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參展科別 **醫學與健康科學**

作品名稱 **Silver Moringa Cloth: Silver Nanoparticle Fabric Based on Moringa Extract (Moringa oleifera) as Antibacterial Against Methicilin Resistant Staphylococcus aureus**

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關鍵詞 **Green Synthesis、Antimicrobial Fabric、Moringa oleifera**

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Abstract

Staphylococcus aureus is addressed as one of the most common pathogens in hospital settings and in the community. This pathogen causes invasive infections, sepsis, and death. The emergence of antibiotic-resistant bacteria is due to bacterial mutations and the use of antibiotic drugs that are not by procedures. Resistance makes MRSA infections difficult to treat, resulting in high healthcare costs. These problems lead to an urgent need to find alternative drugs to control MRSA infection. Therefore, developing new drugs and procedures such as antibacterial nanoparticles, are particularly promising. Indonesia has many medicinal plants with antibiotic activity, including *Moringa oleifera*. *Moringa oleifera* contains several active compounds such as alkaloids, flavonoids, and saponins which are known to have antibiotic activity. Silver nanoparticles or AgNPs are currently used as antimicrobial agents because they are toxic to prokaryotic cells (bacteria) but relatively safe for eukaryotic cells. AgNP synthesis mediated by *M. oleifera* extract has the advantages of being non-toxic, pollution-free, and environmentally friendly. Sisal is a potential source of naturally derived fabric and a prospective source of multifunctional textiles. Recent studies have utilized and functionalized sisal to develop composite materials. However, functionalizing of sisal using nanosilver-based materials has not been studied yet. Bioactive chemicals from plant-extracted nanoparticles also provide additional antimicrobial properties. This study aims to produce AgNPs mediated by *M. oleifera* leaf extract and to analyze its antimicrobial effect on MRSA growth. The powdered Moringa (4g) was boiled with 100 ml of distilled water (550 C) for 15 minutes. The mixture was filtered through Whatman No 1 filter paper and store refrigerated. The nanoparticle was synthesized by rinsing sisal fabric cloth to several concentrations of AgNO3 (1mM, 10mM, and 20mM) with Moringa extract. Nanoparticle synthesis from AgNO3 done with the help of *Moringa oleifera* extract. The resulting AgNPs have MIC values (Minimum Inhibitory Concentration) and MBC (Minimum Bacteriocidal Concentration) of 1.25 mg/ml. The resulting silver nanoparticles showed antibiotic activity against MRSA with an average inhibition zone diameter of 15.677 mm. XRD and SEM studies are going to be held to support the data.

1. BACKGROUND

Antibiotic-resistant bacteria are still a major problem that threatens human health[1]. The emergence of antibiotic-resistant bacteria is due to bacterial mutations and the use of antibiotic drugs that are not by procedures[2]. One of the most attention-grabbing antibiotic-resistant bacteria is MRSA (*Methicillin-Resistant Staphylococcus aureus*). MRSA is one of the main causes of infections such as bacteremia, pneumonia, and endocarditis in the hospital environment (Taylor and Unakal, 2017). MRSA is resistant to all beta antibiotics lactams including penicillins (eg amoxicillin) and cephalosporins (Liang et al., 2022). MRSA resistance is obtained from its ability to produce β-lactamases that hydrolyze β-lactam antibiotics (Taylor and Unakal, 2017). Resistance also arises due to the presence of the PBP2a protein with a much lower affinity for beta-lactams(Peacock and Paterson, 2015). This mechanism allows cells to still carry out cell wall synthesis when beta-lactam antibiotics are administered so that cells remain alive. Resistance makes MRSA infections difficult to treat, resulting in high healthcare costs. These problems lead to an urgent need to find alternative drugs to control MRSA infection.

Indonesia is known as a producer of medicinal plants with antibiotic activity, one of which is the Moringa plant. *Moringa oleifera*) [6]. Moringa is traditionally used to treat dental caries, syphilis, and diarrhea(Dzotam et al., 2016). Moringa contains important secondary metabolites such as alkaloids, flavonoids, and saponins (Dzotam et al., 2016).
Vankwani et al., (2022) reported that moringa extract can inhibit bacterial resistance to antibiotics by inhibiting beta-lactamases and PBP2a enzymes.

On the other hand, silver nanoparticles (AgNP) have been used as antimicrobial agents because they are not toxic to eukaryotic cells including humans, but have high toxicity to prokaryotic cells such as bacteria. (Keshari et al., 2020). In addition, silver nanoparticles are cheaper than gold. AgNP synthesis based on plant extracts has many advantages compared to chemical and physical methods because it is non-toxic, pollution-free, environmentally friendly, economical, and more sustainable. (Ying et al., 2022). Bioactive phytochemical compounds from plant extracts also provide additional antibacterial properties to the nanoparticles(Keshari et al., 2020).

Several researchers have synthesized AgNPs based on plant extracts as antibiotics and antioxidants. Abd Karim et al.(2021) synthesized AgNP based on moringa leaf extract as an antioxidant.Amrulloh and Fatiqin (2020)used moringa extract to produce Magnesium (Mg) nanoparticles with antibiotic activity against the bacteria Escherichia *coli*. Merghni et al., (2022) stated that the production of AgNPs is based on plant extracts that can inhibit MRSA, but the extract used is orange peel. Information regarding the potential of Moringa-based AgNPs against MRSA bacteria is still not available, so research is needed to explore this potential. The antibiotic potential of Moringa extract-based AgNPs, hereinafter referred to as *Silver Moringa* tested on MRSA and is expected to be an effective and inexpensive alternative to treat MRSA bacteria.

One of the main potential spreads of MRSA is in hospitals due to the use of antibiotics that are not by the rules. Some populations tend to have a degree of colonization *S aureus* higher levels such as health workers, people who use needles regularly, and inpatients. *S aureus* can be passed from person to person by direct contact or through contaminated objects(Taylor and Unakal, 2017). One of the innovations that can be used is to use antibacterial fabrics that can prevent the spread of MRSA. In this study, researchers applied Moringa-based silver nanoparticles to fabrics that can be used in hospitals so that they are expected to be a solution to prevent MRSA.

2. METHOD

2.1. Moringa Extract

The plant samples were washed with water, rinsed with distilled water, and air-dried. Prior to extraction, Moringa leaves were blended to form a powder. A total of 4g sample was extracted with 100 mL of distilled water under 550C. Next, the mixture is filtered with Whatman No 1 filter paper. The sample is stored refrigerator for use in nanoparticle synthesis.

2.2. Synthesis of Silver Nanoparticles Based on Moringa Extract

Nanoparticle synthesis was carried out by mixing plant extracts on a sisal fabric cloth with $AgNO_3$. $AgNO_3$ solution concentrations used for synthesis were 1, 10, and 20 mM. The mixture was incubated for 24 hours. After incubation, the absorbance/*Optical Density* (OD) of all mixtures was measured in the range of 320–600 nm with a UV-Vis spectrophotometer (Choi et al., 2021).

2.3. MIC and MBC Test

MIC (Minimum Inhibitory Concentration) is the minimum concentration of an antibiotic substance needed to inhibit bacterial growth, while MBC (Minimum Bacteriocidal Concentration) is the minimum concentration of the antibiotic substance needed to kill bacteria. MIC determination was carried out based on the Microdilution Broth method on a 96-well microplate (Choi et al., 2021). Media Nutrient Broth (NB) 150 µl mixed with 10 µl MRSA bacterial suspension (0.5 McFarland units) and 100 µl AgNP in various concentrations. The mixture was incubated at $37^{O}C$ for 24 hours. The final concentration of AgNPs was serially diluted from 5 mg/ml to 0.045 mg/ml. After incubation, the culture conditions were observed. MIC is defined as the lowest concentration of AgNP solution that results in inhibition of bacterial growth, which is indicated by the color of the culture medium which remains clear. For the MBC test, for every 50 µl of the mixture 96-well microplate, those that did not show growth in the MIC test were subcultured back into the media Mannitol Salt Agar (MSA) at a temperature of $37^{O}C$ for 24 hours. The lowest concentration without growth in MSA was defined as MBC. Data on the diameter of the MRSA growth inhibition zone for each sample (3 replications) were analyzed using the ANOVA statistical test (Analysis of Variance) One-Way with 95% confidence level using the Minitab 17 application. From the ANOVA test, the F and p-value, where if the p-value is less than 0.05, it can be concluded that the diameter of the inhibition zone of the samples tested was significantly different from the control.

2.4. Application of Silver Nanoparticles on Fabric

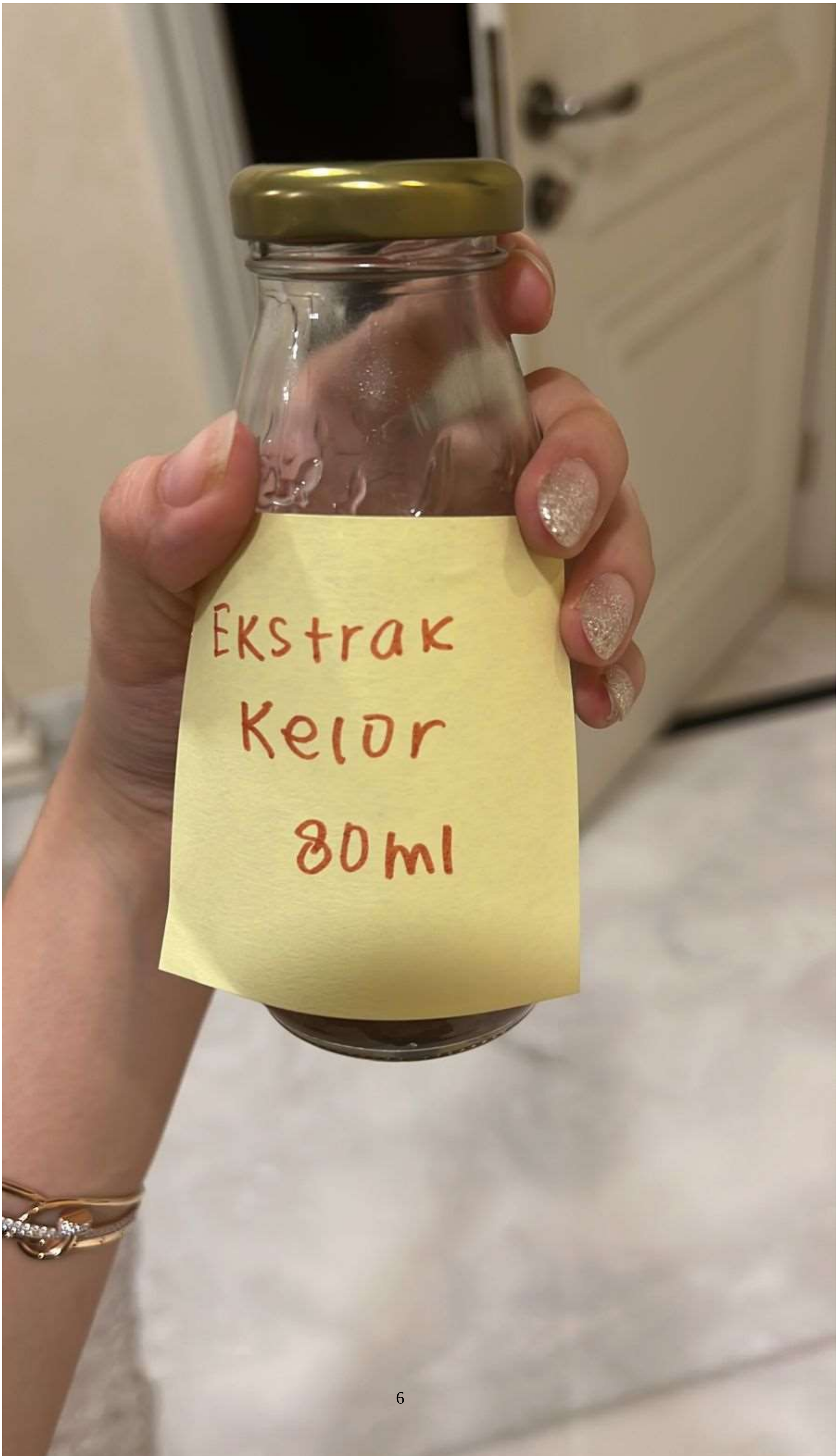
Sisal cloth is washed with a non-ionic detergent, then dried. After that, the cloth is soaked in a solution of silver nanoparticles with concentrations of 1, 10, and 20 mM $AgNO_3$ for 24 hours. Next is to air-dry sisal fabric cloth and the results of nanoparticle cloths were tested for their antibiotic activity.

2.5. Antibiotic Activity Test of Fabric Nanoparticles

MRSA is inoculated on Nutrient Broth at 37°C for 24 hours inside a rotary shaker at a speed of 150 rpm. The MRSA suspension was evenly inoculated into the filled Petri dishes Mueller Hinton Agar (MHA) by swab using cotton bud sterile. Linen cloth that has been formed into a circle with a diameter of 5 mm is soaked in 1 ml of 2.5 mg/ml AgNP solution for 5 minutes and then dried. After that, the linen cloth was placed on MHA which had been inoculated with MRSA. The inoculant was then incubated at $37^{O}C$ for 24 hours. The diameter of the inhibition zone formed around the nanoparticle cloth was measured for each sample along with the control sample. The negative controls in this study were sterile distilled water and the antibiotic cefoxitin which was resistant to MRSA bacteria (Taylor and Unakal, 2017). The positive control in this study was the antibiotic chloramphenicol which is known to be sensitive to MRSA (Taylor and Unakal, 2017). All experiments were carried out in three replicates.

Method Diagram


Lt
X
ml
¼
250
200
150
100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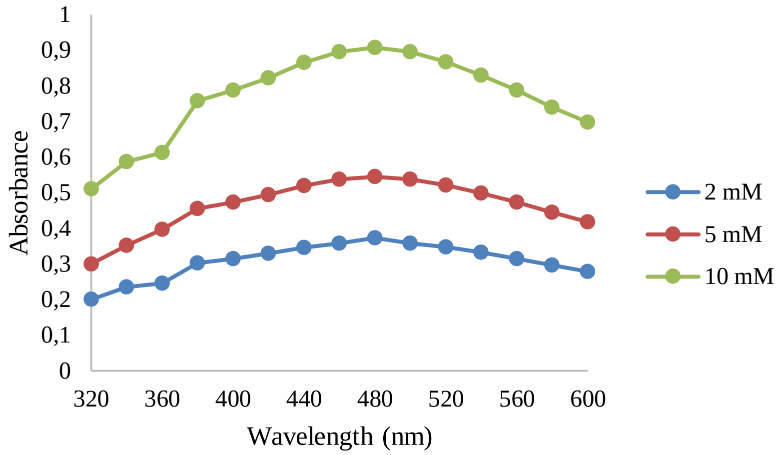
3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles

Synthesis of silver nanoparticles involves mixing a solution of silver nitrate ($AgNO3_)$with a reducing agent extracted from plants. The formation of silver nanoparticles is indicated by a change in the color of the solution to brown (Fig. 2B) [10], [18]. In this study, several concentrations of $AgNO_3$ namely 1 mM, 10 mM, and 20 mM as reagents in the manufacture of silver nanoparticles. The results of the absorbance measurements of the nanoparticles formed for each concentration of $AgNO_3$ shown.



A

B

A: Effect of AgNO3 concentration silver

B. Color change in solution after 24 hours

The absorbance number indicates the concentration of the solute, where the higher the absorbance means the higher the concentration of the substance. The dark brown color of the reaction mixture indicates the synthesis of AgNPs [2]. Bioactive compounds of plant extracts can act as silver ion reducing agents and stabilize nanoparticles [19].

The results of absorbance measurements show that the absorbance peak is at a wavelength of 480 nm. These results are in accordance with previous studies that AgNPs have maximum visible UV absorption in the range of 400-500 nm due to surface plasmon resonance [20].

The method of synthesizing nanoparticles with plant extracts is a potential alternative to replace common methods that use hazardous chemicals and toxic by-products produced during the manufacture of nanoparticles [21]. The advantages of synthesizing AgNPs based on plant extracts compared to chemical and physical methods are non-toxic, pollution-free, environmentally friendly, economical, and more sustainable [10]. Silver is an inorganic antibacterial agent that is non-toxic and safe to use as a substitute for antibiotics. The resulting nanoparticles were then analyzed for their antibacterial activity against MRSA bacteria.

3.2 Confirmation of the MRSA (Methicillin-Resistant Staphylococcus aureus) Bacterial Strand Bacterial

samples were obtained from the Faculty of Health, UNUSA Surabaya. To ensure that the sample used was MRSA (*Methicillin-Resistant Staphylococcus aureus*), several preliminary tests were carried out. Bacterial samples were subjected to preliminary tests using the culture method on MHA media to determine whether there was resistance to the antibiotic cefoxitin 30 µg. The MRSA detection results from the preliminary test in Figure 3 did not show any inhibition zone which indicated that the isolate was resistant to cefoxitin.

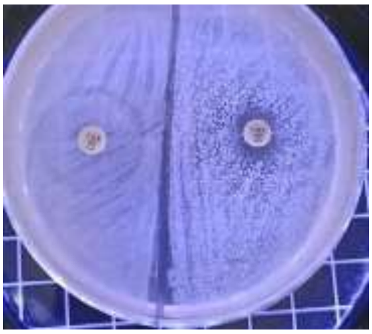


Figure 3: Inhibition zone on MHA media

For testing of *Staphylococcus aureus* , morphological and biochemical observations were carried out. Observation of morphology with Gram staining and observed using a microscope with an
objective lens magnification of 1000x. Based on the results of microscopic observations, the results obtained for round-shaped bacteria are in Figure 4.

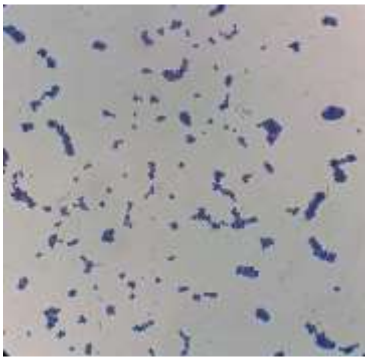
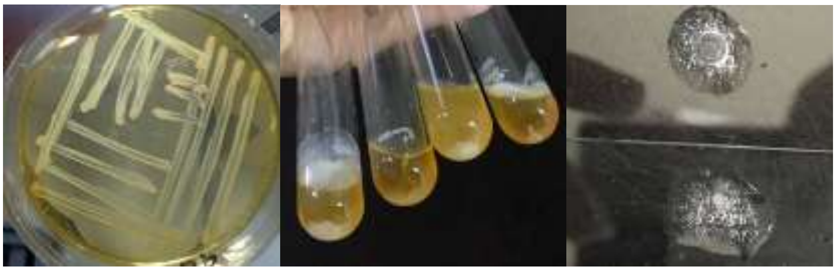


Figure 4: Microscopic morphology of MRSA



(A) (B) (C)

Figure 5: Biochemical test results (A) Manittol test (+), (B) Coagulase test (+), (C) Catalase test (+)

Characteristics *Staphylococcus aureus* based on Taylor and Unakal (2017) is a Gram-positive (purple with Gram stain) bacterium that is cocci-shaped and tends to arrange in clusters similar to grapes. These organisms can grow at up to 10% salinity, and colonies are often colored gold or yellow (aureus means gold or yellow). These organisms can grow aerobically or anaerobically (facultative) and can grow at temperatures between 18°C and 40°C. The key to biochemical identification tests of *S. aureus* was catalase positive (for all *Staphylococcus* pathogenic distinguish *Staphylococcus aureus* from *Staphylococcus*) (Taylor and Unakal, 2017). Results on morphological and biochemical observations (Figure 5) according to the key morphological and biochemical characters of the species *S. aureus.*

3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal

Concentration (MBC)

The MIC test results are shown in Figure 6 below.

Figure 6: Growth of MRSA at various concentrations of silver nanoparticles and Moringa leaf extract

Table 1: MIC and MBC values of the sample

Sample	MIC	MBC
Nanoparticle 1	1,25 mg/ml	1,25 mg/ml
Nanoparticle 2	2,5 mg/ml	5 mg/ml
Moringa extract	5 mg/ml	5 mg/ml

Minimum Inhibitory Concentration (MIC) of the smallest silver nanoparticles is 1.25 mg/ml. Based on Figure 6, it is known that when the nanoparticles were administered at a concentration of 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml the solution remained clear, indicating inhibition of MRSA bacterial growth. These results validate that AgNPs have inhibitory activity on MRSA growth. Silver nanoparticles have high toxicity against prokaryotic cells such as bacteria, viruses, and fungi [9]. AgNPs inhibit the growth and multiplication of many bacteria, including *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio parahaemolyticus*. AgNPs generate reactive oxygen species and free radicals, which cause apoptosis, cell death, and prevention of replication. AgNPs are able to diffuse inside cells and penetrate cell walls because AgNPs are smaller in size than microorganisms [22]. Bioactive phytochemical compounds from plant extracts also provide additional antibacterial properties to nanoparticles [9]. *reactive oxygen species* (ROS) and Ca2+ gradient changes contribute to bacterial cell death. Inner membrane disruption followed by membrane dysfunction is critical in the antibacterial action of AgNPs [23]. Furthermore, AgNPs have the potential to alter cellular communication by dephosphorylating tyrosine residues on important bacterial peptide substrates, thereby preventing microbial development [24]. AgNPs can easily penetrate bacterial cell walls, damage cell membranes, produce reactive oxygen species, interfere with DNA replication and protein synthesis, and cause cell death [2].

An alternative to making silver nanoparticles with the help of Moringa leaf extract (or hereinafter referred to as *Silver Moringa*) can be a potential antibacterial alternative in dealing with MRSA bacteria.

3.4 Antibiotic activity of silver nanoparticle fabrics

The results of the synthesis of silver nanoparticle fabrics are shown in Figure 8.

Figure 8. Results of application of green synthesized nanoparticles (20, 10, and 1 mM) to sisal cloth

The results of the sample's antibiotic activity test using the Kirby-Bauer method are shown in Figure 6. Based on these results it is known that silver nanoparticles cloth produces a larger inhibition zone than cefoxitin. AgNPs can easily enter the bacterial cell wall, disrupt cell membranes, produce reactive oxygen species, interfere with DNA replication and protein synthesis, and cause cell death [2]. Due to the presence of bioactive molecules on the silver nanoparticle surface, green synthetic silver nanoparticles have strong antioxidant and antibacterial activity [9].

Figure 9. Results of the zone of inhibition of growth of MRSA bacteria

The diameter of the zone of inhibition of the growth of MRSA bacteria was then statistically analyzed using the One Way ANOVA followed by Fisher's Follow-up Test (Table 2).

The inhibition zone formed showed that the silver nanoparticles had antibacterial potential against MRSA bacteria, with an average inhibition zone diameter of 15.667 mm. This result is statistically significant with a p-value of 0.001. Fisher's follow-up test showed that the nanoparticle samples and moringa extract produced a larger and significantly different diameter of the inhibition zone compared to cefoxitin. A study by Balashanmugam and Kalaichelvan[25] also found that AgNPs synthesized with plant extracts and then immobilized on cloth showed high antibacterial activity. These results indicate that the silver nanoparticle cloth based on Moringa leaf extract has potential antibacterial activity in controlling the growth of MRSA bacteria.

4. CONCLUSION

The conclusions of this study are:

1. The highest levels of silver nanoparticles (based on UV-Vis absorbance) were obtained by mixing 10 mM $AgNO_3$ and 10 mg/ml *M. oleifera* for 24 hours with stirring at 140 rpm.
2. MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bacteriocidal Concentration) values are 1.25 mg/ml.
3. The silver nanoparticle fabric produced showed antibiotic activity against MRSA with an average inhibition zone diameter of 15.677 mm

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This study sucessfully develops the moringa extract-based silver nanoparticle as a potential antibacterial material against Methicilin-resistant Staphylococcus aureus. This bioactive silver nanoparticle showed stronger anti-bacterial activity than cefoxitin. The researcher applied this moringa-based silver nanoparticles to fabrics and demonstrated its washable and detergent-resistant properties. This Silver Moringa Cloth could be applied to prevent the infection with MRSA in hospitals.