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Original Research Article

Biogenic Synthesis of Silver Nanoparticles using Polygonum minus Fresh and Dried Leaves Extract and Its Antibacterial and Antioxidant Properties

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Abstract: Research reveals that techniques for producing silver nanoparticles through plantmediated synthesis exhibit potent antimicrobial properties. The purpose of this work is to evaluate the antibacterial capabilities of silver nanoparticles made with leaf extract from *Polygonum minus (Kesom)* as the reducing agent. The visual color changes observed the formation of PM-AgNPs and UV-Vis Spectrophotometry was used to validate the production of PM-AgNPs, displaying an absorption peak at about 440 nm. Subsequent characterization using X-ray diffraction (XRD) analysis and Fourier Transform Infrared (FTIR) Spectroscopy revealed the presence of biomolecules from the leaf extract that facilitated the synthesis and stabilization of PM-AgNPs and determining their crystalline nature. Results from the disc diffusion showed that PM-AgNPs inhibited the growth of Gram-negative and Gram-positive bacteria.

Keywords: Polygonum minus; *daun kesum*; silver nanoparticles; green synthesis; antibacterial; antioxidant

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1. Introduction

The exploration of biogenic silver nanoparticles (AgNPs) derived from plant extracts has attracted a lot of attention recently because of their various potential applications including environmental remediation, biotechnology and medicine. Out of many plant species that has been studied, *Polygonum minus*, also referred to as Kesum, has shown great promise because of its rich pharmacological profile and profusion of bioactive chemicals. *Polygonum minus*, a member of the Polygonaceae family, is a widely distributed plant species in Southeast Asia and known for its diverse pharmacological activities. Their aromatic scent properties have attracted research interest and found that this herb has been extensively used traditionally for therapeutic application aside from being utilized in traditional cuisines and

recipes. Flavonoids such as quercetin, myricetin and other methylated flavanols and other phenolics, tannins and terpenoids are part of phytochemical elements that provide *Polygonum minus* its wide range of therapeutic uses in traditional medicine which contribute to their anti-inflammatory, antibacterial, antioxidant and anticancer properties (Baharum *et al.*, 2010; Rusdi *et al.*, 2016)

The abundance of phytochemicals exhibited by this herbaceous plant offers advantages in the synthesis of silver nanoparticles using the green method over the conventional one. Biogenic synthesis is an eco-friendly, cost-effective and non-toxic procedure that involves using natural reducing and capping agents in the plant extracts, hence, minimizing the risk of hazardous chemicals (Fereydani *et al.*, 2023). This made biogenic silver nanoparticles more biocompatible and stable making them suitable for biomedical applications. Further exploring the antioxidative and antibacterial activities against various bacterial strains including drug-resistant pathogens can provide insight into their mechanism and possible benefits when combined with traditional antibiotics. Various studies have been carried out in exploring the biogenic synthesis of nanoparticles using different plant extracts however, there are still limited studies on *Polygonum minus* specifically emphasizing the comparison of the nanoparticles produced from fresh and dried leaf extract

In this study, the antioxidative and antibacterial properties of biogenic silver nanoparticles synthesized from *Polygonum minus* fresh and dried leaf extract were investigated. The synthesized nanoparticles will be characterized using various analytical techniques including UV-vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), photoluminescence spectroscopy and small-angle X-ray scattering (SAXS) analysis to elucidate their structural, optical, and morphological properties. The antioxidative and antibacterial properties will be evaluated using the DPPH radical scavenging assay and disc diffusion method. Through this proposition, this study aims to extract and characterize Polygonum silver nanoparticles and evaluate their potential as antioxidant and antibacterial agents.

2. Materials and Methods

2.1 Extraction Procedure

Fresh leaves of *Polygonum minus* (Kesum) were obtained from the nearby local market, washed thoroughly using clean water and air-dried at room temperature. The dried leaves were prepared by oven-drying the leaves part at 40°C for 24 hours. The aqueous extract of *Polygonum minus* was then prepared using water as a solvent, using ground fresh leaves and oven-dried leaves to produce fresh leaf extract and dried leaf extract of *Polygonum minus*. The extraction method was done using a heat-assisted method where 5 g of the ground leaf and leaf powder were heated in 100 ml distilled water at 65°C for 3 hours in a water bath (Shahar *et al.*, 2015). The extracts were cooled and filtered using Whatman filter paper and stored at 4°C and frozen at -18°C for further analysis.

2.2 Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs) was accomplished through an ecofriendly approach, employing a modified procedure (Sivakumar *et al.*, 2023). Initially, a 0.1 mM silver nitrate solution was meticulously prepared and stored in an amber flask to prevent photoactivation. 10 ml of the solution was combined with 1 ml of fresh and dried extracts of *Polygonum minus*. The resulting mixture has undergone incubation process in darkness at room temperature for 24 hours, facilitating the reaction process in which a noticeable color change was observed. Confirmation of the presence of synthesized silver nanoparticles was established by the distinct shift in solution color from transparent to dark brown, validated visually and further analyzed using UV-vis spectroscopy. The colloidal solution of both fresh and dried extract PM-AgNPs was then refrigerated a 4°C for subsequent analysis.

2.3 Characterization Method

2.3.1 UV-visible Spectroscopy

The evidence formation of silver nanoparticles was based on a noticeable transition in color, shifting from colorless to a deep brown hue of PM-AgNPs which was subsequently confirmed visually. Distilled water acted as a blank, and the PM-AgNPs both fresh and dried were analyzed using a UV-Vis Spectrophotometer (GENESYSTM 180 UV-Vis Spectrophotometer). As for dried PM-AgNP, the solution is measured by diluting silver nanoparticles with distilled water to achieve maximal absorbance, measured within the wavelength range of 300 to 700 nm (Lubis *et al.*, 2022).

2.3.2 Fourier Transform Infrared (FTIR) Analysis

The extract's biomolecules function as silver ions' reducing agents were identified and their quantities were measured using FTIR analysis. An attenuated total reflection (ATR) detector on a Perkin Elmer Spectrum 100 FTIR Spectrometer was used to analyze the chemical compositions of PM-AgNPs. In this process, the instrument emitted infrared radiation ranging from 4000 cm⁻¹ to 650 cm⁻¹, which passed through the silver nanoparticle solution (Iravani *et al.*, 2014). During this interaction, some radiation was absorbed while the rest passed through and the absorbed radiation was then converted into vibrational energy by the molecules present, resulting in a signal detected by the instrument. This signal represents a molecular fingerprint of the sample under examination.

2.3.3 Photoluminescence (PL) Analysis

Photoluminescence refers to the phenomenon where nanoparticles absorb photons of light and emit photons of longer wavelength which involves the excitation of electrons within the silver nanoparticles to higher energy levels followed by relaxation to lower energy levels. A multimode microplate reader (Tecan, Spark) was used to analyze the excitation and emission wavelength of nanoparticles when exposed to light. A small amount of the sample

was prepared in the wells of the microplate and loaded into the microplate reader for measurement. The excitation and emission spectra of the silver nanoparticles were generated and analyzed (Vankudoth *et al.*, 2022a).

2.3.4 Small Angle X-ray Scattering (SAXS)

SAXS measurements were conducted using a Rigaku SmartLab X-ray Diffractometer with SAXS capabilities. A small volume of the AgNP sonicated colloidal solution was loaded into quartz capillary tubes. The sample holder was aligned within the instrument to optimize the X-ray beam-sample interaction. The instrument was calibrated using standard reference materials to ensure accurate determination of scattering angles and intensities. The scattering angle range was set to range 0.06° to 2.00°. X-ray exposure and data acquisition process was controlled using the SmartLab software and the scattering data was collected as a function of the scattering angle. Collected SAXS data was analyzed and background subtraction was performed to remove instrumental noise. The nanoparticle information such as the size and distribution of the sample was extracted by modeling techniques.

2.4 Antioxidant Analysis

Ethanol served as solvent and 1-diphenyl-2-picrylhydrazyl (DPPH) solution was used to evaluate the radical scavenging activity of fresh and dried leaf extract and the synthesized silver nanoparticles (PM-AgNPs). Fresh and dried extracts along with the colloidal solution of synthesized silver nanoparticles were diluted at 1,2,5 and 10 dilution factors. 0.25 ml of each concentration was further diluted with distilled water up to 10 ml. Subsequently, 3 ml of the solution was added into 5 ml of 0.1 mM DPPH solution, well mixed and allowed to at room temperature in the dark for 30 minutes. DPPH solution served as the control and the results were compared with a standard solution of ascorbic acid (0.1 mM) (Marinova & Batchvarov, 2011). The scavenging activity/inhibition percentage was determined by the formula:

$$\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \tag{1}$$

2.5 Antibacterial Analysis

The antibacterial activity of *P.minus* silver nanoparticles (PM-AgNPs) was evaluated using the modified disk diffusion method (Hadi *et al.*, 2024a) where the fresh and dried extract PM-AgNPs were tested against gram-positive (*Staphylococcus aureus*) and gramnegative (*Klebsiella pneumonia*) bacterial cultures. The bacterial strains were cultured on Mueller-Hinton agar plates. Sterile filter paper discs with a diameter of 6 mm impregnated with fresh and dried extract PM-AgNPs were placed on the agar surface and left for incubation at 37°C for 12 to 24 hours. Norfloxacin and gentamicin were used as controls and the diameters of inhibition zones were measured in millimeters and the test is done in triplicates.

3. Results and Discussions

3.1. Characterization of PM-AgNPs

3.1.1. UV-vis Spectroscopy Analysis

The formation of silver nanoparticles was confirmed by visual color changes from light yellow to dark red brownish solution indicating the formation of silver nanoparticles shown in Figure 1 which were then confirmed by the resulting absorbance peak at maximum absorption of 440 to 470 nm shown in Figure 2.



Figure 1. Dark red brownish silver nanoparticles solution after 24 hours incubation.



Figure 2. UV-vis absorbance spectrum of fresh and dried PM-AgNPs

The results from the UV-vis spectroscopy analysis of the *P.minus* silver nanoparticles (PM-AgNPs) synthesized using fresh and dried extracts exhibited peak absorption between 440 nm and 470 nm, aligning with the characteristic absorption range indicative of silver

nanoparticles. This observation emphasizes the reliability of UV-vis spectroscopy as a method for characterizing the optical properties of AgNPs. A previous study also reported a similar peak absorption at 440 nm (Lubis *et al.*, 2022) and another study documented a peak absorption at 426 nm (Ullah *et al.*, 2017). Variations in absorption peaks were attributed to factors such as the size of AgNPs and their surrounding environment which influence the surface plasmon resonance (SPR) intensity. Furthermore, it was noted that the intensity of the absorption peak correlated with the amount of extract used in the synthesis process, suggesting the higher extract amounts might lead to an increased number of particles. Moreover, the activation of SPR during the synthesis process indicated the reduction of silver ions which explained the darkening color solution to a deeper brown hue.

3.1.2. FTIR Analysis

The result of the FTIR analysis from Figure 3 below showed the infrared spectra which indicates the presence of a functional group from the plant extract used that acted as a reducing or stabilizing agent in the synthesis of silver nanoparticles. A similar spectrum can be seen from FTIR spectra of fresh and dried PM-AgNPs which suggested that both dried and fresh extract have similar functional groups that served as reducing agent in the PM-AgNPs synthesis.



Figure 3. Infrared spectra of FTIR analysis. (a) dried extract (b) fresh extract

Both figures showed two major peaks detected at around 3352 and 1638 representing the -OH stretching of alcohols or phenols and C=C stretching vibrations of aromatic compounds. Ullah et al., (2017) suggested that -OH groups presented in the plant extract are responsible for reducing silver nanoparticles. This -OH stretching vibration associated with phenolic groups typically occurs in the region 3200 cm⁻¹ – 3600 cm⁻¹ which also often results in a broad and intense peak. N-H stretching vibration of amines could also possibly be detected at this range of 3300 cm⁻¹ to 3500^{-1} . Meanwhile, the C=C stretching vibration typically occurs in the region of 1600 cm⁻¹ to 1680 cm⁻¹ and is specific to the stretching of carbon-carbon double bonds in organic compounds such as alkenes and aromatic compounds (Nandiyanto *et al.*, 2019). A similar peak around 1632 cm⁻¹ was also detected from a previous study (Lubis *et al.*, 2022) which claimed to be attributed to the C=C vibrations of the phenolic compounds such as polyphenols and flavonoids. The small peak can also be detected in the FTIR spectra specifically at around 2120 cm⁻¹, 1386 cm⁻¹, 1034 cm⁻¹ and 710 cm⁻¹ which represented carbon triple bond of alkyne, C_{sp3} -H bending vibrations of methyl group, C-N stretching of amino group and C-H bending vibration of aromatic benzene respectively. Overall, the FTIR analysis suggests that silver nanoparticles synthesized from *Polygonum minus* extract are likely surrounded or capped by organic molecules that played crucial roles in the reduction of silver ions and stabilization of the nanoparticles, potentially influencing the formation of silver nanoparticles.

3.1.3. Photoluminescence (PL) Analysis

The results suggested that PM-AgNPs contain fluorophores or luminescent compounds that absorb light at 307 nm and emit light at 428 nm. The maximum absorption observed at 307 nm during the excitation scan (Figure 4) suggests the presence of plasmon resonance in the silver nanoparticles. This resonance is typically associated with the collective oscillations of free electrons on the nanoparticle surface when excited by incident light. The emission peak observed at 428 nm during the emission scan (Figure 5) falls within the range of 400 to 500 nm indicating that the nanoparticles are emitting light in the visible spectrum which likely arises from radiative transitions of excited electrons back to lower energy levels, resulting in the release of photons (Kalyani et al., 2017; Wang et al., 2019). Smitha et al., (2008) explained that the absorption band in the visible light region is typical for Ag nanoparticles, where the obtained emission spectrum of AgNPs, which particles emit light with a broad peak at 445 nm when excited at 330 nm. The same result was obtained on synthesized Mc-AgNPs which was then related to the size reduction-induced hole trapping within surface molecules in the unsaturated sp3 orbit. The author also concluded the luminescence enhancement attributed to the presence of biochemicals from the plant extract used in the synthesis as compared with other studies (Vankudoth et al., 2022b).



Dried 60000 Fresh 50000 40000 (a.u.) ntensity 30000 20000 10000 420 440 480 400 460 500 Wavelength (nm)

Figure 4. Excitation scan of PL intensity of PM-AgNPs

Figure 5. Emission scan of PL intensity of PM-AgNPs

3.1.4. Small Angle X-ray Scattering (SAXS)

Small-angle X-ray scattering (SAXS) is a non-destructive technique that provides a fast measurement of nanoparticle size distribution in which the measurements were done automatically from optics alignment to sample alignment and data collection benefiting from the SmartLab multipurpose diffractometer used in this analysis, making SAXS measurement an easy task. This technique can quantify nano-scale density differences in a sample and determine nanoparticle size distributions and average diameter of particle size. To gain insights into both the distribution of nanoparticle size and nanopore sizes, the simulation and the measured curve were modeled and done with NanoSolver software by conducting a least-squares fitting process. Model refinement of the curve provides the result of average diameter and size distribution which is summarized in Table 1.

	Average diameter (nm)	Size RSD, %
Fresh extract PM-AgNPs	8.272	100
Dried extract PM-AgNPs	13.237	79.3

Table 1. Average diameter (nm) and size distribution of PM-AgNPs

The table showed different average diameter sizes obtained for fresh and dried extract PM-AgNps which is around 8 nm and 13 nm respectively. The RSD value of particle size also differs between the two nanoparticle solutions. The factor contributing to the distinct value obtained for the two nanoparticles might be due to the influence of the extract's concentration. Fresh extracts may contain different concentrations of active compounds or reducing agents compared to dried extracts, potentially affecting the rate of nanoparticle formation. Variations in the chemical compositions of both extracts could also impact the nucleation and growth of silver nanoparticles, leading to differences in particle size as mentioned in a previous study that obtained a smaller range diameter size for fresh leaves along with noticeable agglomeration and lack of uniform shape (Bonnia et al., 2018). The quantity and type of the flavonoid group can change depending on the thermal stability during drying and extract preparation process, which can affect the plant extract's capping and stabilizing qualities (Vaidya et al., 2014). Dried extract typically possesses higher content of phenolic and flavonoid which are the main reducing agent acted in the synthesis process and this phytochemical allow more silver nucleation process that allow particles to grow larger at more uniform conditions. This leads to the formation of larger nanoparticle but in a narrower size distribution. On the other hand, fresh leaf extract had a lower concentration of phytochemicals, hence there are fewer donating electrons available to facilitate the complete reduction of metal ions and incomplete reduction results in the presence of both oxidized and reduced species which lead to the formation of smaller nanoparticles due to the limited availability of fully reduced atoms for growth (Pradeep et al., 2022). The RSD percentage of 100% suggested a very wide and uniform distribution of particle sizes within the sample where the particles vary greatly in size with no dominant size population indicating a highly polydisperse sample. Dried extract PM-AgNPs has a higher average size diameter of 13 nm with lower value of RSD which is 79%. This value indicates a certain degree of dispersion in the particle size distribution suggesting a relatively broad distribution of particle sizes but with some degree of clustering around the mean size. In short, both values indicate polydispersity in the sample and fresh extract PM-AgNPs implying a broader and uniform distribution compared to dried extract PM-AgNPs. However, since this method minimized the sum squared residuals, outliers can disproportionately influence the fitted model, leading to inaccuracies. Hence, further validation through additional analysis such as TEM or FESEM may be required to confirm the shape and size of the particles.

3.2. Antioxidant Properties of PM-AgNPs

DPPH is a stable free radical that accepts electron or hydrogen radicals to become a stable diamagnetic molecule. DPPH radical is reduced when a substance with antioxidant properties is added leading to a color change from purple to yellow and can be quantified spectrophotometrically. Several studies proved the antioxidant properties of silver nanoparticles that are demonstrated using DPPH assay which involved electron or hydrogen atoms donation from AgNPs to the DPPH radicals, hence neutralizing their reactivity and preventing oxidative damage. In this study, the antioxidant properties of the PM-AgNPs were evaluated and the results were compared with the extract's antioxidant capacity which summarized in Table 2 and Figure 6.

Sample	DPPH Scavenging Activity (%)							
Dilution Factor	No dilution	DF 2	DF 5	DF 10				
Fresh extract PM-AgNPs	41.78	41.89	39.60	40.75				
Dried extract PM-AgNPs	43.77	45.21	43.78	45.86				
Extract only (fresh)	95.13	92.08	89.56	89.85				
Extract only (dried)	97.33	97.91	98.07	98.00				

Table 2. DPPH scavenging percentage of PM extract and PM-AgNPs



Figure 6. DPPH scavenging percentage chart

The data showed the scavenging activity of the PM-AgNPs synthesized from the fresh and dried extract and the extract only which are compared together with the ascorbic acid which served as standard. The PM-AgNPs colloid solution was diluted with different dilution factors to see the antioxidant activity of the nanoparticles when diluted into different concentrations. The result showed that PM-AgNPs possess antioxidant activity which range from 39 to 45% scavenging activity. A study from (Keshari et al., 2020) conducted an antioxidant analysis of AgNPs synthesized from different plant extracts obtained lower DPPH scavenging activity which is 29.55%. As seen in the table and figure above the level of antioxidants of dried extract PM-AgNPs is slightly higher compared to the fresh extract PM-AgNPs. However, the antioxidant level for both AgNPs when diluted does not show a huge difference or increasing trend which contrasts with a previous study that proved that increasing concentration would increase the DPPH scavenging activity of silver nanoparticles (Ahn et al., 2019). The possible justification for the result in this study might be due to the antioxidant activity of the nanoparticles reaching a state at a certain concentration, where further increases does not result in significant additional antioxidant effects.

The results were also compared with the scavenging activity of the plant's extract only which the extract's antioxidant activity was higher ranging from 89% to 98%. A review study (Bedlovičová *et al.*, 2020) stated that the scavenging activity of the extracts was higher than the AgNPs which contributed to the extract's phytochemicals. The synthesizing AgNPs using the antioxidant power of extract to reduce the silver ions may promote superoxide radicals which consume the antioxidant capacity of the extract. The lower DPPH scavenging activity in AgNPs compared to the extract alone could be attributed to the dual role of the extract as both reducing agent and antioxidant. During the synthesis, some of the active compounds in the extract responsible for antioxidant activity may be involved in the nanoparticle formation, potentially reducing their free radical scavenging efficiency. Thus, we can say that the PM-AgNPs do have antioxidant scavenging activity albeit with lower efficacy, considering the crucial roles of antioxidants as free radical scavengers in potential applications such as drug delivery systems and diagnostic and therapeutic nanoparticles.

3.3. Antibacterial Properties of PM-AgNPs

The antibacterial activity of PM-AgNPs synthesized from the fresh and dried extract was evaluated by observing the inhibition zone using the disc diffusion method. *Staphylococcus aureus* and *Klebsiella Pneumonia* bacterial cultures were observed to exhibit resistance towards standard antibiotic Norfloxacin and Gentamicin. The inhibition zones are shown in Figure 7 below and listed in Table 3. The susceptibility of the PM-AgNPs was observed when the bacterial cultures were exposed to colloidal AgNPs at a concentration of 10 mg/ml. The results showed that both PM-AgNPs synthesized from fresh and dried extract possessed antibacterial properties following the zone inhibition of the nanoparticles against both bacteria cultures. It shows that fresh and dried PM-AgNPs inhibit larger diameter zones of 10.9 mm and 11.06 mm on *Staphylococcus aureus* than *Klebsiella pneumonia* which is 10.84 mm and 10.77 mm.

The observed difference in the zone of inhibition between Gram-negative (*Klebsiella pneumonia*) and Gram-positive bacteria (*Staphylococcus aureus*) when exposed to AgNPs could be attributed to the distinct composition of their cell walls (Rautela *et al.*, 2019). A previous study on the susceptibility of AgNPs on gram-positive and gram-negative bacteria obtained an almost similar result around 10 mm (Hadi *et al.*, 2024b). The result showed that dried extract PM-AgNPs exhibit higher zone diameter on *Staphylococcus aureus* compared to fresh extract PM-AgNPs but lower diameter zone on *Klebsiella pneumonia*. Both AgNPs exhibit inhibition zone diameter, but the value does not exceed the inhibition capabilities of the antibiotics Norfloxacin and Gentamicin which are the standard used in this analysis.



Figure 7. Inhibition zones of AgNPs fresh leaf extract (i), AgNPs dried leaf extract (ii), gentamicin (iii) and norfloxacin (iv); Tested against *Staphylococcus aureus* (**a**) and *Klebsiella pneumonia* (**b**)

	Test Strains Inhibition zone (mm)				
Sample					
	Staphylococcus	Klebsiella			
	aureus	pneumoniae			
Fresh extract	10.0	10.94			
PM-AgNPs	10.9	10.84			
Dried extract	11.04	10 77			
PM-AgNPs	11.00	10.77			
Norfloxacin	21.57	18.91			
Gentamicin	19.76	22.10			

Tahle	3	Inhibition	zone of A	oNPs	(fresh	and	dried	extract)	gentamicin	and	norflovaci	n
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4. Conclusions

In conclusion, the synthesis of silver nanoparticles via *Polygonum minus* extract plays a major development of a realizable process, producing nanoparticles with certain optical, structural, antioxidant, and antibacterial features. The visual color turned from light yellow into dark red-brownish solution along with the corresponding UV-vis spectroscopy analysis confirms the appearance of AgNPs with absorption peaks at 440–470 nm. The existence of functional groups identified while utilizing FTIR analysis proves the participation of plant extract in the form of capping and stabilizing agents in the process of nanoparticle synthesis. Also, the study based on fluorescence assessment determines nanoparticle luminescent properties which suggests their possible utilization in the fields of sensing and imaging.

The SAXS-based size distribution analysis offers insights into the nanoparticle sizes in average which are affected by the extract concentration and composition of the materials, implying that there is some variation in size despite the polydispersities noted in the fresh and dried extract PM-AgNPs. Antioxidant assessment based on the DPPH assay indicates that the particles had modest scavenging effects, demonstrating that the nanoparticles' ability to scavenge free radicals although the efficacy was notably low when compared with the extract only. Furthermore, the antibacterial assessment reveals the inhibitory effects of PM-AgNPs against bacterial strains underscoring their potential as antimicrobial agents. The inhibition zone diameters of gram-positive differ from the gram-negative bacteria's inhibition zone diameter suggesting variations in susceptibility, that is likely due to the differences in cell wall composition.

Overall, the multifaceted properties of PM-AgNPs highlight their potential in various applications including biomedical, environmental and catalysis. Further studies into the physicochemical properties of PM-AgNPs are essential to elucidate their stability, morphology and surface chemistry.

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