Polytechnic Journal

Polytechnic Journal

Volume 15 | Issue 1 Article 2

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How to Cite This Article

Nurfitri, Waode Erimelga; Astuti, Suryani Dyah; Amruloh, Yazid Muhammad; Nurdin, Desy Zahrotul Istiqomah; Zaidan, Andi Hamim; Yaqubi, Ahmad Khalil; and Syahrom, Ardiyansyah (2025) "Silver Nanoparticle Synthesis Using Bay Leaf Extract (Syzygium polyanthum) and Antibacterial Effectiveness Testing Against Staphylococcus Aureus and Escherichia Coli Bacteria," *Polytechnic Journal*: Vol. 15: Iss. 1, Article 2.
DOI: https://doi.org/10.59341/2707-7799.1847

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORIGINAL ARTICLE

Silver Nanoparticle Synthesis Using Bay Leaf Extract (*Syzygium polyanthum*) and Antibacterial Effectiveness Testing Against *Staphylococcus aureus* and *Escherichia coli* Bacteria

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Abstract

The synthesis of silver nanoparticles (AgNPs) using bay leaf extract (*Syzygium polyanthum*) and their testing against bacteria aims to evaluate the antibacterial effectiveness on two types of bacteria, namely *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)*. The synthesis method used is environmentally friendly, with bay leaves serving as a reducing agent. The synthesis process was carried out by mixing silver nitrate ($AgNO_3$) solution with bay leaf extract at concentrations of 2 mM and 3 mM in a ratio of 1:30 and 1:40 between $AgNO_3$ and bay leaf extract. The use of bay leaf extract to create silver nanoparticles (AgNPs) was verified by a yellowish-brown color shift. UV-Vis spectrophotometry revealed that the absorbance was highest between 435 and 442 nm. Increased absorbance on day 7 compared to day 1 indicated better nanoparticle production and stability throughout time. At doses of 3 mM $AgNO_3$, antibacterial activity tests revealed greater inhibition zones; *E. coli* showed 12.7 ± 0.4 mm and *S. aureus* showed 15.4 ± 0.6 mm. Inhibition zones at two mM were 9.3 ± 0.3 mm for *E. coli* and 11.2 ± 0.5 mm for *S. aureus*. These findings demonstrate the potential of bay leaf extract as an environmentally acceptable ingredient for nanoparticle production and antibacterial applications by confirming the increased susceptibility of *S. aureus* to AgNPs. The antibacterial test using the disk diffusion method showed that silver nanoparticles have a significant inhibitory effect on the growth of both bacteria. A larger inhibition zone is formed at a concentration of $AgNO_3$ of 3 mM, with *S. aureus* being more sensitive to changes in concentration compared to *E. coli*. This result shows the potential of bay leaf extract as a natural and environmentally friendly antibacterial agent.

Keywords: AgNPs, Bay leaf (*Syzygium polyanthum*), Green synthesis, *Staphylococcus aureus*, *Escherichia coli*

1. Introduction

There are two types of bacteria that are very commonly known by the public, namely *Staphylococcus aureus* and *Escherichia coli*. *E. coli*, commonly known as *E. coli*, is a pathogenic bacterium often involved in various clinical infections, particularly the most frequent cause of diarrhea. Meanwhile, *S. aureus* is a gram-positive bacterium that can cause skin infections, pneumonia, endocarditis, and food poisoning [1]. On the other hand, *E. coli*, which typically resides in the human intestines, can become pathogenic when certain strains cause urinary tract infections, gastroenteritis,

Received 9 November 2024; accepted 29 December 2024.
Available online 6 February 2025

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https://doi.org/10.59341/2707-7799.1847

and even meningitis. Infections caused by these two bacteria are becoming increasingly difficult to treat due to the emergence of antibiotic resistance, which drives the search for alternative treatments [2,3].

One of the nanoparticles that can be synthesized using the green synthesis method is silver nanoparticles. In recent years, the development of silver nanoparticles (AgNPs) technology has become a research focus, particularly for their potential function as antibacterial agents [4]. Silver nanoparticles are known to have the ability to inhibit the growth of microorganisms through various mechanisms, such as damaging bacterial cell membranes, disrupting enzyme functions, and generating toxic free radicals that are harmful to bacterial cells. However, environmentally friendly synthesis methods based on natural materials are increasingly needed to reduce the toxic impact and high costs of chemical synthesis methods [5].

Silver nanoparticles inhibit bacterial growth through a specific mechanism. The antibacterial mechanism of silver nanoparticles begins with the release of silver ions (Ag^{+}) [6]. These ions bind to the thiol (-SH) groups present on surface proteins, forming a more stable S-Ag group on bacterial cells. As a result, the protein becomes inactive and membrane permeability decreases. Next, silver compounds enter the cells, damaging the DNA structure, which ultimately leads to cell death [7]. Fig. 1 shows the antibacterial mechanism of silver nanoparticles (AgNP) against bacterial cells (AI).

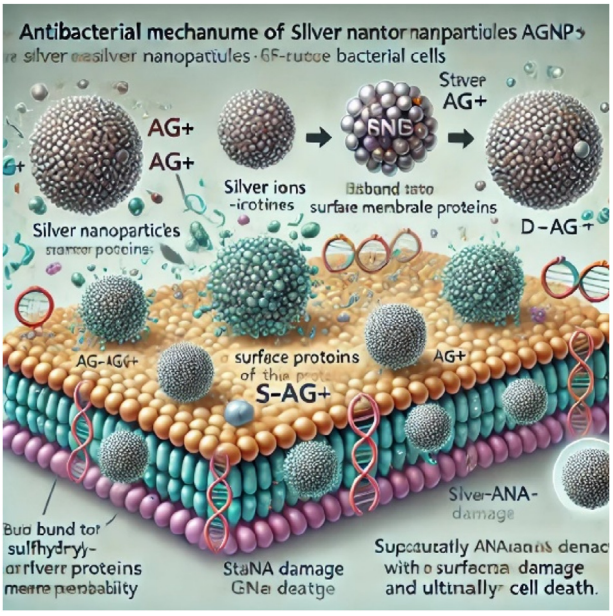


Fig. 1. Illustration of the antibacterial mechanism of silver nanoparticles (AgNP) against bacterial cells (AI).

Antimicrobial compounds can be in the form of synthetic chemical compounds (inorganic) or natural products (organic). Bay leaf (*Syzygium polyanthum*) is one type of medicinal plant that has good antimicrobial properties [8]. The chemical compounds in bay leaves that can have antimicrobial effects include phenolic compounds, quinones, flavonoids, coumarins, essential oils, terpenoids, lectins, polypeptides, alkaloids, polyamines, isothiocyanates, thiosulfinate, glucosides, and polyacetylenes [9].

Staphylococcus aureus is a Gram-positive bacteria that appears purple under a microscope because of its thick peptidoglycan coating, which helps it retain crystal violet stain. Skin infections, pneumonia, and sepsis are among the many illnesses it is known to cause. It usually forms clusters of spherical cocci. Certain bacteria resist many drugs, including methicillin-resistant *S. aureus* (MRSA). On the other hand, the Gram-negative, rod-shaped bacteria *E. coli* stains pink because of its weaker peptidoglycan layer and outer lipid membrane. Some *E. coli* strains, including O157:H7, may cause infections and foodborne diseases, even though most are innocuous. *E. coli* may flourish in aerobic and anaerobic environments because it is a facultative anaerobe [7].

One group of phenolic compounds that has antioxidant properties and plays a role in preventing cellular damage and its components by reactive free radicals is flavonoids [10,11]. The antioxidant role of flavonoids occurs by donating their hydrogen atoms or through their ability to chelate metals, existing in the form of glycosides (containing glucose side chains) or in a free form known as aglycone [12]. The extract of bay leaves, which includes young leaves, semi-mature leaves, and mature leaves, has very strong antioxidant properties. Therefore, bay leaf extract contains bioreductors, making this extract potentially capable of producing metal nanoparticles such as silver nanoparticles [13]. Based on a literature review, the use of this leaf extract to produce silver nanoparticles has not yet been conducted [14,15].

Bay leaves (*S. polyanthum*) in nanoparticle synthesis are not only environmentally friendly but also open up broad application opportunities in the medical field, such as in the production of wound care products, antibacterial coatings for medical instruments, and antimicrobial-based health products [16,17]. Thus, this experiment aims to synthesize silver nanoparticles using bay leaf extract and test their antibacterial activity against *S. aureus* and *E. coli*, which is expected to provide additional literature for future development as an alternative

solution in the treatment of bacterial infections resistant to antibiotics, as well as to support the development of more sustainable and environmentally friendly nanoparticle synthesis methods.

2. Methodology

2.1. Effect of varying $AgNO_3$ concentrations and control comparisons

The specific $AgNO_3$ concentrations of 2 mM and 3 mM were chosen to evaluate the effect of varying silver ion concentrations on the synthesis of silver nanoparticles and their antibacterial activity. The 2 mM concentration was selected to observe the baseline antibacterial effect of silver nanoparticles at a lower concentration. The 3 mM concentration was compared to determine the enhanced antibacterial activity at a higher silver ion concentration. As for the antibacterial tests, controls included blank discs with no silver nanoparticles or bay leaf extract to ensure that any observed antibacterial effect was solely due to the synthesized nanoparticles. Additionally, standard antibiotic discs were used as positive controls to compare the efficacy of AgNPs against *S. aureus* and *E. coli*.

2.2. Preparation of bay leaf extract

5 g of bay leaf powder is weighed and placed into a 250-mL glass beaker, then 100 mL of distilled water is added and heated to a boil for 15 min, after which it is allowed to cool. After reaching room temperature, the boiled water is poured into a centrifuge tube and centrifuged for 30 min at a speed of 3000 rpm. After centrifuging the extract, it was then filtered using Whatman filter paper no. 42. The boiled water can be used directly for the synthesis process of silver nanoparticles.

2.3. Preparation and synthesis of silver nanoparticles from bay leaf extract (S. polyanthum)

Preparation of $AgNO_3$ solution with concentrations of 2 mM and 3 mM. The preparation of $AgNO_3$ solutions with concentrations of 2 mM and 3 mM involves weighing 0.170 g and 0.255 g of $AgNO_3$ powder, respectively, and dissolving them in distilled water to a final volume of 500 mL, followed by mixing until homogeneous. The synthesis process is carried out by mixing 30 mL of the 2 mM $AgNO_3$ solution and 40 mL of the 3 mM $AgNO_3$ solution, measured using graduated cylinders, and placed into separate 250 mL Erlenmeyer flasks. Then, 1 mL of bay leaf extract is added to each of the Erlenmeyer flasks. The mixture was stirred with a magnetic stirrer for 15 min at a speed of 250 rpm at a temperature of 50 °C, then cooled and placed into a vial. It can then be used directly for the characterization process of silver nanoparticles using UV-Vis, FTIR, and PSA testing. Fig. 2 shows the results of the synthesis of silver nanoparticles from variations of concentrations 2 mM and 3 mM with ratios of 1:30 ml and 1:40 ml.

2.4. Testing the antibacterial activity of bay leaf extract against bacteria Staphylococcus aureus and Escherichia coli

The testing method used is the Kirby–Bauer method, which is a diffusion method using paper discs. First, the bacterial suspension to be tested is evenly spread over the surface of Mueller Hinton Agar (MHA) to ensure homogeneous bacterial growth throughout the medium. After that, the disc paper that has been soaked in antibiotics or antimicrobial compounds, specifically the synthesized extract of bay leaves at concentrations of 2 mM and 3 mM, is placed on the media. The dish is then incubated at 37 °C for 16–24 h. After incubation, the inhibition zone around the disk, which indicates the area where bacterial growth has stopped, is measured to assess the effectiveness of the compound, which will then be measured using a caliper. The larger the inhibition zone, the more effective the compound is in inhibiting bacterial growth [18].

2.5. Statistical analysis

The statistical analysis was performed to assess the significance of the observed findings. Data were analyzed using appropriate statistical tests, including analysis of variance (ANOVA) for comparisons between multiple groups, followed by post-hoc Tukey's test for pairwise comparisons. The results were considered statistically significant if the p-value was less than 0.05.

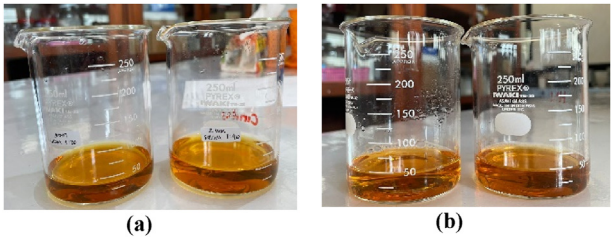


Fig. 2. The results of the synthesis of silver nanoparticles from variations of concentrations 2 mM and 3 mM with ratios of 1:30 ml and 1:40 ml (a) after being subjected to magnetic stirring at a concentration of 2 mM (b) after being subjected to magnetic stirring at a concentration of 3 mM.

3. Results and discussion

3.1. Synthesis of silver nanoparticles

Synthesis of silver nanoparticles using a reducing agent from bay leaf extract by mixing a heated $AgNO_3$ solution at 50 °C with magnetic stirring and then cooling it to room temperature. The purpose of heating and stirring is to accelerate the reaction for the formation of silver nanoparticles in the solution. The color change of the solution is influenced by the reduction process of silver ions in the organic compounds of the plants [19]. The color that indicates the formation of nanoparticles is a pale brownish yellow. Silver nanoparticles (AgNP) that were formed were analyzed using UV-Vis spectrophotometry, PSA, and FTIR and will subsequently be tested for their antibacterial properties. Fig. 3 shows an illustration of the stages of biosynthesis of nanoparticles from bay leaf extract (*S. polyanthum*). The solution obtained with concentration variations of 2 mM and 3 mM as indicators for the formation of silver nanoparticles is visually marked by a color change of the solution from clear to yellowish-brown.

Silver nanoparticles (AgNPs) were created throughout the experiment using bay leaf extract. The solution's color changed from colorless to yellowish-brown to visually demonstrate the nanoparticle creation.

3.2. UV-Vis spectrophotometer analysis

A UV-Vis spectrophotometry analysis was conducted to confirm the formation of silver nanoparticles from the synthesis solution. Absorbance and wavelength measurements were taken using a UV-Vis spectrophotometer in the wavelength range of 200–700 nm. The synthesis process of silver nanoparticles was carried out over 7 days, with measurements taken on day 1 and day 7. Silver nanoparticles can be formed when there is a maximum absorption at a wavelength of 400 nm–450 nm.

UV-Vis spectrophotometry, conducted on days 1 and 7, confirmed the successful synthesis of AgNPs by displaying distinctive absorption peaks between 435 and 442 nm.

These results indicate a shift in wavelength and a change in absorbance over time and with varying concentrations. A slight shift in the maximum wavelength indicates that the size and distribution of the nanoparticles may be stable, but there are minor variations caused by changes in the concentration of $AgNO_3$ and the extract ratio [20]. On the 7th day, the increase in absorbance values indicates better formation of nanoparticles or an increase in the concentration of nanoparticles dissolved in the solution. The increase in absorbance on the 7th day also shows that the particles formed have developed better during the synthesis process.

Thus, the difference in absorbance and wavelength between day 1 and day 7 illustrates the process of formation and stabilization of silver nanoparticles from $AgNO_3$ solution and bay leaf extract. More stabilized nanoparticles tend to have higher absorbance and a slight shift in the maximum wavelength. Table 1 shows the result of the maximum wavelength on day 1.

Table 2 show the wavelength and absorbance of silver nanoparticles as a function of time with concentrations of 2 mM and 3 mM at ratios of 1:30 and 1:40. The maximum wavelength produced varies from 435 to 438 nm on day 1 and 439–442 nm, indicating the formation of silver nanoparticles at all concentrations and compositions used. The nanoscale size produced proves that the extract of bay leaves has the potential as a reducing agent in the synthesis of nanoparticles. Figs. 4 and 5 show the results of UV-Visible on day 1 and results of UV-Visible on day 7.

3.3. Characterization of silver nanoparticles with FTIR

FTIR analysis serves to identify the functional groups involved in the metal ion reduction process.

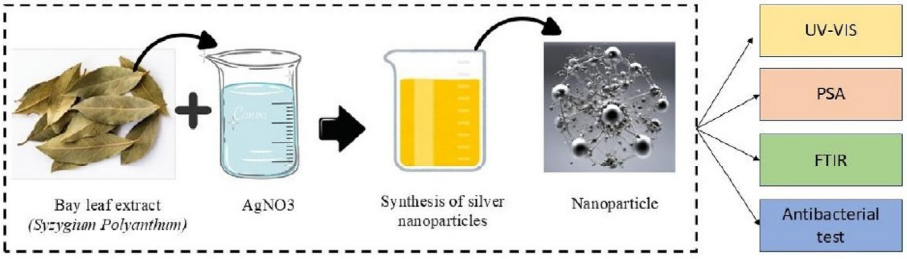


Fig. 3. Stages of biosynthesis of silver nanoparticles from bay leaf extract (Syzygium polyanthum).

Table 1. Absorbance and maximum wavelength for different $AgNO_3$ concentrations and extract ratios on day 1.

Concentration $AgNO_3$ (mM)	Comparison of $AgNO_3$ and extract (mL)	Wavelength (nm)	Abs
2 mM	1:30	438	1.623
	1:40	436	1.266
3 mM	1:30	439	1.643
	1:40	435	1.267

Table 2. Absorbance and maximum wavelength for different $AgNO_3$ concentrations and extract ratios on day 7.

Concentration $AgNO_3$ (mM)	Comparison of $AgNO_3$ and extract (mL)	Wavelength (nm)	Abs
2 mM	1:30	442	2.152
	1:40	441	1.7
3 mM	1:30	442	2.184
	1:40	439	1.764

The characterization results using FTIR are shown in Figs. 6–8.

In the FTIR spectrum of bay leaf powder (Fig. 5), the peak around 3415.93 cm^{-1} indicates the presence of hydroxyl groups (O-H), which are commonly found in phenolic or flavonoid compounds. These compounds are widely recognized for their strong antibacterial activity. This activity occurs through several mechanisms, including damaging the bacterial cell membrane, disrupting cell permeability, or inhibiting essential enzymes in the bacterial life cycle. Hydroxyl groups from phenolic compounds, for example, are capable of forming hydrogen bonds with bacterial cell components, thereby disrupting their structure and function [21].

Another peak at 2922.16 cm^{-1} and 2852.72 cm^{-1} indicates the presence of alkane groups (C-H), which generally originate from terpenoid compounds. Terpenoids are known as bioactive components in medicinal plants that possess antimicrobial activity, either through the inhibition of microbial growth or through synergistic activity with other components to enhance antibacterial effects. Terpenoids can penetrate the lipid layers of bacterial cell membranes, leading to damage to the integrity of the cells [22].

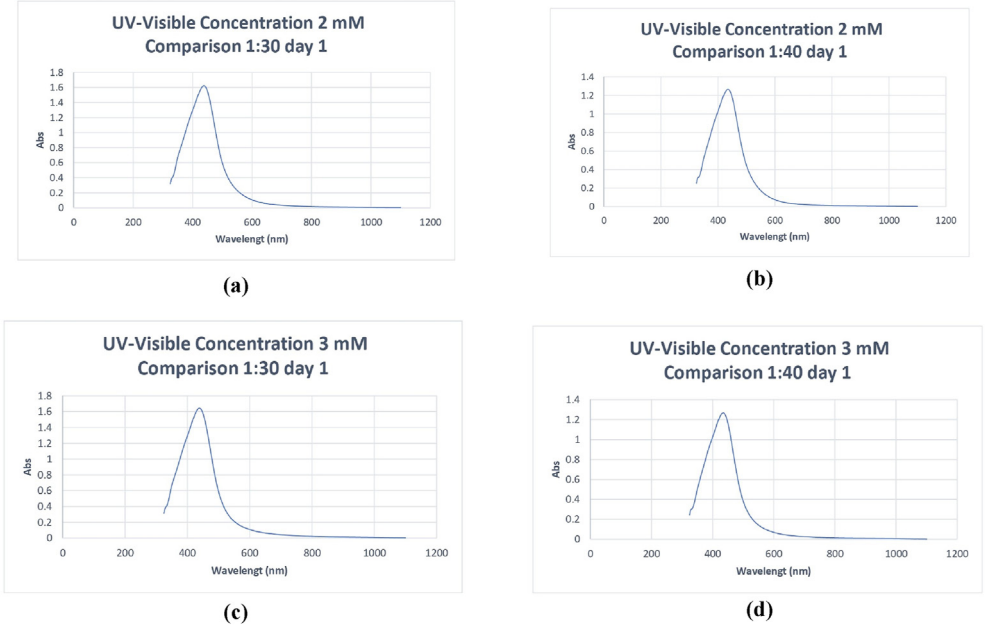


Fig. 4. Results of UV-Visible on day 1: (a) UV-Vis concentration 2 mM ratio 1:30 mL; (b) A) UV-Vis concentration 2 mM ratio 1:40 mL; (c) UV-Vis concentration 3 mM ratio 1:30 mL; (d) UV-Vis concentration 3 mM ratio 1:40 mL.

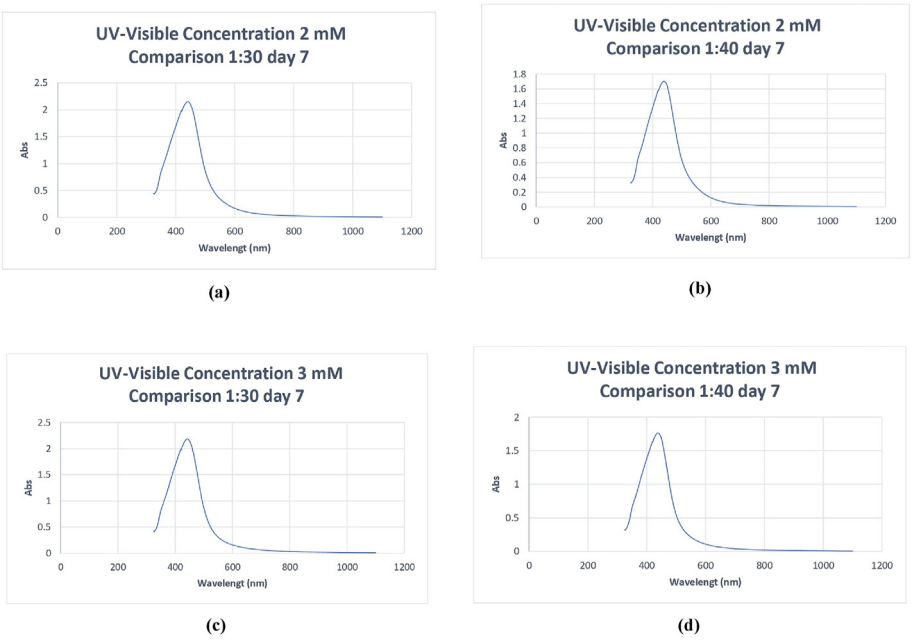


Fig. 5. Results of UV-Visible on day 7: (a) UV-Vis concentration 2 mM ratio 1:30 mL; (b) A) UV-Vis concentration 2 mM ratio 1:40 mL; (c) UV-Vis concentration 3 mM ratio 1:30 mL; (d) UV-Vis concentration 3 mM ratio 1:40 mL.

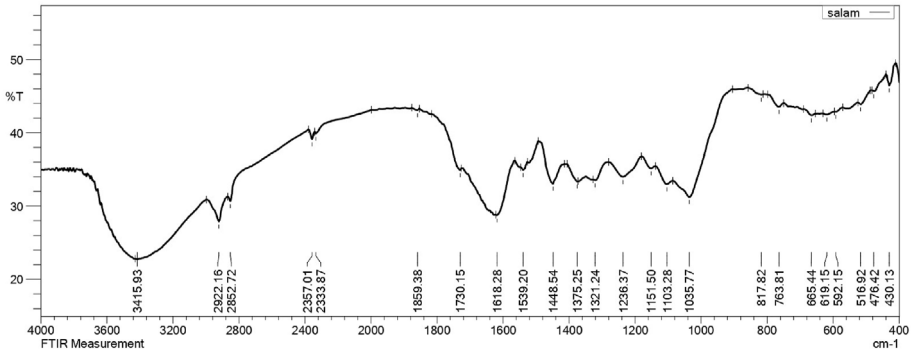


Fig. 6. FTIR spectrum of bay leaves powder.

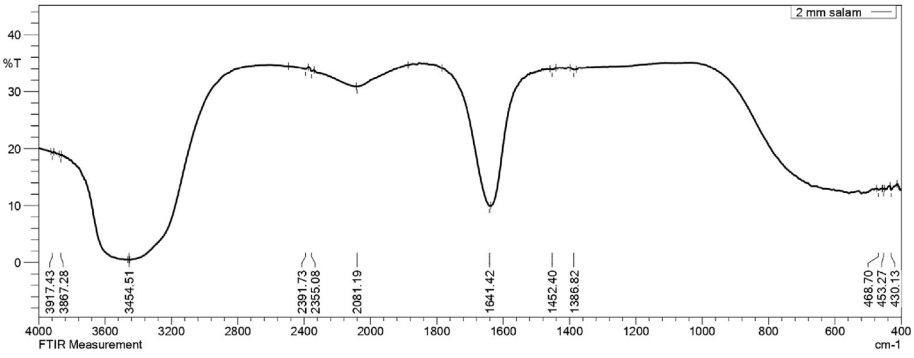


Fig. 7. FTIR spectrum of bay leaf extract in 2 mM $AgNO_3$ solution.

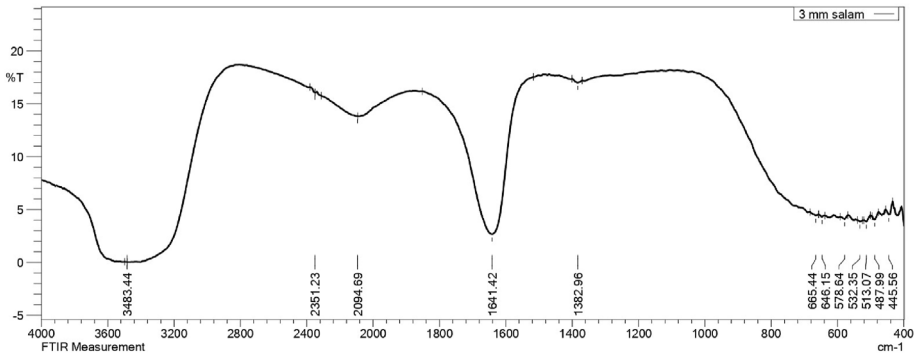


Fig. 8. FTIR spectrum of bay leaf extract in 3 mM $AgNO_3$ solution.

Meanwhile, the carbonyl peak (C=O) at 1730.15 cm^{-1} indicates the presence of compounds such as aldehydes or ketones. Carbonyl compounds play a crucial role in antibacterial mechanisms, particularly through their ability to react with proteins or other components within bacterial cells, resulting in the inactivation of essential enzymes or damage to the cell membrane. Aldehyde, for example, is known to interact with amine groups in bacterial proteins, thereby disrupting the structure and function of those proteins [23].

The FTIR spectrum of the bay leaf extract at a concentration of 2 mM (Fig. 6) shows the presence of hydroxyl groups (O-H) at 3454.51 cm^{-1}, which again confirms the presence of phenolic compounds that have the potential to act as antibacterial agents. The peak at 1641.42 cm^{-1} indicates the presence of a double bond C=C, which is found in aromatic compounds such as flavonoids. This compound is known to interact with bacterial components, such as cell membranes and enzymes, thereby inhibiting the growth of pathogenic microorganisms [24].

Additionally, the spectrum at a concentration of 2 mM also shows peaks at 1452.40 cm^{-1} and 1386.82 cm^{-1}, which correspond to the deformation of the C-H group, commonly found in terpenoid compounds. These terpenoids not only function as antibacterial agents but also play a role in inhibiting bacterial replication by attacking the lipid components of the cell membrane. This compound can disrupt the fluidity and permeability of membranes, ultimately leading to leakage of cell contents and bacterial death [25].

At a higher concentration of 3 mM (Fig. 7), the FTIR spectrum shows a peak at 3483.44 cm^{-1}, indicating the presence of hydroxyl groups (O-H), which is consistent with the presence of phenolic compounds. This group plays an important role in antibacterial activity through mechanisms that damage bacterial cell membranes or inhibit enzymes that are essential in bacterial metabolism. Another peak, such as at 1641.42 cm^{-1}, indicates the presence of a carbonyl group (C=O), which is part of aldehydes or ketones, as well as a nitrile group (C ≡N) at 2094.69 cm^{-1}, which may play a role in microbial inhibition activity [26].

Overall, the presence of various bioactive functional groups such as hydroxyl, carbonyl, and terpenoids in the FTIR spectrum of the bay leaf extract confirms the antibacterial potential of this extract. The combination of phenolic compounds, terpenoids, and aromatics plays a role in disrupting bacterial growth through various mechanisms, including damage to cell membranes, disruption of enzyme functions, and inhibition of bacterial replication. To empirically test the antibacterial activity of bay leaf extract, methods such as agar disc diffusion can be used [27]. This method will provide a clear picture of the extent to which this extract can inhibit the growth of various types of pathogenic bacteria, both gram-positive and gram-negative. In further testing, this extract can be optimized for clinical applications as an antibacterial agent or in the food industry as an effective natural preservative in preventing the growth of harmful microorganisms [28].

Thus, the FTIR spectrum produced from various concentrations of bay leaf extract shows strong evidence of the presence of bioactive compounds with significant potential in the medical and industrial fields, particularly as safe and natural antibacterial agents.

3.4. Determination of silver nanoparticle size using a particle size analyzer (PSA)

The results of the silver nanoparticle synthesis were measured using a Particle Size Analyzer (PSA). The particle size distribution of the sample was then described using particle size analysis. This characterization supports the results obtained using a UV-Vis spectrophotometer. The results of the silver

nanoparticle size distribution determined using PSA are shown in Table 3 and Fig. 9.

Based on the results table of the PSA (Particle Size Analyzer) test displayed, it is evident that the particle size changes in accordance with the increase in concentration. At a concentration of 2 mM, the particle size produced is 39.88 nm, while at a concentration of 3 mM, the particle size decreases to 36.44 nm. Both meet the criteria for nanoparticle size, which ranges from 10 to 100 nm 16. From these results, it can be concluded that an increase in concentration leads to a decrease in particle size. This phenomenon may be caused by more intense interactions between molecules in the solution at higher concentrations, leading to the formation of smaller-sized particles. Smaller particles are generally more stable because they have a larger surface area, which allows for more interactions with their environment [29]. This result is consistent with the theory that an increase in precursor concentration during nanoparticle synthesis can lead to faster nucleation, resulting in smaller-sized particles. The nanoscale size produced proves that bay leaf extract has the potential as a reducing agent in nanoparticle synthesis [30].

3.5. Antibacterial test of silver nanoparticles against Staphylococcus aureus and Escherichia coli bacteria

This research conducted an inhibition zone test to measure the inhibitory power of silver nanoparticles from bay leaf extract against *S. aureus* and *E. coli* bacteria. This test used the disk diffusion method. The concentrations used were 2 mM and 3 mM with a ratio of 30 mL and 40 mL for $AgNO_3$ and 1 mL of bay leaf extract. The obtained inhibition zone was then measured with a caliper and the average measurement was calculated. Table 4 show the average results of the measurement of the clear zone diameter. Table 5 show the antibacterial test results (see Fig. 10).

Larger inhibition zones were seen at a three mM concentration of silver nitrate ($AgNO_3$) when the disk diffusion technique was used to assess the antibacterial activity of the produced AgNPs. The fact that *S. aureus* had a more excellent inhibitory zone than *E. coli* suggests that the bacteria is more susceptible to the effects of AgNPs. The structural variations between Gram-positive and Gram-negative bacteria may cause this outcome. While *E. coli's* outer membrane offers extra protection, *S. aureus's* thicker peptidoglycan layer may render it more susceptible to silver nanoparticles.

Based on the data in Table 4, it shows that the higher the concentration of the test substance and the smaller the ratio, the larger the inhibition zone for both *E. coli* and *S. aureus*. The difference in the inhibition zones produced between the two bacteria is caused by the diameter of the inhibition zones, which is greatly influenced by several factors, including the toxicity of the test substance, the diffusion ability of the test substance in the medium, the interaction between medium components, and the in vitro microenvironment conditions [31,32]. The concentration of a substance that acts as an antibacterial agent is one of the factors that determines its effectiveness in inhibiting or killing the growth of the tested bacteria [33]. Then, the size of the inhibition zone is influenced by several factors, namely the test microorganisms (strain and physiological characteristics of the bacteria), growth

Table 3. The synthesis results of silver nanoparticles from bay leaf extract measured using a particle size analyzer.

No	Concentration	Size of particle
1.	2 mM	39.88 nm
2.	3 mM	36.44 nm

Table 4. Average results of the measurement of the clear zone diameter.

Test bacteria	Comparison	Clear Zone Diameter (cm)	
		2 mM	3 mM
SA	1:30	7.515	7.61
	1:40	5.92	6.5
E. coli	1:30	6.155	7.41
	1:40	6.02	6.555

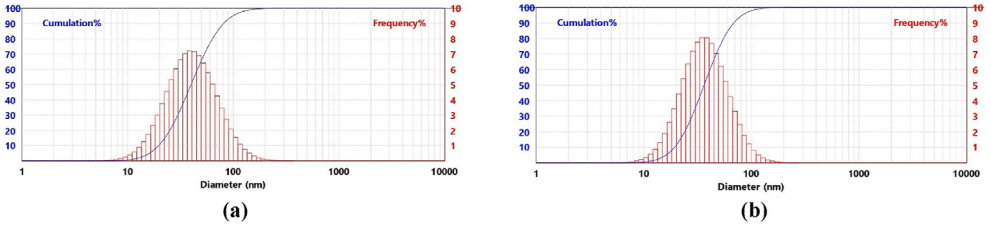
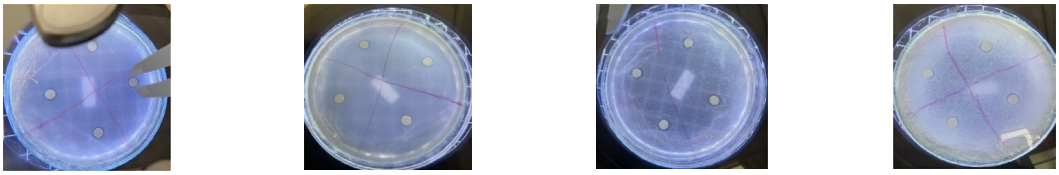
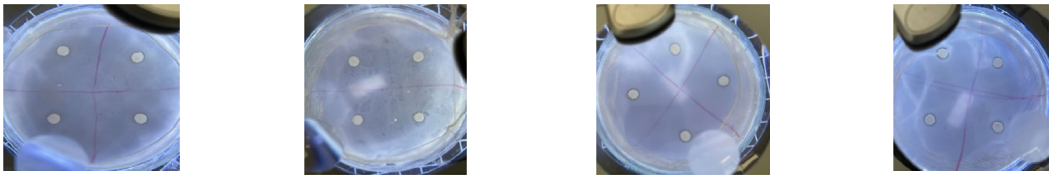


Fig. 9. PSA results (a) synthesis at 2 mM concentration (b) synthesis at 3 mM concentration.

Table 5. Image of antibacterial test results.

Test bacteria	Concentration of Synthesis			
	2 mM		3 mM	
	1:30	1:40	1:30	1:40
S. aureus				
E. coli				

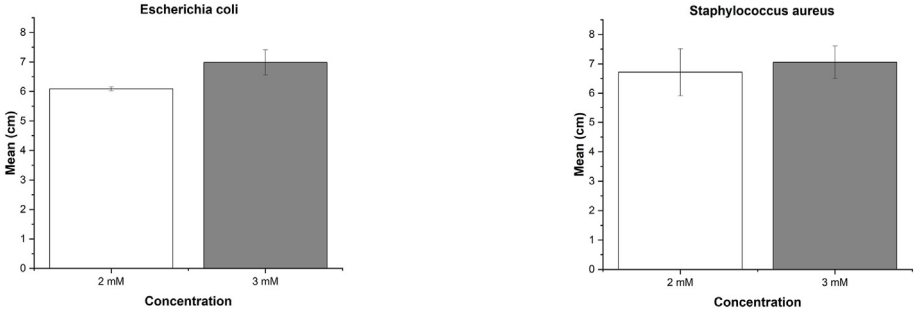


Fig. 10. Statistic of average results of the measurement of the clear zone diameter.

media, testing methods, and the diffusion rate of the substances [34].

Based on Tables 3 and 4, it shows the size of the inhibition zone based on antibacterial testing against *S. aureus* and *E. coli*. The categories of the inhibition zone can be determined as follows: a weak activity inhibition zone has a diameter of $\leq$5 mm, a moderate activity inhibition zone has a diameter of 6–10 mm, and a very strong inhibition zone has a diameter of $\geq$20 mm [35].

The results of the antibacterial test indicate that increasing the synthesis concentration from 2 mM to 3 mM enhances the effectiveness in inhibiting the growth of *S. aureus* (SA) and *E. coli (E. coli)*. At a concentration of 2 mM, SA produced a clear zone with a diameter of 7.515 cm at a ratio of 1:30, which decreased to 5.92 cm at 1:40, while *E. coli* showed a diameter of 6.155 cm, slightly decreasing to 6.02 cm at a more diluted ratio. At a concentration of 3 mM, the diameter of the clear zone for SA increased to 7.61 cm at a ratio of 1:30 and decreased to 6.5 cm at 1:40, whereas *E. coli* showed an increase in the clear zone from 7.41 cm at 1:30, slightly decreasing to 6.555 cm at 1:40. Overall, antibacterial activity is more effective at higher concentrations and more concentrated ratios, with SA being more sensitive to changes in concentration compared to *E. coli*.

The size of the clear zone formed indicates the strength of the inhibitory power; the larger the clear zone produced, the stronger the inhibition of bacterial growth. It can be concluded that the silver nanoparticles produced in this study have antibacterial properties.

4. Conclusion

The extract of Bay Leaves (*S. polyanthum*) has effective antibacterial activity against *S. aureus (SA)* and *E. coli* (*EC*), with effectiveness increasing alongside the concentration of the extract. At higher concentrations (3 mM) and a more concentrated ratio (1:30), a larger clear zone diameter is formed, indicating a stronger inhibitory effect on bacterial growth. *S. aureus* appears to be more sensitive to changes in concentration compared to *E. coli*, as evidenced by the more significant difference in clear zone diameter between the ratios of 1:30 and 1:40. Overall, these results indicate that bay leaf extract

can be a potential antibacterial agent, especially at higher concentrations.

Ethics information

There is no ethics information.

AI usage declaration

This study did not involve the use of AI.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

There is no funding.

Author's contribution statement

Conceptualization, W. E. N; Y. M.A; S.D A; D. Z. I. N; A. H. Z.; A. K. Y; methodology, W. E. N; D. Z. I. N; A. H. Z. software, W. E. N; Y. M.A; S.D A; A. K. Y, validation, W. E. N; Y. M.A; S.D A; D. Z. I. N; A. H. Z.; A. K. Y.; formal analysis, W. E. N; Y. M. A; S.D A; data curation, W. E. N; Y. M.A; S.D A; A. K. Y.; writing, review and editing, S.D A; A. H. Z.; A. K. Y.; supervision, S.D A; A. H. Z. project administration, W. E. N; Y. M.A.

Acknowledgement

The authors would like to thank the Biophysics Laboratory, Faculty of Science and Technology, Universitas Airlangga, for providing access to all the necessary equipment.

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