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# Effectivity of Silver Nanoparticles-Temu Giring (*Curcuma heyneana*) Rhizome on Inhibiting the Growth of Bacteria Causing Nosocomial Infection

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**ABSTRACT**

**Biofilms are a common cause of nosocomial infections that often attack hospitalized patients. The main objective of this study was to examine the efficacy of silver nanoparticles-temu giring rhizomes in combating bacteria and preventing biofilm formation. The antibacterial and antibiofilm properties of these nanoparticles were evaluated against *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The research began with the extraction of temu giring rhizome, synthesis of silver nanoparticles-temu giring rhizome, disk diffusion test, biofilm formation inhibitory activity test, and characterization of silver nanoparticles-temu giring rhizome. In this research, silver nanoparticles-temu giring rhizome were utilized at concentrations of 10, 20, 40, 80, 160 µg/ml, and a control in the form of chlorhexidine. The results showed that the silver nanoparticles-temu giring rhizome produced a larger inhibition zone for bacterial growth compared to the control against the three bacteria. The $IC_{50}$ value of silver nanoparticles-temu giring rhizome required to inhibit biofilm formation was 27.64 µg/ml in *E. coli*, 29.29 µg/ml in *P. aeruginosa*, and 26.21 µg/ml in *S. aureus*. In *P. aeruginosa*, *E. coli*, and *S. aureus*, the $IC_{50}$ for preventing biofilm formation by silver nanoparticles-temu giring rhizome was determined to be 27.64 µg/ml, 29.29 µg/ml, and 26.21 µg/ml, respectively. Evaluation of silver nanoparticles revealed the success of temu giring rhizomes in reducing silver ions. This is shown that silver nanoparticles-temu giring rhizomes can be developed into active ingredients that inhibit the growth of bacteria that cause nosocomial infections.**

## 1. Introduction

Biofilm is a bacterial barrier from carbohydrates which is a common cause of nosocomial infections. This infection often occurs in hospitalized patients with open wounds, intravenous users, catheters, and post-operative conditions (Kesumaputri *et al.* 2021). Biofilms can also worsen the patient's health because of the possibility of causing complications, such as sepsis, endocarditis, and osteomyelitis (Tong *et al.* 2015). *S. aureus*, *P. aeruginosa*, and *E. coli* represent prevalent human pathogenic bacteria known for their ability to form biofilms (Khatoon *et al.* 2018), hence they are able to live in extreme environments and resistant to some antibacterial substances (Hall and Mah 2017).

Nanotechnology is an interdisciplinary field focused on the creation and manipulation of materials within 1 to 100 nm, commonly referred to as nano size (Mohamed *et al.* 2021). This technology is interesting because nano-sized materials have physicochemical properties that are in stark contrast to their original properties, thus displaying unique and new properties (Mohanta *et al.* 2020). Nano-scale materials display improved characteristics as a result of their elevated surface energy, significant surface-to-volume ratio, minimal imperfections, and confinement in space (Sudha *et al.* 2018).

Silver is one of the materials that has antibacterial activity against pathogenic microorganisms (Mohanta *et al.* 2016). Presently, investigations are underway to explore the utilization of silver nanoparticles, such as in the production of ointments and creams, for the purpose of inhibiting microbial infections in

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burns and open wounds. There are various methods available for producing silver nanoparticles, but the biological approach possesses distinct characteristics of its own. Biological approaches can be done in several ways, one of which is mediated by plants. The biological method utilizing plants offers advantages compared to other approaches, as it exhibits lower toxicity towards living cells (Mittal *et al.* 2013). A research study successfully showcased the synthesis of silver nanoparticles using plants (Mohanta *et al.* 2020). Flavonoids and phenolic compounds, which are plant-derived secondary metabolites, have been recognized for their role in the conversion of silver ions to elemental silver (Dauthal and Mukhopadhyay 2016).

Indonesia is a country with great plant diversity. Indonesia also has endemic plants that are still not widely explored. These plants turned out to have potential as herbal plants that can be used to overcome health problems. Temu giring (*Curcuma heyneana*) is one of the native plants of Indonesia (Sugita *et al.* 2018) which contains good phenolic and flavonoid compounds in its rhizome (Marianne *et al.* 2021). However, no one has used this plant for the synthesis of silver nanoparticles, so this research aims to do so. Although some medical devices have been coated with silver, cases of nosocomial infection are still high, so it is necessary to develop antibacterial and antibiofilm materials using nanotechnology. Hence, the objective of this study was to evaluate the antibacterial and antibiofilm efficacy of the silver nanoparticles-temu giring rhizome against *E. coli, P. aeruginosa*, and *S. aureus* bacteria.

## 2. Materials and Methods

The research began with the extraction of temu giring rhizome, synthesis of silver nanoparticles of temu giring rhizome, inoculation of bacterial culture, antibacterial activity testing (disk diffusion inhibition zone test), biofilm formation inhibitory activity test, FTIR analysis, UV-Vis spectrophotometry, data collection, and data analysis. This research is experimental research with six treatments and three replicates. The administered treatments consisted of varying concentrations of silver nanoparticles-temu giring rhizome (independent variables), specifically 10, 20, 40, 80, and 160 µg/ml, along with a control group containing chlorhexidine at a concentration of 160 µg/ml. The data was analyzed by determining the diameter of the inhibition zone and calculating the percentage of inhibition activity for biofilm formation caused by silver nanoparticles-temu giring rhizome against *E. coli, S. aureus*, and *P. aeruginosa*. The results obtained are displayed in graphs and tables. The statistical analysis was analyzed by One Way ANOVA test using IBM SPSS 26.

### 2.1. Synthesis of Silver Nanoparticles-Temu Giring Rhizome

Temu giring (*C. heyneana*) rhizomes were collected from Kebun Percobaan Manoko, Lembang. After washing, the rhizomes were sliced and subjected to drying in an oven. Once dried, the rhizomes were blended and sieved through a 50-mesh size (Mohanta *et al.* 2020). To create a mixture, 5 grams of rhizome powder was combined with 50 ml of sterile deionized water using a 1:10 ratio. The resulting mixture was subjected to sonication in a water bath sonicator (Sibata) at a temperature of 60°C for a period of 4 hours. The mixture was filtered through Whatman filter paper no. 1 and stored at 4°C (Jackson 2019). To isolate plant particles from the extract, centrifugation was performed at a speed of 4,000 rpm for a duration of 10 minutes (Kokusan H-103 N). The resulting supernatant was taken as an extract of the temu giring rhizome, while the pellet precipitate was discarded (Abdelmoteleb *et al.* 2017). The obtained temu giring extract was utilized in the fabrication of silver nanoparticles, where 10 ml of the extract was combined with 90 ml of a 1 mM $AgNO_3$ solution. The solution was kept in a dark environment and incubated for 24 hours at a temperature of 60°C. To separate silver nanoparticles from the mixture, centrifugation was carried out at 12,000 rpm for 30 minutes, followed by two times washing of the pellets with deionized water at the same speed and duration of centrifugation (Khshan and Alkafaje 2021). The pellets were collected for further freeze-drying in order to obtain stable silver nanoparticles (Farzeen and Kumar 2022).

### 2.2. Characterization of Silver Nanoparticles-Temu Giring Rhizome

To confirm the surface plasmon resonance of the silver-rhizome nanoparticles synthesized, an analysis of the synthesis mixture was performed using a UV-Vis spectrophotometer (Thermo Scientific Genesys 10 uv) (Anandalakshmi *et al.* 2016). The maximum absorbance of the silver nanoparticles falls within the

wavelength range of 400-430 nm (Singh *et al.* 2015). To investigate the involvement of phytoconstituents in the synthesis of silver nanoparticles-temu giring rhizome, Fourier transform infrared spectroscopy (FTIR) analysis was performed across the 500-4,000 $cm^{-1}$ range (Mohanta *et al.* 2020). This test was carried out by analytical services (Shimadzu 8400). The size and shape of nanoparticles are known by SEM (Scanning Electron Microscope) with 3,000x and 40,000x magnification with 200 resolution and acceleration voltage of 11 kV. SEM analysis can see the character of both shape and size nanoparticle samples (Romadhan and Pujilestari 2019).

### 2.3. Antibacterial Activity Evaluation

To assess the antibacterial effectiveness of the silver nanoparticles-temu giring rhizome, the three bacterial species were cultivated in Mueller Hinton Broth (MHB) for a duration of 24 hours. The surface of the agar plate is inoculated by evenly spreading a specific volume of the bacterial inoculum across the entire plate. The Kirby-Bauer method was employed to conduct the disk diffusion inhibition zone test. Six discs were used, each containing silver nanoparticles-temu giring rhizome at concentrations ranging from 10 to 160 µg/ml in multiples of 2. Additionally, a control disc containing chlorhexidine at a concentration of 160 µg/ml was included. Subsequently, the samples were placed in an incubator at a temperature of 37°C for a duration of 24 hours. Following the incubation period, the diameter of the inhibition zone was measured. Measurements were made from the horizontal and vertical directions which are then averaged (Cappuccino and Welsh 2019).

### 2.4. Antibiofilm Activity Evaluation

The antibiofilm activity of the temu giring rhizome nanoparticles was evaluated using a 96-well flat-bottom microplate to determine its MIC (minimum inhibitory concentration). In each well, 180 µl of Mueller-Hinton broth and 10 µl of test bacteria (with an optical density of 0.5 McFarland at 600 nm) were added. Subsequently, 10 µl of silver nanoparticles-temu giring rhizome, at concentrations of 10, 20, 40, 80, and 160 µg/ml, were introduced into each well, excluding the control wells that did not receive any treatment. The 96-well flat microplates, containing the samples, were incubated at a temperature of 37°C for a duration of 24 hours in a static environment. Following the incubation period, the contents of each well were carefully aspirated and washed with phosphate-buffered saline (PBS) to eliminate any nonadherent bacterial cells. Subsequently, the 96-well flat microplate was subjected to a drying process lasting 45 minutes. Once dried, the attached sessile bacteria in the well were immobilized by treating them with a solution of 2% sodium acetate, followed by immersion in a 0.1% (w/v) crystal violet solution. The plate was then incubated in a dark environment for a duration of 30 minutes. Following that, each well was rinsed with sterile deionized water until all residual dye was thoroughly washed away. The 96-well flat microplate was then re-dried. Upon completion of the drying process, 200 µl of ethanol (95%, v/v) was introduced into each well, and the absorbance was assessed at a wavelength of 620 nm using an ELISA reader. The proportion of biofilm formation inhibitory activity was calculated using the following formula (Barabadi *et al.* 2021): % biofilm inhibition = [(control $OD_{620}$ - treatment $OD_{620}$) / control $OD_{620}$] × 100 The test was performed three times and the mean was calculated. The MIC for potential inhibitions of biofilm formation was expressed as $IC_{50}$ (Mohanta *et al.* 2020).

## 3. Results

### 3.1. Synthesis and Characterization of Silver Nanoparticles-Temu Giring Rhizome

The initial weight of the temu giring rhizome before drying was 1.384 grams. After drying, the final dry weight of the temu giring rhizome was 138 grams. The acquired temu giring extract (5 grams) was utilized in the production of silver nanoparticles. The synthesis of silver nanoparticles was confirmed by visual observation. Figure 1 shows the transformation wherein the initial yellow color of the mixture containing rhizome extract and $AgNO_3$ changed to a dark brown hue. In the control mixture, which consists of only rhizome extracts and sterile deionized water, no color change was observed.

The existence of the produced silver nanoparticles was additionally verified through UV-Vis spectrophotometry, where an absorbance peak at 410 nm was observed, as depicted in Figure 2.

The synthesis process resulted in 15 ml pellets containing silver nanoparticles. The freeze-drying of pellets resulted in 0.013 g of silver nanoparticles-temu giring rhizome (Figure 3).

The FTIR spectra of silver nanoparticles-temu giring rhizome was recorded to identify functional

groups. Interaction of silver nanoparticles with phytoconstituents from temu giring rhizome extract showed intense peaks at 3688.02, 2366.74, 1539.25, and 1645.33 $cm^{-1}$ (Figure 4).

### 3.2. Morphology of Silver Nanoparticles-Temu Giring Rhizome

SEM analysis can analyze the morphology of the silver nanoparticles-temu giring rhizome which is observed to have a spherical shape (Figure 5). Nanoparticle samples have non-uniform size ranging from 100-1,000 nm.

Figure 1. A noticeable visual change in color is observed in the mixture of rhizome extract and $AgNO_3$, in contrast to the control. Left: mixture of rhizome extract and $AgNO_3$, Right: control mixture of rhizome extracts and sterile deionized water

### 3.3. Antibacterial Activity of Silver Nanoparticles-Temu Giring Rhizome

The antimicrobial activity of silver nanoparticles-temu giring rhizome was evaluated against *E. coli*, *P. aeruginosa*, and *S. aureus* bacteria Figure 6. Based on the disk diffusion inhibition zone test on *E. coli*, the best result was achieved by 80 µg/ml silver nanoparticles-temu giring rhizome solution, which resulted in 4.0±2.9 mm inhibition zone. The best result against *P. aeruginosa* was 2.6±2.4 mm which used 10 µg/ml silver nanoparticles-temu giring rhizome solution, while against *S. aureus*, the best result was 3.3±0.4 mm, using 40 µg/ml solution. The inhibition zone results for all the tested bacteria demonstrated larger zones of inhibition compared to 160 µg/ml

Figure 3. Silver nanoparticles-temu giring rhizome, obtained from freeze-dry

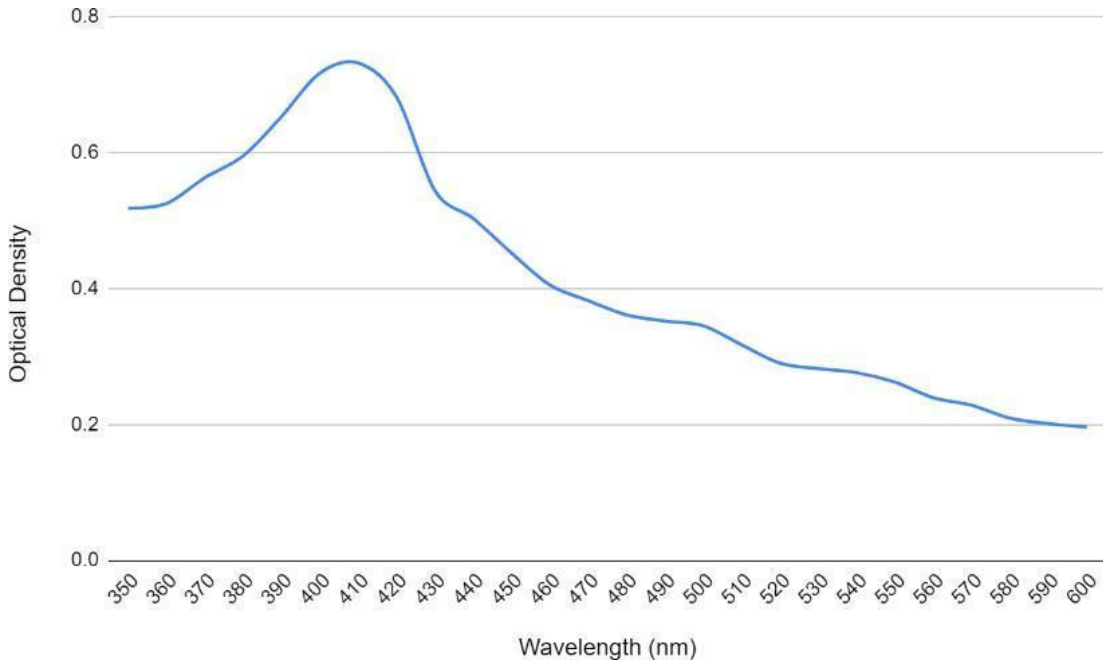



Figure 2. UV-Vis spectrophotometry analysis of silver nanoparticle

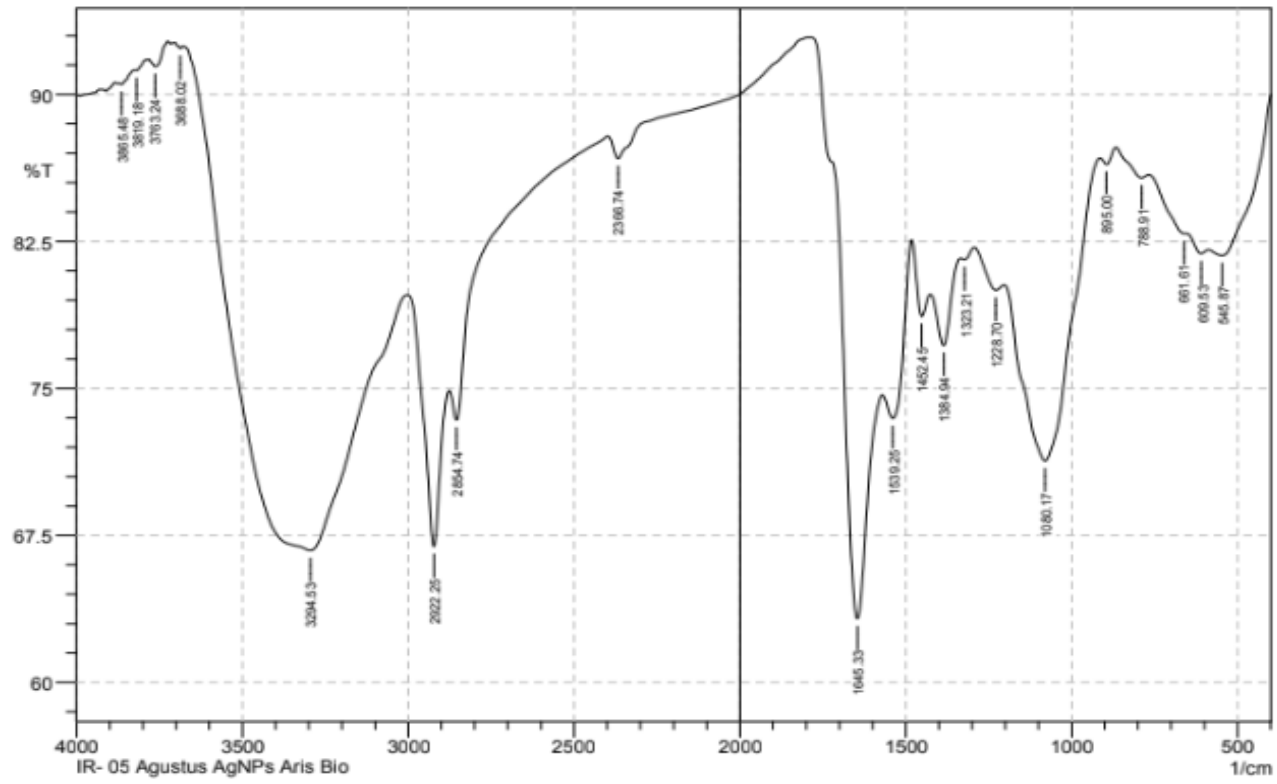
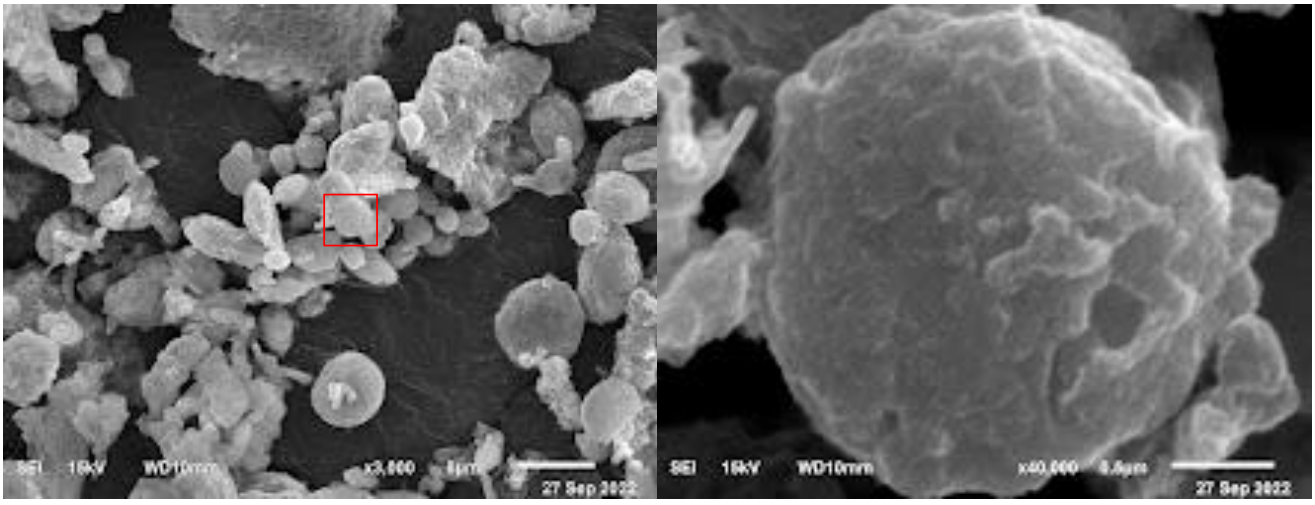



Figure 4. FTIR analysis of silver nanoparticles-temu giring rhizome




A B

Figure 5. Morphology of silver nanoparticles-temu giring rhizome with SEM analysis; (A) Zoom at 3,000x, (B) Zoom at 40,000x

chlorhexidine, measuring 0.6±0.9 mm, 2.0±0.0 mm, and 1.0±0.8 mm against *E. coli, P. aeruginosa*, and *S. aureus*, respectively (Table 1).

### 3.4. Antibiofilm Activity of Silver Nanoparticles-Temu Giring Rhizome

The *in vitro* assessment of the anti-biofilm activity of silver nanoparticles-temu giring rhizome was conducted against three biofilm-forming bacterial species: *P. aeruginosa, E. coli*, and *S. aureus*. The measurement of anti-biofilm activity was conducted by analyzing the ELISA readings to determine the minimum inhibitory concentration (MIC) of anti-biofilm activity, represented as $IC_{50}$. Treatment of *E. coli* with silver nanoparticles-temu giring rhizome at 160 µg/ml was able to reduce biofilm formation by 87.89%, as presented on the graph in Figure 5. Treating *P. aeruginosa* and *S. aureus* with the same concentration resulted in a significant reduction in biofilm formation, with reductions of 94.74% and 95.09%, respectively. The MIC values ($IC_{50}$) of silver-rhizome nanoparticles required to inhibit biofilm formation were 27.64 µg/ml, 29.29 µg/ml, and 26.21 µg/ml in *E. coli, P. aeruginosa*, and *S. aureus*, respectively. The One-Way ANOVA analysis results ($P<0.05$) with a significance level of 0.000 indicated a significant difference in the antibiofilm activity among different concentration treatments for each bacterium.

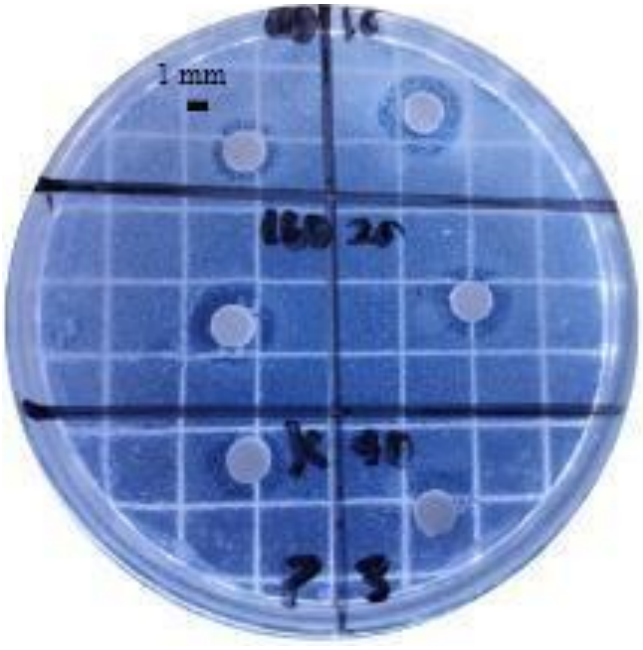



Figure 6. The silver nanoparticles-temu giring rhizome inhibition zones at concentrations of 10, 20, 40, 80, and 160 µg/ml, alongside a control group treated with chlorhexidine at a concentration of 160 µg/ml

## 4. Discussion

Temu giring (*Curcuma heyneana*) is one of the native plants of Indonesia (Sugita *et al.* 2018) which contains phenolic and flavonoid compounds in its rhizome (Marianne *et al.* 2021). Previous studies have documented the ability of these secondary metabolites to convert silver ions into elemental silver (Dauthal and Mukhopadhyay 2016). Due to its capacity, the extract from temu giring rhizome is suitable for use in the plant-mediated synthesis of silver nanoparticles. The emergence of a deep brown color signifies the creation of AgNPs (silver nanoparticles) (Gurunathan *et al.* 2014). The shift in the reaction mixture's color resulted from the existence of phytochemicals within the leaf extract, signifying the reducing capability of the phytoconstituents in producing the silver nanoparticles (Mohanta *et al.* 2020). The detection of the absorption peak associated with silver nanoparticles can be attributed to the resonance vibration of the nanoparticle electrons with specific wavelengths of light. Temu giring extract facilitated the formation of a distinct band of nanoparticles known as the Surface Plasmon Resonance (SPR)

Table 1. The results of disk diffusion inhibition zone test of silver nanoparticles-temu giring rhizome

| Silver nanoparticles-temu giring rhizome concentration (µg/ml) | Inhibition zone diameter ± SD (mm) | | |
|---|---|---|---|
| | *E. coli* | *P. aeruginosa* | *S. aureus* |
| 10 | 1.0±1.4 | 2.6±2.4 | 2.0±1.6 |
| 20 | 1.3±0.9 | 1.3±1.8 | 2.6±0.9 |
| 40 | 0.6±0.9 | 1.6±2.3 | 3.3±0.4 |
| 80 | 4.0±2.9 | 0.6±0.9 | 1.3±0.9 |
| 160 | 1.3±0.9 | 2.0±2.8 | 0.6±0.9 |
| Chlorhexidine (160) | 0.6±0.9 | 2.0±.00 | 1.0±0.8 |

phenomenon. This phenomenon is characterized by a peak observed at a wavelength of 395-420 nm, specifically indicating the reduction of Ag+ to Ag0 by the extract (Creighton and Eadon 1991). The observed surface plasmon resonance characteristic of these particles also suggests the presence of metal nanoparticles within the size range of 2–100 nm (Nayak *et al.* 2015). In our study, the presence of an absorption peak at 410 nm confirmed the successful synthesis of silver nanoparticles using the extract of temu giring rhizome. The intense absorption peak observed at 3688.02 $cm^{-1}$ suggests the robust bonding between silver ions and the hydroxyl group (-OH) present in water. Additionally, the broad spectral peak detected at 2366.74 $cm^{-1}$ indicates a pronounced stretching of the -C≡N (nitrile) group (Mohanta *et al.* 2020). The presence of two additional bands at 1539.25 and 1645.33 $cm^{-1}$ can be attributed to the stretched vibrations of the C=O (ketone) and C=O (amide) functional groups, which are commonly found in proteins involved in the reduction of metal ions. These findings suggest the potential involvement of hydroxyl and carbonyl groups in both the synthesis and stabilization processes of the silver nanoparticles-temu giring rhizome. The size and shape of the silver nanoparticles produced in this study surpass those reported by Mohanta *et al.* (2020), who achieved nanoparticles ranging from 52-96 nm in size and with a spherical shape. However, the findings are comparable to Adebayo-Tayo *et al.* (2019), who also obtained spherical silver nanoparticles measuring 10 nm in size through the green synthesis method utilizing *Oscillatoria* sp. extract.

Comparable findings have been documented in various studies exploring the synthesis of silver nanoparticles utilizing rhizome extracts from diverse plants. For instance, research on the synthesis of silver nanoparticles using *Curcuma longa* and *Zingiber officinale* rhizome extracts observed a peak absorbance within the range of 390-430 nm (Venkatadri *et al.* 2020). Leaf extracts, such as those reported by Mohanta *et al.* (2020), that were utilized in silver nanoparticle synthesis also resulted in the peak absorbance at 420-430 nm. Based on FTIR analysis, the presence of hydroxyl and carbonyl groups in temu giring rhizome might play a role in the synthesis of silver nanoparticles (Khorrami *et al.* 2018). The collected data indicated that the compounds extracted from the rhizome formed a layer over the synthesized nanoparticles, serving as a stabilizing agent.

The molecules found in the temu giring rhizome extract serve a role similar to surfactant molecules by adhering to the surface of the nanoparticles and ensuring colloidal stability (Nayak *et al.* 2016). Moreover, proteins and other organic compounds acting as capping agents prevent the aggregation of nanoparticles within the suspension medium and play a crucial role in the formation of exceptionally stable silver nanoparticles (Khorrami *et al.* 2018). The chosen bacteria for this study, namely *E. coli, P. aeruginosa*, and *S. aureus*, are among the most commonly encountered pathogenic bacteria on various surfaces within healthcare settings (Hammuel *et al.* 2014). Therefore, these bacteria are linked to nosocomial infection, which is a serious threat since many of these bacteria strains are resistant to antibiotic treatment (Oli *et al.* 2013). The outcomes of our antibacterial test, conducted using the disk diffusion method, demonstrated the substantial impact of silver nanoparticles-temu giring rhizome on the growth of all three bacteria, including the lowest concentration of the nanoparticles. Numerous studies have indicated that the antibacterial efficacy of silver nanoparticles stems from their adherence to the bacterial cell wall, leading to significant structural alterations and eventual cell death (Revati *et al.* 2015). Furthermore, the interaction between silver nanoparticles and the thiol groups of enzymes may also play a crucial role in their antibacterial activity (Jardón-Romero *et al.* 2022). The antimicrobial impact of silver nanoparticles-temu giring rhizome was observed against both Gram-positive bacteria (*E. coli, P. aeruginosa*) and Gram-negative bacteria (*S. aureus*).

This discovery indicates that silver nanoparticles-temu giring possess significant potential for use in biomedical applications aimed at preventing nosocomial infections. The three bacteria species tested in this study are known to form biofilms, and our antibiofilm assay demonstrated a noteworthy impact of silver nanoparticles-temu giring rhizome on inhibiting biofilm formation. Silver nanoparticles exhibit anti-biofilm properties by effectively impeding the secretion of exopolysaccharide (EPS), a key constituent involved in the creation of bacterial biofilms (Mohanta *et al.* 2020). In a previous study by Mohanta *et al.* (2020), it was demonstrated that biosynthesized silver nanoparticles derived

from *Semecarpus anacardium* leaf extract exhibited significant anti-biofilm activity against *E. coli, P. aeruginosa*, and *S. aureus*. The study revealed that at a concentration of 100 µg/ml, the biosynthesized silver nanoparticles effectively inhibited over 99% of bacterial biofilm formation. In a study conducted by Singh *et al.* (2021), it was also demonstrated that biogenic silver nanoparticles exhibited potent anti-biofilm activity against *E. coli* and *P. aeruginosa* bacteria at a concentration of 100 µg/ml.

The results of these studies strengthen the evidence for the silver nanoparticles-temu giring rhizome against biofilm formation of *E. coli, P. aeruginosa*, and *S. aureus* at fairly low concentrations. The peak absorbance value of silver nanoparticles-temu giring rhizome, which is in the typical range for silver nanoparticles, indicated the success of synthesizing silver nanoparticles by utilizing temu giring rhizome extract. FTIR analysis revealed the involvement of hydroxyl and carbonyl groups in both the synthesis and stabilization of silver nanoparticles-temu giring rhizome. The findings from these studies provide further support for the efficacy of silver nanoparticles-temu giring rhizome in inhibiting the formation of biofilms by *E. coli, P. aeruginosa*, and *S. aureus* even at relatively low concentrations. The biofilm inhibition test results further confirm the capacity of silver nanoparticles-temu giring rhizome to decrease biofilm formation.

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