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Research Article

PHYSICOCHEMICAL AND ANTIBACTERIAL PROPERTIES FOR GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *POLYGONUM MINUS* EXTRACT

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ABSTRACT

Nanotechnology is an advanced scientific technique and has been widely applied in the 21st century. Connection between plant science and nanotechnology provides an inherently green approach to nanotechnology referred as green nanotechnology. In this study, plant extract from *Polygonum minus* was used for the synthesis of silver nanoparticles from silver nitrate solution. The green synthesis of nanoparticles has been proposed due to the cost effective and environment friendly alternative to chemical and physical. The silver nanoparticles was characterized by UV- vis spectrophotometer and the mean particle sizes were determined by dynamic light scattering. The result showed that the average size of silver nanoparticles was produced in 91.74 ± 0.48nm. UV-Visible absorption spectra of the reaction medium containing silver nanoparticles showed maximum absorbance at 440 nm, which indicated the reduction of Ag^+ to metallic Ag. Field-emission Scanning Electron Microscope (FE-SEM) of silver nanoparticles also showed that the silver nanoparticles has good antimicrobial properties against microorganisms.

Keywords: *Silver Nanoparticles, Polygonum Minus, Green Synthesis*

INTRODUCTION

Nanotechnology is an advanced scientific technique and have been widely applied in the 21st century. It is usually new and fast rising field that involved research and technology development, processing and application of structures, manufacture, sensor devices and control of structures within sizes from 1 to 100 nm (Quintanilla-Carvajal *et al*., 2010; Bouwmeester *et al*., 2009) and can be drastically modified their physic-chemical properties compared to the bulk material (Monica and Cremonini, 2009).

Silver a nobel metal has been known since time immemorial in the form of metallic silver, silver nitrate and silver sulfadiazine for treatment of burns, open wound (Nagati *et al.*, 2012) and several bacterial infections. However, due to the emergence of several antibiotics the use of the silver compounds has been declined remarkably (Raveendran *et al.*, 2003; Tripathy *et al.*, 2009). Silver nanoparticles have attracted intense research interest because of their important application in antimicrobial, catalysis and surface-enhancement (Gokulakrishnan *et al*., 2012). The rapid breakdown of silver nanoparticles release ionic silver that inactivates vital bacterial enzymes by interacting with essential thiol groups. Silver ions can inhibit bacterial DNA replication, damage bacterial cytoplasm membranes, depleting levels of intracellular adenosine triphosphate (ATP) and finally cause cell death (Parveen *et al*., 2012). The high surface to volume ratio of silver nanoparticles increase their contact with microorganisms, promoting the dissolution of silver ions, thereby improving biocidal effectiveness. The ability of silver nanoparticles to release silver ions is a key to their antimicrobial activity.

Recently, biosynthesis of nanoparticles has received considerable attention to obtain nanomaterial such as copper, zinc, titanium (Retchkiman-Schabes *et al.*, 2006), magnesium, gold (Gu *et al*., 2003), alginate

(Ahmad *et al*., 2005) and silver due to the growing need to develop environmentally benign technologies in material synthesis. According to Logeswari *et al.*, (2015) silver have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms. It is well known that the green biological method of synthesizing nanoparticles has materialized as an alternative to overcome the restrain of conventional methods in synthesized nanoparticles such as using several physical and chemical methods including chemical reduction of ions in aqueous solution with or with or without stabilizing agent and reduction in inverse micelles or thermal decomposition in organic solvents. Among the biological alternatives, plants and plants extracts seem to be the best option. Plants are nature's "chemical factory" with cost effective and require little or no maintenance.

Employing plants towards synthesis of nanoparticles has advantages over non biological methods as with the presence of broad variability of biomolecyles in plants which can act as capping and reducing agents and as a result increases the rate of reduction and stabilization of nanoparticles. A wide range of metabolites presented in the plant products/extracts has shown that the nanoparticles produced by plants are more stable and the rate of synthesis is faster in comparison to microorganisms. The reduction of metal ions by a combination of bimolecular found in these extracts such as protein/enzymes, polysaccharide, vitamin and amino acid is environmentally benign, yet chemically complex. Thus, the advantages of using plant and plant derived materials for biosynthesis of metal nanoparticles have investigated researchers to investigate mechanisms of metal ions uptake and bioreduction by plants, and to understand the possible mechanism of metal nanoparticle formation in an by the plants (Dubey *et al.*, 2010; Ahmad and Sharma, 2012; Zahir *et al*., 2012).

In this study, a green method for the synthesis of silver nanoparticles using plant extract of *Polygonum minus* as reducing agent was prepared and analysis of their physical, chemical and antibacterial properties will be studied.

Polygonum minus (synonym *Persicaria hydropiper* or *Polygonum minor* or *Persicariatenella*) which also known as kesum or laksa leaf (Faridah *et al*., 2006; Noor Haslinda *et al*., 2012; Bunawan *et al*., 2011) is a herbaceous plant, belongs to the Polygonacea family and primarily grown in temperature region. Various studies have revealed the different pharmacological potentials of *Polygonum minus* both in vitro and in vivo test models. *Polygonum minus*has demonstrated to possess cytoprotective, antibacterial, antifungal, antiulcer, antiviral and antioxidant activities. A lot of works also claims that kesum promotes high levels of free radical scavenging activity and reducing power as well as an antimicrobial properties (Sumazian *et al*., 2010; Hassim *et al.*, 2014; Qader *et al.*, 2012).

MATERIALS AND METHODS

Collection of Leaves

Polygonum minus (kesum), were purchased from a local wet market in Shah Alam, Selangor. Fresh samples were cleaned and washed under running tap water. The samples were dried in the oven at 37^{o}C overnight. Then, the samples were weighed and blended with Waring blender and stored before further analysis.

Preparation of Leaf Extract

15g of *Polygonum minus* powder leaves were weighed and then 100ml of double distilled water was added and boiled for 15 minutes at 100^{o}C. After cooling the extract was filtered using Whatman No. 1 filter paper and store at 4^{0}C for further use.

Green Synthesis of Plant Silver Nanoparticles

An accurate concentration of 0.1 M silver nitrate (Sigma grade) will be prepared by dissolving 3.058g $AgNO_3$ in 180ml of double distilled water and stored in amber coloured bottle to prevent auto oxidation of silver.

The synthesis of silver nanoparticles was carried out by using 20 ml of the *Polygonum minus* extract in 180 ml of 0.1 M aqueous $AgNO_3$ solution. The mixture was stirred and heated at 80^{0}C for different duration. The concentration was set for 0.1 M which was the optimum concentration of silver nanoparticles to show the smallest particles size according to our previous finding.

Characterization of Polygonum Minus Silver Nanoparticles

UV-Vis spectrophotometer will be used for the spectrometric analysis to confirm silver nanoparticles formation.

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the sample was taken 300 – 700 nm using a UV-vis spectrophotometer (HITACHI, Model U-2800 spectrophotometer). The deionized water was used as the blank. The deionized water will be used as the blank.

The mean particle sizes were determined by dynamic light scattering using a ZetaSizer Nano-ZS 90 (Malvern Instruments, Worcestershire, UK).

Dynamic Light Scattering (DLS) with standard 633 nm laser fitted at a 90 degree angle was used to measure particle size and the molecule size. This technique measured the diffusion of particles moving under Brownian motion, and converted this to size and a size distribution (PDI) using the Stokes-Einstein relationship.

The polydispersity index (PdI) ranges 0 to 1, values between 0 and 0.2 indicate a narrow and homogenous nanoparticles size distribution while values larger than 0.2 indicated a more broadly dispersed nanoparticles size distribution or even presence of different particles populations. Prior to measurement, the nanosuspension of each sample were taken out and adjust to 5% (w/v) concentration and introduced into a disposable cuvette to measured in triplicate.

Observing Bacterial Cells through Field Emission Scanning Electron Microscope (FE-SEM)

To directly observe the morphological changes of bacterial cells treated or not with Ag-NPs, field emission scanning electron microscope (FE-SEM) was used. The sample was cut in 1 cm^3 slices and fixed in 4% glutaraldehyde for 12 -24 hours at 40C.

The fixed cells were washed with phosphate buffered solution (PBS) three times for 10 minutes each sample. After washing with PBS three times, dehydration process was conducted with 30, 50, 70, 80, 90 and 100% of ethanol.

The fixed cell was dried and gold-coated using ion sputter. The pre-treated samples were observed by FE-SEM (SMT-SUPRA 40VP, Carl Zeiss AG), Oberkochen, Germany.

RESULTS AND DISCUSSION

Silver Nanoparticles Analysis

It is well known that silver nanoparticles exhibit brown colour in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. In this experiment silver nanoparticles were successfully synthesized from the aqueous silver nitrate solution using *Polygonum minus* extract in a continuously heated and stirred mixture. The colourless reaction mixture slowly changed from yellowish green to reddish brown indicating of reduction of silver ion after several minutes of reaction (Figure 1).

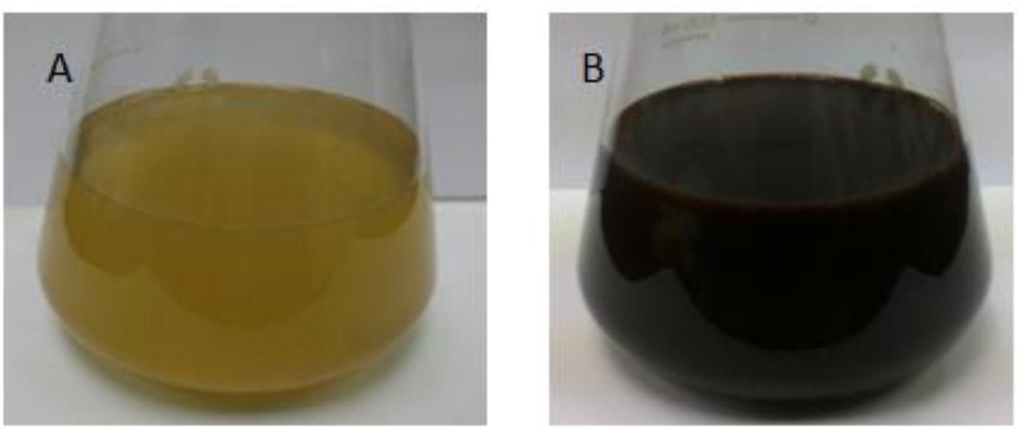

Figure 1: *Polygonum Minus* Extract (A) and Synthesized Silver Nanoparticles (B)

UV-vis Spectroscopy Analysis

UV-vis spectroscopy has been widely used to detect the presence of silver nanoparticles during synthesis (Logeswari *et al.*, 2015; Ali *et al.*, 2016). Particularly, absorbance in the range of 420–450nm has been used as an indicator to confirm the reduction of Ag^+ to metallic Ag (Karuppiah and Rajmoha, 2013; Saraniya Devi and Valentin Bhimba, 2012). The UV–vis spectra showed maximum absorbance at 440 nm, which increased with time of incubation of silver nitrate with the plants extract (Figure 2).

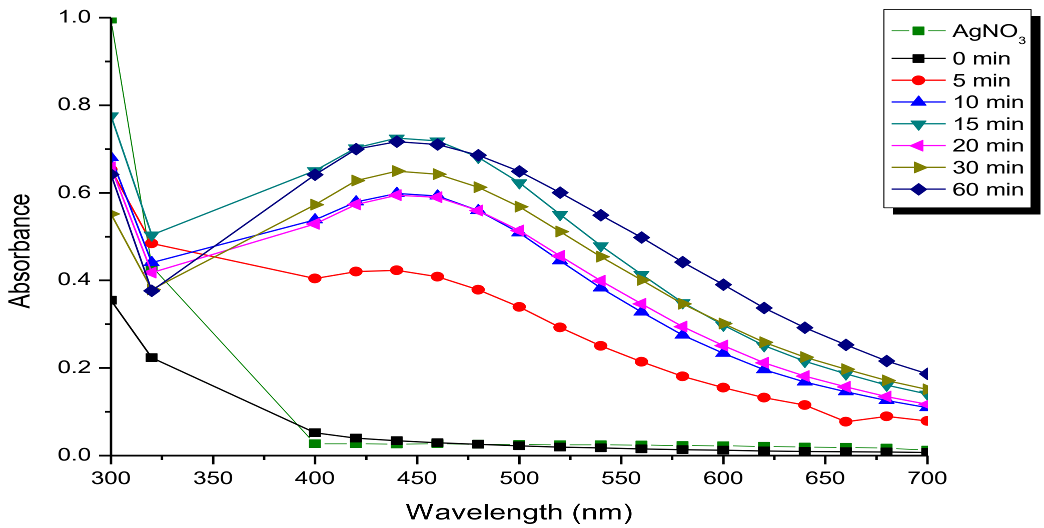

Figure 2 : UV-vis Spectra of Silver Nanoparticles Measured at Different Time Intervals

The curve shows increased absorbance in various time intervals (5 min, 10 min, 15 min, 20 min, 30 min and 60 min) indicating increases in the amount of AgNPs produced from the mixture and the peaks were noticed at 420 nm corresponding to the surface plasmon resonance of silver nanoparticles. Similar changes in colour have also been observed in previous studies (Banerjee *et al.*, 2014; Namratha and Monica, 2013). While the control ($AgNO_3$) and 0 minute showed no colour formation.

Morphological Changes of Bacterial Cells Treated with Silver Nanoparticles

The morphological changes of bacterial cells were observed by FE-SEM (Figure 3). In *E. coli* cells, cells of the control group were typically rod-shaped. Each cell size was almost same and damage on the cell surface was not detected. However, in *E. coli* treated with silver nanoparticles, instead of normal rod-shaped cells, irregular fragments appeared on the cell surface indicating the damage tothe cell surface.

While in *S. aureus* cells, cells of control were typically in graped-shaped where is the cell surface was intact and damage was not seen. However, after treated with with silver nanoparticles there are fragments on the cell surface and agglomerate, indicating the damage of cell surfaces. Increased permeability of the cell membrane or leakage of cell contents could be caused by reactive oxygen species (ROS) (Kim *et al.*, 2011).

FE-SEM morphological showed destruction of bacterial cell of *S.aureus* was feeble than *E.coli*. Its happen due to the difference of the peptidogly can layer of bacterial cell between Gram positive *S. aureus* and Gram negative *E. coli, which* is an essential function of the peptidoglycan layer is to protect against antibacterial agents such as antibiotics, toxins, chemicals and degradative enzymes (Silhavy *et al.*, 2010; Kim *et al.*, 2011).

Normally 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.

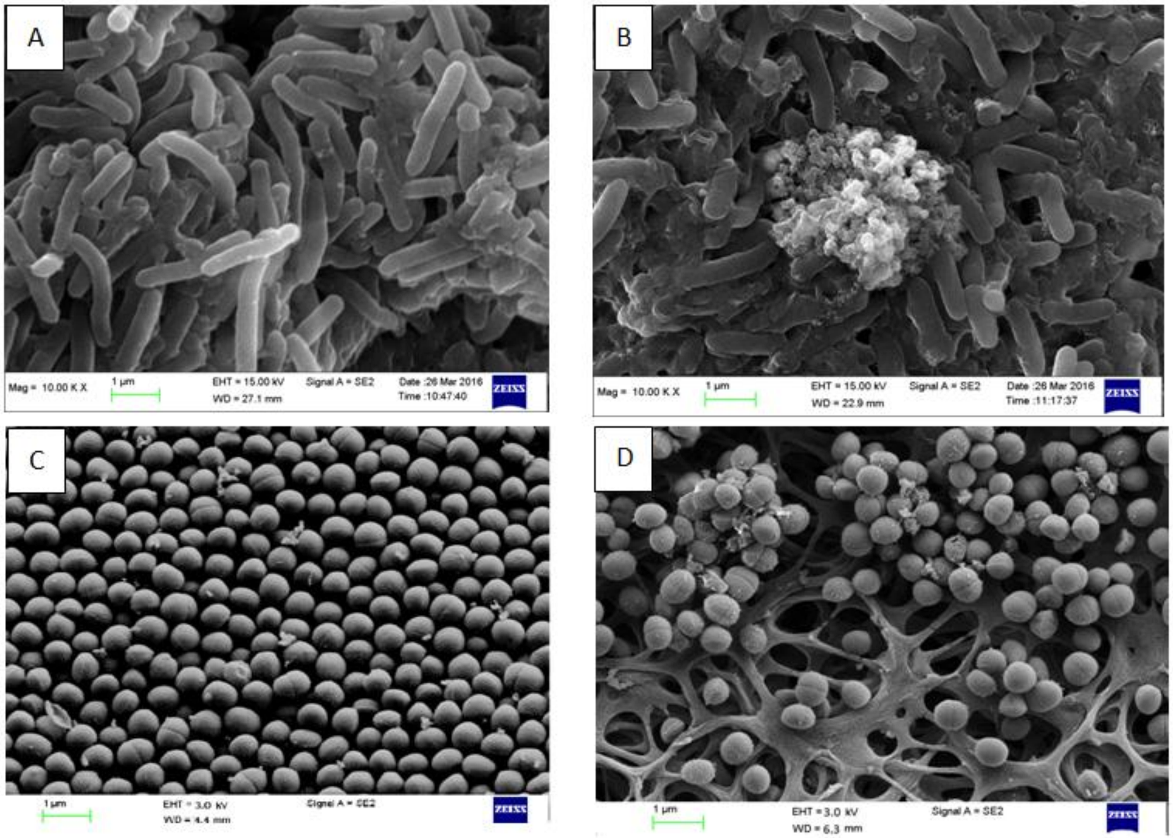

Figure 3: FE-SEM Micrograph of *E. coli* and *S. Aureus* Control (A, C) and *E. coli* and *S. aureus* Treated with Silver Nanoparticles (B, D)

Particles Size Analysis

Figure 4 showed the Z-average of particle size distribution in terms of intensity for silver nanoparticles. The average size of silver nanoparticles was 91.74 ± 0.48 nm where thefine particles could be agglomeration during the centrifuge process. The polydispersity index (PdI)) value of 0.412 indicated the presence of a broad dispersion in the particle size, testified by the presence of some visible agglomerates.

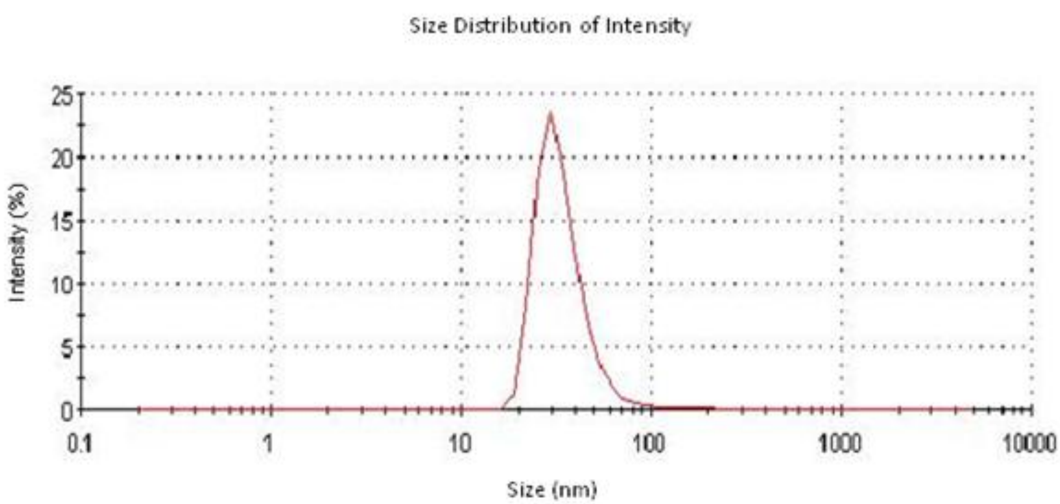

Figure 4: Particle Size Analysis of Silver Nanoparticle Using *Polygonum Minus* Extract

Conclusion

In this study, the silver nanoparticles synthesized using *Polygonum minus* extract with silver nitrate aqueous were successfully produced. This method is environmentally friendly, of low cost, and simple and therefore, can promote the application of green technology for the production of silver nanoparticles. These environmentally benign silver nanoparticles were further confirmed by using UV-vis spectroscopy where the spectra showed maximum absorbance at 440 nm. The average size of the silver nanoparticles showed at 91.74 ± 0.48 nm while the antibacterial study shown that the silver nanoparticles from *Polygonum minus* extract proven as an effective antibacterial agent. Thus, it can be conclude that plant extract being very ecofriendly and cost effective can used for synthesis of silver nanoparticles and promising a small size of molececule and have good antimicrobial activity due to the active molecule present in the plant extract.

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