RJC

RASĀYAN *J. Chem.*
Vol. 14 | No. 1 |9-15| January - March | 2021
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

PREPARATION AND CHARACTERIZATION OF BACTERIAL CELLULOSE SUPPLEMENTED *Centella Asiatica L. Urban* EXTRACT TO IMPROVE MECHANICAL PROPERTIES

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ABSTRACT
Bacterial Cellulose is relatively pure, hydrophilic, has crystallinity index and tensile strength higher. Therefore Bacterial Cellulose has been widely used for biomaterial as woun healing, artificial blood vessel, drug delivery etc. Bacterial cellulose supplemented *Centella Asiatica L. Urban* had been produced from *acetobacter xylinum* using the saturating method. The aim is to enhance the characterization of Bacterial Cellulose by adding *Centella Asiatica L. Urban extract.* Bacterial cellulose (BC) and Bacterial cellulose supplemented *Centella Asiatica L. Urban* (BCCA) were characterized by FTIR test, SEM test, Mechanical Test, and XRD test. The result showed that adding *Centella Asiatica L. Urban extract* enhances the characterization of Bacterial Cellulose. Mechanical Test showed decrease mechanical properties of BC, and XRD showed Supplemented *Centella Asiatic L. Urban* extract did not disturb the regularity of BC chains and increased the crystallinity index.
Keywords: Thermal, Morphology, Bacterial Cellulose, *Centella Asiatica L.Urban,* SEM, FTIR, X-Ray Diffraction (XRD)

RASĀYAN *J. Chem.*, Vol. 14, No.1, 2021

INTRODUCTION

Cellulose is a natural fiber that is very beneficial. Research on cellulose has increasingly attracted the attention of researchers. There is much research has done to prove the benefits of plant cellulose i.e, cellulose bioplastic from bamboo fiber, cellulose biofilm from Gebang leaves, cellulose pulp from Durian peel etc.[1] In addition to plant cellulose, there is cellulose in protozoa, algae and bacteria. One of bacteria that can be produced cellulose is acetobacter xylinum. The cellulose is called bacterial cellulose (BC).[2] Bacterial cellulose (BC) has a different characteristic from plant cellulose, such as high better biodegradability, purity did not mix with lignin and hemicellulose, high molecular weight, high crystallinity, high water-holding ability, a high degree of polymerization and high mechanical strength.[3,4]

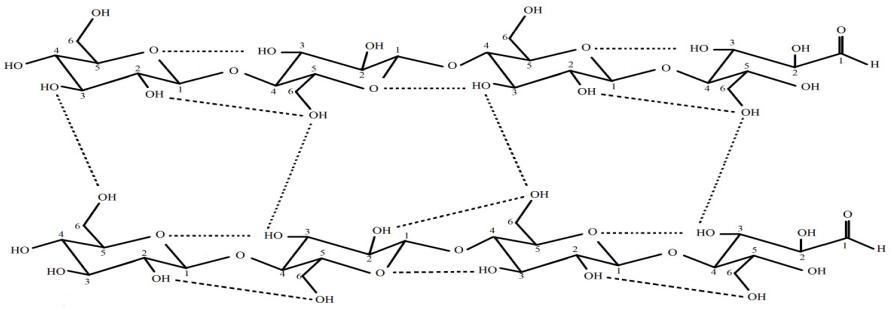

Fig.-1: Structure of Bacterial Cellulose[3]

http://dx.doi.org/10.31788/ RJC.2021.1415810

Based on the advantages of bacterial cellulose, that it has better mechanical properties[5], research on bacterial cellulose has become something of interest as a potential natural resource to be developed in various applications like biomedical[6], pharmaceutical, wound dressing, food ingredient, drug delivery etc.

Centella Asiatica L.Urban is a tropical plant. It was used as a traditional medicinal herbs. The major active compounds of *Centella Asiatica L. Urban* is triterpenoid[7] include asiaticoside, centelloside, madekossida, and asiatic acid.[8,9]

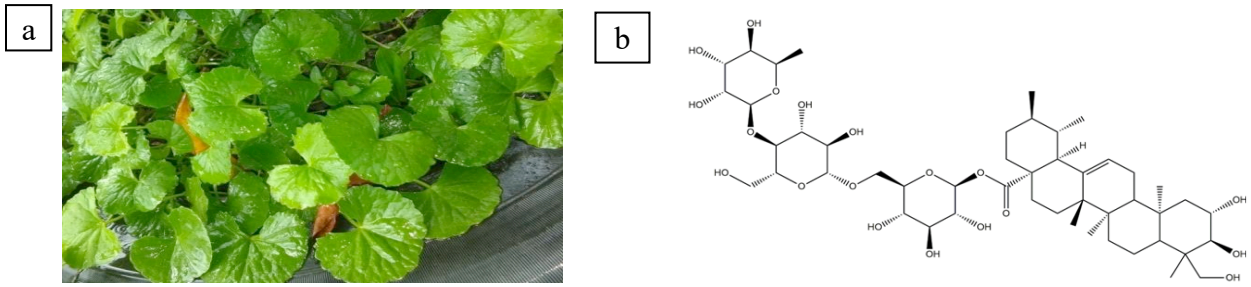

Fig.-2: (a) Centella Asiatica L.Urban Leaf, (b) Structure of Centella Asiatica L.Urban[5,7]

There are many researchers had announced the Bioactivities of *Centella Asiatica L. Urban* i.e. antioxidant[10], antibacterial[11], antifungal[12], anticancer, anti-depressant, for wound healing, treatment of asthma, memory improvement.[13-15] Indonesian mothers are like to eat *Centella Asiatica L. Urban* leaf as a salad after they giving birth.

This research aims to get modified Bacterial Cellulose supplemented plant extract which has good potential antibacterial. This is expected to increase the ability of bacterial cellulose in the health sector, as a medicinal agent, as an anti-bacterial agent as well as to increase existing sources of disease.

EXPERIMENTAL

Materials

In this study used are aquadest, fresh coconut water, stater, oxalic acid, NaOH 4%, *Centella asiatica L. Urban* extract, H2O2 0,25%. aluminium foil, Ammoniumsulfate, sugar, rotary evaporator, Oven, Autoclave, hand press, Thermometer, Analytical Scanning Electron Microscope, Spektrofotometer FTIR, X-ray Difraksi (XRD).

Preparation of *Centella Asiatica L. Urban* Extract

Centella Asiatica L. Urban leaf from Pangkalan Berandan-Sumatera Utara-Indonesia was collected, dried and made into a powder. Furthermore, aqueous extract 50 ppm had made from the powder of Centella Asiatica L. Urban.

Preparation of Bacterial Cellulose supplemented *Centella Asiatica L. Urban* Extract

Heated up about 1 Liter of fresh coconut water, after almost boiling added 5 gram of ammonium sulfate and sugar gradually (10, 20, 30, 40, 50 gram), and then cooled down, glacial acetic acid (p.a) is added to pH 4. Pour into media is sterilized. 10% ml starter of *Acetobacter xylinum* culture is added. Incubation for 7-10 days. Bacterial Cellulose is starts growing on day 4 called Nata. The good nata has a thickness 1-1.5 mm. Nata is washed and saturated by water about 12 hours, then saturated by NaOH solution to removed residual acid and H_2O_2 to bleaching it is called a sample of Bacterial Cellulose (BC), for the second sample nata saturated in *Centella Asiatica L. Urban* extract about 12 hours, called sample Bacterial Cellulose supplemented *Centella Asiatica L. Urban* extract (BCCA), Furthermore, the pellicle is removed the water content by hand press and then dried at oven 60^0C.

Characterization

The FTIR spectrophotometer 8201PC Shimadzu from Japan was used to record the spectra in the 500cm^{-1} – 4000 cm^{-1} at 4 cm^{-1} of resolution[16], The film was crushed and blended with KBr. Then, it was scanned

64 times, ZEISS MA 10 EVO was used to examine the surface and morphological of the sample (SEM test). The sputter coating with an ultrathin layer of gold, then the sample was puted on the holder[17]. XRD analysis using XRD diffractometers. The sample was scanned at 40 kV and 50 mA with a range of 5-60^0 2θ[18]. The crystallinity index (CrI) was defined,

$$CrI(\%) = \frac{I_{002} - I_{100}}{I_{002}} x100$$

Where I_{002} was the intensity peak for the crystalline cellulose and I_{100} was the intensity peak for the amorphous cellulose.

RESULTS AND DISCUSSION

Bacterial cellulose had been produced from *acetobacter xylinum* using the saturating method. It was been good nata with thickness 1-1.5 mm, obtained for 7 days.

Nata is washed and saturated by water for about 12 hours, then saturated by NaOH solution to removed residual acid and H_2O_2 to bleaching it is called a sample of Bacterial Cellulose (BC)

Nata saturated in *Centella Asiatica L. Urban* extract about 12 hours, called sample Bacterial Cellulose supplemented *Centella Asiatica L. Urban* extract (BCCA)

FTIR Analysis

There are much shift occurred peaks of Bacterial Cellulose after supplemented *Centella Asiatica L. Urban* extract. FTIR spectrum of the sample is shown in Fig.-3.

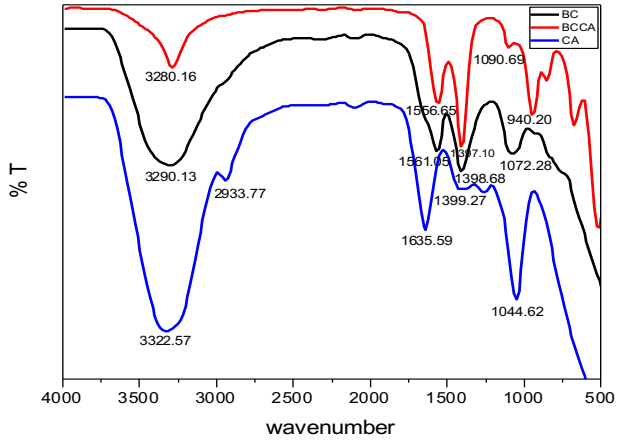

Fig.-3: FTIR Spectra of BC, BCCA and *Centella Asiatica L. Urban*

Centella Asiatica L. Urban extract has interacted successfully to Bacterial Cellulose exhibited the shift peak FTIR spectra of Bacterial Cellulose supplemented *Centella Asiatica L. Urban* extract from Bacterial Cellulose.

The peak at 3290.13 cm^{-1} of Bacterial Cellulose (BC) spectra showed OH stretching[19], shift to 3280.16 cm^{-1} of Bacterial cellulose supplemented *Centella Asiatica L. Urban* extract (BCCA). The peak at 1561.05 cm^{-1} of Bacterial Cellulose (BC) spectra showed C-H group in the bacterial cellulose[20,21], shift to 1556.65 cm^{-1}, peak at 1398.68 cm^{-1} of Bacterial Cellulose (BC) showed OH in-plane bonding, shift to 1397.10 cm^{-1}, peak at 1072.28 cm^{-1} of Bacterial Cellulose (BC) showed C-O stretching[22], shift to 1090.69 cm^{-1}. Refer to the FTIR spectrum, this shift indicated the successful introduction of *Centella Asiatica L. Urban* into BC.

Peaks of BCCA FTIR spectra are located in the range of bacterial cellulose groups and indicate the characteristics of the extract. It was showed the identification of the *Centella Asiatica L. Urban* extract in cellulose bacteria.

Morphology Characterization

The morphology of the sample was tested by SEM test at 1000x and 5000x magnification. It is shown in Fig.-4.

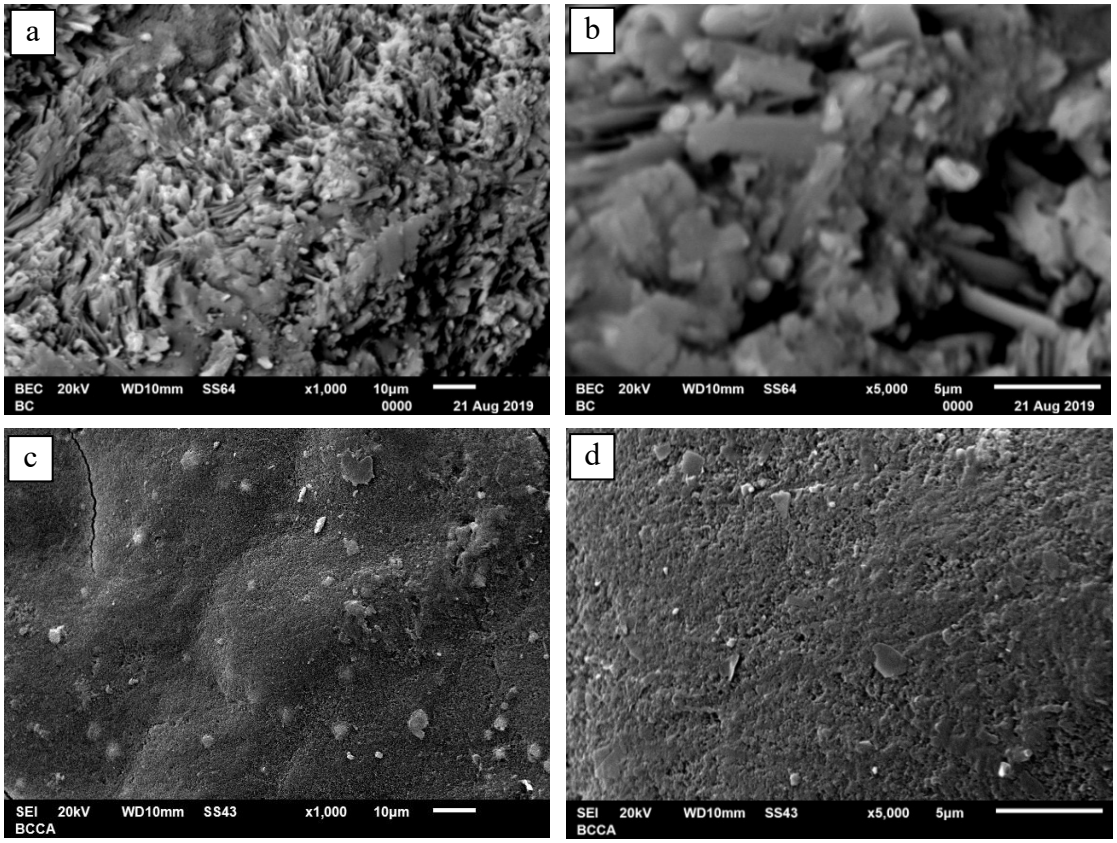

Fig.-4: The SEM Images of, (a) BC at 1000x Magnification, (b) BC at 5000x Magnification, (c) BCCA at 1000x Magnification, (d) BCCA at 5000x Magnification.

Table-1: Diameter Pores of BC and BCC

	BC (µm)	BCCA (µm)
Mean	1.371	0.209
SD	0.389	0.05

The SEM images of BC and BCCA exhibited within 1000x and 5000x magnification. The morphology of bacterial cellulose is shaped three-dimensional structure and porous[18,23]. The surfaces exhibited an interconnected fibril network with varying pore size[24]. Figure 4 shown that BC has pores with diameter pores 1.371 µm. These pores are very useful for controlling chemicals, especially the absorption of the desorption process of bioactive substances[25]. The Bacterial Cellulose supplemented Centella Asiatica L.Urban extract had smaller pores 0.209 µm. It is caused by the absorption process of the extract. It has shown that successfully study of interaction Centella Asiatica L.Urban into BC pore.

Mechanical Test

The result is shown in Fig.-5 and Table-1. Figure-5 is the graphics of elongation-load and strain-stress. The graphic showed decreasing elongation at break of BC while supplemented *Centella Asiatica L. Urban* extract from 25.84091 % decrease till 12.92175 % at BCCA. Interaction from extract believed causing termination of hydrogen bonding in the polymer, decrease mechanical properties of BC[24,25], cause the fibrils of BC to not interact properly with others.[26,27]

Table-2: Mechanical Test of BC and BCC

Sample	Load kgf		Maximum Point Stress MPa	Elongation at Break %	Young's Mpa
	Average Max	Breakpoint Average			
BC	6.71393	2.69648	11.26219	25.84091	43.2692
BCCA	5.51435	5.48554	5.44138	12.92175	42.9587

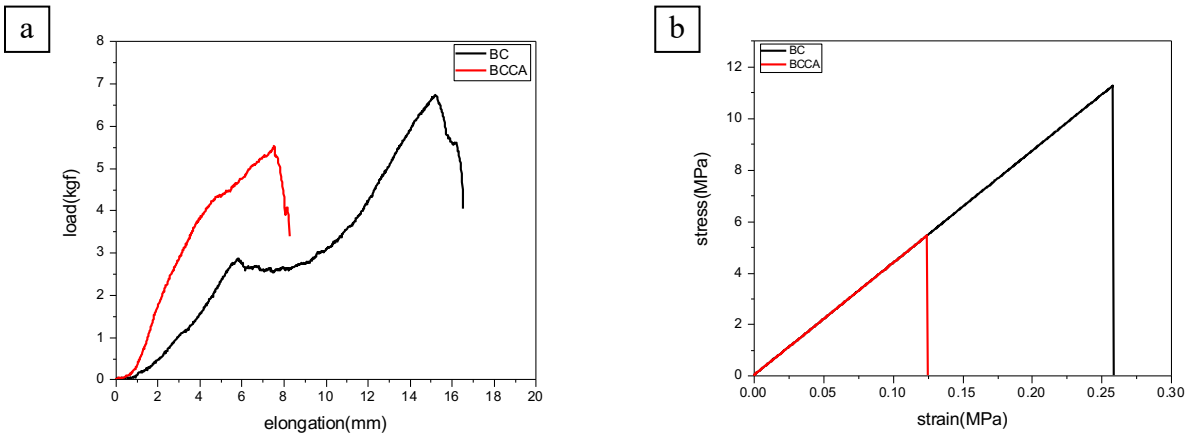

Fig.-5: Mechanical Graphic of BC and BCCA, (a) Elongation-load, (b) Strain-stress

XRD Analysis

The XR-diffractogram of Bacterial Cellulose and Bacterial Cellulose supplemented Centella Asiatica L. Urban extract shown in Fig.-5.

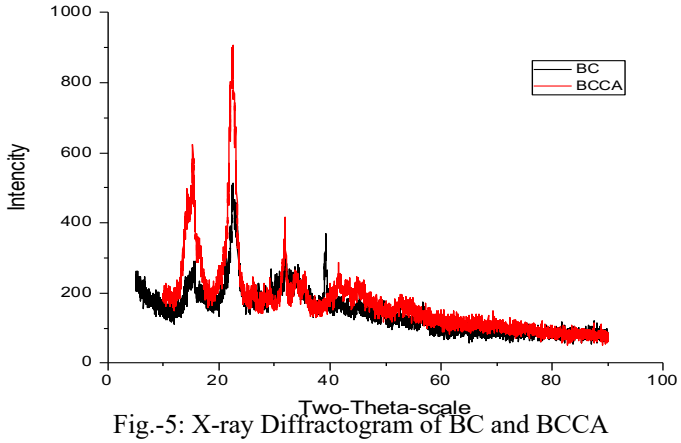

Fig.-5: X-ray Diffractogram of BC and BCCA

Diffractogram of BC showed peaks at 22.44^0, 23.35^0, 21.17^0, and a little lower peak at 15.4^0 which point to 002[23,2] and 110[16], the characteristic of Bacterial Cellulose.[18,21,22] The diffractogram of BCCA also showed the characteristic of Bacterial Cellulose 002 and 110, but there are a shift, which was peaks at 22.51^0, and slightly higher peaks at 15.32^0, 14.17^0, 16.41^0. The shift peak is caused by the lattice plane changes of Bacterial Cellulose.[16]

The crystallinity index (CI) of BC (59.65 %) and amorphous index of BC (40.35 %), while The crystallinity index (CI) of BCCA (71.5 %) and amorphous index of BCCA (28.5 %). All of the samples (BC and BCCA) had a crystallinity index higher than the amorphous index. It points to the samples had good crystal strength.[28,29] The crystallinity index of BCCA was higher than BC, demonstrating that supplemented Centella Asiatic L. Urban extract did not disturb the regularity of BC chains and increased the crystallinity.[21,22] *Centella Asiatica L. Urban* extract was interacted by physical to BC.

CONCLUSION

Centella Asiatica L. Urban extract has interacted successfully to Bacterial Cellulose exhibited the shifted peak FTIR spectra of Bacterial Cellulose supplemented *Centella Asiatica L. Urban* extract from Bacterial Cellulose. Peaks of BCCA FTIR spectra are located in the range of bacterial cellulose groups and indicate the characteristics of the extract. It was showed the identification of the *Centella Asiatica L. Urban* extract in cellulose bacteria.

The SEM images have shown that successfully study of interaction *Centella Asiatica L.Urban* into BC pore with had smaller pore on Bacterial Cellulose supplemented *Centella Asiatica L.Urban* extract. BC has pores with diameter pores 1.371 µm, and the BCCA has pores with diameter pores 0.209 µm. Cause, the *Centella Asiatica L. Urban* extract fills the pores of bacterial cellulose. Mechanical Test showed decrease mechanical properties of BC, the fibrils of BC to not interact properly with others cause interaction from extract believed causing termination of hydrogen bonding in the polymer. XR-Diffractogram showed Supplemented *Centella Asiatic L. Urban* extract did not disturb the regularity of BC chains and increased the crystallinity, it was interacted by physical to BC.

ACKNOWLEDGEMENT

Thanks to Chemistry Doctoral FMIPA Universitas Sumatera Utara and thanks to Promotor and Co-promotor that had been given help and support. Thanks also to Lembaga Pengelola Dana Pendidikan (LPDP) Kemenkeu Indonesia that had been given financial and supports through scholarship and research grant.

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[RJC-5810/2020]