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Antibacterial Properties of Bacterial Cellulose-Quercetin Composite Membrane

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Abstract

Bacterial cellulose has been thoroughly explored as a biomaterial for various applications due to its intrinsic mechanical, physical, and biological features. Its structural characteristics provide ideal conditions for developing composite, expanding its range of applications even further. The current study aims to synthesize quercetin-loaded bacterial cellulose composite as a potential wound dressing by utilizing the antibacterial activity of quercetin and the unique characteristics of bacterial cellulose. The produced bacterial cellulose was *ex-situ* modified with quercetin. The composite was characterized for its functional group by FTIR, its morphology by FE-SEM, and its antibacterial activity was evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli* through the disc diffusion method. Characterization analyses confirmed the successful impregnation of quercetin into the BC matrix. The antibacterial activity of BC-Quercetin showed moderate activity, both against *S. aureus* and *E. coli*. Determining the loading dose of quercetin into bacterial cellulose is a gap for conducting future research. However, this study indicates that combining bacterial cellulose with quercetin could provide a base for developing promising alternatives for the conventional dressing system in wound healing.

1. Introduction

Skin is the largest organ in the body and has a very important role in preventing pathogens from entering the human body. The skin also supports blood vessels and neurons, impacting protection, excretion, temperature regulation, and the perception of external stimuli [1]. When the skin is injured, the potential for infection increases. Hence, appropriate wound dressing is needed to promote and guide the healing process. Optimal wound dressing is characterized by maintaining high humidity at the wound site, removing excess exudate, ensuring non-toxicity and absence of allergic reactions, allowing oxygen exchange, preventing microbial invasion, and providing comfort and cost-effectiveness [2, 3, 4, 5].

Natural polymers have been widely used in wound dressing in recent years because of their biocompatibility, biodegradability, and physicochemical properties. An increasingly popular novel material for wound dressings is bacterial cellulose (BC). BC is a polysaccharide $(C_6H_{10}O_5)_n$, with characteristic microstructures and is derived from microorganisms such as Gram-negative bacterial species of the genera *Gluconacetobacter*, *Sarcina*, *Azobacter Achromobacter*, *Aerobacter*, *Salmonella*, *Rhizobium*, *Pseudomonas* and *Alcaligenes*, as well as oomycetes and green algae [6, 7, 8]. BC exhibits a high water content (98-99%), good liquid sorption capability, strong wet strength, and high chemical purity. Additionally, it features an ultrafine network architecture characterized by distinct tunnels and pores, along with a higher specific surface area featuring numerous hydroxyl groups [9]. It allows the chemical and physical adsorption of various materials and polymers with the BC, resulting in the development of its composites.

Although it has many unique characteristics and wide-ranging applications, it lacks several functional features, including the absence of antibacterial activity.

Much research has been done to negotiate these limitations. In order to develop antibacterial activity, BC has been composited with various polymers and materials by different methods, including *in-situ* and *ex-situ* [10].

Zheng *et al.* [4] reported that BC can acquire effective antibacterial properties through the incorporation of antibiotics, such as ciprofloxacin, ceftriaxone, and amoxicillin. Additionally, BC can be combined with inorganic antibacterial agents, including silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), and copper nanoparticles (CuNPs), as well as organic compounds like curcumin and natural extracts such as oregano, rosemary, and pomegranate [11]. Natural organic antibacterial substances have attracted wide attention due to their good biocompatibility and biodegradability.

The literature demonstrates that combining plant extracts into BC has been widely used. According to Moradian *et al.* [12], the *ex-situ* addition of rosemary water extract to BC may prevent the growth of *Staphylococcus aureus* and *Escherichia coli*. Adding curcumin extract to BC was also found to eliminate *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* [13]. Furthermore, it was shown that the addition of ethanol extracts from oregano [14], gwari leaves [15], and andaliman [16] to BC increased their antibacterial activity. However, the antimicrobial activity achieved was generally lower in intensity. A single active compound was used in this study to increase antibacterial activity.

Quercetin is an important phytochemical that is classified as one of the flavonoid polyphenol group. It can be found in various fruits, vegetables, flowers, leaves, and seeds [16, 17, 18]. Quercetin possesses many pharmacological activities such as antioxidant [16, 19], anticancer, antiviral, antimicrobial, neuroprotection, anti-inflammatory, cardiovascular, and anti-obesity [20]. The US Food and Drug Organization recently granted quercetin the Generally Recognized As Safe (GRAS) designation [21]. Numerous researchers have examined quercetin's antibacterial action in depth, and it has been suggested as a possible treatment for various pathogenic microbes.

The current study aims to develop bioactive BC with additional antibacterial properties by incorporating the natural antimicrobial compound quercetin. Quercetin was impregnated into BC hydrogel to produce a BC-Quercetin composite membrane. This composite was characterized for its functional group by Fourier Transform Infrared (FTIR and for its morphology using Field Emission Scanning Electron Microscopy (FE-SEM). The antibacterial activity of the composite was tested against *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)*.

2. Experimental

2.1. Materials

Coconut water and sugar were bought at a traditional market in Garut, Indonesia. Bacterial inoculum was obtained from a local industry in Serang, Province of Banten, Indonesia. Chemicals, such as glacial acetic acid (CAS-No: 64-19-7), sodium hydroxide (CAS-No: 1316-73-2), and ammonium sulfate (CAS-No: 7783-85-9), were purchased from Merck, Germany. Quercetin, with 99% purity (CAS-No: 117-39-5), was purchased from Xi'an Plant Bio-Engineering Co., Ltd., China.

2.2. Production of BC Sheets

The present study utilized coconut water as the main culture medium for bacterial cellulose (BC) production. A volume of 300 mL of coconut water was filtered and mixed with 15% (w/v) sugar, after which the pH was adjusted to 5.0 using glacial acetic acid. The liquid medium was then sterilized in an autoclave at 121°C for 30 minutes and subsequently cooled to room temperature. Protein denaturation resulting from the heating process is considered negligible, as the growth of bacterial cellulose is not significantly affected by varying levels of nitrogen sources [22].

Subsequently, the medium was put into several sterilized beaker glasses, after which 15% (v/v) of bacterial inoculum was added. Static incubations were conducted at room temperature for seven days. The BC was subsequently harvested and washed repeatedly with running tap water until a neutral pH was achieved. To purify the BC, a 1% (w/v) sodium hydroxide solution was used for 1 hour to remove any residual organic materials embedded in the cellulose. The purified BC was then thoroughly washed with distilled water until the pH was neutral. All washed BC sheets were stored at 4°C until further use.

2.3. Development of BC-Quercetin Composite Membrane

The quercetin was *ex-situ* loaded into the BC. Quercetin was dissolved in analytical-grade ethanol to a concentration of 50 mg/mL. To prepare the BC-Quercetin composite membrane, 6 cm × 6 cm BC pieces were immersed in a 250 mL quercetin suspension and stored for 72 hours at room temperature. The compound was adsorbed onto the surface and impregnated into the matrix of BC. Quercetin-loaded BC membranes were stored at 4°C for further use.

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra were obtained using a Thermo Scientific Nicolet™ iS50 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) diamond accessory. All spectra were derived from 32 scans at a resolution of 4 cm^{-1}, covering a wavenumber range from 400 to 4000 cm^{-1}. The measurements were performed on dry BC sheets placed directly on the ATR crystal.

2.5. Scanning Electron Microscopy (SEM)

The visual structure of the films was established by morphological imaging using a Field Emission (FE)-SEM (SEM JEOL JSM IT3000). The BC was sliced into small pieces and attached to carbon tape-covered SEM sample holders. To improve sample conductivity, film samples on the holders were coated with gold. The magnification used was from 500 to 30,000×.

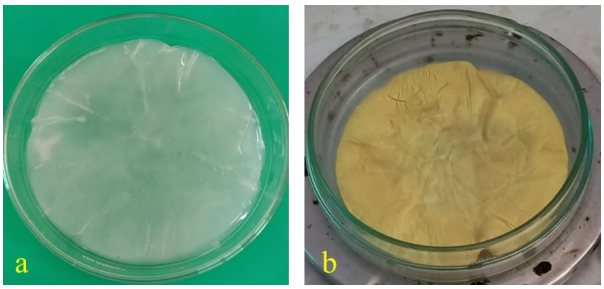

Figure 1. (a) Pristine BC has a white color, (b) BC modified with quercetin turned to yellowish color

2.6. Antibacterial Activity

The antibacterial activities of pristine BC and BC-Quercetin composite membranes were determined against *E. coli* and *S. aureus* by disc diffusion methods. This method was performed on nutrient agar for the respective bacterial strains. To ensure that suspension bacteria are within a specific range, the turbidity of the bacteria was standardized using McFarland 0.5 standard solution. The suspensions of each test organism were prepared and adjusted to 0.5 McFarland turbidity standard.

The antibacterial activity of both membranes was determined by cutting them into disc shapes with a diameter of 6 mm and sterilizing them at 121°C at 103 kPa for 15 minutes. Next, 100 µL of fresh pre-cultures of each *E. coli* and *S. aureus* were spread on respective agar plates, and the disc impregnated with BC-Quercetin (2 mg or 20 µL) was placed on top and incubated at 37°C for 24 h. Finally, the inhibition zones were measured. The disc prepared from dimethyl sulfoxide and 10 µg/mL tetracycline hydrochloride were used as the negative and positive control, respectively. This test was conducted in duplicate.

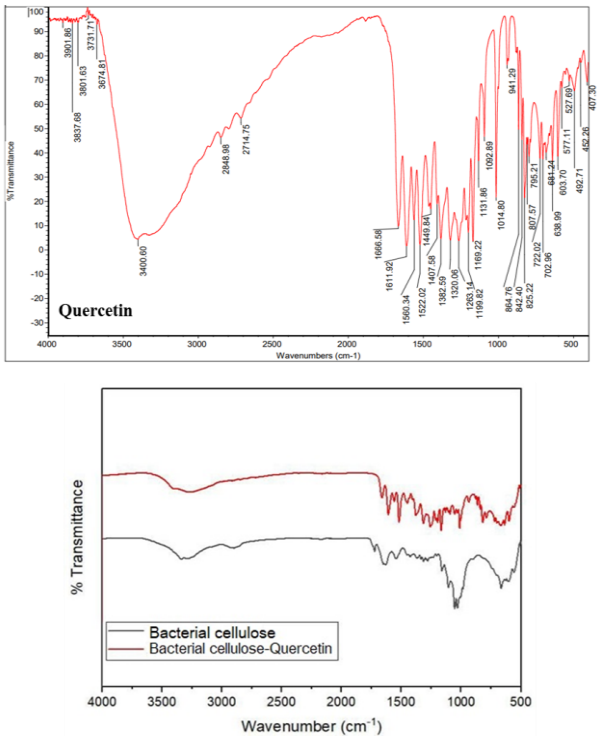

Figure 2. FTIR spectra of prestine BC, quercetin, and BC- Quercetin composite membranes

3. Results and Discussion

3.1. Production of BC and BC-Quercetin Composite Membranes

In this study, bacterial cellulose was manufactured using *Gluconacetobacter xylinus* inoculum with alternative media from coconut water as a substrate, granulated sugar as a carbon source, and ammonium sulfate as a nitrogen source. During fermentation, the bacteria inoculum produces polymers from nutrients in a liquid medium. Since β-glucose is essential for cellulose synthesis, α-glucose is converted into β-glucose by the isomerase enzyme present in the bacterial inoculum. Subsequently, β-glucose molecules are linked via β-1,4-glycosidic bonds, which form between the OH group on the C-1 atom of one β-glucose and the OH group on the C- 4 atom of another β-glucose. The final stage involves the polymerization process, resulting in cellulose formation through the action of polymerization enzymes in the bacterial inoculum [22].

After fermentation, bacterial cellulose forms a white, sour-smelling layer with a chewy and slippery texture. The resulting bacterial cellulose has a thickness of 0.20 cm with a weight of 20.84 g, and the weight after drying is 0.20 g. Thus, the water content of bacterial cellulose is 99.04%. Variability in BC productivity can vary depending on the carbon source used, culture conditions, and duration of fermentation. Based on several pieces of literature, BC productivity obtained from potato peels was 0.78 g [23], vinasse was 0.18 g [24], and rambutan juice was 1.18 g [25].

The BC-Quercetin composite membrane was produced at room temperature for three days. The original white color of BC turned yellow following this modification (Figure 1b), attributed to the yellow quercetin solution. This color change occurs because quercetin and BC form hydrogen bonds throughout the BC chain, causing the BC to adopt quercetin's color. Additionally, ethanol, the solvent used for quercetin, has a density of 0.79360 g/mL, which is lower than that of water (1 g/mL). This difference in solvent density is considered the reason for the change in BC's mass between its pre- and post-modification values, leading to a decrease in the mass of bacterial cellulose after modification. However, the thickness of BC remained unchanged at 0.20 cm before and after the modification. This phenomenon is assumed to be the extract's surface-level interaction with BC rather than internal penetration.

3.2. Functional Group of BC and BC-Quercetin Composite Membranes

The effect of *ex-situ* modification on the BC and BC-Quercetin composite membranes' functional group was determined using FTIR, and the results are compiled in Figure 2. Based on the result, there were absorption peaks in the BC film at about 3341.25 cm^{-1} (–OH stretching) and 2895.81 cm^{-1} due to CH stretching. The C-O-C glycoside group was detected at 1107.31 cm^{-1}. The band at 1031.57 cm^{-1} was present due to the C-O ether group. Meanwhile, another vibration peak was found in the wave number area 1031.57 cm^{-1} for the C-O alcohol group. These results indicated that the synthesis of bacterial cellulose had

been successfully characterized by the presence of the main groups of bacterial cellulose: O-H, C-H, and C-O-C glycoside functional groups.

The characteristic of quercetin was the band at 3400.60 cm^{-1}, which was assigned to the stretching vibration of the hydroxyl of quercetin. Another band at 1382.59 cm^{-1} corresponds to the bending vibration of the phenolic OH group. The wavenumber of aromatic C=C stretching shifted from 1611.92, 1560.34, and 1522.02 cm^{-1}. The absorption stretch of aryl ketone C=O was visible at 1666.58 cm^{-1}. The in-plane C–H bending band in aromatic hydrocarbons was detected at 1449.84 cm^{-1}, and the out-of-plane bending band was observed at 825.22 cm^{-1}. The bands at 1263.14, 1199.82, and 1169.22 cm^{-1} were caused by C–O stretching in the aryl ether ring, C–O stretching in phenol, and C–CO–C stretching and bending in ketones, respectively.

However, the FTIR spectrum of the BC-Quercetin composite membrane almost displayed all the peaks pertaining to BC (Figure 2), with a slight peak shift, indicating an altered hydrogen-bonding pattern, as shown in the wavenumber of 3341.25 (BC), which shifted to 3279.91 (BC-Quercetin). Also, the peak for the C–O group was slightly shifted from 1031.57 to 1032.17 cm^{-1}, which could be attributed to the interaction of BC with quercetin. In addition to the slight peak shift observation, the spectrum of the BC-Quercetin composite membrane exhibited additional peaks around 1400 cm^{-1} to 1550 cm^{-1} due to aromatic ring vibration. Nevertheless, there was also a missing peak at 2895.81 cm^{-1}, which was assumed to indicate whether the interaction in the C-H group successfully created a composite between quercetin and BC.

3.3. Morphological Feature of BC and BC-Quercetin Composite Membrane

The FE-SEM micrographs of BC and BC-Quercetin composite membrane surface view indicate a densely arranged three-dimensional (3D) porous and fibrous web-shaped network (Figure 3). The density and compactness of the BC fibrils are directly associated with the nature and amount of available carbon source, amount of inoculum, type of strain, and culturing conditions, as well as the post-synthesis processing and drying method [26, 27]. In the network structure of the BC, the fibers are interconnected through reversible hydrogen bonding, which can be converted into irreversible hydrogen bonding upon drying [28] through direct conversion of ice into vapors, thus retaining the pore geometry of the BC [29].

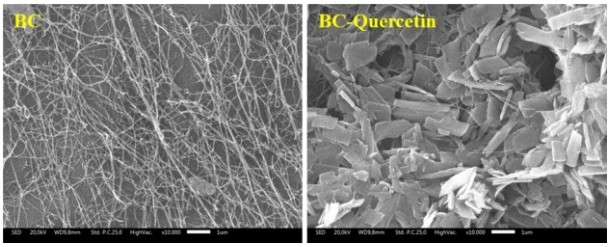

Figure 3. Representative FE-SEM micrographs of surface views of BC and BC-Quercetin composite membrane with 10,000× magnification

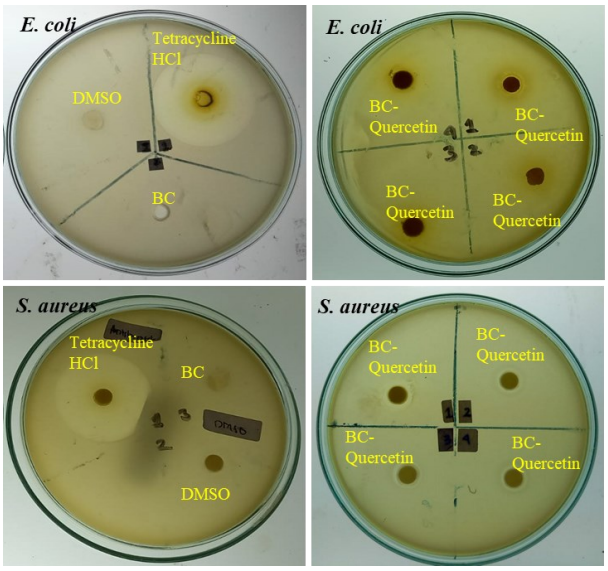

Figure 4. Antibacterial activity of BC-Quercetin composite membrane discs with positive control (tetracycline HCl), negative control (DMSO), pristine BC and BC-Quercetin composites against *E. coli* and *S. aureus* through disc diffusion method

The pores of BC provide an ideal condition for the penetration of different materials, including liquids and solids of specific sizes and different shapes [30]. In this study, the presence of pores in the web-shaped network of the BC, as shown from the surface, ensured the adsorption and penetration of the quercetin under *ex- situ*, which could be potentially retained by the fibrous network through physical attachment or chemical interaction, thus forming the BC-Quercetin composite membrane. Additionally, the impregnation of the quercetin into the BC matrix was confirmed through dry-weight analysis. Overall, the observation of BC morphology indicating successful impregnation of quercetin into BC's matrix justifies the development of its composites with natural bioactive materials.

3.4. Antibacterial Activity

Figure 4 displays the diameters of inhibition zones for antibacterial activity against *S. aureus* and *E. coli*, representative of Gram-positive and Gram-negative bacterial species, respectively, using the disc diffusion method. The result showed that the BC-Quercetin composite membrane produced a clear inhibition zone measuring approximately 9 mm against *S. aureus* and 7 mm against *E. coli*, effectively suppressing their growth. In contrast, BC alone showed no inhibitory effect against either S. aureus or *E. coli*.

The BC is known for its non-bactericidal nature [30], while quercetin has known antibacterial activity [19]. The antibacterial activity of the BC-Quercetin composite membrane against *S. aureus* and *E. coli* could be attributed to the antibacterial activity of the quercetin. The cellulose chains might interact physically or chemically with quercetin, which could cause it to lose quercetin's activity as an active component. Nevertheless, the antibacterial effect of the composite indicated that quercetin retained its antibacterial activity. These findings open a gateway for developing the composites of the BC with different

bioactive compounds to explore their medicinal use for applications in developing medical, cosmetic, and pharmaceutical products.

4. Conclusion

Quercetin was effectively impregnated into the BC matrix owing to the *ex-situ* modification. FTIR spectroscopy confirmed the chemical interaction between BC and quercetin. The BC exhibited a high potential of material holding capacity, conferring it with antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli*. This indicates that improving the BC's existing features and introducing additional features is achievable by supplementing it with suitable materials for specific applications. These studies suggest that the production of BC-Quercetin composite membrane could be used as a potential topical antibacterial patch for wound healing.

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