

Wound-Healing and Antibacterial Activity of the Quercetin–4-Formyl Phenyl Boronic Acid Complex against Bacterial Pathogens of Diabetic Foot Ulcer

Hafiz Muhammad Usman Abid, Muhammad Hanif,* Khalid Mahmood,* Mubashir Aziz, Ghulam Abbas, and Hafsa Latif



Cite This: *ACS Omega* 2022, 7, 24415–24422



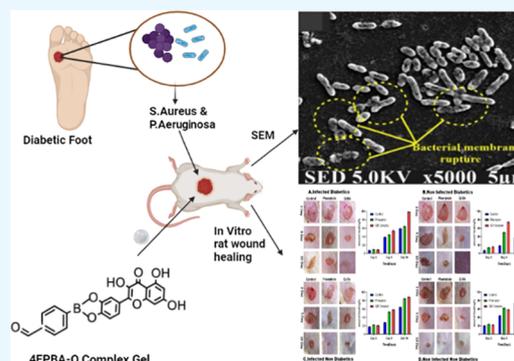
Read Online

ACCESS |

Metrics & More

Article Recommendations

ABSTRACT: Complications of diabetic foot can be prevented using a naturally occurring, efficient, and newly synthesized antimicrobial agent. The purpose of the study was to improve wound healing and antibacterial effects of quercetin and its esterified complex with 4-formyl phenyl boronic acid (4FPBA–Q) compared with phenytoin. The formation of the 4FPBA–Q complex was confirmed by thin-layer chromatography (TLC), Fourier transform infrared (FTIR) spectroscopy, and mass spectrometry (MS). The prepared 4FPBA–Q complex was used against Gram-positive bacteria along with Gram-negative bacteria, and more than 2-fold decrease in minimum inhibitory concentrations (MIC) was observed compared to pure quercetin. Scanning electron microscopic images of *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed their complete destruction after incubation with the 4FPBA–Q complex even after 3 h. Interestingly, wound-healing properties of the 4FPBA–Q complex in infected diabetic rats increased from 64 to 99% as compared to phenytoin, which were increased from those of noninfected diabetic rats. Furthermore, histopathological evaluations showed significantly enhanced wound healing, re-epithelialization, fibroblasts, and angiogenesis in wounds of diabetic rats after 10 days. Conclusively, reduction in the primary irritation index (PDII) and improved antibacterial and wound-healing properties render the 4FPBA–Q complex ideal for diabetic foot ulcer treatment.



INTRODUCTION

Diabetes mellitus (DM) is a significant metabolic problem at present, *i.e.*, 537 million individuals are suffering from this disease overall.¹ Diabetic complications are extensively divided into microvascular and macrovascular, with the latter being predominant compared to the former. Microvascular complications include nephropathy, neuropathy, and retinopathy, while macrovascular complications include stroke, cardiac-related sickness, and disease of the peripheral artery. Diabetic foot has been considered as the presence of foot ulcer associated with neuropathy and peripheral artery disease (PAD), and its contamination is a substantial cause for the removal of the lower appendage.² Diabetic foot ulceration (DFU) is associated with high levels of morbidity and mortality with a significant increase of financial costs.³

It is perceived that diabetic foot infection is polymicrobial, *i.e.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* are the pathogens most frequently involved in DFU.⁴ Due to the regular use of antibiotics, the abovementioned strains have evolved and exhibit resistance against the most profusely used therapeutics, and the handling of diabetic foot ulcer infection is intricated by the fact that

these strains are vulnerable to only limited antimicrobials. As we know, multidrug resistance is a serious and mutual problem in patients with DFU; therefore, research on substitutes for antimicrobials is necessary.⁴

A number of plant species have been implied for antimicrobial characteristics, yet certainly, most of them have not been sufficiently evaluated in the treatment of DFU infection, such as flavonoids like quercetin (Q), which are mostly implied as an antioxidant, having antimicrobial effects and also anti-inflammatory properties.⁵ Recent strategies have focused on the antimicrobial properties of flavonoids.⁶ Similar reports on formyl phenyl boronic acid (FPBA) showed its antimicrobial effects and can be considered beneficial for DFU due to the presence of two hydroxyl groups.⁷ The combination

Received: March 25, 2022

Accepted: June 29, 2022

Published: July 8, 2022



of 4-FPBA with quercetin can be considered an ideal candidate as their boronic ester (BE) can enhance an antimicrobial effect against methicillin-resistant *Staphylococcus aureus* (MRSA), *P. aeruginosa*, and *S. typhi*. To the best of our knowledge, there is no single study reported that showed the cumulative effects of natural and synthetic components against not only Gram-positive but also Gram-negative bacteria.

Therefore, the purpose of the current study was to evaluate the biological capacity of the 4FPBA–Q complex by synthesizing it with a simple esterification method. Validation of the novel complex was done by thin-layer chromatography (TLC), Fourier transform infrared (FTIR) spectroscopy, and mass spectrometry (MS). The zone of inhibition (ZOI), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and scanning electron microscopy (SEM) were considered better tools for the evaluation of antimicrobial studies. The primary dermal irritation index (PDII) and *in vivo* rat wound model and its histopathological studies made the work more interesting and applicable not only in the treatment of DFU in particular but also for diabetes mellitus in general.

MATERIALS

Quercetin and 4-formyl phenyl boronic acid (4-FPBA) were both acquired from Sigma Aldrich, Germany. Ketamine was obtained from Alfasan Corporation, the Netherlands. Phenytoin cream was obtained from the local market, Multan, Pakistan. *P. aeruginosa* (PTCC 1181), *S. aureus* (PTCC 1764), and *S. typhi* (PTCC 1609) were isolated from confirmed patients with diabetic foot ulcers with the permission of the ethical committee. Wistar strain healthy male albino rats (200–250 g) were obtained from the department of pharmacology, Bahauddin Zakariya University. Eosin methylene blue agar, Salmonella Shigella agar, and pseudomonas isolation agar were all purchased from Moltox, United States. Double-distilled water was applied throughout the study, and all reagents were of analytical grade.

METHODS

Preparation Method of the 4FPBA–Q Complex.

Unique, simple, and one-step esterification of quercetin and 4-FPBA was performed. Briefly, 2 ppm methanolic solution (MeOH) of quercetin was prepared at a controlled temperature and pressure with continuous stirring at 1500 rpm for 10 min. The same concentration of 4-FPBA was mixed in a previously prepared solution, and the pH of the reaction was maintained at 8.5 by adding 30 mM phosphate buffer solution (PBS) so as to prepare a 17:5 v/v concentration of MeOH/PBS. The resultant solution was kept in the dark with continuous stirring at 900 rpm for 3 h to prevent any color change. MeOH from the resultant solution was evaporated by increasing the temperature to 37 °C first and then kept in a hot air oven (WHL-25A) for 24 h. The resultant complex (4FPBA–Q complex) was kept in an airtight vessel for further usage. Validation of the QB complex was done by thin-layer chromatography, FTIR, and mass spectrometry. The following equation was used for the calculation of percentage yield.

$$\% \text{ yield} = \frac{\text{weight of the product}}{\text{weight of reactants}} \times 100 \quad (1)$$

Confirmation of the Complex. The Fourier transform infrared spectrum was recorded on a Bruker Alpha (Alpha II).

The spectrum of the 4FPBA–Q complex was recorded in the wavenumber range of 400 to 4000 cm^{-1} , and the reported spectra were an average of 24 scans. All of the spectra were observed in triplicate ($n = 3$) for the mean \pm standard deviation (SD). The molecular mass was determined by electrospray ionization (ESI) mass spectrometry, MS (ESI) m/z : 433.15 $[\text{M} + \text{OH}]^-$, and the spectra were reported in triplicate ($n = 3$). Thin-layer chromatography was performed to confirm the 4FPBA–Q complex. The retention factor (R_f) was calculated using the following equation

$$R_f = \frac{\text{distance traveled by the complex}}{\text{distance traveled by the solvent}} \quad (2)$$

Antimicrobial Effect. Antimicrobial susceptibility analysis of quercetin, 4-FPBA, and the 4FPBA–Q complex against multidrug-resistant *S. typhi*, *S. aureus*, and *P. aeruginosa* was performed by a simple, previously reported, and slightly modified method of RM Humphries et al.⁸ Briefly, the medium was sterilized at 120 °C. Mueller–Hinton agar (20 mL) was transferred aseptically into each of the sterilized Petri plates and kept for solidification at 37 °C. In the nutrient broth, bacterial cultures were diluted and attained the optical density corresponding to 0.5. All bacterial cultures were grown in nutrient broth for 24 h at 37 °C. After the preparation of the agar plates of Muller–Hinton (MH), a bacterial suspension of 100 μL (1×10^5 CFU/mL) was spread on these plates with a sterilized cotton swab. In each MH agar plate, wells of 3 mm diameter were prepared. Quercetin (4 mg/mL, 100 μL), 4-FPBA, and the 4FPBA–Q complex solutions were applied to the bore, and the plates were incubated at 37 °C for 24 h. Ciprofloxacin (10 μg) was used as a positive control in one well and incubated in for 24 h. After 24 h, the zone of inhibition of every sample was measured. Bacterial ZOI with ciprofloxacin (positive control) was compared. The growths of those strains affected by the complex were selected for further experiments and repeated in triplicate.⁹

MIC and MBC of Quercetin, 4-FPBA, and the 4FPBA–Q Complex. The minimum inhibitory concentration (MIC) values previously selected for quercetin, 4-FPBA, and the 4FPBA–Q complex against *S. typhi*, *S. aureus*, and *P. aeruginosa* were calculated using the already reported method of dilution. Briefly, nutrient broth (5 mL) was mixed with a loop filled with bacterial culture in test tubes. Different amounts, *i.e.*, 2, 5, 10, 15, 20, and 30 $\mu\text{g}/\text{mL}$ samples, were mixed in previously prepared broth and incubated at 37 °C for 24 h. The optical density was observed using a UV–visible spectrophotometer at 260–557 nm. The lowest concentration of samples used against each bacterial species was considered the MIC, and the assay was repeated in triplicate to obtain the mean \pm SD ($n = 3$).⁹

A similar procedure was applied for the minimum bactericidal concentration (MBC) assay. Briefly, from the MIC tubes, 1 mL of culture was transferred into the nutrient agar and incubated at 37 °C for 24 h. The MBC of all samples was obtained from the amount at which 100% of the bacterial growth inhibition occurred. Ciprofloxacin (10 $\mu\text{g}/\text{mL}$) was used as a positive control against all bacterial species.¹⁰

Primary Dermal Irritation Index (PDII). The primary dermal irritation index (PDII) was determined on albino rats. Briefly, four groups of rats were divided in such a way that each group comprised of four rats ($n = 4$). 2 cm^2 area of the abdomen surface of each rat was used for the application of

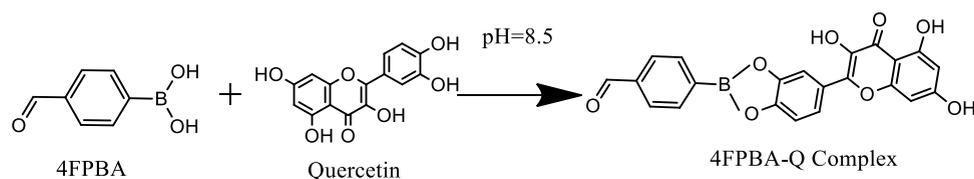


Figure 1. Chemical interaction of 4-FPBA and Q for the preparation of the 4FPBA–Q complex.

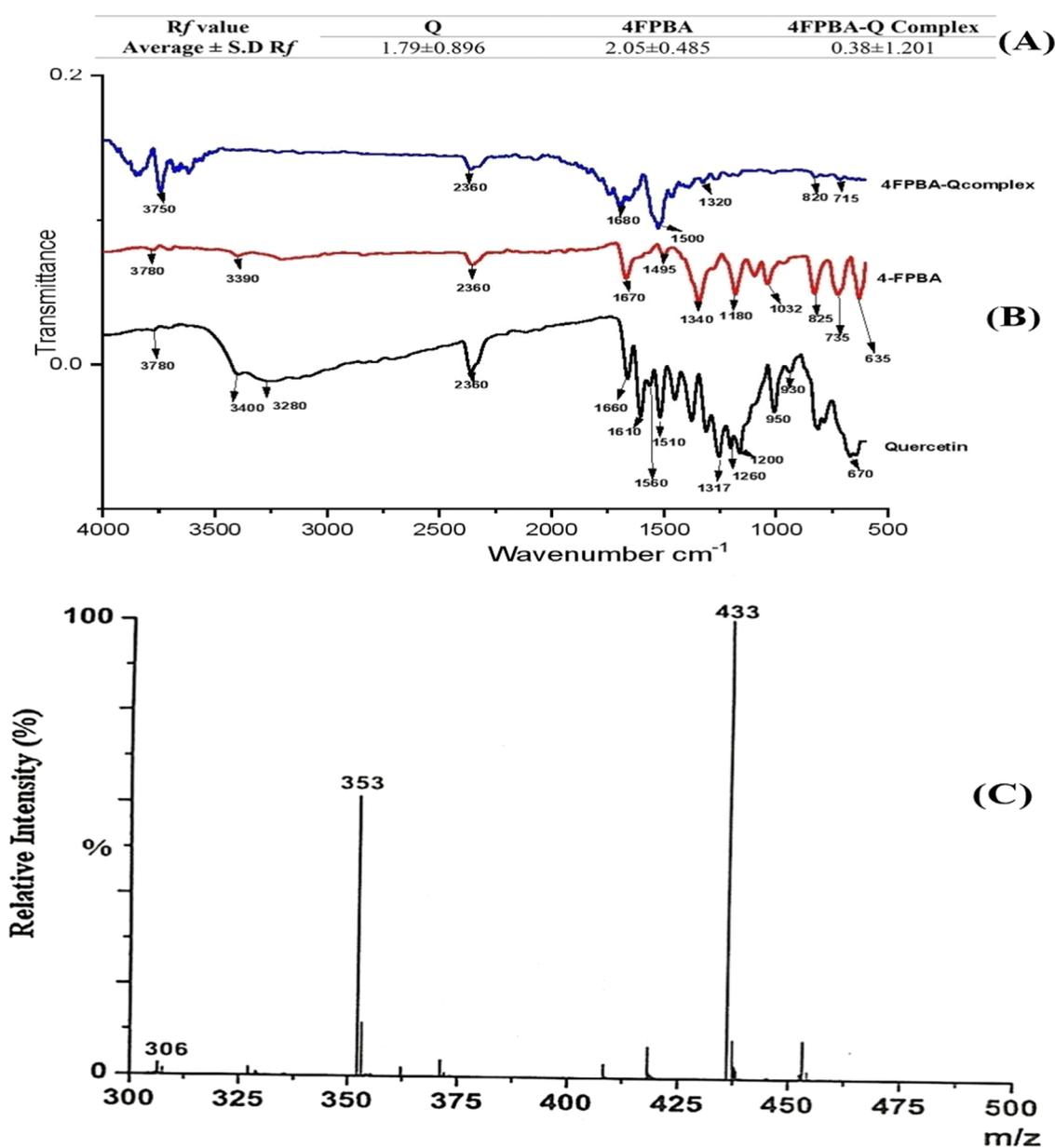


Figure 2. (A) Confirmation of complex formation by TLC (R_f factor) and (B) FTIR spectra of Q, 4-FPBA, and the 4FPBA–Q complex. (C) ESI mass spectrometry of the 4FPBA–Q complex.

samples. The first group was considered as the control and standard skin sensitizing agent (chloro dinitrobenzene in 10% propylene glycol) was applied. The second group was named as the 4-FPBA-treated group and was treated with 1 mL of 4-FPBA solution (5 $\mu\text{g}/100 \mu\text{L}$), and the third group was treated with 1 mL (5 $\mu\text{g}/10 \mu\text{L}$) of quercetin solution. The 4FPBA–Q complex solution (1 mL, 5 $\mu\text{g}/100 \mu\text{L}$) was applied to the skin of the fourth group. Erythema and edema were observed at predetermined time intervals of 0, 8, and 24 h. Skin

sensitization was repeated three times ($n = 3$), and the average \pm SD was reported.¹¹

On the skin, noticeable changes were observed, which were classified according to the mean values of erythema, *i.e.*, no erythema was indicated with 0; light pink with 1; dark pink with 2; light red with 3; and dark red with 4. The mean values of 0–0.49 indicated no irritation, 0.5–2.99 indicated light irritation, 3.0–5.99 indicated modest irritation, and 6–8 indicated severe irritation.¹² The grade of irritancy was

Table 1. ZOI, MIC, and MBC of Quercetin, Boronic Acid, and the 4FPBA–Q Complex against Pathogenic Bacteria^a

sample	bacterial strains	MIC ($\mu\text{g}/100 \mu\text{L}$)	MBC ($\mu\text{g}/100 \mu\text{L}$)	zone of Inhibition
quercetin	<i>S. typhi</i> (Gram-negative)	3.125 \pm 0.02	6.25 \pm 0.01	13.1 \pm 0.02
4FPBA		3.125 \pm 0.03	6.25 \pm 0.03	17.4 \pm 0.04
4FPBA–Q complex		1.5625 \pm 0.04	3.125 \pm 0.03	19 \pm 0.01
quercetin	<i>P. aeruginosa</i> (Gram-negative)	3.125 \pm 0.03	6.25 \pm 0.03	14.5 \pm 0.01
4FPBA		3.125 \pm 0.02	6.25 \pm 0.02	26.5 \pm 0.02
4FPBA–Q complex		1.5625 \pm 0.04	3.125 \pm 0.04	28.4 \pm 0.02
quercetin	<i>S. aureus</i> (Gram-positive)	12.5 \pm 0.04	25 \pm 0.02	21.1 \pm 0.01
4FPBA		6.25 \pm 0.02	12.5 \pm 0.02	18.5 \pm 0.03
4FPBA–Q complex		3.125 \pm 0.02	6.25 \pm 0.04	24.4 \pm 0.03

^aMIC = Minimum inhibitory concentration, MBC = minimum bactericidal concentration.

obtained by calculating the primary dermal irritation index (PDII).

$$\text{PDII} = \frac{\text{PDII for 8, 16, and 24 h}}{3} \quad (3)$$

In Vivo Wound-Healing Study. Animal studies were performed to assess the capability of the 4FPBA–Q complex for *in vivo* wound healing in accordance with the experimental procedures permitted by the guiding principles of the Ethical Committee of Bahauddin Zakariya University, Multan, Pakistan. Male Wistar albino rats were kept in a cage in a room with a constant temperature ($28 \pm 2 \text{ }^\circ\text{C}$). The rats were preliminarily divided into two groups A and B. Diabetes was induced in Group A using alloxan 300 mg/mg previously dissolved in saline sodium citrate buffer, while group B was considered as nondiabetic. Diabetes was confirmed by monitoring the blood glucose content after 48 h, and the rats with $250 \pm 5 \text{ mg/dL}$ glucose were added in the diabetic group, *i.e.*, A. For wound creation, first, all of the animals were anesthetized using an intraperitoneal injection of 10% ketamine (80 mg/kg). Then, they were shaved at the backside, and the skin was disinfected with alcohol wipes. A 2 cm diameter circular wound was created on the dorsum of all rats with a perforator. Furthermore, diabetic rats from group A were divided into diabetic infected (Group I) and diabetic noninfected (Group II). Similarly, group B was also divided into nondiabetic infected (Group III) and nondiabetic noninfected (Group IV) comprising ten rats in each group. For the infected rats (both diabetic and nondiabetic), the wounds were infected *via* bacterial suspension containing *S. typhi*, *S. aureus*, and *P. aeruginosa* ($1 \times 10^5 \text{ CFU/mL}$). Among the nine rats in each group, three were treated with phenytoin cream considered as a controlled drug, three were exposed to the QB complex (50 mg/mL), and the remaining three were left untreated for 10 days. The wounds were photographed using a 48 MP mobile camera (Huawei company) on different days (2, 5, and 10) post wounding. The wound closure percentage was calculated using the following equation

$$\text{wound closure (\%)} = \frac{A_0 \times A_t}{A_0 \times 100\%} \quad (4)$$

In the above equation, A_0 is the primary wound area and A_t is the wound area at time t .¹³

Histopathological Analysis. For a better understanding of the pathophysiology of wounds, the histopathology of wounds is a very supportive tool. When *in vivo* studies were performed, tissue samples were placed into particular solutions such as 10% percent buffered formaldehyde to keep their integrity without cellular structure changes. The samples were

fixed in paraffin, and sections of 5 mm thickness were obtained. The tissue was then exposed to the numerous steps of histological processing, including fixing, sectioning, and staining. The most extensively applied stains in wound pathology are hematoxylin and eosin (H&E), and then the sections were observed with a routine light microscope.

SEM Analysis. In Mueller–Hinton broth, bacterial suspensions (10 mL) of *S. aureus*, *P. aeruginosa*, and *S. typhi* were prepared ($1 \times 10^5 \text{ CFU/mL}$) under the condition that Q, 4-FPBA, and the QB complex have a half value of their respective MIC in beakers. Glass slides were dipped vertically and incubated for 48 h at $37 \pm 0.5 \text{ }^\circ\text{C}$. Glass slides were removed, gently washed with phosphate buffer saline (pH 7.0) for the removal of unbound material, and fixed with acetic acid for 15 min at $37 \pm 0.5 \text{ }^\circ\text{C}$. Staining was done with 3% crystal violet, and the slides were taped onto gold-coated stubs and observed under a scanning electron microscope (model: JSMS910 JEOL, Japan). The experiment was repeated three times, and the results of the mean \pm SD ($n = 3$) were reported.¹⁴

Statistical Analysis. Statistical studies were done using GraphPad Prism (Software Inc., La Jolla, CA). Data were assessed using analysis of variance (ANOVA). Data were reported as the mean \pm SD at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The 4FPBA–Q complex (Figure 1) was prepared by one-step esterification of quercetin and 4-formyl phenyl boronic acid (4-FPBA). The percentage yield of the 4FPBA–Q complex was $98 \pm 0.5\%$. Figure 2A shows the R_f values of TLC measurements, while Figure 2B shows the FTIR spectra of quercetin, 4-FPBA, and the 4FPBA–Q complex. The R_f (average \pm SD) value of quercetin was 1.79 ± 0.895 , which decreased to 0.38 ± 1.201 in the case of the 4FPBA–Q complex and may be due to the formation of ester linkages between the diol groups of quercetin and 4-FPBA. In FTIR, quercetin exhibited the characteristic intensities of O–H stretch at 3400 cm^{-1} , C=O stretch at 1660 cm^{-1} , aromatic C=C stretch at 1510 and 1610 cm^{-1} , and aromatic C–O stretch at 1200 cm^{-1} . Compared to quercetin, 4-FPBA exhibited characteristic peaks of O–H stretch at 3780 and 3390 cm^{-1} , C=O stretch at 1670 cm^{-1} , aromatic C=C stretch at 1495 cm^{-1} , C–O stretch at 1180 cm^{-1} . Due to the presence of the halide group (bromine), a stretch at 635 cm^{-1} was observed,¹⁵ while in the 4FPBA–Q complex, the peak of the bromine-containing halide group shifted toward 715 cm^{-1} . The shifting of the bromine peak in the 4FPBA–Q complex from 635 to 715 cm^{-1} rendered the resultant complex more

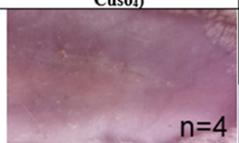
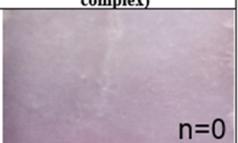
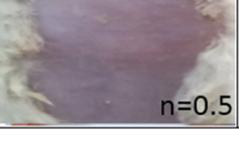
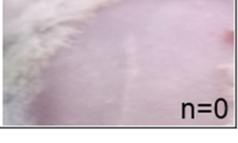
Time (h)	Group 1 (Treated with Cuso ₂)	Group 2 (Treated with Quercetin)	Group 3 (Treated with 4FPBA-Q complex)
8 h	 n=4	 n=3	 n=0
16 h	 n=4	 n=2	 n=0
24 h	 n=4	 n=0.5	 n=0

Figure 3. Visible changes on the skin were observed and marked according to the mean values of erythema (no erythema, 0; light pink, 1; dark pink, 2; light red, 3; and dark red, 4).

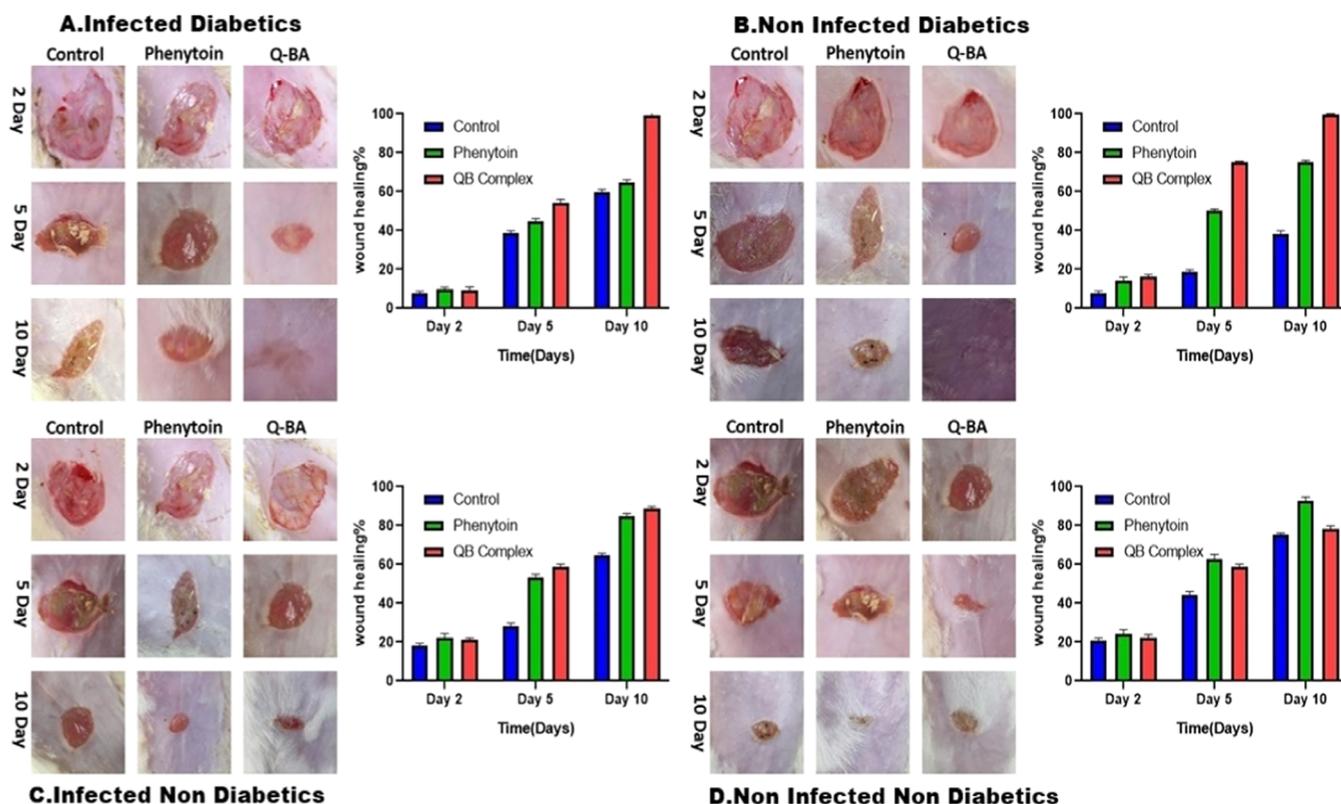


Figure 4. Animal study of wound healing by G-BA at different time frames for (A) infected diabetic, (B) noninfected diabetic, (C) infected nondiabetic, and (D) noninfected nondiabetic wounds.

ideal for antibacterial studies. The results are attributed to previously reported results of the FTIR spectrum.¹⁶

The results revealed that most of the isolated foot ulcer bacteria were extremely resistant to numerous antibiotics. In the present work, *S. typhi*, *P. aeruginosa*, and *S. aureus* were isolated from foot ulcer wounds.¹⁷ The minimum and maximum zone of inhibition (ZOI) of quercetin, 4-FPBA, and the 4FPBA-Q complex is shown in Table 1. In the case of Gram-negative bacteria, *i.e.*, *S. typhi* and *P. aeruginosa*, the maximum zone of inhibition of the 4FPBA-Q complex was 19 ± 0.01 (76%) and 28.4 ± 0.02 (113.6%), respectively. However, in the case of Gram-positive bacteria, *i.e.*, *S. aureus*,

the maximum zone of inhibition of the 4FPBA-Q complex was 24.4 ± 0.03 (97.6%). An increase in the ZOI of the 4FPBA-Q complex against *P. aeruginosa* may be due to the presence of pores in the outer membrane of Gram-negative bacteria. Gram-positive bacteria, *i.e.*, *S. aureus*, lack the outer membrane, which makes Gram-negative bacteria more susceptible to the complex than Gram-positive ones.¹⁸ The ZOI of the complex against *S. typhi* is low as compared to against *P. aeruginosa*. Further analysis was done for *P. aeruginosa* and *S. aureus*, revealing that the 4FPBA-Q complex was more effective against *S. typhi* and *P. aeruginosa*, presenting an MIC of $1.562 \pm 0.04 \mu\text{g}/100 \mu\text{L}$, being superior to that found in the

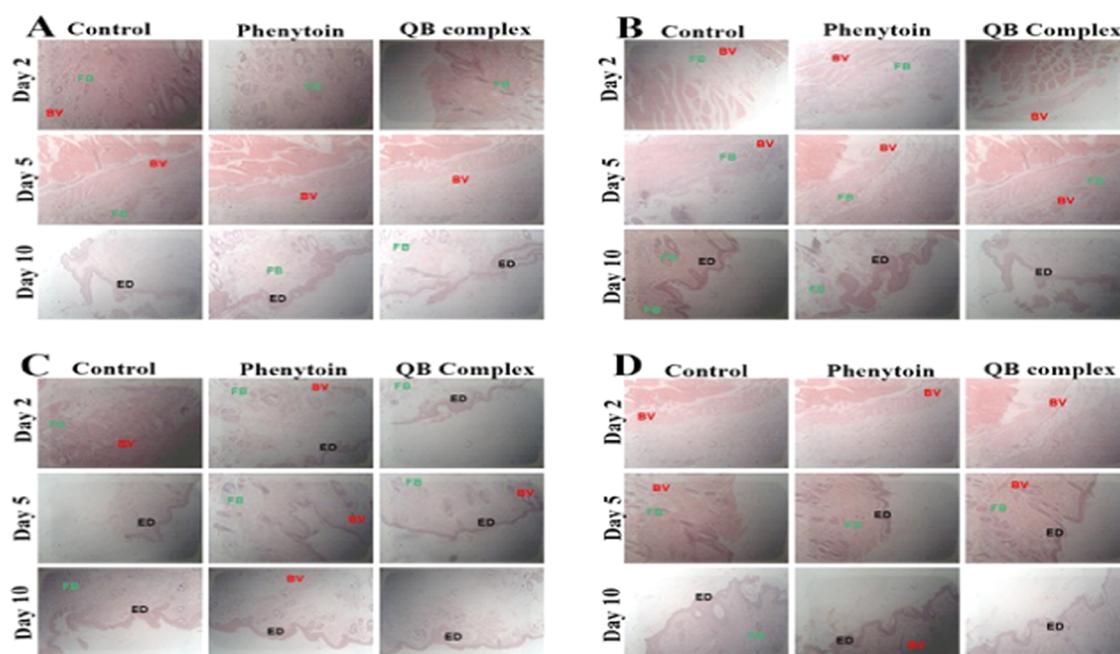


Figure 5. Histological image analysis of the hematoxylin–eosin (HE)-stained tissues (wounds) of diverse groups of rats: (a) infected diabetic wound, (b) noninfected diabetic wound, (c) infected nondiabetic wound, and (d) noninfected nondiabetic wound. Border of the epidermis layer of the skin is represented as ED, blood vessels are indicated as BV, and fibroblasts are indicated as FB.

study for the same strain.¹⁹ The MBC of the 4FPBA–Q complex against *S. typhi* and *P. aeruginosa* was similar, *i.e.*, 3.125 ± 0.03 , and for *S. aureus* it was 6.250 ± 0.04 . Due to the increased ZOI and decreased MIC and MBC, the *P. aeruginosa* from Gram-negative and *S. aureus* from Gram-positive bacteria were considered to be optimal species for further antibacterial mechanistic study by SEM analysis.

The safety of the reported complex was evaluated by the primary dermal irritation index (PDII) in 12 albino rats, which did not exhibit any clinical indications such as making noise instantly after application, swelling, or any obvious signs of irritation on the tested area. Skin irritation score was found to be less than 2 related to the control group (treated with CuSO_4) animals from the first day till the end of the experiment. Noticeable variations on the skin were observed and marked according to the mean values of erythema (no erythema indicated by 0; light pink indicated by 1; dark pink indicated by 2; light red indicated by 3; and dark red indicated by 4). The mean values of 0–0.9 indicated no irritation, 1.0–2.99 indicated light irritation, 3.0–5.99 indicated moderate irritation, and 6–8 indicated severe irritation. Figure 3 shows that rats treated with CuSO_4 remain dark red ($n = 4$) after 8, 16, and 24 h and those treated with quercetin are 3, 2, and 0.5 after 8, 16, and 24 h, while those treated with the 4FPBA–Q complex did not exhibit any sign of erythema, demonstrating that there was no skin irritation in treated rats.

In vivo rat wound model was used for the improvement of healing of wounds in diabetic as well nondiabetic rats. The group with infected diabetic wounds treated with the 4FPBA–Q complex showed the finest results and ample healing within 10 days. However, the control and phenytoin-treated groups with an infected diabetic wound exhibited contraction of wounds up to 59 and 64%, respectively; it was 99% for rats treated with the 4FPBA–Q complex for 10 days (Figure 4A). A significant increase in the contraction of the wounds favored the use of the 4FPBA–Q complex not only as an antimicrobial

agent but also improvement of skin regenerations. The contraction of wounds for control, phenytoin, and 4FPBA–Q complex-treated rats (for the noninfected diabetic group) was 38, 75, and 99% in 10 days, respectively (Figure 4B). The 4FPBA–Q complex enhanced the contraction of wounds in the infected nondiabetic group by up to 88%. However, it was 64% and 84% in the control and phenytoin-treated groups in 10 days, respectively. These results disclosed that the antibacterial actions of the 4FPBA–Q complex are significant but not the mere reason for their therapeutic efficiency (Figure 4C). Remarkably, the finest results were attained by phenytoin in the noninfected nondiabetics group (92% contraction of the wound), and the results for the 4FPBA–Q complex-treated group were very close to the control (untreated) group, *i.e.*, 78% contraction of the wound in the 4FPBA–Q complex compared with 75% in the control group. This can be due to the presence of the phenyl boronic acid segment of the 4FPBA–Q complex in complications of the diabetic wound-healing process such as a reduction in angiogenesis compared with nondiabetic wounds. This consequence revealed that the diabetic wound-healing characteristic of the 4FPBA–Q complex is linked with the suppression of adverse diabetic effects (Figure 4D).

The tissues were examined *via* the hematoxylin–eosin (HE) staining assay (Figure 5). Histological image examination of the hematoxylin–eosin (HE) stained tissues of diverse groups of the rats, *i.e.*, infected diabetic wound, noninfected diabetic wound, infected nondiabetic wound, and noninfected nondiabetic wound. New blood vessels as well as hair follicles started to grow, exhibiting a healing process superior to the other groups within 6–10 days. At the late phase of the healing process, the 4FPBA–Q complex-treated wounds exhibited the highest similarity to normal skin, a thick epidermis, collagen regeneration, neovascularization, and hair follicles. These observations demonstrated that the 4FPBA–Q complex improves diabetic wound healing efficiently.

SEM images of the control and the 4FPBA–Q complex are shown in Figure 6. Due to the functionality of phenyl boronic

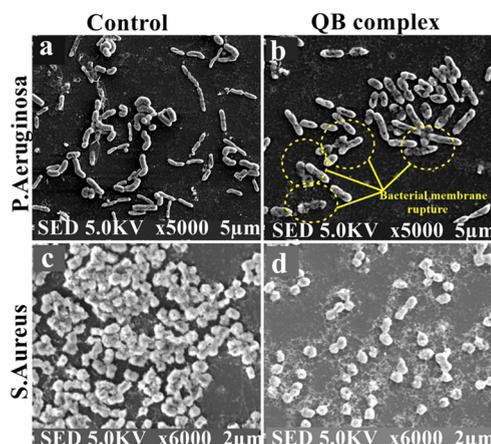


Figure 6. Scanning electron microscopic (SEM) images of antimicrobial activity of the QB complex against different strains of bacteria. Images (a) and (c) represent *P. aeruginosa* and *S. aureus* before treatment, while (b) and (d) represent rupturing of the membrane of *P. aeruginosa* and reduction in the number of *S. aureus* colonies after treatment, respectively.

acid, the 4FPBA–Q complex is supposed to attach to *P. aeruginosa* and *S. aureus* covalently (Figure 6). The 4FPBA–Q complex inhibits the growth of *P. aeruginosa* and *S. aureus* due to the presence of phenyl boronic acid and the capability of this compound for the covalent interactions with these pathogens. The establishment of colonies for both *P. aeruginosa* and *S. aureus* was inhibited by the 4FPBA–Q complex. According to SEM images, *P. aeruginosa* and *S. aureus* were completely demolished after incubation with the 4FPBA–Q complex for 3 h. Bacteria were completely wrapped or bacterial membrane rupture was observed, confirming the robust attachment of the 4FPBA–Q complex to their membranes (Figure 6).²⁰

CONCLUSIONS

Complications of diabetic foot ulcers and resistance to the available antibiotics remain a challenge for pharmaceutical scientists. In the present research work, naturally occurring polyphenols and their complexes with the 4-FPBA (4FPBA–Q) showed a remarkable effect against Gram-positive as well as Gram-negative bacteria, which are involved in the diabetic foot ulcer. The formation of the 4FPBA–Q complex, its confirmation by FTIR and MS, reduction in the primary irritation index (PDII), antibacterial effect, diabetic foot wound healing, and SEM results showed the complete eradication of *S. aureus*. Re-epithelialization, fibroblasts, and angiogenesis in histopathological evaluations showed enhanced wound healing in diabetic rats after 10 days. The reported complex can be used not only for the treatment of diabetic foot ulcers but also can provide an alternative to the available resistant antibiotics.

AUTHOR INFORMATION

Corresponding Authors

Muhammad Hanif – Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan 60800, Pakistan; orcid.org/0000-0002-2303-3804; Email: muhammad.hanif@bzu.edu.pk

Khalid Mahmood – Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan; Email: khalidmahmood@bzu.edu.pk

Authors

Hafiz Muhammad Usman Abid – Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan 60800, Pakistan; Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan

Mubashir Aziz – Department of Microbiology, Bahauddin Zakariya University, Multan 60800, Pakistan

Ghulam Abbas – Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad 38000, Pakistan; orcid.org/0000-0002-4531-3911

Hafsa Latif – Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan 60800, Pakistan

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsomega.2c01819>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are thankful to the Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University Multan, for providing the research facilities. Special thanks to Dr. Muhammad Sami Ullah from Sami's lab for providing the histopathological facilities of reported research work. On the other hand, the Higher Education Commission (HEC) of Pakistan is also acknowledged for the approval of the reported study.

REFERENCES

- Lotfy, M.; Adeghate, J.; Kalasz, H.; Singh, J.; Adeghate, E. Chronic complications of diabetes mellitus: a mini review. *Curr. Diabetes Rev.* **2016**, *13*, 3–10.
- Papatheodorou, K.; Edmonds, M.; et al. Complications of Diabetes 2017. *J. Diabetes Res.* **2018**, *2018*, 1–4.
- Bus, S. A.; Lavery, L. A.; Monteiro-Soares, M.; Rasmussen, A.; Raspovic, A.; Sacco, I. C.; van Netten, J. J.; on behalf of the International Working Group on the Diabetic Foot. Guidelines on the prevention of foot ulcers in persons with diabetes (IWGDF 2019 update). *Diabetes/Metab. Res. Rev.* **2020**, *36*, No. e3269.
- Kim, H. K.; Thamavongsa, V.; Schneewind, O.; Missiakas, D. Recurrent infections and immune evasion strategies of *Staphylococcus aureus*. *Curr. Opin. Microbiol.* **2012**, *15*, 92–99.
- Moon, T.; Wilkinson, J.; Cavanagh, H. Antibacterial activity of essential oils, hydrosols and plant extracts from Australian grown *Lavandula* spp. *Int. J. Aromather.* **2006**, *16*, 9–14.
- González-Lamothe, R.; Mitchell, G.; Gattuso, M.; Diarra, M. S.; Malouin, F.; Bouarab, K. Plant antimicrobial agents and their effects on plant and human pathogens. *Int. J. Mol. Sci.* **2009**, *10*, 3400–3419.
- Adamczyk-Woźniak, A.; Gozdalik, J. T.; Wiczorek, D.; Madura, I. D.; Kaczorowska, E.; Brzezińska, E.; Sporyński, A.; Lipok, J. Synthesis, properties and antimicrobial activity of 5-trifluoromethyl-2-formylphenylboronic acid. *Molecules* **2020**, *25*, 799.
- Humphries, R. M.; Ambler, J.; Mitchell, S. L.; Castanheira, M.; Dingle, T.; Hindler, J. A.; Koeth, L.; Sei, K.; et al. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. *J. Clin. Microbiol.* **2018**, *56*, No. e01934-17.

- (9) Appapalam, S. T.; Panchamoorthy, R. Aerva lanata mediated phytofabrication of silver nanoparticles and evaluation of their antibacterial activity against wound associated bacteria. *J. Taiwan Inst. Chem. Eng.* **2017**, *78*, 539–551.
- (10) He, Z.; Zhang, X.; Song, Z.; Li, L.; Chang, H.; Li, S.; Zhou, W. Quercetin inhibits virulence properties of *Porphyromonas gingivalis* in periodontal disease. *Sci. Rep.* **2020**, *10*, No. 18313.
- (11) Sohail, T.; Yasmeen, S.; Imran, H.; Ferheen, S.; Rehman, A. U.; Khan, R. A. Standardization and skin irritation potential of herbal analgesic cream containing *Nigella sativa* seed oil. *Bangladesh J. Med. Sci.* **2019**, *19*, 163–168.
- (12) Ghazali, A. R.; Rajab, N. F.; Zainuddin, M. F.; Ahmat, N.; Surien, O. Assessment of Skin Irritation and Sensitisation Effects by Topical Pterostilbene. *Biomed. Pharmacol. J.* **2021**, *14*, 1917–1927.
- (13) Shrivastav, A.; Mishra, A. K.; Ali, S. S.; Ahmad, A.; Abuzinadah, M. F.; Khan, N. A. In vivo models for assesment of wound healing potential: A systematic review. *Wound Med.* **2018**, *20*, 43–53.
- (14) Buluş, E.; Buluş, G. S.; Şahin, Y. M. Production and Characterization of Nanotechnological Tape for Wounds Caused by Diabetes. *J. Mater. Electron. Devices* **2020**, *5*, 20–24.
- (15) Alugoju, P.; Narsimulu, D.; Bhanu, J. U.; Satyanarayana, N.; Periyasamy, L. Role of quercetin and caloric restriction on the biomolecular composition of aged rat cerebral cortex: An FTIR study. *Spectrochim. Acta, Part A* **2019**, *220*, No. 117128.
- (16) Yang, X.; Wu, D.; Du, Z.; Li, R.; Chen, X.; Li, X. Spectroscopy study on the interaction of quercetin with collagen. *J. Agric. Food Chem.* **2009**, *57*, 3431–3435.
- (17) Lekshmi, P.; Shobi, M. HPTLC profile of quercetin in three cultivars of *Allium sativum* and its antimicrobial activity against bacterial cultures. *J. Chem. Pharm. Res.* **2015**, *7*, 38–43.
- (18) Breijyeh, Z.; Jubeh, B.; Karaman, R. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules* **2020**, *25*, 1340.
- (19) Sun, D.; Li, N.; Zhang, W.; Yang, E.; Mou, Z.; Zhao, Z.; Liu, H.; Wang, W. Quercetin-loaded PLGA nanoparticles: a highly effective antibacterial agent in vitro and anti-infection application in vivo. *J. Nanopart. Res.* **2016**, *18*, No. 3.
- (20) Beyranvand, S.; Pourghobadi, Z.; Sattari, S.; Soleymani, K.; Donskyi, I.; Gharabaghi, M.; Unger, W. E.; Farjanikish, G.; Nayeibzadeh, H.; Adeli, M. Boronic acid functionalized graphene platforms for diabetic wound healing. *Carbon* **2020**, *158*, 327–336.