

Antibacterial activity and underlying mechanism of Meconopsis quintuplinervia Regel extract against the acne-causing bacteria Propionibacterium acnes and Staphylococcus aureus

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Abstract

The aim of this study was to evaluate the antibacterial activity and underlying mechanism of ethanol extracts of *Meconopsis quintuplinervia* Regel (EMQ) against the acne-causing bacteria *Propionibacterium acnes* and *Staphylococcus aureus*. The diameter of the inhibition zone (DIZ), minimum inhibitory concentration (MIC), and minimum bactericide concentration (MBC) were determined and growth curves were generated to assess the antibacterial activity of EMQ. The antibacterial mechanism was explored based on morphological observations, staining of the cell membrane and wall, protein analysis, and biofilm formation. The study results indicated that EMQ was an effective antibacterial agent against *P. acnes* and *S. aureus*, with a DIZ of 14.5 and 13.2 mm, MIC of 12.5 and 12.5 mg/mL, and MBC of 100 and 50 mg/mL, respectively. EMQ induced morphological changes to bacterial cells, as determined by electron microscopy. Leakage of alkaline phosphatase and nucleic acids confirmed that EMQ compromised the membrane integrity of bacterial cells. Furthermore, protein analysis revealed that EMQ hindered total protein expression and lowered adenosine triphosphatase activity, while crystal violet staining revealed suppressed biofilm production. Bacterial adhesion analysis demonstrated that EMQ lowered the adhesive capacity of bacterial cells. Collectively, these findings revealed high antibacterial activity of EMQ for treatment of acne vulgaris.

Important Notes

This study was to evaluate the antibacterial activity and underlying mechanism of EMQ against the acne-causing bacteria *Propionibacterium acnes* (*P. acnes*) and *Staphylococcus aureus* (*S. aureus*). EMQ induced morphological changes to bacterial cells, as determined by electron microscopy. Leakage of alkaline phosphatase and nucleic acids confirmed that EMQ compromised the membrane integrity of bacterial cells. Furthermore, protein analysis revealed that EMQ hindered total protein expression and lowered adenosine triphosphatase activity, while crystal violet staining revealed suppressed biofilm production. Bacterial adhesion analysis demonstrated that EMQ lowered the adhesive capacity of bacterial cells. Collectively, these findings revealed high antibacterial activity of EMQ for treatment of acne vulgaris.

1 Introduction

Acne vulgaris (AV) is a chronic pilosebaceous inflammatory skin condition characterized by the appearance of blackheads, papules, pustules, nodules, and cysts on the face, chest, and back (Hazari N 2021; Kurokawa I et al. 2020). With an estimated global prevalence of 9.4%, AV is the eighth most prevalent disease worldwide (Heng AHS et al. 2020). Despite the ability to self-heal, AV can leave scars and pigmentary changes that can last a lifetime (Connolly D et al. 2017).

AV is mainly caused by overcolonization of *Propionibacterium acnes* (*P. acnes*) and *Staphylococcus aureus* (*S. aureus*) and subsequent inflammation (Kurokawa I et al. 2021). *P. acnes* stimulates Langerhans cells, infundibular keratinocytes, and sebocytes via Toll-like receptor 2, leading to the production of

interleukin (IL)-12, IL-8, IL-6, and interferons, resulting in the formation of inflammatory lesions, such as papules and pustules(Li Y et al. 2018; Su Y et al. 2020). Overcolonization of *P.acnes* and *S.aureus* is involved in the occurrence of AV and associated inflammation *via* the release of extracellular toxins and enzymes(Poomanee W et al. 2018). Therefore, inhibition of the growth of *P. acnes* and *S. aureus* is considered the most efficacious treatment of AV(Marson JW et al. 2019).

Currently, antibiotics for the treatment of AV mainly include erythromycin, tetracycline, and clindamycin(Xu H et al. 2019). However, these antibiotics can cause irritation and drying of the skin, immunological hypersensitivity, organ damage, and photosensitivity(Weiner DM et al. 2021). In addition, long-term antibiotic treatment can lead to bacterial resistance, rendering some medications ineffective against AV(Karadag AS et al. 2021; Aslan Kayiran M et al. 2020). Therefore, the development of plant-based antibacterial drugs with better safety and lower drug resistance has important practical significance for treatment of AV(Winkelman WJ et al. 2018).

Meconopsis quintuplinervia Regel (MQ) is a perennial herb used as a traditional folk medicine in Tibet, China, for treatment of a variety of ailments, including headache, hepatitis, pneumonia, and edema. MQ, which is mainly distributed in Qinghai, Shanxi, Tibet, Gansu, and other regions of China, contains various bioactive components, including alkaloids, flavonoids, and volatile oils(Xu B et al. 2019). In addition, extracts of MQ (EMQ) contain the antibacterial substances quercetin and luteolin and these two substances have been used to inhibit various bacteria, such as *Escherichia coli*, *Bacillus subtilis*, *Salmonella* and *Enterococcus faecalis*, etc.(Guo Y et al. 2020; Wang S et al. 2018). However, the antimicrobial effectiveness of EMQ against *P. acnes* and *S. aureus* remains unclear.

Therefore, the aim of the present study was to assess the antibacterial effect and underlying mechanism of EMQ against *P. acnes* and *S. aureus* *via* analysis of cell morphology, staining of the cell membrane and wall, protein analysis, and biofilm production to provide a scientific foundation for the development of herb-based antibacterials for treatment of AV.

2 Materials And Methods

2.1 Materials

P. acnes strain source GDMCC 1.243 and *S.aureus* strain source GDMCC 1.1220 were purchased from Guangdong Microbial Culture Collection Center. (Guangzhou, China). BHI Broth medium, Tryptic Soy Broth medium, Reinforced Clostridium Agar medium, Tryptic Soy Agar (TSA) medium were purchased from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). 2.5L Round Bottom Vertical Anaerobic Culture Bag, 2.5L Anaerobic Gas Generating Bag were purchased from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). The AKP enzyme assay kit and ATP content determination kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2 Strain activation and suspension preparation

Using a sterile syringe, lyophilized bacteria were gently resuspended in liquid culture media in seeding tubes. *P. acnes* was cultivated in 100 mL of tryptic soy broth for 48 h at 37 °C in an anaerobic gas-generating bag, while *S. aureus* was cultivated in 100 mL of brain heart infusion growth medium at 37 °C for 24 h. For the following assays, the bacterial suspensions were diluted with phosphate-buffered saline (PBS) to appropriate concentrations.

2.3 Preparation of EMQ

MQ was provided and authenticated by Professor Zhuoma Dongzhi (Medical College, Tibet University, China) in August 2020. The certificate sample (No.CBTM-E124) is kept in the Herbarium of Pharmacy Department of Qingdao University of Science & Technology. Pulverized leaves of MQ were extracted twice with 70% EtOH under reflux for 1.5 h each round. The obtained liquid was filtered with filter paper, and the obtained extractive solution was filtered and evaporated in vacuum to obtain extract powder. For antibacterial experiments, ethanol extract was dissolved in dimethyl sulfoxide (DMSO), and then kept at -20 °C for subsequent studies.

2.4 Agar diffusion assay

The antibacterial activity of EMQ was determined based on the agar diffusion method. Briefly, 20 mL of reinforced Clostridium agar medium (for cultivation of *P. acnes*) or tryptic soy agar medium (for cultivation of *S. aureus*) were added to Petri dishes, allowed to solidify, and then uniformly coated with 100 µL of the appropriate bacterial suspension. Afterward, a sterilized steel rod was used to punch holes in the test plate, then 80 µL of the bacterial suspensions were injected through the holes and the plates were incubated for 24 h at 37 °C for *S. aureus* or 48 h for *P. acnes*, which was cultured in an anaerobic gas-generating bag. Finally, the diameter of the inhibition zone (DIZ) was calculated to determine the antibacterial activity of EMQ.

2.5 Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC)

EMQ was diluted with liquid medium to concentrations of 0.78–100 mg/mL by the double dilution method. Then, 100 µL of the microbial suspensions at 10^7 colony-forming units (CFU)/mL were incubated at 37 °C. The MIC was calculated as the EMQ concentration required to inhibit bacterial growth. Next, 100 µL of the bacterial suspension were collected from each tube without visible bacterial growth and cultured on agar plates at 37 °C. The MBC was calculated as the concentration of EMQ in the medium that inhibited bacterial growth.

2.6 Bacterial growth curves

Bacterial growth curves were generated to assess the antibacterial activities of EMQ. Briefly, both strains (10^7 CFU/mL, 0.1 mL) were inoculated into 100 mL of sterile medium containing EMQ at MICs of 0.5 and 1.0. Cultures of *P. acnes* and *S. aureus* in aseptic distilled water without EMQ were used as controls. The

bacterial suspensions were incubated at 37°C and growth curves were generated from the optical density at 600 nm (OD₆₀₀) at 0, 2, 4, 6, 8, 10, 12, 14, 24, and 30 h.

2.7 Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

SEM was used to investigate alterations to the bacterial cell surface after treatment with EMQ at MICs of 0.5 and 1.0. After 12 h of EMQ treatment, the bacterial cells were pelleted by centrifugation at 4500 rpm for 10 min, then washed twice with PBS, fixed in 2.5% glutaraldehyde for 24 h, and washed twice again with PBS, followed by a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100%) for 15 min at each concentration. Afterward, the ethanol was replaced with isoamyl acetate as an intermediate medium. The dehydrated samples were freeze-dried and stored as a powder. Finally, images of all samples were obtained using a field emission scanning electron microscope (JSM-6700F; JEOL, Ltd., Tokyo, Japan).

TEM was used to observe alterations to the cytoplasm and membrane of bacterial cells after treatment with EMQ at MICs of 0.5 and 1.0. The bacteria were pretreated as described above for SEM. Finally, images of all samples were obtained using a transmission electron microscope (HT7800; Hitachi High-Technologies Corporation, Tokyo, Japan).

2.8 Extracellular alkaline phosphatase (AKPase) analysis

Bacterial cells were treated with EMQ at MICs of 0.5 and 1.0 for 12 h, then centrifuged for 10 min at 4500 rpm. The supernatants were collected for analysis. An untreated control group was included for comparisons. AKPase activity was measured using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the manufacturer's instructions.

2.9 Measurement of nucleic acid leakage

Bacterial cells were treated with EMQ at MICs of 0.5 and 1.0 and subsequently incubated for 12 h at 37°C. During the incubation period, 1 mL of each culture was collected every 2 h and centrifuged at 12000 rpm for 10 min. Then, the supernatants were collected and the OD₂₆₀ was measured using a microplate reader.

2.10 Analysis of bacterial proteins

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to assess the effect of EMQ on bacterial proteins. Briefly, bacterial cells (10⁷ CFU/mL) were treated with EMQ at an MIC of 1.0 for 3, 6, 9, and 12 h, respectively, at 37 °C while untreated cells were utilized as negative controls. At the indicated times, 2 mL of the cultivation media were collected and centrifuged for 10 min at 4°C. The harvested cell pellets were resuspended in PBS, heated for 5 min at 95°C, and centrifuged. The supernatant was collected for SDS–PAGE with the use of a 5% stacking gel and a 10% separating gel, which were stained with 0.1% Coomassie Brilliant Blue R250 and destained with glacial acetic acid and

methanol in distilled water. The decolorized gels were imaged using a Gel Documentation & Analysis Systems (WD-9413B; Beijing Liuyi Biotechnology Co., Ltd., Beijing, China).

2.11 Measurement of intracellular adenosine triphosphatase (ATPase) concentrations

Bacterial cells (10^7 CFU/mL) were treated with EMQ at MICs of 0.25, 0.5, 1.0, and 2.0 for 12 h at 37°C, while untreated cells were employed as negative controls. The protein extraction method is described in section 2.9. ATPase activity was measured using a commercial kit (Nanjing Jiancheng Bioengineering Institute) in accordance with the manufacturer's instructions.

2.12 Crystal violet (CV) staining

Biofilm formation was assessed by staining with CV. Bacterial cells (10^5 CFU/mL) were dispensed into the wells of a 96-well plate and treated with EMQ at MICs of 0.25, 0.5, 1.0, and 2.0 for 24 h at 37 °C. Untreated cells were included as negative controls. After 24 h, planktonic cells and spent growth media were removed from each well and the plate was washed three times with sterile water. Biofilms were then stained with 150 μ L of 0.1% CV for 15 min at room temperature. The plate was washed with sterile water to remove the excess dye and then air-dried. Absorbance of the CV solubilized in 150 μ L of 95% ethanol was measured at an OD₅₉₅ using a microplate reader.

2.13 Effect of EMQ on bacterial adhesion

Bacterial suspensions (100 μ L) in the wells of a 96-well plate were cultured overnight in culture medium supplemented with 2% glucose, followed by an equivalent amount of culture medium containing EMQ at MICs of 0.25, 0.5, 1.0, and 2.0. Untreated cells were used as control. After incubation at 37 °C for 4 h, the plates were rinsed with sterile water. Then, planktonic cells were removed and the OD₆₀₀ was measured to determine the number of cells adhering to the wells.

2.14 Statistical analysis

All data are displayed as the mean and standard deviation. The differences between the two groups were analysed by a double-tailed T test. When $p < 0.05$, there was a significant difference between the groups.

3 Results

3.1 Determination of DIZ, MIC, and MBC of EMQ against *P. acnes* and *S. aureus*

As indicators of antibacterial activity, the DIZ, MIC, and MBC of EMQ against *P. acnes* and *S. aureus* were 14.5 and 13.2 mm, 12.5 and 12.5 mg/mL, and 100 and 50 mg/mL, respectively (Fig. 1, Table 1). These results demonstrate that EMQ had an inhibitory effect on both bacterial species.

Table 1

MICs and MBCs against *P.acnes* and *S.aureus* for EMQ .

Microbial strain	<i>P.acnes</i>		<i>S.aureus</i>	
	MIC(mg/ml)	MBC(mg/ml)	MIC(mg/ml)	MBC(mg/ml)
EMQ	12.5	100	12.5	50

3.2 Effects of EMQ on bacterial growth

As further verification of the antibacterial activities of EMQ, growth curves of *P. acnes* and *S. aureus* cells treated with different concentrations of EMQ were generated. As shown in Fig. 2, the OD₆₀₀ of the control group increased rapidly, while that of *P. acnes* remained relatively unchanged after about 14 h and that of *S. aureus* remained constant for about 12 h, primarily due to the bacterial life cycle. However, as compared to the control group, the OD₆₀₀ of cells treated with EMQ at an MIC of 0.5 was considerably decreased and remained at a value of about 0.2 at an MIC of 1.0, indicating inhibited growth of the bacterial cells.

3.3 SEM observations

Morphological changes to bacterial cells treated with EMQ and untreated control cells were observed by SEM. As shown in Fig. 3A(a) and B(a), the untreated control cells had regular morphologies, smooth surfaces, and good refraction. *P. acnes* cells treated with EMQ displayed major deformations and damage (Fig. 3A(b) and A(c)), while *S. aureus* cells had obvious vesicle formation and irregular surface processes (Fig. 3B(b) and B(c)). Overall, morphological changes and damage to the cells increased with the concentration of EMQ. SEM demonstrated that EMQ damaged the bacterial wall and membrane, thereby allowing the release of proteins, nucleic acids, and other biomolecules from the cytoplasm.

3.4 TEM observations

TEM verified alterations to the structures of bacterial cells treated with EMQ, while the control cells had intact membranes entirely surrounding the cytoplasm (Fig. 4A(a) and B(a)). EMQ caused various abnormalities, including separation of the cytoplasmic membrane from the cell wall, lysis of the cell wall, leakage of the cytoplasm, and morphological deformations (Fig. 4A(b), A(c), B(b), and B(c)). Notably, the degree of cell damage increased with the concentration of EMQ. The TEM observations confirmed that EMQ damaged the cell membrane and organelles of *P. acnes* and *S. aureus*, resulting in leakage of the cytoplasm.

3.5 Effects of EMQ on the cell wall and membrane

AKPase is located between the cell wall and membrane. Considerable leakage of intracellular AKPase suggests damage to the cell wall. The OD₅₂₀ was used to calculate the quantity of AKPase leaked from *P. acnes* (Fig. 5A) and *S. aureus* (Fig. 5B) cells. At 5 h, the OD₅₂₀ of *P. acnes* increased from 0.014 (control) to 0.166 (MIC of 0.5) and 0.232 (MIC of 1.0), while that of *S. aureus* increased from 0.018 (control) to 0.182 (MIC of 0.5) and 0.243 (MIC of 1.0). These findings confirmed that EMQ damaged the cell wall.

Measurement of the release of macromolecular components, such as nucleic acids, is a useful strategy to assess membrane permeability and cellular wall integrity. The OD₂₆₀ was measured to assess the amount of nucleic acids leaked from *P. acnes* (Fig. 6A) and *S. aureus* (Fig. 6B) cells. At 12 h, the OD₂₆₀ of *P. acnes* increased from 0.02 (control) to 0.307 (MIC of 0.5) and 0.480 (MIC of 1.0), while that of *S. aureus* increased from 0.042 (control) to 0.339 (MIC of 0.5) and 0.485 (MIC of 1.0). These findings clearly indicate that EMQ damaged the cell wall and membrane of *P. acnes* and *S. aureus*.

3.6 Effects of EMQ on the proteins of *P. acnes* and *S. aureus*

Proteins are critical to metabolism of bacterial cells. SDS-PAGE was used to determine changes to the protein profiles of *P. acnes* (Fig. 7A(a)) and *S. aureus* (Fig. 7A(b)) cells treated with EMQ at an MIC of 1.0 for 12 h. The protein bands of the control cells of both bacteria were comparatively clearer and more plentiful than those of cells treated with EMQ. These results demonstrate that EMQ hindered the proliferation and protein production of bacterial cells.

Cellular energy levels are inextricably linked to cell development and metabolism. Adenosine triphosphate (ATP) is essential to energy metabolism of cells and concentrations signify the degree of cellular growth. Therefore, the ATP content can be used to determine the effectiveness of bacteriostatic/bactericidal agents. The intracellular ATP concentrations of *P. acnes* and *S. aureus* cells treated with EMQ at MICs of 0.25, 0.5, 1.0, and 2.0 are shown in Fig. 7B(a) and 7B(b), respectively. The results show that EMQ suppressed cell growth by reducing ATP generation.

3.7 Effects of EMQ on biofilm formation

Changes to biofilm formation by *P. acnes* and *S. aureus*, as detected by staining with CV, are shown in Fig. 8A(a) and 8(b), respectively. As compared to the control cells, biofilm formation was significantly inhibited in cells treated with EMQ at all tested levels. Notably, the inhibitory effect of EMQ increased with the concentration. These findings suggest that EMQ specifically inhibited biofilm production by *P. acnes* and *S. aureus*.

The capability of a bacterium to attach to a substrate or surface is crucial for biofilm formation and, thus, presents a potential target to prevent biofilm formation. The anti-adhesive properties of *P. acnes* and *S. aureus* are shown in Fig. 8B(a) and 8B(b), respectively. The results show that EMQ at MICs of 0.25 and 2.0 significantly reduced the capability of *P. acnes* and *S. aureus*, respectively, to adhere to solid surfaces.

4 Discussion

The antibacterial activity of natural products has drawn considerable attention in recent years(Dai J et al. 2020). For instance, the MIC and MBC of an ethanol extract of *Angelicae dahuricae* against *P. acnes* and *S. aureus* are reportedly 50 and 12.5 mg/mL, and 100 and 25 mg/mL, respectively(Li QL et al. 2021). The MIC of a water extract of *Zanthoxylum bungeanum* is reportedly 100 mg/mL against both *P.acnes* and *S.aureus*(Wang Y et al. 2017). In the present study, the antibacterial indices DIZ, MIC, and MBC, as well as growth curves, were utilized to evaluate the antibacterial activity of EMQ against *P.acnes* and *S.aureus*. The results showed that the MIC, MBC, and DIZ of EMQ against *P. acnes* and *S. aureus* were 12.5 and 12.5 mg/mL, 100 and 50 mg/mL, and 14.5 and 13.2 mm, respectively, demonstrating that EMQ has a significant inhibitory effect against *P. acnes* and *S. aureus*, similar to other natural products.

A growing body of evidence indicates that the mechanisms underlying the capability of natural compounds to inhibit the proliferation of bacterial cells involves morphological damage and leakage of intracellular components(Zeng Y et al. 2021). In the present study, SEM and TEM were used to observe morphological changes to *P. acnes* and *S. aureus* cells treated with EMQ. The findings revealed that EMQ caused severe morphological changes, including cell collapse, leakage of intracellular contents, and cell disintegration, demonstrating the effect of EMQ on the bacterial cell wall and membrane, thereby laying groundwork for further research into the mechanisms of herb-derived antibacterials.

The bacterial cell wall and membrane are critical to enclose the cell interior and maintain a reasonably stable internal environment to facilitate metabolic events(Zhang Y et al. 2021). Leakage of the cytoplasmic contents is a hallmark of disruption to the bacterial membrane(Wang S et al. 2018). AKPase leakage is an established marker of damage to the cell wall and membrane(Zhou Y et al. 2021). Likewise, nucleic acids in the cytoplasm of bacterial cells are released by disruption to the permeability of the cell membrane(Gao F et al. 2020). In this study, leakage of AKPase and nucleic acids was adopted as an indicator of the integrity and permeability of the cell wall and membrane. The levels of AKPase and nucleic acids in the extracellular environment increased after EMQ treatment, thereby demonstrating damage to the cell wall and membrane, as verified by SEM and TEM.

Bacterial proteins, as vital cell components, are tightly linked to important physiological functions and, thus, are useful indicators of cell metabolism(Wang S et al. 2021). Previous studies have used changes in bacterial protein profiles to investigate the mechanisms underlying the antibacterial activities of various natural products(Li JF et al. 2020). The SDS-PAGE results in this study showed that the protein bands of *P. acnes* and *S. aureus* cells were significantly darker or even vanished after treatment with EMQ, illustrating that protein synthesis was inhibited and the metabolic capacity of the cells was decreased. ATP is a critical component of intracellular energy production and required for cell development, metabolism, and proliferation. Hence, ATP levels are sensitive to changes in energy metabolism(Jia Y et al. 2021). The results of the present study showed that EMQ severely inhibited the production of ATP, which led to a significant decrease in cell viability. These findings suggest that EMQ inhibits bacterial growth by interfering with ATP production and, subsequently, protein synthesis.

Biofilm not only acts as a barrier to create a stable internal environment of cells, but also mediates transmission of signals among cells and substrates, transmembrane transport, and energy conversion, and plays a key role in maintaining the physiological functions of bacterial cells(Han Q et al. 2021). Therefore, the capability of biofilm to inhibit the activities of antimicrobials has been widely investigated in recent years(Yang Z et al. 2021). The results of CV staining and bacterial adhesion assays conducted in this study showed that EMQ altered the adhesion capabilities of *P. acnes* and *S. aureus*, thereby preventing biofilm production. Similar findings have been reported for an ethanol extract of *Australian propolis* against methicillin-resistant *S. aureus*, while the plant-derived antibiotic rhodomyrone and the synergistic combination of sapindoside A and B were reported to inhibit biofilm production by *P. acnes*(Wang F et al. 2021). In fact, practically all antimicrobial drugs that inhibit biofilm production are significantly weaker against bacterial cells in biofilms, implying that biofilms are responsible for antimicrobial resistance, at least to some extent(Matsugishi A et al. 2021). Collectively, these findings suggest that antibacterials with strong inhibitory effects against biofilm formation will have a wider range of applications.

5 Conclusion

EMQ showed superior antibacterial activity against *P. acnes* and *S. aureus*. Furthermore, the antibacterial mechanism of EMQ involves causing damage to the bacterial cell wall and membrane, which leads to leakage of macromolecular substances, including proteins and nucleic acids, resulting in decreased metabolic activity and eventual cell death (Fig. 9). In addition, EMQ inhibited bacterial cell adhesion and biofilm formation. These results show that EMQ is effective against biofilm production by *P. acnes* and *S. aureus*, suggesting that EMQ has potential for clinical treatment of AV and lays a foundation for the development of new anti-AV agents.

Declarations

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Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Authors Contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; MX performed biochemical assays, conducted data analysis, and wrote the

manuscript. ZP performed biochemical assays. LYG, RYY and ZMDZ assisted in data analysis. TD and SH assisted in design of the experiments. BL designed the experiments.

Data Availability

The data that support the findings of this study are available from the corresponding author, [B.L.], upon reasonable request.

References

1. Aslan Kayiran M, Karadag AS, Al-Khuzaei S, Chen W, Parish LC(2020). Antibiotic Resistance in Acne: Mechanisms, Complications and Management. *Am J Clin Dermatol* 21(6):813-819.
2. Connolly D, Vu HL, Mariwalla K, Saedi N(2017). Acne Scarring-Pathogenesis, Evaluation, and Treatment Options. *J Clin Aesthet Dermatol* 10(9):12-23.
3. Dai J, Han R, Xu Y, Li N, Wang J, Dan W(2020). Recent progress of antibacterial natural products: Future antibiotics candidates. *Bioorg Chem* 101:103922.
4. Guo Y, Liu Y, Zhang Z, Chen M, Zhang D, Tian C, Liu M, Jiang G.(2020) The Antibacterial Activity and Mechanism of Action of Luteolin Against *Trueperella pyogenes*. *Infect Drug Resist* 10;13:1697-1711.
5. Gao F, Zhou H, Shen Z, Zhu G, Hao L, Chen H, Xu H, Zhou X(2020). Long-lasting anti-bacterial activity and bacteriostatic mechanism of tea tree oil adsorbed on the amino-functionalized mesoporous silica-coated by PAA. *Colloids Surf B Biointerfaces* 188:110784.
6. Hazarika N(2021). Acne vulgaris: new evidence in pathogenesis and future modalities of treatment. *J Dermatolog Treat* 32(3):277-285.
7. Heng AHS, Chew FT(2020). Systematic review of the epidemiology of acne vulgaris. *Sci Rep* 1;10(1):5754.
8. Han Q, Feng L, Zhang Y, Zhang R, Wang G, Zhang Y(2021). Effect of Juglone against *Pseudomonas syringae* pv *Actinidiae* Planktonic Growth and Biofilm Formation. *Molecules* 26(24):7580.
9. Jia Y, Hao C, Yang Q, Zhang W, Li G, Liu S, Hua X(2021). Inhibition of *Haemophilus parasuis* by berberine and proteomic studies of its mechanism of action. *Res Vet Sci* 138:62-68.
10. Kurokawa I, Nakase K(2020). Recent advances in understanding and managing acne. *F1000Res*. 9:F1000 Faculty Rev-792.
11. Kurokawa I, Layton AM, Ogawa R(2021). Updated Treatment for Acne: Targeted Therapy Based on Pathogenesis. *Dermatol Ther (Heidelb)* 11(4):1129-1139.
12. Li Y, Zhou Y(2018). The therapeutic effect of tanshinone IIA on *Propionibacterium acnes*-induced inflammation in vitro. *Dermatol Ther* 31(6):e12716.
13. Li JF, Zhang JX, Li G, Xu YY, Lu K, Wang ZG, Liu JP(2020). Antimicrobial activity and mechanism of peptide CM4 against *Pseudomonas aeruginosa*. *Food Funct* 11(8):7245-7254.
14. Li QL , Xiong ZT, Yang H, Huang R, Wang HB, Luo C, Liu J(2021). Antimicrobial Activity of Extract from *Angelicae Dahuricae* on Acne-associated Bacteria. *The Chinese Journal of Dermatovenereology*

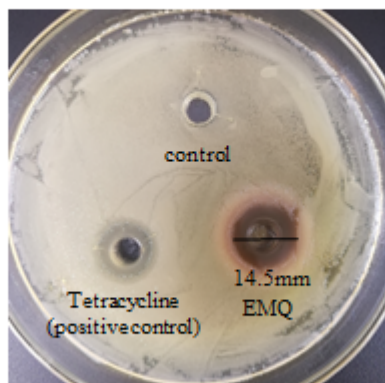
35(01):26-29.

15. Marson JW, Baldwin HE(2019). An Overview of Acne Therapy, Part 1: Topical therapy, Oral Antibiotics, Laser and Light Therapy, and Dietary Interventions. *Dermatol Clin* 37(2):183-193.
16. Matsugishi A, Aoki-Nonaka Y, Yokoji-Takeuchi M, Yamada-Hara M, Mikami Y, Hayatsu M, Terao Y, Domon H, Taniguchi M, Takahashi N, Yamazaki K, Tabeta K(2021). Rice peptide with amino acid substitution inhibits biofilm formation by *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. *Arch Oral Biol* 121:104956.
17. Poomanee W, Chaiyana W, Mueller M, Viernstein H, Khunkitti W, Leelapornpisid P(2018). In-vitro investigation of anti-acne properties of *Mangifera indica* L. kernel extract and its mechanism of action against *Propionibacterium acnes*. *Anaerobe* 52:64-74.
18. Su Y, Cui W, Wei H(2020). Influence of lactoferrin on *Propionibacterium acnes*-induced inflammation in vitro and in vivo. *Dermatol Ther* 33(6):e14483.
19. Winkelman WJ(2018). Aromatherapy, botanicals, and essential oils in acne. *Clin Dermatol* 36(3):299-305.
20. Weiner DM, James WD(2021). Acne and antibiotics: a look back. *Int J Dermatol* 60(8):1019-1027.
21. Karadag AS, Aslan Kayiran M, Wu CY, Chen W, Parish LC(2021). Antibiotic resistance in acne: changes, consequences and concerns. *J Eur Acad Dermatol Venereol* 35(1):73-78.
22. Wang Y, Chen YH, Pang WW, Chen JY, Zheng TL, Zhou T, Pei XF(2017). Antimicrobial effect and the related mechanism of the extracts from *zanthoxylum bungeanum* against acne-inducing bacteria. *Modern Preventive Medicine* 44(09):1692-1697.
23. Wang S, Yao J, Zhou B, Yang J, Chaudry MT, Wang M, Xiao F, Li Y, Yin W(2018). Bacteriostatic Effect of Quercetin as an Antibiotic Alternative In Vivo and Its Antibacterial Mechanism In Vitro. *J Food Prot* 81(1):68-78.
24. Wang S, Deng H, Wang Y, Rui W, Zhao P, Yong Q, Guo D, Liu J, Guo X, Wang Y, Shi C(2021). Antimicrobial Activity and Action Mechanism of Thymoquinone against *Bacillus cereus* and Its Spores. *Foods* 10(12):3048.
25. Wang F, Liu H, Li J, Zhang W, Jiang B, Xuan H(2021). Australian propolis ethanol extract exerts antibacterial activity against methicillin-resistant *Staphylococcus aureus* by mechanisms of disrupting cell structure, reversing resistance, and resisting biofilm. *Braz J Microbiol* 52(4):1651-1664.
26. Xu H, Li H(2019). Acne, the Skin Microbiome, and Antibiotic Treatment. *Am J Clin Dermatol* 20(3):335-344.
27. Xu B, Kang H, Luo S, Yang Q, Zhao Z, Peng F, Liu ZL(2019). Complete chloroplast genome of the *Meconopsis quintuplinervia* (Papaveraceae), a traditional medicine of Tibetan. *Mitochondrial DNA B Resour* 12;4(2):2335-2336.
28. Zhang Y, Gao K, Wang C, Liu S(2021). A Novel Antibacterial Component and the Mechanisms of an *Amaranthus tricolor* Leaf Ethyl Acetate Extract against *Acidovorax avenae* subsp. *citrulli*. *Int J Mol Sci* 28;23(1):312.

29. Yang Z, Ma X, Li Y, Xu H, Han X, Wang R, Zhao P, Li Z, Shi C(2021). Antimicrobial Activity and Antibiofilm Potential of Coenzyme Q0 against Salmonella Typhimurium. Foods 10(6):1211.
30. Zeng Y, Yu Q, Cheng S(2021). Antibacterial Mechanism of Liangguoan against Staphylococcus aureus and Escherichia coli. Arch Microbiol 203(7):4025-4032.
31. Zhou Y, Zhang Y, Zong H, Lu X, Shen W, Zhuge B(2021). Chemical constituents, antibacterial activity and mechanism of Paeonia suffruticosa Andr. buds extract against Staphylococcus aureus and Escherichia coli O157:H7. Nat Prod Res 35(6):1005-1009.

Figures

(A) *P.acnes*



(B) *S.aureus*

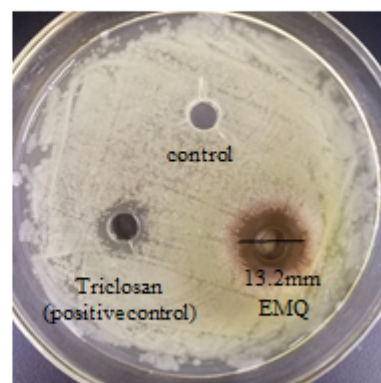
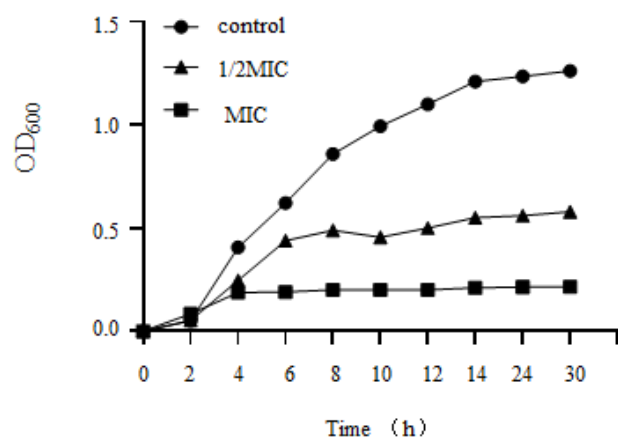


Figure 1

Agar disc diffusion method showing (A) the inhibition zone of 200mg/ml of EMQ and 10ug/ml tetracycline against *P.acnes*, (B) the inhibition zone of 200mg/ml of EMQ and 10ug/ml triclosan against *S.aureus*.

(A)*P.acnes*



(B)*S.aureus*

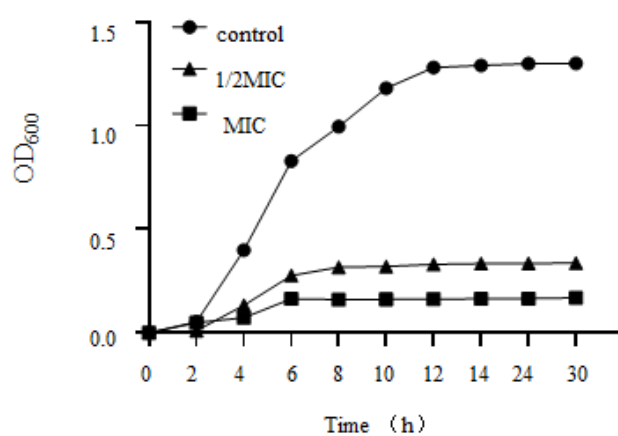


Figure 2

Growth curves for the bacteria exposed to various concentrations of EMQ and the untreated controls. Growth curves for (A) *P. acnes* and (B) *S. aureus*.

(A)*P.acnes*

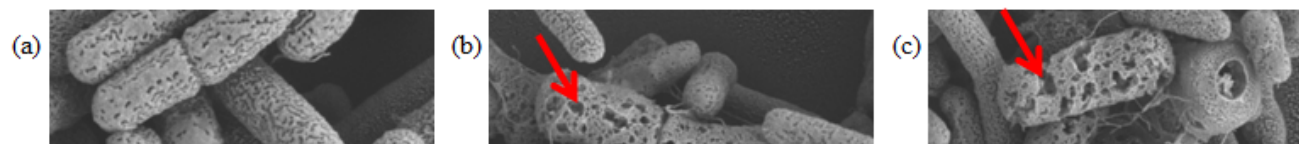
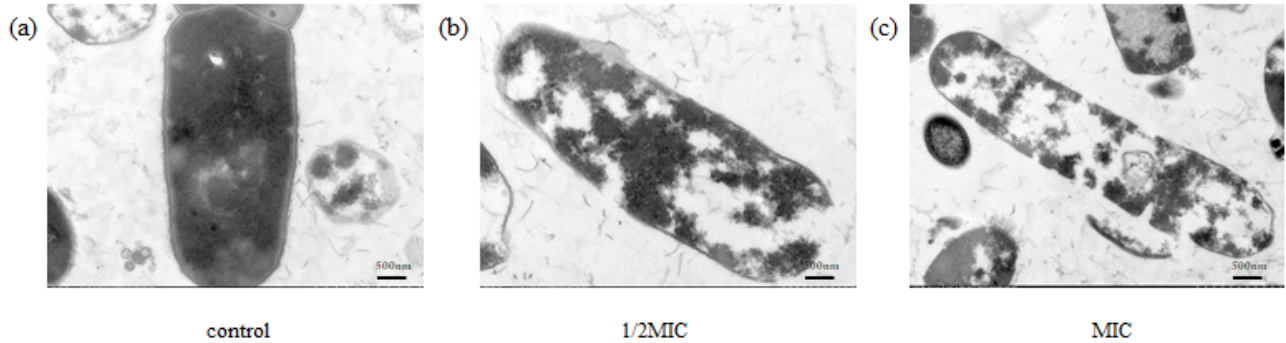


Figure 3

SEM observations (23000×magnification) of (A) *P.acnes* and (B) *S.aureus* after different treatments. (a) untreated for 12 h, (b) treated with 1/2 MIC of EMQ for 12 h, and (c) treated with 1 MIC of EMQ for 12 h.

(A)*P.acnes*



(B)*S.aureus*

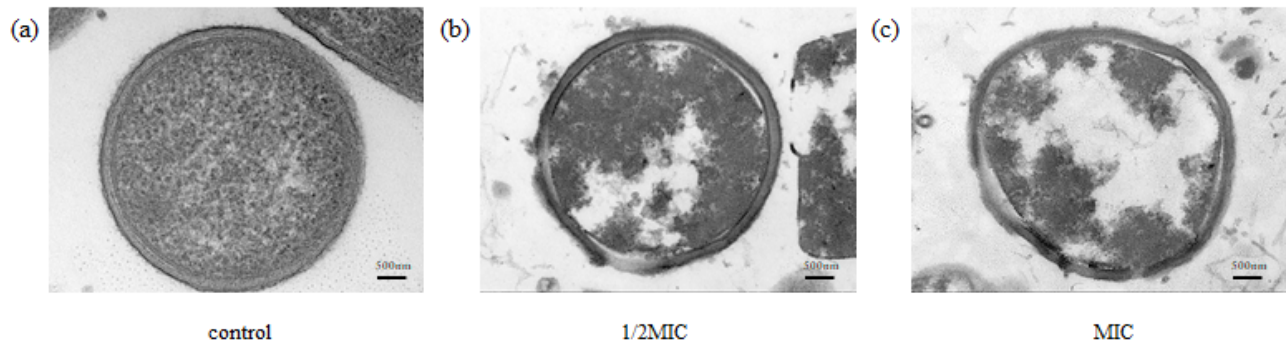
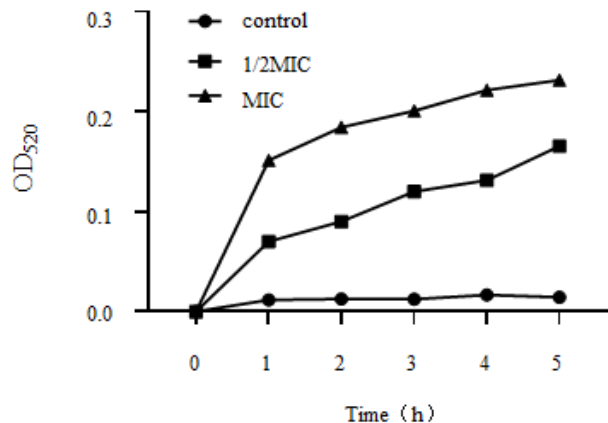
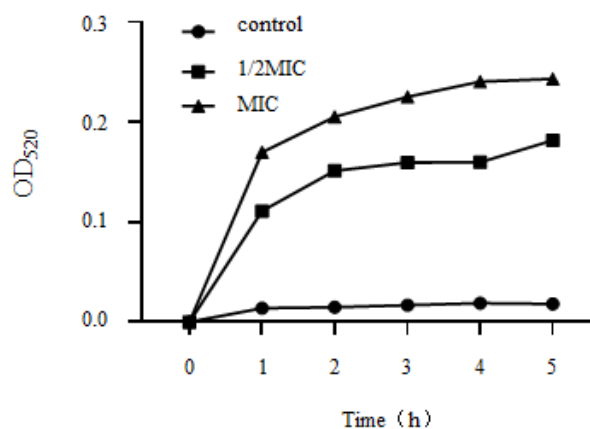
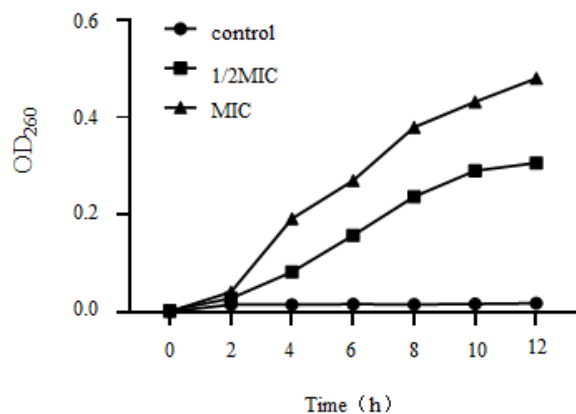
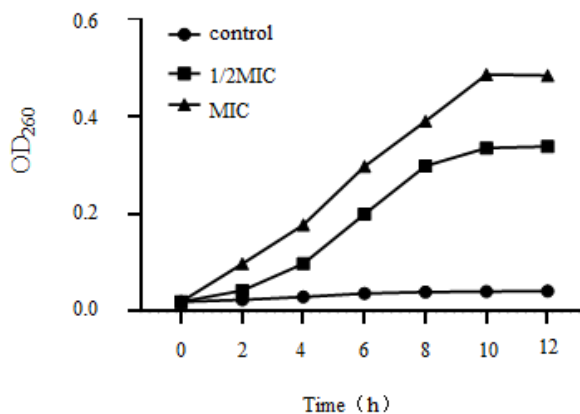


Figure 4

TEM observations (40000×magnification) of (A) *P.acnes* and (B) *S.aureus* after different treatments. (a) untreated for 12 h, (b) treated with 1/2 MIC of EMQ for 12 h, and (c) treated with 1 MIC of EMQ for 12 h.

(A) *P.acnes*(B) *S.aureus***Figure 5**

Changes of extracellular AKPase content of (A) *P.acnes* and (B) *S.aureus* after 12 h of treatment with various concentrations of EMQ.

(A) *P.acnes*(B) *S.aureus***Figure 6**

The nucleic acid leakage of (A) *P.acnes* and (B) *S.aureus* after treatments with different concentrations of EMQ.

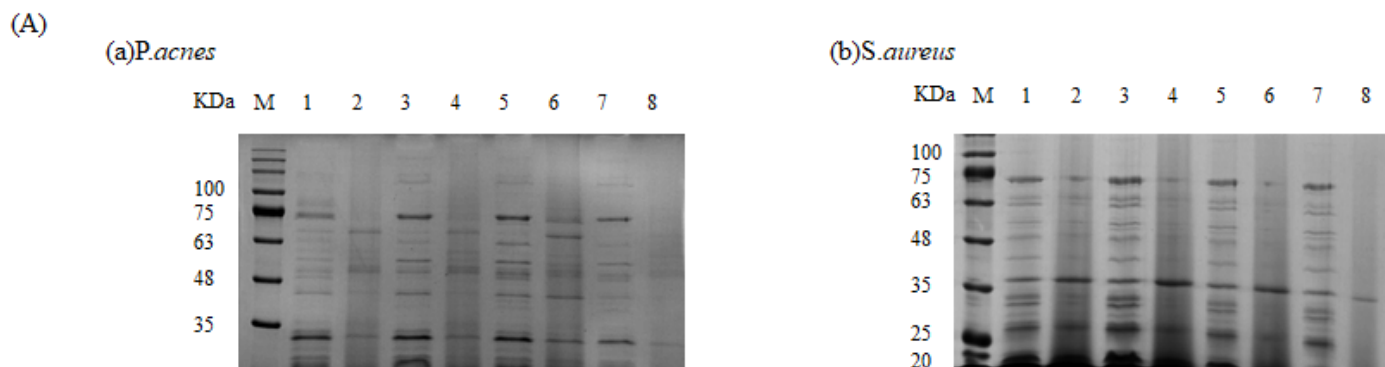


Figure 7

Changes of intracellular protein content in *P.acnes* and *S.aureus* treated with EMQ. (A) SDS-PAGE analysis of (a) *P.acnes* and (b) *S.aureus* proteins treated with 1 MIC of EMQ. Lane M: marker. Lanes 1, 3, 5, and 7: control for 3, 6, 9, and 12 h, respectively; Lanes 2, 4, 6, and 8: treated sample for 3, 6, 9, and 12 h, respectively. (B) Effects of EMQ on the intracellular ATP concentrations of (c) *P.acnes* and (d) *S.aureus*. Values are the mean $\pm$ SD of 3 independent experiments with triplicate samples. * $p < 0.05$ or ** $p < 0.01$ compared to the group treated with control.

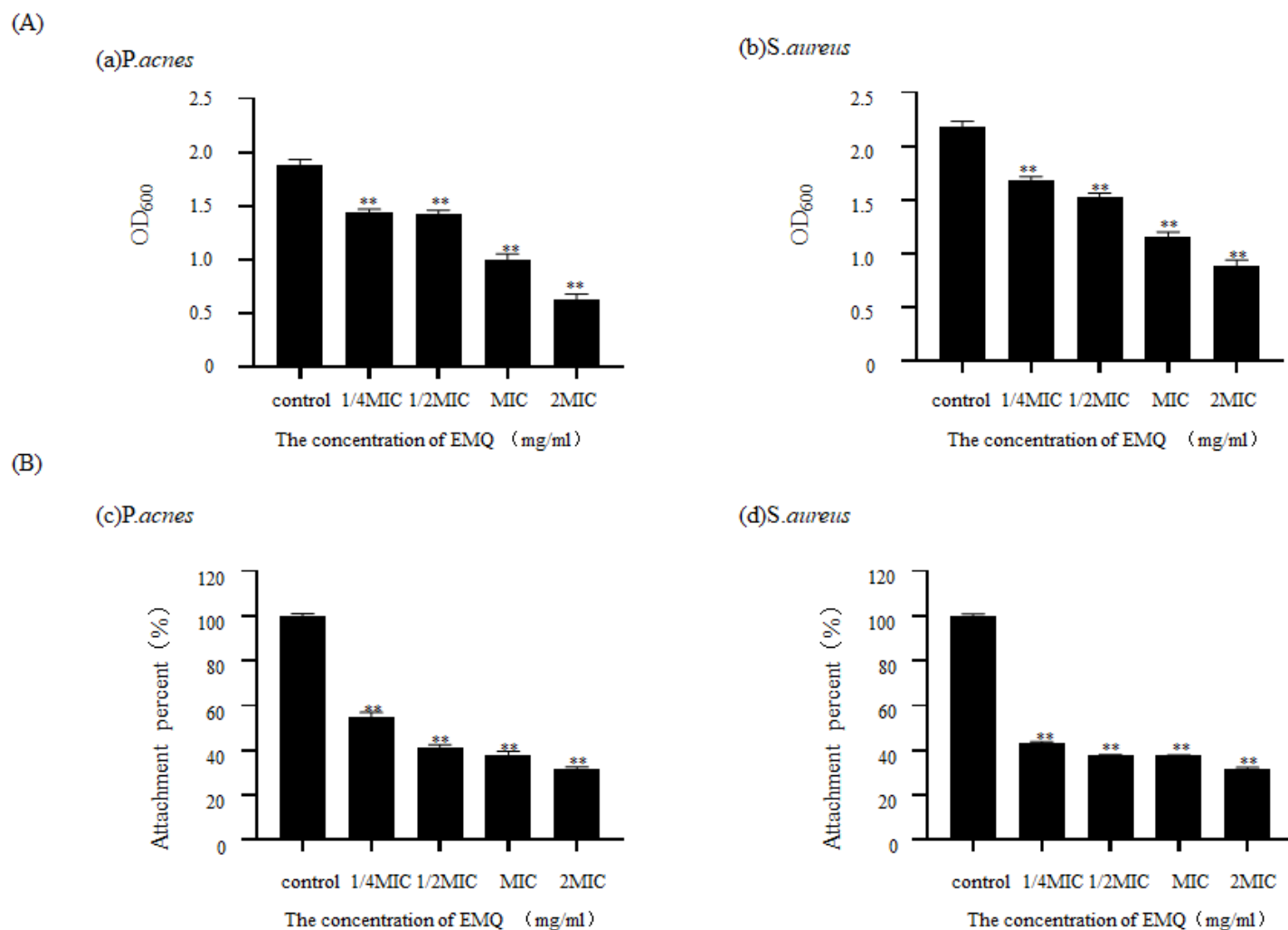


Figure 8

Effect of different concentrations of EMQ on (A) biofilm biomass of (a) *P.acnes* and (b) *S.aureus* quantified by CV assay, (B) attachment percent of (c) *P.acnes* and (d) *S.aureus* detected by attachment assay (the control was set to 100%). Values are the mean $\pm$ SD of 3 independent experiments with triplicate samples. * $p < 0.05$ or ** $p < 0.01$ compared to the group treated with control.

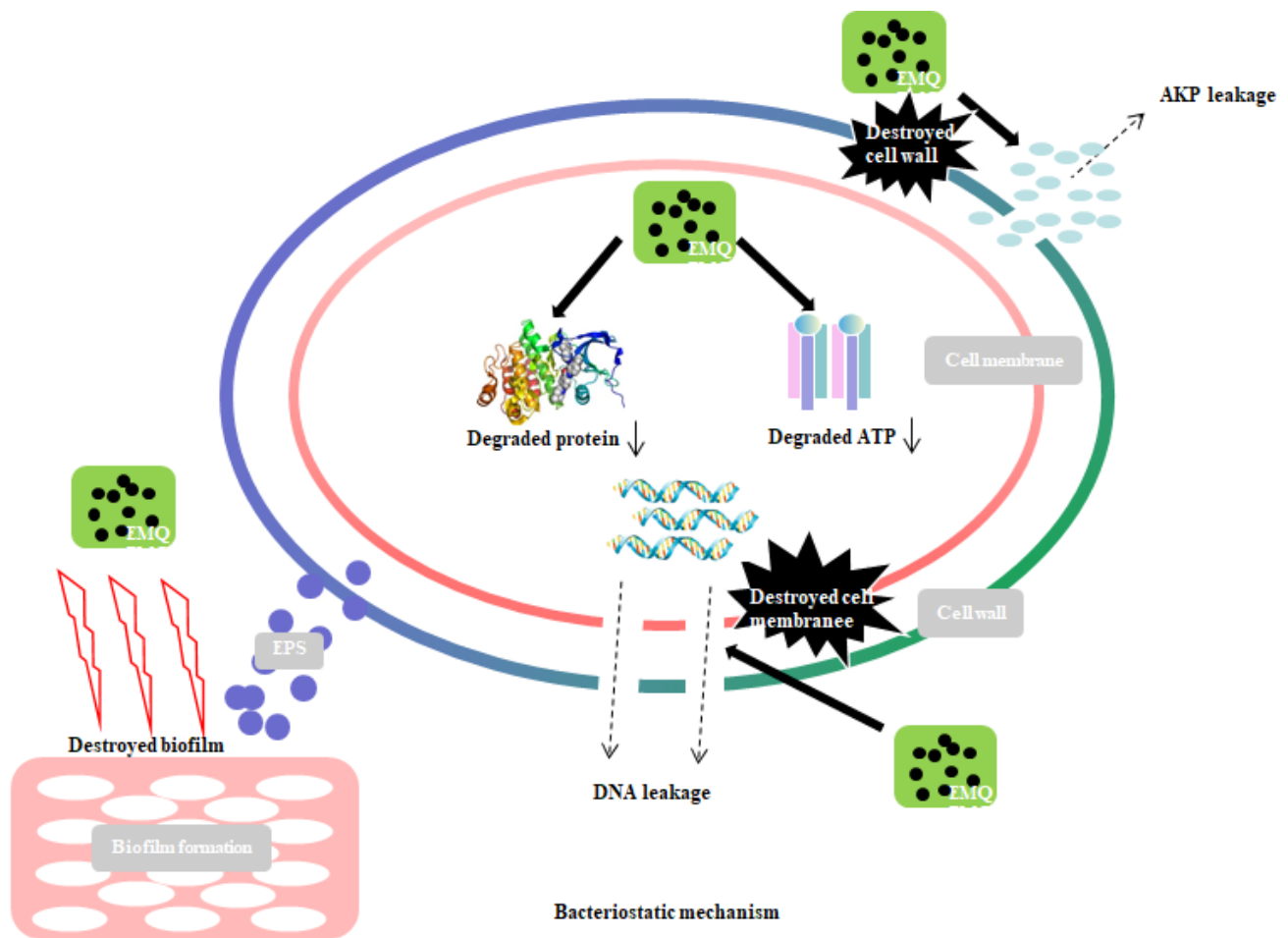


Figure 9

Possible antibacterial mechanism of action of EMQ.