



Phytosynthesis and Antibacterial Activity of Silver Nanoparticles of Aqueous Extracts of Gandaria (*Bouea macrophylla* G.) Leaves and Stem Bark

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ABSTRACT

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Nanoparticles, with size ranging from 1-100 nm, have numerous applications, particularly as antimicrobial agents. The present study was designed to synthesize silver nanoparticles (AgNPs) using aqueous extracts of gandaria (*Bouea macrophylla* G.) leaves and stem bark as bioreductants and investigate their antibacterial activity. Gandaria leaves and stem bark were extracted by boiling with water. Silver nanoparticles were prepared from each of the extracts, and the optimal concentrations of AgNO₃ for nanoparticle formation were determined with and without the addition of 1% polyvinyl alcohol (PVA). The phytosynthesized AgNPs were characterized using ultraviolet-visible (UV-Vis) spectrophotometry and Fourier transform infrared (FTIR) spectroscopy. The antibacterial activity of the AgNPs was evaluated against *Escherichia coli*, and *Stapilococcus aureus*. The results showed that the optimal AgNO₃ concentrations for AgNPs synthesis in the leaf and stem bark extracts were 1.5 and 2.0 mM, respectively. The addition of PVA affected AgNPs synthesis in the leaf extract, but not in the stem bark extract. The best time for the formation of AgNPs in the aqueous extract of gandaria leaves without, and with PVA were 3 days, and 5 days, respectively. In the aqueous extract of gandaria stem bark with and without PVA, the optimal time for AgNPs formation was 5 days. The antibacterial sensitivity test showed that AgNPs from the leaf and stem bark extracts of gandaria had the same inhibitory potential against the test bacteria. The findings from this study suggest that AgNPs with antibacterial potential can be prepared using gandaria extracts as bioreductants.

Keywords: Antibacterial activity, Gandaria bark, Gandaria leaves, Phytosynthesis, Polyvinyl alcohol, Silver nanoparticles

Introduction

Nanotechnology is a field of engineering science that focuses on creating materials and functional structures at the nanoscale. It includes the synthesis of materials, one of which is nanoparticles, which have properties or characteristics different from their bulk size. Nanoparticles have a size range of 1–100 nm and are used in electronics, agriculture, and medicine, especially as antibacterial and antifungal agents. Their synthesis can be carried out using several methods. The green synthesis (phytosynthesis) method is a frequently employed technique that produces metal nanoparticles by utilizing naturally occurring components produced from plants, as bioreductants.^{1,2} One of the nanoparticles that can be synthesized using the green synthesis method is silver nanoparticles (AgNPs).^{3–5} Plant extracts containing secondary metabolites can be used to produce AgNPs which have antibacterial and antifungal properties.^{6–8} The gandaria plant (*Bouea macrophyll* G.) is one of the plants that can be employed as a bioreductor in the synthesis of AgNPs.

Silver nanoparticles are unstable because they tend to aggregate during the process of synthesis. This aggregation results in clumping of silver nanoparticles, the clumps grow larger and become large particle (bulk). The stability of silver nanoparticles plays a very important role in their characterization and application. Therefore, it is necessary to incorporate stabilizing agents or coating molecules such as polyvinyl alcohol (PVA) in the synthesis of AgNPs to prevent clumping, and control the size of the AgNPs formed.^{9–12} A previous study by Bijang *et al.* (2023) employed water extract of gandaria (*Bouea macrophyll* G.) seeds as a bioreductant in the synthesis of AgNPs. The plant extract acts as a reducing agent for converting AgNO₃ Ag⁺ into Ag⁰ at different concentrations of AgNO₃.¹³ The present research aimed to synthesize and investigate the antibacterial activity of AgNPs using a bioreductant from the water extract of gandaria (*Bouea macrophyll* G.) leaves and bark.

Materials and Methods

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The present research aimed to synthesize and investigate the antibacterial activity of AgNPs using a bioreductant from the water extract of gandaria (*Bouea macrophyll* G.) leaves and bark.

Materials and Methods

Reagents and Equipment

Some of the reagents used include distilled water (aquabides), silver nitrate (AgNO_3), polyvinyl alcohol (PVA). The equipment used include a set of glassware (Pyrex), a magnetic stirrer, an analytical weighing balance (Ohaus), a micropipette (DragonLAB), UV-Vis spectrophotometer (Shimadzu 1800), Fourier transform infrared (FTIR) spectrophotometer.

Plant collection and identification

Fresh leaves and stems of gandaria tree were collected on July 7, 2023, from Poka Village, Teluk Ambon District, Maluku, Indonesia, located at coordinates -3.659057° latitude and 128.187583° longitude. The plant materials were identified and authenticated at the Laboratory of Botany, Faculty of Mathematics and Natural Sciences, Pattimura University, and was deposited with a voucher code 32.

Extraction of gandaria leaves

Ten grams (10 g) of freshly harvested gandaria leaves were thoroughly washed with water and dried. The dried leaves were cut into pieces and ground with a blender. A total of 0.5 g of finely ground gandaria leaf powder was poured into an Erlenmeyer flask, 100 mL of distilled water was added. The mixture was heated to boiling at 110°C with constant stirring with the aid of a magnetic stirrer until complete extraction. The extract was filtered to obtain an extract with a concentration of 0.5% (w/v).

Extraction of the gandaria tree stem bark

Ten grams (10 g) of fresh gandaria tree stem bark were thoroughly washed and air-dried. The dried bark was cut into pieces and ground with a blender. Distilled water (100 mL) was added to 0.5 g of the powdered stem bark of gandaria tree in an Erlenmeyer flask. The mixture was boiled while stirring using a magnetic stirrer until completely extracted. The extract was filtered to obtain aqueous extract of gandaria tree bark with a concentration of 0.5% (w/v).

Preparation of a silver nitrate solution

A total of 1.062 g of AgNO_3 powder was dissolved in 250 mL of distilled water and stirred to make a 2.5 mM AgNO_3 stock solution. To prepare 0.5, 1.0, 1.5, and 2.0 mM solutions of AgNO_3 , aliquots of 10, 20, 30, and 40 mL of the 2.5 mM AgNO_3 stock solution were pipetted into a 50 mL volumetric flask, and distilled water was added up to the mark.

Preparation of a 1% polyvinyl alcohol solution

A total of 5 g of PVA powder was heated with distilled water in a 250 mL volumetric flask to make a 2% (v/v) PVA solution. Then, 25 mL of the 2% PVA solution was pipetted into a 50 mL volumetric flask to

make a PVA concentration of 1% (w/v). Aquabides were then added up to the mark.

Determination of the optimal concentration of AgNO_3 for nanoparticle synthesis

The synthesis of AgNPs was carried out by mixing AgNO_3 solution with water extract of the leaves and bark of gandaria tree. The optimal concentration of AgNO_3 solution at 0.5, 1.0, 1.5, 2.0, and 2.5 mM was determined for subsequent nanoparticle synthesis. Determination of the optimal concentration of AgNO_3 was carried out by mixing each AgNO_3 solution concentration with water extract of the leaves and bark of the gandaria tree in a ratio of 10:3 (v/v). The mixture was reacted by stirring for 15 minutes, after which a yellowish-brown colouration was observed. Then, the maximum absorption wavelength was determined using a UV-Vis spectrophotometer.

Synthesis of silver nanoparticles

Two approaches were used in the synthesis of AgNPs. In the first approach, PVA was not used (sample A). A total of 3 mL of a 0.5% (w/v) water extract of gandaria tree leaves and bark were transferred into a glass beaker marked AgNO_3 1.5 and 2.0 mM. Then, 10 mL of each concentration of AgNO_3 solution was added drop by drop using a burette, and the mixture was stirred for 15 minutes. A yellowish-brown colouration was observed. The absorbance of the resulting mixture was measured on day 1, 2, 3, 4, 5, and 6 using a UV-Vis Spectrophotometer. In the second approach, PVA was added to the reaction mixture (sample B). An aliquot of 5 mL of 1% PVA solution was added to 3 mL of water extract from the leaves and bark of the gandaria tree in a glass beaker. The mixture was stirred using a magnetic stirrer and 1.5 mM AgNO_3 solution was added in a dropwise manner using a burette until a colour change occurred. The same procedure was repeated using 2.0 mM AgNO_3 solution. The volume ratio of AgNO_3 : water extract of leaves and bark of the gandaria tree: PVA was 10:3:5 (v/v/v).

UV-Vis spectrophotometric analysis of silver nanoparticles

After preparing 1 mL of AgNPs at two different concentrations of 1.5 and 2.0 mM AgNO_3 , they were placed in a cuvette, and their absorbance was measured between 380 and 800 nm. The formation of AgNPs was indicated by the appearance of an absorbance peak at wavelength of 410 nm. Each the samples was subjected to the same UV-Vis characterization procedure from day one to day six of storage. Distilled water was used as the blank.

Fourier transform infrared spectroscopic analysis of silver nanoparticles

Silver nanoparticles formed from water extracts of the leaves and bark of gandaria tree were centrifuged. The resulting pellets were dried using an oven. The AgNPs powder was then analyzed using a Shimadzu-FTIR spectrometer. FTIR spectroscopy was used to determine the functional groups contained in the water extract of the leaves and bark of gandaria tree that are responsible for reducing Ag^+ to Ag^0 in the process of silver nanoparticles synthesis.

Evaluation of the antimicrobial activity of the silver nanoparticles

Test organisms

The test microorganisms namely; *Escherichia coli* and *Staphylococcus aureus* were obtained from the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Pattimura University collection. The organisms were selected based on their pharmacological and clinical significance. An ose of each microbes was taken into test tubes containing 5 mL of 0.9% NaCl. The microbial solution was standardized to 0.5 McFarland in 15 minutes to be used as inoculum.

Antibacterial activity screening

The antibacterial activity of the synthesized AgNPs was evaluated against the test bacteria using the agar well diffusion method. Molten

nutrient agar was prepared, and about 20 mL of the molten agar was poured into Petri dishes, and allowed to set.

Wells of about 6-7 mm in diameter each were bored on the agar using a sterile cork borer. The total wells were three each for the agar inoculated with the test organisms, and two each for the negative and positive control agar. AgNPs of Gandaria leaf and stem bark water extracts (40 μ L each) was added into the wells and then incubated at 37°C for 24 hours. The photographs were taken and the inhibition zone diameter was measured. DMSO (40 μ L) was used as the negative control, while 40 μ L of amoxicillin (2%) was used as the positive control.

Results and Discussion

Optimal concentration of silver nitrate solution for nanoparticle synthesis

Optimization of the AgNO₃ solution was carried out using a UV-Vis spectrophotometer to determine the best concentration for the synthesis of silver nanoparticles. In this study, concentration range of 0.5, 1.0, 1.5, 2.0, and 2.5 mM of AgNO₃ was used. The UV-Vis absorption spectrum of AgNPs as a function of AgNO₃ concentration is presented in Figure 1. Based on the results of UV-Vis absorption measurements of the various concentrations at a wavelength of 400-500 nm, maximum absorbance was recorded in the range of 430-440 nm. This is consistent with the surface plasmon resonance (SPR) characteristics of AgNPs, which appeared in the 380-450 nm range.¹⁴ The results of the optimization of the AgNO₃ concentration of the two samples showed that 1.5 mM was the optimal concentration for the gandaria leaf extract (EADG), and 2.0 mM was the optimal concentration for the gandaria stem bark extract (EABG). These optimal concentrations were used for further analysis.

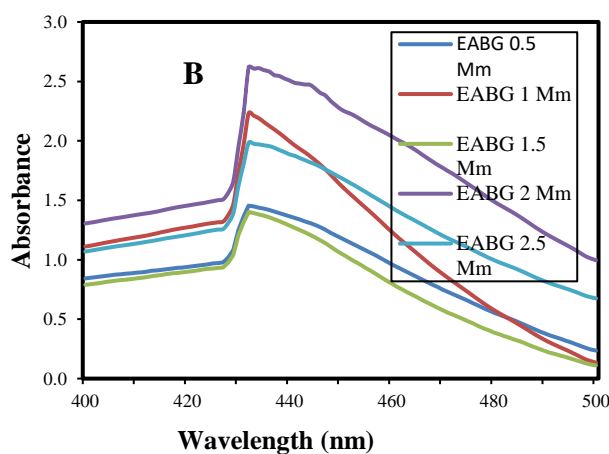
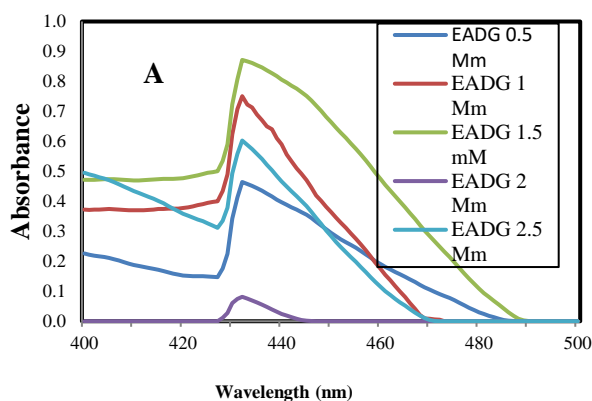


Figure 1: Ultra violet-visible (UV-Vis) spectra of different concentrations of silver nanoparticles (AgNPs).

A: Gandaria leaf aqueous extract; B: Gandaria stem bark aqueous extract

Biosynthesized silver nanoparticles

Silver nanoparticles were successfully synthesized using water extracts of the leaves and stem bark of the gandaria tree. When AgNO₃ solution as a precursor of AgNPs at a volume ratios of extract:AgNO₃ (3:10), and extract:AgNO₃:PVA 1% (3:10:5) were reacted using the different concentrations of AgNO₃, it was found that 1.5 mM of AgNO₃ for the leaves, and 2 mM of AgNO₃ for the stem bark gave the desired colour change. This indicates that the functional groups in the aqueous extract of the gandaria tree leaves and stem bark initiated a bioreduction process, reducing Ag⁺ ion to Ag⁰. It is proposed that the reduction reaction occurred between phenolic compounds in the extracts and Ag⁺ ions. Figure 2 shows the proposed reduction reaction facilitated by the phenolic compounds in the extracts to form AgNPs. The resulting colour changes are depicted in Figure 3.

Optimal time for silver nanoparticle formation

From the UV-Vis spectra as presented in Figure 4, silver nanoparticles of gandaria leaf extract prepared from 1.5 mM AgNO₃ without the addition of PVA resulted in a wavelength of maximum absorption (λ_{max}) of 430 nm with a maximum absorbance value of 2.55 on the third day (H3). Meanwhile for the nanoparticles of gandaria stem extract prepared with 2.0 mM AgNO₃, maximum absorbance value of 3.00 was obtained on the fifth day (H5) at λ_{max} 430 nm. On the other hand, silver nanoparticles of gandaria leaf extract prepared with 1.5 mM AgNO₃, and modified with 1% PVA produced the same absorbance value of 2.55 at 430 nm as for the unmodified sample, but this was attained on the fifth day (H5). For the AgNPs of gandaria stem extract prepared with 2.0 mM AgNO₃, and modified with 1% PVA resulted in a higher absorbance value of 4.30 at 430 nm in the same time frame of five days (H5) as for the unmodified sample. It can be seen that silver nanoparticles prepared with 1.5 and 2.0 mM AgNO₃ without the addition of PVA had different AgNPs formation times, with AgNPs produced from 1.5 mM AgNO₃ having a faster formation time than those obtained from 2.0 mM AgNO₃. Addition of PVA influenced the AgNPs formation times for the leaf extract of gandaria. In this sample, PVA prolonged the silver nanoparticle formation time to 5 days (H5), whereas, sample without PVA formed AgNPs on the third day (H3). In contrast, PVA did not influence AgNPs formation time for the stem bark extract of gandaria. For this sample, silver nanoparticles were formed on the fifth day (H5) with or without PVA modification. This is because more Ag⁰ is produced at a higher concentration of AgNO₃ used in the stem bark extract. Figure 5 showed that AgNPs of the stem bark extract has a higher UV-Vis absorption than that of the leaf extract. Furthermore, Ag⁰ nanoparticle aggregation in the sample produced from gandaria stem bark extract could not be stopped using PVA at the same concentration as that used for gandaria leaf extract. This is because concentration has a great influence on reaction rate. A higher concentration of AgNO₃ was used in the stem bark extract, so it is expected that more Ag⁰ will be produced (Figure 5), as evident in the absorbance value of the AgNPs of the stem bark extract which was higher than that obtained for AgNPs of the leaf extract. For this reason, the use of PVA at the same concentration for both the leaves and stem bark samples will not be effective in preventing Ag⁰ nanoparticle aggregation in the stem bark AgNPs.

FTIR spectra of silver nanoparticles

Characterization of AgNPs using FTIR was one of the analyses used to compare the functional groups in the water extracts of the leaves and bark of gandaria tree. Apart from comparing functional groups, it can also be used to determine the functional groups that play a role in the bioreduction process of Ag⁺ to Ag⁰. Figure 6 shows the FTIR spectra of EADG and EABG modified with PVA and without PVA. Silver nanoparticles synthesized from PVA-modified EABG exhibited a broad absorption at 3437 cm⁻¹ which is characteristic of OH group (Figure 6a). The alkyl group (-CH) is indicated by the strong absorption at a wave number of 2912 cm⁻¹, while the aromatic C=C absorption is characterized by the weak absorption at wave number of 1643 cm⁻¹. Meanwhile, strong absorptions at wave numbers of 1446 and 1382 cm⁻¹ indicate -CH₃ group, and absorption of the -CO group at wave numbers of 1139 and 1093 cm⁻¹.

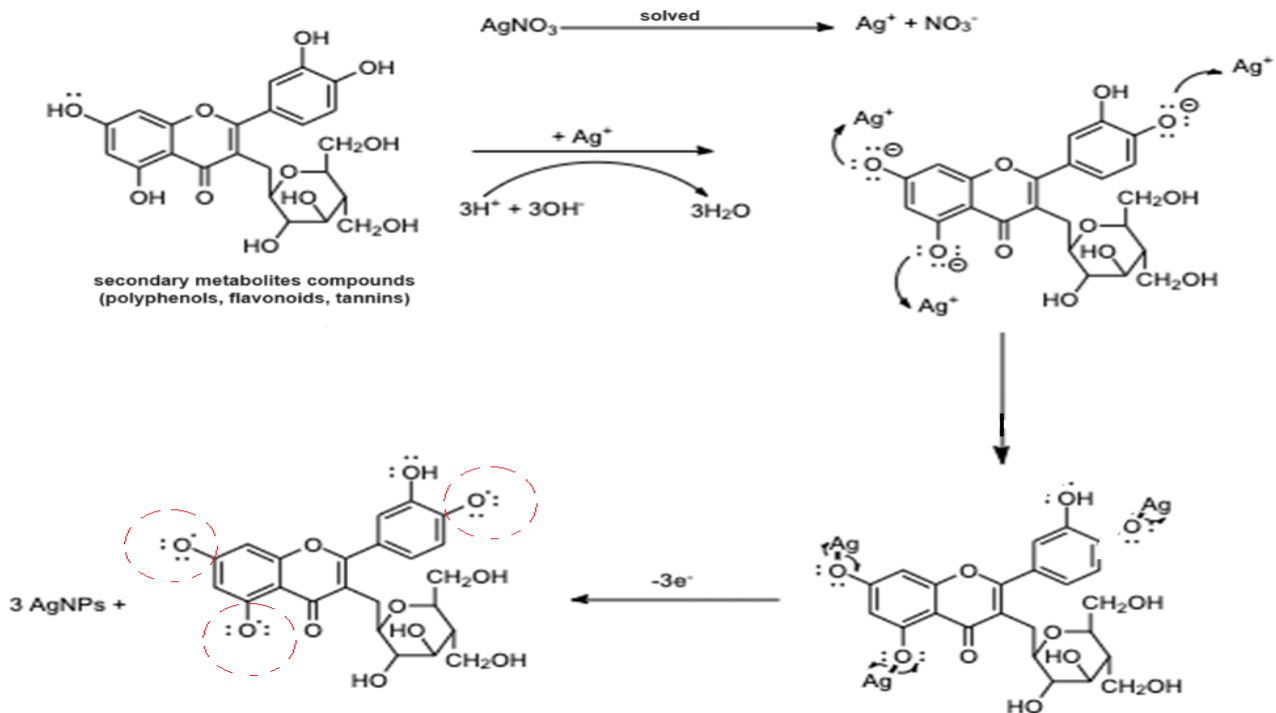


Figure 2: Proposed chemical reaction for the formation of silver nanoparticles (AgNPs) from plant bioactive compounds

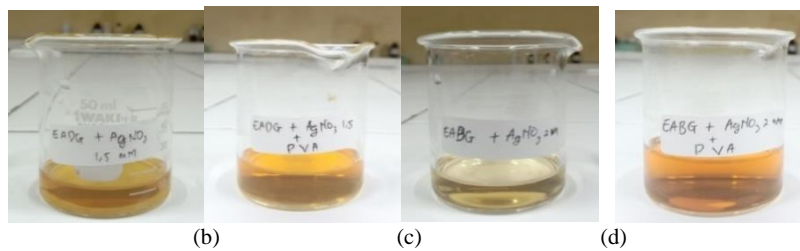


Figure 3: Colour change in silver nanoparticle synthesis

Synthesis using an aqueous extract of gandraia leaves as bioreductant without PVA (A); with the addition of 1% PVA (B); to a 1.5 mM AgNO_3 solution, and aqueous extract of gandraia tree stem bark without PVA (C); with the addition of 1% PVA (D) to 2 mM AgNO_3 solution.

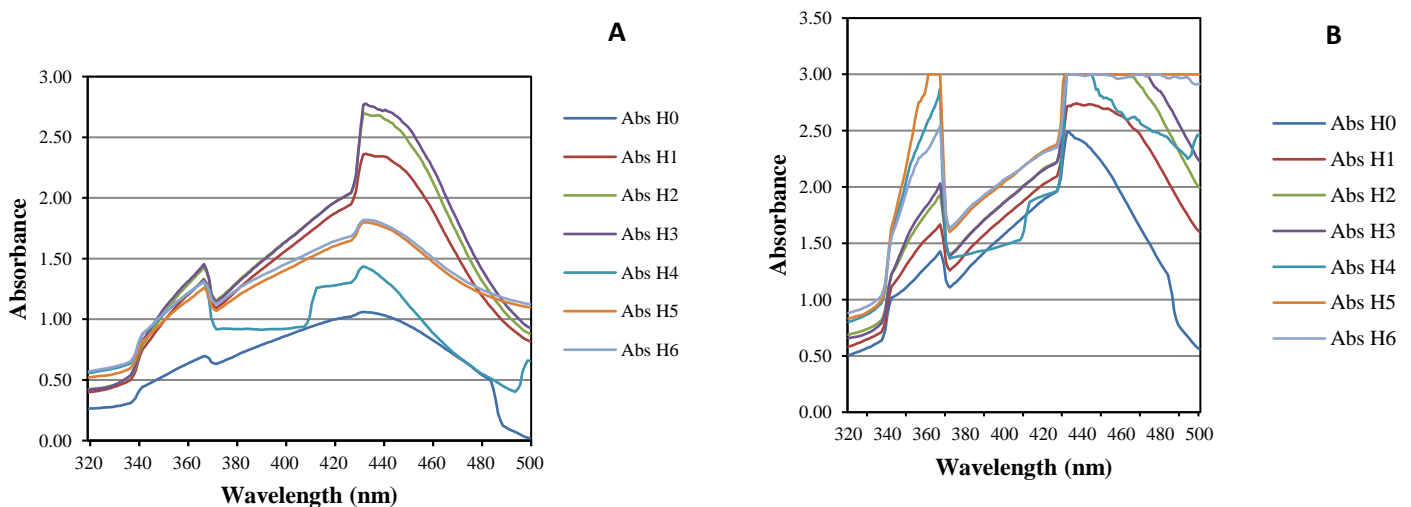


Figure 4: Ultra violet-visible (UV-Vis) spectra of silver nanoparticles without PVA at various storage times (H0-H6)
A: Gandaria leaf aqueous extract 1.5 mM; B: Gandaria stem bark aqueous extract 2.0 mM

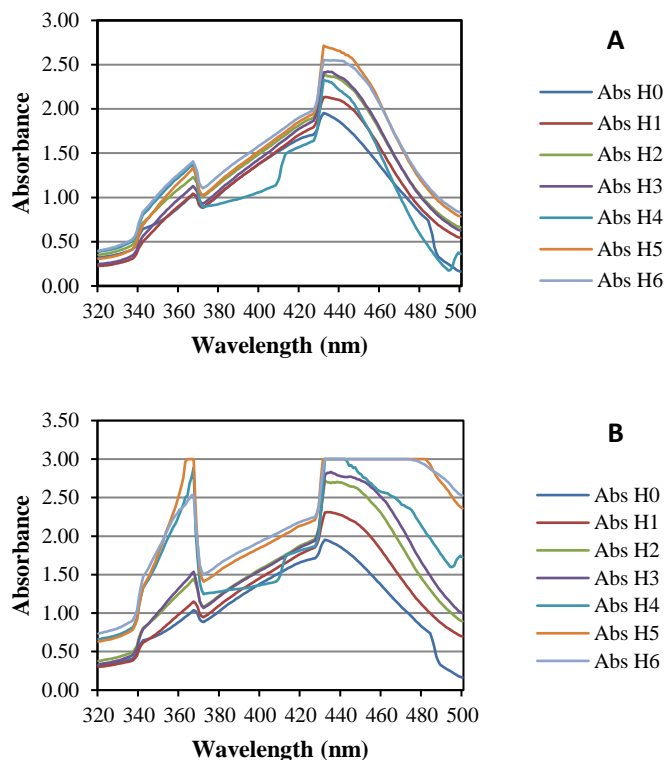


Figure 5: Ultra violet-visible spectra of PVA-modified silver nanoparticles (AgNPs) at different storage times (H0-H6). A: Gandaria leaf aqueous extract 1.5 mM PVA; B: Gandaria stem aqueous extract 2 mM PVA

The FTIR spectrum of AgNPs synthesized from EABG without PVA showed a broad but weak absorption at a wave number of 3425 cm^{-1} , which is typical of absorption from a phenolic $-\text{OH}$ group (Figure 6b). Additionally, the $-\text{CH}$ group exhibited absorption at 2956 , 2920 , and 2848 cm^{-1} ; the $\text{C}=\text{C}$ group exhibited absorption with a broad peak at 1629 cm^{-1} ; the $-\text{CH}_3$ group exhibited sharp absorption at 1382 cm^{-1} , and the $-\text{CO}$ group exhibited absorption at 1020 cm^{-1} . As illustrated in Figure 6c, the IR spectrum of AgNPs synthesized from PVA-modified EADG had an absorption characteristic of the stretching vibration of the $-\text{OH}$ group, which was shown at a wave number of 3415 cm^{-1} . The polar group in the $-\text{OH}$ bond can interact with silver ions quite effectively. Furthermore, the weak absorption at wave numbers of 2953 , 2920 , 2848 cm^{-1} are indicative of alkyl ($-\text{CH}$) group, the weak absorption at 1629 cm^{-1} was due to aromatic $\text{C}=\text{C}$ absorption. Meanwhile, the strong absorption at 1384 cm^{-1} indicates the $-\text{CH}_3$ group, and the $-\text{CO}$ group absorption was displayed at 1018 cm^{-1} . The FTIR spectrum of AgNPs synthesized using EADG without the addition of PVA showed a similar pattern but a different intensity as that obtained for EADG AgNPs modified with PVA. This indicates that both samples have the same functional groups. The spectrum of EADG AgNPs without the addition of PVA showed absorption peak at 3402 cm^{-1} , which is characteristic of $-\text{OH}$ stretching vibrations in alcohols and phenolic compounds. Furthermore, the absorption of alkyl groups was visible around 2920 cm^{-1} . The $\text{C}=\text{C}$ group exhibited a broad peak at 1625 cm^{-1} , the $-\text{CH}_3$ group displayed sharp absorption at 1381 cm^{-1} , and the $-\text{CO}$ group exhibited absorption at 1018 cm^{-1} . The IR spectra of the two samples showed that PVA-modified EABG had a larger absorption with higher intensity than EABG without PVA (Figure 6). The wave number shift between EABG and EADG without PVA as well as samples modified with PVA are visible in Figure 6. The wave number shift indicate an interaction between AgNPs and functional groups in the plant extracts, resulting in the redox reaction that occur during AgNPs synthesis. Functionalities such as $-\text{OH}$, $\text{C}=\text{C}$, $-\text{CO}$, and $-\text{CH}_3$ groups play a role in the silver metal reduction reaction. Previous studies have suggested that carbonyl and carboxylate groups play a role in nanoparticle

stability, and that $-\text{OH}$ group take part in the oxidation-reduction processes.^{16,17}

Antibacterial activity of silver nanoparticles

The antibacterial activity of the silver nanoparticles against *Escherichia coli* and *Stapilococcus aureus* was examined using the agar well diffusion method. As shown in Figure 7, the synthesized AgNPs inhibited the growth of the test bacteria. This was evident from the diameter of the clear zone of inhibition. The wider the inhibition zone diameter, the stronger the microbial growth inhibitory activity. The inhibitory capacity of the AgNPs from gandaria leaves and stem bark is relatively the same for the two bacteria tested (Table 1). The antibacterial mechanism of silver nanoparticles involves nanoparticle adhesion to the bacterial cell surface, which changes the properties of the cell membrane.¹⁸⁻²⁰ Due to their small size and large surface area, nanoparticles can easily penetrate cell membrane with a high contact surface area. Silver nanoparticles enter bacterial cells, causing DNA damage due to the Ag^+ released from the AgNPs. Released silver ion (Ag^+) interact with cell wall and cytoplasmic proteins especially sulfur-containing proteins.

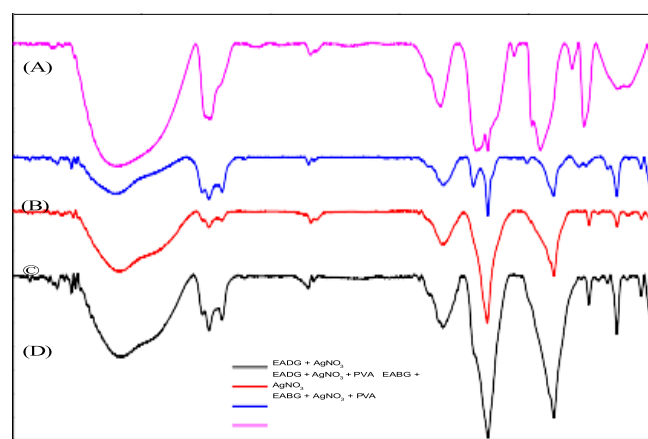


Figure 6: Fourier transform infrared (FTIR) spectrum of silver nanoparticles of EADG and EABG without PVA and modified with PVA.

A: PVA modified EABG; **B:** EABG without PVA; **C:** PVA modified EADG; **D:** EADG without PVA; PVA: Polyvinyl alcohol; EADG: Gandaria leaf aqueous extract; EABG: Gandaria stem aqueous extract

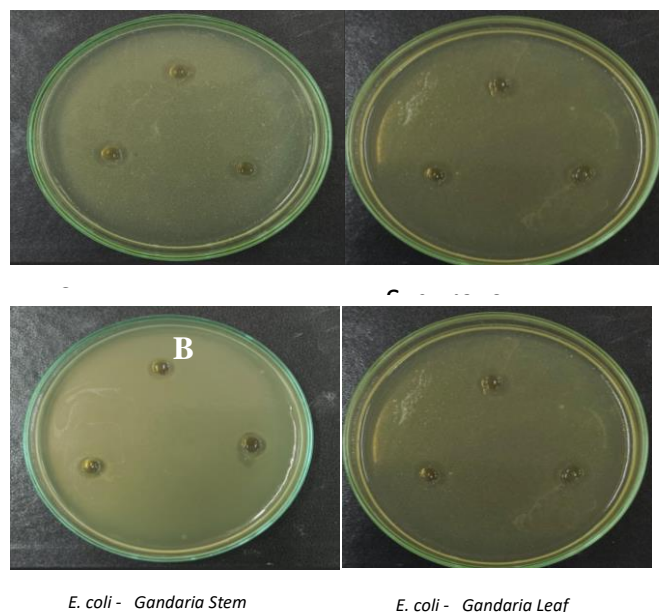


Figure 7: Antibacterial potential of silver nanoparticles (AgNPs) from gandaria leaf and stem bark extracts against *S. aureus* (A) and *E. coli* (B)

Table 1: Antibacterial activity of silver nanoparticles against *E. coli* and *S. aureus*

Sample	Inhibition Zone Diameter (mm)		Method
	Replicate 1	Replicate 2	
PP Leaf extract		6	5.5
AgNPs stem bark extract	5.5		4.5
Negative Control (Seed extract + water)	0	0	Agar well diffusion (<i>S. aureus</i>)
Negative Control (Leaf extract + water)	0	0	
Negative Control (stem bark extract + water)	0	0	
Positive Control (Amoxicilin 2 %)		35.5	35.5
AgNPs Seed extract	5		5
AgNPs Leaf extract	6		5
AgNPs stem bark extract	5.5		5.5
Negative Control (Seed extract + water)		0	0
Negative Control (Leaf extract + water)	0		0
Negative Control (stem bark extract + water)	0		0
Positive Control (Amoxicilin 250 mg)		32	32
			Agar well diffusion (<i>E.coli</i>)

Conclusion

The findings from the present study revealed that the optimal concentrations of AgNO₃ for AgNPs synthesis from the leaf and stem extracts were 1.5 and 2.0 mM, respectively. In contrast to stem extract, the inclusion of PVA influenced AgNPs synthesis in the leaf extract by potentially extending the AgNPs production time. The optimal time for the formation of AgNPs in the water extract of gandaria leaves without PVA, and with PVA modification were 3 days, and 5 days, respectively. In the water extract of gandaria stems without PVA and with PVA, the optimal time was the fifth day. The findings of this research suggest that AgNPs with antibacterial activity can be successfully produced using aqueous extracts of gandaria leaves and stem bark as bioreductants.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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