Received: 24 February 2024 | Revised: 17 July 2024 | Accepted: 12 October 2024 | Published online: 17 October 2024

RESEARCH ARTICLE

Antibacterial Effect of Silver Nanoparticles Prepared Using Pterospermum Diversifolium Semi-Solid Extract

Journal of Optics and Photonics Research
yyyy, Vol. XX(XX) 1–9
DOI: 10.47852/bonviewJOPR42022684
BON VIEW PUBLISHING

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Abstract: A green technique was used to create silver nanoparticles (AgNPs) using Pterospermum diversifolium semi-solid extract. The leaves were processed into a soft extract to retain the active ingredients of the leaves for a long time. The extract acts as a reducing agent to form Ag atoms and as a surfactant to create a stable environment for AgNPs. Spherical silver nanoparticles with a diameter of about 20 - 50 nm ± 5 nm were formed and were well stable in the solution. Optical properties, as well as structure, size, size distribution, and composition of elements and functional groups of AgNPs, were investigated through absorbance spectra, transmission electron microscopy (TEM), dynamic light scattering (DLS), Energy-dispersive X-ray spectroscopy (EDS) and Fourier transform infrared spectroscopy (FTIR) measurements. The extract and AgNPs were used to study the antibacterial effect on bacterial strains Ecoli, P. aeruginosa, and S. Aureus, with the positive control being Amoxicillin. The results showed that AgNPs and leaf extract with a 100 μg/mL concentration have better antibacterial ability than antibiotic amoxicillin 50 μg/mL.

Keywords: Pterospermum diversifolium extract, silver nanoparticles (AgNPs), antibacterial effect

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1. Introduction

Recently, nanotechnology has increasingly been proving its position in different areas of life. Metallic nanomaterials, including gold and silver nanoparticles, are candidates with the most applications, especially biomedical applications. Silver nanoparticles (AgNPs) are used as biological probes [1-3], applied in the fields of composite fiber synthesis [4], antibacterial [1, 5, 6], anti-cancer [7-10], anti-inflammatory [11-13], wastewater treatment [14, 15], food packaging [16], and many consumer products [17].

Silver nanostructures with unique optical properties and antibacterial properties have been widely studied by using many techniques [18, 19]. Chemical reduction techniques mostly created AgNPs with many different shapes and structures, such as spheres, triangles, semicircles, corals, flowers, etc. [20, 21] and photochemical methods [22-26].

Besides, AgNPs were also synthesized from green materials such as plant extracts [27-29], bacteria [30, 31], fungi [32], i.e., The green methods synthesized AgNPs to create new friendly products with high biocompatibility that were advantageous in the applications. Using plant extracts to synthesize AgNPs was considered the most effective method because of its few by-products and rich raw material sources. Spherical AgNPs with diameters of 100 nm and 35 nm were synthesized using Eucalyptus camaldulensis and Terminalia arjuna extract [28]. C. prophetarum extract was used to synthesize AgNPs 30 nm- 50 nm in diameter [27]. Prosopis farcta [33], Aloe vera [34], Eclipta alba [35] have also been used to synthesize AgNPs. Pterospermum

Diversifolium leaves are one of the plants with good antibacterial activities and show different effects when extracted in different solvents [36].

However, plant extracts are usually only active for a short period of time (about a few weeks). To maintain the activity of the extract over time while optimizing the synthesis process. The novelty of our work is using the Pterospermum Diversifolium Semi-Solid Extract which had a long-term activity to synthesize AgNPs with high efficiency. We use the Pterospermum Diversifolium leaves to create a Pterospermum Diversifolium Semi-Solid Extract, which serves as a starting point for the synthesis of AgNPs and study the antibacterial activity of the Pterospermum Diversifolium Semi-Solid Extract and synthesized AgNPs.

2. Materials and Methods

2.1. Materials

Pterospermum Diversifolium leaves were gathered in Dong Nai province, Vietnam. Silver nitrate ($AgNO3$, >99%), Sodium hydroxide (NaOH, >99%), Ethanol (99.5%) and Hydrochloric acid (HCl, 38%) were provided by Merck. The strains of Escherichia coli (E. coli), Staphylococcus Aureus (S. aureus -S.A), Pseudomonas aeruginosa (P. aeruginosa -P.A), Amoxicillin (Amo) 500 mg, and Dimethylsulfoxide (DMSO) were provided by Sigma-Aldrich. Without additional purification, all compounds were utilized. Milli-Q water was used throughout this study.

2.2. Method

The collected leaves were washed several times to remove the dirt. 100 g of leaves were dried at 50 ° C for 24 h, then refluxed in 99.5% Ethanol at 80 ° C for 4 h. The extract was concentrated until the solution had a honey-like consistency, containing about 20-25% water. That mixture was called Pterospermum Diversifolium semi-solid extract. This extract is stored at 4 °C for long-term use.

AgNPs were synthesized using the green method using Pterospermum Diversifolium semi-solid extract. Factors such as pH, AgNO3 concentration, and extract volume were changed to optimize the synthesis process. The first 10 mL of 3 mM AgNO3 was adjusted to pH by adding NaOH and HCl so that the solution pH reached values of 5, 6, 7, 8, 9, 10. Then, 3 mL of 50 ppm extract was added into the solution, stirring at 1000 rpm, 60 °C for 2 h. The experiment was repeated with the procedure in which the solution pH was adjusted to 9; the AgNO3 concentration was adjusted to the corresponding values of 1, 2, 3, 4, 5, and 6 mM. 2 mL of the extract was used equally in the reaction vessels. The final experiment was performed by varying the extract volume with values of 0.5, 1, 3, 4, 5, and 6 mL, while the AgNO3 concentration was kept constant at 2 mM and pH = 9.

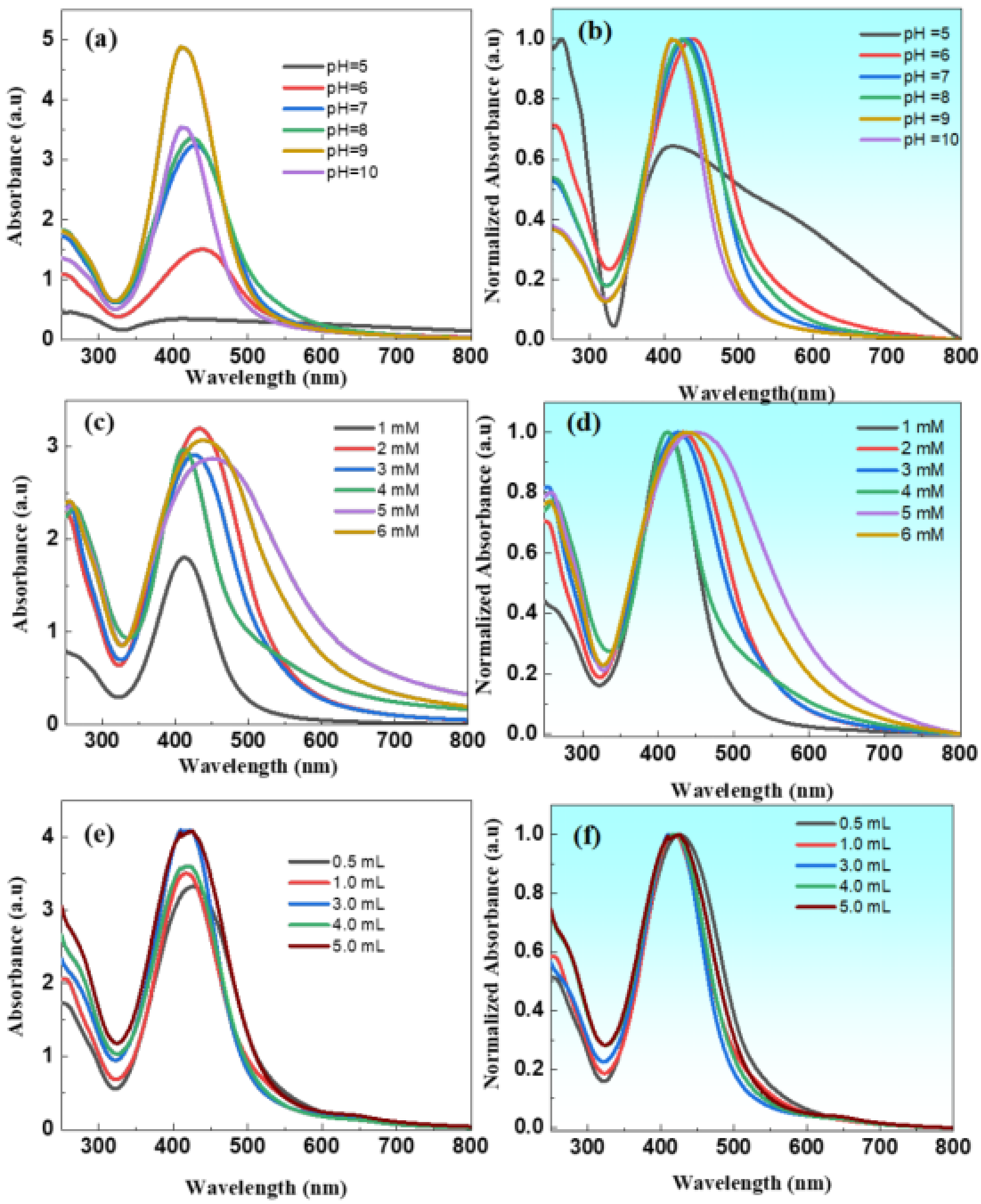
2.3. Application

The AgNPs were centrifuged 3 times, and the pellet was redispersed with DMSO. Antibacterial assay was carried out by disc diffusion technique [37]. The antibacterial activity of the extracts and AgNPs was carried out on agar plates with 3 strains of E.coli - Gram (-)), Pseudomonas aeruginosa (P.A - Gram (-)) and Staphylococcus aureus (S.A – Gram (-)). All bacteria were pre-cultured in agar overnight in a shaker at 37 °C. Make 5 holes 0.6 cm in diameter on the plate and inject 100 µL of sample solution into each hole corresponding to each type of bacteria. The sample solution included samples of P.AgNP particles with 25, 50, and 100 g/mL concentrations. Dimethyl sulfoxide (DMSO) was the negative control, and Amo 50 µg/mL was the positive control sample. The plates were placed in the refrigerator for an hour and then incubated at 37 °C for 18 h. The zone of inhibition that developed following the incubation time was measured to identify the antibacterial activity. The diameter of the inhibition zone was determined by the formula: H = D - d (mm), where H was the inhibition zone (mm), D was the sum of the diameters of the agar hole and the inhibition zone (mm), d was the diameter of the agar hole (mm).

3. Results and Discussions

Figure 1(a)-(b) shows the absorption spectra and normalized absorption spectra of AgNP solutions with varying solution pH.

Figure 1
(a-b) Absorption spectra and normalized absorption spectra of AgNP solutions when pH changes, (c-d) $AgNO_3$ concentration changes and (e-f) extract volume changes



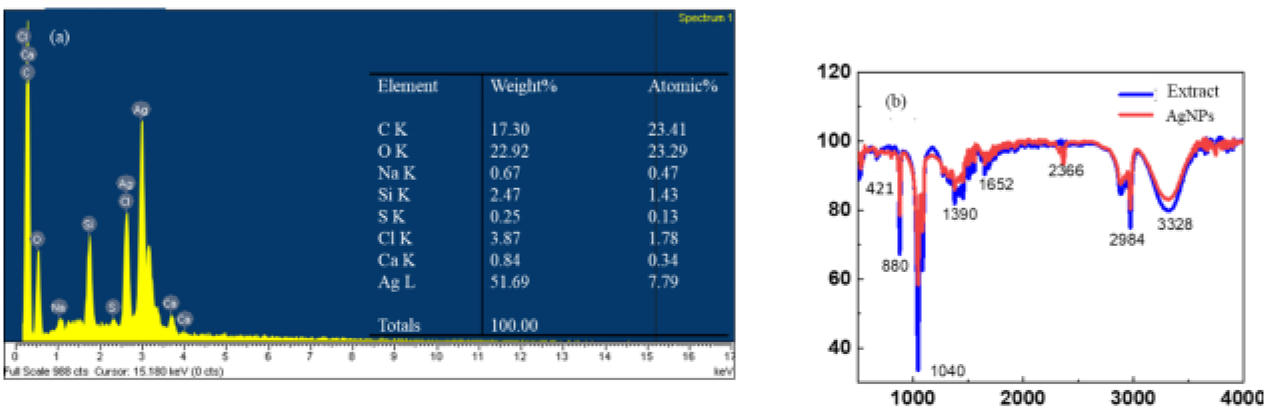
The absorption spectra of the synthesized AgNP solutions with different pH showed that when pH = 5, the absorbance was mainly in the ultraviolet region due to the contribution of the extract, while the absorbance in the visible region was very low. This proved that the efficiency of AgNPs synthesis was very poor at low pH. When the pH exceeds 6, the absorption spectra have the characteristic shape of AgNPs with a plasmon resonance peak in the wavelength range of 412 to 443 nm [38, 39] (Figure 1(a)). However, with pH = 6, the absorbance of the solution is low, and the full width at half maximum (FWHM) is large (FWHM = 112.5 nm). This proves that AgNPs have a wide size range and the AgNPs are inconsistent. The high absorbance and narrow spectra of the AgNPs solution when pH values from 7 - 10 are suitable for synthesizing AgNPs (FWHM = 90.5 – 84.5 nm). Which pH = 9 gives the highest synthesis efficiency (FWHM = 84.5 nm) (Figure 1(b)). Therefore, subsequent surveys adjust the solution to pH = 9.

To find the optimal AgNO3 concentration for the synthesis process, the AgNO3 concentration was adjusted with 6 values from 1 to 6 mM. Figure 1(c)-(d) shows the absorption and normalized absorption spectra of AgNP solutions obtained with the different AgNO3 concentrations. It can be seen that the AgNO3 concentration affects the concentration and size of the AgNPs. This is reflected in the maximum absorbance and half-broadness. When the AgNO3 concentration changes from 1 to 6 mM, the maximum absorption wavelength changes from 414 to 456 nm, corresponding to 20 to 50 nm in the diameter of AgNPs.

Figure 1(e)-(f) shows the absorption and normalized absorption spectra of the AgNP solutions depending on the extract volume. The results show that the particle size is almost unchanged with the resonance peak at wavelength 420 nm when the extract volume changes. This shows that the volume of extract is excess in the reactions. The results show that 3 mL of Pterospermum Diversifolium semi-solid extract performs best.

Figure 2

(a) EDS analysis of AgNPs and (b) FT-IR spectra of AgNPs synthesized using Pterospermum Diversifolium Semi-Solid Extract



At 3 keV, a strong peak signal is seen (Figure 2a). It is characteristic of the absorption of Ag metal [40]. Other peaks, such as O, Si, Na, S, Cl, and Ca, are components in the glass substrate that holds the sample. The C component in the EDS analysis is due to the technique of measuring the sample spread on a Carbon grid [41]. The functional groups in the extract and AgNPs are determined by FT-IR spectra (Figure 2b), thereby identifying the compounds that play a crucial part in the synthesis of AgNPs. The absorption peaks of the extract and AgNPs are similar. The display of the peak at 421 cm-1 in the spectrum of AgNP is only different, indicating the formation of AgNPs. The peak positions are listed in Table 1 and compared with standard wave numbers to identify the corresponding functional group and compound (Table 1). Comparing the FT-IR spectra of the extract and AgNPs, we can see a strong decrease in intensity at the peak of 1040 cm-1. This proves that the Carboxylic acids group contributes significantly to reducing AgNO3 to produce AgNPs.

Table 1
Primary functional categories of AgNPs' surface

Functional groups	Standard wave number (cm^{-1})	The wave number of synthesized AgNPs (cm^{-1})
O–H (Monomeric—Alcohols, Phenols)	3640–3160 (stretch) [42]	3328
C–H (Alkanes)	2960–2850 (stretch) [42]	2984
$-NH2^{+}$ $-NH^{+}$	2366-2320 (Stretching primary and secondary amines) [42]	2366
N–H (Amines)	1650–1580 (bend) [43]	1652
C–H (Alkanes)	1470–1350 (scissoring and bending) [43]	1390
C–O (Carboxylic acids)	1260–1000 (stretch) [43]	1040
C-H	838 (Out of plane bending) [43]	880
Ag-OH	455 And 410 (Banding silver-hydroxyl group) [43]	421

Figure 3
(a) TEM image of AgNPs with a scale of 100 nm and (b) size distribution spectrum according to particle number

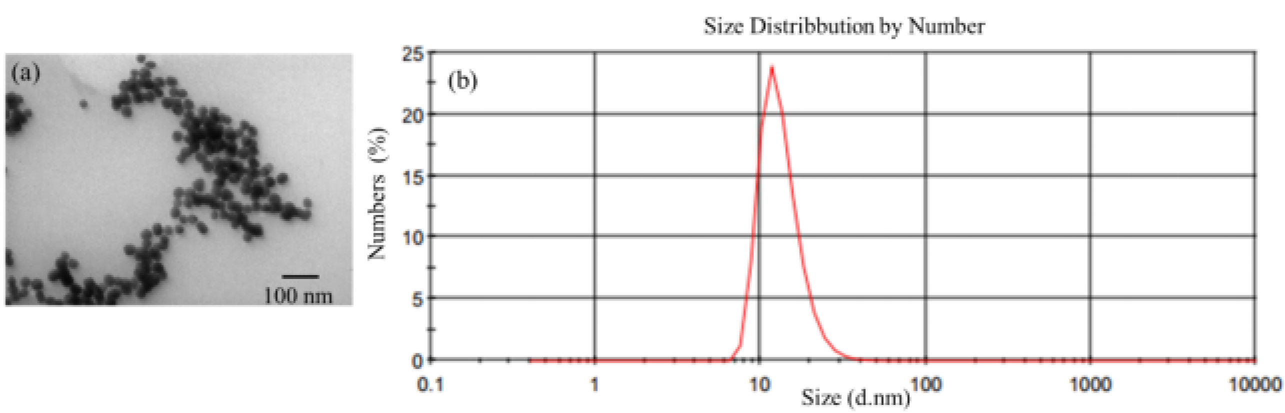
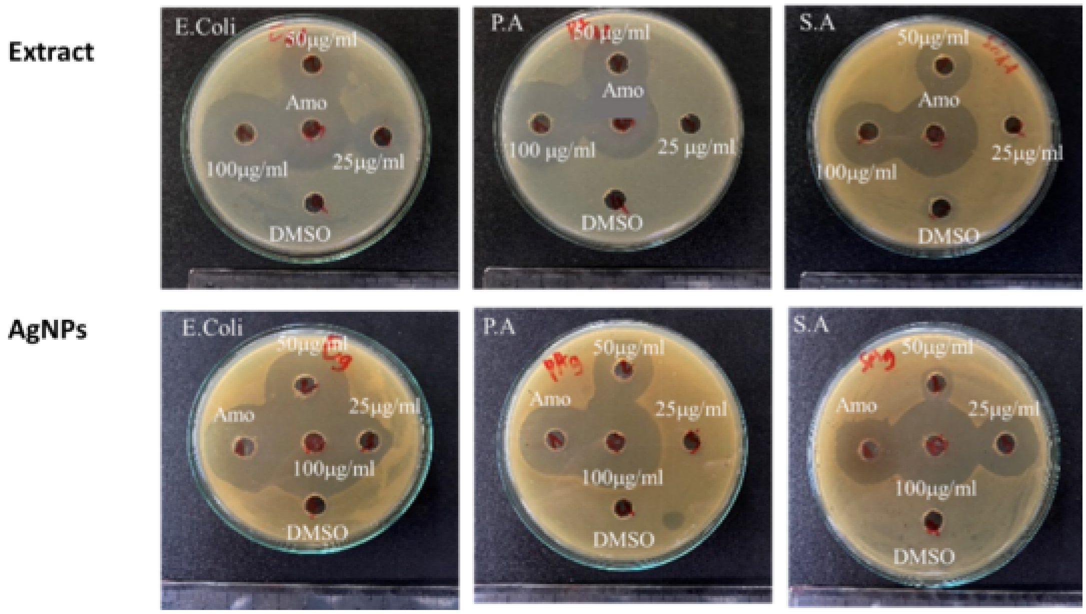


Figure 3(a) displays a TEM image of AgNPs. The particles are spherical and relatively uniform in size. The average diameter of AgNPs is 20 nm ± 5 nm. This is also shown in the size distribution spectrum according to the percentage of particles (Figure 3(b)). Compared with the previous publications on the synthesis of AgNPs applying plant extracts, the use of Pterospermum Diversifolium Semi-Solid Extract synthesizes the AgNPs with few byproducts and a narrow size distribution spectrum. It is easy to control the size of AgNPs.

Figure 4
Image of antibacterial rings when using AgNPs with various concentrations (25, 50, 100 μg/mL), positive control (Amo), and negative control (DMSO)



The results on the antibacterial activity of extracts and AgNPs were determined on three bacterial strains: E.coli, P. A and S.A. The evaluation of the antibacterial activity is based on the width of the sterile ring. Figure 4 shows the antibacterial rings when using Pterospermum Diversifolium Semi-Solid Extract and AgNPs with 25, 50, and 100 μg/mL concentrations. The results showed the radius of sterility on the culture plates of gram (-) bacteria E.coli and P.A when using leaf extract and AgNPs 100 μ g/mL were equal and larger than when using Amo 50 μ g/mL. For gram (+) S.A bacteria, the antibacterial activity of Pterospermum Diversifolium Semi-Solid Extract and AgNPs was not as good as that of Amo.

The parameters of antibacterial ring diameter when using antibiotics Amo, extracts, and AgNPs on three types of bacteria, E. Coli, P. aeruginosa, and S. aureus, are listed in Table 2. Pterospermum Diversifolium Semi-Solid Extract and AgNPs have the ability to inhibit all three strains of bacteria but with different levels depending on concentration and bacteria strain. The antibacterial mechanism of Pterospermum Diversifolium Semi-Solid Extract is explained by the fact that Pterospermum Diversifolium Semi-Solid Extract contains various active ingredients such as phenols, flavonoids, tannins, alkaloids. Pterospermum Diversifolium is active with a broad spectrum of activity against various bacteria, including Gram-negative bacteria such as E. Coli and P.A and gram-positive bacteria such as S.A. Meanwhile, the antibacterial mechanism of AgNPs is mainly based on Ag^+ ions, which are released from AgNPs and caused by the buildup of AgNPs on the cellular membrane. Ag^+ ions are able to cross the cytoplasmic wall of bacteria and membrane to disrupt the cell membrane, denature Ribosomes, interrupt ATP production, and impede DNA replication [1, 5, 6].

Table 2
Diameter of antibacterial rings E. coli, P. aeruginosa, S. aureus when using antibiotics Amo, extract, and AgNPs with different concentrations

Samples	Concentrations (μg/mL)	Antibacterial ring diameter E. coli (mm)	Antibacterial ring diameter P. Aeruginosa (mm)	Antibacterial ring diameter S. aureus (mm)
Amoxicillin	50	26.2	25.7	25.7
Leaf extract	50	28.1	26.2	18.1
	25	20.2	19.7	13.9
	12.5	14.3	-	-
AgNPs	50	28.3	26.3	13.7
	25	20.3	19.3	13.7
	12.5	8.3	-	

4. Conclusion

This work synthesized uniform-spherical AgNPs with controllable size in the 20 – 50 nm range using Pterospermum Diversifolium semi-solid extract as a reduction and surface stabilizer solvent. The FT-IR spectra analysis shows that the extract's functional group of carboxylic acids plays an important role in creating AgNPs. The results of investigating the influence of reaction factors on AgNP synthesis efficiency show that the solution's pH greatly influences particle synthesis. pH = 9 gives the best AgNP synthesis efficiency. High concentrations of $AgNO_3$ and extract also affected the size and dispersion of the particles. Depending on the size of AgNPs, the concentration of precursor and extract can be adjusted accordingly. The Pterospermum Diversifolium semi-solid extract and AgNPs have good antibacterial activity when used at a 100 μg/mL concentration and are better against gram (-) bacteria such as E.coli and P.A. This study give us the scientific idea of using plants with similar functional groups prepared as semi-solid extract to synthesize AgNPs on a large - scale and towards their many other applications.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author Contribution Statement

Hue Thi Do: Conceptualization, Software, Validation, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Trung Anh Le**: Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing.

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How to Cite:
Do, H. T., & Le, T. A. (2024). Antibacterial Effect of Silver Nanoparticles Prepared Using Pterospermum Diversifolium Semi-Solid Extract. *Journal of Optics and Photonics Research*. https://doi.org/10.47852/bonviewJOPR42022684