

Antibacterial Evaluation of Saponins Extracted from Fresh Fruit of *Luffa acutangula* against Multidrug Resistance *Staphylococcus aureus* and their Mechanism in Ultrastructural Surface

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Abstract

Background

Bacterial resistance leads to the dilemma in the treatment with synthetic antibiotics. A new alternative strategy is to develop some safe and non-toxic natural antibiotics as alternative therapeutic drugs. The aims of this research were to determine the saponins extracted from fresh fruit of *Luffa acutangula*, to evaluate their antibacterial property by inhibiting the formation of biofilm, and to reveal the mechanism in ultrastructural surface.

Methods

the saponin components were extracted and isolated from Fresh Fruit of *Luffa acutangula* by organic solvents extraction; their chemical structures were identified by HPLC-MS/MS. The growth inhibition curves and the 50% inhibitory concentrations (IC₅₀) were used to evaluate the antibacterial activity of the extract. Then, crystal violet staining method was accepted to investigate the capacity of the saponins to inhibit biofilm formation. Anti-biofilm activity was assessed with minimum biofilm inhibitory concentration (MBIC). The interaction of the chemical compounds with saponins and biofilm were observed by SEM in ultrastructural surface.

Results

the extract by n-butanol was mainly saponins and the total content of saponins in the extract was 322.58 mg/g. nine saponin compounds were identified in n-butanol extract and the saponin compounds were mainly triterpene aglycone and triterpene glucosides. The extracted saponins have great bacteriostasis with IC₅₀ value 3mg/ml, and the saponins can also inhibit the biofilm formation with MBIC value 16mg/ml.

Conclusions

the saponins extracted from fresh fruit of *Luffa acutangula* could inhibit multidrug resistance *Staphylococcus aureus* and substantially eradicate biofilm, it may be a potential to be developed as antibacterial agent.

Background

Staphylococcus aureus is a common pathogenic bacterium, which cause serious infection in skin, superficial tissue and respiratory, or even some fatal diseases such as bacteremia, septicemia and CLS[1]. Synthetic antibiotics such as β -lactams, fluoroquinolones, and cephalosporins are usually used

as the first-line drugs in treatment of serious infections caused by *Staphylococcus aureus*[2]. Unfortunately, these synthetic antibiotics are easy to develop resistance during treatment. Stimulated by drug molecules, SA secret extracellular polymeric substance to form biofilm surrounding it, which protect bacteria from the drugs, thus minimizes therapeutic efficacy[3]. Multidrug resistance leads to the dilemma of no drugs available in antibacterial treatment. Therefore, some safe and non-toxic natural antibiotics as alternative therapeutic drugs attracted attention [4]. Many herbs such as *Moringa oleifera*, *Vernonia amygdalina*, *Azadirachta indica* and *Acalypha wilkesiana* were reported to possessing high antimicrobial activities as alternative antibiotics[5–8]. These plant extracts are natural, safe, non-toxic with little or no side-effects, and with a significant level of phytochemicals compounds such as saponins, flavonoids, tannins and phenols[9]. These natural compounds can prevent the formation of *Staphylococcus* biofilms and have anti-drug resistance effects. Because of their wide distribution and low cost, these herbs are widely used to treat bacterial infections in Asia, Africa and elsewhere[10].

Luffa acutangula is one member of the family Cucurbitaceae. In tropical and subtropical region, the fresh fruit of *Luffa acutangula* is a common vegetable as well as the young leaves can be eaten. The seeds can be roasted to produce oil. Its leaves were used in atherosclerosis therapy because of its antioxidant components. The fruit contains triterpenoid, saponins, lucyosides, the biological and medicinal application of *Luffa* plant has been discussed in anti-inflammation, anti-fungus and anti-asthma [11, 12]. But the chemical constitutes was not so clear. Therefore, in this study, the saponins extracted from the fresh fruit were identified, and their antibacteria activities against multi-resistant *Staphylococcus aureus* were investigated to explore the mechanism of inhibiting the biofilm formation by Fluorescence microscope and SEM in ultrastructural surface.

Materials And Methods

2.1 Materials and reagents:

Fresh Fruit of *Luffa acutangula* were collected in Hainan province, south China, N20°02'45.97", E110°11'38.39", in June 2022. The fruit was identified by Prof. Zhanping Gou, full professor of plant taxonomy at Guangdong Medical University.

The *Staphylococcus aureus* strain was isolated from clinic patients that be in line with ethical guidelines and approved by administrative department. This strain was chosen for its multidrug resistance and it was cultured at 37°C using Luria-Bertani (LB) broth and LB agar plates[13].

2.2 Separation And Identification Of Saponins

Phytochemical components such as anthocyanin, flavonoids, phenols, saponins, and tannins were isolated with organic solvents according their chemical polarity[14]. Fresh fruit of *Luffa acutangula* (1.1kg) was cut into small pieces and then juiced, ethanol was added into the juice to a concentration of 50%, the mixture was then filtrated, the filtrate was distilled under reduce pressure to remove ethanol, the

concentrate was partitioned in turn by liquid-liquid extraction process into hexane (Wa 0.2 g), ethyl acetate (Wb 7.7 g), n-butanol (Wc 5.6 g) and aqueous extract (Wd 2.2 g). The n-butanol extracts of *Luffa acutangula* was analyzed using LC-MS/MS in the positive and negative modes. The identification of compounds was based on the accurate comparison of MS profiles with reference standard of the natural product database, which including the data information of accurate molecular mass, MS/MS fragmentation patterns, relative retention times [15].

2.3 Growth inhibition of *Staphylococcus aureus*

Bacterial cultures were carried by inoculating 1ml bacterial sample into conical flask containing 100mL LB broth, 1ml of the fruit juice, the crude extract and the saponins solution were added to the flasks, respectively. PBS was used as negative control instead of the plant extracts. The flasks were then incubated at 37°C for 32 h in an incubator and kept them shaking. OD values at 610 nm were measured at sampling time points [16].

2.5 Determination Of IC₅₀

The inhibitory concentration (IC) of the extracted saponins was determined using microdilution methods as literature with some modifications and improvements [17]. Briefly, the extracted saponins were prepared with Luria-Bertani (LB) liquid medium at concentrations of 24, 12, 6, 3, 1.5, 0.75 mg/ml, 10 μ l series of saponins solutions were added into a 96-well microplate with 480 μ l LB broth, then 10 μ l bacterial solution (10⁶ CFU/ml) was added into every well. The control group was only added LB and bacterial solution. After culturing at 37°C for 24 h, the OD values were determined at 610nm and compared with the control group. IC₅₀ means at the concentration, 50% inhibitory rate was achieved. Inhibition rate was calculated by the following equation.

$$\text{Inhibition rate} = (\text{OD}_{\text{control}} - \text{OD}_{\text{drug}}) / \text{OD}_{\text{control}} \times 100\%$$

2.6 Acridine Orange (AO) Stain

Acridine Orange (AO) can penetrate the intact living cell membrane. Therefore, live *S. aureus* was stained by AO. Live bacteria observed green fluorescence at 488nm by rotary table super-resolution laser confocal microscope [18]. Brief and to the point, *S. aureus* cultures (10⁶ CFU/ml) were added with two saponin solutions with concentration of 3mg/ml (1/2IC₅₀) and 12mg/ml (2IC₅₀), respectively. After culturing at 37°C for 18 h, cells were collected by centrifugation at 3000rpm for 6min, washed by PBS once, and then stained by AO solution for 15min at room temperature. Taking cell suspension on the adhesive cover glass after staining is completed. Last but not least, observed green fluorescence which were live bacteria and capture the fluorescent picture.

2.7 SEM Analysis

To be brief, bacteria cells were cultured for 18h then added saponins at IC₅₀ concentration and still incubated for 30min at 37°C, collected the cells by centrifuging at 3000rpm for 6min. After that, added paraformaldehyde to fixed cell state and washed it with PBS once. Cell suspension was diluted by a million times. At last, 5µl cell suspension was dropped on the cover glass. After drying the cell, coated with gold in a sputter coater, the bacterial cells were observed under a scanning electron microscope (SEM), according to a previously reported method [19].

2.8 Minimum Biofilm Inhibitory Concentration (MBIC)

MBIC means the minimum biofilm inhibition concentration, at which concentration the inhibition rate was over 50%[7, 20]. MBIC values of the extracted saponins on *S. aureus* were determined by modified crystal violet assay [21]. The extracted saponins were prepared with Luria-Bertani (LB) liquid medium at concentrations of 32, 16, 8, 4, 2, 1 mg/ml. 10µl series of saponins solutions were added into a 96-well microplate with 480µl LB broth, then 10µl bacterial solution (10⁶ CFU/ml) was added into every well. The control group was only added LB and bacterial solution. After culturing at 37°C for 24 h, crystal violet assay was performed to quantify biofilm biomass. OD values were determined at 570nm and compared with the control group. Calculated the inhibition rate from the data and concluded the minimum biofilm inhibition concentration (MIBC).

$$\text{Inhibition rate} = (\text{OD}_{\text{control}} - \text{OD}_{\text{drug}}) / \text{OD}_{\text{control}} \times 100\%$$

2.9 Biofilm Staining With DAPI

Biofilm of the *S. aureus* were visualized by fluorescence microscopy using 4,6-diamidino-2-phenylindol (DAPI). Briefly, the extracted saponins were prepared at concentrations of 1/2MBIC, MBIC. After incubation at 37°C for 18h, the bacterial biofilms were washed by PBS once. Then added DAPI solution, which covered for 8min in the dark. Washing the biofilms once and visualized the anti-biofilm activity by rotary table super-resolution laser confocal microscope [22].

3.0 Statistical Analysis

The mean values and standard deviations of each quantitative variable were calculated after repeating each experiment three times. All the quantitative data were represented as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel. Two-tail Student's t test was performed to compare the percent of biofilm inhibition/eradication by each concentration and control group. Differences were considered significant at $p < 0.05$.

4 Results

4.1 Phytochemical identification

The primary phytochemical composition in fresh fruit of *Luffa acutangula* revealed significant level of alkaloid, flavonoids, phenol, tannins and saponin, the extract by n-butanol was mainly saponins and the total content of saponins in the extract was 322.58 mg/g according to the colorimetric method. The identification of individual saponin compound extracted from *Luffa acutangula* was carried out by HPLC-MS/MS, and the structures of compounds were determined by means of the accurate comparison of MS data with the available literature information, accurate mass, MS/MS fragmentation patterns, relative retention times, and by comparison with the literature data. In this study, 84 compounds were analyzed and matched in the natural product database of Agilent technology Q-TOF B.08.00 (B8058.0), nine saponin compounds were identified in n-butanol extract listed in Table 1, and the chemical structures of these compounds were shown in Fig. 1.

According to the molecular skeleton, these compounds were identified as 5-methylchromone (**Compound 1**), pentacyclic triterpenoids (**Compound 2, 3**) and tetracyclic triterpenoids (**Compound 4, 5, 6, 7, 8, 9**). Four of these compounds (**Compound 2, 3, 4, 5, 6, 7 and 8**) were also found in leaves of *Luffa acutangula* [11, 23], **Compound 4** was ginsenoside Rd, which was reported in the fruit of *Luffa acutangula* [24], **Compounds 1 and 9** were the first reported in this plant.

In these compounds, the triterpene skeleton was contacted with or without glucoside. Although without triterpene skeleton, **Compound 1** is a derivant of 7-O-Methylaloeresin A (CAS NO. 329361-25-3), which is 5-methylchromone glycoside [25]. 7-O-Methylaloeresin A exhibits good activity against multiple drug resistant *Staphylococcus aureus* [26]. Obviously, compound 1 antibacterial properties can be attributed to its chemical structure.

Table 1
LC-MS/MS spectral data of saponin compounds extracted from *Luffa acutangula*

No	Compound name	MS	MS fragmentation pattern (m/z)	Retention time(min)	Molecular Formula	Target MS
1	7-O-Methylaloeresin	408.1413	408.1415, 409.1493, 426.1759	5.008	C ₂₀ H ₂₄ O ₉	408.1420
2	3"-O-Acetylsaikosaponin D	822.4773	822.4760, 823.4794, 823.4838, 824.4872	8.909	C ₄₄ H ₇₀ O ₁₄	822.4776
3	Glyeursaponin	821.3953	821.3954, 822.3988, 822.4032, 823.4066	9.261	C ₄₂ H ₆₁ O ₁₆	821.396
4	Ginsenoside Rd	946.5523	946.5496, 947.5530, 947.5574, 948.5608	10.043	C ₄₈ H ₈₂ O ₁₈	946.5501
5	Tokoronin	594.3773	594.3762, 595.3796, 595.3841, 596.3875	10.907	C ₃₃ H ₅₄ O ₉	594.3768
6	24-Methylcholesta-5,7,22-trien-3beta-ol	396.3397	396.3387, 397.3421, 397.3465, 398.34	12.498	C ₁₇ H ₂₅ NO ₄	396.3398
7	Betulafolientriol	460.3912	460.3911, 461.3945, 461.3989, 462.4023	12.559	C ₂₈ H ₄₄ O	460.3917
8	3alpha-Hydroxy-6-oxo-5alpha-cholanic acid	390.2772	390.2765, 391.2843, 408.3108	12.666	C ₃₀ H ₅₂ O ₃	390.277
9	Gypenoside L	770.4828	770.4811, 771.4845, 771.4889, 772.4923	10.019	C ₂₄ H ₃₈ O ₄	770.4816

4.2 Bacteria Growth Curve

SA was cultured with or without the extract of *Luffa acutangula*, and the bacteria growth curve was shown in Fig. 2. Within the initial time between 0-6h, the growth of SA in all groups is slow, while it was rapidly increased during 6-18h in the control group, and the OD value is the highest at 24h, which indicated the bacterial quantity is the maximum. In contrast, the other groups were lower than that, which

means the growth of SA was inhibited, and the OD value of saponin group was little increasing within 0-24h, it indicated the bacterium was mainly inhibited by the saponins. The inhibitory activity at the same concentration (10mg/ml) is the saponins > crude extract > ordinary luffa. The results suggested the saponins extracted from FF were the mainly chemical components with antibacterial activity.

4.3 Determination Of IC₅₀

IC₅₀ was considered at which saponin concentration the inhibition rate is more than 50% [27]. In Fig. 3, the inhibition rate was increased when the saponins concentration increasing, at the concentration of 3mg/ml, the inhibition rate was of 58%. Results suggest that the extracted saponin was potent as an antibiotic agent with IC₅₀ value of 3mg/ml. The plant extracts of *Moringa oleifera*, *Acalypha wilkesiana*, *Vernonia amygdalina* and *Azadirachta indica* were reported with significant anti-staphylococci activities with inhibition of *S. aureus*, and their IC₅₀ (mg/ml) were 4.80, 3.23, 11.10, 3.75, respectively [5, 28]. These plant extracts have been reported to be valuable medicinal plant with anti-staphylococci activity. Therefore, the saponins extracted from FF could be potent as an antibiotic agent.

4.4 Live Bacterial Cells Evaluation

Live bacterial cells were evaluated by AO staining; the results were shown in Fig. 4–5. In AO staining, live cells were stained to give green fluorescence. Every green fluorescence spot represents the live cell; the number of spots in control group was more than that in drug groups, In the concentration of 1/2MIC, saponin obviously decreased the number of living cells, and the increase the drug concentration, the decrease the spots. These results indicated that the antibacterial activity of the saponins was concentration-dependent.

4.5 Ultrastructure Of SA

Scanning electron microscopy was applied to visualize the effect of the saponins on cell morphology of SA. It was observed the SA cells in the control group had an intact structures with a round, plump spherical shape (Fig. 6A), in contrast, the cell membrane of SA was damaged and morphology broken in the saponin-treated group (Fig. 6B), it indicated the cell membrane lysis with the saponins treatment. The results revealed the saponins induced SA cell membrane disruption in ultrastructure surface.

4.6 Determination Of MBIC

MBIC was defined as which concentration shows $\geq 50\%$ inhibition rate. In Fig. 4, it shows 64% inhibition rate at the concentration of 16mg/ml. It can conclude that the MBIC of saponin was 16mg/ml.

4.7 Ultrastructure Of Biofilm

Biofilm were marked by staining with DAPI (**Fig. 8**). The biofilm of control group were fairly complete, and its blue fluorescence was bright and intense. When treated with the saponins, the fluorescence intensity of biofilm gradually dim until to be dark. Moreover, the biofilm treated at the concentration of MBIC seem to be cracked. The results indicated the saponins inhibit the formation of biofilm through inhibiting the growth of bacteria.

5 Discussions

In this study, the saponins inhibit the growth of SA with IC_{50} value of 3mg/ml, live SA cells died and the fluorescent spot dramatically decrease in AO staining assay, these results demonstrated the saponins from *Luffa acutangula* were potencial natural antibiotics. Natural compound with special bioactivity is tightly relative with its molecular structure; Saponin molecular is usually composed of hydrophilic and hydrophobic moieties, which make saponin an amphipathic compound [29]. Natural saponins grant strong antibacteria properties by inhibiting the growth of both Gram negative and Gram positive bacteria, which antimicrobial properties can be attribute to the hydrophilic-lipophilic amphipathic chemical structure of saponin molecules, that amphipathic chemical structure lead to bacterial cell death by penetrating or disrupting the bacterial plasma membrane [30].

The saponins extracted from *Luffa acutangula* were mainly triterpene aglycone (compound 6, 7, 8) and triterpene glucosides (compound 2, 3, 4, 5, 9). These saponins have the same triterpene skeleton, which is similar with lucyoside B except for the side chains [23]. Triterpenoid saponins are known to be a class of compounds with antimicrobial, anti-inflammatory, and antitumor properties [31]. Previous studies showed that lucyosides (total saponins) has excellent antimicrobial property [32]. Martins et al. [33] reported the strong antibacterial activity of oleanolic against MDR *Staphylococcus aureus*. Moreover, Fontanay et al. [34] showed the inhibitory effect of oleanolic acid against *E. faecalis*. Obviously, all the triterpene saponins are similar in antibacterial activities because of their similar chemical structures.

The antibacterial mechanism of saponin against SA was intuitively revealed in ultrastructural surface. In this study, SEM observation found that the morphology of SA cells treated with the saponins was cracked and the membrane integrity was damaged, in contrast, cells in the control group had an intact cell structures with a round, plump spherical shape. The saponin disrupts the outer phospholipidic membrane of *S. aureus* cell wall, lead to permeability of lipophilic solutes rendering the cell wall inactive [35]. Li et al [36] observed with SEM & TEM that natural compound ethyl gallate had significant inhibitory effects on KP by rupturing completely the cell walls and membranes. Sun et al [37] reported that alkali-transformed saponin lead to the bacterial *Fusobacterium nucleatum* death, and observed with TEM that the bacterial membrane structure was destroyed. These reports demonstrated the antibacterial mechanism of natural saponins.

Bacterial resistance is a difficult problem in treatment. Multidrug resistance *Staphylococcus aureus* secretes exopolysaccharides to form biofilm, which is able to niches bacteria and cut off the contact with drugs, thus it is the critical strategy to preventing and disrupting the biofilms formation. In this study,

Biofilm were observed with DAPI staining. When treated with the saponins at the concentration of 16mg/ml (MBIC), the biofilm was gradually dim and cracked. The results indicated the saponins inhibit the formation of biofilm.

6 Conclusions

This study has shown that rich saponins were extracted from fresh fruit of *Luffa acutangula*. The extracted saponins have great bacteriostasis at the concentration of 3 mg/ml (IC₅₀). The antibacterial mechanism of saponin revealed by SEM may be that it acts on bacterial membrane, causing its rupture and the outflow of bacterial contents. The effect of saponin on the biofilm formation of *Staphylococcus aureus* was detected by crystal violet staining, and it was found the saponins at 16 mg/ml (MBIC) could inhibit the biofilm formation. The morphology of biofilm was observed by DAPI, and it was found that the formation of biofilm under MBIC was inhibited. It is worth looking forward the extracted saponins might be potencial antibiotic agents against multidrug resistance *Staphylococcus aureus*.

Abbreviations

SA
Staphylococcus aureus
LB
Luria-Bertani
IC₅₀:the concentration of the saponin that inhibits 50% of bacterial
MBIC
the concentration of the saponin that inhibits 50% of biofilm
DAPI
4,6-diamidino-2-phenylindol
SEM
scanning electron microscope
AO
Acriding Orange

Declarations

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Declarations of interest

None.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

Miaoen Huang: performed the experiments and analyzed the data; writing-original draft, data curation ; Tianji Wang: performed part of the experiment; experimental process communication and discussion; Xiaoxuan Cai,Xixiang Huang Yinghao Wang, Jinjun Chen: proofreading the manuscript; LiLi: partial experimental guidance; Yingnian Lu: experimental design and guidance; revised the manuscript.

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References

1. Olaniyi R, Pozzi C, Grimaldi L, Bagnoli F. Staphylococcus aureus-Associated Skin and Soft Tissue Infections: Anatomical Localization, Epidemiology, Therapy and Potential Prophylaxis. *Curr Top Microbiol Immunol*. 2017;409:199–227.
2. Jin T, Mohammad M, Pullerits R, Ali A. Bacteria and Host Interplay in Staphylococcus aureus Septic Arthritis and Sepsis. *Pathogens* 2021, 10(2).
3. Bouvet C, Gjoni S, Zenelaj B, Lipsky BA, Hakko E, Uckay I. Staphylococcus aureus soft tissue infection may increase the risk of subsequent staphylococcal soft tissue infections. *Int J Infect Dis*. 2017;60:44–8.
4. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of multidrug resistance. *Crit Care*. 2016;20(1):136.
5. Akinduti PA, Emoh-Robinson V, Obamoh-Triumphant HF, Obafemi YD, Banjo TT. Antibacterial activities of plant leaf extracts against multi-antibiotic resistant Staphylococcus aureus associated with skin and soft tissue infections. *BMC Complement Med Ther*. 2022;22(1):47.
6. Wado TE, Suleman S, Mohammed T. Antimicrobial Evaluation of Sequentially extracted Leaf of *Vernonia auriculifera* Hiern (Rejicho). *BMC Complement Med Ther*. 2022;22(1):219.
7. Guchhait KC, Manna T, Barai M, Karmakar M, Nandi SK, Jana D, Dey A, Panda S, Raul P, Patra A, et al. Antibiofilm and anticancer activities of unripe and ripe *Azadirachta indica* (neem) seed extracts. *BMC Complement Med Ther*. 2022;22(1):42.

8. Guefack MF, Ngangoue MO, Mbaveng AT, Nayim P, Kuete JRN, Ngaffo CMN, Chi GF, Ngameni B, Ngadjui BT, Kuete V. Antibacterial and antibiotic-potential activity of the constituents from aerial part of *Donella welwitschii* (Sapotaceae) against multidrug resistant phenotypes. *BMC Complement Med Ther.* 2022;22(1):194.
9. Demgne OMF, Damen F, Fankam AG, Guefack MF, Wamba BEN, Nayim P, Mbaveng AT, Bitchagno GTM, Tapondjou LA, Penlap VB, et al. Botanicals and phytochemicals from the bark of *Hypericum roeperianum* (Hypericaceae) had strong antibacterial activity and showed synergistic effects with antibiotics against multidrug-resistant bacteria expressing active efflux pumps. *J Ethnopharmacol.* 2021;277:114257.
10. Subramani R, Narayanasamy M, Feussner KD. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. *3 Biotech.* 2017;7(3):172.
11. Zhang L, Shi M, Ji J, Hu X, Chen F. Gut microbiota determines the prevention effects of *Luffa cylindrica* (L.) Roem supplementation against obesity and associated metabolic disorders induced by high-fat diet. *FASEB J.* 2019;33(9):10339–52.
12. Raut P, Dhawale S, Kulkarni D, Pekamwar S, Shelke S, Panzade P, Paliwal A. Pharmacodynamic findings for the usefulness of *Luffa cylindrica* (L.) leaves in atherosclerosis therapy with supporting antioxidant potential. *Future Journal of Pharmaceutical Sciences* 2021, 7(1).
13. Karmakar A, Dua P, Ghosh C. Biochemical and Molecular Analysis of *Staphylococcus aureus* Clinical Isolates from Hospitalized Patients. *Can J Infect Dis Med Microbiol* 2016, 2016:9041636.
14. Madl T, Sterk H, Mittelbach M, Rechberger GN. Tandem mass spectrometric analysis of a complex triterpene saponin mixture of *Chenopodium quinoa*. *J Am Soc Mass Spectrom.* 2006;17(6):795–806.
15. Chinnadurai V, Viswanathan P, Kalimuthu K, Vanitha A, Ranjitha V, Pugazhendhi A. Comparative studies of phytochemical analysis and pharmacological activities of wild and micropropagated plant ethanol extracts of *Manihot esculenta*. *Biocatalysis and Agricultural Biotechnology* 2019,19.
16. Akinduti AP, Osiyemi JA, Banjo TT, Ejilude O, El-Ashker M, Adeyemi AG, Obafemi YD, Isibor PO. Clonal diversity and spatial dissemination of multi-antibiotics resistant *Staphylococcus aureus* pathotypes in Southwest Nigeria. *PLoS ONE.* 2021;16(2):e0247013.
17. JN. E: A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. *Planta Med* 1998.
18. Khan I, Bahuguna A, Kumar P, Bajpai VK, Kang SC. Antimicrobial Potential of Carvacrol against Uropathogenic *Escherichia coli* via Membrane Disruption, Depolarization, and Reactive Oxygen Species Generation. *Front Microbiol.* 2017;8:2421.
19. Lee S-Y, Kim K-B-W-R, Lim S-I, Ahn D-H. Antibacterial mechanism of *Myagropsis myagroides* extract on *Listeria monocytogenes*. *Food Control.* 2014;42:23–8.
20. Biswas S, Mukherjee P, Manna T, Dutta K, Guchhait KC, Karmakar A, Karmakar M, Dua P, Panda AK, Ghosh C. Quorum Sensing Autoinducer(s) and Flagellum Independently Mediate EPS Signaling in *Vibrio cholerae* Through LuxO-Independent Mechanism. *Microb Ecol.* 2019;77(3):616–30.

21. Gupta P, Mankere B, Chekkoora Keloth S, Tuteja U, Pandey P, Chelvam KT. Increased antibiotic resistance exhibited by the biofilm of *Vibrio cholerae* O139. *J Antimicrob Chemother.* 2018;73(7):1841–7.
22. Peele KA, Ch VR, Kodali VP. Emulsifying activity of a biosurfactant produced by a marine bacterium. *3 Biotech.* 2016;6(2):177.
23. Han Y, Zhang X, Qi R, Li X, Gao Y, Zou Z, Cai R, Qi Y. Lucyoside B, a triterpenoid saponin from *Luffa cylindrica*, inhibits the production of inflammatory mediators via both nuclear factor-kappa B and activator protein-1 pathways in activated macrophages. *Journal of Functional Foods* 2020;69.
24. Liu X, Zhao M, Wang J, Yang B, Jiang Y. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. *J Food Compos Anal.* 2008;21(3):219–28.
25. Blitzke T, Schmidt J, Masaoud M. 7-O-methylaloeresin A—a new chromone glycoside from *Commiphora socotrana*. *Nat Prod Lett.* 2001;15(1):27–33.
26. Asamenew G, Bisrat D, Mazumder A, Asres K. In vitro antimicrobial and antioxidant activities of anthrone and chromone from the latex of *Aloe harlana* Reynolds. *Phytother Res.* 2011;25(12):1756–60.
27. Pham HNT, Sakoff JA, Bond DR, Vuong QV, Bowyer MC, Scarlett CJ. In vitro antibacterial and anticancer properties of *Helicteres hirsuta* Lour. leaf and stem extracts and their fractions. *Mol Biol Rep.* 2018;45(6):2125–33.
28. Hong Ngoc Thuy P, Vuong QV, Bowyer MC, Scarlett CJ. Phytochemical profiles and antioxidant capacity of the crude extracts, aqueous- and saponin-enriched butanol fractions of *Helicteres hirsuta* Lour. leaves and stems. *Chem Pap.* 2017;71(11):2233–42.
29. Mercan DA, Niculescu AG, Grumezescu AM. Nanoparticles for Antimicrobial Agents Delivery-An Up-to-Date Review. *Int J Mol Sci* 2022, 23(22).
30. Arabski M, Wegierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Effects of saponins against clinical *E. coli* strains and eukaryotic cell line. *J Biomed Biotechnol.* 2012;2012:286216.
31. Seki H, Tamura K, Muranaka T. P450s and UGTs: key players in the structural diversity of triterpenoid saponins *Plant and Cell Physiology* 2015.
32. Shendge PN, Belemkar S. Therapeutic Potential of *Luffa acutangula*: A Review on Its Traditional Uses, Phytochemistry, Pharmacology and Toxicological Aspects. *Front Pharmacol.* 2018;9:1177.
33. Martins A, Vasas A, Viveiros M, Molnar J, Hohmann J, Amaral L. Antibacterial properties of compounds isolated from *Carpobrotus edulis*. *Int J Antimicrob Agents.* 2011;37(5):438–44.
34. Fontanay S, Grare M, Mayer J, Finance C, Duval RE. Ursolic, oleanolic and betulinic acids: antibacterial spectra and selectivity indexes. *J Ethnopharmacol.* 2008;120(2):272–6.
35. LEE WSSDG. The Combination Effect of Korean Red Ginseng Saponins with Kanamycin and Cefotaxime against Methicillin-Resistant *Staphylococcus aureus*. *Biol Pharm Bull.* 2008;318:1614–7.).

36. Li K, Lin Y, Li B, Pan T, Wang F, Yuan R, Ji J, Diao Y, Wang S. Antibacterial constituents of *Fructus Chebulae Immaturus* and their mechanisms of action. *BMC Complement Altern Med*. 2016;16:183.
37. Sun X, Yang X, Xue P, Zhang Z, Ren G. Improved antibacterial effects of alkali-transformed saponin from quinoa husks against halitosis-related bacteria. *BMC Complement Altern Med*. 2019;19(1):46.

Figures

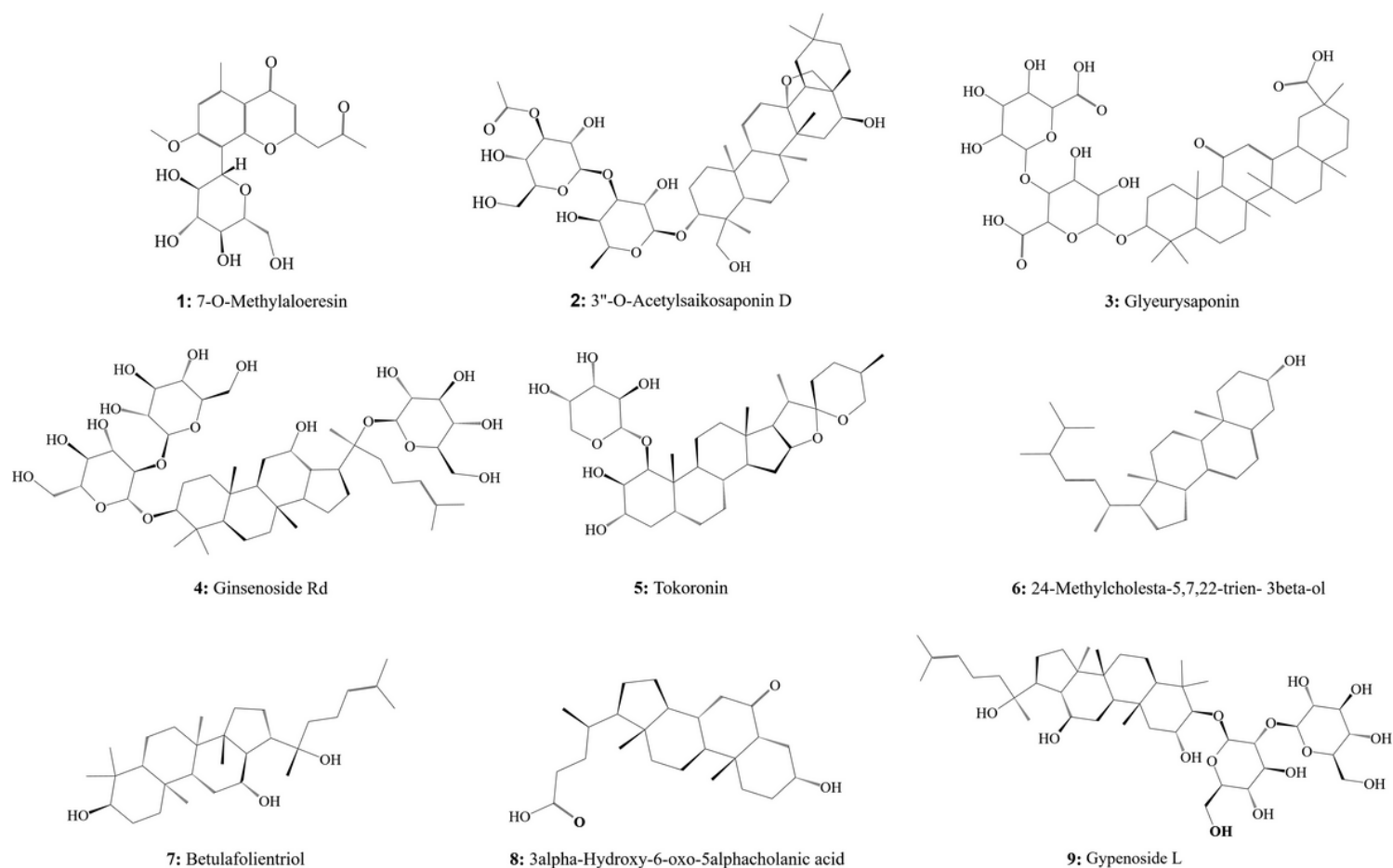


Figure 1

chemical structure diagram of saponin compounds

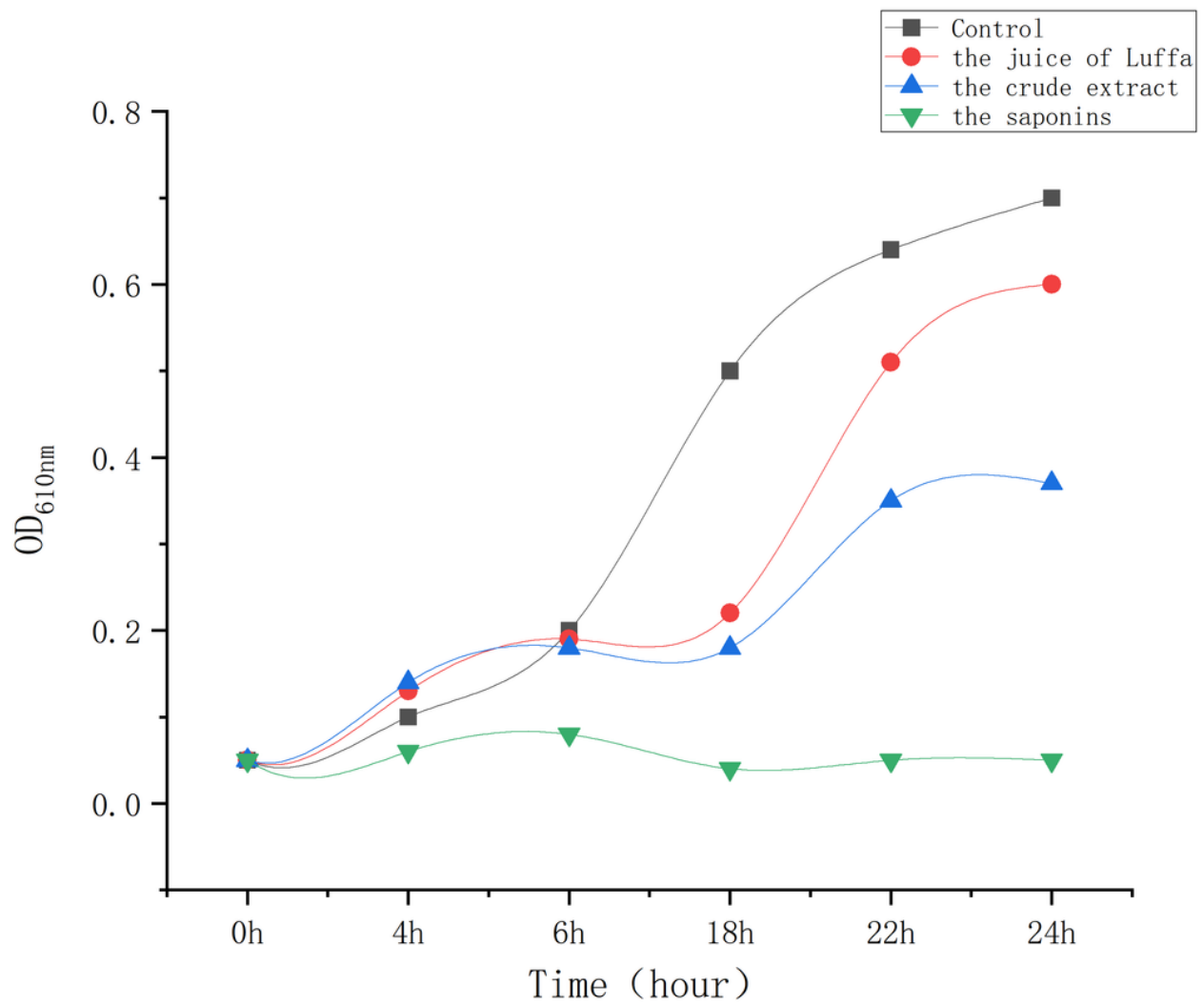


Figure 2

growth inhibition of *S. aureus* in 24 hours

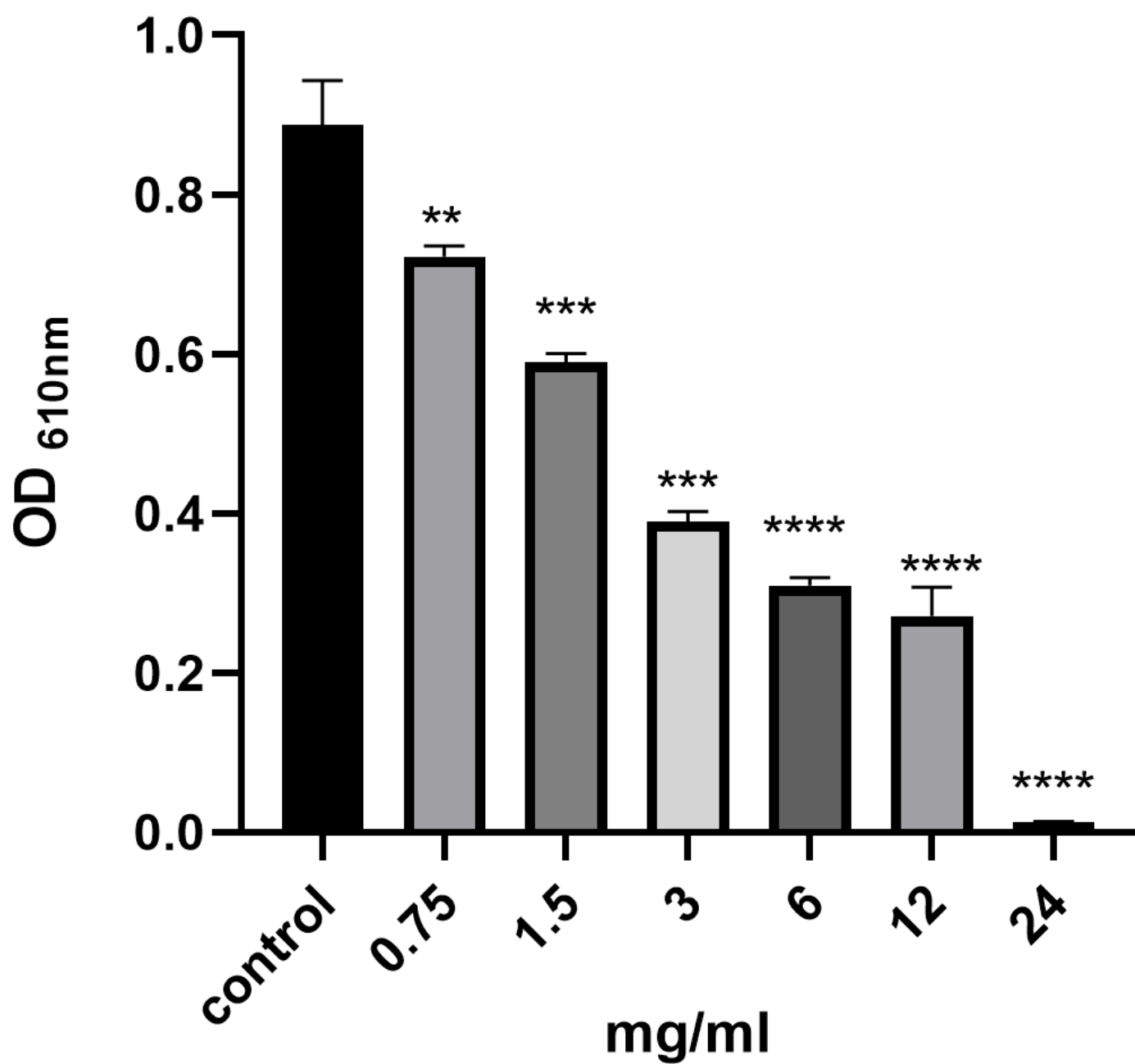


Figure 3

IC₅₀ of saponin

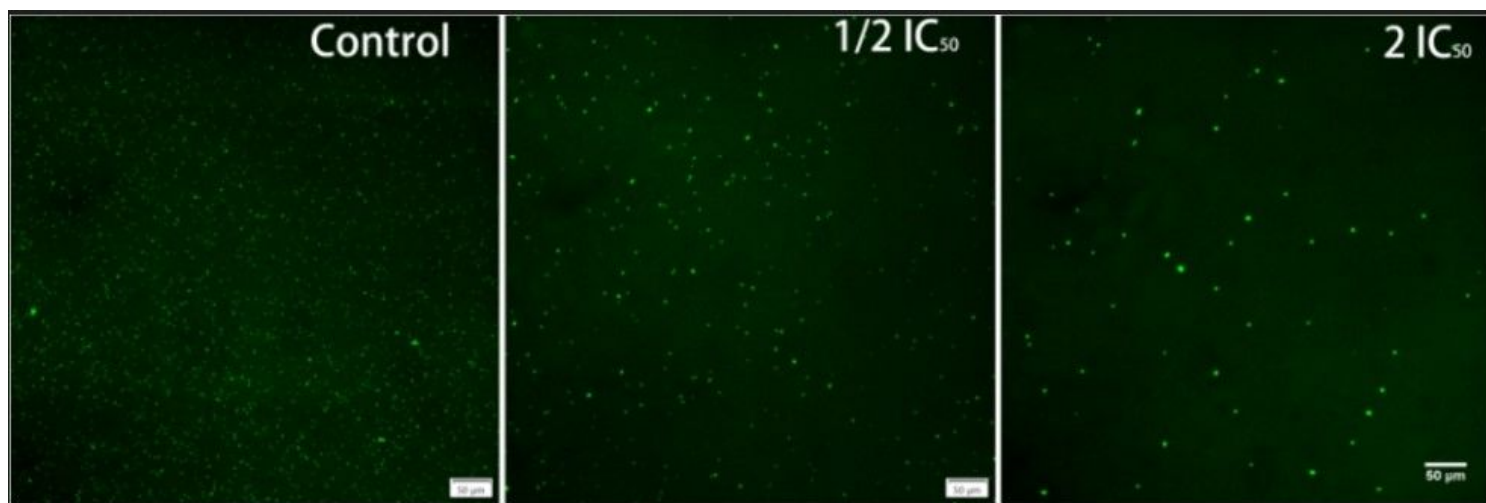


Figure 4

Acridine orange stain live bacteria

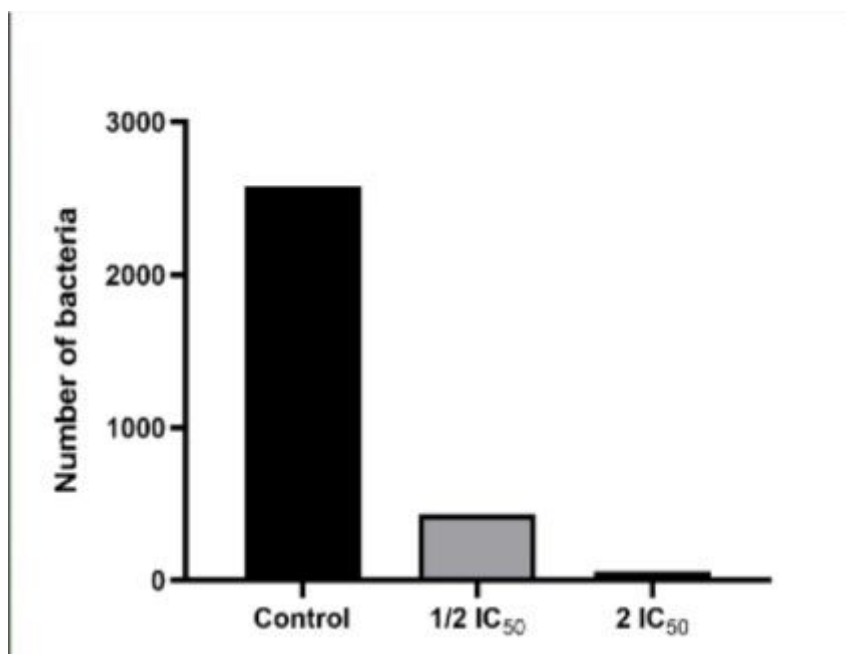


Figure 5

the number of live bacteria stained by Acridine orange

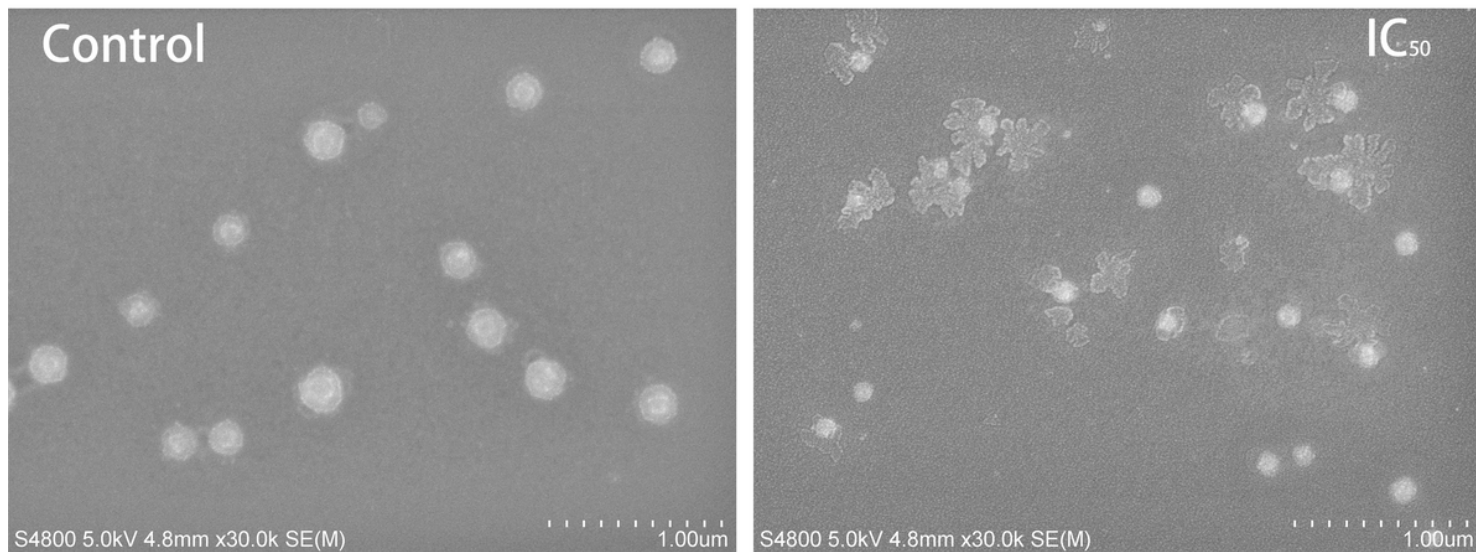


Figure 6

SEM of *S. aureus* at control group and saponin-treated group

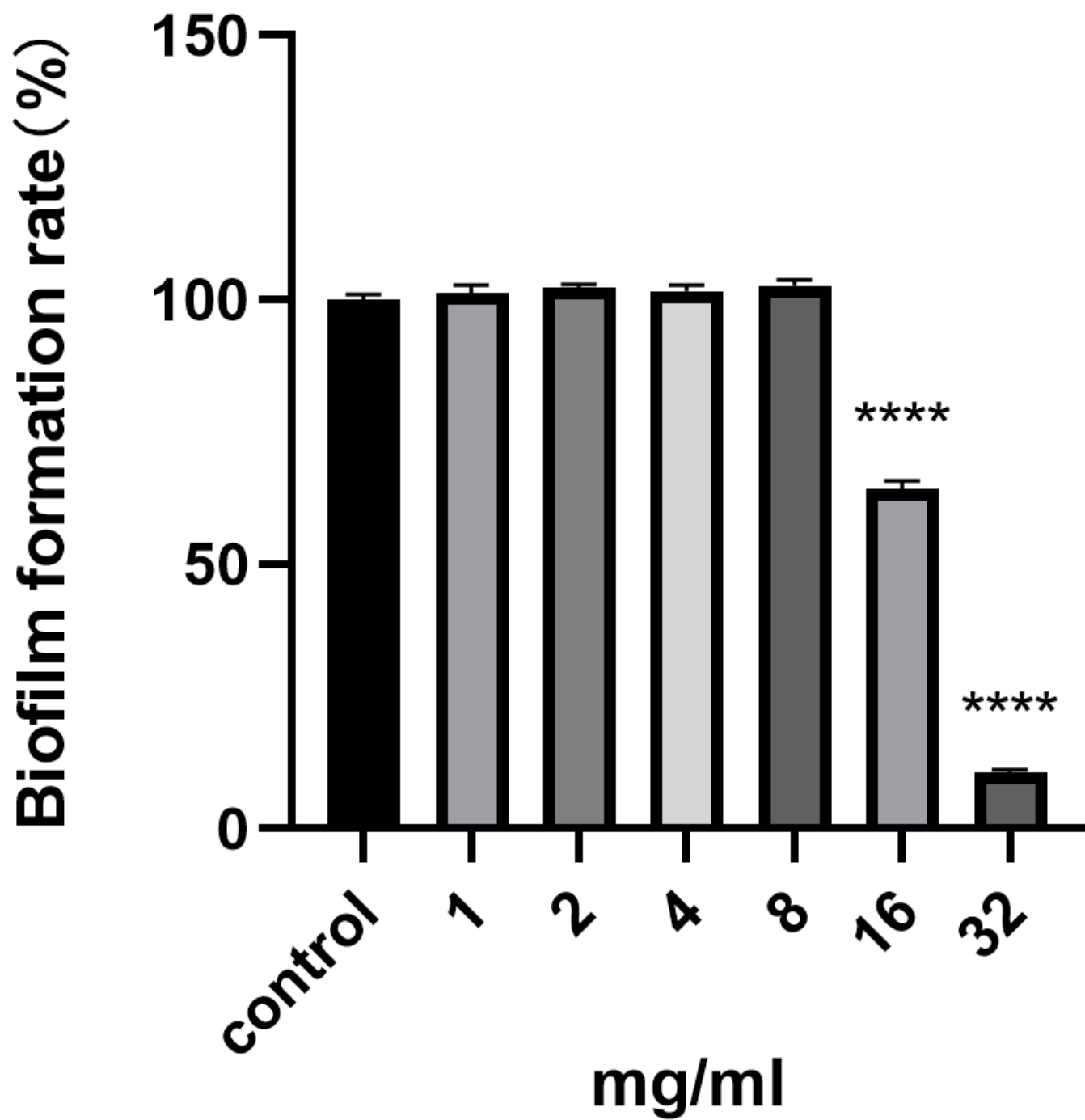


Figure 7

Inhibitory effects of the saponins on biofilm formation in *S. aureus*

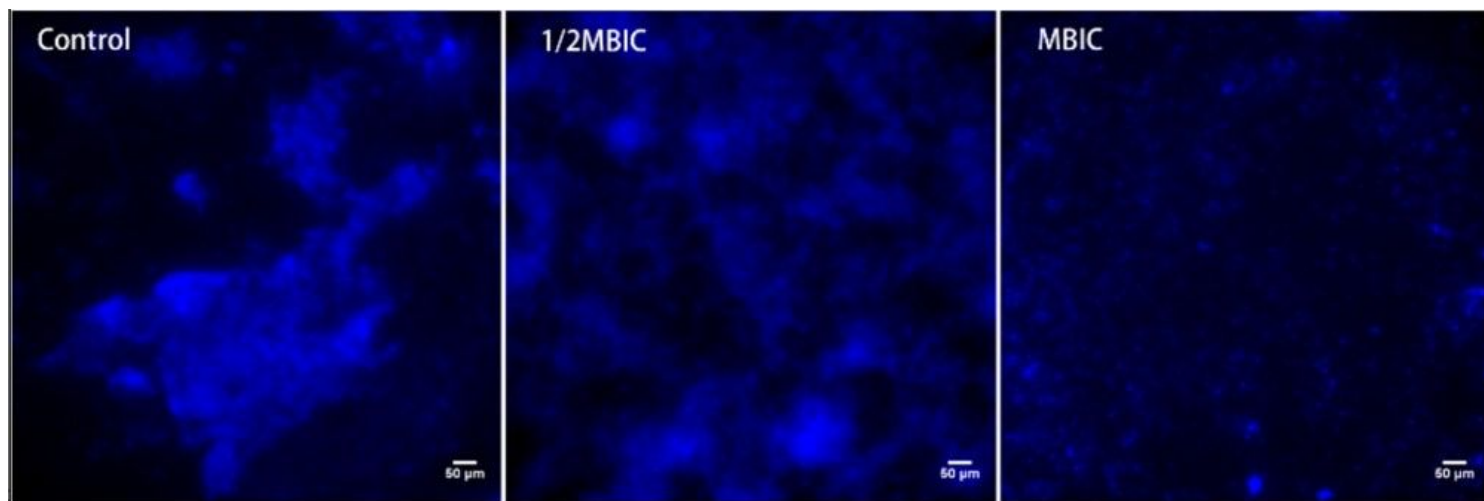


Figure 8

Fluorescence microscopic images of DAPI stained biofilm morphology treated by the saponins at the concentration of 0 (control), 1/2MBIC and MBIC

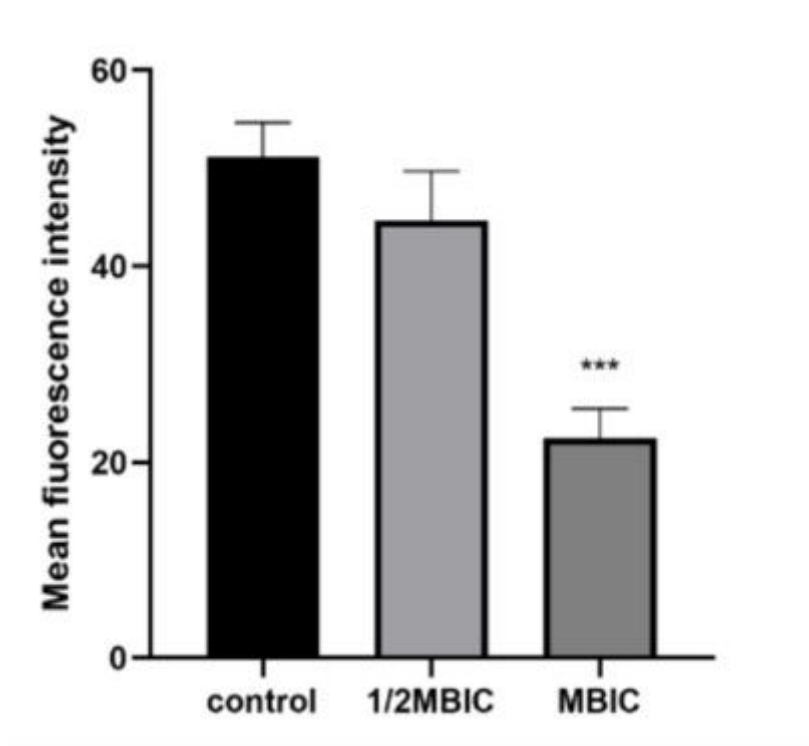


Figure 9

Mean fluorescence intensity