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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 study results indicated that EMQ was an effective antibacterial agent against P. acnes and S. aureus, with a DIZ of 14.5 and 13.2mm, MIC of 12.5 and 12.5mg/mL and MBC of 100 and 50mg/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. The main chemical components of EMQ, identified by LC-MS, seem to have important roles in the antimicrobial effects against P. acnes and S. aureus, suggesting EMQ is a promising therapeutic for acne treatment.

Keywords: *Meconopsis quintuplinervia* Regel; *Propionibacterium acnes*; *Staphylococcus aureus*; antibacterial activity; antibacterial mechanism

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 (Hazarika 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 (Sood S *et al*., 2020).

AV is mainly caused by over colonization 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). Over colonization 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, which have been shown to inhibit the growth of *Escherichia coli*, *Bacillus subtilis*, *Salmonella* sp. and *Enterococcus faecalis*, among others (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*

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scientific foundation for the development of herb-based antibacterials for treatment of AV.

MATERIALS AND METHODS

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 and tryptic soy agar (TSA) medium were purchased from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). Round-bottom vertical anaerobic culture bags (2.5 L) and anaerobic gas generating bags (2.5 L) were purchased from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). An AKP enzyme assay kit and ATP content determination kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

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 100mL of tryptic soy broth for 48h at 37oC in an anaerobic gas-generating bag, while *S. aureus* was cultivated in 100mL of brain heart infusion growth medium at 37°C for 24h. For the following assays, the bacterial suspensions were diluted with phosphate-buffered saline (PBS) to appropriate concentrations.

Preparation of EMQ

MQ was provided and authenticated by Professor Zhuoma Dongzhi (Medical College, Tibet University, China) in August 2020. The certificate sample (CBTM-E124) was stored in the Herbarium of Pharmacy Department of Qingdao University of Science and 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 under vacuum to obtain a powder extract. To test for antibacterial activities, the ethanol extract was dissolved in dimethyl sulfoxide (DMSO) and stored at -20°C for subsequent studies.

LC-MS Analysis

LC-MS/MS analyses were performed using an UHPLC system (Vanquish; Thermo Fisher Scientific, Waltham, MA, USA) with a UPLC BEH Amide column (2.1mm× 100mm, 1.7μm) coupled to a Q Exactive HFX mass spectrometer (Orbitrap MS; Thermo Fisher Scientific). The mobile phase consisted of 25mM ammonium acetate and 25mM ammonia hydroxide in water (pH 9.75) and acetonitrile. The auto-sampler temperature was set at 4°C and the injection volume was 2μL.

Agar diffusion assay

The antibacterial activity of EMQ was determined based on the agar diffusion method. Briefly, 20mL 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 100μL of the appropriate bacterial suspension. Afterward, a sterilized steel rod was used to punch holes in the test plate, then 80μL of the bacterial suspensions were injected through the holes and the plates were incubated for 24h at 37ºC for *S. aureus* or 48h 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.

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

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

Bacterial growth curves

Bacterial growth curves were generated to assess the antibacterial activities of EMQ. Briefly, both strains (10^7 CFU/mL, 0.1mL) were inoculated into 100mL 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 600nm (OD_{600}) at 0, 2, 4, 6, 8, 10, 12, 14, 24 and 30h.

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 12h of EMQ treatment, the bacterial cells were pelleted by centrifugation at 4500rpm for 10min, then washed twice with PBS, fixed in 2.5% glutaraldehyde for 24h 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).

Extracellular alkaline phosphatase (AKPase) analysis

Bacterial cells were treated with EMQ at MICs of 0.5 and 1.0 for 12h, then centrifuged at 4500rpm for 10 min. 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) in accordance with the manufacturer's instructions.

Measurement of nucleic acid leakage

Bacterial cells were treated with EMQ at MICs of 0.5 and 1.0 and subsequently incubated for 12h at 37°C. During the incubation period, 1mL of each culture was collected every 2h and centrifuged at 12000rpm for 10 min. Then, the supernatants were collected and the OD_{260} was measured using a microplate reader.

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 (10^7 CFU/mL) were treated with EMQ at an MIC of 1.0 for 3, 6, 9 and 12h, respectively, at 37oC, while untreated cells were utilized as negative controls. At the indicated times, 2mL 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).

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 12h 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.

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 24h at 37oC. Untreated cells were included as negative controls. After 24h, 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 150µL 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 150µL of 95% ethanol was measured at an OD_{595} using a microplate reader.

Effect of EMQ on bacterial adhesion

Bacterial suspensions (100µL) 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 37oC for 4h, the plates were rinsed with sterile water. Then, planktonic cells were removed and the OD_{600} was measured to determine the number of cells adhering to the wells.

STATISTICAL ANALYSIS

All data are presented as the mean±standard deviation. The significance of differences between two groups was determined with the two-tailed *t*-test. Statistical analysis was carried out using the GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA).A probability (*p*) value of <0.05 was considered statistically significant.

RESULTS

Measurement of the representative components of EMQ by LC-MS

The molecular components present in the EMQ were identified by LC-MS (fig. 1). Among them, muscomosin is reported to inhibit the growth of *Alternaria alternata, Monographella cucumerina* and *Aspergillus fumigatus* (Cheel J *et al*., 2018). The flavonoids betagarin, 5,6,7,8-tetrahydroxy-3',4'-dimethoxyflavone and 2'-hydroxygenistein are reported to significantly inhibit the growth of *E. coli, S. aureus* and *Pseudomonas aeruginosa* (Gutiérrez-Venegas G *et al*., 2019). Additional chemical components of EMQ with antibacterial activities include quercetin, 3-hydroxycoumarin, isorhamnetin, luteolin, quercetin and eugenol (table 2) (Wang S *et al*., 2018; Šimat V *et al*., 2022). EMQ contains high levels of phytochemicals, which may contribute to the *antimicrobial* effects.

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 13.2mm, 12.5 and 12.5mg/mL and 100 and 50mg/mL, respectively (fig. 2, table 2). These results demonstrate that EMQ had inhibitory effects against both bacterial species.

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. 3, the OD_{600} of the control group increased rapidly, while that of *P. acnes* remained relatively unchanged after about 14h and that of *S. aureus* remained constant for about 12h, primarily due to the bacterial life cycle. However, as compared to the control group, the OD_{600} of cells treated with EMQ at an MIC of 0.5 was considerably decreased and remained at about 0.2 at an MIC of 1.0, indicating inhibited growth of the bacterial cells.

SEM observations

Morphological changes to bacterial cells treated with EMQ and untreated control cells were observed by SEM. As shown in fig. 4A(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. 4A(b) and A(c)), while *S. aureus* cells had obvious vesicle formation and irregular surface processes (fig. 4B(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.

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. 5A(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. 5A(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.

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 OD_{520} was used to calculate the quantity of AKPase leaked from *P. acnes* (fig. 6A) and *S. aureus* (fig. 6B) cells. At 5h, the OD_{520} 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 OD_{260} was measured to assess the amount of nucleic acids leaked from *P. acnes* (fig. 7A) and *S. aureus* (fig. 7B) cells. At 12h, the OD_{260} 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*.

***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. 8A(a)) and *S. aureus* (fig. 8A(b)) cells treated with EMQ at an MIC of 1.0 for 12h. 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. 8B(a) and 8B(b), respectively. The results show that EMQ suppressed cell growth by reducing ATP generation.

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. 9A(a) and 9(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. 9B(a) and 9B(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.

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 12.5mg/mL and 100 and 25mg/mL, respectively (Li QL *et al*., 2021). The MIC of an organic extract of *Asphodelus tenuifolius* is reportedly 18.75mg/mL against *Salmonella typhimurium* (Khalfaoui A *et al*., 2021). 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 12.5mg/mL, 100 and 50mg/mL and 14.5 and 13.2mm, respectively, demonstrating that EMQ has a significant inhibitory effect against *P. acnes* and *S. aureus*, similar to other natural products.

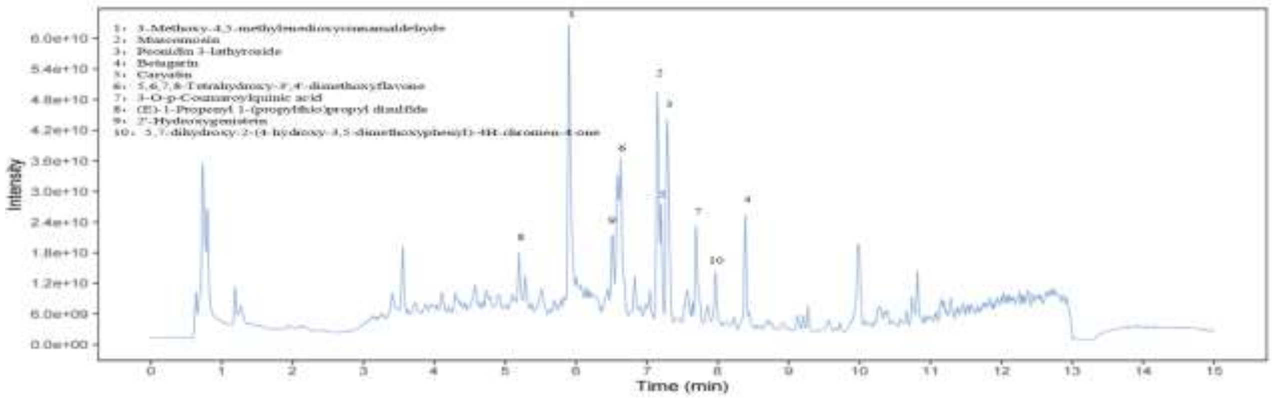

Fig. 1: LC-MS of EMQ.

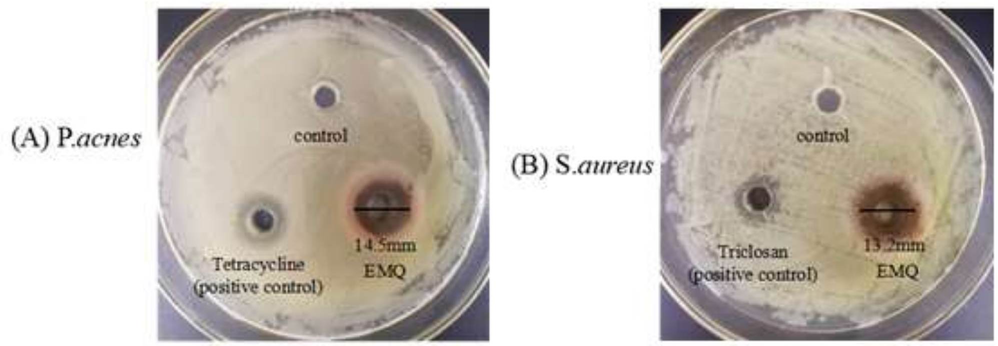

Fig. 2: Agar disc diffusion method showing the inhibition zone of (A) 200mg/mL of EMQ and 10µg/mL of tetracycline against *P. acnes* and (B) 200mg/mL of EMQ and 10µg/mL of triclosan against *S. aureus*.

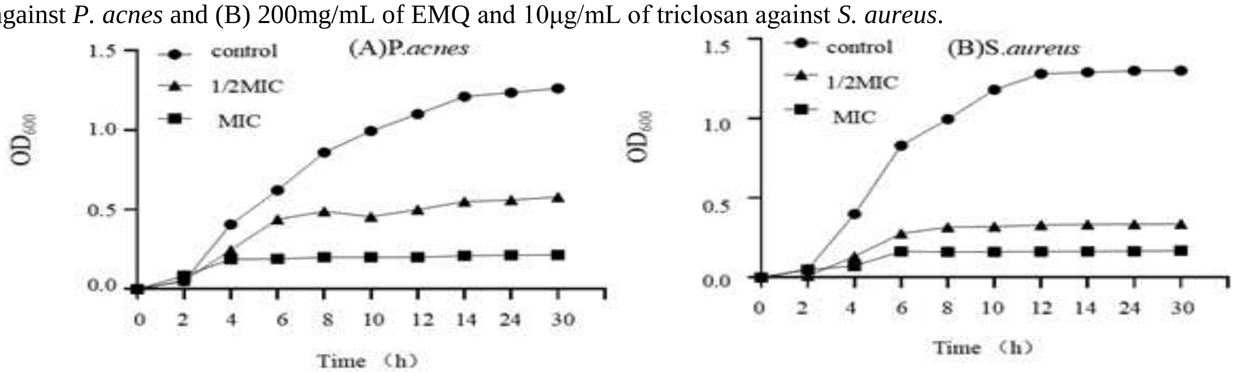

Fig. 3: Growth curves of (A) *P. acnes* and (B) *S. aureus* exposed to various concentrations of EMQ and the untreated controls.

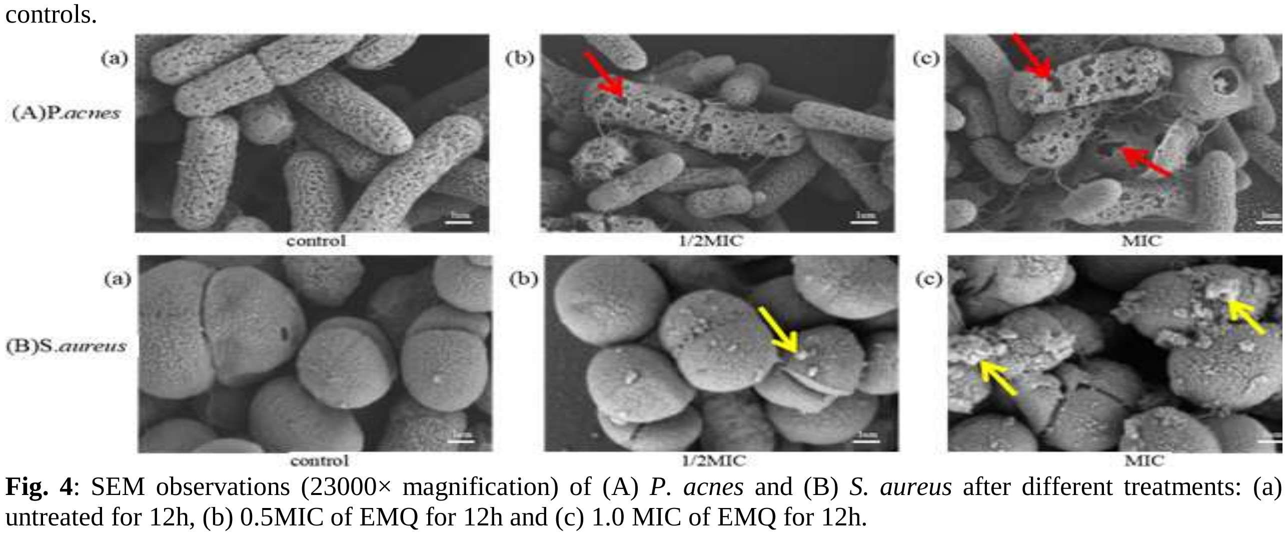

Fig. 4: SEM observations (23000× magnification) of (A) *P. acnes* and (B) *S. aureus* after different treatments: (a) untreated for 12h, (b) 0.5MIC of EMQ for 12h and (c) 1.0 MIC of EMQ for 12h.

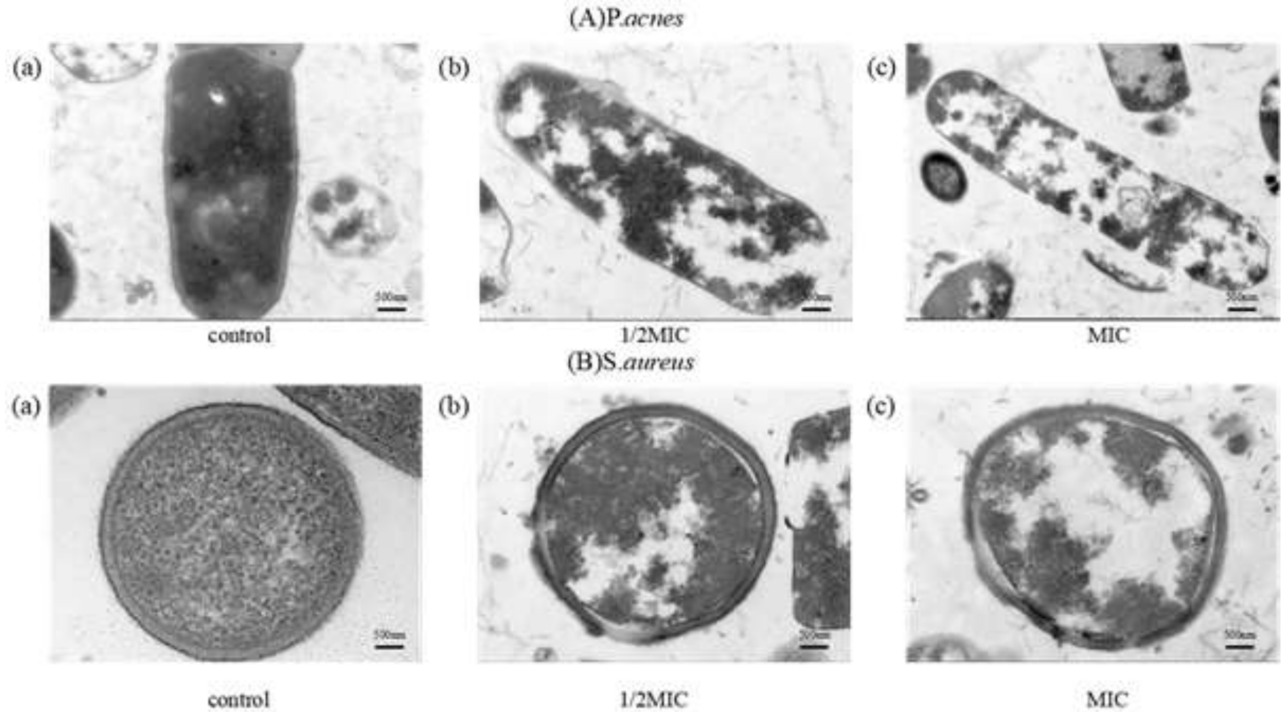

Fig. 5: TEM observations (40000× magnification) of (A) *P. acnes* and (B) *S. aureus* after different treatments: (a) untreated for 12h, (b) 0.5 MIC of EMQ for 12h and (c) 1.0 MIC of EMQ for 12h.

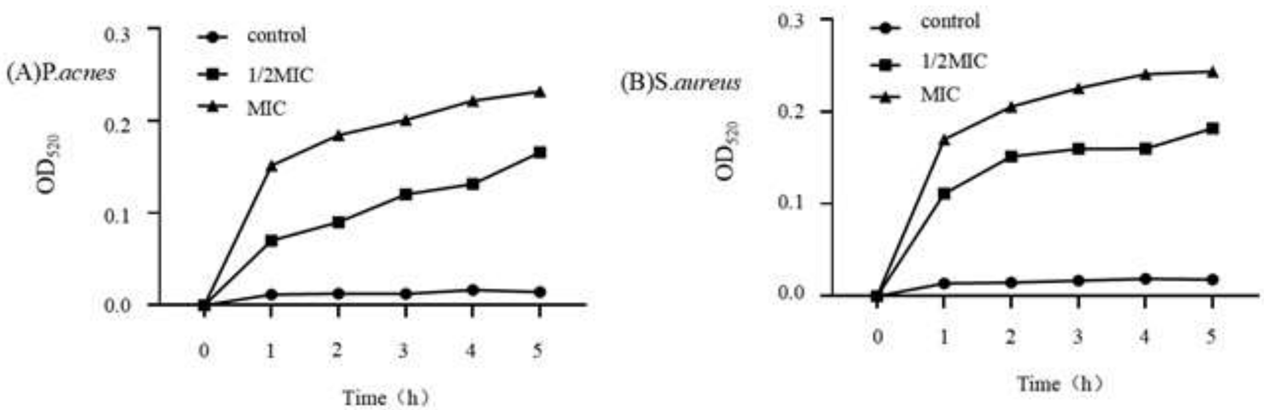

Fig. 6: Changes to the extracellular AKPase content of (A) *P. acnes* and (B) *S. aureus* after 12h of treatment with various concentrations of EMQ.

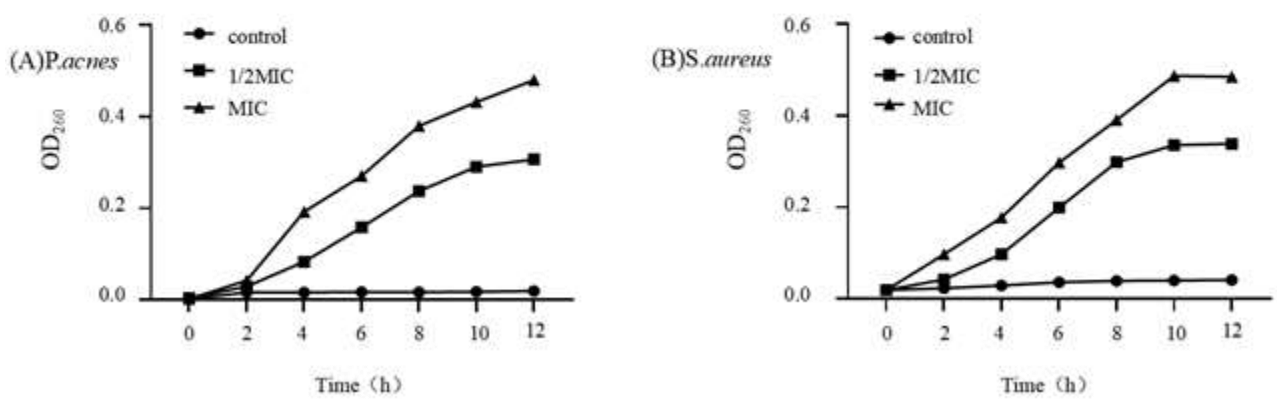

Fig. 7: Leakage of nucleic acids by (A) *P. acnes* and (B) *S. aureus* after treatment with different concentrations of EMQ.

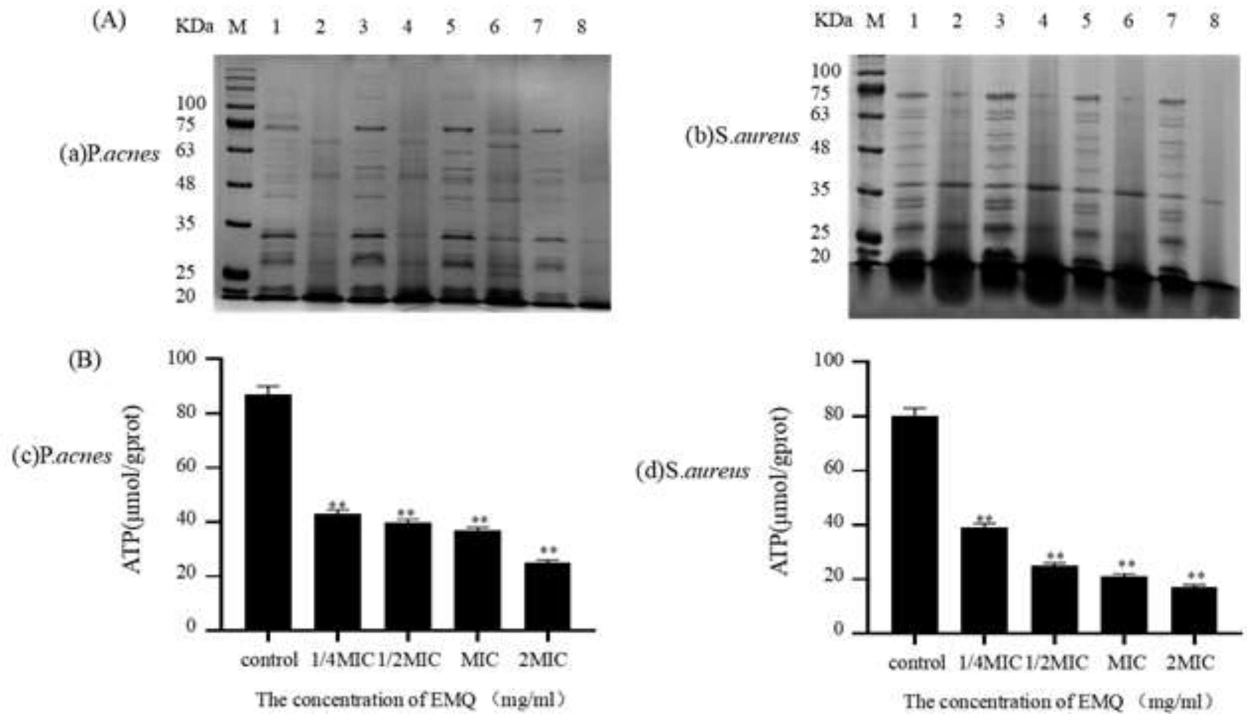

Fig. 8: Changes to the intracellular protein contents of *P. acnes* and *S. aureus* treated with EMQ. (A) SDS-PAGE analysis of (a) *P. acnes* and (b) *S. aureus* proteins treated with 1.0 MIC of EMQ. Lane M: marker; Lanes 1, 3, 5 and 7: Control for 3, 6, 9 and 12h, respectively; Lanes 2, 4, 6 and 8: Treated sample for 3, 6, 9 and 12h, respectively. (B) Effects of EMQ on the intracellular ATP concentrations of (c) *P. acnes* and (d) *S. aureus*. Values are presented as the mean±SD of three independent assays of triplicate samples. *p<0.05 and **p<0.01 vs. the control group.

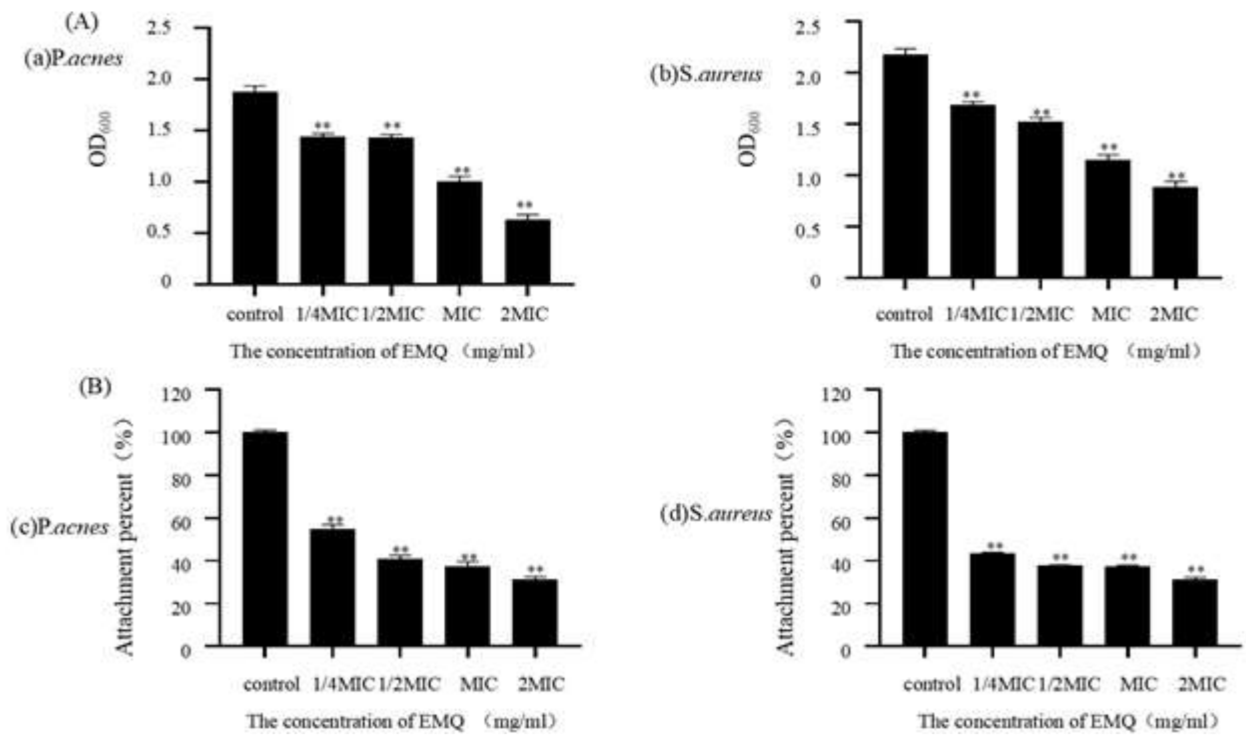

Fig. 9: Effect of different concentrations of EMQ on (A) biofilm biomass of (a) *P. acnes* and (b) *S. aureus* as determined by staining with CV. (B) Percent of attached (c) *P. acnes* and (d) *S. aureus* cells as determined with the attachment assay (the control was set to 100%). Values are presented as the mean±SD of three independent assays of triplicate samples. *p<0.05 and **p<0.01 vs. the control group.

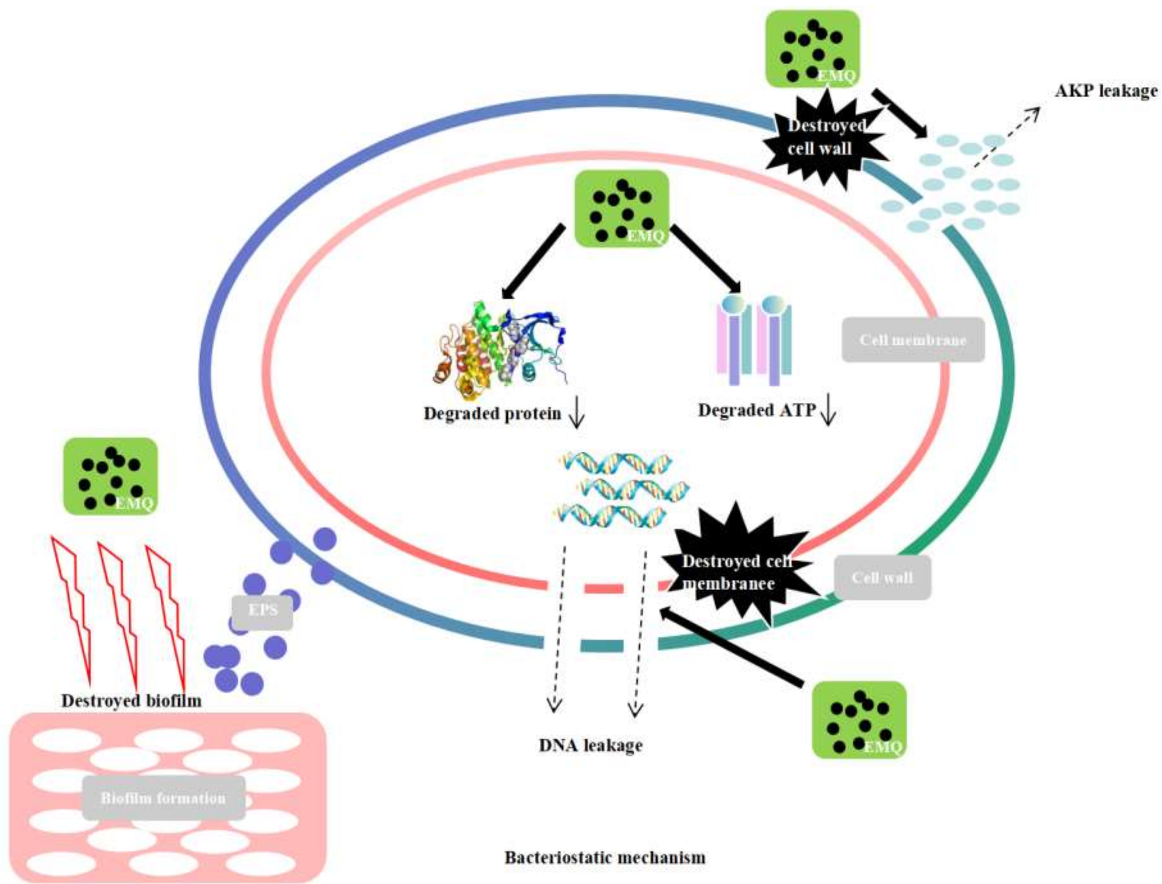

Fig. 10: Proposed antibacterial mechanism of action of EMQ.

Table 1: The major components of EMQ as determined by LC-MS.

No.	Retention time (min)	Tentative Identification	Mass m/z	Molecular Formula	% of Total
1	8.38	Betagarin	329.1000	C18H16O6	4.50
2	6.51	2'-Hydroxygenistein	287.0538	C15H10O6	2.15
3	4.82	Quercetin	303.0488	C15H10O7	0.63
4	3.74	3 Hydroxycoumarin	163.0385	C9H6O3	0.50
5	5.09	Isorhamnetin	317.0646	C16H12O7	0.34
6	6.01	Luteolin	287.0537	C15H10O6	0.30
7	5.19	Quercitrin	449.1054	C21H20O11	0.24
8	5.49	Eugenol	165.0903	C10H12O2	0.15

Table 2: MICs and MBCs of EMQ against *P. acnes* and *S. aureus*.

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

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 investigated changes to bacterial protein profiles to elucidate 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 rhodomyrtone 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.

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. In addition, EMQ inhibited bacterial cell adhesion and biofilm formation (fig. 10). These results show that EMQ has potential for clinical treatment of AV and lays a foundation for the development of new anti-AV agents.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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