ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 27 (3) (2024): 101-109

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Synthesis of Silver Nanoparticles from Extract of Epiphytic Shrub Leaves (*Ficus heteropleura* Blume) on Oil Palm Plant and Evaluation of Their Antibacterial Activity Against *Escherichia coli*

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https://doi.org/10.14710/jksa.27.3.101-109

Article Info

Article history:

Received: 23rd October 2023
Revised: 16th January 2024
Accepted: 19th February 2024
Online: 8th April 2024

Keywords:
epiphytic shrub leaves; silver nanoparticles; antibacterial; *Escherichia coli*

Abstract

Silver nanoparticles are silver metal particles with a size of less than 100 nm. Extract of epiphytic shrub leaves (*Ficus heteropleura* Blume) serves as a bioreductant in producing silver nanoparticles, which are utilized for their antibacterial properties against *Escherichia coli*. This study aims to synthesize silver nanoparticles and assess their activity against *Escherichia coli*. Silver nanoparticles (AgNPs) were synthesized using the green synthesis method with *Ficus heteropleura* leaf extract with silver nitrate ($AgNO_3$). AgNPs were synthesized using a 1 mM solution of $AgNO_3$ and different concentrations (2, 4, 6, 8, and 10%) of *Ficus heteropleura* extract. The AgNPs were characterized using UV-Vis spectrophotometer, FTIR, and PSA. UV-Vis analysis revealed that AgNPs at concentrations ranging from 2% to 10% exhibited absorbance peaks within the wavelength range of 201.2–473.5 nm. FTIR analysis identified functional groups such as OH, CH, C=C, C≡C, C≡N, and CO, which played a role in reducing silver nanoparticles. The size distribution of AgNPs was determined, with AgNPs synthesized at a concentration of 6% exhibiting a size range of 1–100 nm, constituting 40.77% of the total. For AgNPs synthesized at concentrations of 8% and 10%, the percentage of nanoparticles in the 1–100 nm size range was found to be 26.845% and 1.28%, respectively. The antibacterial activity of AgNPs at concentrations of 6% and 8% against *Escherichia coli* demonstrated moderate effectiveness.

1. Introduction

Nanotechnology research has recently seen significant developments, particularly in silver nanoparticles. Silver nanoparticles are one of the most widely synthesized metal nanoparticles [1]. Despite having the same chemical composition as larger particles (bulk), nano-sized particles exhibit superior properties. These enhanced properties contribute positively to the versatility of silver nanoparticles, rendering them applicable across various fields of life. In water treatment, silver nanoparticles serve as potent disinfectants, while in chemical measurements, they function as effective metal ion sensors. Moreover, silver nanoparticles are utilized in the medical field for their anti-inflammatory and antibacterial properties [2].

Silver nanoparticles are metallic silver particles with sizes smaller than 100 nm. They offer distinct advantages over antimicrobial compounds or antibiotics, notably their bacteriostatic activity, which inhibits the growth of microorganisms [3]. Chemical reduction is a commonly employed method in nanosynthesis due to its simplicity, ease, and effectiveness in producing silver nanoparticles. The silver metal precursor commonly utilized is $AgNO_3$. However, it is important to note that such chemicals can be hazardous and cause harmful effects due to the adsorption of toxic properties, particularly inorganic solvents, onto the material's surface [4].

In the realm of research, scientists have developed a method known as green synthesis, which involves synthesizing nanoparticles using biological agents, with

plant extracts being one of the key components [5]. Green synthesis also referred to as a method for producing metal nanoparticles, relies on natural materials derived from organisms, including plants and microorganisms from both terrestrial and marine environments [6]. The use of bioreductors based on plant extracts holds promise as an environmentally friendly alternative to traditional reducing agents. Plant extracts have demonstrated effectiveness as reductants in the synthesis of silver nanoparticles, offering the potential to reduce reliance on chemical reductants while minimizing environmental impact [7].

Ficus heteropleura leaves have the potential as a reducing agent in nanoparticle synthesis, offering a natural source of antioxidants and antibacterial agents [8]. This plant is recognized for its abundance of secondary metabolite compounds, including flavonoids, triterpenoids, polyphenols, tannins, alkaloids, and saponins [9]. Antibacterial agents are compounds employed to impede bacterial growth. Typically, the mechanism of action of antibacterial compounds involves damaging the cell walls of bacteria such as *Escherichia coli*, altering membrane permeability, disrupting protein synthesis, and inhibiting enzyme activity [10].

Various factors influence particle size during synthesis, including solution temperature, salt concentration, reducing agent, and reaction time. Metal nanoparticles encompass a range of elements, such as silver, zinc, copper, iron, and gold [11]. Silver was employed for this study. In research on silver nanoparticle synthesis, silver nitrate ($AgNO_3$) is frequently utilized as the silver salt precursor. The concentration of $AgNO_3$ as a precursor significantly impacts the shape and size of silver nanoparticles and their concentration, which in turn affects bacterial growth [10].

A previous study utilized key lime peel extract (*Citrus microcarpa* Bunge), which is rich in flavonoids, terpenoids, alkaloids, tannins, and saponins. The resulting silver nanoparticles had average sizes of 253.8 nm (10%), 254.2 nm (15%), and 253.9 nm (20%). However, the synthesis of nanoparticles using key lime peel extract still yielded particles larger than 100 nm [12].

This study employed epiphytic shrub leaves (*Ficus heteropleura* Blume) on oil palm plants due to their potential reduction agent in nanoparticle synthesis and as a source of natural antioxidants and antibacterial agents. *Ficus* spp. plants are known to contain secondary metabolite compounds, including flavonoids, polyphenols, tannins, alkaloids, and saponins [9]. Generally, *Ficus* spp. plants have been used in traditional medicine for various treatments, such as cancer, diarrhea, and dysentery. Thus, they are anticipated to be a bioreductant in synthesizing silver nanoparticles. Given the limited research on *Ficus heteropleura* leaf extract as a bioreductant in silver nanoparticle synthesis, there remains significant potential for further exploration and development in this area. Therefore, this study aims to synthesize silver nanoparticles using *Ficus heteropleura* leaf extract as the bioreductant.

2. Experimental

2.1. Materials and Tools

The materials employed in this study comprised *Ficus heteropleura* leaves sourced from Kijang Jaya Village, Tapung Hilir District, Kampar Regency. Silver nitrate ($AgNO_3$), nutrient broth (NB), nutrient agar (NA), distilled water, NaCl 0.9%, and *Escherichia coli* bacteria. The equipment utilized included glassware (Pyrex), filter paper discs, a UV-Vis spectrophotometer (Shimadzu), an FTIR spectrometer, a particle size analyzer (PSA), a magnetic stirrer (IKA), Petri dishes, an incubator, and an autoclave.

2.2. Sample Preparation

The collected *Ficus heteropleura* leaves were rinsed with running water to remove any adhered dust particles. Subsequently, the leaves were air-dried for a week to reduce moisture content. Once dried, the leaves were finely ground using a mixer grinder or blender, and the resulting powder was stored in a sealed container at room temperature.

Plant extracts were prepared by pouring 500 g of the dry leaf powder into separate dark bottles, each containing 250 g of powder, and adding distilled water until the sample was fully submerged. The mixture was allowed to soak for 24 hours. The mixture was then filtered using Whatman filter paper to separate the solid components from the liquid extract. The obtained filtrate was then concentrated using a rotary vacuum to yield a concentrated distilled water extract. This extract was subsequently stored at a temperature of 4°C for further utilization [13]. The *Ficus heteropleura* leaves, once collected, underwent a cleaning process by rinsing under running water to eliminate any adhered dust particles. Subsequently, the leaves were air-dried for one week to reduce moisture content. Once dried, the leaves were finely ground using a mixer grinder or blender, and the resulting powder was stored in a sealed container at room temperature.

To prepare the $AgNO_3$ solution, varying concentrations of 1 mM were prepared by weighing 0.017 g of $AgNO_3$ and dissolving it in 100 mL of distilled water. Additionally, extract solutions were prepared at concentrations of 2, 4, 6, 8, and 10% by mixing the *Ficus heteropleura* extract with 25 mL of distilled water [13].

2.3. Phytochemical Test

2.3.1. Alkaloids

The procedure involved adding 50 mg of the sample to a test tube and dissolving it in 10 mL of 1 M HCl solution, followed by filtration. Subsequently, the filtrate underwent testing with various Meyer's reagents: 4 mL of the filtrate was transferred into a test tube, and 1 mL of Meyer's reagent was added. A positive test result was indicated by forming a yellowish-white precipitate [14].

2.3.2. Triterpenoids and Steroids

The process began by placing 2 g of extract into a test tube and diluting it with 70% ethanol. Subsequently, five drops of ether were added until two distinct layers of

water and ethanol solution were formed. The top layer (ethanol) was carefully separated and evaporated in a drip plate. Concentrated H_2SO_4 was added to the evaporated ethanol solution. A positive result was indicated by the formation of a blackish-green precipitate.

2.3.3. Flavonoids

A total of 200 mg of plant samples were extracted with 5 mL of ethanol and heated for ± 5 minutes in a test tube. Subsequently, three drops of concentrated HCl were added to the mixture. Then, 0.2 g of Mg powder was added. A positive result was indicated by the appearance of a dark red color, indicating the presence of flavonol compounds or red precipitates indicative of flavone compounds.

2.3.4. Saponins

The procedure commenced with the addition of 2 g of ground plant samples into a test tube, followed by dilution with 70% ethanol. Warm water was added to the mixture, and the contents were shaken for 30 minutes. The foam formation was observed, and its height in centimeters was measured. After allowing the mixture to stand for 10 minutes, concentrated HCl was added if the foam persisted without dissipating [14]. If the foam remained constant even after adding concentrated HCl, it indicated a positive result.

2.3.5. Tannin

Tannins content was tested by placing 0.5 g of ground plant samples into a test tube and adding 10 mL of boiling distilled water. The mixture was then filtered, and the resulting filtrate was mixed with 2-3 drops of 1% $FeCl_3$ solution. A positive test was indicated by the appearance of a brownish-green or blackish-blue coloration in the solution.

2.3.6. Phenolic

The extraction process began by combining 1 g of the sample with 20 mL of 70% ethanol. Following extraction, 1 mL of the resulting extract was mixed with two drops of 5% $FeCl_3$ solution. A positive indication of phenolic compounds in the material was denoted by the formation of a green or blue-green color [14].

2.4. Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized by reacting 10-3 M silver solution and *Ficus heteropleura* extract solution (5:5 mL ratio) with different concentrations (2, 4, 6, 8, and 10% in 25 mL distilled water). The reaction mixture was heated in boiling water for 90 minutes. Then, it was cooled in tap water, stored at room temperature, and analyzed using a UV-Vis spectrophotometer [2].

2.5. Characterization of Silver Nanoparticles

The synthesized AgNPs were characterized using various analytical instruments. A UV-Vis spectrophotometer was employed to detect wavelength shifts indicated by color changes, an FTIR instrument was utilized to identify alterations in functional groups, and a PSA was used to assess the distribution of silver nanoparticle sizes.

2.6. Antibacterial Activity

The qualitative antibacterial activity testing utilized the disc diffusion method. Initially, *Escherichia coli* bacteria suspended in NB media (0.2 mL) were inoculated into sterile NA media (200 mL). The NA media containing the bacterial culture was then poured into sterile 20 mL Petri dishes and allowed to solidify. Once solidified, paper discs were placed on the agar surface, and each disc was laden with 10 µL of the test solution, repeated three times for consistency. Subsequently, the Petri dishes were incubated at 37°C for 24 hours. The inhibitory effect was determined by a clear zone surrounding the paper discs. The diameter of the inhibition zones was measured using a caliper for further analysis [10].

3. Results and Discussion

The synthesis of AgNPs in this study employed the green synthesis method, utilizing plant extracts as reducing agents to convert Ag^+ ions to Ag^0. The aqueous extract of *Ficus heteropleura* leaves is the Ag^+ reducing agent [15]. Phenolic compounds in the extract contain - OH groups capable of metal binding. This functional group donates electrons to Ag^+ ions, leading to the formation of Ag nanoparticles. Initially, phenolic compounds in solution undergo a transformation from R-OH groups to RO^- groups, primed for reaction. Phenolics then bind with Ag^+ ions to form RO-Ag groups. Subsequently, the bound Ag^+ ions undergo resonance, releasing them to form Ag^0 nanoparticles [16].

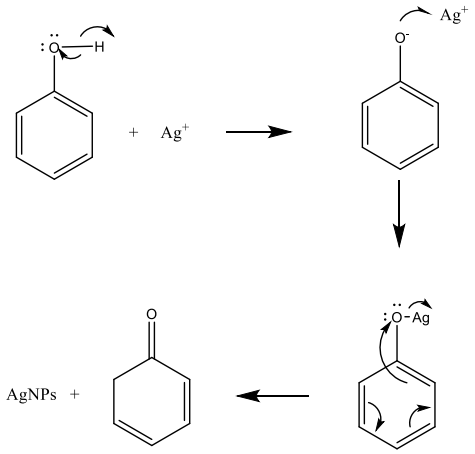

Figure 1. Reaction mechanism for the formation of AgNPs

Table 1. Results of *Ficus heteropleura* leaf extract

Sample	Extract weight (g)	Yield (%)
Ficus heteropleura leaves	5000	-
Dried *Ficus heteropleura* leaves	500	10
The obtained extract	46.31	9.26

Table 2. Results of *Ficus heteropleura* leaf extract

Compound class	Reactor	Change	Result
Alkaloids	HCl and Mayer	Yellowish white precipitate	+
Terpenoids and steroids	Ether and H_2SO_4	Blackish green precipitate	-
Flavonoids	Ethanol, HCl, and 0.2 g Mg	Red precipitate	+
Saponins	Hot distilled water	Foamy	+++
Tannin	Hot distilled water and 1% $FeCl_3$	Brownish green color	++
Phenolic	5% $FeCl_3$	Green or blue-green	+

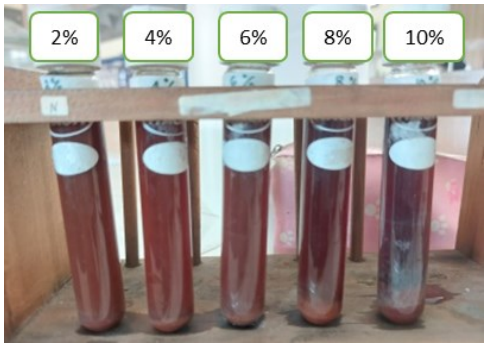

Figure 2. AgNPs solution with *Ficus heteropleura* leaf bioreductant

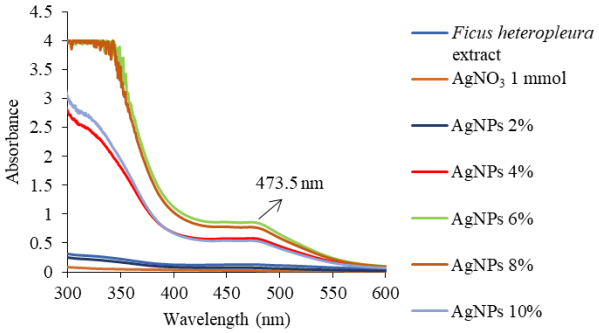

Figure 3. Maximum wavelengths of $Ag(NO_3)$ solution, aqueous extract, and AgNPs

3.1. Sample Preparation

The extraction process aims to isolate the compounds in the *Ficus heteropleura* leaves. The extracted compounds from *Ficus heteropleura* leaves are shown in Table 1. Initially, the *Ficus heteropleura* leaves undergo drying to eliminate moisture content. Subsequently, the dried leaves were subjected to maceration, which involved soaking the sample in distilled water as a solvent. Distilled water, being a polar solvent, facilitates the dissolution of polar compounds in the leaves. The resulting macerate was filtered, and the filtrate was concentrated using a rotary evaporator. This process yielded an extract weighing 46.31 g, yielding 9.262%. The extract exhibited a brick-red color.

3.2. Phytochemical Test

Phytochemical tests were conducted on the *Ficus heteropleura* leaves extract to ascertain the presence of various compound groups, as detailed in Table 2. These tests aim to determine the secondary metabolite content in the samples qualitatively. The phytochemical analysis of the aqueous extract revealed the presence of various compound groups. Alkaloids were identified by forming a yellowish-white precipitate, while a red precipitate indicated the presence of flavonoids. Saponins were detected by the continuous formation of froth or foam. Tannins were present, as evidenced by a shift to a brownish-green color. Furthermore, phenolic compounds were identified by their green or bluish-green color [14]. However, the aqueous extract of *Ficus heteropleura* tested negative for terpenoid and steroid compounds.

3.3. Synthesis of Silver Nanoparticles

A key indicator of AgNPs formation is the alteration in solution color. In this study, the synthesis of AgNPs has been distinguished by a noticeable change in color. The observed color variations are documented in Table 2. Synthesis of AgNPs using a reductant from *Ficus heteropleura* leaf extract mixed with $AgNO_3$ silver solution, heated in boiling water for 90 minutes, and cooled using tap water. The heating process aims to accelerate the formation of AgNPs.

One indication of AgNPs formation within this solution is the transition in color from yellowish to reddish-brown as time increases [5]. This alteration in colloid color during silver nanoparticle formation is attributed to the oxidation-reduction process. Organic compounds, including flavonoids, alkaloids, phenolics, and tannins found in *Ficus heteropleura* leaf extract, serve as reducing agents, facilitating the reduction of Ag^+ to Ag^0. Initially, the mixture of extract and $AgNO_3$ exhibited a light-yellow color, which gradually evolved into darker shades of yellow, reddish-brown, and, eventually, dark brown, signifying the initiation of nanoparticle formation [2].

The color observed during the AgNPs synthesis process arises from the oxidation of organic compounds. The intensity of the color produced corresponds to the degree of oxidation of organic compounds and the reduction of Ag^+ to Ag^0, forming more AgNPs [6]. In Figure 1, the color changes of the solution in the extract concentrations of 2, 4, 6, 8, and 10% from minute 0 to minute 90 exhibit a transition from brick red to brown, dark brown, and finally to a very dark brown color. These color changes are attributed to the variations in extract solution concentration during the AgNPs synthesis process, resulting in differing color outcomes.

Table 3. AgNPs synthesis results

Extract variation	Initial color	Final color
2%	Brick red	Dark red
4%	Brick red	Dark brownish red
6%	Brick red	Deep brownish red
8%	Brick red	Deep brownish red
10%	Brick red	Deep brownish red

Table 4. UV-Vis spectrum wavelength of AgNPs

Extract concentration	Wavelength	Absorbance
2%	201.2 nm	2.019
4%	469.2 nm	0.576
6%	473.5 nm	0.897
8%	471.2 nm	0.681
10%	468.3 nm	0.588

3.4. UV-Vis Spectrophotometer Characterization

The maximum wavelength for AgNPs formation typically falls within the range of 400-500 nm [17]. This study establishes the maximum wavelength of AgNPs with various extract concentrations, as illustrated in Figure 2. The aqueous extract of *Ficus heteropleura* leaves and $AgNO_3$ solution were utilized as monitoring solutions for synthesizing AgNPs. The UV-Vis spectra in Figures 3 and 4 reveal the maximum wavelength of AgNPs with several extract concentration variations, as outlined in Tables 4 and 5.

UV-Vis spectrophotometer analysis was employed to verify the formation of nanoparticles resulting from the synthesis process. Absorbance and λ_{max} measurements of AgNPs were conducted using a UV-Vis spectrophotometer within the wavelength range of 190-700 nm. The synthesis of AgNPs involved observations conducted under boiling water for 90 minutes.

In Tables 4 and 5, the λ_{max} measurements show the size distribution of the produced nanoparticles. For the λ_{max} measurement of the 2% extract concentration, the maximum absorption occurred at a wavelength of 201.2 nm. In the case of the 4% extract concentration, the maximum absorption was observed at a wavelength of 469.2 nm. Conversely, for the 6, 8, and 10% extract concentrations, the maximum absorption wavelengths were recorded at 473.5 nm, 471.2 nm, and 468.3 nm, respectively.

AgNPs typically exhibit maximum absorption within the wavelength range of 400 nm to 500 nm [17]. Analysis of the maximum wavelength reveals that a 2% concentration variation does not exhibit the presence of formed AgNPs, as the resulting λ_{max} falls below 400 nm. Conversely, the maximum wavelength results obtained from concentration variations of 4, 6, 8, and 10% lie within the 400-500 nm range, indicating the successful formation of AgNPs for these concentrations.

Additionally, the change in color of AgNPs for each concentration variation indicates a darker color, which correlates with higher absorbance values. A darker color suggests a higher absorbance value of AgNPs, indicating increased nanoparticle concentration. The absorbance values obtained through UV-Vis spectrophotometer analysis can be used to estimate the quantity of AgNPs formed [18]. Observations demonstrate that concentrations of 10, 8, 6, and 4% exhibit progressively higher absorbance values, indicating that the concentration of the extract influences the formation of AgNPs. Moreover, the duration of contact time appears to impact the biosynthesis process, with a concentration of 6% yielding superior AgNPs, as evidenced by a tendency towards higher absorbance values in the absorption spectrum.

3.5. FTIR Characterization

The FTIR results (Figure 4 and Table 6) exhibit various functional groups. The absorption band observed in *Ficus heteropleura* leaves extract at 3365.12 cm^{-1} corresponds to OH stretching vibrations, indicating the presence of hydroxyl groups in the reducing agents derived from compounds such as flavonoids, tannins, terpenoids, saponins, and polyphenols [7]. This observation is further supported by a strong intensity absorption at 1064.92 cm^{-1}, representing the stretching vibration of the CO aldehyde bond. Additionally, the stretching vibration of the C≡C bond is noted at 2138.77 cm^{-1}, while the stretching vibration of the C=C bond in the aromatic ring of *Ficus heteropleura* extract is observed at 1557.45 cm^{-1} [18].

Table 5. UV-Vis spectra of aqueous extract of *Ficus heteropleura* leaves and $AgNO_3$

Monitoring solution	Wavelength	Absorbance
$AgNO_3$ solution	217.2 nm	3.375
Ficus heteropleura extract solution	196.6 nm	2.454

Table 6. FTIR characterization of aqueous extract, 6% AgNPs, and $AgNO_3$

Peak	Peak wavenumber (cm^{-1})	Functional group [6]
Ficus heteropleura extract	3365.12	O-H
	2138.77	C≡C
	1557.45	C=C aromatic
	1407.85	C-H
	1064.92	C-O aldehyde
6% AgNPs	3324.64	O-H
	2180.70	C≡C
	2068.70	C≡N
	1636.73	C=O
$AgNO_3$	3371.30	O-H
	1629.53	O-N=O
	1345.22	N-O
	802.04	O-N=O

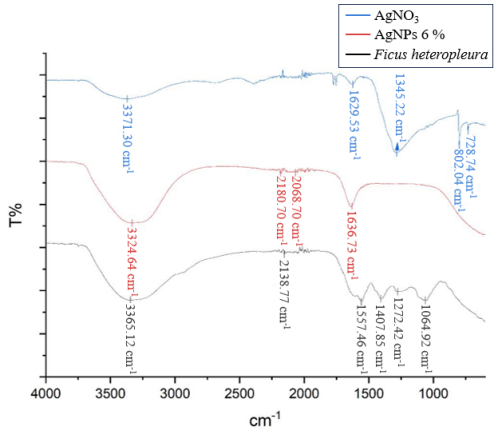

Figure 4. FTIR characterization results of aqueous extract, 6% AgNPs, and $AgNO_3$

In the synthesis results of AgNPs, a notable shift is observed in the peak of the absorption band from the wave number 3365.12 cm^{-1} to 3324.64 cm^{-1}, indicating the presence of OH stretching vibrations (red) and thereby signifying the existence of hydroxyl groups within the reducing agent. Phenolic compounds within this extract contain –OH groups capable of metal binding, serving as electron donors to Ag^+ ions for Ag nanoparticles. Additionally, the wavenumber 2180.70 cm^{-1} signifies the presence of C≡C stretching vibrations, indicative of chemical constituents in *Ficus heteropleura* leaf extract coordinating with metal surfaces, forming small quantities of silver nanoparticles.

Moreover, the peak wavenumber of 1636.73 cm^{-1} illustrates the vibration of the C=O bond, representing a carbonyl group formed in the reductant post-reduction of silver nanoparticles. Furthermore, the free electrons on the O atom of C=O offer a capping agent function in the formed silver nanoparticle bioreductor [19], indicating the potential for protein binding to the nanoparticle surface through free amine groups or cysteine residues, acting as stabilizing agents for AgNPs. Lastly, the abundance of polyphenol groups in *Ficus heteropleura* leaf extract significantly contributes to reducing Ag^+ ions to Ag^0 (Ag nanoparticles) and plays a pivotal role in forming stable AgNPs [3].

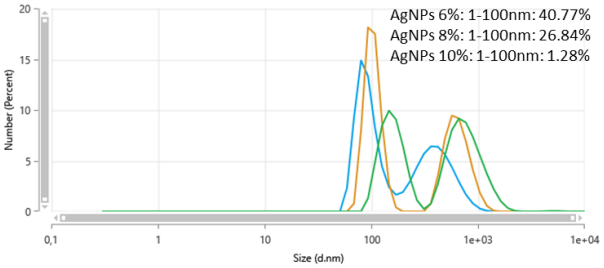

Figure 5. PSA results of AgNPs in different variations

Figure 4 displays a wavenumber shift, suggesting an interaction between the functional groups and the nanoparticles. The spectrum shift observed in mistletoe palm leaf extract following the formation of silver nanoparticles, particularly in the OH, CH, C=C, C≡C, and CO groups, indicates the involvement of these groups in the reduction reaction of silver metal [20].

3.6. PSA Characterization

The results of the synthesis of AgNPs were characterized by PSA at varying concentrations of 6, 8, and 10% and aqueous extract from *Ficus heteropleura* leaves. The characterization results can be seen in Figure 5. Characterization utilizing PSA was conducted to ascertain the size distribution of silver nanoparticles synthesized from *Ficus heteropleura* extract. The characterization results (Figure 5) reveal that 6% AgNPs exhibit a silver nanoparticle size within the range of 1- 100 nm, accounting for 40.77% of the total. For 8% AgNPs, the proportion of silver nanoparticles with sizes ranging from 1-100 nm is 26.84%, whereas for 10% AgNPs, it is only 1.28%.

The variability in particle sizes indicates that the produced AgNPs exhibit diverse sizes owing to nanoparticle agglomeration, wherein particles aggregate, resulting in non-uniform particles [6]. Agglomeration is driven by a mechanical-chemical process associated with the physical binding mechanism facilitated by a high-speed centrifuge. The increased rotational speed generates higher kinetic energy, leading to more frequent particle collisions, enabling particles to interact and

amalgamate into larger agglomerates [6]. The presence of AgNPs within the size range of 1-100 nm exhibits strong antimicrobial activity against *Escherichia coli*. The small particle size facilitates the adhesion of AgNPs to bacterial cell walls and enables penetration into bacterial cells, thereby disrupting bacterial activity.

3.7. Antibacterial Activity Test

The results of the *Escherichia coli* antibacterial activity test from AgNPs with concentrations of 4, 6, and 8% using the disk diffusion method used to determine antibacterial activity can be seen in Table 7. Figure 6 illustrates the ability of AgNPs to impede bacterial growth, as evidenced by the width of the clear zone surrounding the paper disc on the cultured media. A broader clear zone indicates more potent inhibitory activity against bacterial growth. Table 7 confirms the inhibitory effect of colloidal AgNPs against *Escherichia coli* bacteria, resulting in clear zones around the paper discs. The average clear zone for 6% AgNPs measured 8.9 mm. The average clear zones at 12% and 18% concentrations were 9.86 mm and 11.03 mm, respectively. In contrast, the positive control (Chloramphenicol 2%) exhibited an average clear zone of 23.3 mm.

The 8% AgNPs exhibited inhibitory activity against *Escherichia coli* bacteria, resulting in a 3.03 mm average clear zone. Meanwhile, the 12% and 18% AgNPs showed average clear zones of 8.8 mm and 9.7 mm, respectively. The positive control (2% chloramphenicol) revealed an average clear zone of 24.0 mm. Additionally, the negative control showed no inhibition zone against *Escherichia coli*.

The large diameter of the clear zone indicates the strong inhibitory activity of 8% AgNPs against *Escherichia coli*. The effectiveness of AgNPs against *Escherichia coli* underscores their potential as antimicrobial agents. The resistance observed in Gram-negative bacteria, such as *Escherichia coli*, may be attributed to the structure of their cell walls. Gram-negative bacteria possess a robust permeability barrier, characterized by a thin layer of lipopolysaccharide on the outer membrane, which can impede the penetration of AgNPs solutions [21].

Several studies have been conducted to investigate the antibacterial activity of AgNPs synthesized from extracts of oil *Ficus heteropleura* leaves against Gram- negative bacteria, such as *Escherichia coli*. These investigations suggest that AgNPs may adhere to the surface membrane of bacterial cells, potentially disrupting cell permeability and penetrating into the cell interior, thereby affecting membrane permeability and respiration [22]. The primary components of bacterial cell membranes are proteins, with sulfur being a predominant element. These proteins interact with AgNPs, subsequently engaging with phosphorus-containing compounds like DNA, resulting in damage that culminates in a lethal effect on microorganisms [23].

The antibacterial activity of AgNPs relies on electrostatic interactions. Positively charged amino groups on AgNPs interact with negatively charged molecules on the bacterial cell surface, resulting in the permeabilization of the cell membrane and leakage of intracellular substances, culminating in cell demise. Additionally, AgNPs induce structural and physiological alterations in membrane permeability and potential, disrupting cellular homeostasis and ultimately leading to bacterial cell death [12].

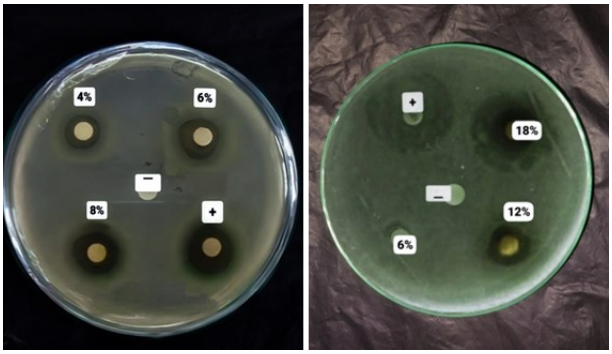

Figure 6. Activity test results for 6% and 8% AgNPs

Table 7. Test of the antibacterial activity of AgNPs of *Ficus heteropleura* against *Escherichia coli*

AgNPs sample	Concentration	Diameter of zone of inhibition			Average (mm)
		Petri dish 1 (mm)	Petri dish 2 (mm)	Petri dish 3 (mm)	
6% AgNPs	6%	9.4	9.1	8.2	8.9
	12%	10.2	9.2	10.2	9.86
	18%	11.0	11.05	11.05	11.03
	Chloramphenicol (+)	26.1	23.4	20.4	23.3
	DMSO (-)	-	-	-	-
8% AgNPs	6%	4.7	2.3	2.1	3.03
	12%	8.9	8.2	9.3	8.8
	18%	9.3	9.1	10.7	9.7
	Chloramphenicol (+)	23.6	25.4	23.1	24.0
	DMSO (-)	-	-	-	-

Antibacterial activity is greatly influenced by the particle size of the sample. Small AgNPs possess a larger surface area, facilitating greater interaction with numerous bacteria than larger ones. Consequently, small nanoparticles can exert a more pronounced antibacterial effect. Conversely, increasing particle size diminishes the surface area available for interaction with bacteria, thereby reducing the rate of bacterial elimination [24].

4. Conclusion

Silver nanoparticles (AgNPs) were successfully synthesized, evidenced by noticeable color changes and the emergence of specific wavelengths at 201.2 nm, 469.2 nm, 473.5 nm, 471.2 nm, and 468.3 nm from different concentrations of AgNPs. Among these concentrations, 6% AgNPs exhibited the most optimal wavelength at 473.5 nm. The functional groups identified from 6% AgNPs include O-H, C≡C, C≡N, and C=O. *Ficus heteropleura* extract displayed several functional groups: O-H, C≡C, aromatic C=C, C-H, and C-O aldehydes. The particle sizes of 6%, 8%, and 10% AgNPs within the range of 1-100 nm were found to be 40.77%, 26.845%, and 1.28%, respectively. *In vitro* antibacterial testing against *Escherichia coli* bacteria demonstrated moderate antibacterial activity for 6% and 8% AgNPs.

Acknowledgment

We thank the Directorate of Learning and Student Affairs, Ministry of Education, Culture, Research and Technology, for supporting and funding the 8 Sector Student Creativity Program (PKM) 2022, under grant number 2489/E2/KM.05.01/2022.

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