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THE ROLE OF NANOPARTICLE IN ENHANCING QUALITY CELLULOSE MICROFIBER AS AN ANTIMICROBIAL MATERIAL

E. Rohaeti[1]✉, R. Joronavalona[2], Isana SYL[1], D. Rohmawati[1], I. Yunita[1], S. Kristianingrum[1], and M. F. Rakotondramanga[2]

[1]Department of Chemistry Education, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, Yogyakarta 55281, Indonesia

[2]Department of Organic Chemistry, University of Antananarivo, Madagascar

✉Corresponding author: eli_rohaeti@uny.ac.id

ABSTRACT

This study investigates silver nanoparticles synthesised via bioreduction of water hyacinth leaf extract using ultrasound, and their incorporation into cellulose microfiber for antimicrobial purposes. Water hyacinth leaves served as the cellulose source and bioreductor for nanoparticle synthesis. Ultrasonication facilitated the process, with characterisation involving FTIR, UV-Vis, SEM, and XRD for cellulose microfibers, and UV-Vis and PSA for nanoparticles. Antimicrobial tests targeted *Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Candida albicans*. The UV–VIS analysis revealed an absorption peak at 241 nm, while particle size analysis (PSA) indicated an average size of 70.6 nm, confirming the formation of silver nanoparticles synthesised using water hyacinth leaf extract as a reducing agent. The cellulose microfibers prepared from water hyacinth stems and modified with silver nanoparticles exhibited a lighter, paler, and whiter appearance. UV–Vis characterisation of the cellulose microfibers showed an absorption peak at 281 nm. XRD analysis identified crystalline regions at 2θ = 22.08° with a crystallinity index of 37.385%. FTIR spectra confirmed the presence of –OH functional groups at approximately 3410 cm^{-1} and –C–H groups at around 2908 cm^{-1}. SEM observations revealed fibrillar structures forming fibres with diameters of approximately 1 µm. The 1:1 cellulose-nanoparticle sample showed the highest activity against *Pseudomonas aeruginosa* and *Candida albicans,* while the 1:2 sample was most effective against *Staphylococcus epidermidis.*

Keywords: Antimicrobial, Water Hyacinth, Cellulose Microfiber, Modification, Ultrasonication

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INTRODUCTION

Water hyacinth *(Eichhornia crassipes)* is a fast-growing aquatic plant that is often classified as an invasive weed due to its negative impact on aquatic ecosystems. The problem caused by the high water hyacinth population is a decrease in water quality because the oxygen levels and the intensity of the light entering the water body decrease, so that the water ecosystem becomes disrupted.[1-4] One of the efforts made to overcome these problems is to use water hyacinth. Water hyacinth plants contain the structure of lignocellulose. Lignocellulose is a component of the cell wall constituent of water hyacinth, consisting of hemicellulose, cellulose, and lignin. The water hyacinth contains 48% hemicellulose, 18% cellulose, and 3.5% lignin.[5] In recent years, microtechnology has become one of the fields of physics, chemistry, biology, and exciting and essential engineering. One of the developments of microtechnology is the manufacture of microfiber.[6-8] Microfiber is a fibre with a diameter of 1-10 µm. The advantages of microfiber are a porous and elastic structure and a high surface area.[9] The current application of microtechnology is in the development of antimicrobial material. Research to apply microtechnology in producing antimicrobial products, namely through metal particle engineering, one of which is silver nanoparticles. Silver nanoparticles in research results can be applied to various antimicrobial products, such as pulp, textiles, and so on.[10] Because silver nanoparticles attach to protein molecules in microbial cells, they can disrupt microbial metabolism and ultimately kill microorganisms. The nature of silver nanoparticles is also not toxic to human skin.[11] The trend of microfiber research uses a lot of natural fibres to make the material as a substitute for synthetic fibres. The advantages of cellulose microfiber

include the availability of abundant natural materials that can be renewed, in the environment, strength and high modulus, and a low thermal expansion coefficient.[9] Another advantage in terms of the economy is the low price of natural fibre; it can reduce the cost of researching raw materials. Methods that can be used for cellulose microfiber preparation include chemical techniques, ultrasonication, and enzymatic techniques. The conducted research aligns with SDG 3 (Good Health and Well-Being) and SDG 12 (Responsible Consumption and Production). The use of ultrasonic energy can gradually destroy cellulose fibres into microns. The fibre results obtained from the ultrasonication method are aggregated microfibers with a wide distribution.[4] This study aimed to modify cellulose microfibers derived from water hyacinth stems (*Eichhornia crassipes*) through the incorporation of silver nanoparticles and to assess their antimicrobial activity.

EXPERIMENTAL

Tools and Materials

The tools used are digital scales, sample bottles, test tube racks, beaker cups, dropper pipettes, measuring flask, magnetic stirrer with hot plate, thermometer, Erlenmeyer flask, funnel, ultrasonic cleaner, UV-Vis Spectrophotometer Shimadzu UV-3600 Plus, Instrument XRD Rigaku Miniplex 600, FTIR Shimadzu 8201PC Instrument, SEM Instrument, PSA Microtac Nanotrac Wave II Instrument, Aluminum Foil, Refrigerator, and Label. As the material used is the stem of the water hyacinth plant, a 2.5% sodium hydroxide solution, 1.7% hypochlorite sodium solution, solution, 5 M hydrochloric acid, $AgNO_3$ solids, distilled water, Whatmann filter paper No. 41, tissue, plastic wrap, blank disc, gram-negative *Pseudomonas aeruginosa* bacteria, gram-positive *Staphylococcus epidermidis* bacteria, and *Candida albicans* fungi. The variation of the sample is presented in Table-1.

Table-1: Variations of the Sample Cellulose Microfiber

Variation	Code
Addition of Silver Nanoparticles (AGNP) Variation 1: 1	MA
Addition of Silver Nanoparticles (AGNP) Variation 1: 2	MB
Addition of Silver Nanoparticles (AGNP) Variation 1: 3	MC
Without modification	MD

Methods

This experiment aimed to modify microfiber cellulose on water hyacinth stems with the addition of silver nanoparticles and to evaluate the antimicrobial properties of cellulose microfibers of water hyacinth stems *(Eichhornia crassipes)*. In addition, the XRD, FTIR, and SEM tests were conducted to identify the characteristics of the cellulose microfiber formed. Silver nanoparticles were characterised using UV–Vis spectroscopy and PSA to evaluate their formation. Water hyacinth stems (*Eichhornia crassipes*) were prepared by cleaning, drying, and cutting, followed by pulping using 2.5% NaOH (1:10 ratio) under pressure. The obtained pulp was washed, dried, and bleached with 1.7% NaOCl at 60°C to remove lignin. Acid hydrolysis was then carried out using 5 M HCl at 60°C, followed by washing and ultrasonication at 40°C to obtain cellulose microfibers. The microfibers were characterised using UV–Vis, XRD, FTIR, and SEM. Modification was performed by immersing the microfibers in $AgNO_3$ colloid containing water hyacinth leaf extract as a bioreductor. Antimicrobial activity was evaluated against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Candida albicans* by measuring inhibition zones over 48 hours.

RESULTS AND DISCUSSION

Preparation and Characterisation of Silver Nanoparticles

Under saturated conditions, the existence of Ag leads to the formation of silver nanoparticles (AgNPs), which is indicated by a change in the solution's colour.[9]

The results of silver nanoparticle synthesis use the bioreduction of water hyacinth leaves. This solution has a light-yellow characteristic (Fig.-1b). Based on the UV-Vis spectrum, it is known that the colloidal wavelength of $AgNO_3$ $1x10^{-3}$ M (Fig.-1a) produces an absorption peak of 241 nm (Fig.-1c). This is different from the theory that the uptake peak for silver nanoparticles ranges from 400 to 450 nm.[10,11] The results of the PSA analysis are shown in Fig.-2.

(a) (b) (c)

Fig.-1: (a) Silver Nitrate Solution; (b) Silver Nanoparticle Colloidal; (c) UV-Vis Spectrum of AgNPs

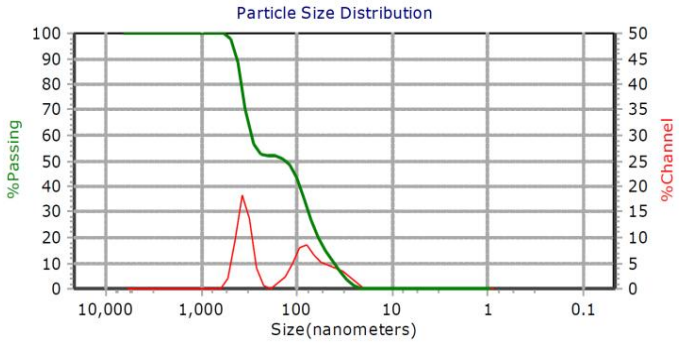


Fig.-2: Silver Nanoparticle Distribution (AgNP) Ultrasonic Method

PSA analysis in Fig.-3 shows that AgNPs synthesised by the ultrasonic method have a peak size of 70.6 nm, confirming their nanoscale range of 1–100 nm.

Preparation and Characteristics of Cellulose Microfiber

The optimal condition to obtain cellulose fibres from water hyacinth stems is chemical pulping with 2.5% NaOH, followed by bleaching using 1.7% NaClO at 60 °C for 2 hours to remove lignin, colour, and hemicellulose. Lignin binds strongly to cellulose and hemicellulose through a hydrogen and covalent bond to form lignocellulose.[8] NaClO in water will produce hydroxyl ions (OH-) and hypochlorite (HClO), potent oxidising agents that can break lignocellulose as well as the ether-type bonds that constitute lignin. The reactions that occur during the bleaching process are shown in the reaction equations (1) to (4):

$$NaClO_{(aq)} \rightarrow Na^{+}_{(aq)} + ClO^{-}_{(aq)} \quad (1)$$

$$ClO^{-}_{(aq)} + H_2O_{(l)} \rightarrow HClO_{(aq)} + OH^{-}_{(aq)} \quad (2)$$

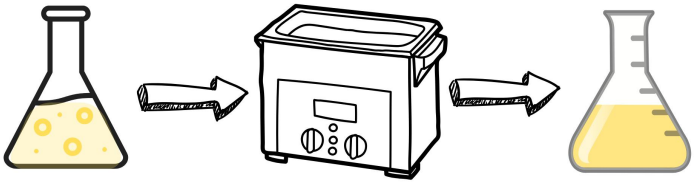
$$\text{Lignocellulose}_{(s)} + HClO_{(aq)} + OH^{-}_{(aq)} \longrightarrow \text{Lignin}_{(aq)} + \text{cellulose}_{(aq)} \quad (3)$$

$$\text{Lignin}_{(aq)} + HClO_{(aq)} + OH^{-}_{(aq)} \longrightarrow \text{Lignin degraded}_{(aq)} \quad (4)$$

Bleaching samples are then adjusted to a neutral pH (7) using distilled water. The cellulose fibre is hydrolysed by the reflux method using a 5 M HCl (hydrochloric acid) solution for 2 hours at 60° and a speed of 550 rpm.[9] The acid hydrolysis process aims to break down cellulose and hemicellulose compounds into glucose. The acid hydrolysis process results in hydrolysed fibre with a texture like porridge with a pale white colour, as shown in Fig.-3(a). After passing through the acid hydrolysis process, the sample enters the ultrasonic technique. The sample was put into an ultrasonic cleaner for 30 minutes at 45° C. Then the suspension was rinsed with aquades in the Buchner funnel, as illustrated in Fig.-3.

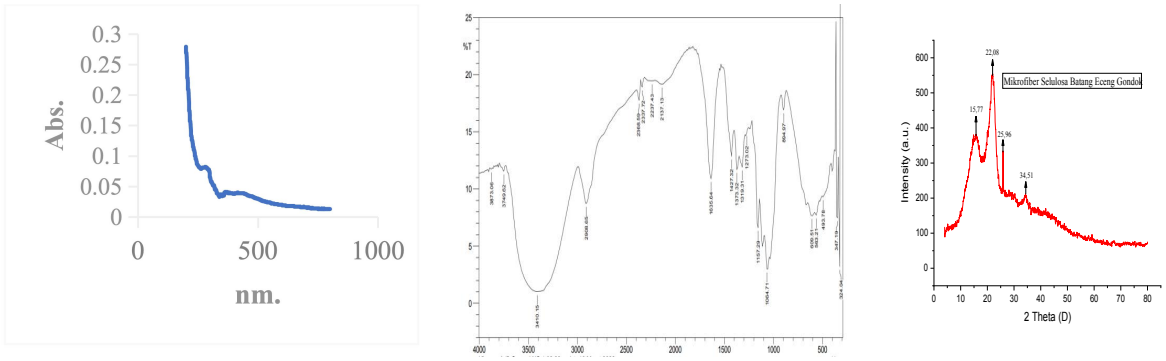
The synthesis results are obtained as microfiber cellulose stems of water hyacinth, which are white, have a soft texture and are fibrous. The UV-VIS, FTIR spectrum, and XRD diffractogram of cellulose

microfiber are shown in Fig.-4. The cellulose microfiber wavelengths produce 281 nm of absorption peak.[12]



(a) (b) (c)

Fig.-3: Hydrolysed Fibre, Ultrasonication, and the Sample Washing Process



(a) (b) (c)

Fig.-4: UV-VIS, FTIR Spectrum, and Diffractogram XRD of Cellulose Microfiber

The results of the FTIR testing obtained include the FTIR spectrum, and a table can be made to determine the absorption of cellulose microfiber functional groups, as shown in Table-2.

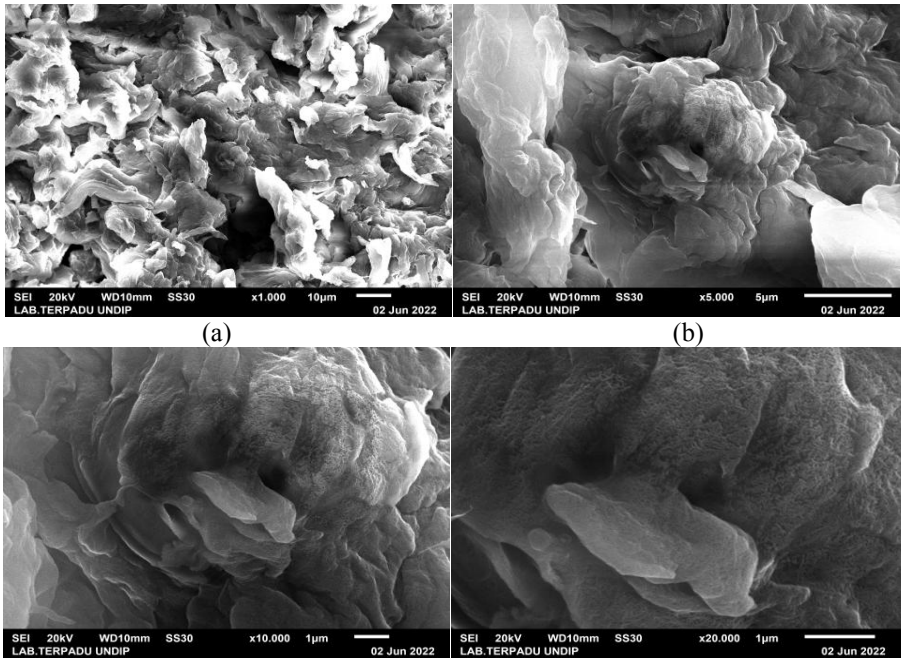
Table-2: The Functional Groups Present in Cellulose Microfibers, as Determined by FTIR Analysis

Peak	Vibration	Wave Number (cm^{-1})	Reference
A	-OH *stretching*	~3410	[10]
B	-C-H *stretching*	~2908	[10]
C	-OH dan C=O; C=C *vibration*	~1635	[11]
D	C-H dan C-O	~1373	[12]
E	C-O-C	~1064	[12]

Based on the analysis of FTIR, all the absorption of the functional groups identified in the FTIR spectra in Fig.-4 are consistent with the cellulose structure reported in several previous references, as summarised in Table-2.[12–17] The ultrasonication method conducted in this study proves that it can be used to synthesise cellulose microfibers from water hyacinth stems. The XRD test results showed 4 characteristic peaks in the 15-40°. The first, third, and fourth peaks, respectively, at 2θ = 15.77°, 25.96°, and 34.51° according to the amorphous area, while the second peak at 2θ = 22.08° indicates the presence of the crystalline area.[12] Based on data processing using the 2018 Origin, the area of the crystal fraction and the area of the cellulose microfiber diffractogram can be determined. The area of the crystal fraction is 2858,567, while the diffractogram area is 7646,302, so the crystallinity value can be determined at 37.385%. Based on JCPDS data (ICDD), cellulose $(C_6H_{10}O_5)_x$ has a monoclinic structure because A = 7.87; B = 10.31; C = 10.13, and $\alpha = \gamma = 90° \neq \beta = 122$, HKL (Cellulose Crystal Position) is at 22.08, which is in the JCPDS database. This illustrates quality cellulose microfibers produced to be applied as an antimicrobial material. The SEM test results obtained showed 4 images consisting of 1000x magnification in Fig.-5a, 5000x in Fig.-5b, 10000x in Fig.-5c, and 20000 in Fig.-5d.

Based on the SEM test results shown in Fig.-5, it appears that the morphological structure of cellulose microfiber fibres, shown in Fig.-5a and 5b, is in the form of fibril threads that form cellulose microfiber fibres in 1000x and 5000x magnification. After elaboration of 10000x and 20000x in Fig.-5c and 5d,

cellulose microfibers have smaller dimensions and are hollow.[12] This indicates that the pulping process using 2.5% NaOH solution successfully breaks bonds between lignin, hemicellulose, and cellulose. Cellulose microfiber fibres also look fibrillar because of the ultrasonication process, so the results are obtained by micrometre-sized fibres of 1 μm.



(a) (b)

(c) (d)

Fig.-5: The SEM Morphology of the Cellulose Microfibers

The Mechanism of Silver Nanoparticles in Cellulose Microfiber

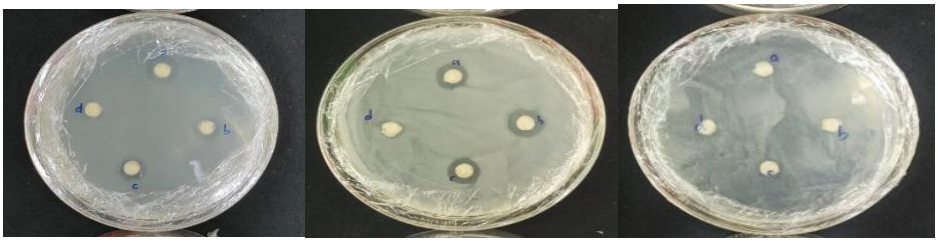
Cellulose microfiber modified by the ultrasonic method becomes paler, indicating the formation and attachment of silver nanoparticles (AgNPs) onto its surface through coordination, covalent and ionic interactions with hydroxyl groups.[18,19] In addition to the interaction between nanosilver and cellulose of water hyacinth stems, solvents in nanocolloids, which are water hyacinth leaf extracts, also allow interaction. The role of water hyacinth leaf extract *(Eichhornia crassipes)* is indirectly involved in the change in cellulose microfiber colour and mechanical properties.[20–23] The content of secondary metabolites found in water hyacinth leaves and commonly used as dyes is flavonoids. The observed colour change confirms the successful incorporation of silver nanoparticles into cellulose fibres, leading to the modification of cellulose microfibers.

Antimicrobial Test on Modified Cellulose Microfiber

Observation of the measurement of the inhibition zone in testing the antibacterial activity of *Pseudomonas aeruginosa* bacteria, as in Fig.-6a, *Staphylococcus epidermidis* bacteria, as in Fig.-6b, and *Candida albicans* fungi, as in Fig.-6c.

Microfiber cellulose BEG with a 1:1 silver nanoparticle variation shows the highest inhibition against *Pseudomonas aeruginosa*, reaching 5.93 mm at the 15th hour of incubation at 25 °C, with inhibition dynamics fluctuating between 18 and 30 hours. The death phase may have occurred because the food reserves have been used up, and so many bacteria have died. In addition, parameters influencing antibacterial activity include pH, temperature, the stability of the antibacterial agents, bacterial population density, incubation time, and bacterial metabolic activity.[24–26]

Cellulose microfiber modification (1:2) showed the most significant antibacterial inhibition (12.01 mm) with maximum effect at 15 hours and dynamic inhibition over time, while in antifungal testing against Candida albicans, cellulose microfiber with silver nanoparticle variation (1:1) gave the best inhibition (15.63 mm at 18 hours), influenced by factors such as pH, temperature, substance stability, bacterial load, incubation time, and metabolic activity.



(a) (b) (c)

Fig.-6: The Clear Zone of Cellulose Microfiber Against (a) *Pseudomonas aeruginosa* Bacteria; (b) *Staphylococcus epidermidis* Bacteria; (c) *Candida albicans* Fungi

Statistical analysis using ANOVA revealed a p-value of 0.000, indicating statistical significance ($p \leq 0.05$). A sig value ≤ 0.05 indicates that antibacterial activity is affected by incubation time, variations among samples, and the interaction between these two factors.[27–29] The activity of Candida albicans, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* is impacted by simultaneous differences in sample type and incubation duration. The MA sample's kind of variation yields the greatest results based on *Pseudomonas aeruginosa's* antibacterial activity. Conversely, the MB sample exhibits the best outcomes on *Staphylococcus epidermidis*. Producing better antibacterial material and clean production is possible using the microcellulose combination approach.[30,31] It is a new way to combat microbial resistance bacteria to prevent bacterial infections and is utilised in a variety of everyday products and medical devices. In addition to the many ways that silver and items containing silver can be used as antibacterial agents in a variety of industries, the main danger factor associated with silver is its real toxicity to humans. Additionally, researchers are working to comprehend how AgNPs may affect long-term health and how they may cause bacterial resistance, which will open up new research avenues.[32] Ag ions' positive charge is essential to their antibacterial properties. Silver needs to be ionised in order to possess any antibacterial qualities. Silver is inert while it is ionised, but it releases silver ions when it comes into contact with moisture.[33] Rather than interacting with the phosphate groups of nucleic acids, Ag+ ions can form complexes with nucleic acids and nucleosides. As a result, silver ions (Ag+) can originate from any form of silver or silver-containing compounds that have been shown to have antimicrobial qualities. These ions can be integrated into the material and released gradually over time, as in the case of silver sulfadiazine, or they can originate from ionising the surface of a solid piece of silver, as in the case of silver nanoparticles.[34, 35] Positively charged nanoparticles are thought to be the best bactericidal agents because of the electrostatic attraction they have with negatively charged bacterial cells.[36]

CONCLUSION

A peak of UV-VIS absorption is obtained at 241 nm, and measuring 70.6 nm for PSA test results, based on the properties of silver nanoparticles, with the reduction of the water hyacinth leaf extract that has been carried out. Characteristics of cellulose microfibers, the preparation results using water hyacinth stems modified by adding silver nanoparticles, give colour to the cellulose microfiber, making it pale and whiter. The peak of cellulose microfiber absorption based on the UV-Vis test shows 281 nm. XRD results indicate the presence of crystalline areas at $2\theta = 22.08°$, and crystallinity is 37.385%. The FTIR test results show the-OH group in the wave number ~ 3410 cm^{-1} and the-CH group in the wave number ~ 2908 cm^{-1}. The SEM test results show fibril threads that form a fibre and measure 1 μm. The antimicrobial activity of cellulose microfiber resulting from preparation using the water hyacinth stem produced without and by modification of silver nanoparticles has a significantly different effect based on the tests carried out. Cellulose samples-silver nanoparticles with 1:1 (m/v) variations show the highest antimicrobial activity in inhibiting *Pseudomonas aeruginosa* and the greatest level of antifungal efficacy against *Candida albicans.* The cellulose sample - nanoparticles 1:2 (m/v) offers increased antimicrobial activity, inhibiting *Staphylococcus epidermidis.*

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Eli Rohaeti https://orcid.org/0000-0002-0930-732X
Rasamimanana Joronavalona https://orcid.org/0009-0001-4535-3024
Isana SYL https://orcid.org/0000-0002-7227-2668
Dini Rohmawati https://orcid.org/0009-0001-9877-8026
Isti Yunita https://orcid.org/0000-0003-0199-509X
Susila Kristianingrum https://orcid.org/0000-0003-4787-1199
Maonja Finaritra Rakotondramanga https://orcid.org/0000-0003-1623-4760

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