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Optimization of Bacterial Cellulose-Quercetin Biocomposite Synthesis Using The Ex-Situ Method and Its Application as an Antibacterial Agent

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ABSTRACT.

Bacterial cellulose is a natural polymer synthesized by bacteria, possessing significant potential for biomedical and pharmaceutical applications. However, it lacks inherent antibacterial properties, necessitating modification with additional materials. Quercetin, a flavonoid compound, is known for its pharmacological activities, including antibacterial effects by disrupting bacterial cell walls. This study aims to identify the antibacterial activity of bacterial cellulose-quercetin biocomposite by optimizing the concentration and modification time, and to characterize their functional groups. Bacterial cellulose was modified using an ex-situ method by immersing it in quercetin solution. Antibacterial testing was performed using the disk diffusion method against *Staphylococcus aureus* and *Escherichia coli*. The optimal biocomposite was achieved at 6% quercetin concentration and 48 hours modification time, showing inhibition zones of 18.5 mm for *S. aureus* and 18 mm for *E. coli*. This indicated strong antibacterial activity. FTIR analysis confirmed the main functional groups of cellulose (O-H, C-H, and C-O-C glycosidic bonds) in both bacterial cellulose and the composite, showing no significant changes after immersion in distilled water. This suggests that quercetin binds strongly to the bacterial cellulose matrix, resulting in a stable biocomposite with potential as an effective antibacterial material for wound healing.

Keywords: bacterial cellulose, quercetin, antibacterial

1. INTRODUCTION

Natural polymers or biopolymers are compounds in plants, animals, and microorganisms. One of the most common and abundant in nature is cellulose [1], [2]. Cellulose is a linear homopolysaccharide composed of β-D-glucopyranose units linked by β-1,4 glycosidic bonds. In addition to serving as the main structural component of plant cell walls, cellulose is also found in bacteria [3]. Generally, bacterial cellulose is purer than plant cellulose because it is not associated with hemicellulose and lignin. This allows it to maintain a high degree of polymerization, crystallinity, and water absorption capacity [4]. Moreover, due to its good biocompatibility and biodegradability, bacterial cellulose has excellent potential in biomedical and pharmaceutical applications such as wound dressings, organ tissue engineering, synthetic organs, and more [5], [6], [7]. However, due to its lack of antibacterial properties, it often requires incorporation (compositing) with other materials such as polymers (e.g., chitosan), nanomaterials (e.g., CNT), solid materials (e.g., silica), metals (e.g., Au), and bioactive compounds from plants (e.g., various phenolic compounds) [6], [8], [9].

Flavonoids are generally known as bioactive compounds with antimicrobial activity [10]. Quercetin is a flavonol compound from the polyphenol group (flavonoids) that exhibits antibacterial properties [11]. Quercetin has antibacterial effects against almost all types of bacteria, affecting the digestive, respiratory, urinary, and skin systems [12]. It is effective against gram-positive bacteria such as *Enterococcus faecalis* (MIC = 125 µg/mL) with an inhibition zone of 9.60 ± 0.38 mm, *Bacillus subtilis* (MIC = 62.5 µg/mL) with an inhibition zone of 18.76 ± 0.41 mm, and *Staphylococcus aureus* (MIC = 700 µg/mL) with an

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inhibition zone of 12 ± 0.9 mm, as well as gram-negative bacteria such as *Escherichia coli* (MIC = 300 µg/mL) with an inhibition zone of 14 ± 0.5 mm, *Klebsiella pneumoniae* (MIC = 500 µg/mL) with an inhibition zone of 25 ± 1.0 mm, and *Pseudomonas aeruginosa* (MIC = 125 µg/mL) with an inhibition zone of 12.82 ± 0.32 mm [13], [14], [15]. Studies also indicate that quercetin exhibits a bacteriostatic effect by damaging the cell wall and membrane of *S. aureus* at 10 × MIC and *E. coli* at 50 × MIC [16]. Compared to other flavonoids such as naringin (MIC = 3000 µg/mL against *S. aureus* and MIC > 2000 µg/mL against *E. coli*) and catechins (MIC = 1000 µg/mL against *S. aureus* and MIC = 640 µg/mL against *E. coli*), quercetin exhibits strong antibacterial activity, making it widely used in various medical applications, including wound healing [17], [18], [19], [20]. Therefore, the combination of bacterial cellulose and quercetin composites is expected to enhance its antibacterial activity and other properties such as anti-inflammatory and antioxidant effects, supporting its potential use in medical applications like wound healing [10].

The synthesis of the bacterial cellulose-quercetin biocomposite is conducted using an ex-situ method. This method's advantage lies in the simplicity of the synthesis process, which preserves the integrity of the bacterial cellulose structure and ensures that the included antimicrobial material remains undamaged. This study aims to synthesize and characterize the bacterial cellulose-quercetin biocomposite and evaluate its antibacterial activity. In this study, optimization of quercetin solution concentration and modification time was carried out. Characterization involves analyzing its functional groups. Antibacterial activity is tested using the disc diffusion method against gram-positive bacteria, namely *Staphylococcus aureus*, and gram-negative bacteria, specifically *Escherichia coli*.

2. MATERIALS AND METHOD

In this study, the bacterial cellulose-quercetin composite method begins with synthesizing bacterial cellulose as the base material. Subsequently, quercetin solutions with various concentrations are prepared to determine the minimum inhibitory concentration (MIC) against the target bacteria. After determining the MIC, the selected concentration modifies the bacterial cellulose through immersion in the quercetin solution for varying periods. The antibacterial activity of the formed biocomposite is tested to identify the combination with the best activity. The biocomposite with optimal antibacterial activity is then analyzed using Fourier Transform Infrared (FTIR) spectroscopy to examine the interactions between bacterial cellulose and quercetin. Additionally, the biocomposite is immersed in distilled water to identify any changes in the FTIR spectrum that indicate interactions or changes in chemical structure after modification.

2.1. Tools and Materials

The equipment used in this study includes an autoclave, analytical balance, hot plate, oven, incubator, inoculating needle, tweezers, watch glass, spirit burner, funnel, Erlenmeyer flask, graduated cylinder, beakers, stirring rod, spatula, dropper pipette, petri dishes, volumetric flask, micropipette, vortex mixer, dry bacterial cellulose cutter, thermometer, caliper, test tubes, Fourier Transform Infrared (FTIR) spectrophotometer Bruker Alpha II, filter paper, vials, yellow pipette, blue pipette, pH indicator, filter paper, cotton, newspaper, aluminum foil, and thread.

The materials used in this study include coconut water and granulated sugar were bought at a traditional market in Garut, Indonesia; the bacterial starter *Gluconacetobacter xylinus* was obtained from a local industry in Serang, Indonesia; ammonium sulfate (CAS-No: 7783-85-9), sodium hydroxide (NaOH) (CAS-No: 1316- 73-2), glacial acetic acid (CAS-No: 64-19-7) were purchased from Merck, Germany; McFarland solution 0.5; distilled water; quercetin powder 99% purity (CAS-No: 117-39-5) was purchased from Xi'an Plant Bio-Engineering Co., Ltd., China; analytical-grade ethanol, nutrient agar (NA), nutrient broth (NB), 1% tetracycline hydrochloride antibiotic, and isolates of *Escherichia coli* and *Staphylococcus aureus*.

2.2. Procedure

2.2.1. Synthesis of Bacterial Cellulose

The glassware was prepared and sterilized using an autoclave. A volume of 100 mL of coconut water was filtered into an Erlenmeyer flask, then transferred to a sterile beaker and heated until it reached

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a gentle boil. The liquid medium was mixed with 15% sucrose solution as a carbon source and 4% ammonium sulfate as a nitrogen source. The pH was then adjusted to 5 by adding glacial acetic acid and a 1% NaOH solution. The medium was cooled in running water and inoculated with 10% of the bacterial starter culture *Gluconacetobacter xylinus*. The beaker was covered with newspaper or aluminum foil, pierced with 3-5 air holes using a needle, and left to stand for 7-10 days at room temperature. After 7 to 10 days, bacterial cellulose formed on the surface of the medium, which was harvested and purified by soaking it in a 1% NaOH solution for 2 hours. It was then neutralized with distilled water and dried in an oven at 45-50°C. The bacterial cellulose was weighed and its thickness measured before and after drying.

2.2.2. Preparation of Quercetin Solution

To prepare a 6% quercetin solution, 0.6 grams of quercetin powder is weighed and placed into a beaker. Then, 10 mL of ethanol (p.a.) is added.

2.2.3. Determination of Minimum Inhibitory Concentration (MIC)

This study determined the Minimum Inhibitory Concentration (MIC) using the macro dilution method with Nutrient Broth (NB) medium. The total volume of each test tube was 5 mL (containing nutrient broth and quercetin solution). A volume of 4.7 mL of nutrient broth was added to each tube. Serial dilutions were then performed to achieve concentrations of 6%, 3%, 1.5%, 0.75%, and 0.375%. In the first medium (6%), 0.3 mL (300 µL) of the quercetin solution was added, vortexed, and an equal volume was transferred from the first medium to the second (3%), repeating this process until the last medium with a concentration of 0.375%. Afterward, 100 µL of bacterial suspension, standardized to a turbidity equivalent to a McFarland standard of 0.5, was added to each medium. As a comparison, positive control (nutrient broth and test bacteria) and negative control (nutrient broth and 1% tetracycline HCl) were prepared. The mixtures were then incubated at 37°C for 24 hours, after which turbidity was assessed.

2.2.4. Modification of Bacterial Cellulose-Quercetin

The modification was carried out after the bacterial cellulose was formed and purified. Bacterial cellulose was immersed in a quercetin solution with varying concentrations and modification times: 6% for 6 hours, 6% for 24 hours, 6% for 48 hours, 3% for 6 hours, 3% for 24 hours, and 3% for 48 hours. Residual quercetin solution on the surface of the bacterial cellulose-quercetin composite was removed by rinsing with distilled water and then dried in an oven at a temperature of 45-50°C. It is important to note that before and after drying, the biocomposite was weighed and its thickness was measured.

2.2.5. Antibacterial Activity Testing

Antibacterial testing was conducted using the disc diffusion method. The medium used was Nutrient Agar (NA), which was previously sterilized. A total of 20 mL of the sterilized nutrient agar solution was aseptically poured into Petri dishes and allowed to solidify. Once the medium solidified, 100 µL of bacterial suspension, standardized to a turbidity equivalent to McFarland standard 0.5, was added to each medium and spread using a sterile cotton swab or spreader. Bacterial cellulose and quercetin-bacterial cellulose discs, which had been dried and cut into circular shapes, were placed on the agar surface. Additionally, paper discs impregnated with 1% tetracycline HCl antibiotic as a positive control and absolute ethanol as a negative control were also included in each Petri dish. Paper discs soaked in 6% and 3% quercetin solutions were placed in separate dishes for comparison. The plates were then incubated at 37°C for 24 hours, after which the total diameter of the clear zone around each disc was measured.

2.2.6. Immersion of Biocomposite in Distilled Water

The bacterial cellulose-quercetin biocomposite with a concentration of 6% and a modification time of 48 hours was selected for immersion in distilled water, with variations in immersion times of 12 and 48 hours. This method ensures that the bacterial cellulose remains well-composited with quercetin after immersion in distilled water.

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2.2.7. Functional Group Analysis using FTIR

The functional group analysis of the bacterial cellulose-quercetin biocomposite was conducted using a Bruker Alpha II Fourier Transform Infrared (FTIR) spectrophotometer with the Attenuated Total Reflectance (ATR) method, over a wavelength range of 4000 cm^{-1} to 400 cm^{-1}. This analysis confirmed bacterial cellulose was successfully synthesized and effectively composited with quercetin.

3. RESULTS AND DISCUSSION

3.1. Minimum Inhibitory Concentration (MIC) of Quercetin Solution

Quercetin exhibits good antibacterial properties by disrupting the integrity of bacterial cell membranes, leading to cell wall lysis, cytoplasmic leakage, and separation of the plasma membrane from the cell wall, ultimately resulting in cell death. The antibacterial capability of quercetin is associated with its solubility and its interaction with bacterial cell membranes, which is primarily determined by the presence of hydroxyl groups on the quercetin molecule [11]. This study used a quercetin solution as an antibacterial agent for the ex-situ modification of bacterial cellulose. Therefore, it is essential to determine the minimum concentration of the quercetin solution required to inhibit the growth of the test bacteria (*E. coli* and *S. aureus*). The Minimum Inhibitory Concentration (MIC) was determined using a liquid dilution method with nutrient broth as the medium. The principle of the liquid dilution method involves serial dilutions of the antimicrobial agent in liquid media and observation of microbial growth under those conditions. The MIC values for the quercetin solution obtained are presented in Table 1.

Based on the observational data presented in Figure 1, after incubation with *S. aureus* and *E. coli*, it was observed that quercetin concentrations of 0.375%, 0.75%, and 1.5% still allowed bacterial growth, as evidenced by the turbid appearance of the medium. In contrast, at concentrations of 3% and 6%, no bacterial growth was detected, indicated by the clear appearance of the medium. Therefore, it can be concluded that the minimum inhibitory concentration (MIC) of quercetin solution against *S. aureus* and *E. coli* is 3%.

Table 1. MIC Values of Quercetin Solution

No.	Concentration	*S. aureus*	*E. coli*
1.	6%	-	-
2.	3%	-	-
3.	1.5%	+	+
4.	0.75%	+	+
5.	0.375%	+	+

Note: (+) = bacterial growth present
(-) = no bacterial growth present

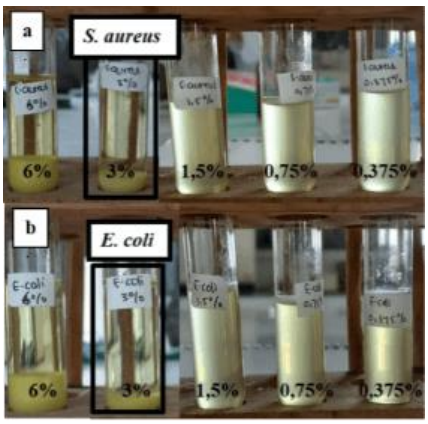

Figure 1. Minimum Inhibitory Concentration (MIC) a. *S. aureus*; b. *E. coli*

3.2. Modification of Bacterial Cellulose-Quercetin

The modification of bacterial cellulose with quercetin was carried out using an ex-situ method. The principle of the ex-situ modification method involves the absorption and distribution of the additive material into the bacterial cellulose matrix after purification through immersion in a selected concentration and modification time. In this study, quercetin was chosen as the modifying agent for bacterial cellulose, expecting to enhance its antibacterial activity. The ex-situ modification process of bacterial cellulose with quercetin was performed by optimizing both concentration and modification time. Based on the Minimum Inhibitory Concentration (MIC) tests, the selected concentration variations were 3% and 6%. The modification times were 6, 24, and 48 hours for each concentration.

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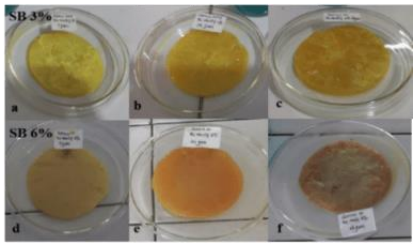

Figure 2. Bacterial cellulose after modification a. 3% for 6 hours; b. 3% for 24 hours; c. 3% for 48 hours; d. 6% for 6 hours; e. 6% for 24 hours; f. 6% for 48 hours

Table 2. Conditions Bacterial Cellulose Before and After Modification

Variation Concentration and Time Modification	Wet Mass		Thickness		Dry Mass	Water Absorption
	Before Modification	After Modification	Before Modification	After Modification		
Non-modif	28.57 g	-	1.07 cm	-	0.63 g	97.7%
3% (6 hour)	20.23 g	16.33 g	1.04 cm	1.04 cm	0.19 g	98.8%
3% (24 hour)	25.17 g	18.95 g	1.09 cm	1.09 cm	0.21 g	98.8%
3% (48 hour)	29.09 g	22.24 g	1.13 cm	1.13 cm	1.07 g	95.2%
6% (6 hour)	38.90 g	34.67 g	1.17 cm	1.17 cm	1.86 g	94.6%
6% (24 hour)	40.31 g	34.56 g	1.18 cm	1.18 cm	2.38 g	93.1%
6% (48 hour)	42.22 g	36.24 g	1.21 cm	1.21 cm	2.56 g	92.9%

Based on Table 2, the thickness of bacterial cellulose remained unchanged before and after modification. The wet mass of bacterial cellulose decreased following modification instead of prior to it. Water absorption decreased with increasing quercetin content.

Based on the observation data shown in Figure 2, bacterial cellulose changed color and mass after modification. Initially, the bacterial cellulose layer was white, with a chewy texture and a smooth surface, accompanied by a sharp sour odor. After modification, the original color of the bacterial cellulose changed to yellow due to the yellow-colored quercetin solution. As the concentration increased and the modification time extended, the color of the bacterial cellulose layer became more intense. This color change occurs due to hydrogen bonding interactions between the bacterial cellulose chains and quercetin, resulting in the bacterial cellulose binding to the yellow color of quercetin [21]. Bacterial cellulose also experienced a decrease in mass after modification compared with mass before modification, as indicated in Table 2. This is likely because the ethanol solvent used to dissolve quercetin replaced some of the water content in the bacterial cellulose. Ethanol has a lower density (0.79360 g/mL) compared to water (1 g/mL), which means that the modified bacterial cellulose is lighter than the unmodified bacterial cellulose [21]. Nevertheless, the thickness of the bacterial cellulose did not change before and after modification, likely because the interaction between quercetin and bacterial cellulose is predominantly at the surface level rather than involving internal penetration of the bacterial cellulose.

3.3. Antibacterial Activity of Bacterial Cellulose-Quercetin

The antibacterial activity test of the bacterial cellulose-quercetin biocomposite was conducted using the disc diffusion method. The principle of the disc diffusion method is based on the diffusion of antimicrobial agents from discs into a solid medium inoculated with the test bacteria. This method was chosen due to its advantages, including being more economical, flexible, requiring no special equipment, and allowing easy interaction between the antimicrobial agents and the bacteria through the disc

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or other matrices, as they are located on the surface of the medium [22]. Figures 3, 4, and 5 show the antibacterial test results.

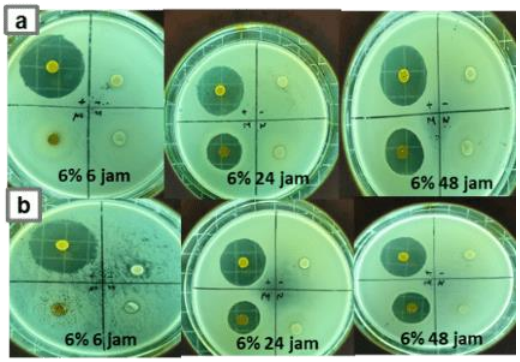

Figure 3. Antibacterial test of 6 % bacterial cellulose-quercetin against bacteria: a. *S. aureus*; b. *E. coli*

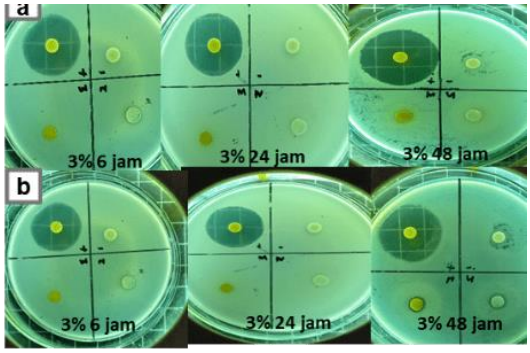

Figure 4. Antibacterial test of 3 % bacterial cellulose-quercetin against bacteria: a. *S. aureus*; b. *E. coli*

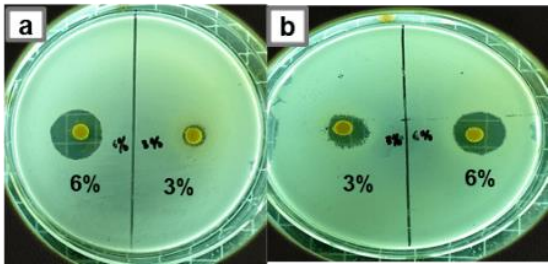

Figure 5. Antibacterial test of 6% and 3% quercetin solution against bacteria: a. *S. aureus*; b. *E. coli*

Figures 3, 4, and 5 show the antibacterial test results. Based on the observations, pure bacterial cellulose exhibited no antibacterial activity, as evidenced by the absence of inhibition zones on the medium. In contrast, the bacterial cellulose-quercetin composite demonstrated antibacterial activity by forming inhibition zones on the medium. However, not all bacterial cellulose-quercetin samples with varying concentrations and modification times produced inhibition zones, as indicated in Table 3.

Table 3. Results of Antibacterial Testing

Sample		*S. aureus* (mm)	*E. Coli* (mm)	Inhibition Response
3% Quercetin Solution		8.75	13.75	Strong
6% Quercetin Solution		19.5	18	Strong
BC-Quercetin 3% 6 hours	Modif	-	-	Not present
	Non-modif	-	-	Not present
	Control +	30	27	Not present
	Control -	-	-	Not present
BC-Quercetin 3% 24 hours	Modif	-	-	Not present
	Non-modif	-	-	Not present
	Control +	28	28	Very strong
	Control -	-	-	Not present
BC-Quercetin 3% 48 hours	Modif	6.5	8	Moderate
	Non-modif	-	-	Not present
	Control +	35	30	Very strong
	Control -	-	-	Not present
BC-Quercetin 6% 6 hours	Modif	6.5	7	Moderate
	Non-modif	-	-	Not present
	Control +	33	33	Very strong
	Control -	-	-	Not present
BC-Quercetin 6% 24 hours	Modif	17	17.75	Strong
	Non-modif	-	-	Not present
	Control +	29	33	Very strong
	Control -	-	-	Not present
BC-Quercetin 6% 48 hours	Modif	18.5	18	Strong
	Non-modif	-	-	Not present
	Control +	29	31	Very strong
	Control -	-	-	Not present

Note: BC is Bacterial Cellulose

The bacterial cellulose-quercetin composite at a concentration of 6% with a modification time of 48 hours exhibited the best antibacterial activity, with inhibition zones measuring 18.5 mm against *S. aureus* and 18 mm against *E. coli*. This suggests that

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increasing the concentration of the quercetin solution and extending the modification time likely enhance the interactions between quercetin and the surface of bacterial cellulose, resulting in a greater antibacterial effect. The enhanced antibacterial activity at 6% concentration and 48 hours is likely due to stronger and more numerous molecular interactions (e.g., hydrogen bonding, π–π stacking) between quercetin and cellulose, leading to better quercetin loading, retention, and activity on the surface.

Antibacterial activity was also tested on quercetin solution. At a concentration of 3%, the inhibition zones measured 8.75 mm against *S. aureus* and 13,75 mm against *E. coli,* while at a concentration of 6%, the zones were 19,5 mm for *S. aureus* and 18 mm for *E. coli*. The combination of functional groups in quercetin, such as hydroxyl (O-H), carbonyl (C=O), C-O-C, and aromatic ring structures (A and B), enables quercetin to interact with various targets in bacterial cells, thereby enhancing its antibacterial activity [23].

At the same concentration, the diameter of the inhibition zone for the bacterial cellulose-quercetin composite was smaller than that of the quercetin solution alone. The suboptimal quality of the bacterial cellulose matrix may have resulted in uneven distribution of the quercetin solution, which was likely limited to the surface, contributing to the smaller inhibition zones or even the absence of inhibition zones in other concentration and modification time variations compared to the quercetin solution tested using disk diffusion. Additionally, the antibacterial activity of quercetin can be influenced by chemical modifications or by the chemical environment in which quercetin is applied. The interaction of quercetin with other materials may alter its antibacterial effectiveness.

3.4. Characteristics of Functional Groups using FTIR

In this study, the analysis of functional groups in bacterial cellulose and bacterial cellulose-quercetin was conducted using an FTIR spectrophotometer. The principle of FTIR operates on the interaction between energy and matter. Infrared light passes through a gap that controls the amount of energy delivered to the sample. Then, at the interferometer, the sample absorbs some of the light and transmits through its surface. The light then proceeds to the detector, generating a measurable interferogram signal sent to a computer for recording in the form of peaks [24]. The FTIR spectra of pure bacterial cellulose and bacterial cellulose-quercetin are shown in Figure 6.

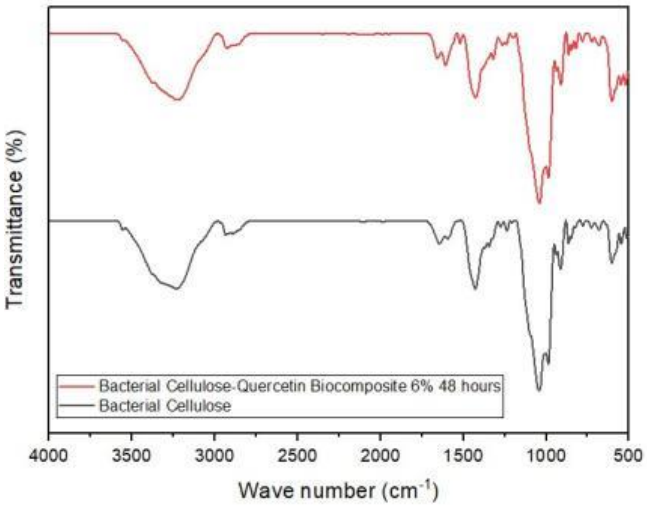

Figure 6. FTIR Spectrum a. bacterial cellulose and bacterial cellulose-quercetin at a concentration of 6% for 48 hours

Based on the analysis results presented in Figure 6, the FTIR spectrum of bacterial cellulose exhibits a moderate intensity at a wavenumber of 3230.15 cm^{-1}, indicating the presence of O-H stretching, while the wavenumber of 2934.38 cm^{-1}corresponds to aliphatic C-H stretching. Additionally, the peak at 1042.92 cm^{-1} can be associated with either C-O-C groups and C-O-H ring sugar stretching. The peaks in the 1200-1300 cm-1 range indicate C-O stretching and O-H in-plane bending, which are associated with crystalline regions within the structure [25], [26]. These results demonstrate that bacterial cellulose synthesis has been successfully achieved, marked by the presence of key functional groups of bacterial cellulose, namely O-H, C-H, and C-O-C glycosidic bonds [21].

The FTIR spectrum of the bacterial cellulose-quercetin composite displays similar absorption peaks but with slight shifts compared to bacterial cellulose. The O-H group shows a wavenumber shift to 3219.14 cm^{-1}, while the aliphatic C-H peak shifts to 2928.37 cm^{-1}. The C-O-C peak also shifts to 1041.16 cm^{-1}. These shifts are likely due to interactions between quercetin and bacterial cellulose. When quercetin interacts with

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bacterial cellulose, changes occur in the chemical environment of bacterial cellulose, affecting the vibrational frequency of the molecules. New absorption peaks corresponding to aromatic ring C=C groups appear at wavenumbers 1611.52 and 1522.55 cm^{-1} [21]. Thus, bacterial cellulose has been successfully composited with quercetin.

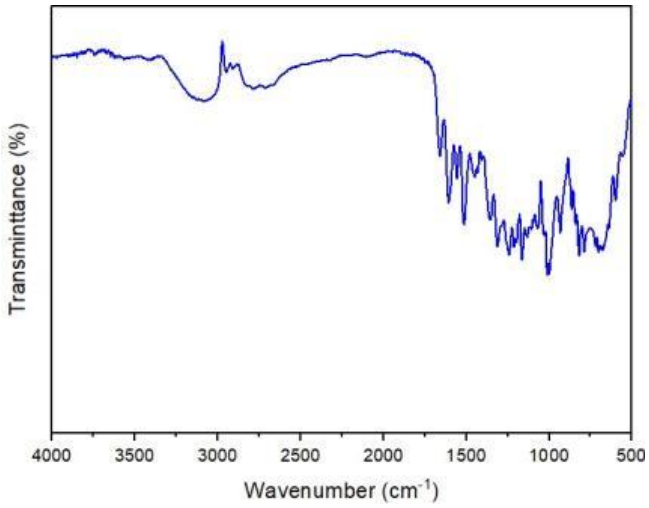

Figure 7. FTIR Spectrum bacterial cellulose-quercetin at a concentration of 6% for 48 hours after immersion in distilled water for 12 hours

Furthermore, the FTIR spectrum of the 6% bacterial cellulose-quercetin composite after being immersed in distilled water for 12 hours in Figure 7, shows similar absorption peaks with slight shifts compared to both the bacterial cellulose and the bacterial cellulose-quercetin composite before immersion. The O-H group shifts to 3082.38 cm^{-1}, the aliphatic C-H peak shifts to 2948.03 cm^{-1}, and the C-O-C peak shifts to 1012.03 cm^{-1}. Thus, quercetin binds strongly to the bacterial cellulose matrix, potentially an effective antibacterial material, such as for wound healing.

4. CONCLUSION

Based on the results, the bacterial cellulose-quercetin biocomposite demonstrated good antibacterial activity, showing significant inhibition zones against *S. aureus* and *E. coli* at a concentration of 6% with a modification time of 48 hours. FTIR analysis before and after immersion in distilled water revealed no significant changes in the absorption spectrum of the main functional groups of bacterial cellulose. In contrast, new functional groups from quercetin appeared. This indicates that quercetin binds strongly to the bacterial cellulose matrix, resulting in a stable biocomposite with potential as an effective antibacterial material for wound healing. A potential future direction includes conducting in-vivo testing to evaluate the biocomposite's effectiveness and biocompatibility in real biological environments and exploring scalability and manufacturing feasibility for practical applications in wound healing and other biomedical uses.

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