# Characterization of Bacterial Cellulose Composite–Gardenia Leaf Extract (*Gardenia Jassminoides* J. Ellis) with Addition of Crosslinker

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**Abstract.** Bacterial cellulose (BC) can be applied in various fields such as biomedical, separation membranes, artificial blood vessels, and substrates for cartilage tissue engineering. BC still has low mechanical properties, so a bacterial cellulose composite was formed with Gardenia Leaf Extract (BC-GLE) to obtain higher mechanical properties. The purpose of this study was to determine the effect of adding a crosslinker, namely 1, 2 and 3% tapioca flour soaked with CBC-GLE to form (CBC-GLEC) by knowing the mechanical, physical, and structural properties (CBC-GLEC). BC is produced from a mixture of coconut water, sugar, and urea. BC was fermented with *Acetobacter xylinum* for 14 days. Composed with gardenia leaf extract and called bacterial cellulose-gardenia leaf extract composite (CBC-GLE). BC, CBC-GLE, and CBC-GLEC were characterized by testing water content, tensile strength, compressive strength, structural analysis using FTIR, and degree of crystallinity using XRD. The addition of Crosslinker can reduce the percentage of the water content of CBC-GLEC by 3% with a value of 90.73%, CBC-GLE 95.69%, and BC 99.21%. The best tensile strength test results were the addition of cross-linked starch with a concentration of 3% (CBC-GLEC) with a value of 121.45 MPa, CBC-GLE 49.81 MPa, and BC 32.09 MPa. The best compressive strength test results were the addition of starch crosslinker with a concentration of 3% (CBC-GLEC) with a value of 4.38 mm, CBC-GLE 3.42 mm, and BC 2.65 mm. The results of the FTIR spectrum showed that the functional groups contained in cellulose only experienced a shift, while the results of the analysis of the degree of crystallinity showed the percentage of the degree of crystallinity of BC was 75.47%, CBC-GLE was 94.42%, CBC-GLEC starch 3% was 67.26%.

**Keywords:** Gardenia Leaf Extract · Crosslinker · Cellulose


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## 1 Introduction

Cellulose is a biopolymer that is commonly found in nature. This cellulose is biodegradable, hydrophilic, and can be applied in chemical modification [1]. In nature, cellulose is widely obtained in animals, plants, and bacteria [2]. Plants are the largest source of cellulose production, but plant cellulose has less pure properties compared to cellulose derived from bacteria due to the high amount of lignin and hemicellulose possessed [3]. In addition to plant cellulose, bacterial cellulose is also found in nature and produced by microbes [4].

Bacterial Cellulose (BC) is cellulose produced by bacteria in the form of a homopolymer consisting of -D-1,4 glucose units bonded to each other through the first and carbon atoms fourth. The bond that occurs is a -glycosidic bond [5]. The properties possessed by bacterial cellulose are crystallinity, good purity, high porosity, mechanical properties, ease to decompose, and do not cause allergies [6]. The properties possessed by bacterial cellulose can be applied in several medical fields, such as wound dressings and cartilage tissue modeling [7].

BC use in biomedical science has weaknesses and constraints, namely the lack of elasticity properties. BC which was initially pressed using a finger, the water in the gel came out but the gel could not return to its original shape. This is because the compressive modulus is low, despite the high overall tensile strength of the fiber layer [8]. Based on the weaknesses and shortcomings of bacterial cellulose, a study was carried out to increase high elasticity, namely by combining SB with other materials into a new material called composite [9]. Composite materials generally consist of two elements, namely matrix, and filler [10].

One of the natural composites used is gardenia leaves, The chemical constituents in plate glass are flavonoid compounds, saponins, iridoid glycosides, and essential oils. Composites can be made using natural materials as has been done by Dewi [11], one of which is the manufacture of composites from BC and Gardenia Leaf Extract (GLE) into BC-GLE to obtain new materials used in biomedical applications, one of which is as an alternative to cartilage. The bacterial Cellulose composite of Gardenia Leaf Extract (BC-GLE) produced by Dewi does not meet the standards of cartilage, where the compressive strength and tensile strength are still low. To increase the modulus of elasticity and achieve the standard of cartilage in biomedical applications, a composite material is added by adding a crosslinker.

Crosslinkers can form cross-links and in other molecules can attract certain functional groups. Can be covalent bonds or ionic bonds in cross-links. Crosslinker used a compound that contains the -OH group or -NH2 [12]. The crosslinker used is tapioca flour. This flour contains starch and protein which has an active group so that it can bind the material. Starch in tapioca flour is a glucose homopolymer with α-glycosidic bonds. Starch consists of two fractions that can be separated by hot water. The dissolved fraction is called amylose and the insoluble fraction is called amylopectin. Amylose has a straight structure while amylopectin has branches [13]. Amylose contributes to gel characteristics because the presence of amylose affects gel formation [14].

## 2 Materials and Methods

### 2.1 Equipment and Chemicals

The equipment used in the preparation and characterization of BC-GLEC are Glassware (1000 mL volumetric flask, 2000 mL beaker, 100 mL measuring cup), stir bar, funnel, spatula, watch glass, balance analytical, pH meter, shaker (LaMaS modification), Samsung brand UV lamp, blender (Phillips), Compressive Strength (Outside Micrometer 0–25 × 0.01 mm) Tricle Brand and Tensile Strength (Buchel BV Horizontal Tensile Tester model No. K465 with item 84- 58-00-0002 range 500N, 230V-50Hz), glass, Fourier Transform Infra-Red (PerkinElmer), XRD (UNP Physics Lab) XPERT PRO PANalytical PW30/40 the Year 2012 Nederland Netherlands production wavelength 0.154 Armstrong, anode Copper, voltage 40 kV, current 30 mA, scan step 0.02 degrees, diffraction angle 10 to 100 degrees, iron (Sanyo) and plastic containers, stainless steel pans, stoves, knives, scissors, filters, cloth, plastic, rags, newspapers, rubber bracelet, tissue and label paper.

### 2.2 Bacterial Cellulose (BC) Preparation

Put 600 ml of coconut water into a stainless steel pot and heat it, then add 60 g of C6H11O6 and 6 g of CO(NH2)2. This solution is heated to boiling then add CH3COOH (± 12 mL) to pH 4-4.3. in a hot state, transfer the solution into a plastic container measuring 24 cm × 17 cm × 4 cm as much as 600 mL [15], then covered with sterilized newsprint. The media was left to a temperature of ±28 °C (room temperature), adding 10% v/v starter *A. xylinum* aseptically. Fermented at room temperature until BC is formed with a thickness of ±1–2 cm. After the SB is formed, the BC can be harvested.

### 2.3 Purification and Washing of Bacterial Cellulose

BC washing was carried out for 24 h under running water. The BC was then purified with 2% NaOH for 24 h. After soaking and purification, the BC has then washed again with running water, and then the SB was stored until the BC was ready for use. BC in storage is done by soaking SB in water and replacing 1 × 24 h.

### 2.4 Making Gardenia Leaf Extract (GLE)

Weighing 100 g of gardenia leaves, then wash the gardenia leaves with running water. Put the gardenia leaves into the blender for ±5 min until the leaves are mixed and produce Gardenia Leaf Extract (GLE). The resulting GLE was filtered using a filter cloth. A filler is used for the preparation of BC-GLE which is produced from the filtrate carried out.

### 2.5 Immersion of BC into GLE

BC cut with sizes of 15 × 2 × 1 cm and 2 × 2 × 1 cm were immersed into GLE with variations in immersion time, namely 1, 2, 3 and 4 days at ±28 °C (room temperature). Shaking during immersion with the use of a shaker and use with UV light. BC-GLE collection was carried out aseptically and cleaned using a tissue. The BC-GLE can be used for further characterization.

### 2.6 BC-GLE Immersion with 1, 2, and 3% Starch Crosslinker (BC-GLEC)

BC-GLE was soaked with tapioca flour solution with several concentration variations, namely 1, 2 and 3% (100 mL distilled water). The immersion was carried out by shaking using a shaker and UV light for 3 days to obtain BC-GLEC starch 1, 2 and 3%. Then, the BC-GLEC surface was cleaned with tissue on each surface and the mechanical properties and characteristics were tested.

### 2.7 Characterization of BC, BC-GLE, and BC-GLEC Starch 1, 2 and 3%

#### 2.7.1 Characterization of Physical Properties (Water Content)

The resulting BC, BC-GLE, and BC-GLEC starch 1%, 2%, and 3% were weighed initially (Wb) using an analytical balance. After that, the sample was put into the oven at a temperature of 105 °C until the sample was dry and the dry weight (Wk) was re-weighed until it was constant. The formula for finding the water content is as follows:

$$Wc(\%) = \frac{Wb - Wk}{Wb} x100\%$$

Description:
Wb: Gross weight
Wk: Dry weight
Wc (%): Water Content

#### 2.7.2 Tensile Strength Test

BC, BC-GLE, and BC-GLEC starch 1, 2 and 3%, cut into 15x2x1 cm sizes. The sample is pressed by placing it on a glass and ironed until it is moist and thin. The sample was put into an oven at 105 °C to dry. After drying, the sample is placed between the sample clamps on the tool (Tensile Strength). Operate the tool until the sample breaks. The results of the magnitude of the tensile strength value (MPa) and the value of strain and modulus of elasticity (MPa) will be seen on the monitor.

#### 2.7.3 Compressive Strength Test

BC, BC-GLE, and BC-GLEC starch 1%, 2%, and 3%, were cut to size (2 × 2 × 1 cm) to test the compressive strength of the samples. The sample thickness was measured initially before being pressed using a micrometer screw (mm). Then on top of the sample, the sample is pressed using a 1 kg weight placed on a flat surface and given 1 min for each sample, after the load is applied, the thickness is measured again, the difference between the thickness of the initial and final samples is the result of testing the compressive strength of the sample.

#### 2.7.4 Functional Group Analysis with Fourier Transform Infrared (FT-IR)

BC, BC-GLE, and BC-GLEC starch 3%, with a size (2 × 2 × 1 cm) that has been removed from the oven and analyzed using FTIR (Fourier Transform Infra Red). Prior

to analysis, the holder was washed using CH2OH, then the test sample was placed on the cell holder. Select the option to save the data on the computer, enter a file name and save it in a folder. Scan the wave number from 4000–400 $cm^{-1}$. The spectrum will be visible on the monitor to see the functional group of the relationship between wave number and intensity.

#### 2.7.5 Crystallinity Analysis Using X-ray Diffraction (XRD)

BC, BC-GLE, and BC-GLEC starch 3%, using XRD to see the degree of crystallinity. Samples were cut to size (2 × 2 × 1 cm) in the form of dry samples, each of which had been removed from the oven and then cut to the size of the cell holder. The sample that has been cut is then placed on the holder and ready to be tested. Select the option to save the data on the computer, enter a file name and save it in a folder. To determine the degree of crystallinity of SB, KSB-EDKP, and KSB-EDKPC 3% on the monitor screen will display the obtained diffractogram. The calculation of the percent degree of crystallinity can be seen in the formula:

$$Percent.ofCrystalinity = \frac{height[cts] - low[cts]}{height[cts]} x100\%$$

## 3 Result and Discussion

### 3.1 Bacterial Cellulose (BC) Preparation

The results of the study obtained BC with a yellowish color and it takes ±15 days to get SB results with a thickness of ±1 cm. In the fermentation process, *A. xylinum* bacteria will produce cellulose fibers on the surface of the liquid. This SB growth process from the surface of the medium is followed by the growth of the tissue underneath the fermentation medium which will eventually produce a thicker layer of cellulose (Fig. 1).

#### 3.1.1 Purification and Washing of Bacterial Cellulose

Immersion of BC using NaOH aims to remove non-cellulose components so that the BC obtained is purer so that the cellulose structure becomes denser, the links between chains

a b

**Fig. 1.** Well-formed BC (a) and moldy BC (b)

**Fig. 2.** BC results after soaking with 2% NaOH

**Fig. 3.** Gardenia Leaf Extract (GLE)

in cellulose are getting stronger through hydrogen bonds between chains. Erosion on the bottom layer of SB is caused by the use of NaOH solution so that the BC is obtained soft and not stiff. After being immersed in a 2% NaOH solution, the remaining nutrients (non-cellulose components) and bacterial residues disappeared, this can be seen by the reduced thickness of the SB from the original (Fig. 2).

### 3.1.2 Making Gardenia Leaf Extract (GLE)

The preparation of gardenia leaf extract obtained a dark green and thick gel, the results of the gardenia leaf extract can be seen in the picture (Fig. 3).

### 3.1.3 Immersion of BC into GLE

The BC was then soaked with GLE using a shaker and UV light with a wavelength of 380–315 for 4 days. BC-GLE can be seen in Fig. 4.

### 3.1.4 BC-GLE Immersion with 1, 2, and 3 Percent Starch Crosslinker (BC-GLEC)

The crosslinker can be a link between the matrix and the filler. The absorption process occurs at first with the filler with the matrix, now it is bound by the addition of a crosslinker. in the picture, it can be seen that there is a color change in BC that has been given a filler, namely GLE, when it is added with a tapioca flour crosslinker it turns yellowish.

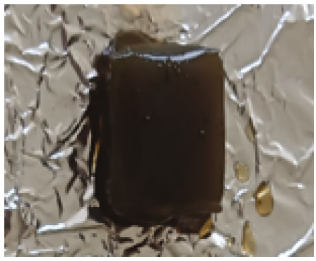

**Fig. 4.** BC-GLE

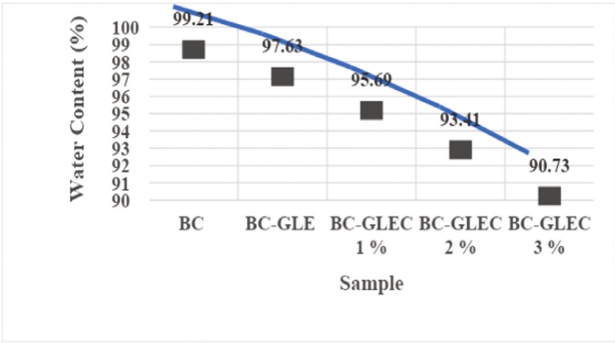


**Fig. 5.** Water Content

### 3.2 Characterization of BC, BC-GLE, and BC-GLEC Starch 1, 2 and 3%

#### 3.2.1 Characterization of Physical Properties (Water Content)

The percentage content of water obtained in the research results is 99.21%. This is in accordance with the research conducted by [7] and [16] that the percentage of water contained in SB is greater than 90%. so that SB has a higher percentage of water content than its total weight (Fig. 5).

The percentage of water content in BC-GLE is lower than in BC, which is 97.63%. Because the water in the BC is replaced by GLE which enters through the pores of the BC which is called the absorption process. The water content of BC-GLE which had been immersed in the starch crosslinker 1, 2 and 3% decreased each time the concentration was added. At a concentration of 1%, namely 95.69%, the concentration of 2% was 93.41% and the concentration of 3% was 90.73%. The addition of crosslinker concentration in BC-GLE can reduce the water content, so it is expected to strengthen the mechanical properties of BC-GLEC.

#### 3.2.2 Tensile Strength Test

The factor that affects the tensile strength value is the strain owned by each test sample. The strain value is the ratio of the change in sample length to the initial length. The greater the change in sample length, the greater the strain value and the smaller the tensile

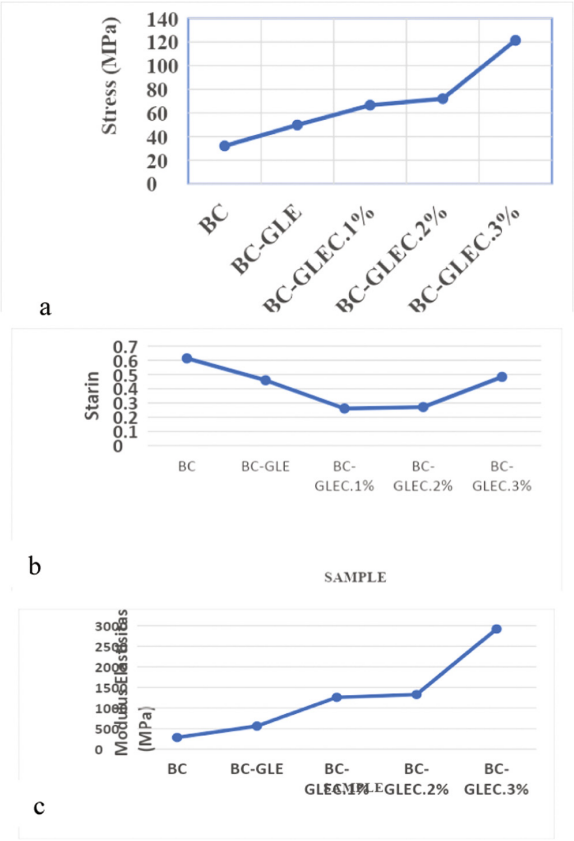


**Fig. 6.** Tensile strength (a), strain (b), modulus of elasticity (c)

strength of the sample. While the value of the tensile strength is directly proportional to the elasticity of a material (Fig. 6).

### 3.2.3 Compressive Strength Test

Tests that have been carried out on samples of BC, BC-GLE, and BC-GLEC starch 1, 2, and 3%. BC and BC-GLE results obtained from physical testing the results are not much different, where when pressed using a finger, the sample does not tear but only becomes flat when the water inside comes out. Of the three different crosslinker concentrations used, 3% starch BC-GLEC was the sample with higher strength than the other two crosslinkers. This can be seen and felt. When pressed using a finger, the higher the concentration of starch crosslinker given to the sample, the higher the compressive strength given. The results of the compressive strength can be seen in the picture (Fig. 7).

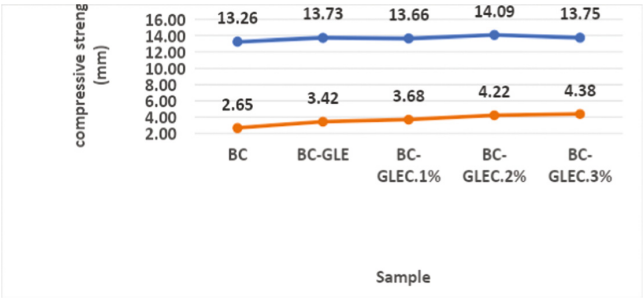


**Fig. 7.** Compressive strength

### 3.2.4 Functional Group Analysis with Fourier Transform Infrared (FT-IR)

Analysis by FTIR is to determine the functional groups of samples BC, BC-GLE, and BC-GLEC 1, 2, and 3%. The spectrum that will be used is with a wavelength of 4000–600 $cm^{-1}$. The results of the FTIR spectrum were then analyzed qualitatively to determine the functional groups of the sample. Figure 8 shows the FTIR spectrum of the sample.

According to [17] the wave numbers in the BC vibration are 3100–3800 $cm^{-1}$ (O-H), and 2901 $cm^{-1}$ (C-H). While C-O-C at wave numbers 1163 $cm^{-1}$ and 1068 $cm^{-1}$. As

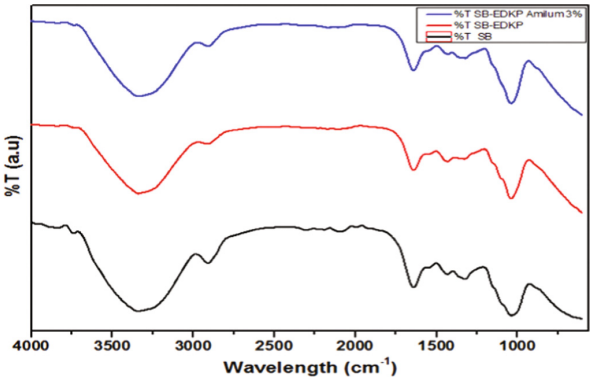


**Fig. 8.** FT-IR spectrum

**Table 1.** Each functional group on vibration wavenumber

| Sample | Peak ($cm^{-1}$) | | | |
|---|---|---|---|---|
| | O-H | C-H | C-O-C | C-O |
| BC | 3340,63 | 2910,89 | 1640,78 | 1035,67 |
| BC-GLE | 3338,57 | 2901,04 | 1639,99 | 1038,97 |
| BC-GLEC AM. 3% | 3338,93 | 2910,04 | 1640,81 | 1037,81 |

described in Table 1, the results of this study are almost the same as previous studies, namely [11] and [16] where BC, BC-GLE, and BC-GLEC showed OH absorption at vibration wave numbers 3550–3200 $cm^{-1}$, and CO absorption (-glycosidic bonds) was around 1500–1000 $cm^{-1}$.

### 3.2.5 Crystallinity Analysis Using X-Ray Diffraction (XRD)

Crystallinity test on BC, BC-GLE, and BC-GLEC starch 1, 2, and 3% XRD analysis was used to determine the degree of crystallinity. Figure 9 shows the comparison of patterns in SB, KSB-EDKP and KSB-EDKP added with 3% tapioca flour (Table 2).

The results of the diffractogram on the SB at the peak of 2θ are located at 47°. The peak of BC-GLE is located at 33° and the peak of BC-GLE in tapioca flour solution is located at 23°. It can be said that the cellulose produced is cellulose I. According to [15] that the peak typical of cellulose I is located at 2θ namely at 14°, 16°, 23°, and 34°. This proves that 2% NaOH can stop the activity of bacteria that can convert cellulose I into cellulose II. Cellulose I is a natural product and has a different degree of crystallinity from cellulose II. The degree of crystallinity of SB in the table is 75.47% and KSB-EDKP is 94.42%. This is in accordance with a study conducted by [11] that the percentage of crystalline KSB-EDKP was higher than that of SB. The crystalline percentage can also be seen for the amorphous percentage, namely for SB of 24.53% and KSB-EDKP of 5.58%. Meanwhile, 3% starch KSB-EDKP has a lower percentage than SB and KSB-EDKP which is 67.26% and the amorphous percentage is 32.74%. The factors that affect the % crystallinity and % amorphous obtained depend also on the selection and preparation carried out because the robustness of the sample will affect the physical and mechanical properties and characteristics of the test sample [18].

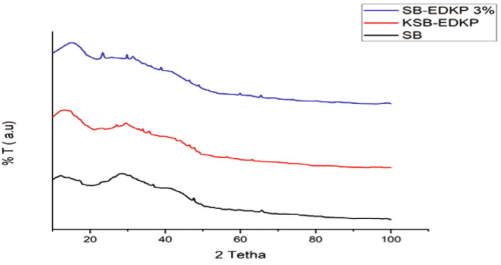


**Fig. 9.** XRD diffractogram

**Table 2.** Crystalline percentage BC, BC-GLE, and BC-GLEC starch 3%

| Sample | Highest intensity | Peak 2θ | Height [cts] | Low [cts] | Crystallinity (%) |
|---|---|---|---|---|---|
| BC | 100 | 47.55 | 18.47 | 4.53 | 75,47% |
| BC-GLE | 100 | 33.98 | 13.81 | 0.77 | 94,42% |
| BC-GLEC 3% | 100 | 23.32 | 31.01 | 10.15 | 67,26% |

## 4 Conclusion

Based on the research that has been done, the addition of a crosslinker soaked with BC-GLE can reduce the percentage of the water content of BC-GLE by 97.63% to 90.73%. The crosslinker that can increase the mechanical properties the highest in this study is 3% starch BC-GLEC. The addition of this crosslinker does not change the structure of KSB-EDKP, but only affects the location of the functional groups found in KSB-EDKPC 3%.

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