescence exhibited a continuous increase up to the 30 min, after which it stabilized and remained constant until the final observation at the 60 min. At the MBC of Dendrocin-ZM1, the intensity of fluorescein was significantly greater compared to its MIC. These results demonstrate that Dendrocin-ZM1 has a concentration-dependent effect on the polarization status of the *S. aureus* cell membrane.

Leakage of Intracellular Contents

The intracellular ATP level of *S. aureus* was determined. As shown in Fig. 4a, after 5 min, Dendrocin-ZM1 released 75% and 92% of ATP at concentrations of 8 μg/mL (1 × MIC) and 16 μg/mL (2 × MIC), respectively. The release of ATP by Dendrocin-ZM1 was found to be dose-dependent.

Figure 4b illustrates the release of *S. aureus* DNA/RNA (260 nm absorbing materials). After 5 min of incubation with Dendrocin-ZM1, the release of *S. aureus* DNA/RNA was significantly elevated compared to the negative control. The release of DNA/RNA by Dendrocin-ZM1 was also found to be dose-dependent.

TEM Observations

To gain a more comprehensive visual understanding of the mechanism of action of Dendrocin-ZM1 on *S. aureus*, TEM was utilized to observe the morphological changes and internal structure of *S. aureus* after 30 min of treatment with Dendrocin-ZM1. As shown in Fig. 5a, the surface of untreated *S. aureus* appeared smooth and bright, with an intact structure and complete contents. In contrast, the cell membrane of *S. aureus* exposed to Dendrocin-ZM1 exhibited significant

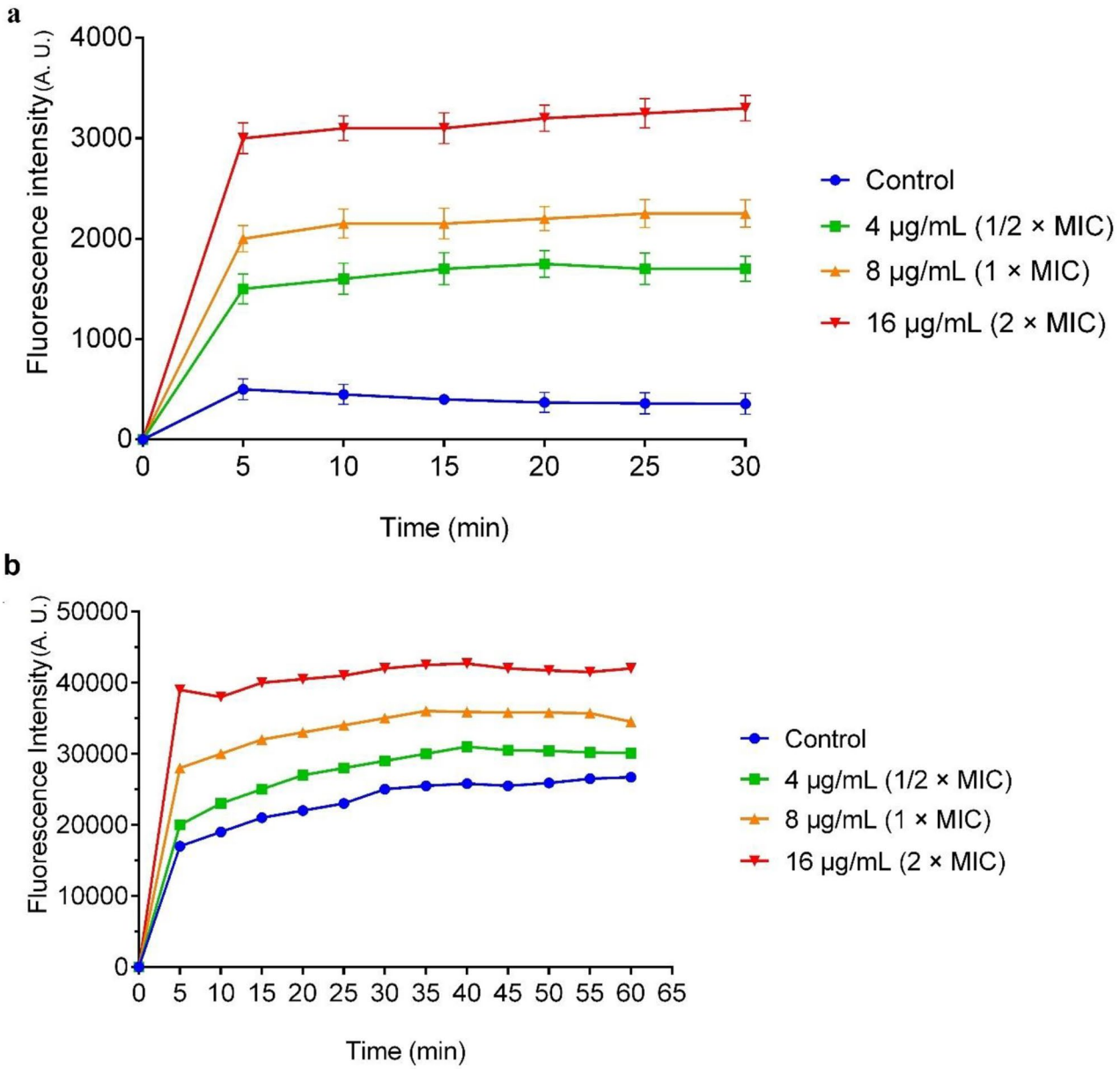

Fig. 3 (a) Uptake of NPN by *S. aureus* ATCC 25,923 treated with Dendrocin-ZM1 at different concentrations. (b) Cytoplasmic membrane depolarization of *S. aureus* ATCC 25923by Dendrocin-ZM1, as assessed by release of the membrane potential-sensitive dye DiSC3-(5) measured spectroscopically at 620 to 670 nm excitation and emission wavelength, and corresponding bacterial survival as determined by plate counts. Data presented as means (±SD) of three independent repeats in triplicate cells

damage, along with leakage of cell contents and disruption of the structure (Fig. 5b). These experimental findings demonstrate that Dendrocin-ZM1 enhances the permeability of Propidium Iodide (PI), alters the integrity of the cellular membrane, and facilitates the release of intracellular contents, ultimately resulting in a bactericidal effect.

Discussion

S. aureus is capable of inducing a diverse spectrum of infections, some of which may result in organ dysfunction and even mortality in severe cases (Assis et al. 2017; Guo et al. 2020; Tong et al. 2015). This microorganism is prevalent in both community and hospital settings globally, making it one of the most frequently encountered pathogens. The increasing and rapid dissemination of multidrug-resistant (MDR) *S. aureus* strains has presented significant challenges

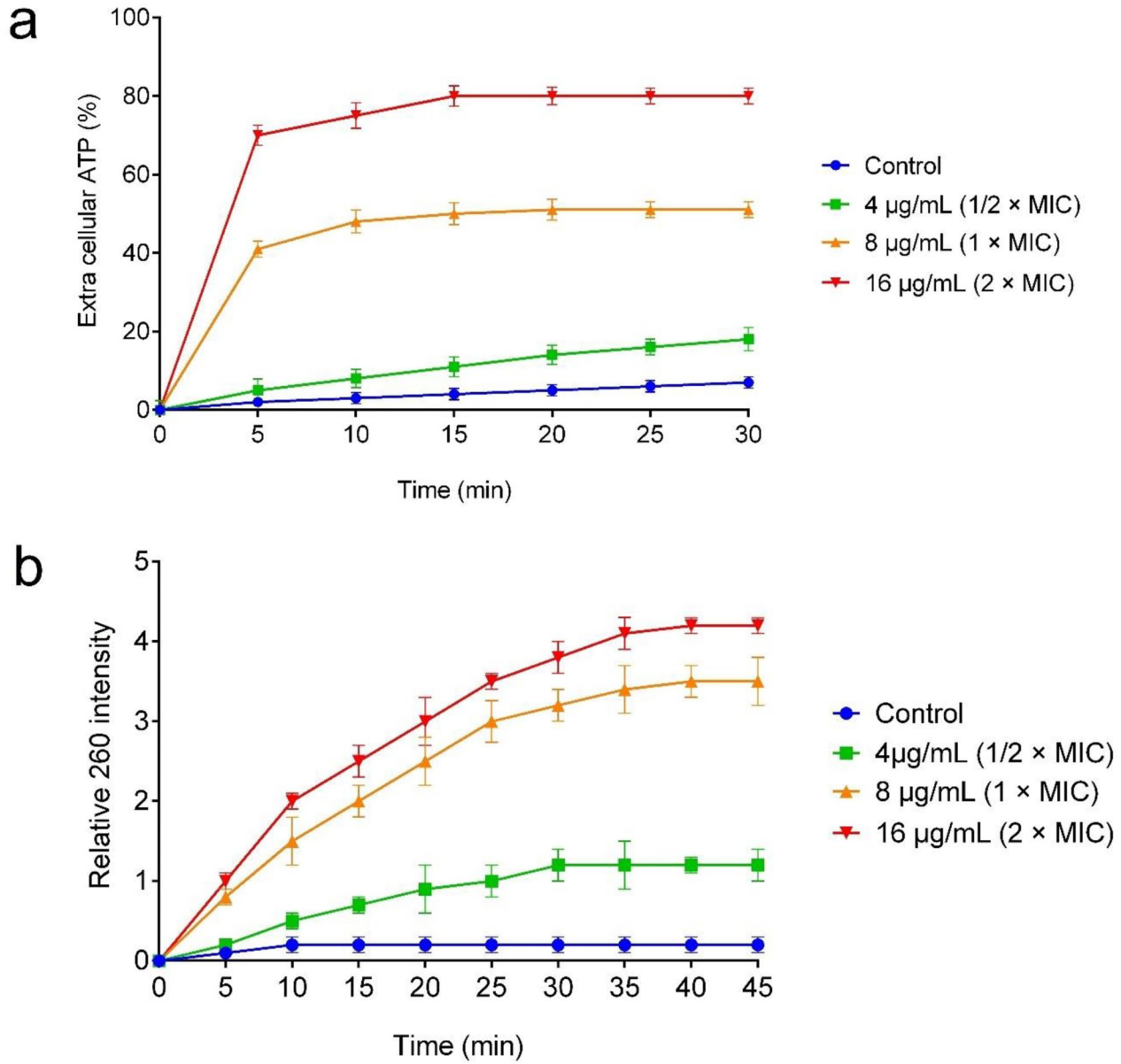

Fig. 4 (a) Leakage of ATP. The leakage of cellular ATP (in percentage) from *S. aureus* ATCC 25,923 after treatment with different concentrations (4–32 µg/mL) of Dendrocin-ZM1 shown at X axis. (b) Release of DNA/RNA from *S. aureus* ATCC 25,923 due to action of Dendrocin-ZM1 at different concentrations (4–16 µg/mL) determined spectroscopically at OD260 nm. Data presented as means (±SD) of three independent repeats in triplicate compared with buffer-treated control

in clinical treatment (Guo et al. 2020; Tong et al. 2015). According to reports, individuals affected by infections caused by MDR *S. aureus* strains are at a heightened clinical risk, particularly in terms of mortality (Tong et al. 2015).

Thus, it is imperative to enhance preventive measures against MDR *S. aureus* infection, explore effective options for antibiotics, and discover therapeutic alternatives. Antimicrobial peptides (AMPs), known for their strong antibacterial activity against various pathogens, have a high potential to replace antibiotics in the treatment of infections caused by MDR strains (Kluytmans and Wertheim 2005; Lei et al. 2019; Luo and Song 2021). In this study, we demonstrate that Dendrocin-ZM1, a newly discovered AMP derived from *Zataria multiflora Boiss*, induces changes in the membrane structure of *S. aureus*, leading to the release of intracellular contents and providing potent antibacterial efficacy. Despite ongoing research efforts, the exact mechanism of action of AMPs remains incompletely understood. However, most researchers agree that the bactericidal effect of AMPs primarily involves the creation of pores on the bacterial surface, disruption of the membrane structure, and

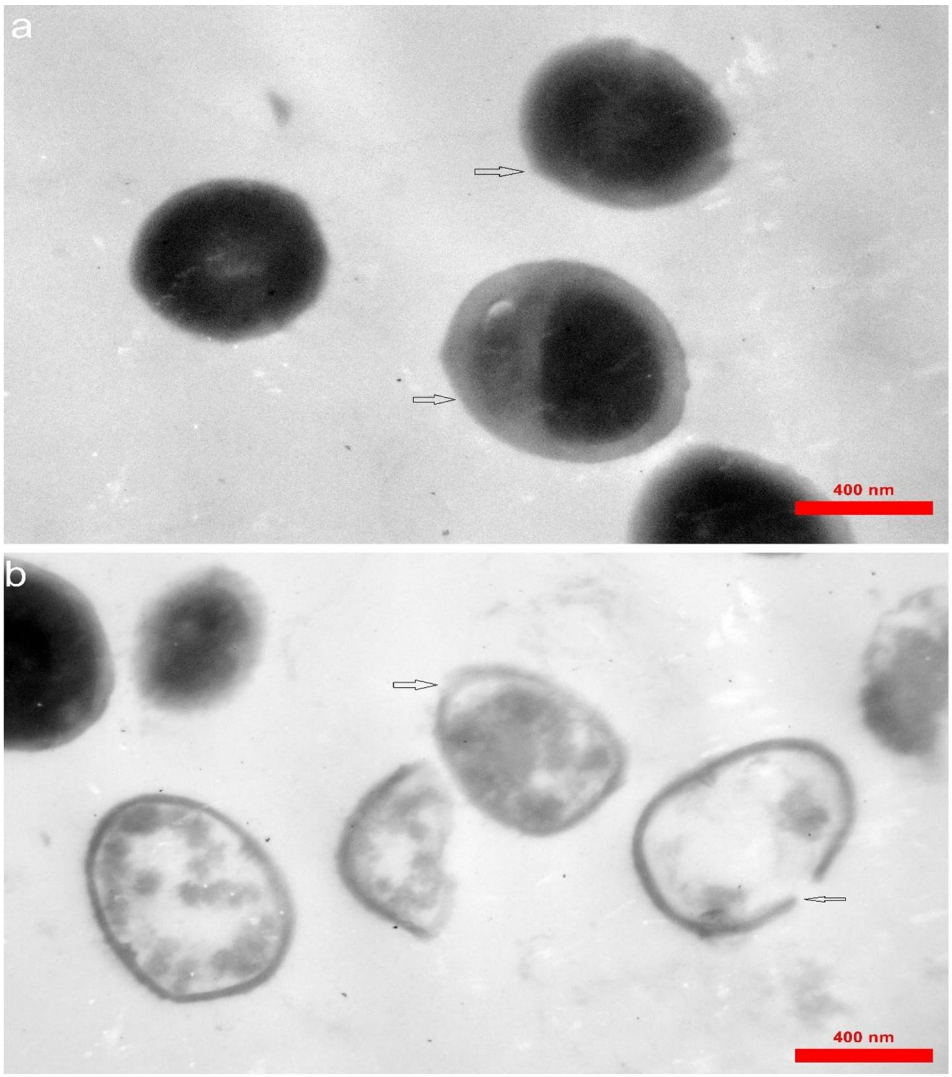

Fig. 5 Transmission Electron microscopic analysis. Transmission electron microscopic micrographs of treated with Dendrocin-ZM1. (**a**) Untreated *S. aureus* ATCC 25,923 as control. These arrows indicate intact septa. (**b**) Treated *S. aureus* ATCC 25,923 after 30 min exposed to the 16 µg/mL of Dendrocin-ZM1. These arrows indicate damage of cytoplasmic membrane and cell membrane disintegration and dispersion of intracellular contents

subsequent release of cell contents, ultimately resulting in bacterial death (Rima et al. 2021; Lei et al. 2019).

Reports suggest that AMPs have the ability to enhance membrane permeability, modify cellular membrane integrity, and induce ion release (Huang and Charron 2017). Furthermore, AMPs can penetrate cellular membranes, interact with DNA, promote bacterial cell death, and interfere with biofilm formation (Erdem Büyükkiraz and Kesmen 2022; Huang and Charron 2017; Luo and Song 2021). Our study demonstrates that Dendrocin-ZM1 exhibits bactericidal properties against MDR bacterial strains. This effect is achieved through disruption of the cell membrane structure, increased membrane permeability, alteration of bacterial membrane potential, destruction of the bacterial membrane, and subsequent leakage of cellular contents, ultimately leading to bacterial death. The use of Dendrocin-ZM1 resulted in an increase in fluorescence intensity of diSC3-5, indicating depolarization of the cytoplasmic membrane.

The findings obtained from TEM analysis revealed that Dendrocin-ZM1 induced significant morphological changes in *S. aureus* and cellular membrane disruption was observed, leading to the release of intracellular contents. Additionally, the uptake of the PI dye into the bacterial cytosol and the rapid increase in PI fluorescent signaling suggest that the bactericidal mechanism of Dendrocin-ZM1 involves compromising cellular membrane integrity.

To develop AMPs as viable treatment options, it is imperative to address several limiting factors. The sensitivity of AMPs to salt is a significant limitation in their potential application as therapeutic agents (Park et al. 2004). The efficacy of AMPs may be influenced by the presence of salt cations (Kwon et al. 2019). The presence of cations results in a reduction of the electrostatic interaction between the peptides with positive charge and the bacterial surface with negative charge, ultimately leading to a decrease in antimicrobial activity (Cantor et al. 2019).

To ensure that Dendrocin-ZM1 remains effective even in the presence of higher levels of cations, we conducted tests using higher concentrations of salts. The results, shown in Table 1, revealed no difference in the minimum inhibitory concentration (MIC) of Dendrocin-ZM1 when exposed to Na^+, K^+, and Mg^+. However, when a physiological concentration of Ca^{2+} and Fe^{3+} was present, we observed a one-fold increase in the MIC of Dendrocin-ZM1. Dendrocin-ZM1 exhibits a synergistic response when combined with Zn^{2+}, leading to a notable enhancement in its activity.

The exceptional ability of Dendrocin-ZM1 to withstand high salt levels can be attributed to its specific composition of amino acids. Dendrocin-ZM1 consists of tryptophan and arginine residues, which are known to enhance antimicrobial activity even in challenging salt conditions (Chan et al. 2006; Deslouches et al. 2013). Research findings indicate that the inclusion of Ca^{2+} and Mg^{2+} in the growth medium leads to a significant reduction of up to 80% in bactericidal activity (Qian et al. 2018).

In summary, a systematic evaluation was conducted to determine the antibacterial mechanism of Dendrocin-ZM1, with a focus on its effects on *S. aureus*. It was found that Dendrocin-ZM1 has the ability to disrupt the integrity and later the potential of the cell membrane, subsequently leading to the release of ATP and DNA, which ultimately results in cellular demise. Furthermore, Dendrocin-ZM1 exhibited a notable degree of biocompatibility and maintained stability in the presence of salt. Given the significant antibacterial properties exhibited by Dendrocin-ZM1, it presents a promising candidate for therapeutic applications in the treatment of clinically severe bacterial infections. The results of this study demonstrate that Dendrocin-ZM1 exhibits notable efficacy against *S. aureus*, thereby establishing a foundation for further investigation and innovation in the field of antibacterial therapeutics.

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Author Contributions S.S.S., M.R.A., and M.G. conducted the experiments. S.S.S., M.R.A., and M.G. performed the data analysis. S.S.S. and M.G. conceived and designed the experiments, and prepared the manuscript. P.B. prepared all figures and tables and wrote the main manuscript text. M.G. was the project leader. All authors have read and agreed the final manuscript.

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Data Availability Data will be made available on request.

Declarations

Competing interests The authors declare no competing interests.

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