

Antibacterial Evaluation of Sophoraflavanone G from *Sophora pachycarpa* Against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* Biofilm

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Abstract

Antimicrobial resistance, particularly among biofilm-forming pathogens, poses a serious threat to public health and drives the urgent need for new therapeutic agents. Sophoraflavanone G, a prenylated flavonoid derived from *Sophora pachycarpa*, has shown notable antimicrobial activity, especially against Gram-positive bacteria. In this study, Sophoraflavanone G was isolated and purified using chromatographic techniques and structurally confirmed via $^1\text{H-NMR}$. Its antibacterial and anti-biofilm properties were evaluated against several bacterial strains, including *Pseudomonas aeruginosa* PAO1 and *Staphylococcus epidermidis* DSMZ 3270. The compound exhibited potent inhibitory and bactericidal activity against all tested Gram-positive bacteria, with *Listeria monocytogenes* being the most sensitive (MIC = 0.98 $\mu\text{g/mL}$). However, it showed no activity against *P. aeruginosa* in planktonic form (MIC > 1000 $\mu\text{g/mL}$). While it failed to inhibit *P. aeruginosa* biofilm formation or enhance tobramycin penetration at low doses, a higher concentration (1 mg/mL) of Sophoraflavanone G significantly improved antibiotic penetration into the biofilm. In contrast, the compound demonstrated strong inhibitory, disruptive, and biofilm-penetrating effects against *S. epidermidis*, with a clear dose-dependent response. These findings underscore the potential of Sophoraflavanone G as a candidate for managing Gram-positive and biofilm-associated infections, particularly those involving *S. epidermidis*, while highlighting the need for further development to improve its efficacy against Gram-negative pathogens.

Introduction

Antibiotic resistance (AMR) is a critical global public health issue that threatens the effectiveness of antibiotics in treating bacterial infections. This phenomenon is not only a modern challenge but has ancient origins, as bacteria have evolved resistance mechanisms over millions of years [1]. The widespread use of antibiotics in human medicine, veterinary practice, and agriculture has accelerated the development and spread of resistant bacteria, creating a complex web of resistance elements that are genetically diverse and mechanistically sophisticated [1, 2]. Biofilm-forming bacteria play a significant role in persistent infections due to their unique structural and functional characteristics that enhance survival against antimicrobial treatments and host defenses. These bacteria, such as *Pseudomonas aeruginosa*, form complex communities encased in an extracellular polymeric substance (EPS) matrix, which not only protects them from antibiotics but also facilitates genetic exchange, including antibiotic resistance genes [3, 4]. Moreover, the EPS matrix acts as a physical barrier, creating gradients of nutrients and oxygen that contribute to the metabolic dormancy of some cells, making them less susceptible to antimicrobial agents [3, 5]. Additionally, biofilms can impair the activation of immune responses, such as phagocytosis and the complement system, allowing bacteria to evade host defenses [6, 7].

P. aeruginosa PAO1 is a significant pathogen in clinical settings due to its robust antibiotic resistance mechanisms and ability to cause persistent infections. This bacterium is particularly problematic in hospital environments, where it contributes to nosocomial infections, especially in immunocompromised and cystic fibrosis patients. The resistance of *P. aeruginosa* to antibiotics is multifaceted, involving

intrinsic mechanisms such as efflux pumps and acquired resistance through gene mutations and adaptive responses [8]. [9]. Therefore, innovative treatment strategies are being explored, including non-antibiotic approaches like quorum sensing inhibition, phage therapy, and nanoparticle-based treatments, although these approaches face challenges related to cost and safety [10].

Quorum sensing (QS) in *P. aeruginosa* plays a critical role in regulating virulence and biofilm formation, significantly contributing to its pathogenicity. This process involves the production of signaling molecules that coordinate gene expression related to virulence factors and biofilm development, enabling the bacteria to adapt and thrive in hostile environments, such as during infections in immunocompromised patients [16, 17]. Disrupting QS can effectively reduce the pathogenicity of *P. aeruginosa* without exerting additional selective pressure for antibiotic resistance, as QS inhibitors (QSIs) can attenuate virulence without inhibiting bacterial growth [17, 18].

Staphylococcus epidermidis, a common skin commensal, has emerged as a significant opportunistic pathogen, particularly in healthcare settings, due to its ability to cause infections such as septicaemia and endocarditis. The prevalence of antibiotic resistance among *S. epidermidis* strains is alarming, with studies indicating high resistance rates against penicillin (95.65%), tetracycline (91.30%), and methicillin (92.2%) among hospital isolates [11, 12]. The bacterium's capacity to form biofilms enhances its virulence and resistance to antibiotic treatment, complicating management strategies [13]. Furthermore, the presence of resistance genes, such as *mecA* and various efflux pumps, contributes to its multi-drug resistant phenotype, making infections difficult to treat [14]. This multidrug resistance poses significant challenges for treatment, particularly in immunocompromised patients, highlighting the need for ongoing surveillance and novel therapeutic strategies [15].

In *S. epidermidis*, QS is a critical mechanism that regulates biofilm formation and virulence, primarily through the accessory gene regulator (AGR) system. This system is activated by autoinducing peptides (AIPs) that bind to the AgrC receptor, leading to the expression of virulence factors and biofilm dispersal agents such as phenol-soluble modulins [16]. Inhibiting QS in *S. epidermidis* is a promising strategy to combat biofilm-associated infections, particularly those involving drug-resistant strains. Various approaches have been explored, including the use of synthetic peptides and natural compounds [17].

Conventional antibiotics are often ineffective against biofilms due to several inherent challenges. Biofilms, which are communities of bacteria encased in a protective matrix, exhibit reduced susceptibility to antibiotics as these drugs typically target planctonic cells, while many cells within biofilms are metabolically inactive or in a persister state. In addition, the resistance is amplified by efflux pumps and physiological changes [18, 19].

The exploration of plant-derived compounds as alternative antimicrobial agents has gained significant traction due to the rising threat of AMR and the limitations of conventional antibiotics. Various studies highlight the diverse antimicrobial properties of these compounds, which include essential oils, extracts, and bioactive substances from both medicinal and non-medicinal plants, as well as marine sources [20–22]. These plant-derived agents exhibit multi-target mechanisms, making them less prone to resistance

compared to traditional single-target antibiotics [20]. Additionally, they have shown efficacy against biofilms, which are notoriously difficult to treat due to their protective matrices [23].

Sophora pachycarpa, a member of the Fabaceae family, has been utilized in traditional medicine for its diverse therapeutic properties, primarily attributed to its rich content of flavonoids, particularly prenylated flavonoids. Research has identified several bioactive compounds, including Sophoraflavanone G and Sophoraisoflavanone A, which exhibit significant biological activities such as anti-inflammatory, antibacterial, and cytotoxic effects [24, 25]. Sophoraflavanone G, a prenylated flavonoid predominantly isolated from plants of the genus *Sophora*, has garnered attention for its notable antimicrobial properties. Recent studies have demonstrated its efficacy against a range of bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecium* [26, 27]. Its mode of action is believed to involve disruption of bacterial cell membrane integrity and interference with peptidoglycan synthesis, making it a promising candidate for novel antimicrobial therapies [28].

Despite growing evidence supporting the broad-spectrum antibacterial activity of Sophoraflavanone G, its specific effects on biofilm formation and eradication remain inadequately characterized and the potential of Sophoraflavanone G to act synergistically with conventional antibiotics, particularly tobramycin against *P. aeruginosa* has not been systematically evaluated. These knowledge gaps underscore the necessity for targeted investigations into the anti-biofilm and combinatory antibacterial properties of this compound, especially in the context of biofilm-mediated antimicrobial resistance. This study, tries to introduce the ability of Sophoraflavanone G in inhibition and disruption of biofilm, in both Gram-positive and Gram-negative bacteria, including *P. aeruginosa* and *S. epidermidis*, as well as its capacity to potentiate the effect of antibiotics.

Material and Method

Chemicals and Materials

All solvents and reagents used in this study were of analytical grade. Silica gel GF254 plates and silica gel (230–400 mesh) for column chromatography were obtained from Merck (Germany). Ethyl acetate, petroleum ether (60–80°C), dichloromethane, methanol, and acetone were purchased from Dr. Mojallali Co. (Iran). Vanillin, sulfuric acid, sodium chloride (NaCl), glucose, crystal violet, and 2,3,5-triphenyl tetrazolium chloride (TTC) were also sourced from Merck (Germany). Dimethyl sulfoxide (DMSO) was obtained from Merck (Germany), and absolute ethanol was purchased from Simin Tak (Iran). Tryptic Soy Agar (TSA) was acquired from Himedia (India), while Mueller-Hinton Broth (MHB) was provided by Liofilchem (Italy). Tobramycin was sourced from Sigma (USA), and cloxacillin was obtained from Farabi (Iran).

The bacterial strains used in this study included *Pseudomonas aeruginosa* PAO1 (Wild type Nottingham), *Staphylococcus epidermidis* (DSMZ 3270, Germany), *Listeria monocytogenes* (PTCC 1298, Iran), *Bacillus subtilis* (PTCC 1247, Iran), methicillin-sensitive *Staphylococcus aureus* (MSSA, PTCC 1431,

Iran), methicillin-resistant *S. aureus* (MRSA, PTCC 1112, Iran), and *Micrococcus luteus* (PTCC 1110, Iran). All strains were maintained and handled according to standard microbiological practices.

Extraction and Purification

The roots of *S. pachycarpa* were collected in June 2019 from the Kalat region, Razavi Khorasan Province, Iran. Botanical identification was performed by Mrs. Soozani, and a voucher specimen (No. 13275) was deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. The air-dried roots (1350 g) were powdered using an electric grinder and macerated in 4 L of acetone for 24 hours at room temperature. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to afford a reddish-brown residue (35.5 g).

Isolation and Purification of Sophoraflavanone G and Structure Elucidation

The crude extract was subjected to column chromatography on silica gel using a polarity-gradient elution system comprising petroleum ether, ethyl acetate, and methanol. A total of 65 fractions (200 mL each) were collected and concentrated under reduced pressure. The solvent ratios used for each fraction are listed in Table 5. Further purification of the target compound was achieved by preparative thin-layer chromatography (PTLC). Development was carried out in a petroleum ether:ethyl acetate (6:4) system. The resolved band corresponding to the standard was visualized under UV light, marked, scraped off, and extracted with acetone. The extract was filtered and the solvent was removed under reduced pressure to obtain the purified compound. The structure of compound was confirmed through spectral interpretation (¹H-NMR) and comparison with previously published data [29].

Determination of MIC and MBC

The MIC (Minimum Inhibitory Concentration) of sophoraflavanone G was tested against *S. aureus*, *S. epidermidis*, *B. subtilis*, *L. monocytogenes*, *M. luteus*, and *P. aeruginosa* PAO1. A 2 mg sample of the compound was dissolved in 70 µl DMSO (3.5%) and 930 µl MHB (Mueller-Hinton Broth). Serial dilutions (1000 – 0.49 µg/ml) were prepared in a 96-well plate, with each concentration tested in duplicate. Positive control wells contained only separate bacteria in MHB; negative controls contained no bacteria. After adding 20 µl bacterial suspension (10⁶ CFU/ml), the plates were incubated for 24 h. Then, 20 µl TTC (5 mg/ml) was added and incubated for another 1 h. The lowest concentration with no color change was reported as MIC. Following MIC assays, samples from wells with no red color were streaked on TSA agar and incubated for 24 h. The lowest concentration with no bacterial growth was reported as MBC (Minimum Bactericidal Concentration).

Biofilm Assays Involving *P. aeruginosa* PAO1

To investigate the inhibitory effect of sophoraflavanone G on biofilm formation by *P. aeruginosa* PAO1, a suspension containing 10⁶ CFU/mL of the bacterium was prepared. A total of 20 µL of this suspension was added to 180 µL of glucose-enriched Mueller-Hinton broth (MHB) containing sophoraflavanone G at concentrations corresponding to ½ MIC (0.5 mg/mL). For the positive control, 180 µL of medium and 20 µL of bacterial suspension were used. The negative control wells contained only the medium. Plates were incubated at 37°C to allow biofilm formation.

After 24 and 36 hours, the content of each well was gently aspirated and refreshed with medium containing the same concentration of sophoraflavanone G. At 48 hours, the wells were emptied and washed twice with 200 µL of normal saline. Subsequently, 50 µL of 0.03% crystal violet solution was added to each well and allowed to incubate for 10 minutes. Excess dye was then removed, and wells were washed at least twice to remove unbound stain. After drying, 200 µL of 95% ethanol was added to each well to solubilize the bound dye, and absorbance was measured at 590 nm using an ELISA reader to quantify the biomass of the formed biofilm.

To investigate whether sophoraflavanone G facilitates the penetration of tobramycin into the constructed biofilm, first, biofilms were allowed to develop by adding 20 µL of bacterial suspension (10⁶ CFU/mL) to 180 µL of glucose-supplemented MHB in 96-well plates, followed by a 24-hour incubation. After this period, the medium was removed and replaced with fresh medium. At 36 hours, the wells were treated with either 1 mg/mL or 0.5 mg/mL of sophoraflavanone G in combination with tobramycin at its MIC level (2 µg/mL). At the 48-hour mark, the wells were washed and then received 180 µL of enriched medium and 20 µL of 5 mg/mL tetrazolium salt (TTC). Following a 4-hour incubation, absorbance at 450 nm was measured to determine metabolic activity, reflecting penetration and antibacterial activity within the biofilm. Wells treated with tobramycin only were used as control (2 µg/mL). The results compared with the positive controls which received no treatment.

Biofilm Assays Involving *S. epidermidis* DSMZ 3270

In evaluating the ability of sophoraflavanone G to inhibit biofilm formation by *S. epidermidis*, a suspension containing 10⁶ CFU/mL was added (20 µL) to 180 µL of glucose-enriched MHB containing sophoraflavanone G at ½ MIC (3.9 µg/mL) and MIC (7.8 µg/mL). The plates were incubated, and after 24, and 36 hours, the medium was gently replaced with fresh medium of identical composition, preserving any forming biofilm. After 48 hours, the wells were washed and stained with crystal violet, followed by measurement of absorbance at 590 nm.

In evaluating biofilm disruption, 20 µL of the bacterial suspension was combined with 180 µL of glucose-enriched MHB in 96-well plates. After 24-hours incubation, the medium was replaced, and biofilms were allowed to continue forming. At 36 hours, the wells were treated with sophoraflavanone G at ½ MIC and MIC concentrations. Positive controls received only fresh medium. After 48 hours, standard crystal violet staining was carried out and absorbance was measured at 590 nm.

For determining compound penetration into established biofilms, the same procedure was followed, with treatments administered with the similar concentrations of sophoraflavanone G, after 36 hours. Again, at 48 hours, all wells were emptied and received 180 μ L of fresh glucose-enriched MHB along with 20 μ L of 5 mg/mL TTC solution. After a 4-hour incubation period, absorbance was read at 450 nm to assess bacterial metabolic activity within the biofilm matrix, serving as an indirect indicator of drug penetration.

Statistical Analysis

The results of each experiment were evaluated after three repetitions as the means $\pm$ standard deviation. All data were analyzed by SPSS software (version 27.0.1) using one-way ANOVA (Tamhane/Tukey's Multiple Comparison Tests) at significant level of p-value < 0.05.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals. All the plant species were collected from various locations by Dr. Milad Iranshahy following proper guidelines and legislation procedures and identified by Ms. Souzani (MSc of Systematic Botany, Department of Pharmacognosy, School of Pharmacy) and have been deposited at the Herbarium of School of Pharmacy, Mashhad University of Medical Sciences, Iran. No specific license was required for the collection of the plants.

Results and Discussion

Sophoraflavanone G characterization

Sophoraflavanone G, is a bioactive compound that exhibits significant antibacterial properties, particularly against *E. faecium*, by disrupting the bacterial cell wall. This disruption is evidenced by the compound's ability to bind to peptidoglycans, leading to cell wall damage and subsequent cell lysis, as confirmed by transmission electron microscopy [26]. Additionally, Sophoraflavanone G demonstrates synergistic antibacterial effects when combined with other antimicrobial agents, such as ampicillin and gentamicin, against various oral bacteria, significantly reducing the minimum inhibitory and bactericidal concentrations required for efficacy [30, 31]. Despite its promising pharmacological activities, including anti-inflammatory and antiproliferative effects, SFG's clinical application is limited by its poor water solubility and bioavailability, necessitating further research to enhance its therapeutic potential [32]. In this study, Sophoraflavanone G was isolated and purified using Silica gel column chromatography and preparative thin-layer chromatography (PTLC) using a petroleum ether:ethyl acetate system at a 6:4 ratio. The chemical structure of the compound is shown in Fig. 1.

Based on the $^1\text{H-NMR}$ data (Fig. 2), proton number 2 appears as a doublet of doublets (dd) at 5.68 ppm with coupling constants $J = 13$ and 2.7 Hz. The axial and equatorial protons at position 3 are observed at 3.0 and 2.79 ppm, respectively, with corresponding coupling constants of $J = 13$ and 17 Hz for the axial proton, and $J = 2.7$ and 17 Hz for the equatorial proton. These values confirm the presence of the flavanone scaffold with an alpha-positioned B-ring.

Three methyl signals were appeared at 1.5, 1.58, and 1.67 ppm, corresponding to the C-9, 10, and 4, respectively. The hydroxyl proton at position 5 (H-5) showed a sharp singlet at 12.21 ppm. Proton number 6 (H-6) appears as a singlet at 6.05 ppm. The methylene protons at position 5 appear as two doublets at 4.56 and 4.61 ppm. Aromatic protons on the B-ring are observed at 6.46 (5'), 6.5 (3'), and 7.4 ppm (6'). A detailed summary of the ¹H-NMR assignments is provided in Table 1.

Table 1. ¹H-NMR Data for Sophoraflavanone G

Position	¹ H-NMR (ppm, <i>J</i> in Hz)
2	5.67 (dd 13.2, 2.7)
3 a	3.00 (dd 13.2, 17),
3 b	2.79 (dd 2.7, 17)
4	–
5	12.21
6	6.05 (s)
3'	6.50 (d 2.2)
5'	6.46 (dd 8.3, 2.2)
6'	7.40 (dd 8.2)
1'' a	2.64 (dd 1.2)
1'' b	2.66 (d 2.3)
2''	2.58 (m)
4''	1.67 (s)
5''	4.56, 4.61 (brs)
6''	2.05 (m)
7''	5.01 (brt)
9''	1.50 (s)
10''	1.58 (s)

Antibacterial Activity of Sophoraflavanone G

MIC and MBC of Sophoraflavanone G against the selected bacterial strains are shown in Table 2. The compound exhibited potent activity against the all tested Gram-positive bacteria, including MRSA. In contrast, the Gram-negative bacterium, *P. aeruginosa* PAO1, was unaffected at concentrations up to 1000 µg/mL.

Table 2. MIC and MBC of sophoraflavanone G against the tested microorganisms (all values are expressed in µg/ml).

Microorganism	MIC (µg/mL)	MBC (µg/mL)
<i>Pseudomonas aeruginosa</i> PAO1	>1000	>1000
<i>Staphylococcus epidermidis</i> DSMZ 3270	7.8	7.8
<i>Listeria monocytogenes</i> 1298	0.98	7.8
<i>Bacillus subtilis</i> 1247	7.8	7.8
<i>Staphylococcus aureus</i> 1431 (MSSA)	7.8	7.8
<i>Staphylococcus aureus</i> 1112 (MRSA)	7.8	7.8
<i>Micrococcus luteus</i> 1110	7.8	7.8

Among Gram-positive bacteria, *L. monocytogenes* exhibited the greatest sensitivity (MIC = 0.98 µg/mL). Identical MIC and MBC values (7.8 µg/mL) were observed for *S. epidermidis*, MRSA, MSSA, *B. subtilis*, and *M. luteus*. The MBC values confirmed that Sophoraflavanone G has bactericidal activity against all tested Gram-positive bacteria. This lower effect against Gram-negative bacteria could be due to the presence of a rich hydrophilic lipopolysaccharides (LPS) barrier in outer membrane of such pathogens, which prohibits the penetration of strong hydrophobic compounds [33, 34].

Biofilm Inhibition and Synergistic Activity in *P. aeruginosa*

The anti-biofilm potential of Sophoraflavanone G against *P. aeruginosa* PAO1 was assessed using the crystal violet staining method. As shown in Fig. 3, the compound was ineffective in inhibiting biofilm formation at any tested concentration.

In additional experiments, the ability of Sophoraflavanone G to enhance the penetration of tobramycin into mature *P. aeruginosa* biofilms was evaluated. Co-treatment with tobramycin and 0.5 mg/mL of Sophoraflavanone G showed no significant improvement in biofilm penetration compared to tobramycin alone or untreated controls (Fig. 4).

However, at 1 mg/mL, Sophoraflavanone G significantly enhanced the activity of tobramycin, leading to improved antibiotic penetration and increased killing of biofilm-embedded bacteria (P < 0.001). These findings suggest a concentration-dependent synergistic effect between Sophoraflavanone G and tobramycin. In analysis of the biofilm degradation, the intensity of the color formed was so high and there were no differences between the control and treated wells (data are not shown). Hence, the mechanism of this effect is not due to the destruction and removal of the biofilm layer.

Inhibitory Effects on Biofilm Formation, Disruption of Constructed Biofilm, and Eliminating Bacteria Protected within the Biofilm of *S. epidermidis*

The results of the compound's effect on inhibiting *S. epidermidis* biofilm formation are presented in Fig. 5. Based on Fig. 5, unlike *P. aeruginosa*, sophoraflavanone G was able to inhibit biofilm formation of *S. epidermidis* DSMZ 3270, significantly. Interestingly, this effect was also seen on the constructed biofilm of *S. epidermidis* (Fig. 6). In other words, Sophoraflavanone G at both concentrations of 1/2 MIC and MIC was significantly effective in disrupting the biofilm of *S. epidermidis* DSMZ 3270.

Therefore, it was expected that this compound, even on its own, would be able to destroy bacteria sheltering in biofilms. The results of the compound's effect on penetrating the biofilm of *S. epidermidis* are presented in Fig. 7.

Based on this figure, sophoraflavanone G, at both MIC and 1/2 MIC concentrations was significantly effective in eliminating bacteria protected within the biofilm. This figure shows that combination treatment with an appropriate antibiotic can lead to the complete elimination of the cells protected within the biofilm.

Jia et al. demonstrated that alkaloids from *Sophora alopecuroides* interfere with AI-2 signaling in *S. epidermidis* biofilms, suggesting that similar bioactive compounds, such as sophoraflavanone G, may exert anti-biofilm effects by disrupting quorum-sensing pathways [35].

According to the study by Chan et al., Sophoraflavanone G exhibited notable antimicrobial activity against Gram-positive bacteria. However, it demonstrated no antimicrobial effect against Gram-negative bacteria such as *Escherichia coli*, *E. coli* O157, *Vibrio vulnificus*, *Shigella*, and *Salmonella typhi* [36]. Therefore, its lack of activity against the resistant *P. aeruginosa* PAO1 strain observed in this study is understandable.

On the other hand, regardless of the Staphylococcal strain tested (MRSA or MSSA), similar results were obtained for MIC and MBC. Tsuchiya et al. confirmed that among the flavanones extracted from *Sophora* species, Sophoraflavanone G and Exiguaflavanone D exhibited the highest activity against various MRSA strains, with MIC values ranging from 13.3 to 25.6 µg/ml. The MICs of these flavanones against MSSA and MRSA were determined to be similar, suggesting that the anti-MRSA activity of the flavanones is independent of the degree of antibiotic resistance in Staphylococcal strains [37]. However, according to the studies, the MIC values for various *S. aureus* strains in different studies ranged from 0.05 to 8 µg/ml, which is consistent with the 7.8 µg/ml value obtained in this study [36, 38-40].

In the experiments conducted in this study, the MIC of Sophoraflavanone G against *S. epidermidis* was found to be 7.8 µg/ml, which closely aligns with the MIC reported by Wan et al. [41]. An et al. also demonstrated the antibacterial activity of prenylated flavonoids from *Sophora flavescens* against the plant pathogen *Xanthomonas oryzae*, suggesting a broad-spectrum effect that extends beyond human pathogens [42]. Therefore, the MIC values obtained for Sophoraflavanone G against the microorganisms listed in Table 1 are consistent with the experimental data from previous studies

Various studies have proposed antimicrobial mechanisms for the compound Sophoraflavanone G. Tsuchiya and Iinuma investigated the antibacterial activity mechanism of Sophoraflavanone G and suggested that the compound reduces the fluidity of both the outer and inner layers of bacterial membranes. Specifically, the presence of the aliphatic lavandulyl group at the C8 position enables Sophoraflavanone G to interact with bacterial cell membranes more effectively than Naringenin (5,7,4'-trihydroxyflavanone), thereby enhancing its membrane-disruptive activity. Sophoraflavanone G affects membrane fluidity at concentrations ranging from 0.05 to 5 µg/ml, whereas Naringenin requires higher concentrations above 2.5 µg/ml to exhibit similar effects. Notably, Sophoraflavanone G reduces the fluidity of both the outer and inner membrane layers equally, while Naringenin predominantly affects the outer membrane layer more than the inner one [43].

In one study aimed at confirming cell wall disruption in *Enterococcus faecium* caused by Sophoraflavanone G, the compound was added to bacterial strains at concentrations ranging from 0 to 50 µg/ml. Subsequently, UV absorbance of the supernatant was measured at 260 nm and 280 nm using a spectrophotometer. A significant increase in absorbance was observed following the addition of Sophoraflavanone G, indicating leakage of intracellular components such as proteins and nucleic acids. Based on transmission electron microscopy (TEM) images and additional assays, the proposed antimicrobial mechanism of Sophoraflavanone G in this article involves its binding to bacterial cell wall peptidoglycans, leading to cell wall disintegration and ultimately bacterial cell lysis [26].

These mechanisms were also confirmed by Weng et al. Their comprehensive mechanistic studies revealed that Sophoraflavanone G could destruct MRSA bacterial membrane integrity, increase permeability, and change the membrane potential. It could also disrupt cell wall synthesis, induce hydrolysis in the cell wall, and inhibit construction of bacterial biofilms by reducing the biosynthesis of PIA (the major component in the biofilm matrix of Gram-positive bacteria). In addition, it can interfere with the energy production of MRSA and interrupt the normal physiological activities. In vivo studies have shown that treatment of skin infections caused by MRSA with Sophoraflavanone G, promoted wound healing, and inhibited purulent secretion through remarkable reduction of the bacteria in the infected areas and suppression of the of pro-inflammatory cytokine, IL-6, levels [27]. The majority of these antibacterial mechanisms of Sophoraflavanone G were also established by An et al. in the case of *Xanthomonas oryzae* pv *oryzae* (*Xoo*) including inhibition of biofilm formation and metabolic activity, reducing extracellular enzyme activity and surface hydrophobicity, inducing the production of ROS, decreasing ATP generation, disrupting membrane potential of mitochondria, and finally triggering apoptosis [42].

Moreover, several studies have investigated the synergistic effect of Sophoraflavanone G in combination with antibiotics. According to the study by Fakhimi et al., the MIC values of Sophoraflavanone G and gentamicin against *S. aureus* were determined to be 0.05 µg/ml and 32 µg/ml, respectively. However, in the presence of a sub-MIC concentration of Sophoraflavanone G (0.03 µg/ml), the MIC of gentamicin for this strain was reduced from 32 µg/ml to 8 µg/ml—a fourfold decrease. These findings suggest that the antibacterial effect of gentamicin is enhanced by Sophoraflavanone G [38].

Additionally, Sophoraflavanone G and gentamicin demonstrated significant PAE (post-antibiotic effect) and PA-SME (post-antibiotic sub-MIC effect) at the tested concentrations against *S. aureus*. The prolongation of PAE induced by Sophoraflavanone G and gentamicin was dose-dependent. Moreover, Sophoraflavanone G at sub-MIC concentrations enhanced both the PAE and PA-SME of gentamicin in a dose-dependent manner. The most pronounced potentiating effect for gentamicin was observed at synergistic MIC (8 µg/ml) and half-synergistic MIC (4 µg/ml) levels in the presence of 0.03 µg/ml Sophoraflavanone G (a concentration below its MIC of 0.05 µg/ml). This synergy increased the PA-SME of gentamicin at 4 µg/ml from 15 minutes to 80 minutes—a sixfold extension. Sophoraflavanone G at 0.03 µg/ml not only enhanced gentamicin's antibacterial activity but also significantly prolonged its PA-SME duration at synergistic MIC levels. At present, the underlying reason for these enhancements is not fully understood. It has been hypothesized that Sophoraflavanone G's antibacterial effect may be attributed to a reduction in bacterial cell membrane fluidity [44].

Sophoraflavanone G, isolated from *Sophora alopecuroides*, exhibited a significant synergistic effect with the antibiotic norfloxacin at a low concentration (1 mg/ml) against the fluoroquinolone-resistant *S. aureus* strain SA1199B (MRSA) under in vitro conditions. The MIC of norfloxacin combined with Sophoraflavanone G was found to be sixteen times lower than that of norfloxacin alone, decreasing from 32 µg/ml to 2 µg/ml. Since the SA1199B strain overexpresses the NorA efflux protein, it is hypothesized that the synergistic mechanism between Sophoraflavanone G and norfloxacin involves inhibition of this pump. Results from ethidium bromide (EtBr) efflux assays supported this hypothesis, showing that Sophoraflavanone G exhibited NorA-efflux inhibitory activity in *S. aureus* SA1199B compared to the positive control [45]. Moreover, it has been shown that this compound enhances the effect of further antibiotics as either additivity or synergy including ciprofloxacin, erythromycin, fusidic acid, ampicillin and oxacillin [46]. In the experiments conducted in this study, Sophoraflavanone G also exhibited promising synergistic activity with tobramycin against *P. aeruginosa* PAO1.

In a study by Wan et al. Sophoraflavanone G demonstrated notable antibacterial and anti-biofilm activity against *S. epidermidis* (ATCC 35984, BF⁺). The compound's anti-biofilm effects against this bacterium were also evaluated at concentrations of 3.125, 6.25, 12.5, 50, and 100 µg/ml. the results revealed that Sophoraflavanone G effectively inhibited biofilm formation of *S. epidermidis* at all tested concentrations except 3.125 µg/ml [41]. Additionally, Fan et al. showed that alkaloids from *Sophora flavescens* are effective against metronidazole-resistant *Gardnerella vaginalis* under both planktonic and biofilm conditions, reinforcing the biofilm-targeting potential of this genus [47]. Future investigations should also explore its mechanism of synergy at the molecular level and evaluate its in vivo therapeutic potential, In this context, Sychrová et al. emphasized the value of prenylated flavonoids like sophoraflavanone G in treating topical infections and promoting wound healing, due to their dual antimicrobial and anti-inflammatory properties [46].

Conclusion

In conclusion, sophoraflavanone G is a potent antibacterial agent, especially for Gram-positive bacteria, and a promising anti-biofilm compound, particularly against *S. epidermidis*. The ability of this compound to penetrate biofilms and kill microorganisms is also a notable point that could provide combination therapy with antibiotics. On the other hand, it has been shown sophoraflavanone G has no cytotoxic effects (up to 8 µg/ml), that is important for wound healing [48]. However, its limited efficacy against Gram-negative bacteria, principally in biofilm-associated states, highlights the need for further structural optimization or smart formulation strategies to enhance its activity. Future investigations should explore its mechanism of synergy and probable drug interactions at the molecular level, possible toxicity to various human cells, and also evaluate its in vivo therapeutic potential.

Declarations

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

N.Z. Initiated the study, conducted the experimental workG.H. Drafted the original manuscriptA. S .Revised the manuscriptB. F. B. Provided supervisionM.I. Provided supervision, developed the conceptual framework, and performed critical revisions V. S. Developed the overall research objectives and methodologies, provided supervision, and conducted critical revisions

Data Availability

Data is provided within the manuscript

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Figures

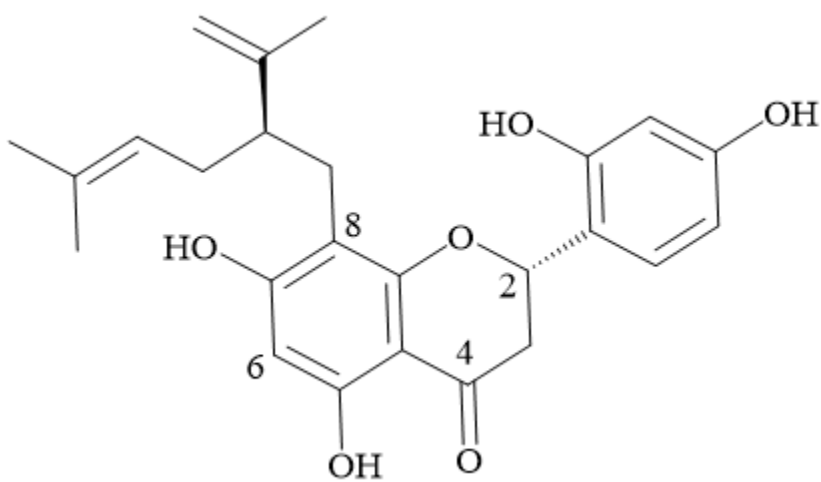


Figure 1

Chemical structure of Sophoraflavanone G

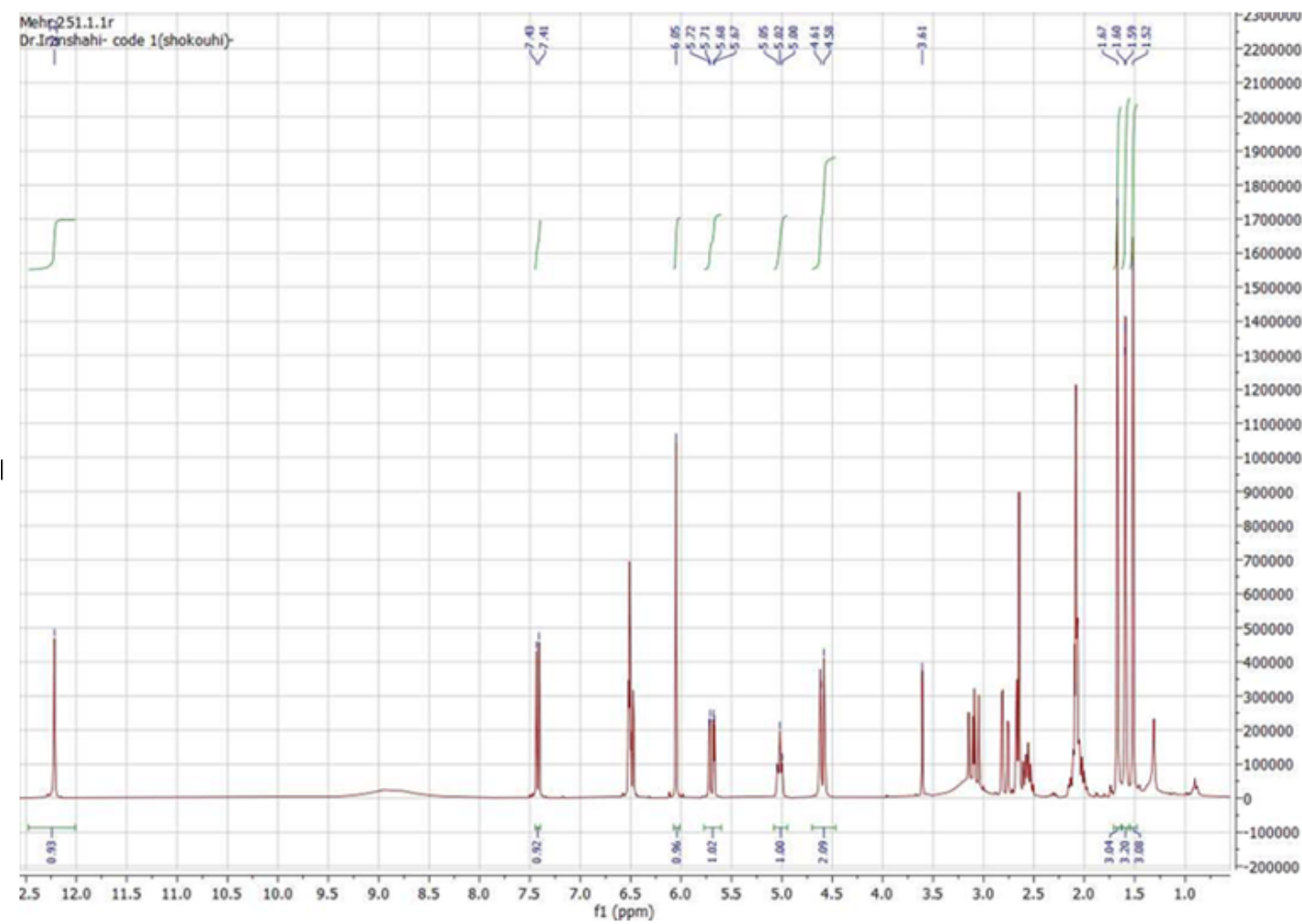


Figure 2

The ¹H-NMR data of Sophoraflavanone G

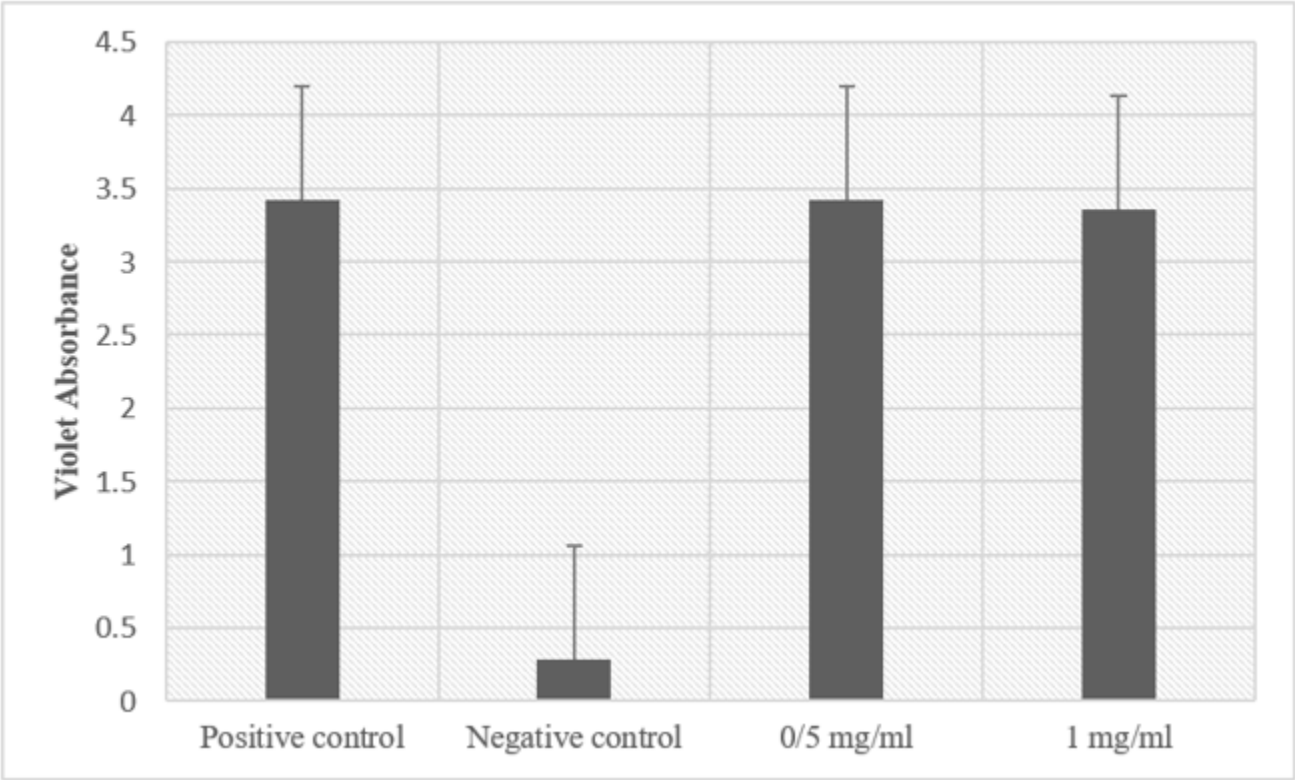


Figure 3

The effect of Sophoraflavanone G on the inhibition of biofilm formation in *Pseudomonas aeruginosa* PAO1

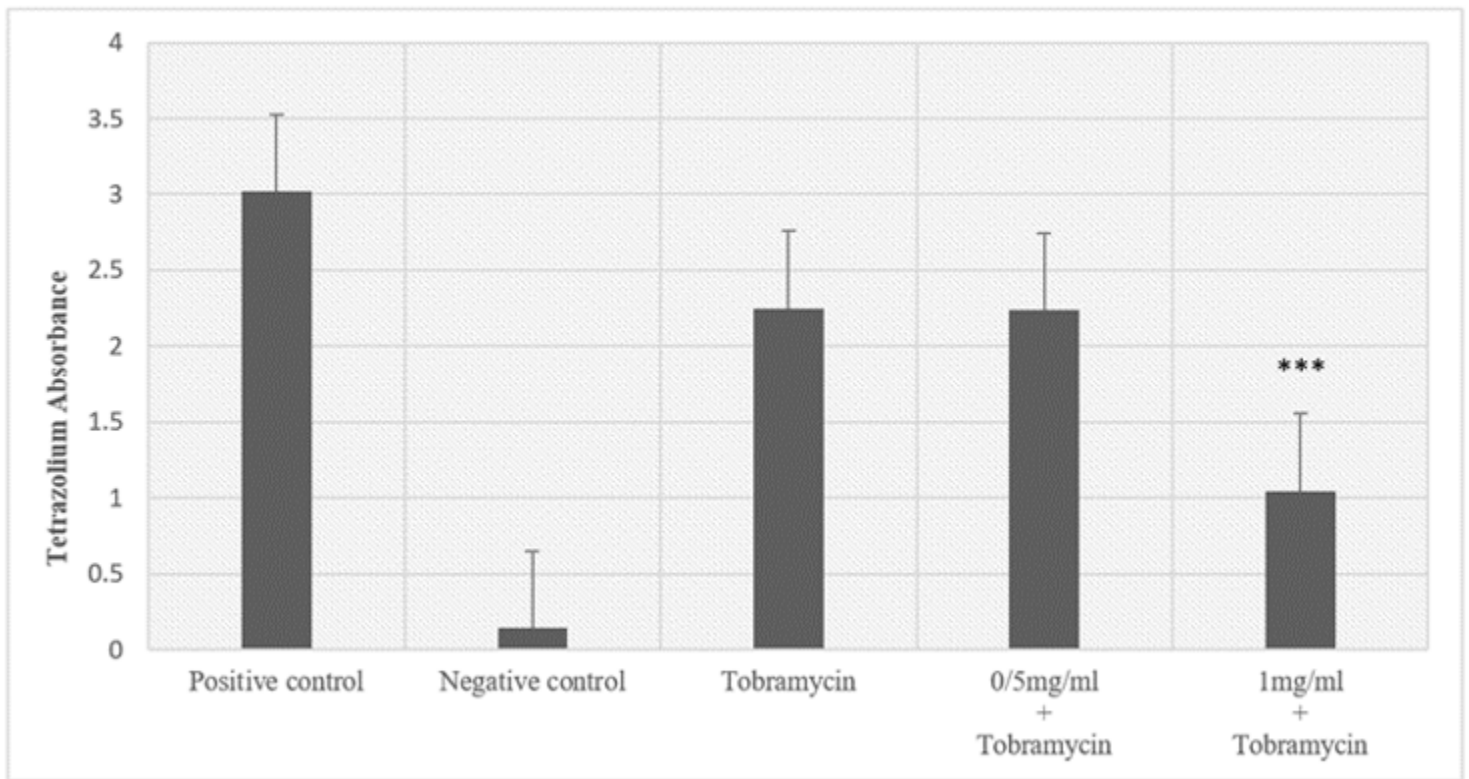


Figure 4

The synergistic effect of Tobramycin and sophoraflavanone G (at 0.5 mg/ml and 1 mg/ml concentration) on biofilm penetration of *Pseudomonas aeruginosa* PAO1

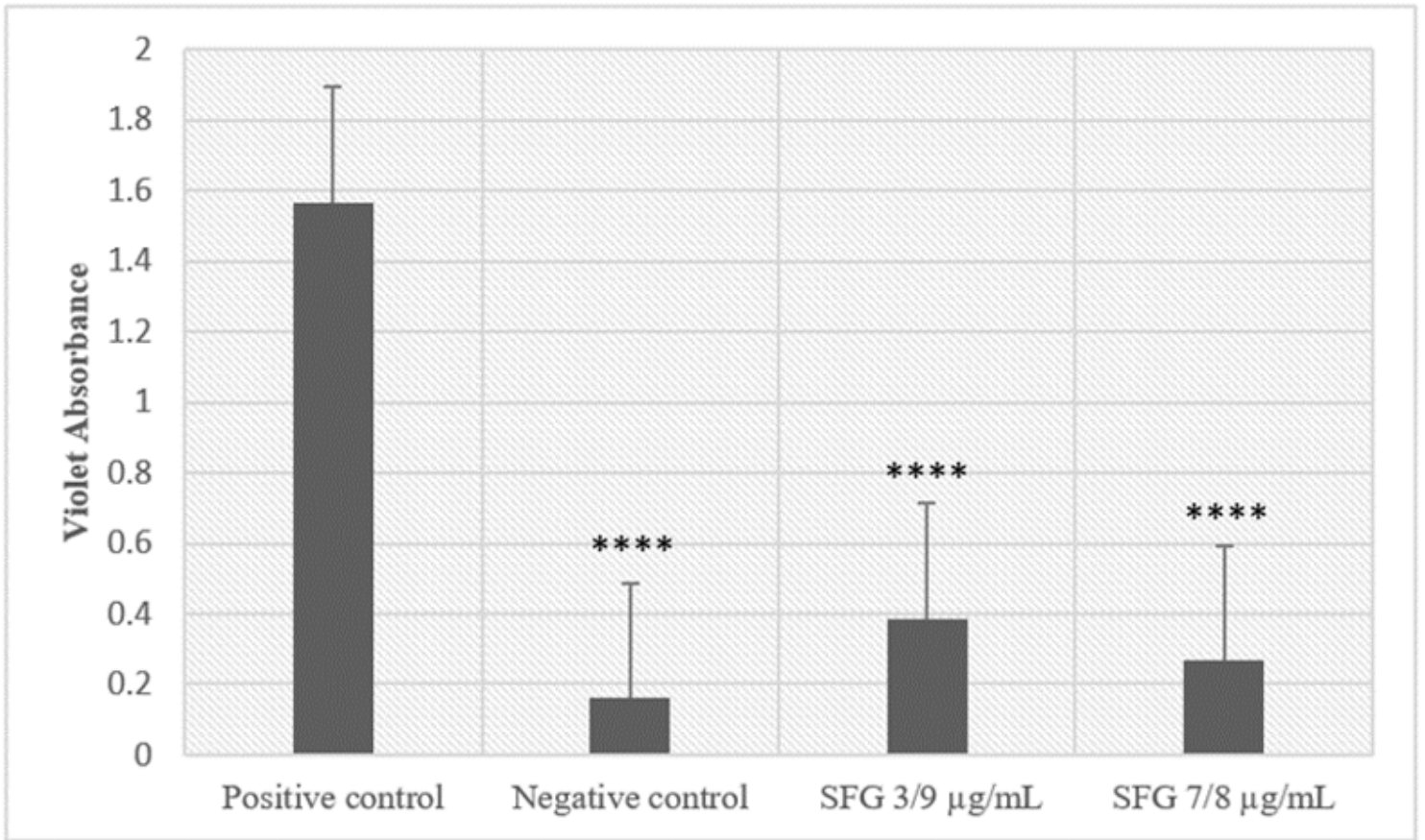


Figure 5

The effect of sophoraflavanone G on inhibiting biofilm formation of *Staphylococcus epidermidis* DSMZ 3270

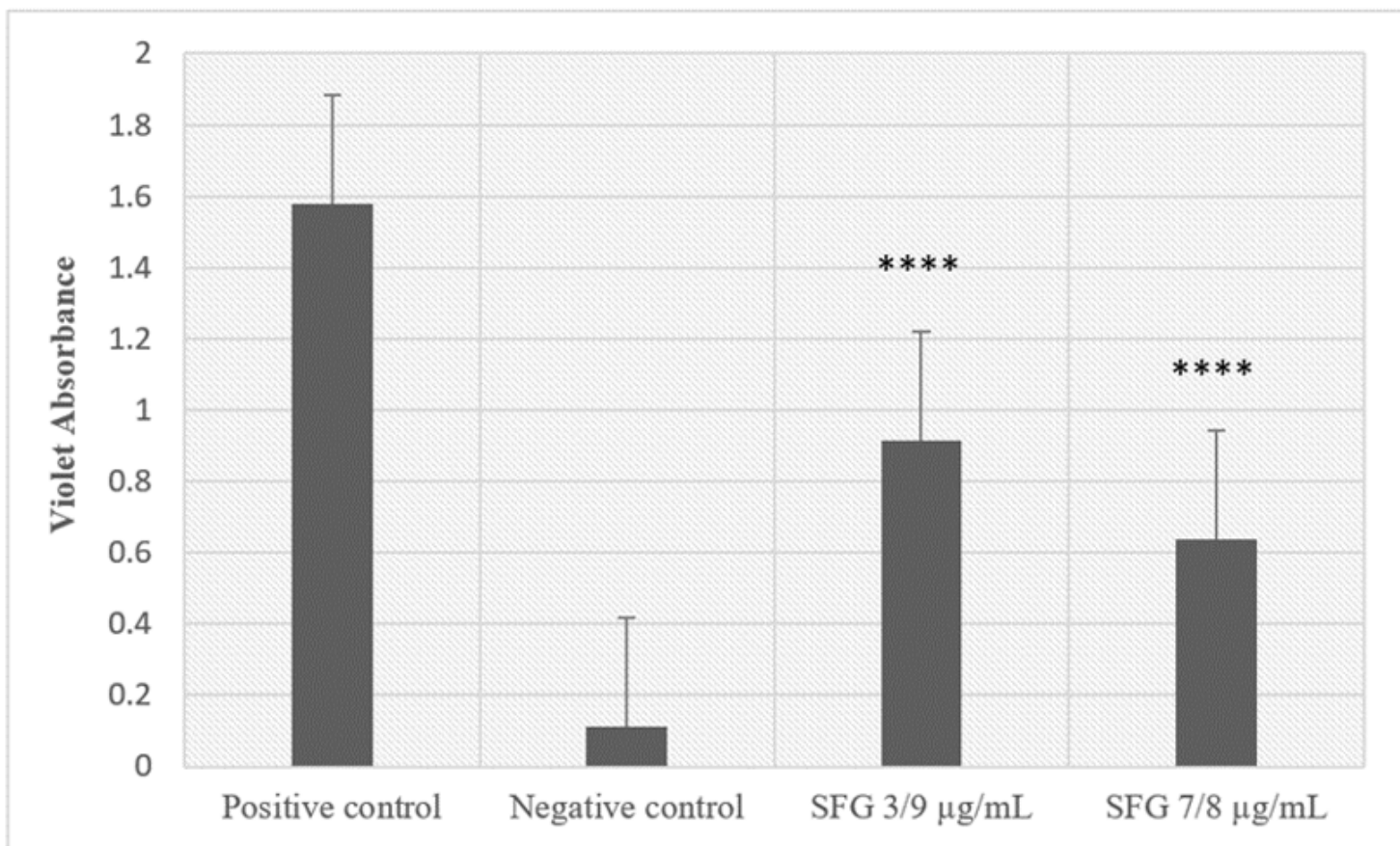


Figure 6

The effect of Sophoraflavanone G on the disruption of *Staphylococcus epidermidis* DSMZ 3270 biofilm

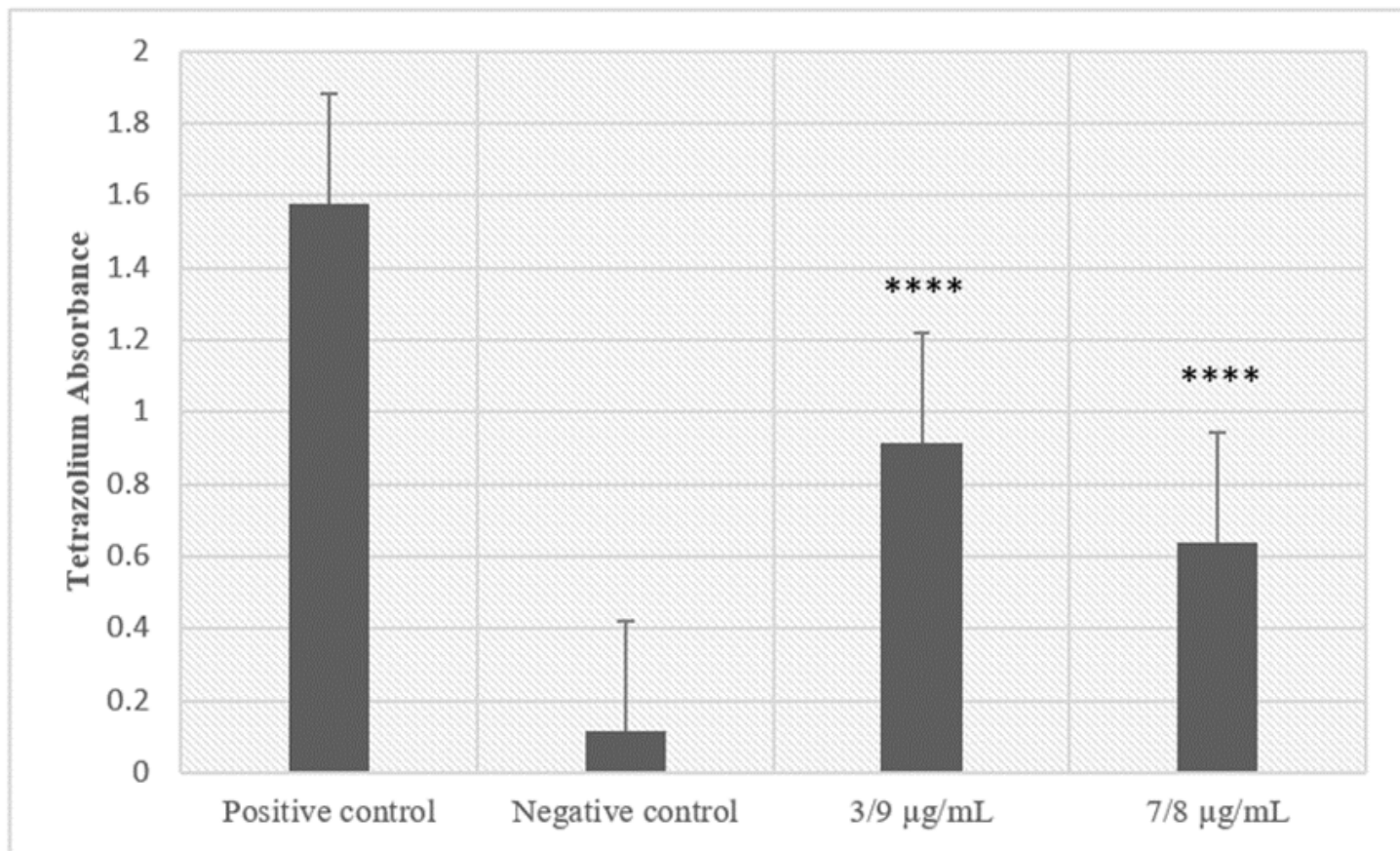


Figure 7

The effect of Sophoraflavanone G on penetration of *Staphylococcus epidermidis* DSMZ 3270 biofilm