Research Article

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Green synthesis methods and characterization of bacterial cellulose/silver nanoparticle composites

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Abstract: Bacterial cellulose (BC) is a microbiologically produced cellulose with high purity and excellent biocompatibility, allowing it to be used alone or in combination with other materials, including polymers and nanoparticles. This study was conducted to incorporate silver nanoparticles (AgNPs) into a BC matrix using simple and environmentally friendly methods in order to create a composite with superior industrial properties. The fabricated composites were characterized with Fourier transform infrared, X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive X-ray (EDX), while the thermal stability was investigated by thermogravimetric analysis. The antimicrobial activity of the composites was determined by observing the formation of an inhibition zone during the incubation of *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative). The SEM, EDX, and XRD analysis confirmed the presence of AgNPs. The composites also exhibit excellent thermal stability and significant antimicrobial activity against *S. aureus* and *E. coli*.

Keywords: bacterial cellulose, characterization, composites, silver nanoparticle

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1 Introduction

Nanotechnology is a scientific discipline that describes the chemistry of nanoparticles. Richard P. Feynman was the first to introduce the concept of nanotechnology in his lecture *There's plenty of room at the Bottom* in 1959 [1], and the term nanotechnology was first introduced by Taniguchi in 1974 [2] to describe the production of materials in nanometer size. Nanoparticles combine a group of atoms or molecules of different materials with nanoscale sizes of about 10^{-9} m [3]. In one dimension, the size of nanoparticles can range from 1 nm to several tens of nm. Nanoparticles and minerals exhibit unique catalytic, electronic, magnetic, chemical, photochemical, and optical properties compared to conventional materials [4]. Many types of nanoparticles, including metals, metal oxides, polymers, carbon-based, and composites, can be manufactured according to the desired application [5].

Gold and silver are the most popular metal nanoparticles used in various applications, including electronics, textiles, sensors, and biomedicine applications. Silver nanoparticles (AgNPs) have several unique properties depending on their size, morphology, and surface charge, inspiring a vast range of applications. Additionally, AgNPs are recognized for their enhanced antimicrobial properties against pathogenic microbes such as bacteria, fungi, algae, and viruses [6]. On the other hand, the rapid oxidation of AgNPs and their dependence on the surface charge of the suspension medium severely restrict the use of AgNPs [7]. Therefore, numerous AgNP applications are incorporated into nanocomposites to enhance their stability. So far, numerous studies have been conducted on the synthesis and properties of silver nanocomposites. AgNPs can be incorporated into a matrix of materials to form nanocomposites, such as polymers [5,7], graphene/graphene oxide [8,9], chitosan [10,11], and cellulose.

Bacterial cellulose (BC) is a microbiologically produced cellulose that is a natural biopolymer. Compared to conventional cellulose, utilizing BC has remarkable advantages such as higher purity, greater mechanical strength,

and relatively more straightforward obtainability [12,13]. Because of its purity and biocompatibility, BC can be used alone or in combination with other materials to alter its structure and function, providing a perspective for using BC in industrial and medical applications [14]. Composites of cellulose have been frequently used as templates to create inorganic nanoparticles. Because cellulose contains abundant hydroxyl groups, it is compatible with polar matrices and enhances the homogenous dispersion of inorganic nanoparticles [15]. In addition, BC is relatively easy to undergo various chemical reactions and structural transformation due to three free hydroxyl groups located at C_2, C_3, and C_6 [16].

Recent studies have been conducted to incorporate AgNPs into cellulose structure to form a composite to obtain new materials with superior properties in industrial applications, for example, in generated cellulose membrane by dip coating [17], in BC by *in situ* [18–20] and *ex situ* preparation [21,22]. However, all the aforementioned studies utilized chemicals to boost the reduction of silver ions into cellulose matrix to form AgNPs; some of those chemicals are hazardous and may be dangerous if released into the environment. This study focused on producing BC/AgNP composites by greener methods than previously conducted studies. In this study, BC was made from a fermentation process by *Acetobacter xylinum* bacteria in a simple coconut water medium to produce low-cost BC sheets. Then BC was utilized as a support and dispersion media for AgNPs. A natural reducing agent, *Averrhoa bilimbi* fruit extract, and a hydrothermal method assisted the reducing process of silver ions into AgNPs in the BC matrix. *Averrhoa bilimbi* was chosen as a reducing agent because of the high content of phytochemicals such as flavonoids, saponins, terpenoids, and tannins [23–25], which play a significant role in the bioreduction and stabilization of AgNPs [26,27]. Those methods were simple, cost-effective, and chemical-free, which have not been studied before. These methods were expected to broaden the technology to generate metal cellulose composites, particularly those with antimicrobial properties for biotechnology applications. The eco-friendly BC AgNP composite was also beneficial for antimicrobial applications, especially those involving direct human contact. Green AgNPs offer pharmacological and biological potential, including antiviral, antimicrobial, antifungal, antiparasitic, antioxidant, anticoagulant, and biofilm inhibition activity, as well as anticancer activity against various cancers [28,29]. The most significant contribution of this research was the development of sustainable nanoparticle composite biomaterials that utilize inexpensive and indigenous materials.

2 Experimental

2.1 Materials and methods

All chemicals used in this study were of analytical grade and purchased from Merck. BC samples for this study were obtained, as reported in our previous study [30]. The BC samples resulted from the fermentation of *Acetobacter xylinum* in a simple coconut water medium at a static condition and room temperature. After 4 days of fermentation, BC was harvested and cleaned under running water. The BC was then boiled in water for 5 min to kill the remaining *Acetobacter xylinum* bacteria, and the sample was ready for the following treatment, as displayed in Figure 2a.

Averrhoa bilimbi was obtained from local parks. Fruits were crushed and then boiled for 5 min. After cooling down, the stew was pressed and filtered for the liquid-only extract. The *Averrhoa bilimbi* fruit and its extract are displayed in Figure 1.

Figure 1: *Averrhoa bilimbi* fruit and its extract.

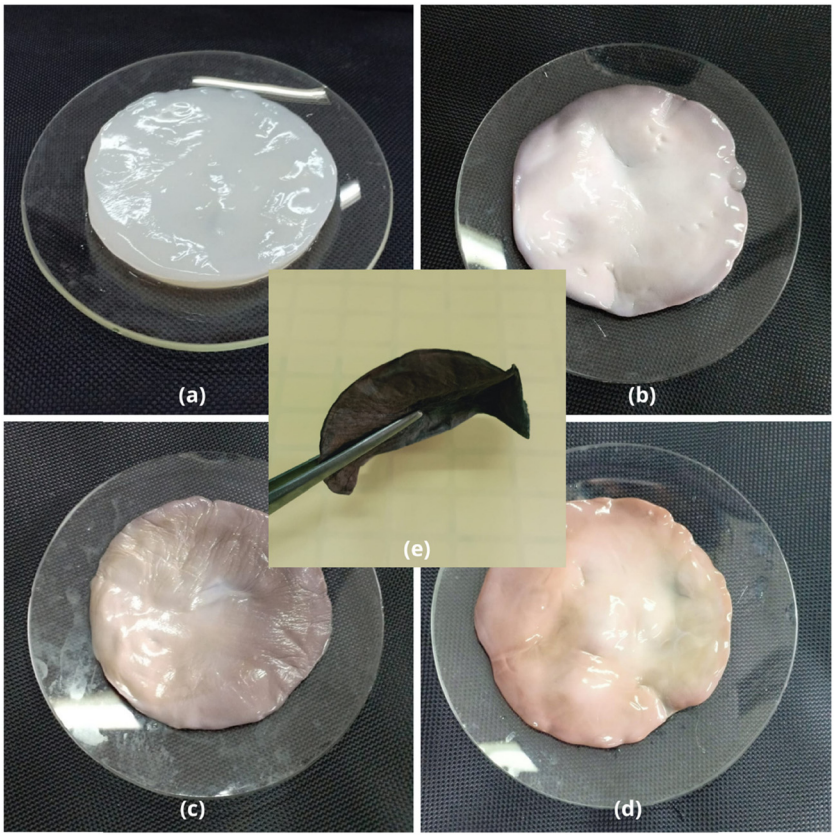

Figure 2: (a) Pristine BC *nata de coco*, (b) BC after treated by immersing in $AgNO_3$ solution, reduction of Ag ions in BC by the support of (c) hydrothermal treatment, (d) *Averrhoa bilimbi* extract, and (e) dried obtained composite sheet.

2.2 Impregnation of Ag into BC

The impregnation of Ag ions into BC involved simple steps. First, pristine BC was immersed in an $AgNO_3$ solution with a concentration of 10 mM. The mixture was stirred for 1 h, then rested for a night. Afterward, BC was picked and rinsed multiple times with distilled water to eliminate the remaining $AgNO_3$ solution. BC sample was ready for the reduction process, as seen in Figure 2b. BC color became thicker and grayish at this point compared to pristine BC.

2.3 Green reduction methods

2.3.1 Immersion in *Averrhoa bilimbi* extract

In this reduction method, the BC sample previously immersed in $AgNO_3$ solution was immersed in *Averrhoa bilimbi* extract for 1 h. As depicted in Figure 2d, a grayish hue change was observed. The BC sample was then rinsed and dried at 65°C in an oven. This method's dry sheet sample was designated as BC/AgNPs-A.

2.3.2 Hydrothermal treatment

The reduction method by a hydrothermal treatment was conducted by isolating the BC sample in a glass bottle and then putting it in a water bath at a static condition at 75°C for 1 h. Similar to the previous reduction method, the sample was dried in the oven until a dried sheet was obtained. The dry sheet sample of this method was denoted as BC/AgNPs-H.

2.4 Characterization of BC/AgNP composite

The Fourier transform infrared (FTIR) spectra were collected with the Nicolet Avatar 360 IR instrument, measured at a 4,000–500 cm^{-1} wavelength. The X-ray diffraction (XRD) patterns were obtained with a Bruker D2 Phaser XRD instrument. The diffractograms 2θ were recorded between 10° and 80° and λ = 1.54060 nm. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) Phenom Desktop ProXL were utilized to analyze the composite samples' morphological characteristics and elemental composition. The samples

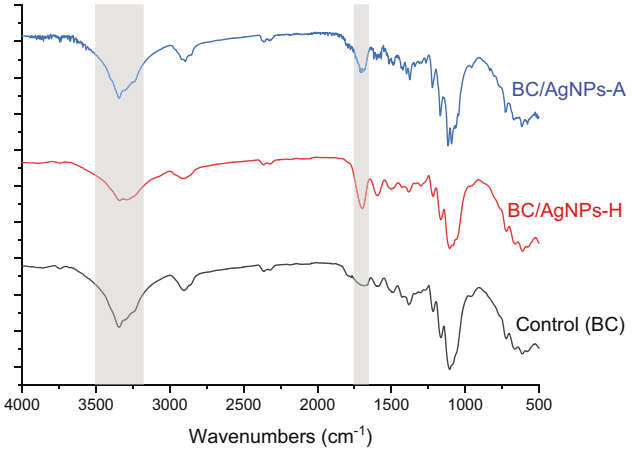

Figure 3: The FTIR spectra of BC and composite samples.

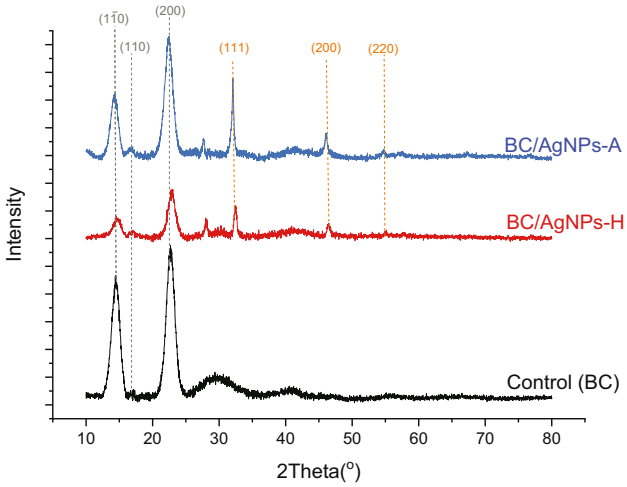

Figure 4: The XRD spectrum of BC and composite samples.

were made conductive by coating them with a thin layer of gold using a sputtering technique before SEM and EDX analysis. A Linseis PT1000 instrument measured the thermal stability of the samples with an analytical temperature from 0°C to 600°C and a heating rate value of 10°C·min^{-1}. A nitrogen gas flow rate of 50 mL·min^{-1} was used to avoid burning of the sample.

2.5 Antimicrobial activity of BC/AgNP composite

Staphylococcus aureus (gram-positive) and *Escherichia coli* (gram-negative) were precultured in agar nutrients placed in a 10 cm diameter petri dish at 37°C for 24 h to reach a

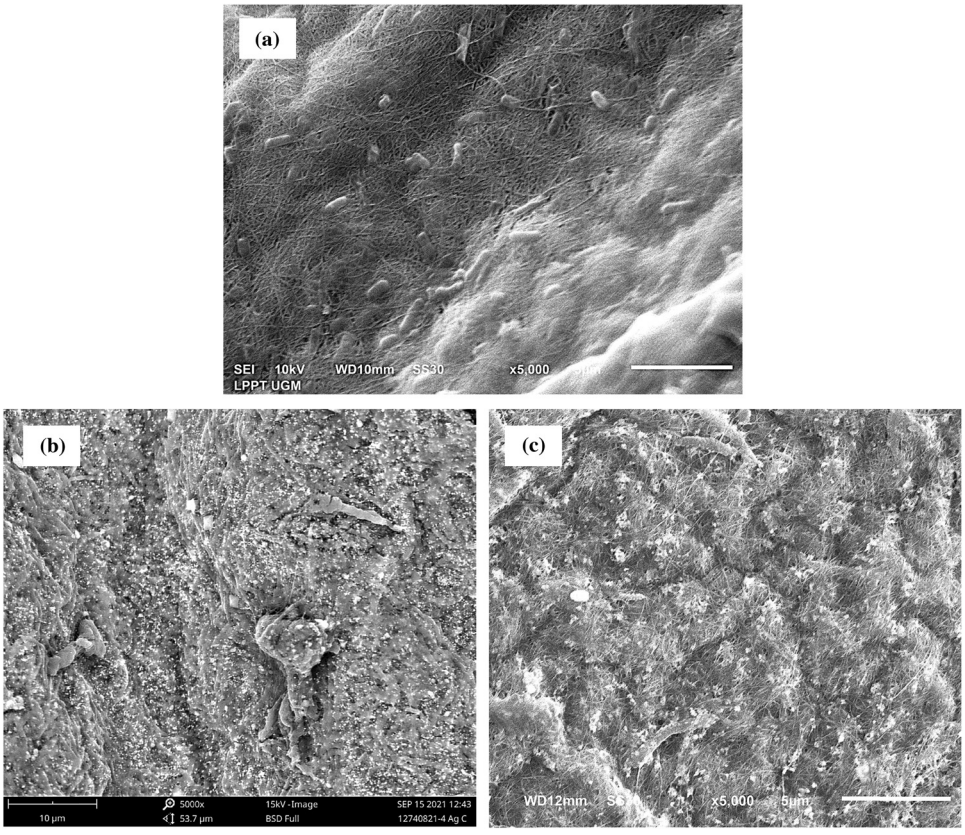

Figure 5: The SEM image of (a) BC, (b) BC/AgNPs-A, and (c) BC/AgNPs-H with 5,000× image magnification.

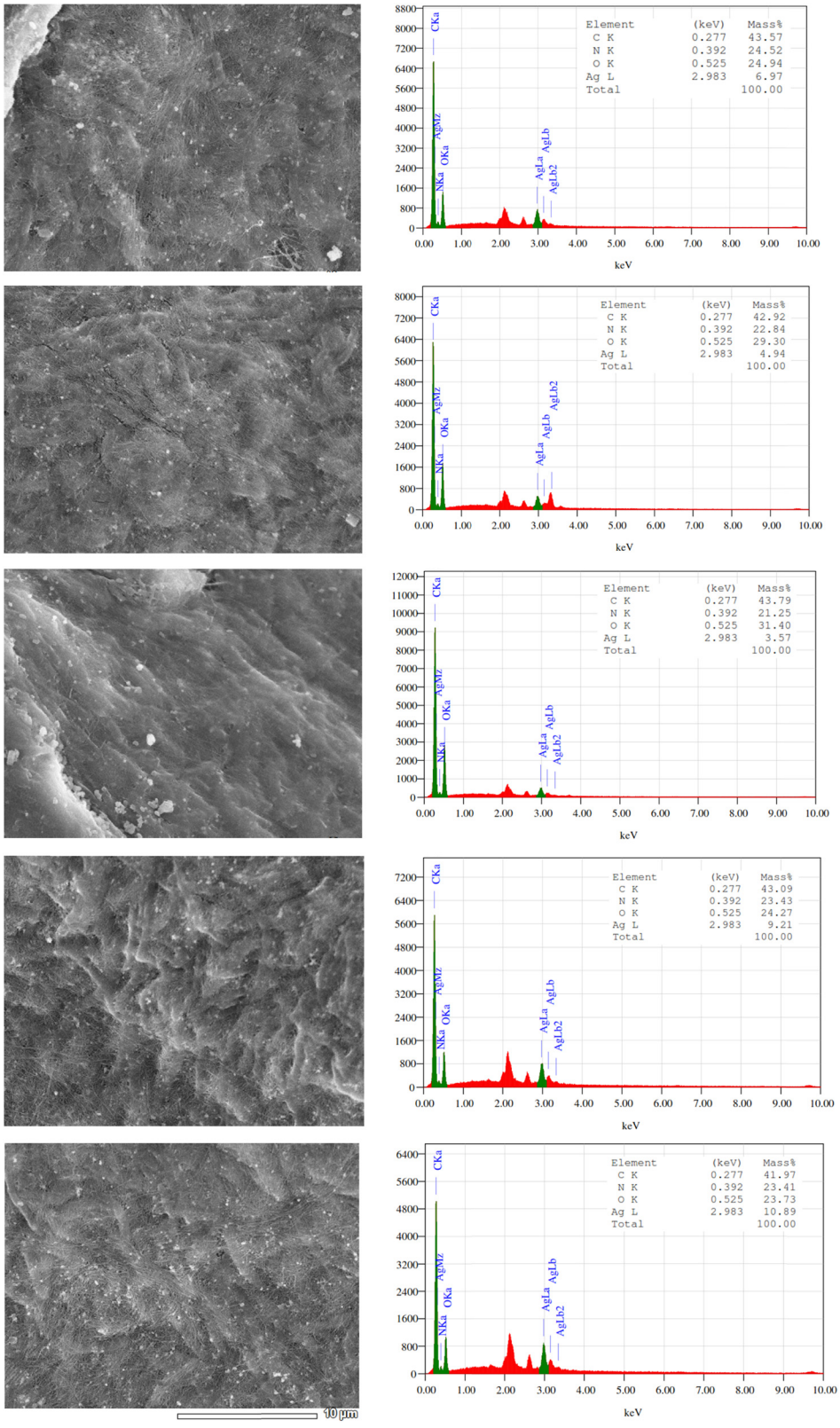

Figure 6: Various selected areas for EDX analysis of BC/AgNPs-A sample.

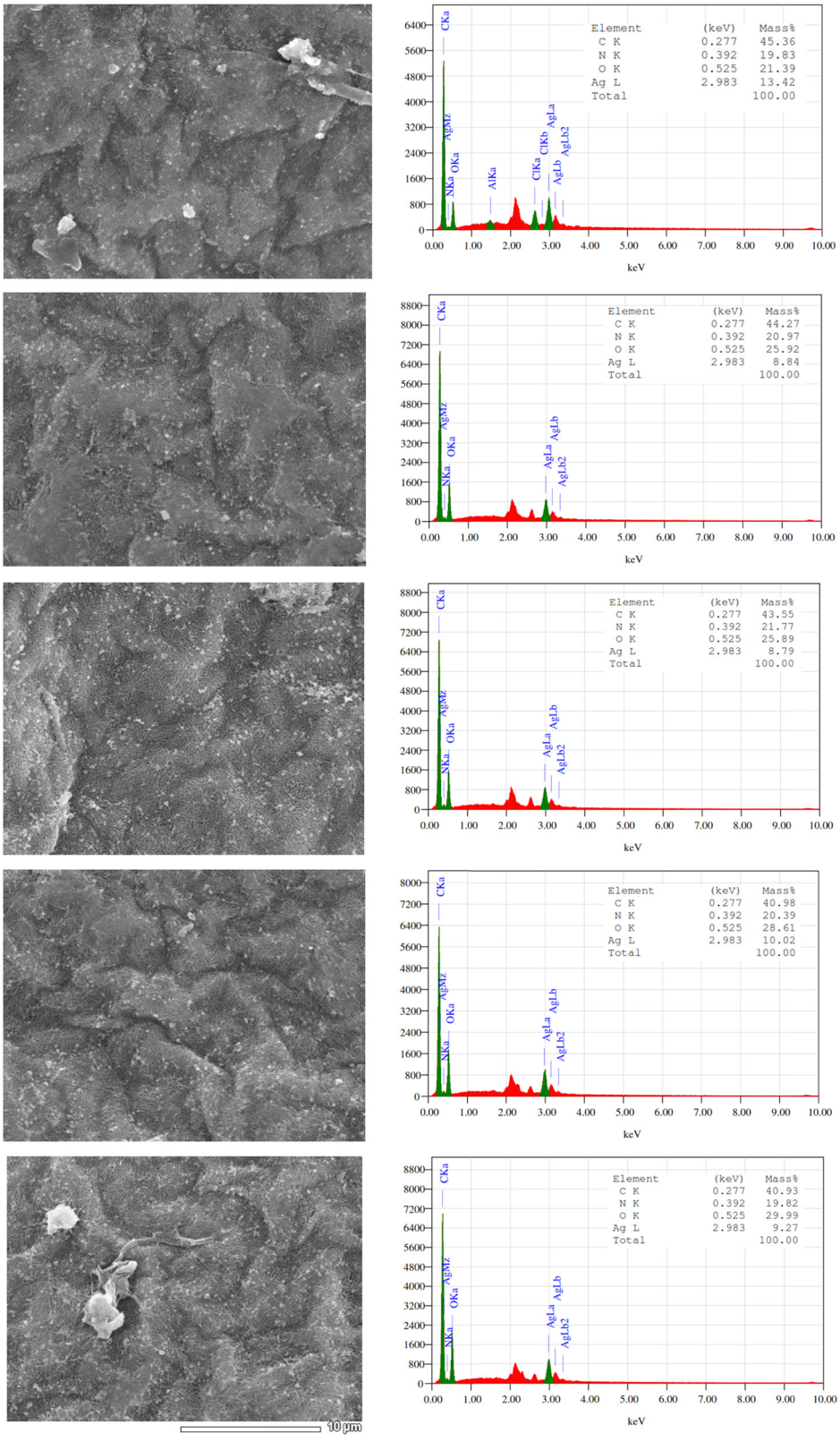

Figure 7: Various selected areas for EDX analysis of BC/AgNPs-H sample.

Table 1: Details of elements contained in composite samples

Area	BC/AgNPs-A elements (%mass)				BC/AgNPs-H elements (%mass)			
	C	N	O	Ag	C	N	O	Ag
1	43.57	24.52	24.94	6.97	45.36	19.83	21.39	13.42
2	42.92	22.84	29.3	4.94	44.27	20.97	25.92	8.84
3	43.79	21.25	31.4	3.57	43.55	21.77	25.89	8.79
4	43.09	23.43	24.27	9.21	40.98	20.39	28.61	10.02
5	41.97	23.41	23.73	10.89	40.93	19.82	29.99	9.27
Mean	43.07	23.09	26.73	7.12	43.02	20.56	26.36	10.07
Deviation	±0.71	±1.19	±3.42	±3.00	±1.99	±0.83	±3.29	±1.94

concentration of approximately 10^7 colony forming unit·mL^{-1}. Around 1 cm × 1 cm composite samples were placed on the surface of the medium, and the formation of the inhibition zone was measured for each sample.t

3 Results and discussion

3.1 Chemical and morphological characterization

FTIR spectra data were used to determine which functional group shifts are essential in forming AgNPs in the BC structure. Figure 3 compares the FTIR spectra of pure BC to composite samples. FTIR spectra of both pure BC and composites displayed a typical cellulose spectrum. A strong broad spectrum at 3,550–3,200 cm^{-1} indicated the O–H stretching of alcohol. This hydroxyl group was crucial in converting Ag^+ to Ag^0 and stabilizing AgNPs formed in the BC matrix [31,32]. The reduction process of Ag^+ ions in the BC structure was proved by the appearance of a peak at a wavelength of around 1,700 cm^{-1}, which indicates the presence of C═O from the carbonyl group. In contrast, the spectrum's peak at the mentioned wavelength is not significant enough in the BC sample. The notable change in the FTIR spectrum reflects the main factor in synthesizing metal nanoparticles: the oxidation of hydroxyl groups to form carbonyl groups [31,33].

XRD analysis was performed to validate and characterize the presence of AgNPs in the BC matrix. The X-ray spectra of BC and composite samples are shown in Figure 4. All three samples' diffraction spectra show peaks at 14.5°, 16.7°, and 22.7°, which respectively correspond to the planes (1$\bar{1}$0), (110), and (200) of crystalline Cellulose I [30]. Additional peaks of 2θ from 30° to 60° corresponded to AgNPs in BC/AgNPs-A and BC/AgNPs-H spectra, whereas no such diffraction was observed in BC. The diffraction peaks at 32.5°, 46.5°, and 54.7° are assigned to the reflection of crystal planes (111), (200), and (220) of AgNPs [34–36]. These peaks clarify the presence of the face-centered cubic structure of AgNPs. As the intensity of AgNP peaks was relatively small, SEM and EDX analysis was performed to ensure the existence of AgNPs in the composite samples.

As displayed in Figure 5, the morphological surface of BC and composite samples was studied using SEM images. Figure 5a shows a clear 3D network of BC microfibril. The BC sample is composed of microfibril networks arranged in such a way as to form a dense matrix network. The remaining oval-shaped *Acetobacter xylinum* bacteria in the BC matrix is also spotted. The presence of white spots in Figure 5b,c confirms the attachment of AgNPs to the BC matrix of composite samples. The shape of the AgNPs can be spherical or cubical in one BC/AgNP composite sample [20].

Figures 6 and 7 display the area selected from the composite sample for chemical element analysis, whereas Table 1 presents the mass percentage of chemical elements determined by the EDX quantitative analysis spectrum.

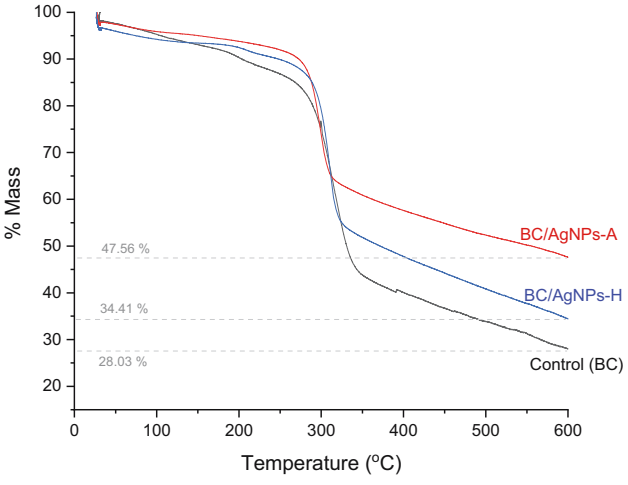

Figure 8: The TGA graph corresponding to the BC and composite samples.

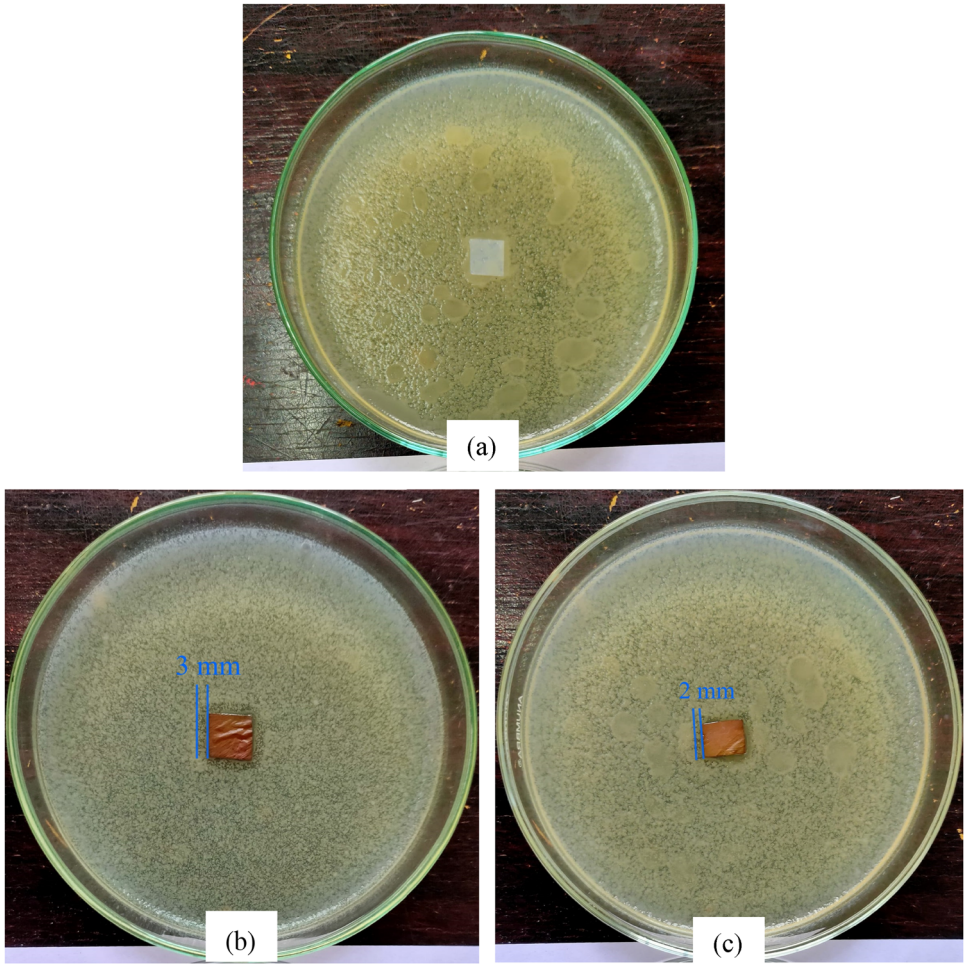

Figure 9: Antimicrobial activity of (a) BC, (b) BC/AgNPs-A, (c) BC/AgNPs-H against *E. coli*.

The results show that carbon and oxygen are the elements with the first and second-highest mass percentages, corresponding to cellulose's primary constituents [17]. On the other hand, the presence of Ag elements was detected quite significantly in all areas analyzed. The peaks around 2.98 keV correspond to the binding energy of Ag_L, proving that the synthesized composites were composed of AgNPs. Table 1 shows that the AgNPs could be synthesized in the BC matrix by the reduction method with the support of *Averrhoa bilimbi* extract and hydrothermal treatment.

$AgNO_3$ dissolved in water will form the ion $(Ag(H_2O)_2)^+$, and the diffusion of hydrated Ag ions $(Ag(H_2O)_2)^+$ into the BC matrix leads to coordination with the hydroxyl groups of cellulose [37]. Ionic interactions occur between these Ag ions and the hydroxyl groups of cellulose. AgNPs form when Ag ions are reduced from Ag^+ to Ag^0, and the hydroxyl groups of cellulose are oxidized to form carbonyl groups. In forming metal nanoparticles, the surface of BC is abundant in hydroxyl groups capable of lowering the energy demand for metal reduction. Additional energy or an external reducing agent might be needed to ease the reaction. The hydrothermal treatment was conducted at 75°C in order to facilitate the reduction reaction. BC, which had been soaked in $AgNO_3$ solution and incubated at room temperature for 2 days, did not undergo a color change. By increasing the temperature, a slow reaction was observed as a color change [38]. Using plant extracts as a reducing agent is the second environmentally friendly method for synthesizing metal nanoparticles. It has been demonstrated that *Averrhoa bilimbi* contains high levels of flavonoids and terpenoids [25,39]. Plant phytochemicals with more than two hydroxyl groups, such as saponin, flavonoid, terpenoid, and tannin, play a role in the bioreduction and stabilization of AgNPs [26,27].

The highest Ag content was found in the BC/AgNPs-H sample with a value of 13.42% mass and an overall average of 10.07 ± 1.94%. Compared to previously published studies [20,36,40], the composites of this study are quite promising

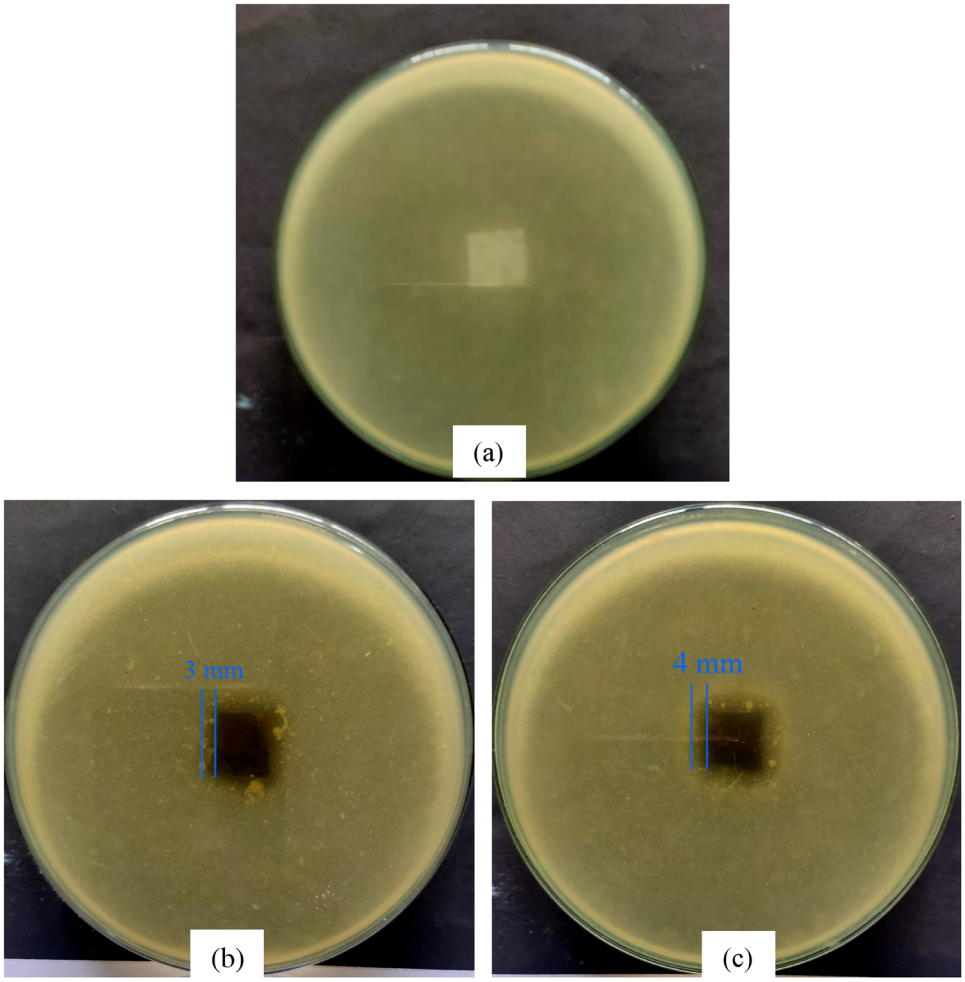

Figure 10: Antimicrobial activity of (a) BC, (b) BC/AgNPs-A, (c) BC/AgNPs-H against *S. aureus*.

as sustainable nanoparticle composite biomaterials. The cellulose matrix is a suitable host material for metal nanoparticle interactions. Cellulose's abundant hydroxyl groups serve as active sites for electropositive metal cations [36].

Thermogravimetric analysis (TGA) was carried out to investigate the thermal stability of the samples, as shown in Figure 8. There were three stages of weight reduction during temperature increase for BC and composite samples. The first stage was a minor weight reduction at a temperature below 260°C, probably due to the evaporation of water and other liquid contained in the samples. A significant weight loss was observed in heating temperatures 260°C to 330°C, indicating the degradation process of the cellulose components due to thermal oxidation [41]. The exothermic effect due to the intense heating was observed in weight reduction at the temperature above 330°C and it finally produced char. Significant weight loss difference was observed for each sample. At the end of the TGA process, the remaining weight was 28.03% for BC, 47.56% for BC/AgNPs-A, and 34.41% for BC/AgNPs-H. For the BC sample, the remaining weight corresponded to produced char, consistent with previous studies' findings [12,30]. On the other hand, for BC/AgNPs-A and BC/AgNPs-H, there was a way more residual mass on heating up to 600°C, this probably indicating the presence of AgNPs in the matrix of the two samples, which were not degraded during the heating process. Overall, both BC and BC composite materials of this study had good temperature resistance, as evidenced by their high stability at heating over 250°C, which has met the requirements of industrial applications for biopolymer materials [12].

3.2 Assay of antimicrobial activity

Due to their ability to generate reactive oxygen species (ROS), nanoparticles have significant antimicrobial potential. The formation of ROS inhibits the antioxidant defense system and damages the cell membrane mechanically [42].

As shown in Figures 9 and 10, the antimicrobial activity of composite samples was tested against pathogenic microbes *E. coli* and *S. aureus*. During the incubation period, the microbes around the BC membrane grew normally. On the other hand, a distinct inhibition zone formed around the composite sample. The BC/AgNP-H composite was proven to be able to form a larger inhibition zone against *S. aureus* compared to *E. coli,* whereas the BC/AgNP-A sample exhibited the same antimicrobial strength against both microbes. There are several factors that may influence the biological activity of AgNPs, such as concentration [30,43], size and shape [44,45], surface charge, silver ion release rate, and stability [46]. These factors should be the primary concern while synthesizing AgNPs and their clinical use.

4 Conclusions

In conclusion, BC/AgNP composites were successfully prepared using green methods: treatment with the bioreduction agent *Averrhoa bilimbi* extract and increasing reduction temperature through hydrothermal treatment. The FTIR spectrum reflects that the oxidation of hydroxyl groups to form carbonyl groups is essential in forming AgNPs. The XRD spectrum confirms the presence of the face-centered cubic structure of AgNPs. The SEM images exhibit the attachment of AgNPs to the BC matrix, while EDX quantitative analysis detailed the elements contained in composite samples. The highest Ag content was found in the BC/AgNP-H sample with a value of 13.42% mass. The TGA graph showed that both BC and composite materials of this study had good temperature resistance and met the requirements of industrial applications. Finally, the BC/AgNP composites demonstrated significant antimicrobial activity by forming a clear inhibition zone against *S. aureus* and *E. coli.*

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Conflict of interest: The authors declare that they have no conflict of interest.

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