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Synthesis of Silver Nanoparticles Using *Polygonum minus* Extract and Analysis of Their Antimicrobial Properties

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Abstract Nanotechnology is a rapidly developing and promising field that makes best use of inert metals like silver, gold and platinum to synthesize metallic nanoparticles with high potential for various applications. Among all metal nanoparticles, silver nanoparticles (AgNPs) have much attention due to the surface plasmon resonance (SPR) (strong absorption in the visible region), which can be easily observed by UV–visible spectrophotometer. This study aims to investigate an antimicrobial activity of silver nanoparticles (AgNPs) synthesize using *Polygonum minus* extract as a reducing agent and aqueous silver nitrate as a precursor. Based on the observation, the colorless reaction mixture slowly changed from yellowish green to reddish brown and further confirmed by surface plasmonic resonance (SPR) band at 440 nm using UV–visible spectroscopy indicating of reduction of silver ion after several minutes of reaction. The AgNPs was characterized by Field-emission Scanning Electron Microscope (FE-SEM) and Transmission Electron Microscopy (TEM). The observation of FE-SEM showed the size of AgNPs was produced in the range of 15 nm – 25 nm while TEM image shows a well-dispersed silver nanoparticles with roughly spherical shape and size ranging particle size 6 – 21 nm. Three bacteria such as *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 and showed that the AgNPs has good antimicrobial properties against microorganisms and it is proven by low of MIC value. Thus, the ability of AgNPs to release Ag ions is a key to their antimicrobial activity.

Keywords Silver Nanoparticles; Polygonum minus; green synthesis, antimicrobial

1. Introduction

Nanotechnology is rapidly growing and becoming the important tool to be applied in many areas and industry due to increase the population size and increasing incomes in developing countries. BCC Research in 2012 reported that the global market for nanotechnology was valued at nearly US$20. 1 billion in 2011 and will reach US$20. 7 billion in 2012. This report expects the total sales of nanotechnology product will reach to US$48. 9 billion in 2017. According to the National Nanotechnology Initiative (2013), United States in the federal fund budget has been spent, nearly $1.8 billion on the advancement of nanotechnologies in 2013 and it is 4% increased than year 2012. While the latest report by PRNewswire (2018) expected the global market for nanotechnology will be exceeded to US$ 125 Billion by 2024. It is in line with Roco et al. (2010) reported that nanotechnology are projected to impact at least $3 trillion across the global economy by 2020, and nanotechnology industries worldwide may require at least 6 million workers to support them by the end of the decade.

Recent advancement in nanotechnology and nanobiotechnology has expanded our ability to design and construct nano materials with targeting, therapeutic, and diagnostic

functions. These multifunctional materials have attracted our attention to be used as the promising tool for selective bacteria sensing and therapy without the current drugs. The most effective vicinity of nanotechnology is the competence of modulating metals into their nano size (Jeevanandam et al., 2018) which offers unique approaches to probe and control a wide variety of biological and medical processes that occur at nanometer length and is believed to have a successful collision on biology and medicine (Zarina and Nanda, 2014). Owing to their high antibacterial properties, nanoparticles of silver, oxides of zinc, titanium, copper, and iron are the most commonly used nanoparticles in antimicrobial studies. Furthermore, these nanoparticles have been used to deliver other antimicrobial drugs to the site of pathological process (Gatto and Bardi, 2018). Metallic nanoparticles provide an attractive alternative to antibiotics in the pharmaceutical field by developing novel applications. The synthesis of nanomaterials of specific composition and size is a burgeoning area of materials science research. The properties of these materials in applications as diverse as catalysis, sensors and medicine depend critically on the size and composition of the nanomaterial (Navya and Daima, 2016). Thus, researchers have used biological synthesis, since this technique provides the particles with good control over the size distribution. The main reason for this may be that the processes devised by nature for the synthesis of inorganic materials on nano- and micro- scales have contributed to the development of a relatively new and largely unexplored area of research based on green chemistry.

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*., 2014). 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.

2. Material and Methods

2.1 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.

2.2 Preparation of Leaf 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.

2.3 Green Synthesis of Plant Silver Nanoparticles and Characterization

An accurate concentration of 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 amber colored bottle to prevent auto oxidation of silver. The concentration was set for 0.1 M which was the optimum concentration of AgNPs to show the smallest particle size according to our previous finding.

The synthesis of AgNPs was carried out by added about 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 color 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 maximum production of AgNPs, the absorption spectra of the sample was taken 300 – 700 nm using a UV-vis spectrophotometer (Thermo Fisher Scientific, Model Biomate 3 spectrophotometer). The deionized water was used as the blank. Proceed with collected of AgNPs, the solution was centrifuged at 10,000 rpm for 30 minutes. The separated nanoparticles settled at the bottom was collected and washed for three time with double distilled water at 10,000 rpm for 10 minutes of each sample. The collected AgNPs was dried in the oven at 60 ^oC for until achieve to the constant weight. The stabilized powder forms of the nanoparticles were stored for further characterization.

2.4 Field Emission Scanning Electron Microscope (FE-SEM) and Transmission Electron Microscopy (TEM)

The particle size and nanostructural studies of AgNPs were investigated using a SUPRA 55VP Carl Zeiss Model field emission scanning electron microscope (Germany). The dried AgNPs powder was coated with gold to prevent electric charging during investigation. Further analysis was carried out by observing the morphology of bacterial cells treated with AgNPs through FE-SEM (three selected bacteria, i.e. *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 15442)).

The TEM analysis also perform to determine the morphology and size of AgNPs on the CM12 Phillip Model transmission electron microscopy (Holand). For this purpose , one drop of dispersed AgNPs was placed on a copper grid and allowed to evaporate completely under infrared light before investigation.

2.5 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 hours 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 (SUPRA 55VP, Carl Zeiss, Germany).

2.6 Antimicrobial Property of Silver Nanoparticles

2.6.1 Culture Condition and Cell Inoculum

Six bacteria, i.e. *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella typhi (*ATCC 14O28), *Klebsiella*, and *Bacillus subtilis ATCC®11774™* were chosen to be tested in this study. The pure cultures of these bacteria were obtained from the Microbial Culture Laboratory, Institute of Bio-IT Selangor, Universiti Selangor (UNISEL). Each bacteria strain was respectively cultured on Mueller Hinton Agar (MHA) media (Oxoid, UK) at 37 °C for 24 h. The turbidity of the suspension was adjusted to an optical density (OD550nm) of 0.144 which is equivalent to 1 × 106 cells/mL. A stock solution of 1 mg/mL 0.1, 0.01 and 1 mM silver naniparticles (Ag NPs) was prepared in sterile distilled water (sdh20) and stored at 4 °C until used.

2.6.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacteriacidal Concentration (MBC)

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. This was determined from readings on the culture plates after incubation. The minimum inhibitory concentrations (MIC) of silver nanoparticles (Ag NPs) against Gram-positive and Gram-negative bacteria was determined according to the microdilution method of the CLSI M38-A2 using 96-well microtiter plates with some modifications (Clinical and Laboratory Standards Institute 2008). A broth microdilution method was recommended by CLSI as a general standard methodology for testing active compound or commercialized antimicrobial agent.

Thus, this method was employed to analysed the MIC of 0.1 M, 0.01 M and 1 mM of silver nanoparticles (Ag NPs). Each well contained the 10 µL of bacteria at a final concentration of 1.0×10^6 cells/mL, 100 µL of MH broth, and 100 µL of 0.1 M, 0.01 M and 1 mM Ag NPs. Mueller Hinton (MH) broth without test agents was included as an agent-free control, and MH broth was used as a medium blank. All plates were incubated in an aerobic incubator at 37 °C for 24 h, after which the growth was determined spectrophotometrically at 550 nm by means of a microplate reader (PowerWave 200, Bio-Tek Instruments, and Winooski, VT, USA). The data were reported as the median of at least 3 independent tests.

While the MBCs is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates that do not contain the test agent. In this method, the MBCs were determined by spreading aliquots of 50 μL from the well showing no visible growth on MH agar plates. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The absence of viable growth following 24 to 48 h incubation indicated the MBC of compound on the respective strains.

3. Result and Discussion

3.1 Silver Nanoparticles Analysis

It is well known that AgNPs exhibit brown color in aqueous solution due to excitation of surface Plasmon vibrations in AgNPs (Bonnia *et al*., 2016). In this experiment AgNPs were successfully synthesized from the aqueous $AgNO_3$ solution using *P. minus* extract in a continuously heated and stirred mixture. Figure 1 shows the colorless reaction mixture slowly changed from yellowish green to reddish brown indicating of reduction of the Ag ion after several minutes of reaction.

3.1.1 *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). The possible reduction mechanism of silver ion in this reaction can be carry out by the involvement of phenolic compound through oxidation of aldehyde groups (H-C=O) in the *P.* minus to carboxylic acids (-COOH). It's probably happening with the dealings of carbonyl groups or pi-electrons in the absence of other powerful chelating agents, create flavonoids and terpenoids adsorbed on the surface of AgNPs and act as capping agents (Anjum *et al.*, 2016).

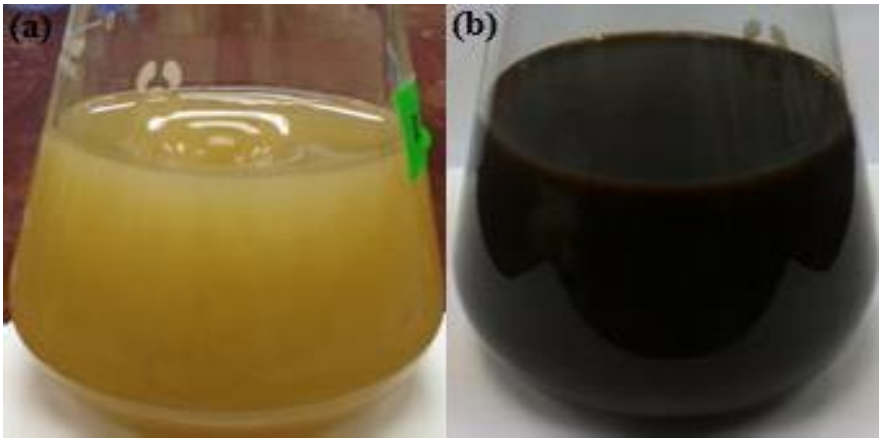

Figure 1. The colour changes of (a) *P. minus* extract and (b) synthesized AgNPs

3.2 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.

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 the 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.

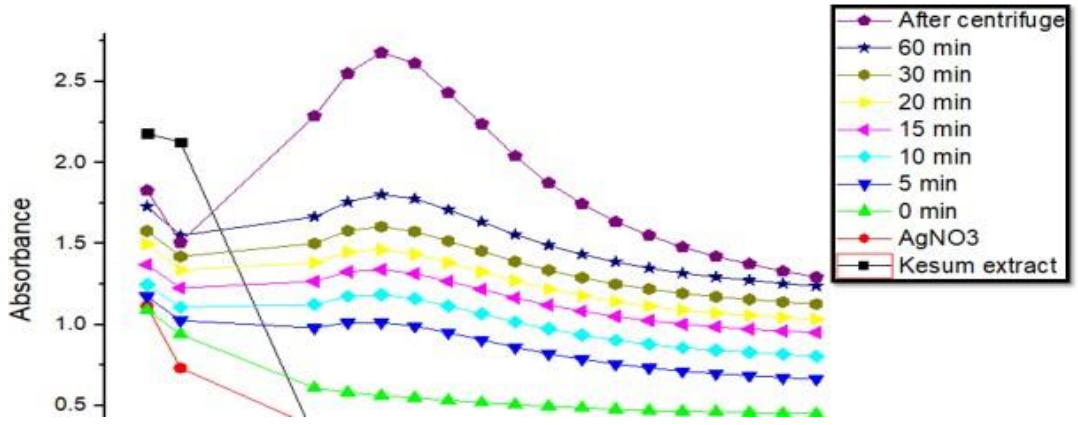

Figure 2. UV-Vis Spectra of AgNPs measured at different time intervals

3.3 FE-SEM and TEM 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. 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.

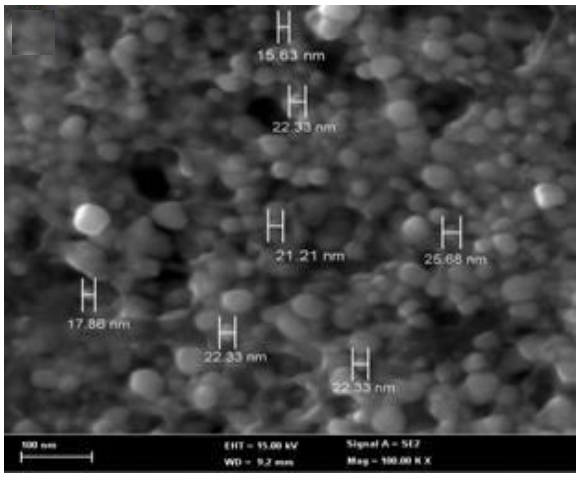

Figure 3. FE-SEM image of AgNPs

Transmission electron microscope (TEM) study was performed to estimate the shape and size of the nanoparticles. The particles are predominantly spherical shape with smooth surface morphology with size ranging particle in 6 – 21 nm (Figure 4). Some of the AgNPs were found to be oval and the scenario in variation of shape and size of nanoparticles is common when using a biological system (Rao *et al.*, 2016*).*

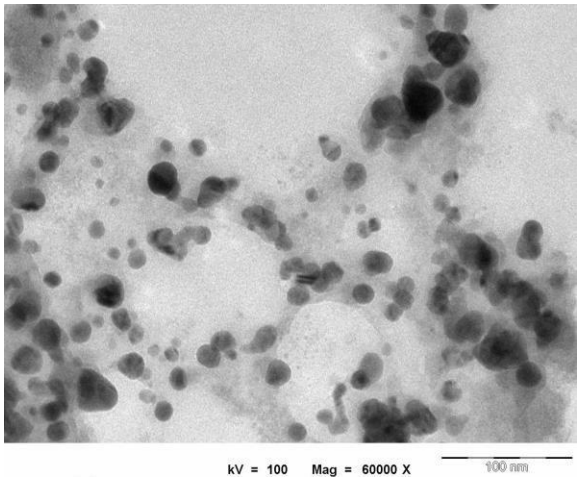

Figure 4. TEM image of AgNPs

3.4 MIC and MBC Determination

Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. The MIC and MBC of AgNPs towards all six bacterial species showed variations (Table 1). The MICs of 0.1 M of AgNPs were determined within the range of 31.25 to 100 µg/mL, while the MBC values were from 62.5 to 100 µg/mL. For *Pseudomona aeruginosa* and *Staphylococcus aureus* the MBCs were found to be greater than 100 µg/mL Among the six bacterial species *S. aureus* was the most susceptible to the 0.1 M AgNPs. While the *Bacillus subtilis* showed a negative result with AgNPs.

Table 1: MIC and MBC (µg/ml) inhibitory results of silver nanoparticles (Ag NPs)

No.	Bacterial strain (1×10^6 cells/ml)	MIC of AgNPs (µg/ml)	MBC of AgNPs (µg/ml)
1	*Staphylococcus aureus ATCC 43300*	50	>100
2	*Pseudomonas aeruginosa ATCC 15442*	100	>100
3	*Escherichia coli 25922*	50	100
4	*Salmonella typhi* ATCC 14028	50	100
5	*Klebsiella*	31.25	62.50
6	*Bacillus subtilis ATCC 11774*	>1000	N.D.

3.5 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 5). 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.

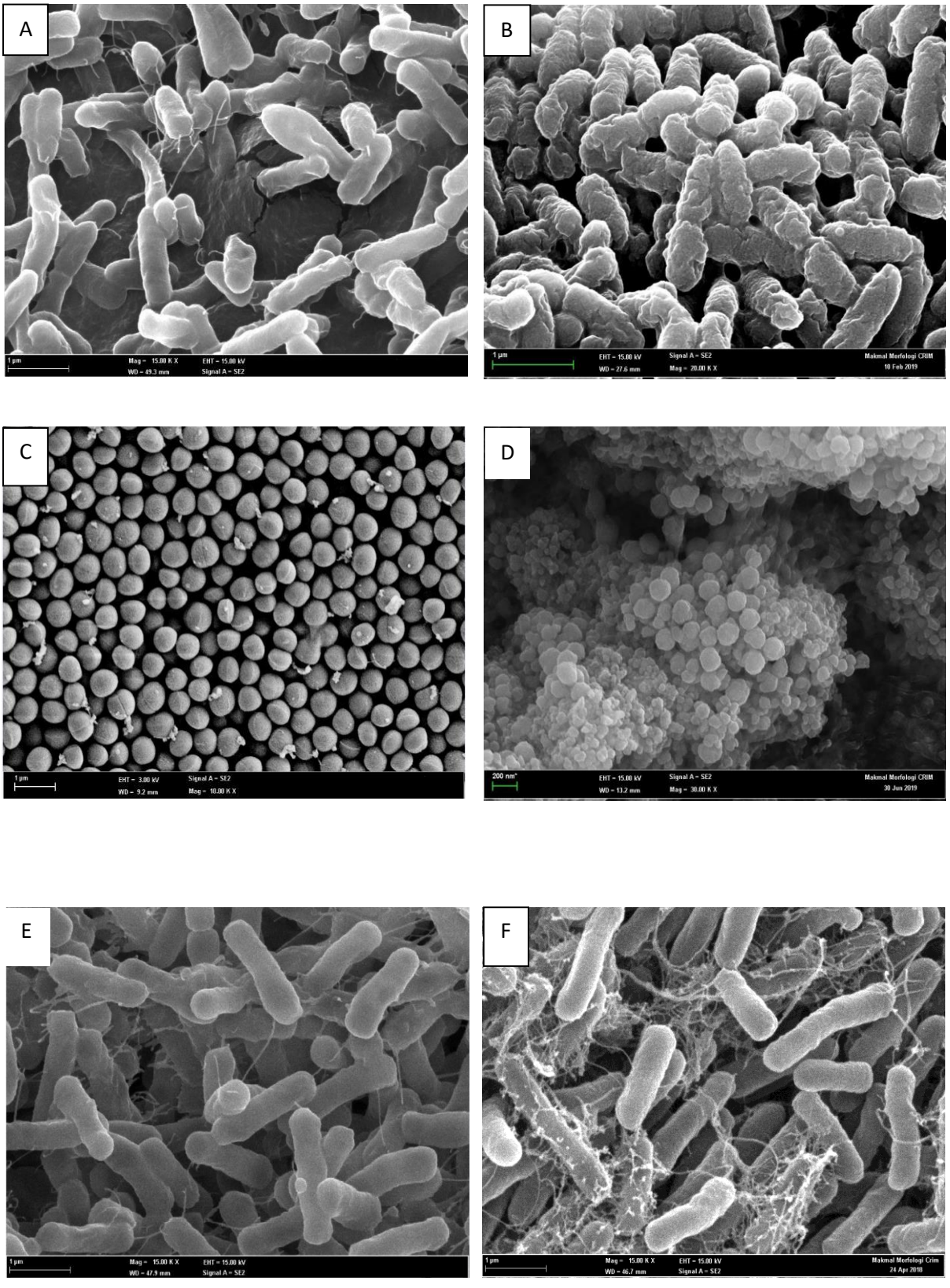

Figure 5: FE-SEM micrograph of *P. aeruginosa, S. aureus* and *E. coli* control (A, C, E) and *P. aeruginosa, S. aureus* and *E. coli* treated with silver nanoparticles (B, D, F).

4. Conclusion

In this study, the synthesized AgNPs using *P. minus* extract with $AgNO_3$ aqueous were successfully produced. It can be concluded that plant extract being very ecofriendly, cost effective, promising a small size of molecules and as effective antibacterial materials against various microorganisms and this method is potentially exciting for the large-scale synthesis of nanoparticles.

5. Acknowledgements

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6. 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.

Anjum, S., Abbasi, B. H. & Shinwari, Z. K. (2016). Plant-mediated green synthesis of silver nanoparticles for biomedical applications: Challenges and opportunities. *Pak. J. Bot.*, 48(4), 1731-1760.

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.

BCC Research Market Forecasting (2012). Nanotechnology: A Realistic Market Assessment. Retrieved October 10, 2019. http://www.bccresearch.com/report/nanotechnology-market-applications-products-nan031e.html.

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.

Gatto, F. & Bardi, G. (2018). Metallic nanoparticles: General research approaches to immunological characterization. *Nanomaterials (Basel)*, 8(10),753.

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.

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A. & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: History, Sources, Toxicity and Regulations. *Beilstein J Nanotechnol*, 9, 1050–1074.

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.*

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.

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.

National Nanotechnology Initiative (2013). National nanotechnology initiative: NNI manufacturing and job creation focus reflected in strong budget numbers for FY 2013.Washington, DC: Office of Science and Technology Policy. Retrieved October 10, 2019. http://www.nano.gov/about-nni/what/funding.

Navya, P. N. & Daima, H. K. (2016). Rational engineering of physicochemical properties of nanomaterials for biomedical applications with nanotoxicological perspectives. *Nano Converg.*, 3(1), 1.

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.

PRNewswire. 2018. The Global Nanotechnology Market (by Component and Applications), Funding & Investment, Patent Analysis and 27 Companies Profile & Recent Developments - Forecast to 2024. Retrieved October 10, 2019. https://www.prnewswire.com/news-releases/global-nanotechnology-market-2018-2024-market-is-expected-to-exceed-us-125-billion-300641054.html.

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.

Rao, M. V., Kishore, M. & Rao, Y. H. (2016). Green nanoparticles synthesized from roots of *Datura metel* and evaluation of antimicrobial activity. *Journal of Pharmacy Research*, 10(11),730-734.

Roco, M.C. Mirkin, C.A. & Hersam, M.C. (2010). Nanotechnology Research Directions for Societal Needs in 2020: Retrospective and Outlook, World Technology Evaluation Center (WTEC) and the National Science Foundation (NSF), Springer, 2010. Retrieved October 11, 2019. http://www.wtec.org/nano2/Nanotechnology_Research_Directions_to_2020.

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

Zarina, A. & Nanda, A. (2014). Combined efficacy of antibiotics and biosynthesised silver nanoparticles from Streptomyces Albaduncus. *International Journal of PharmTech Research*, 6, 1862-1869.