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Phytosynthesis, characterization of silver nanoparticles using *Pentapetes phoenicea* leaf extract and their antimicrobial effects on some food pathogens

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Abstract

The AgNPs used for this work were obtained from ethanolic extract of *Pentapetes phoenicea* leaves and AgNO_3 via the green synthesis method (natural reducing agent), which are found in a dark brown colour, which is attributed to the reduction reaction of Ag^+ ions to Ag^0 . As determined by TEM and XRD analyses, the nanoparticle size ranges from 10 to 40 nm and exhibits an approximately spherical morphology. The ethanolic extract of *P. phoenicea*, analysed through phytochemical screening, contains metabolites that act as reducing and stabilizing agents. Silver nanoparticles were formed by reacting the 5% ethanol extract at pH 12 for 15 min, resulting in an SPR peak at 403 nm. UV–Vis analysis over 2 days showed that the SPR peak remained stable between 400 and 403 nm. FTIR analysis shows silver nanoparticles' reduced absorption peak spectrum at a wave number of 760 cm^{-1} . The XRD and TEM results show that silver nanoparticles have an average crystal size of $17.9 \pm 2.03\text{ nm}$. Silver nanoparticles have a spherical shape with an average particle size of 18 nm, which can be seen in the TEM results' morphology. The silver nanoparticles synthesized with a low concentration of *P. phoenicea* extract demonstrated stronger inhibition of *E. coli* (MIC: $0.41\text{ }\mu\text{g/mL}$, MBC: $1.2\text{ }\mu\text{g/mL}$) than *S. aureus* (MIC: $0.20\text{ }\mu\text{g/mL}$, MBC: $0.6\text{ }\mu\text{g/mL}$). A delayed bactericidal effect was observed, with time-dependent inhibition noted through colony-forming unit counts at various intervals. While AgNPs are promising antibacterial agents for food-related applications, potential cytotoxicity to human cells or animals must be considered. Thus, AgNPs from *P. phoenicea* leaf extract show potential as an antibacterial agent against food-pathogenic bacteria.

Keywords Green synthesis, Characterization, Silver nanoparticles, *Pentapetes phoenicea* leaf extract, Antibacterial activity

Introduction

The current trend of nanoscience is experiencing rapid development; nanoscience is getting much attention because it is claimed to have unique optical and catalytic properties. The synthesis of silver nanoparticles (AgNPs) is a growing research field in materials science (Bamal et al. 2021). AgNPs have the potential to be utilized in many fields, namely technology, science, optics and biomedicine, because they exhibit unique properties, sizes and shapes. AgNPs have strong conductive and

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antibacterial properties and are suitable for use in the pharmaceutical field (Yu et al. 2021).

Process through chemical synthesis causes significant exposure to the environment and is toxic to mammals or other microorganisms where the composition of the water or soil microbial community changes after exposure to chemical residues left over from the synthesis. The development of safer nanoparticle synthesis methods has led to the production of nanoparticles by utilizing bioactive compounds as reducing agents, including plant extracts. This method is called green synthesis, namely environmentally friendly synthesis that utilizes secondary metabolites of plants (Rehman et al. 1677). Secondary metabolites are reported to be reducing agents, including flavonoids, hydroxyl groups and phenolics. Compounds from the extract have been reported to play a role in the rapid synthesis process, stabilizing agents and shape directors in the silver reduction process. Plant extracts are more profitable because of easier accessibility, both in obtaining bioactive compounds and during the synthesis process. In many cases, using plant bioactive compounds is relatively nontoxic, so it can help reduce the risk of exposure to silver ions (Nogueira et al. 2020). In the green synthesis process, it is essential to know that the concentration or amount of extract influences the formation time of nanoparticles, physical and chemical properties as a reducing agent, the concentration of AgNO_3 as a precursor, pH and temperature. Alkaline pH and Ag concentration significantly affect the average size distribution of AgNPs and can increase the reduction ability. Therefore, it is necessary to optimize the optimal conditions in the reaction process, which is expected to produce good synthetic conditions to produce silver nanoparticles that are relatively small in size and stable (Nkosi et al. 2024).

In nanoparticle studies, silver, nickel, gold, iron, zinc, platinum, selenium and palladium are frequently used metals. It is known that silver (Ag), in particular, is an essential factor that suppresses the proliferation of bacteria. "Ag" ion can prevent cell division and DNA replication. In chemistry and materials science (Nogueira et al. 2020; Nkosi et al. 2024), producing silver nanoparticles through ecologically friendly means is gaining popularity. Due to their small size, AgNPs bind to cell membrane proteins and catalyse the formation of reactive oxygen species in bacterial cells. Thus, they cause cell death due to oxidative stress. Many previous researchers have successfully synthesized silver nanoparticles using natural reducing agents from Malvaceae family plant extracts. From existing references and literature, the green synthesis method using plant extracts has excellent potential in synthesizing silver nanoparticles. The reduction reaction of Ag^+ to Ag^0 is carried out at room temperature until the

colour of the solution changes to brown (Miranda et al. 2022). Therefore, studying the influence of each material used in the green synthesis process is necessary.

Epidemiological concerns have arisen in recent years due to the emergence of bacterial strains resistant to antibiotics. Recent research indicates (Miranda et al. 2022; Butucel et al. 2022) that some bacteria that cause foodborne illnesses have acquired resistance to commonly used disinfectants, such as triclosan and quaternary ammonium compounds. As a result, there has been increasing interest in finding new, environmentally friendly and nontoxic antimicrobial agents for use as disinfectants. A valuable tool for developing new antimicrobial agents is nanotechnology, whose objective is the design of materials at the nanometric scale. The synthesis of nanoparticles has led to a wide range of applications, including biosensors, catalysts, anticancer agents, antimicrobial agents and targeted drug delivery systems. In recent years, the antimicrobial properties of various metallic nanoparticles, such as gold, zinc, platinum and other elements, have been reported. Silver is particularly notable for its long-established antimicrobial properties (Sánchez-López et al. 2020). Therefore, it is of great interest to evaluate the antibacterial activity of these nanoparticles.

In this study, AgNO_3 aqueous solution was reduced using *P. phoenicea* leaf ethanolic extract through a green synthesis method, where the extract acts as both the reducing and stabilizing agent in an environmentally friendly process. *P. phoenicea*, a weed belonging to the Malvaceae family. In this study, AgNPs obtained from *P. phoenicea* leaf were characterized, and their antibacterial and anticandidal activities against some foodborne pathogens were revealed by the MIC method. There is a lack of research on *P. phoenicea* leaves, so further research is being carried out regarding bioactivity based on secondary metabolite content and metallic nanoparticle synthesis.

Materials and methods

All chemicals used in this work were obtained from SD fine chemicals, Merck India, with 98% 99% purity and AR grade quality. Solutions were prepared with double distilled water with pH 7.02. This study used AgNPs with a 107 $\mu\text{g/mL}$ concentration in ethanolic extract of *P. phoenicea* leaves extract. *E. coli* O157:H7 and *Staphylococcus aureus* ATCC 23235 strains obtained from HI Media, India, were used and characterized in the Microbiology Laboratory.

Obtaining *P. phoenicea* plant leaf extract: Fresh *P. phoenicea* plant leaf collected from the agricultural fields, Guntur rural areas, Andhra Pradesh, India (Longitudes 16.257464, 80.420379). The leaf

powder was cut into small pieces to increase the contact surface, speeding up the extraction process. To carry out the process of obtaining the extract, around 143 g of plant material was weighed on an analytical balance, Mettler Toledo Balance XPR105DR (India), and placed in a 500-mL Erlenmeyer flask as suggested by Benjamin et al. (2022). A volume of 350 mL of 90% Ethanol was added in another flask of the same capacity. Then, the *P. phoenicea* leaf powder was added. The plant material immersed in the alcohol was kept under constant stirring on a magnetic stirrer (Remi Made 2L model) for 2 h at room temperature (29 ± 1 °C) (Fig. 1).

Preliminary phytochemical analysis: The phytochemical test for active compound content was carried out qualitatively (Muhamad et al. 2023). The qualitative test was a reagent test from the ethanol extract of *P. phoenicea* leaves dissolved in a small amount of solvent; then, phytochemical screening was carried out, which included steroids, flavonoids, alkaloids, saponins, tannins, quinones and triterpenoids.

Synthesis of silver nanoparticles

The optimization of extract pH was performed to identify conditions that can reduce Ag^+ to Ag^0 . SPR was monitored in the range of 400–500 nm immediately after the synthesis reaction began, then selected for use in the optimization process of extract concentration variations and Ag concentration variations with the formula; namely, 20 ml of 5% (w/v) ethanol extract each was reacted with 100 ml of 1 mM Ag, from now on referred to as (AgNP-1), 10% (w/v) ethanol extract was reacted with 100 ml of 1 mM Ag (AgNP-2), 5% ethanol extract with 2 mM Ag (AgNP-3), and 10% ethanol extract with Ag

2 mM (AgNP-4) (Fig. 2) (Hasnain et al. 2019). After optimizing the determination of extract and Ag, the effect on reaction time was optimized. Each formula was heated with a magnetic stirrer at a temperature of 80 °C with the stirrer stirring for 1 h, then continued with storage at room temperature for 1×24 h; UV–Vis spectro monitoring was carried out by sampling every 15 min for the first 1 h after mixing and after storage for 24 h to determine the SPR and absorbance. Synthesis is confirmed to successfully form AgNPs if SPR shows an absorption peak between 400 and 500 nm. The selected formula was centrifuged at 6000 rpm for 25 min to collect the AgNPs. The precipitate was collected and dried using freeze-dry.

pH optimization: pH optimisation was carried out as extract preparation to obtain optimal conditions in the reaction process, namely by adding 1% NaOH to the extract, which had been dissolved in distilled water and then reacted with 2 mM Ag; then, the wavelength was then measured using UV-Vis spectrophotometry, and the pH of the extract was adjusted. The absorption peak at

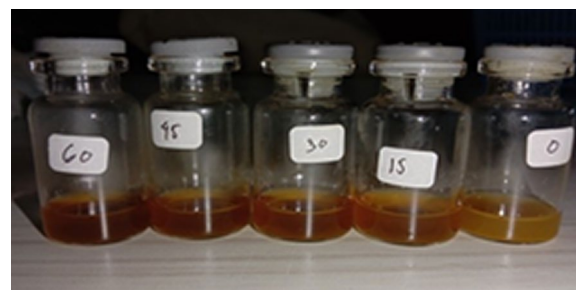


Fig. 2 Prepared AgNPs observed visually

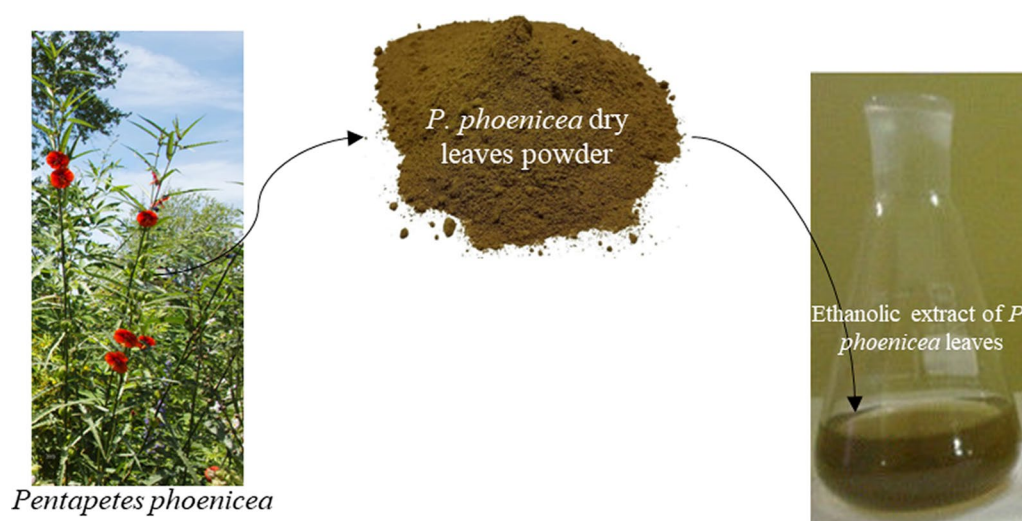


Fig. 1 Preparation of Ethanolic Extract from *Pentapetes phoenicea* leaves

400 nm, showing the fastest response, was selected for the next step (Manosalva et al. 2019).

Optimisation of Ag concentration: The Ag concentration was optimized based on the results of wavelength and absorbance checks during the synthesis process, namely checking each synthesis solution formula periodically every 20 min for 1 h and after storage for 1×24 h at room temperature. The optimal formula is determined by a red shift in the absorption peak, observed in the 400–500 nm range on day two.

Optimisation of extract concentration: Optimization of the extract aims to determine the optimal concentration of the extract to form AgNPs in large quantities, with a small size and stability indicated by a significant absorbance. During the checking process, SPR monitoring of absorption peaks and absorbance is carried out on day 2 using a solution (Fig. 3). AgNPs were sampled and examined using UV–Vis spectrophotometry; the formula that showed the smallest SPR in the 400–500 nm range with the largest absorbance on day 2 was selected as the optimal extract concentration (Ajlouni et al. 2023).

Effect of contact time: Each reaction formula is sampled every 15 min for 1 h to be examined using UV–Vis spectrophotometry and after the second day. This aims to monitor contact time during the reaction by observing wavelength absorption intensity and absorbance changes. The results will be displayed in a tabulation during the process and storage take place, and the optimal result is a formula that shows the SPR value is in the range of 400–500 nm, does not increase in size and shows an absorbance value that gets more significant each time. Contact time is closely related to the stability of AgNPs, where AgNPs can be declared stable if, during the storage period, the SPR does not shift towards a larger one. The absorbance value continues to increase; conversely, if the SPR absorption peak increases and the absorbance

value decreases, the agglomeration process or nanoscale growth is more significant (Fierascu et al. 2022).

Characterization of synthesized nanoparticles

Characterization of silver nanoparticle colloids using a UV–Vis spectrophotometer (Shimadzu 2600), wavelength 200–800 nm with a tungsten lamp source, FTIR spectroscopy (Bruker Alpha II model) wave number $4000\text{--}400\text{ cm}^{-1}$ with a Nernst lamp source, X-ray diffraction (BRUKER D8 ADVANCE) with a cobalt electron source, particle size analyser (PSA Malvern ZEN 1600) with a dynamic light scattering process, surface chemistry and chemical composition done with scanning electron microscope with EDS system (JSM-7610F) and transmission electron microscopy (TEM JEM 1400) using an energy beam of 350 eV. Energy-dispersive X-ray spectroscopy (EDX) was used to determine the elemental composition of the synthesized silver nanoparticles (AgNPs). The analysis was conducted under standard operating conditions using a scanning electron microscope (SEM) equipped with an EDX detector. EDX spectra were collected at an accelerating voltage of 15 kV to ensure accurate elemental detection. The detection limits of the EDX technique for silver were in the range of 0.1–1 atomic per cent, allowing for precise identification of silver in the nanoparticles. The EDX spectrum showed a strong peak for silver at approximately 3 keV, confirming the successful reduction of silver ions and the presence of silver in the synthesized nanoparticles. Thermal gravimetric analysis (TGA) and differential thermal analysis (DTA) (Linseis STA PT 1750 brand TGA) analyses were performed to examine the thermal degradation behaviour of AgNPs. DLS studies of AgNPs diluted were measured with the Malvern Zetasizer (Malvern MPT.2 Instruments).

Identification of the strains: Gram staining, catalase and oxidase tests were performed for the phenotypic

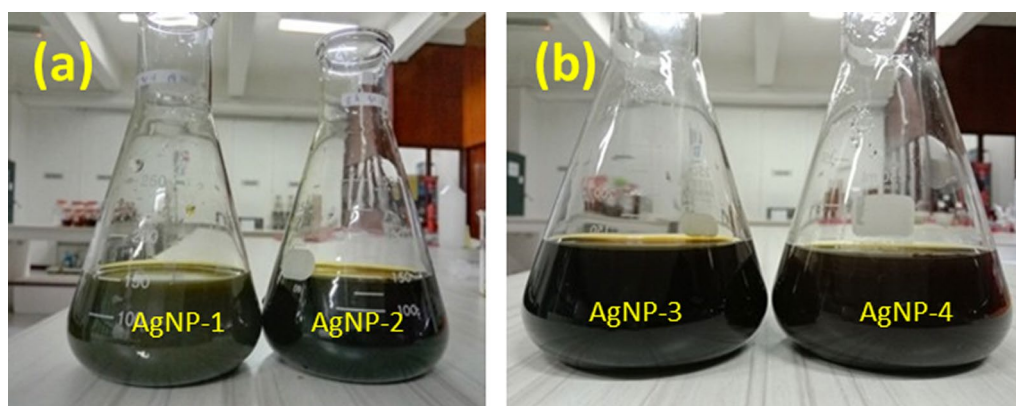


Fig. 3 Formula optimization process. **a** AgNP-1 and AgNP-2; **b** AgNP-3 and AgNP-4

identification of the strains. In the case of *Staphylococcus aureus*, it was cultured in Oxford, PALCAM, chromogenic, bile esculin, gelose sheep blood media, the CAMP test (Ferreira et al. 2014) was carried out and inoculated by sting in TSI (Triple Sugar Iron) medium and SIM (sulphide indole motility) medium. For the biochemical identification of *E. coli* O157:H7, it was shown in MacConkey medium with lactose, MacConkey with sorbitol, chromogenic agar and inoculated by pitting in TSI, MIO, LIA, citrate and urea medium. Subsequently, the strains were reseeded in nutrient media to obtain pure cultures. To preserve the strains, broth added with 20% glycerol was used and kept at -20°C .

Antimicrobial activities of silver nanoparticles

Determination of the MIC and MBC of AgNPs (Biliuta et al. 2022): Before testing, bacterial strains were cultured on trypticase soy agar and incubated at 37°C for 24 h. To obtain the MIC, serial dilutions of half of the antimicrobial test agent (AgNPs $107\ \mu\text{g/mL}$) were made, and a bacterial standard equivalent to 5×10^5 CFU/mL was prepared for each bacterial strain. The methodology is based on the microdilution or tube dilution technique. The MIC value corresponds to the tube with the lowest concentration of AgNPs where no visible growth has been observed, that is, the absence of turbidity due to the complete inhibition of the bacteria. The methodology follows the CLSI M07-A9 (2012) guidelines for antimicrobial susceptibility testing.

To determine the MBC, the dilutions of the tubes made for the MIC were used, and the tubes that did not show visible growth were poured onto plates. The methodology used was the continuation of the microdilution technique. The MBC value corresponds to the plate where the content of the tube with the lowest concentration of AgNPs has been added and in which no visible colony growth is observed (CLSI M07-A9 2012).

Results and discussion

Determination has been done to ensure that the plant used in the research is *P. phoenicea*. Based on the identification results carried out at the Biology Laboratory at Makassar State University (UNM), information was obtained that the plant used was the *Pentapetes phoenicea* species, or what is usually called kitchen *P. phoenicea*. The *P. phoenicea* leaf extraction process produces 15.08% of the extract weight of 22.69 g.

The green synthesis mechanism involves a reduction process, namely the functional group of secondary metabolites, which acts as a reducer that donates electrons to the Ag^+ ion so that Ag^+ is reduced to Ag^0 (Bukhari et al. 2021). The parameters greatly influence the shape and size of the AgNP material formed during

the reaction, namely pH, concentration of AgNO_3 and extract (Gemishev et al. 2022). The results of this research show that visually, AgNPs begin to form when the colour of the synthetic solution changes, namely from yellow to dark brown, over time, when there is contact between the extract and the Ag^+ solution. The colour difference in each mixture is influenced by the content of organic reducing bioactive compounds used; a colour change indicates the formation of AgNPs (Fernando et al. 2024).

A material is said to be in the form of a nanoparticle if the material changes size to become smaller and has different properties and shapes from the bulk material. The typical characteristic of AgNPs is an absorption peak in the visible region between 400 and 500 nm (Bukhari et al. 2021); this peak is called the SPR peak (surface plasmon resonance). SPR is the interaction of electromagnetic rays and the sample with a metal surface. The colour change in the AgNP solution apparently also occurs due to the SPR phenomenon or surface plasmon resonance (Bukhari et al. 2021).

pH optimization: pH optimization was carried out as outlined in Table 1. The first experiment was performed by reacting 20 ml of 5% ethanol extract pH 8 with 100 ml of 2 mM Ag. The UV–Vis absorption peak appeared at 299 nm at 45 min after mixing. The second experiment using an extract with pH 10 showed an absorption peak of 398 nm at 45 min, and the third experiment using pH 12 showed an absorption peak with characteristics of AgNP formation, namely 402 nm at 20 min. In experiments 1 and 2, based on UV–Vis absorption peaks, no AgNPs were formed, while in the third experiment, with the extract pH being 12, it showed that the reduction reaction was running, which was marked by an SPR peak of 402 nm. Alkaline pH causes the functional groups of the extract to be deprotonated so that its ability to chelate metals is more vital, as in previous research, it was reported that alkaline pH mediates the rapid synthesis of AgNPs, where immediately after adding NaOH as a function of pH it becomes more alkaline causing accelerated reduction in the mixture of Ag and extract, this is due to changes in the electrical charge of the biomolecules, which enhances their reactivity as reducing agents (Azarbani and Shiravand 2020). At pH 12, the higher alkalinity facilitates the reduction of silver ions (Ag^+)

Table 1 Effect of extract pH on the formation time of silver nanoparticles

Formulas	pH	Time (Minutes)	Wavelength (nm)	Information
Ag 2 mM + 5% extract	8	45	292	Not yet formed
Ag 2 mM + 5% extract	10	45	398	Not yet formed
Ag 2 mM + 5% extract	12	20	402	Formed

to silver nanoparticles (AgNPs), as the increased negative charge on the silver ions enhances their interaction with the reducing agents in the *P. phoenicea* extract. This pH-induced environment promotes the efficient formation of AgNPs, whereas, at low pH, aggregation often occurs above nucleation, leading to large-sized nanoparticles forming (Bukhari et al. 2021). However, at specific pH values, it can cause agglomeration of AgNPs, which is why pH optimization is necessary. Based on pH optimization, pH 12 extract is selected for the next stage.

Green synthesis was carried out using reducing *P. phoenicea* extract pH 12 at a concentration of 5% and 10%, which was reacted in an Ag solution with a concentration of 1 mM and 2 mM so that the results were obtained as shown in Fig. 4 and given the identities of AgNP-1, AgNP-2, AgNP-3 and AgNP-4, the optimisation process can be seen in Fig. 3. Day 2 AgNP-3 showed typical AgNP formation characteristics at the SPR peak of 403 nm using a 5% concentration extract in 2 mM Ag solution, as shown in Fig. 4; monitoring the comparison of Ag concentrations showed a positive effect with an increase in concentration on In this study, formulas other than AgNP-3 did not show any AgNP formation, possibly due to the dominant absorption of extract components (Miranda et al. 2022); therefore, an Ag concentration of 2 mM was chosen as the optimal concentration.

As the literature states, the greater silver concentration causes a more significant shift in wavelength; a greater shift in UV–Vis wavelength absorption indicates the formation of larger-sized nanoparticles because less energy is needed to attract electrons to the plasma resonance

surface (Miranda et al. 2022; Bukhari et al. 2021). The increase in size is likely caused by particle aggregation in response to high substrate concentrations.

The concentration of the extract determines the amount of AgNP that can be formed, and the research results can be seen in Table 2. The research results show that the greater the amount of reducers, in this case, the ethanol extract shows a reduced absorbance value, which means the amount of AgNP formed is smaller; this can happen because the amount of reducers is too high. This is also widely proven by the SPR peak, which does not show the typical AgNP peak; this phenomenon caused by the ratio between the amount of Ag^+ ion solution is less than the amount of reductant so that the absorption wavelengths that can be read using UV–Vis spectrophotometry are other compounds which can be components of complex compound extracts. Or impurities (Iglesias 2022), based on this explanation, the optimal extract concentration to form AgNPs is 5% in the AgNP-4 formula, because the extract concentration of 5% shows a

Table 2 Optimisation of extract concentration

Sample	Time (Minutes)	Wavelength (nm)	Absorbance
AgNP-1	Day 2	370	1.181
AgNP-2	Day 2	378	0.830
AgNP-3	Day 2	402	2.234
AgNP-4	Day 2	398	1.589

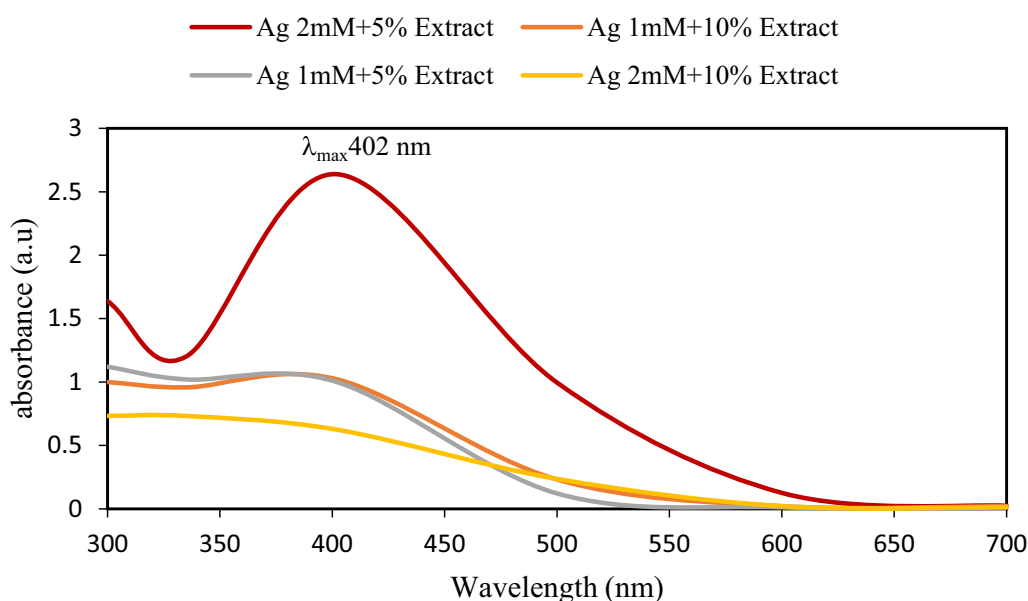


Fig. 4 SPR Peak Optimization of Ag Concentration

considerable absorbance value and the SPR peak is by the characteristics of AgNPs, namely 402 nm.

Effect of contact time

Contact time is closely related to the stability of AgNPs, where the stability of AgNPs is expressed in the context of aggregation; aggregation is the phenomenon of agglomerates or the reassembly of particles to form a more significant size which occurs as a result of storage age that exceeds the maximum limit which is marked by the SPR peak shifting towards a larger or on the contrary and the absorbance value becomes less as time goes by Iglesias (2022) the effect of contact time was tested through UV–Vis spectrophotometry examination which was sampled every 15 min for 1 h and on the second day for all formulas, the research results can be seen in Table 3 which is based on the absorbance value indicates that synthesis is continuing, suggesting that AgNP-1 and AgNP-2 indicate that green synthesis stopped at 15 min, so it is stated that the formula cannot be used to obtain AgNPs, the most stable formula is AgNP-4 because it shows that the green synthesis process continues longer than the others, this is in accordance with the results of optimizing the concentration of Ag and extract, namely the formula using 5% ethanol extract with an Ag concentration of 2 mM. In this formula, the absorbance value is experienced. This research shows limitations on the effect of the ratio of the formula amount between the bioreductor and the AgNO_3 precursor so that it cannot be explained what a good ratio of amounts to use in the formula is, and further research should be carried out to test the characteristics to determine the condition and characteristics of the AgNP produced, to get better results.

Results of qualitative phytochemical analysis: The following are the results of qualitative tests for the seven compounds which are presented in Table 3.

Of the seven compounds tested, it can be seen that *P. phoenicea* leaves positively contain steroid, flavonoid, tannin and quinone compounds. Meanwhile, the three

other compounds, namely alkaloids, triterpenoids and saponins, showed negative results. Steroids are organic compounds of non-hydrolysed sterol fats which can be produced by degradation reactions from terpenes or squalene. Flavonoids are polar compounds that generally dissolve easily in polar solvents such as ethanol, methanol, butanol and acetone. (Chávez-González et al. 2020). Flavonoids are polar compounds that generally dissolve easily in polar solvents such as ethanol, methanol, butanol and acetone. Flavonoids are the largest group of phenolic compounds, phenolic compounds have effective properties in inhibiting the growth of viruses, bacteria and fungi. Tannins are a class of active plant compounds that are phenolic in nature, have an astringent taste. Tannins have antibacterial activity, in general the mechanism is by damaging the bacterial cell membrane (Czerkas et al. 2024). Molnar et al. (2024) explained that the antibacterial activity of tannin compounds is by contracting the cell wall or cell membrane, thereby disrupting the permeability of the cell itself. Quinones are coloured compounds and have basic chromophores such as the chromophore in benzoquinone, which consists of two carbonyl groups conjugated with two carbon–carbon double bonds. Anthraquinone and quinone compounds have the ability to act as antibiotics and pain relievers and stimulate the growth of new cells in the skin (Czerkas et al. 2024). From the discussion above, it can be seen that *Pentapetes phoenicea* leaves are proven to contain chemical compounds that have the potential to be used as medicines. Further research to prove and support the results of this research is very necessary to develop *Pentapetes phoenicea* leave as a safe and effective medicinal ingredient.

In this research, the bioreductant used in the synthesis of silver nanoparticles was *Pentapetes phoenicea* plant leaf extract. From the preliminary phytochemical study, it was stated that leaf extract contains tannin and flavonoid compounds which are phenolic derivatives and can work actively in reducing silver nitrate (Bukhari et al. 2021). The reaction mechanism for reducing phenolic compounds to silver compounds can be seen in Fig. 5. In principle, the functional groups in plant extracts cause Ag^+ to be reduced to Ag^0 .

Fourier Scanning Infrared (FTIR) Spectroscopy analysis: Comparison of the FTIR spectra of the plant extract and AgNPs reveals potential interactions between bioactive molecules in the extract and the nanoparticles, which may contribute to the synthesis and stability of AgNPs. This suggests that some bioactive molecules bind to the nanoparticles and thus become stabilized by surrounding them. In our study, 13 bands were obtained from FTIR analysis with the *P. phoenicea* leaf obtained plainly and the silver nanoparticle we obtained through

Table 3 Results of qualitative phytochemical testing of *Pentapetes phoenicea* leaf extract

Types of compound groups	Test results
Alkaloids	Negative
Steroids	Positive
Triterpenoids	Negative
Flavonoids	Positive
Saponin	Negative
Tannin	Positive
Quinones	Positive

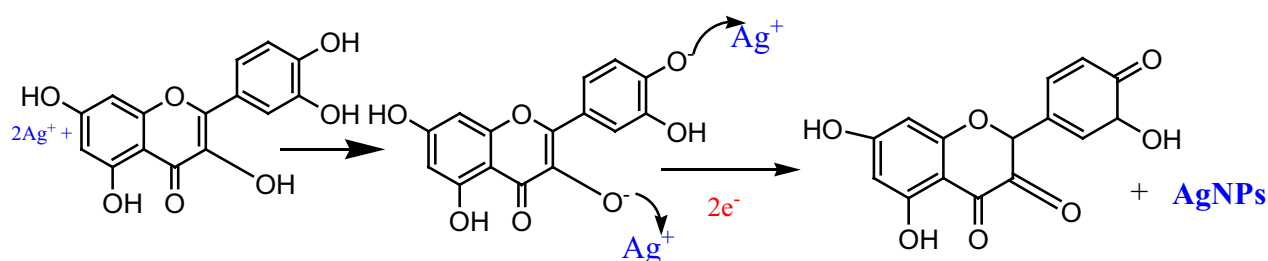
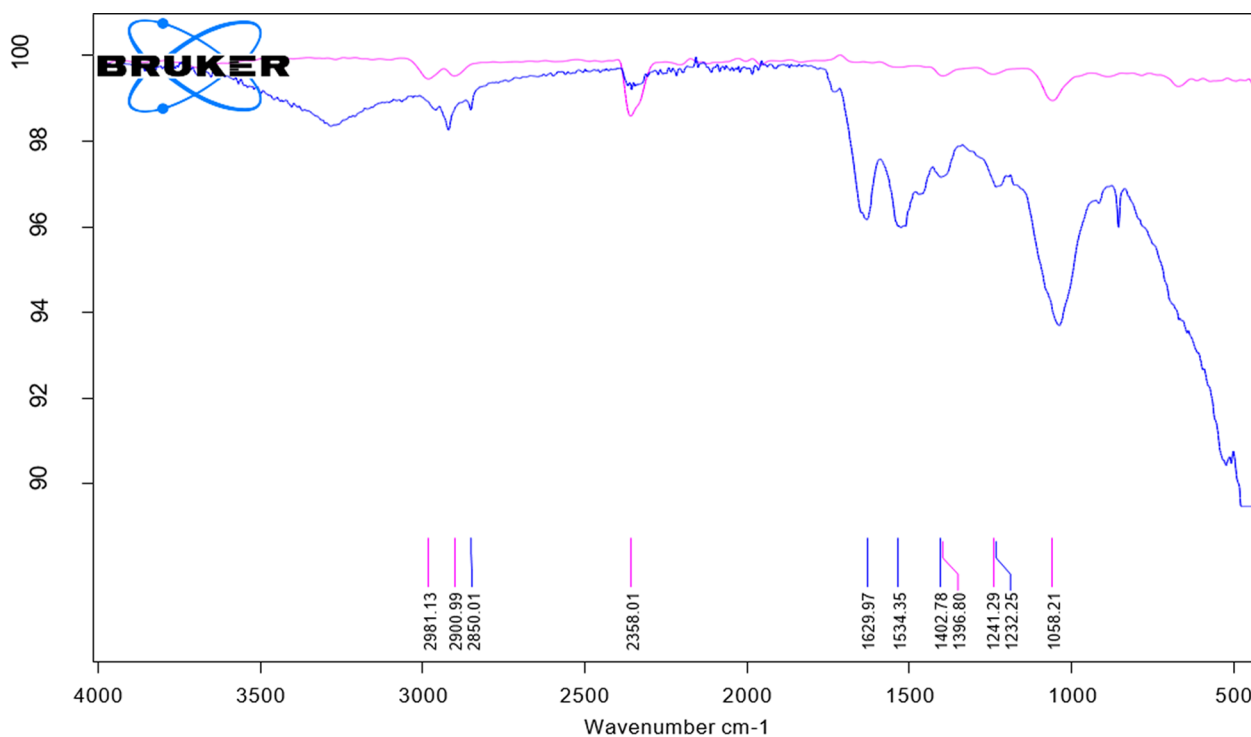


Fig. 5 Silver ion reduction mechanism (Mikhailova 2020)

green biosynthesis (Fig. 6), these results are; 2981, 2900, 2850, 2358, 1629.97, 1534.35, 1402.78, 1396.80, 1241.29, 1232.25, 1059.21, 886.81 and 671.74 cm^{-1} . These results indicate that this value indicates the- NH group according to the vibration mode at 2981 cm^{-1} . The presence of protein was predicted with the upward peak occurring at 2900 cm^{-1} and it was expected to be a methylene group. It was estimated that they were $\text{C}=\text{O}$ and $\text{C}-\text{O}$ based on the peak formation between 1629.97 and 1534.35 cm^{-1} , respectively. According to the vibration at 1402.78 cm^{-1} and 1396.80 cm^{-1} , it was determined that

the ethyl group represents CH_2 . According to our results, the specific frequency between 1250 and 1020 cm^{-1} indicates the presence of $\text{C}-\text{N}$, aliphatic amines and $\text{O}-\text{H}$. The peak at 671.74 cm^{-1} is estimated to represent the presence of alkyl halides, and the peak at 886.81 cm^{-1} is indicative of $((\text{CH})\text{C}=\text{CH}_2)$. However, the result at 886.81 cm^{-1} is not a meaningful indicator. It shows the mechanism of silver nanoparticle synthesis by reducing silver ions (Ag^+) to become silver nanoparticles (Ag^0) (Khan et al. 2023).



C:\OPUS_7.0.129\MEAS\moss	extracted_3.0	P.phoenicea leaf extract	Instrument type and / or accessory	17.04.2024
C:\FTIR DATA\AgNPs33.0	AgNPs33	Instrument type and / or accessory		17.04.2024

Fig. 6 FT IR spectra of Plant extract and AgNPs

FTIR analysis was used to identify functional biomolecules that cause the reduction and stabilization of Ag(I) cations in AgNO₃ solution to the neutral Ag⁰ species. *P. phoenicea* leaf extract rich in phenolic components was taken and the spectra were performed at room temperature (Khan et al. 2023).

X-ray diffraction (XRD) analysis results: Crystal structure analysis and phase analysis of the *P. phoenicea* leaf extract-based AgNPs sample were performed using the X-ray diffraction (XRD) technique. The X-ray diffraction pattern obtained for the AgNPs sample synthesized with green extract is shown in Fig. 7. In the resulting diffraction pattern, characteristic peaks of the AgNPs structure ($2\theta = 37.04^\circ$, 44.17° , 66.43° and 78.35°) were obtained. These peaks show that AgNPs are cubic (Savvidou et al. 2024). Sarwer et al. (2022) and Naveed et al. (2022) synthesized AgNPs from *Myrsine africana* leaf extract and *Acer oblongifolium*, respectively, and reported that the mentioned peaks belonged to silver.

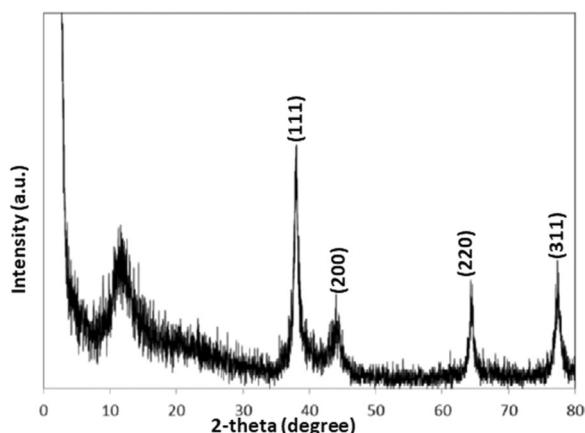


Fig. 7 XRD analysis of AgNPs from *P. phoenicea* leaves extract

Scanning electron microscope (SEM) analysis: Characterization using SEM aims to show the morphology of the particles. Apart from that, analysis using SEM can also provide size distribution information. The sample analysed using SEM was the best and most stable sample from the synthesis of silver nanoparticles, namely an AgNO₃ concentration of 1.0 mM on the 48 h of formation, which had the smallest average particle size, namely 19.24 ± 16 nm. The results of observations using SEM can be seen that the nanoparticles formed are slightly round (spherical) (Sarwer et al. 2022; Naveed et al. 2022). SEM analysis of *phoenicea* leaf extract-based AgNPs was measured with a magnification of 5000 $\times$, and the corresponding image is shown in Fig. 8a. The morphology of the silver nanoparticles does not appear very clear and displays an out-of-focus image.

Energy-dispersive X-ray spectroscopy (EDX) is commonly used to determine the elemental composition of AgNPs. To conduct this analysis, the AgNP sample was placed on grids coated with copper. Figure 8b shows the EDX analysis results for AgNPs. The presence of pure silver was also shown in the EDX pattern. This shows that silver has a high elemental composition.

This is likely due to the surface plasmon resonance (SPR) effect of the silver nanoparticles (AgNPs), which contributes to the observed absorbance. The EDX analysis revealed that the sample contained elements such as calcium (Ca), chlorine (Cl), oxygen (O) and carbon (C), likely originating from the surrounding medium. Notably, the EDX spectrum showed that 78% of the material was composed of silver, confirming the high silver content in the nanoparticles.

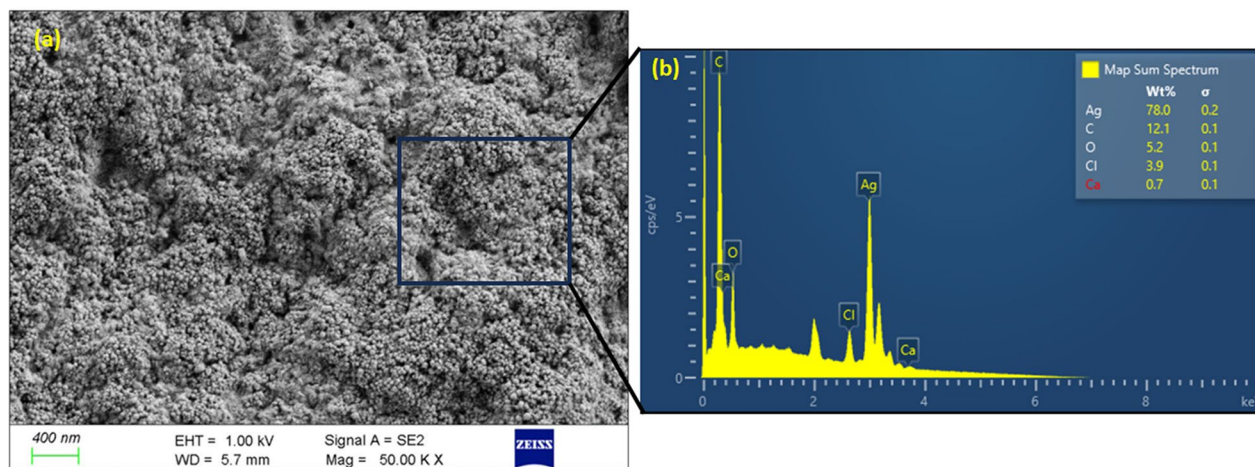


Fig. 8 **a** SEM analysis of AgNPs, **b** EDX spectra of AgNPs

TEM analysis

The study of the physical properties of silver nanoparticles included studying the size, shape and distribution characteristics of silver nanoparticles using TEM (Gemishev et al. 2022). The TEM images of the synthesized AgNPs using *P. phoenicea* leaf ethanolic extract show spherical particles with sizes ranging from 12 to 32 nm, with an average size of 16.3 ± 1.8 nm and shows that the particles are nanoscale and homogeneous. Therefore, AgNPs are expected to have a stronger antimicrobial activity. As can be seen from the TEM images, most of the synthesized AgNPs have a size around 20 nm (Fig. 9). HRTEM was used to monitor the shape and size of nanoparticles. For this purpose, AgNPs were analysed by HRTEM. The results show that the particles are generally spherical in shape and their average size is around 24 nm between 7 and 45 nm with green extract application and around 24 nm between 23 and 25 nm with dry extract application (Fig. 9). Similar results have been by Castañeda-Aude et al. (2023) AgNPs with 15 nm in spherical shape based on the TEM analysis, Calderón-Jiménez et al. (2022) developed AgNPs with 4–24 nm size, spherical shape and Saeb et al. (2014) also developed that AgNPs with an average size of 16–38 nm and 24–35 nm in size.

The size ranges from 10 to 50 nm as shown in Fig. 9, which is consistent with the experiment of Kandasamy et al. (2013) who conducted an experiment to synthesize silver nanoparticles using extracts from the coastal plant *Prosopis chilensis*. It was found that the substances are extracted from plant leaves, it can synthesize silver nanoparticles with a size of 5–25 nm, most

with an average spherical shape of 16.69 nm, and has the effect of inhibiting the bacteria that cause vibriosis in the shrimp *Penaeus monodon* after being treated with Keep infected for 30 days.

According to the SEM and TEM analysis results, it is seen that the biosynthesized AgNPs mostly have a spherical morphology (Figs. 8 and 9). AgNPs have been reported to have a spherical morphology by many different researchers.

Particle Size Analysis (PSA): PSA analysis is carried out to determine the size distribution and uniformity of the particles.

Based on Table 4, it shows that the size distribution of the nanoparticles produced is still not evenly distributed, around 10% of the total nanoparticle size distribution has a size of 22.12 nm, where this size is in the nanometre range, namely 0–50 nm. The resulting average diameter is 20.21 nm and within the nano-size (> 100 nm). The resulting sample is more stable so that little agglomeration occurs which causes the particle size uniform.

Table 4 PSA analysis results

AgNO ₃ concentration (mM)	0.5
Synthesis time (seconds)	300
Average diameter (nm)	20.21
D10% (nm)	22.12

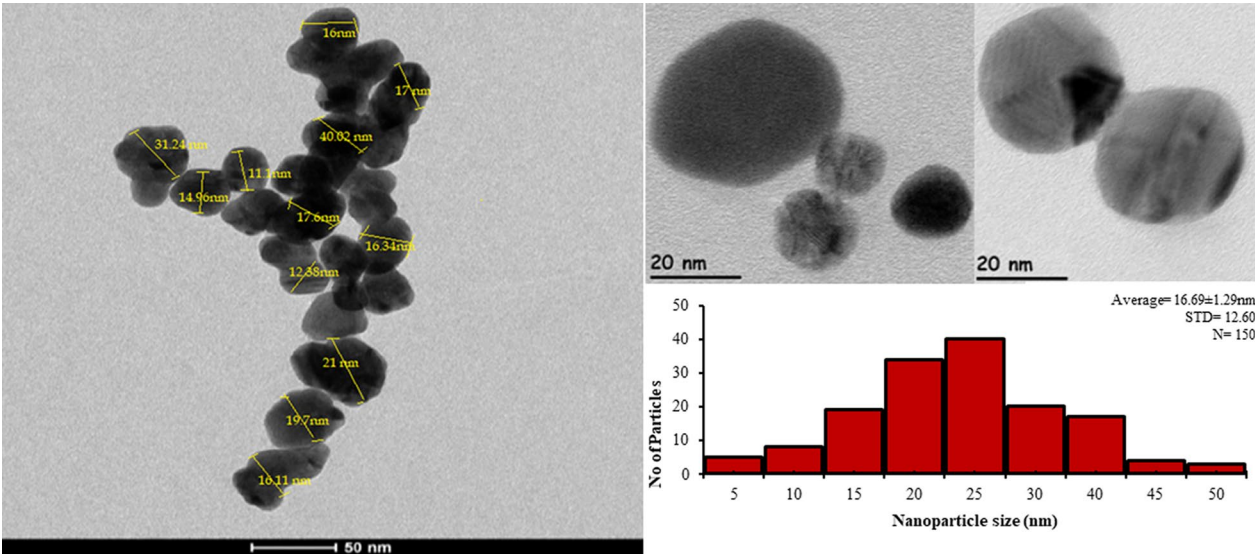


Fig. 9 TEM images and Histogram of AgNPs synthesized with *P. phoenicea* leaves extract

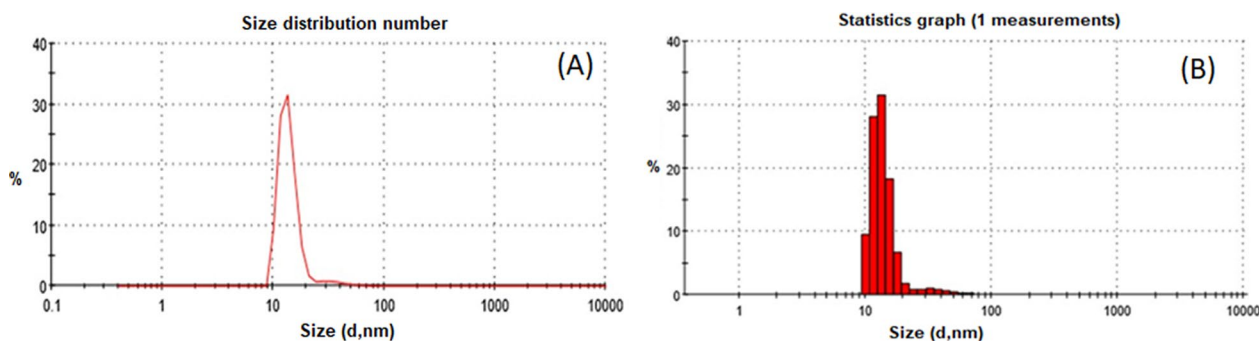


Fig. 10 Size distribution curve of AgNPs (A), statistical curve of AgNPs (B)

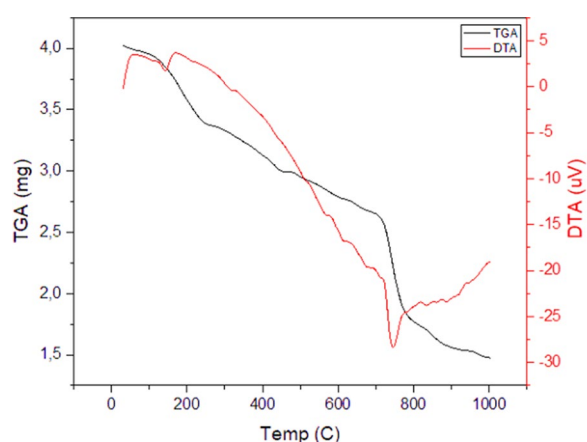


Fig. 11 TGA and DTA analysis of AgNPs prepared from

Zeta potential

The results showed that the average size of the silver nanoparticle was 17.57 ± 1.023 nm (Fig. 10). It was found that the PDI value of the nanoparticles was 0.337 under these synthesis conditions. Low stability of silver nanoparticles occurs when the value of a polydispersion index (PDI) is greater than 0.5

TGA and DTA analysis of AgNPs

To further examine the properties of nanoparticles, TGA-DTA analyses were carried out under N_2 gas in the thermal range of 50–1000 °C (Figs. 11). The TGA curve indicates AgNPs mass loss against temperature, and the DTA curve indicates the highest decomposition temperature at each stage of degradation (Fig. 11) (Farivar et al. 2021). The data in Fig. 11 show that mass loss at 30–60 °C is due to moisture, at 60–148 °C the mass loss is due to hydrated water, at 148–300 °C it is due to phytochemicals coming from leaf extract, and at 300–900 °C the organic structure that is deteriorated can be seen.

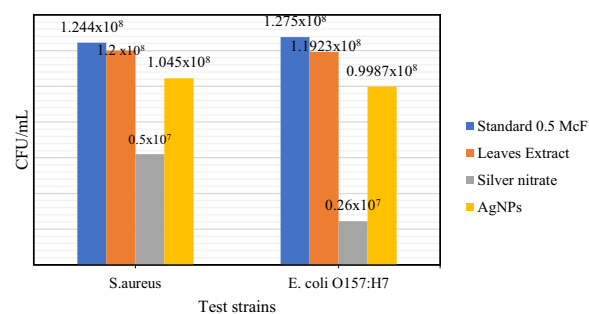


Fig. 12 Comparison of bacterial growth (CFU/mL) against the AgNPs precursors

Antibacterial study

According to the study results, AgNPs demonstrated strong inhibitory effects against food pathogens such as *S. aureus* and *E. coli* O157:H7. Both bacterial strains were identified using various microbiological criteria outlined in the methodology. The antimicrobial effects of the AgNPs were evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), with descriptive statistics and graphs used to analyse the results. It is important to note that, due to the natural origin of the AgNPs, they exhibit inherent turbidity. To accurately assess the antibacterial effects, the AgNPs were diluted to minimize their turbidity, allowing for clearer observation of the bacterial inoculum's turbidity during testing.

Figure 12 compares the assays based on bacterial growth (CFU/mL). In the test with *Staphylococcus aureus*, the silver salt presented a decrease in the bacterial load of 88%, ethanolic extract of 13%, and the AgNPs a decrease of 99.99% compared to the 0.5 McFarland standard. On the other hand, in the test with *E. coli* O157:H7, the silver salt showed a decrease in the bacterial load of 93%, and the ethanolic extract of 23%, while the AgNPs showed a decrease of 99.99% was observed compared to the 0.5 McFarland standard. The results shown in Fig. 12 correspond to the colony count

within the range of representative plates (10^{-5} and 10^{-6} dilutions).

The MIC of AgNPs in the strains evaluated and reported that the MIC of AgNPs in *Staphylococcus aureus* and *E. coli* O157:H7 was 0.41 $\mu\text{g/mL}$ and 0.20 $\mu\text{g/mL}$, respectively. These results correspond to the average of the triplicate tests and are expressed in units of $\mu\text{g/mL}$.

To determine whether a compound is a bacteriostatic or bactericidal agent, the MBC/MIC parameter can be used. When the MBC is less than or equal to four times the MIC ($\text{MBC/MIC} \leq 4$), the compound is considered a bactericidal agent. If the MBC is greater than four times the MIC ($\text{MBC/MIC} > 4$), the compound is considered a bacteriostatic agent.

The MBC/MIC ratio values of AgNPs for *Staphylococcus aureus* (ratio 2) and *E. coli* O157:H7 (ratio 4) indicate that the AgNPs exhibited a bactericidal effect on both bacteria, as the MBC/MIC ratio for *Staphylococcus aureus* is ≤ 4 , and for *E. coli* O157:H7, it is also ≤ 4 . (Table 5). While AgNPs showed bactericidal effects on both *S. aureus* and *E. coli* O157:H7, the study demonstrated that the antibacterial activity of AgNPs was not due to the plant extract of *P. phoenicea*, as the inhibition percentage from the extract alone was minimal. Furthermore, AgNO_3 displayed bactericidal activity at the same concentration as AgNPs (107 $\mu\text{g/mL}$), but AgNPs significantly enhanced the bactericidal effect of AgNO_3 .

Regarding the toxic effects of silver on mammalian cells, prolonged exposure to high concentrations of AgNO_3 (lethal dose, 308 mg/kg) has been associated with argyria, neurotoxicity and immunotoxicity. Cytotoxicity tests comparing AgNPs and AgNO_3 have shown differences, with AgNPs demonstrating lower toxicity. Based on these findings, AgNPs can be considered for use as antimicrobial agents at

concentrations lower than those that would be toxic to living organisms.

From the MIC tests, it was concluded that AgNPs from *P. phoenicea* leaves exhibit a bacteriostatic effect. However, due to the microdilution technique used (CLSI M07-A9 2012), the percentage of inhibition and the quantification of the bacteriostatic effect could not be determined. Some studies have reported the MIC of AgNPs from other plant extracts against *S. aureus* and *E. coli* O157:H7 using similar techniques, providing further insight into the antimicrobial potential of plant-based AgNPs. Zorraquín-Peña et al. (2020) determined by microdilution that the MIC of AgNPs was different for *Staphylococcus aureus* and *E. coli* O157:H7, reporting 6.25 and 3.12 $\mu\text{g/mL}$, respectively.

In relation to the MBC assays, the bactericidal effect of AgNPs could be verified in *Staphylococcus aureus* and *E. coli* serotype O157:H7. In addition to the above, differences in the bactericidal capacity were observed for AgNPs, it showed their bactericidal effect observed at lower concentrations. Likewise, it was possible to observe differences in the bactericidal effect between the bacterial species evaluated since *E. coli* O157:H7 was notably more sensitive to AgNPs than *Staphylococcus aureus*. From the MBC results, it is inferred that the bactericidal effect of the AgNPs is dependent on the AgNPs' concentration. Various studies evaluating the effect of AgNPs on different bacterial strains showed that the antibacterial activity was directly proportional to the nanoparticle concentration. As the AgNP concentration increased, greater inhibition of bacterial growth was observed (Yu et al. 2021). In a comparative study, Butucel et al. (2022) found that Gram-negative bacteria (*P. aeruginosa*, *E. coli*) required a lower MBC of AgNPs than Gram-positive bacteria (*B. subtilis*, *S. aureus*), with values of 5.1 and 10.2 $\mu\text{g/mL}$, respectively. This suggests that AgNPs face greater challenges in permeating the cell walls of Gram-positive bacteria.

Conclusion

Pentapetes phoenicea leaf extract with ethanol solvent has the ability to act as a reducer in alkaline conditions, namely pH 12, with the results of optimization of concentration variation which shows that the optimal conditions are the reaction between Ag concentration of 2 mM, and extra 5% which is confirmed to form silver nanoparticles in 20 min, the wavelength intensity shows the typical characteristics of AgNPs formation in the SPR peak area of 400–402 nm. The AgNPs have an intrinsic antibacterial effect not attributable to the ethanolic extract of *P. phoenicea*. The AgNPs evaluated act as a bacteriostatic agent against *S. aureus* and a bactericidal agent in *E. coli* O157:H7 according to the determined MIC and MBC

Table 5 The results of the bacterial count (CFU/mL) of the three successive dilutions selected for determination of the MBC of AgNPs in *Staphylococcus aureus* and *E. coli* O157:H7

Bacteria	AgNPs concentration ($\mu\text{g/mL}$)	Bacterial control (CFU/mL)	Bacterial growth (CFU/mL)
<i>S. aureus</i>	1.7	5.2×10^5	0
	0.85	5.2×10^5	663
	0.42	5.2×10^5	1122
<i>E. coli</i> O157:H7	1.7	5.2×10^5	0
	0.85	5.2×10^5	0
	0.42	5.2×10^5	0

parameters. The AgNPs act as a bactericidal agent in *S. aureus* and in *E. coli* O157:H7. According to the MIC and MBC parameters obtained, AgNPs have a delayed bactericidal effect (which was observed over time through bacterial growth monitoring) that can be attributed to the gradual release of silver ions. However, AgNPs act in a significantly shorter exposure time against the two bacteria evaluated and have a significantly greater bactericidal effect, when comparing the parameters of MIC and MBC against the two bacteria evaluated.

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Declarations

Competing interests

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