Preparation of Silver-Incorporated *Rhynchospora corymbosa* (L.) Cellulose via *in-situ* Green Reduction and Its Antibacterial Study

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**Abstract.** Silver incorporated *R. corymbosa* (L.) cellulose was obtained through the direct attachment (insertion) of silver ions to natural cellulose, followed by the green reduction of ions to metallic silver, using aqueous extract of fresh *R. corymbosa* (L.). In addition, Fourier transform infra-red (FTIR) analysis of the isolated cellulose showed some peaks at 3330 cm⁻¹, 2890 cm⁻¹, 1320 cm⁻¹, 1030 cm⁻¹, 895 cm⁻¹ corresponding to OH stretching, methylene (−CH₂−) stretching, OH bonding, C-O bonding, and 1,4 β-glycoside, respectively. The silver incorporated *R. corymbosa* (L.) cellulose was prepared by impregnating silver ions obtained from silver nitrate solution, followed by ion reduction to the metallic silver *in-situ*. Furthermore, another interesting outcome of this study is seen in the products’ ability to show inhibition zones in the growth medium of *Escherichia coli*.

1. **Introduction**

There has been rapid development in the adoption of green technologies during the preparation of sustainable materials. This is possibly implemented through the physical methods, by direct deposition of silver nanoparticles on cellulose matrices, or chemical methods, which include the *in-situ* formation of silver nanoparticles within the mixture of cellulose-Ag salts, and also covalent binding [1]. Furthermore, some of the current development approaches are described using the following examples: the adoption of spun-cellulose fiber was used in the preparation of silver/cellulose composites, obtained by the dispersion of cellulose in silver nitrate solution, followed by heating at 80 °C to form silver nanoparticles, without the addition of chemical reductors. The reaction product had greater effectiveness as an antibacterial material against *Staphilococcus aureus* than *Eschericia coli* [2].

Paper–cellulose was used as a supporting material in the preparation of Au-Ag-cellulose nanocomposites, which was performed using tri-sodium citrate dihydrate as a reductor, in the presence of silver enhancer, and the product had better activity against *E.coli* (AATCC 100) [3]. The synthesis silver-cellulose nanocomposites involved the *in-situ* bioaccumulation of cellulose matrices in bio-flocculants, followed by a reaction using some variation of silver ions. The reduction process was successfully conducted using the bio-flocculants identified by the reaction color and supported by some characterization analysis, and the product possesses good antibacterial activity against *E. coli* [4].
The dispersion of antibacterial silver nanoparticle in deacetylated cellulose was obtained using NaBH₄ as a reductor, while KMnO₄ as an oxidator was used to reduce the particle size, and the resulting activity was markedly higher than the un-oxidized composites [5]. In addition, other studies have demonstrated the insertion of silver and silica nanoparticles in the matrices of cellulose pulp, in a 50% water solution of N-methylmorpholine-N-oxide, and the material produced possesses effective antibacterial activity, due to the synergetic work between both components [6]. Meanwhile, synthetic method of producing cellulose-supported silver nanoparticles was also developed through the use of paper cellulose as the main support, and adding a small amount of stabilizers, e.g., β-cellulose, carboxymethyl cellulose or amino cellulose, to decrease the incidence of aggregation. NaBH₄ was adopted as a synthetic reductor, and the material produced conferred effective antibacterial activity, especially against methicillin-resistant S. aureus (MRSA) [7].

It is important to develop new sustainable materials with higher biocompatibility in the production of antibacterial agents, using simple treatment. This report is, therefore, aimed at providing new information regarding the use of R. corymbosa as sustainable cellulose in the preparation of silver incorporated cellulose and its possible application as an antibacterial agent. Furthermore, green reduction of silver ions was performed to form metallic silver, using the aqueous extract of fresh R. corymbosa.

2. Material and Methods

2.1. Materials

Silver nitrate (AgNO₃) was purchased from Merck and used without further purification for the generation of metallic silver. Also, R. corymbosa(L.) was collected from Bengkulu coastal area, while Escherichia coli (ATCC 8739) was used as an antibacterial assay representative to investigate the prepared composites, and demineralized water was used in most of the experimental procedures.

2.2. Procedure

R. corymbosa (L.) cellulose was extracted using the modification of a known technique [8-10], where R. corymbosa (L.) powder (10 gram) was placed in a round bottom flask, followed by the addition of 120 mL NaOH (1 M). Therefore, the mixture was refluxed for 3 hours, followed by the immersion of solid materials in 100 mL of 5% H₂O₂, at 80 °C for 30 minutes. The product was then soaked in 100 mL sulfuric acid solution (1 N), stirred vigorously for 2 hours, subsequently decanted, and washed using demineralized water every 24 h for 4 days. Finally, the solid materials obtained were then dried at 60 °C for 6 h.

Aqueous extract of fresh R. corymbosa (L.) was obtained by heating the fresh plant powder (10 gram) in 100 mL demineralized water, and the filtrate obtained using Whatman paper (Number 3) was freshly adopted in the reduction of silver ions. On the other hand, R. corymbosa (L.) cellulose obtained from the previous step was immersed in AgNO₃ solution (0.01 M) for 24 hours, and the as-prepared aqueous extract of fresh R. corymbosa (L.) was added dropwise and stirred at room temperature. This reaction mixture was preserved for 24 hours, followed by the separation of solid materials from the reaction flask, and washing using demineralized water.

The bacterial target used was Escherichia coli ATCC 8739, of which one loop was cultured in trypticase soy broth (TSB) and incubated with a shaker for 24 hours at 27 °C. Furthermore, about 500 µL of E. coli suspension was inoculated into 100 mL of melted trypticase soy agar (TSA) medium and poured into sterile plates. After solidification, the separated sterile paper discs were sprinkled with R. corymbosa (L) powder, and silver-R. corymbosa (L) cellulose, while 20 µL of 15 mg silver-sulfadiazine cream, diluted in 1 mL of demineralized water was dropped into the sterile paper disk and adopted as a positive control. Conversely, demineralized water (20 µL) was applied as a negative control, and the plates were incubated for 24 hours at 300°C. These assays were conducted in duplicates, and the
materials’ antimicrobial activity was indicated by formation of a clear zone around the bacterial colonies.

3. Results and Discussions

The use of simple methods in the extraction of cellulose, following the modified procedure creates a white powder form as shown in Figure 1. This isolation was initiated by grinding the dried pieces of *R. corymbosa* (L.) to produce powder samples as shown in Figure 1(b), followed by treatment with NaOH, H$_2$O$_2$, and H$_2$SO$_4$ to give the product shown in Figure 1(c). Specifically, the addition of NaOH is to remove lignin and other compounds, followed by a bleaching treatment with hydrogen peroxide, and then the sample preparation was terminated by hydrolyzing the powder with sulfuric acid, producing a final product yield of 22.6%.

![Figure 1. Cellulose isolation processes of *R. corymbosa* (L.), (a) dried *R. corymbosa* (L.) plant, (b) powder of *R. corymbosa* (L.), (c) final product.](image1)

The fresh pieces of *R. corymbosa* (L.) were warmed up in hot water for 30 minutes, followed by filtration, and the clean extract was added to silver nitrate (AgNO$_3$) solution. Interestingly, the mixture demonstrates a specific peak at 449 nm, using UV-Vis spectrophotometry analysis, as shown in Figure 2, therefore providing preliminary evidence on the reduction and stabilizing capability of the metallic silver particles produced in the corresponding solution.

![Figure 2. UV–vis spectra of (a) fresh *R. corymbosa* (L.) extract solution, (b) AgNO$_3$- *R. corymbosa* (L.) extract reaction mixture after 2 h, (c) AgNO$_3$- *R. corymbosa* (L.) extract reaction mixture after 24 h, peak observed at 449 nm.](image2)

Based on these results in Figure 1 and 2, further experiments were performed to obtain *in-situ* incorporated silver-cellulose through a green approach. The final powder product shown in Figure 1(c) was treated with AgNO$_3$ solution for 24 h to provide enough time for the attachment of silver ions to the
cellulose surface as well as for entry into the matrix [11]. This was then treated with the fresh extract of *R. corymbosa* (L.), causing a color change from clear solution to brown after 24 hours, and a red-brown solid material was obtained as shown in Figure 3 after further treatment indicating the change from silver ions to metallic silver, as seen in Figure 2. Figure 3(b) shows the color of silver-incorporated cellulose powder, which was different from the initial cellulose shown in Figure 3(a).

![Figure 3](image)

**Figure 3.** (a) *R. corymbosa* (L.) cellulose powder, (b) *R. corymbosa* (L.) cellulose treated with AgNO₃ and fresh aqueous extract of *R. corymbosa* (L.)

The identification of functional group changes required an investigation on both powders obtained using the Fourier transform infra-red (FTIR) spectrophotometer, and the results are shown in Figure 4.

![Figure 4](image)

**Figure 4.** FTIR spectra of (a) *R. corymbosa* (L.) cellulose powder, (b) *R. corymbosa* (L.) cellulose treated with AgNO₃ and fresh extract of *R. corymbosa* (L.)

Based on Figure 4, the *R. corymbosa* (L) cellulose powder gives peaks at 3330 cm⁻¹, 2890 cm⁻¹, 1320 cm⁻¹, 1030 cm⁻¹, 895 cm⁻¹ and silver-fiber *R. corymbosa* (L) peaks at 3330 cm⁻¹, 2870 cm⁻¹, 1320 cm⁻¹, 1030 cm⁻¹, 888 cm⁻¹, which corresponds to OH stretching, methylene stretching, O-H bending, C-O bonding, and 1,4 beta-glicoside. This FTIR analysis demonstrates the appearance of a new peak at 599 cm⁻¹ in *R. corymbosa* (L) cellulose treated with silver nitrate in the presence of the fresh extract of *R. corymbosa* (L) extract. Despite the absence of exact evidence, the new peak was assumed to have resulted from the interaction between silver and the cellulose functional group.

The experimental product was applied as an antibacterial agent against the gram-negative bacteria *E. coli*, and the results are shown in Figure 5 and Table 1 (two repetitions). The results showed an average inhibition zone of 3.97 mm for silver incorporated- *R. corymbosa* (L) cellulose, which indicates a lesser activity than the control material (silver sulfadiazine), measuring 5.35 mm. This clearly indicates the
propensity to use the current material as an alternative antibacterial agent, as well as the potential for further development in aspects of wound healing and water filtration [12].

Figure 5. Antibacterial assay of filter paper (a) immersed in silver sulfadiazin cream as control positive, (b) sprinkled with silver incorporated *R. corymbosa* (L) cellulose powder, (c) sprinkled with fiber *R. corymbosa* (L) powder, (d) immersed in demineralized water

Table 1. Results of antibacterial activity toward *E. coli* growth represented by zone of inhibition of as-prepared materials and standard antibiotic by Diffusion Disc Methodology

| No | Material | Inhibition Zone | Average (mm) |
|----|----------|----------------|--------------|
|    |          | Repetition I (mm) | Repetition II (mm) | |
| 1  | Filter paper immersed in silver sulfadiazin cream as control positive | 5.50 | 5.20 | 5.35 |
| 2  | Filter paper sprinkled with silver incorporated *R. corymbosa* (L) cellulose powder | 4.03 | 3.90 | 3.97 |
| 3  | Filter paper sprinkled with fiber *R. corymbosa* (L) powder | 0 | 0 | 0 |
| 4  | Filter paper immersed in demineralized water | 0 | 0 | 0 |

These findings provide evidence stipulating the ability for *R. corymbosa* (L) plant grown in coastal area to valorize as an alternative cellulose source for further research. Interestingly, the metallic silver synthesized from AgNO₃ salt in this study required the use of aqueous extract sourced from the same plant, which possibly attaches to the cellulose matrices.

4. Conclusion

Silver-incorporated *R. corymbosa* (L.) cellulose was successfully produced using *R. corymbosa* (L) as a source of cellulose, while the fresh aqueous extract was adopted as a reducing agent in the conversion of AgNO₃ to Ag (0). In addition, FTIR analysis demonstrated the occurrence of a new peak on the cellulose-Ag composite at 599 cm⁻¹, and the final product displayed characteristics of a growth inhibitor against *E. coli* (ATCC 8739). Furthermore, product effectiveness is shown by the presence of an inhibition zone, which was almost equal to that of the control, silver-sulfadiazine (volume 20 µL; concentration 15 mg/mL).
5. Acknowledgement
The research fund was obtained from Direktorat Riset dan Pengabdian Masyarakat (DRPM), Direktorat Jenderal Penguatan Riset dan Pengembangan, Kementerian Riset, Teknologi, dan Pendidikan Tinggi, Republik Indonesia, under the scheme of Penelitian Tim Magister Fiscal Year 2019.

References
[1] Xu Y, Li S, Yue, X and Lu W 2018 BioResources 13(1) 2150 – 2170.
[2] Li R, He M, Li T and Zhang L 2015 Carbohydrate Polymers 115 269 –275
[3] Tsai T.-T, Huang T.-H, Chang C.-J, Ho N, Y.-J, Tseng, Y.-T and Chen C.-F 2017 Scientific Reports 7 3155 DOI:10.1038/s41598-017-03357-w
[4] Muthulakshmi L, Rajini N, Rajalu A.V, Siengchin S and Kathiresan T 2017 Intl. J. Biological Macromolecules 103, 1113 – 1120
[5] Kalwar K, Bhutto M A, Dali L dan Shan D 2017 Mater. Res. Express 4 105405 https://doi.org/10.1088/2053-1591/aa925b
[6] Smiechowicz E, Niekraszewicz B, Kulbinski P and Dzitko K, 2018 Cellulose 25 3499 – 3517.
[7] Alahmadi N S, Betts J W, Heinze T, Kelly S M, Koschella A and Wadhawan J D 2018 RSC Adv., 8 3646 – 3656.
[8] Hamisan A F, Abd-Aziz S, Kamaruddin K, Shah U K, Shahab N and Hassan M A 2009 J. Agricultural Res. 4, 250 – 256.
[9] Ahmad Z, Roziaizan N N, Rahman R, Mohamad A F and Wan Ismail W I N 2016 MATEC Web of Conferences 47(Mcc), hal. 05013. doi: 10.1051/matecconf/20164705013.
[10] Yuningsih L M, Mulyadi D and Aripandi I 2017, American J. Mat. Sci. 7(3) 59 – 63.
[11] Basta A H, El-Saied H, Hasanin M S and Deftar M M, 2018 Intl. J. Biological Macromolecules 107 Part A 1364 – 1372.
[12] Praveena S M, Han L S, Than L T L and Aris A Z 2016 J. Experimental Nanosci. 11(17) 1307 – 1319.