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ORIGINAL ARTICLE

Antibacterial and antibiofilm activities of taxifolin against vancomycin-resistant *S. aureus* (VRSA)

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ABSTRACT

Background and objective: The medicinal effects of flavonoids are widely described in the literature; however, their antimicrobial effects against antibiotic resistant bacteria are yet to be highlighted. This study was aimed at investigating the growth and biofilm inhibitory effects of taxifolin, a flavonoid, against vancomycin-resistant *Staphylococcus aureus* (VRSA).

Methods: Seven VRSA isolates were used to assess the antimicrobial and antibiofilm influence of taxifolin. The agar-well diffusion method was used to determine the zones of inhibition caused by taxifolin, and resazurin-based microdilution technique was used to assess the minimum inhibitory concentration. Crystal violet staining technique was used to assess the biomass of biofilms formed by the microorganisms. GraphPad Prism software was used to present the data in figures.

Results: Taxifolin inhibited bacterial growth in a dose-dependent fashion and reduced bacterial viability. It similarly attenuated the biofilm production activity of bacterial isolates in a dose-dependent manner.

Conclusions: Current findings suggest the antibacterial and antibiofilm influence of taxifolin against VRSA in a dose-dependent manner.

Keywords antibacterial, biofilm, resazurin, staphylococcus aureus, taxifolin

INTRODUCTION

In recent years, many classic antimicrobials become ineffective due to the increased prevalence of multidrug-resistant (MDR) bacteria. This is mostly attributed to the misuse of antimicrobials.[1,2] Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are mostly treated with vancomycin.[3,4] Alongside the increasing occurrence of MRSA infections, vancomycin consumption has also risen.[5] Accordingly, vancomycin-resistant *Staphy-*

lococcus aureus (VRSA) stains started to emerge.[5] Thus, exploring new antimicrobial agents is urgently needed.

Accumulated evidence showed that natural products, by their bioactive constituents, can be used to inhibit pathogenic bacteria.[2,6,7] Flavonoids are large class of naturally occurring bioactive compounds with significant antibacterial activities.[7] Taxifolin (or dihydroquercetin) is a flavonoid abundant in natural products such as honey,[8] onion,[9] and citrus.[10] Previous reports showed that the taxifolin have many medicinal activities such as anti-Alzheimer,[11] anti-angiogenic,[12] antibacterial,[13] anti-inflammatory,[14] anti-leukemic,[15] antioxidant,[15,16] anti-toxoplasmosis,[17] and hepatoprotective effects[18,19]. Moreover, anti-cancer and anti-tumor effects of taxifolin have widely been assessed and suggested.[20–26] But, the antibacterial activity of taxifolin against many MDR bacterial strains is yet to be investigated.

The expansion of antibiotic resistance among *S. aureus* isolated from humans can be ascribed to their ability to form biofilms. Therefore, targeting biofilms can be good approach to fight staphylococcal infections.[5,27] In this context, Lopes *et al.* (2017) and Matilla-Cuenca *et al.* (2020) suggested that the flavonoids can eradicate bacterial biofilms and result in antibacterial activity.[28,29] Based on these facts, we hypothesized that the taxifolin, as a flavonoid, can inhibit bacterial growth and biofilm formation of VRSA.

To test our hypothesis, we investigated the antibacterial and antibiofilm activities of taxifolin against VRSA isolates collected from clinical specimens.

MATERIALS AND METHODS

Materials and chemicals

Microplate reader (Bio-Rad, USA), Mueller-Hinton agar and broth (Oxoid, UK), resazurin (Sigma-Aldrich, Germany), taxifolin (Sigma-Aldrich, Germany), Vancomycin (Bioanalyse, Turkey), Vitek® 2 system (bioMérieux, USA).

Microorganisms

Seven VRSA isolates were obtained from Baquba Teaching Hospital (Baqubah, Iraq). These isolates were reactivated and the confirmation of diagnosis was made at Microbiology Laboratory of the Technical Institute of Baquba using Gram's staining, catalase, and coagulase results. The speciation was additionally confirmed by Vitek® 2 system (bioMérieux, USA).

Antibacterial activity

Antibacterial activity of taxifolin was preliminarily assessed by measuring the diameters of zones of inhibition (in millimeters, mm) using agar well diffusion method on Mueller-

Hinton agar (MHA).[30] In addition, the minimum inhibitory concentration (MIC) was determined by resazurin-based microdilution technique developed by Elshikh *et al.* (2016) to assess the antibacterial activity of taxifolin with starting inocula of 5×10^5 CFU/mL on Mueller-Hinton broth (MHB) according to guidelines of CLSI.[31]

A 96-wells plate was used to determine the MIC. Wells with bacterial inocula (positive control) and without inoculation or treatment (MHB only or negative control) as well as those with vancomycin (VM) were used to compare the results.

Bacterial viability was also assessed according to Krishnamurthi *et al.* (2021) method.[32] Briefly, a 96-well plate was used and microplate absorbance reader (Bio-Rad, Germany) to evaluate the bacterial growth for a duration of 10 hours (each 2 hours the absorbance was recorded). The tested wells were positive control (3 wells), negative control (3 wells), vancomycin (0.02 mM, 3 wells), taxifolin 1 (0.1 mM, 3 wells), and taxifolin 2 (0.21 mM, 3 wells).

Antibiofilm activity

A single colony of each isolate, cultivated overnight on MHA, was suspended in 0.85% saline. The suspension was then vortexed to ensure homogeneity. Bacterial suspension was adjusted to 5×10^5 CFU/mL by diluting with MHB, and then 200 μl of it were dispensed into wells of a 96-wells plate. Three wells, for each isolate, were left with bacterial suspension only, 3 wells were included bacterial suspension and taxifolin 1 together, 3 wells were included bacterial suspension and taxifolin 2 together and 3 wells were included bacterial suspension and vancomycin alone as well as 3 wells with MHB and saline only. Two plates were used to cover the testing required wells and both were incubated for 24 hours at 37°C.

To assess the biofilm mass produced by VRSA isolates, the crystal violet staining was used as described by Stepanović *et al.* (2000)[33] and Cruz *et al.* (2018)[34] . In brief, about 200 μl of 0.01% crystal violet was added to each testing well of a 96-wells plates. The mixture was incubated in room temperature for 30 min. Then, the crystal violet solution is discarded and each well was washed 3 times with 200 μL water, carefully, and left to dry at 50°C for 30 min. Once the wells dried, 200 μl of 96 ethanol was added to each well. Microplate absorbance reader was used at 570 nm and the absorbance values were obtained. The optical density of negative control was reduced from other densities. The below formula was used to evaluate the biofilm formation activity, of each isolate, after treatment with taxifolin 1, taxifolin 2, and vancomycin in comparison with positive (growth) control biofilm.

$$Biofilm\ inhibition\ (\%) = \left(\frac{OD_{PC} - OD_{Test}}{OD_{PC}}\right) \times 100$$

Whereas; OD= optical density at 570 nm, PC= positive (growth control), Test= taxifolin or vancomycin

Data analysis

Data was statistically evaluated using GraphPad Prism 8 (GraphPad software, USA). The zones of inhibition and viability of bacterial isolates were presented as mean and standard deviation. Zones of inhibition and biofilm inhibition effects of test agents were analysed by using one way ANOVA (or Kruskal-Wallis as appropriate). A *p*-value of 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

Antibacterial activity

Antimicrobial sensitivity test showed a dramatically increased zones of inhibition as the concentration of taxifolin is increased (Figure 1). Although the lower taxifolin 1 zones of inhibition against VRSA was significantly lower than that of vancomycin, taxifolin 2 revealed significantly wider zones than that resultant from vancomycin's action.

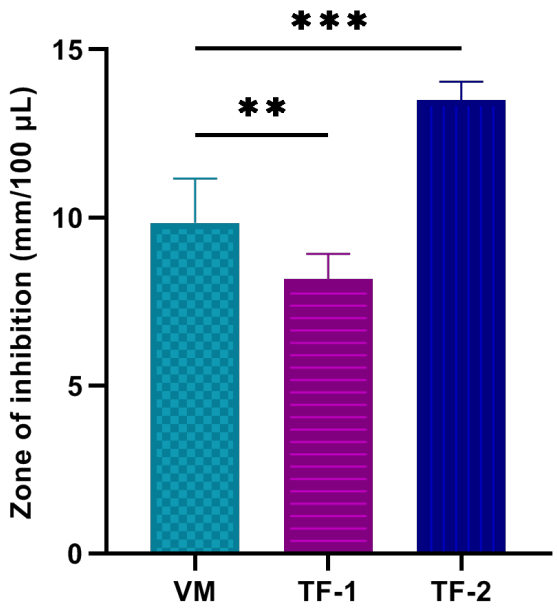

Figure 1 The diameters of zonesof inhibition (in mm) of tested agents. VM: vancomycin (=0.02 mM), TF-1: taxifolin 1 (=0.1 mM), TF-2: taxifolin2 (=0.21 mM).

The MIC results showed that taxifolin of 0.02 mM can inhibit six out of seven VRSA isolates. Bacterial cell viability curves revealed notable reduction accompanied with taxifolin 2 treatment in a period of 10 hours (Figure 2).

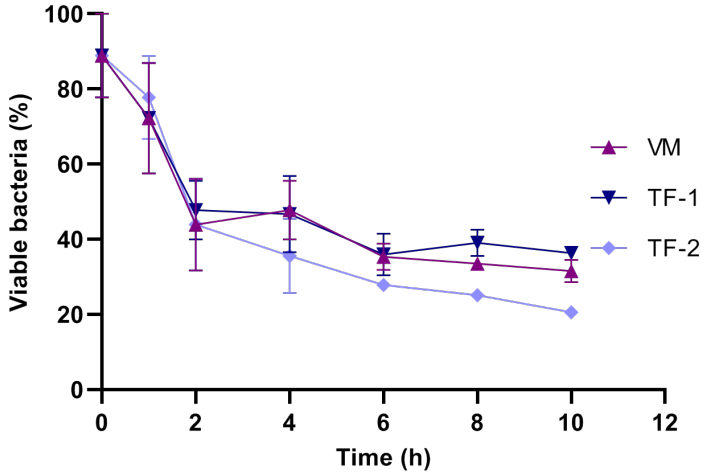

Figure 2 **Percentage ofbacterial viability after incubation with vancomycin and taxifolin. VM:vancomycin (0.02 mM),TF-1: taxifolin 1 (=0.1 mM),TF-2: taxifolin 2 (=0.21mM).**

Antibiofilm activity

Taxifolin of 0.21 mM concentration inhibited biofilm of VRSA isolates significantly when compared with vancomycin. The increased concentration of taxifolin showed increasable effects (Figure 3).

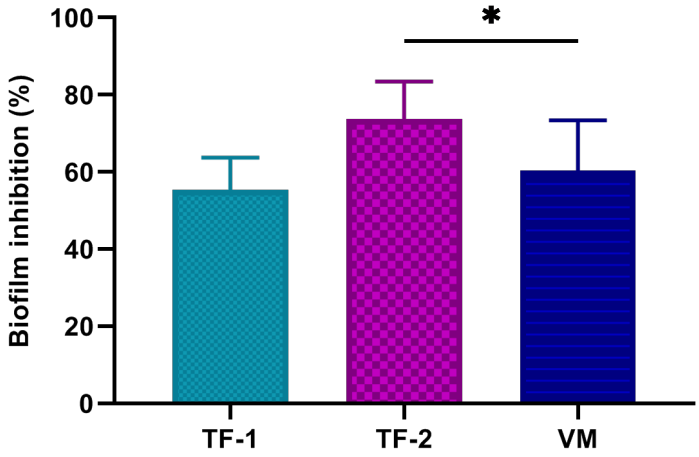

Figure 3 **Biofilm formationactivity after treatment with vancomycin and taxifolin. VM: vancomycin (=0.02 mM), TF-1:taxifolin 1 (=0.1 mM),TF-2: taxifolin 2 (=0.21mM).**

Our findings confirmed the significant inhibitory effects of taxifolin on bacterial viability and biofilm formation as well as its influence in a dose-dependent manner. These

findings were consistent with that widely reported by other researchers.[13,35–38] Aires *et al* (2016), found taxifolin-rich plant extracts inhibit *S. aureus*.[36] They suggested that extracts can act against MRSA and methicillin-sensitive *S. aureus* (MSSA) due to their high content of different classes of flavonoids, including taxifolin, which can work synergistically with each other against tested bacteria. Taxifolin and related isomers isolated from *Hypericum japonicum* Thunb. ex. Murray (Guttiferae), in another study, slowed the protein synthesis of *S. aureus*, disrupting the production of nucleic acids and enzymatic systems required for the growth of bacteria.[39] These processes makes membranes more permeable to medicines, which reduces bacterial viability and suggests that taxifolin may have a bacteriostatic effect rather than bactericidal activity. Furthermore, flavonoids and oligomers of flavonoids, can bond with bacterium cell walls and form complexes affecting the bacterial growth and survival.[40,41] Thus, and due to their bacteriostatic activity, taxifolin can be particularly effective when used as a supplemental therapy with commercial medications.

CONCLUSIONS

To sum up, this study investigated the antibacterial activity of taxifolin against vancomycin-resistant *S. aureus* (VRSA). The findings confirmed the inhibitory effects of taxifolin against growth, viability and antibiofilm formation activities of VRSA. The influence was dose-dependent. Further research to explore the underlying molecular mechanism is needed.

ABBREVIATIONS

ANOVA: analysis of variance, MDR: multidrug-resistant, MHA: Mueller-Hinton agar, MHB: Mueller-Hinton broth, MIC: minimum inhibitory concentration, MRSA: methicillin-resistant *S. aureus*, OD: optical density, TF: methicillin-sensitive *S. aureus*, VM: vancomycin, VRSA: vancomycin-resistant *S. aureus*.

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DECLARATIONS

Authors' contributions

Conceptualization: NAA, HAA. Data curation: NAA, HAA. Formal analysis: NAA, EMH, MAI. Funding acquisition: N/A. Investigation: NAA, HAA. Methodology: NAA, EMH,

MAI, HAA. Project administration: NAA. Resources: NAA. Supervision and validation: HAA. Writing-original draft, review & editing: NAA, EMH, MAI, HAA. All the authors reviewed and approved the final draft before publishing.

Conflict of interest

The authors have no conflict of interest.

Ethical approvals

This work does not include any human or animal participants. However, institutional approval was obtained from the College of Medicine, Al-Nahrain University, and the Technical Institute of Baquba, Middle Techincal University.

Data availability

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

Funding resources

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