Journal of Academia Vol.7, Issue 2 (2019) 46-55

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *POLYGONUM MINUS* EXTRACT AND ITS ANTIMICROBIAL PROPERTIES

Nadzirah Abu Samah[1,2*], Massuri Mahamudin[2], Salina Muhamad[3], Abdul Latif Ibrahim[2]

[1]*Centre for Foundation and General Studies, Universiti Selangor, Bestari Jaya Campus, 45600 Bestari Jaya, Selangor, Malaysia*
[2]*Institute of Bio-IT Selangor, Universiti Selangor, Shah Alam Campus, 40000 Shah Alam, Selangor, Malaysia*
[3]*Faculty of Engineering and Life Sciences, Universiti Selangor, Bestari Jaya Campus, 45600 Bestari Jaya, Malaysia*

**Corresponding author: naziee976@gmail.com*

Abstract

Nanotechnology is the study and advanced/modern application of small object that can be practised across various scientific fields, such as physics, chemistry, biology, material science, engineering, etc. It has been widely applied in the 21st century, and it also paved the way to the green approach in technology in the form of green nanotechnology. The field of nanotechnology enables silver nanoparticles (AgNPs) to be widely used as novel therapeutic agents in the semblance of antibacterial, antifungal, antiviral, anti-inflammatory, and anti-cancerous agents. In this study, plant extract of *Polygonum minus* (known as *kesum*) was used for the synthesis of AgNPs from silver nitrate ($AgNO_3$) solution. The green synthesis, which is an alternative way to produce silver nanoparticles, was proposed because it is cost-effective and environmentally friendly. The colourless reaction mixture was observed to slowly change from yellowish-green to reddish-brown, indicating the reduction of silver ion after several minutes of reaction. The AgNPs were characterised by Ultraviolet-visible (UV-Vis) spectrophotometer, Field-emission Scanning Electron Microscope (FE-SEM), and Energy-Dispersive X-Ray Spectroscopy (EDX). The results obtained from the UV-Vis spectrophotometer showed a sharp peak absorbance at 440 nm, which indicated the reduction of Ag^+ to metallic Ag. Meanwhile, the size of AgNPs observed via FE-SEM was in the range of 15–25 nm. Accordingly, based on the EDX analysis, 82.6% of AgNPs were determined to show strong peaks for silver (Ag). Three bacteria, i.e. *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 15442) were chosen to be tested in this study. The morphological changes of bacterial cells treated with AgNPs were observed by FE-SEM, showing that the AgNPs have excellent antimicrobial properties against microorganisms. Thus, the ability of AgNPs to release Ag ions is a critical factor in its antimicrobial activity.

Keywords: silver nanoparticle, Polygonum minus, antibacterial, green synthesis

Article history:- Received: 18 April 2019; Accepted: 10 October 2019; Published: 16 December 2019

Introduction

Intense attention is directed to nanomaterials at the nano level due to the implication of nanotechnology. Due to their unique physicochemical properties, metal nanoparticles with a dimension of approximately 1–100 nm have received considerable attention in the last few decades (Gurunathan *et al*., 2014; Khan *et al*., 2017) besides their chemical stability, good conductivity, and catalytic abilities (Lee *et al.*, 2011; Simsikova, 2016; Cedillo-Alvarez *et al*., 2017). Nanotechnology is used widely in the advanced application of small object that can be practised across all fields of science, including physics, chemistry, biology, material science, engineering, and medicine. Today, it is not surprising to see nanoparticle technologies being used in the quest against antimicrobial resistance as a global problem that is affecting modern healthcare. The field of nanotechnology has enabled silver nanoparticles (AgNPs) to be widely used as

novel therapeutic means in the forms of antibacterial, antifungal, antiviral, anti-inflammatory, and anti-cancerous agents (Motitswe *et al.*, 2019).

According to Bouqellah *et al.* (2018), various types of nanomaterials such as copper (Cu), zinc (Zn), titanium (Ti; Retchkiman-Schabes *et al.*, 2006), magnesium (Mg), gold (Au; Gu *et al.*, 2003), and silver (Ag) are widely used but AgNPs have been proven to be the most effective as an antimicrobial agent against bacteria, viruses, and other eukaryotic microorganisms. Ag has been known for many of years as the metal that exhibits excellent medical property and used in numerous antimicrobial applications. The high surface to volume ratio of AgNPs increases their contact with microorganisms, promoting the dissolution of Ag ion, thereby improving biocidal effectiveness. The ability of AgNPs to release Ag ion is a critical factor in its antimicrobial activity.

Different types of method for the synthesis of nanoparticles have been used, such as laser ablation, microwave, wet chemical methods, microemulsion, and gamma irradiation (Motitswe *et al.*, 2019). AgNPs can be produced using conventional or unconventional methods, by two different approaches: "top-down" and "bottom-up". The top-down approach is a process that breaks up bulk materials using milling, nanolithography, or precision engineering to generate nano-level structures, while the bottom-up approach is a process in which nanoparticles are built from individual atoms or molecules that are capable of self-assembly. Several nanoparticles syntheses or the production methods of nanoparticles involved the use of hazardous chemicals, low material conversions, and high energy requirements. Thus, green chemistry and biosynthetic methods have become the mode of choice and gained importance in developing an environmentally friendly process for nanoparticle synthesis without the use of toxic chemicals. It is well known that the green biological method of synthesising nanoparticles has materialised as an alternative to overcome the shortcomings of conventional methods in synthesising nanoparticles such as using several physical and chemical methods, including chemical reduction of ions in aqueous solution with or without stabilising agent and reduction in inverse micelles or thermal decomposition in organic solvents. Among the natural alternatives, plants and plant extracts seem to be the best option. Plants are nature's "chemical factory" that are cost-effective and require little or no maintenance. Employing plants in the synthesis of nanoparticles proved to be advantageous over non-biological methods, especially with the presence of variable biomolecules in plants that can act as the capping and reducing agents; hence, increasing the rate of reduction and stabilisation of nanoparticles. According to Vidya *et al*. (2016), the synthesis of AgNPs by plant extracts are more stable with a higher rate of synthesis than other organisms. Furthermore, the combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids, and vitamins are responsible for the reduction and stabilisation of Ag ion. Additionally, it is presumed that flavanone and terpenoid components from leaf broth can stabilise the formation of AgNPs, while the reduction of Ag ions is the result of the combination of polyol and water-soluble heterocyclic components (Alexandra Sorescu *et al*., 2016).

In this study, a green method for the synthesis of AgNPs using the plant extract of *Polygonum minus* (*kesum*) as a reducing agent was prepared and analysed for their physical, chemical, and antibacterial properties. Widely known in Malaysia, *kesum* is used as spice condiment, and it is one of the herbs that were identified potentially as a source of essential oils, especially in the fragrance industry (Hassim *et al*., 2015). Traditionally, the leaves have been used to treat maladies such as skin fungal infection, indigestion, dandruff, postnatal tonic, sprains, and body aches (Nurain *et al.*, 2012; Mohamad *et al*., 2017; Ahmad *et al.*, 2018). *P. minus* has been demonstrated to possess cytoprotective, antibacterial, antifungal, antiulcer, antiviral, and antioxidant activities. Several works also claimed that *P. minus* promotes high levels of free radical scavenging activity and reducing power as well as antimicrobial properties (Qader *et al.*, 2012; Hassim *et al.*, 2014, 2015). Various studies have revealed the different pharmacological potentials of *P. minus* both in vitro and in vivo test models.

Methods

Collection of Leaves

P. minus or *kesum* was purchased from a local wet market in Shah Alam, Selangor. The plant sample was washed and rinsed with running tap water to remove dirt and contaminants. The cleaned sample was dried in the oven at 60 °C for two days. Then, the dried plant sample was weighed and ground with a high-speed blender and stored at room temperature for further analysis.

Preparation of Plant Extract

An amount of 10 g *P. minus* powder was weighed and added to 100 mL of double-distilled water. The mixture sample was boiled for 15 min at 100 °C and left to cool. The extract solution was filtered using a vacuum pump, and the filtrate was used as the reducing agent for the preparation of AgNPs.

Green Synthesis of Plant Silver Nanoparticles and Characterisation

$AgNO_3$ (99.98%), which was applied as a silver precursor, was obtained from R & M Chemicals (UK). A 0.1 M $AgNO_3$ was prepared by dissolving 3.058 g of $AgNO_3$ in 180 mL of double-distilled water and stored in an amber-coloured bottle to prevent auto-oxidation of silver. The concentration was set to 0.1 M, which was the optimum concentration of AgNPs to show the smallest particles size according to our previous finding.

The synthesis of AgNPs was carried out by the addition of 20 mL *P. minus* extract in 180 mL of 0.1 M aqueous $AgNO_3$ solution. The mixture solution was stirred and heated at 80 °C. The colour change of the solution was observed and recorded. UV-Vis spectrophotometer was used for the spectrometric analysis to confirm the formation of AgNPs. To determine the time point of the maximum production of AgNPs, the absorption spectra of the sample was taken at 300–700 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific, Model Biomate 3 spectrophotometer) with deionised water as the blank.

After the synthesis of AgNPs, the solution containing nanoparticles was centrifuged at 10,000 rpm for 30 min to separate AgNPs from other compositions of the solution. The sedimented nanoparticles were collected and washed three times with double-distilled water before drying in a hot air oven at 60 °C for 24 h. The dried AgNPs were stored for further analysis.

Field Emission Scanning Electron Microscope (FE-SEM) and Energy Dispersive X-ray (EDX) Analysis

The particle size and nanostructural studies of AgNPs were investigated by FE-SEM. The dried AgNPs powder was coated with gold approximately 10 nm thick and was placed onto an adhesive tape on the FE-SEM stub. Meanwhile, the analysis of EDX was used to find the elemental composition of the reaction mixture. Further analysis was carried out by observing the morphology of bacterial cells treated with AgNPs through FE-SEM.

Observing Bacterial Cells through FE-SEM

FE-SEM was used to directly observe the surface morphological changes of untreated or treated (with AgNPs) bacterial cells. The sample was cut to 1 cm^3 dimension and fixed in 4% glutaraldehyde for 12–24 h at 40 °C. The fixed cells were washed three times with phosphate-buffered solution (PBS) for 10 min of each sample. After washing with PBS, the dehydration process was conducted with 30, 50, 70, 80, 90, and 100% of ethanol. The fixed cell was dried and gold-coated using an ion sputter. The pre-treated samples were observed by FE-SEM (SMT-SUPRA 40VP, Carl Zeiss AG, Oberkochen, Germany).

Antimicrobial Property of Silver Nanoparticles

Bacterial Isolates

Three bacteria, i.e. *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 15442) were chosen to be tested in this study. The pure cultures of these bacteria were obtained from the Microbial Culture Collection Unit (UNiCC), Institute of Bioscience, Universiti Putra Malaysia (UPM).

Well Diffusion Assay

The well diffusion assay was conducted to determine which antibiotic or sample given are the most successful in treating bacterial infections. Wells with the diameter of 6 to 8 mm were punched into the agar medium on Mueller-Hilton (MH) plates, and a volume of 40 µL of the antimicrobial agent and *P. minus* extract solution at the desired concentrations were introduced into the wells. The microbial culture was standardised according to 0.5 McFarland standards (1×10^8 CFU/mL), and the streptomycin standard was used for each bacterium. All plates were incubated at 30–37 °C for 18–24 h. The diameters of the zones of complete inhibition were measured using a vernier calliper and interpreted according to the National Laboratory Standard Institute (NLSI, 2010).

Results and Discussion

Silver Nanoparticles Analysis

Figure 1 shows the appearance of the colour change of the mixture from yellowish to reddish-brown after several minutes indicating the reduction of Ag ion due to excitation of surface plasmon vibrations in AgNPs (Bonnia *et al*., 2016). In this experiment, AgNPs were successfully synthesised from the aqueous $AgNO_3$ solution using *P. minus* extract in a continuously heated and stirred mixture. *P. minus* has been reported to have a large group of flavonoid content in its polyphenolic compound, which can actively chelate and reduce metal ions into nanoparticles. Various functional groups of flavonoids can also form nanoparticles (Makarov *et al.*, 2014). The formation of AgNPs was confirmed by the change in the colour of the solution mixture by the bioreduction of Ag^+ to Ag^0 (Khan *et al*., 2017) using possible chemical reactions as follows:

$$Ag^+ (aq) + \textit{Polygonum minus} \rightarrow [Ag (\textit{Polygonum minus})]^+ \quad (1)$$

$$[Ag (\textit{Polygonum minus})]^+ + R\text{-}CHO \rightarrow [Ag (\textit{Polygonum minus})] + R\text{-}COOH \quad (2)$$

The reaction between $AgNO^3$ and *P. minus* extract occurred when the dispersion of silver ion in the *P. minus* aqueous solution mixture (1) reacted with the Ag to form $[Ag (P. minus)]^+$ complex, which reacted with aldehyde in the molecular structure to form [Ag (*P. minus*)] due to the reduction of silver ions (2).

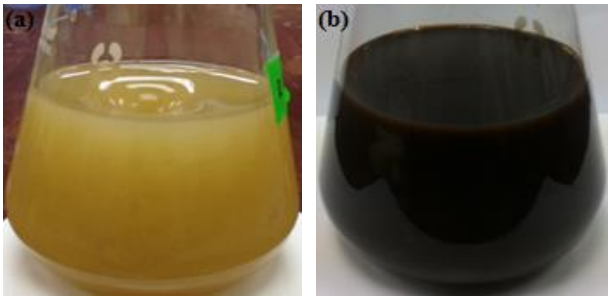

Figure 1. The colour changes of (a) *P. minus* extract and (b) synthesised AgNPs

UV-Vis Spectroscopy Analysis

UV-Vis spectroscopy has been widely used to detect the presence of AgNPs during synthesis (Logeswari *et al*., 2015; Ali *et al.*, 2016). Surface plasmon absorption peaks in the range from 420 to 470 nm have been used as an indicator to confirm the reduction of Ag^+ to metallic Ag in AgNPs (Hyllested *et al.*, 2015; Motitswe *et al.*, 2019). In this study, the formation of AgNPs was monitored by measuring UV-Vis spectra at different time intervals.

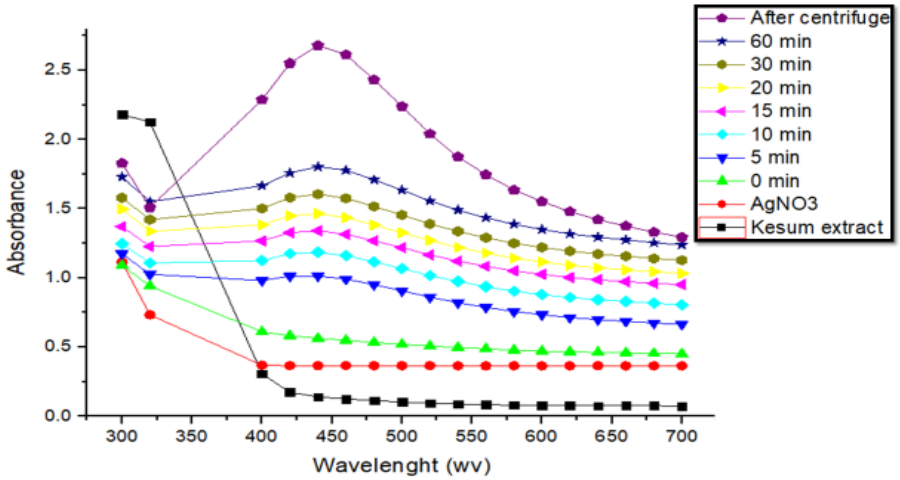

Figure 2. UV-Vis Spectra of AgNPs measured at different time intervals

The UV-Vis spectra showed a strong peak absorbance at 440 nm corresponding to the surface plasmon resonance (SPR) of AgNPs, which increased with the time of incubation of $AgNO^3$ (5 min, 10 min, 15 min, 20 min, 30 min, and 60 min) with the plants extract indicating increased amount of AgNPs produced from the mixture (Figure 2). This characteristic colour variation is due to the excitation or the SPR in the metal nanoparticles (Khan *et al.*, 2017). Similar changes in colour have also been observed in previous studies (Banerjee *et al.*, 2014; Namratha & Monica, 2013). On the contrary, the control experiment ($AgNO^3$) and the time point of 0 min showed no colour, indicating the absence of AgNPs.

FE-SEM and EDX Analysis

The particle size and nanostructural studies of AgNPs were investigated by FE-SEM. The spherical size of AgNPs is in the range of 15–25 nm, as shown in Figure 3 (a). Detected too are the larger size of nanoparticles. According to Khan *et al.* (2017), some nanoparticle size is more substantial because AgNPs tend to agglomerate due to their high surface energy and high surface tension of the ultrafine nanoparticles. Meanwhile, the elemental composition in the reaction mixture of AgNPs was measured by EDX analysis. The EDX analysis confirmed the presence of AgNPs in *P. minus* extract by the observation of strong signal energy peaks of Ag atoms (82.6%), as shown in Figure 3 (b) below. The synthesised AgNPs is formed by the assistance of *P. minus* extract, which act as a good bio-reductant for $AgNO_3$ in the process of AgNPs biosynthesis (Bouqellah *et al.*, 2018). The EDX result confirms the presence of Ag at a very high percentage of 82.3%. Other peaks of carbon (C), chloride (Cl), oxygen (O), and phosphorus (P) were also observed, which might be attributed to the presence of ambient air interference during the centrifugation process.

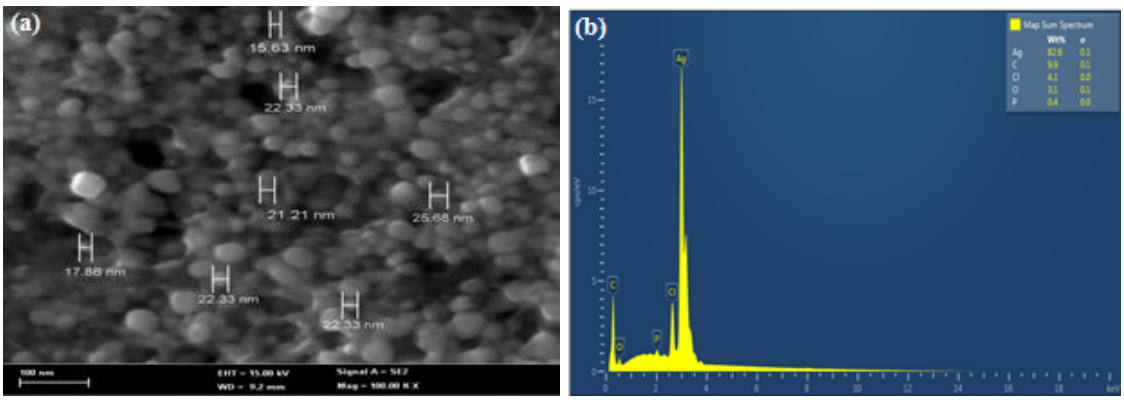

Figure 3. (a) FE-SEM image of AgNPs and (b) EDX analysis of AgNPs

Morphological Changes of Bacterial Cells Treated with Silver Nanoparticles

The morphological changes of bacterial cells between untreated and treated with AgNPs were observed by FE-SEM, respectively (Figure 4, 5, and 6). In the *E. coli* and *P. aeruginosa* cultures, cells of the control group were typically rod-shaped. Each cell size was almost the same and damage on the cell surface was not detected. However, in *E. coli* and *P. aeruginosa* treated with AgNPs, instead of the normal rod-shaped cells, irregular fragments appeared on the cell surface indicating the damage to the cell surface. Meanwhile, in the *S. aureus* culture, cells of control were typically grape-shaped, with an intact cell surface and no damage was seen. However, after treatment with AgNPs, fragments were detected on the cell surface, and it became agglomerated, indicating a damaged cell surface. Increased permeability of the cell membrane or leakage of cell contents could be caused by the reactive oxygen species (ROS; Kim *et al.*, 2011). FE-SEM morphological micrographs showed that the destruction of the bacterial cell of *S. aureus* was feebler compared to *E. coli* and *P. aeruginosa*. It could be due to the difference of the peptidoglycan layer of bacterial cell between Gram-positive *S. aureus* and Gram-negative *E. coli* and *P. aeruginosa*, where the essential function of the peptidoglycan layer is to protect the bacteria against antibacterial agents such as antibiotics, toxins, chemicals, and degradative enzymes (Silhavy *et al.*, 2010; Kim *et al.*, 2011). Typically, the Gram-positive cell envelope consists of lipoteichoic acid-containing thick peptidoglycan layer and cell membrane while the Gram-negative cell envelope consists of the outer membrane, thin peptidoglycan layer, and cell membrane.

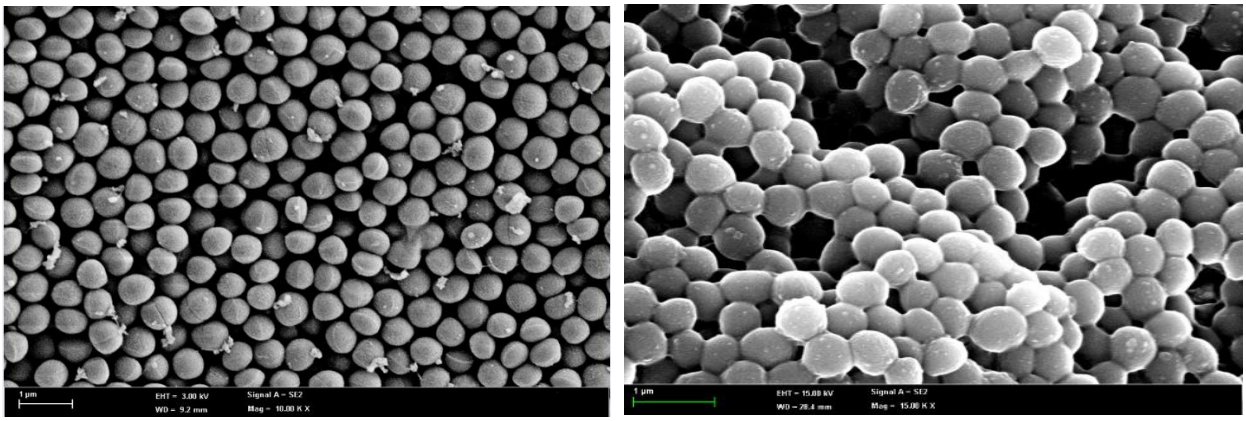

Figure 4. The FE-SEM study of bacteria (*S. aureus*) untreated and treated with AgNPs.

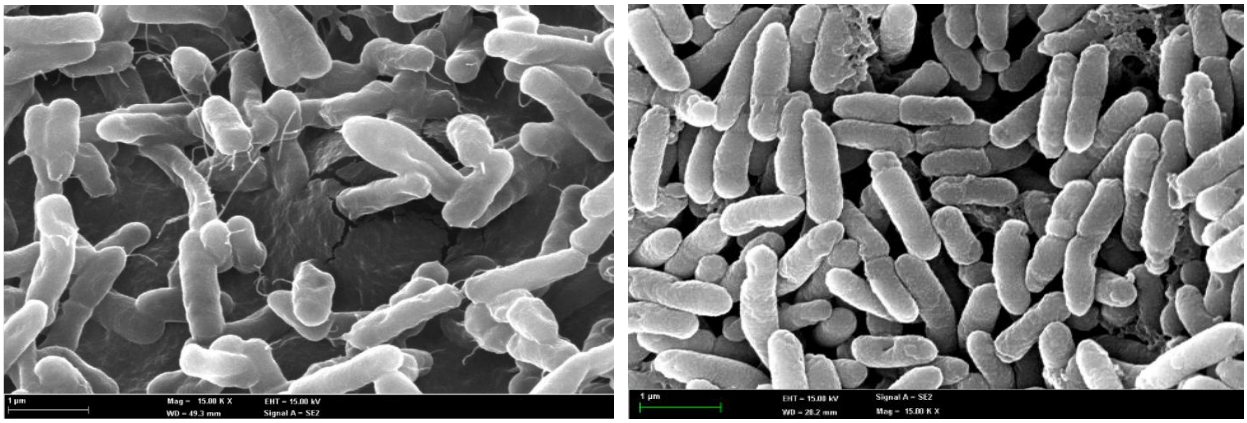

Figure 5. The FE-SEM study of bacteria (*P. aeruginosa*) untreated and treated with AgNPs.

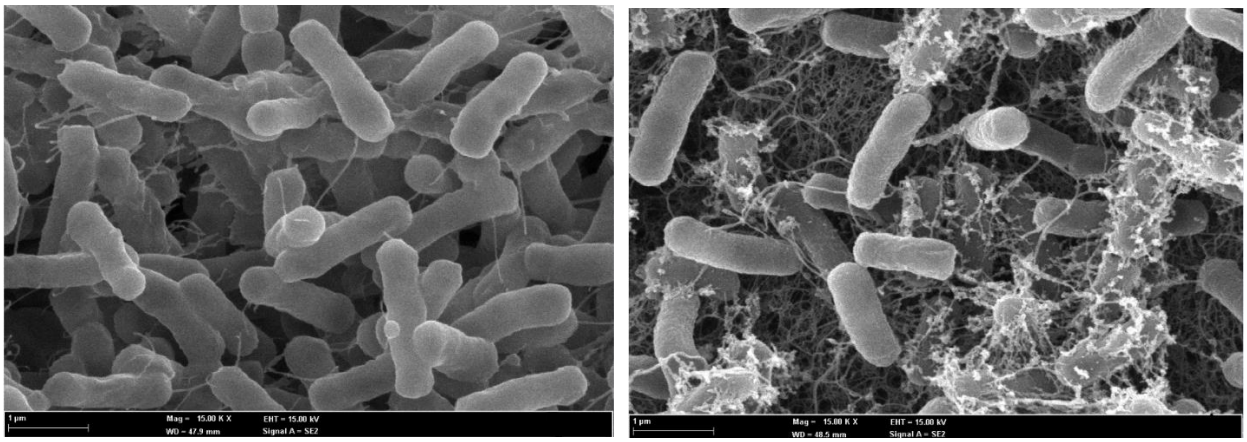

Figure 6. The FE-SEM study of bacteria (*E. coli*) untreated and treated with AgNPs.

Antimicrobial Activity of Silver Nanoparticles

After incubation for 24 h, growth inhibition was observed in samples impregnated with AgNPs and the positive control (streptomycin) with the inhibition zone of 27 mm, 30 mm, and 31 mm, respectively (Table 1), while the negative control showed no inhibition zone. AgNPs exhibited strong antimicrobial activity against both Gram-positive, (*S. aureus*) and Gram-negative bacteria, (*E. coli* and *P. aeruginosa*) and formed inhibition zones of 16 mm, 17 mm, and 17 mm, respectively (Table 1). Higher inhibition zones were noticed for *E. coli* and *P. aeruginosa* compared to *S. aureus*. From the results obtained, Gram-negative bacteria showed a larger inhibition zone compared to Gram-positive bacteria (Figure 7). The Ag mode of action is presumed to be dependent on Ag^+ ions, which strongly inhibit bacterial growth through the suppression of respiratory enzyme and electron transport components, as well as the interference with DNA functions (Hassan *et al.*, 2014; Cedillo-Alvarez *et al*., 2017). Thus, AgNPs have been demonstrated to exhibit antimicrobial properties against bacteria with a close attachment of the nanoparticles with the microbial cell.

Table 1. Well diffusion assay of AgNPs against bacteria

Sample	**Target Microbes**		
	S. aureus (ATCC 43300)	*P. aeruginosa* (ATCC 15442)	*E. coli* (ATCC 25922)
	Zone of inhibition (mm)		
0.1 M	16	17	17
-ve control	-	-	-
+ve control	27	31	30

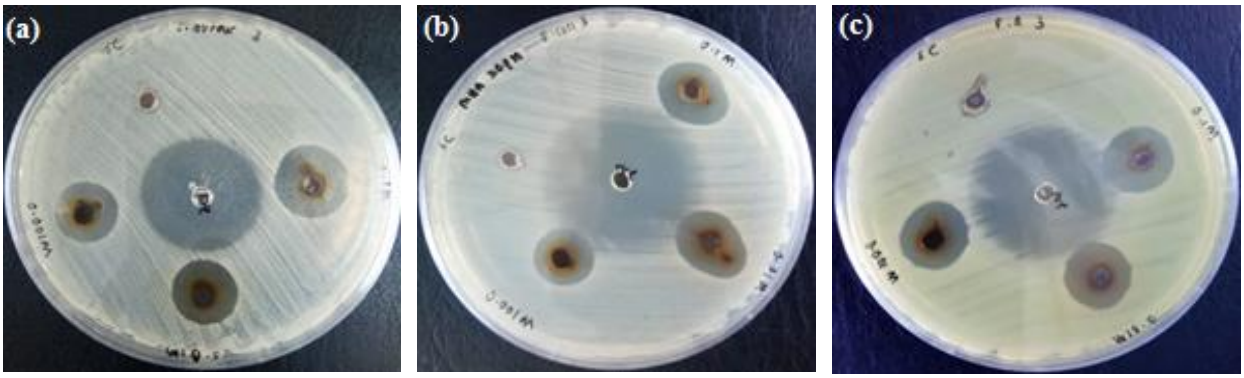

Figure 7. The observations of growth inhibition zone on each bacterial plate (a) *S. aureus*, (b) *E. coli*, and (c) *P. aeruginosa*

Conclusion

In this study, the synthesised AgNPs using *P. minus* extract with aqueous $AgNO_3$ were successfully produced. The synthesised AgNPs were characterised by UV-Vis spectroscopy, FE-SEM, and EDX measurements. The UV-Vis spectra showed a strong peak absorbance at 440 nm that indicated the reduction of Ag^+ to metallic Ag. The size of AgNPs produced were in the range of 15–25 nm with strong peaks for silver (Ag) is 82.6% of AgNPs. For the antimicrobial property assessment, Gram-negative bacteria showed more significant inhibition zones compared to Gram-positive bacteria. The morphological changes of bacterial cells treated with AgNPs were observed by FE-SEM and showed that the AgNPs has excellent antimicrobial properties against microorganisms. Thus, it can be concluded that besides being eco-friendly, the application of plant extracts in the synthesis of nanoparticles is also cost-effective, small in size, and effective as an antibacterial agent against various microorganisms, and it holds the potential to be utilised at a larger scale.

Acknowledgement

The authors would like to thank the Research Grant Scheme (GPNS – 01/UNISEL/18-006) and Universiti Selangor (UNISEL) for supporting this research.

References

Ahmad, R., Sahidin, I., Taher, M., Low, C., Noor, N. M., Sillapachaiyaporn, C., Chuchawankul, S., Sarachana, T., Tencormnao, T., Iskandar, F., Rajab, N. F. & Baharum, S. N. (2018). Polygonumins A, a newly isolated compound from the stem of Polygonum minus Huds with potential medicinal activities. *Scientific reports*, *8*(1), 4202.

Ali, Z. A., Yahya, R., Sekaran, S. D., & Puteh, R. (2016). Green synthesis of silver nanoparticles using apple extract and its antibacterial properties. *Advances in Material Science and Engineering, 2016*, 1-6.

Ana-Alexandra, S., Alexandrina, N., Rodica-Mariana, I., & Suică-Bunghez, I. -R. (2016). Green synthesis of silver nanoparticles using plant extracts. *The 4th International Virtual Conference on Advanced Scientific Results, Romania*, 188-193.

Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, 1(3), 1-10.

Bonnia, N. N., Kamaruddin, M. S., Nawawi, M. H., Ratim, S., Azlina, H. N. & Ali, E. S. (2016). Green biosynthesis of silver nanoparticles using '*Polygonum Hydropiper*' and study its catalytic degradation of methylene blue. *Procedia Chemistry*, *19*, 594 – 602.

Bouqellah, N. A., Mohamed, M. M., & Yasmine, I. (2018). Synthesis of eco-friendly silver nanoparticles using *Allium sp.* and their antimicrobial potential on selected vaginal bacteria. *Saudi Journal of Biological Sciences.*
Cedillo-Alvarez, C. P., Gupta, D., & Chauhan, P. (2017). Green synthesis of silver nanoparticles involving extract of plants of different taxonomic groups. *J. Nanomed Res., 5(2),* 00110.

Gu, H., Ho, P. L., Tong, E., Wang, L., & Xu, B. (2003). Presenting vancomycin on nanoparticles to enhance antimicrobial activities. *Nano Lett., 3(9),* 1261-1263.

Gurunathan, S., Han, J. W., Kwon, D. -N., & Kim, J. -H. (2014). Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale Research Letters, 9, 1,* 1-17.

Hassan, M., Walter, M., & Moseler, M. (2014). Interactions of polymers with reduced graphene oxide: van der waals binding energies of benzene on graphene with defects. *Phys. Chem. Phys., 16(1),* 33-37.

Hassim, N., Markom, M., Anuar, N., Dewi, K. H., Baharum, S., & Noor, N. (2015). Antioxidant and antibacterial assays on *Polygonum minus* extracts: different extraction methods. *International Journal of Chemical Engineering,* 2015, 1-10.

Hassim, N., Markom, M., Anur, N., & Baharum, S. N. (2014). Solvent selection in extraction of essential oil and bio active compounds from *Polygonum minus*. *Journal of Applied Sciences, 14(13),* 1440-1444.

Hyllested, J. A. E., Palanco, M. E., Hagen, N., Mogensen, K. B., & Kneipp, K. (2015). Green preparation and spectroscopic characterization of plasmonic silver nanoparticles using fruits as reducing agents. *Beilstein J. Nanotechnol***.,** *6,* 293-299.

Khan, M. Z. H., Tarek, F. K., Nuzat, M., Momin, M. A., & Hasan, M. R. (2017). Rapid biological synthesis of silver nanoparticles from *Octimum sanctum* and their characterization. *Journal of Nanoscience.*

Kharissova, O. V., Dias, H. V. R., Kharisov, B. I., Perez, B. O., & Perez, V. M. J. (2013). The greener synthesis of nanoparticles. *Trends in Biotechnology, 31, 4,* 240-248.

Kim, S. H., Lee, H. S., Ryu, D. S., Choi, S. J., & Lee, D. S. (2011). Antibacterial activity of silver-nanoparticles against *Staphylococcus aureus* and *Escherichia coli*. *Korean Journal of Microbiology and Biotechnology, 39(1),* 77-85.

Lee, D. Y., Khatun, Z., Lee, J. H., Lee, J. K., & In, I. (2011). Blood compatible graphene/heparin conjugate through noncovalent chemistry. *Biomacromolecules, 12(2),* 336-341.

Logeswari, P., Silambarasan, S., & Abraham, J. (2015). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. *Journal of Saudi Chemical Society, 19,* 311-317.

Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., & Kalinina, N. O. (2014). "Green" nanotechnologies: Synthesis of metal nanoparticles using plants. *Acta Naturae, 6(1),* 35 – 44.

Mohamad, Z. A., Johari, M. A., Mitra, A., Puteri, S. A. -R., & Onn, H. (2017). Anti-proliferative, *in vitro* antioxidant, and cellular antioxidant activities of the leaf extracts from *Polygonum minus* huds: Effects of solvent polarity. *International Journal of Food Properties, 20, sup1,* 846-862.

Motitswe, M. G., & Fayemi, O. E. (2019). Characterization of Green Synthesized Silver Nanoparticles Doped in Polyacrylonitrile Nanofibers. *American Journal of Nanoscience & Nanotechnology Research, 7,* 32-48.

Namratha, N., & Monica, P. V. (2013). Synthesis of silver nanoparticles using *Azadirachta indica* (Neem) extract and usage in water purification. *Asian Journal of Pharmaceutical Technology, 3,* 170–174.

Nurain, A., Noriham, A., Zainon, M. N., Khairusy Syakirah, Z., & Wan Saifatul Syida, W. K. (2012). Phytochemical constituents and in vitro bioactivity of ethanolic aromatic herb extracts. *Sains Malaysiana*. 41, 1437–1444.

Qader, S. W., Abdulla, M. A., Chua, L. S., & Salehhuddin, H. (2012). Potential bioactive property of *Polygonum minus* huds (kesum) review. *Scientific Research and Essays, 7,* 90–93.

Retchkiman, S., Canizal, P. S., Becerra-Herrera, G., Zorilla, R., Liu, C., & Ascencio, H. B. (2006). Biosynthesis and characterization of Ti/Ni bimetallic nanoparticles. *Opt. Mater., 29*, 95-99.

Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, 2(5), a000414.

Simsikova, M. (2016). Interaction of graphene oxide with albumins: Effect of size, pH and temperature. *Arch. Biochem. Biophys, 593*, 69-79.

Vidya, C. M., Krinsha, K. R., Rajendra, A. L., Dipak, A. K., Sanjay, S. S., & Kokare, B. N. (2016). Green synthesis of silver nanoparticles from plants. *Proceeding of International conference on Advances in Materials Science, India,* 347 – 352.