# Green Synthesis of Silver Nanoparticles Using Moringa oleifera sp. (Malunggay) Seed Aqueous Extract and Its Antibacterial Activity

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**Abstract** – Silver nanoparticles, in recent years have gained interest due to their applications in various fields, such as in medicine, as a result of its antibacterial properties. The synthesis of nanoparticles involves reduction and capping processes. The current processes, however, involve the use of toxic chemicals. A solution for this is the use of green synthesis, which is done through the utilization of biological components which acts as both a reducing and capping agent. The study used *M. oleifera sp.* seed extract to synthesize silver nanoparticles as they possess various biomolecules that make the process effective. Ultraviolet-Visible spectrophotometry was used to monitor the formation of the silver nanoparticles. The synthesized silver nanoparticles were viewed under a Transmission Electron Microscope and had a mean size of 12 nm and spherical shape. The silver nanoparticles also showed antibacterial activity against the *S. aureus* bacterial culture. The study concluded that silver nanoparticles can be synthesized using *M. oleifera sp.* seed extract.

**Introduction.** – Silver nanoparticles, in recent years have gained interest due to their applications in medicine, as a result of its antibacterial properties. It has also been acknowledged to have strong inhibitory and bactericidal effects along with antifungal, anti-inflammatory, and anti-angiogenesis activities[1].

It is required for silver nanoparticles to have a reducing and a capping agent for it to be synthesized and stabilized. Reduction takes place when silver ions ($Ag^+$) are reduced in aqueous or non-aqueous solutions and lead to the formation of metallic silver ($Ag^0$)[2]. The capping agent stabilizes the nanoparticle by limiting the size, controlling the nanoparticle morphology, and protecting the surface from aggregation of nanoparticles[3]. Both the reduction and the capping processes are essential for the synthesis of the silver nanoparticles; however, these processes would require a variety of chemical and physical methods which are potentially environmentally hazardous which involve use of toxic and perilous chemicals such as sodium borohydride or hydrazine that are responsible for various biological risks[4].

A solution for this is the use of an alternative method of synthesizing silver nanoparticles called green synthesis, a method that does not employ toxic chemicals but is done through the utilization of biological components which acts as both a reducing agent and a capping agent. This method cost effective, eco-friendly, and safer. It has been shown that among the candidates for green synthesis: plants, bacteria, and fungi; plants proved to be the most favorable because it contains effective biomolecules that enhance the synthesis rate[3].

The *Moringa oleifera sp.* tree is a candidate for the use of green synthesis, as it is highly nutritious and is a significant source of biomolecules that are necessary for the use reduction and capping of the silver nanoparticles[5]. A study by Prasad and Elumalai (2011)[6] was able to synthesize silver nanoparticles using *M. oleifera sp.* leaf extract and concluded that the leaf extract can demonstrate strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions. However, according to the phytochemical evaluation between the leaf and the seed, both of them had flavonoids and phenolics but only the seeds had alkaloids and proteins which are highly advantageous biomolecules that can act as capping agents[7]. According to the study of Jain et al. (2015)[8], the presence of a protein shell is highly

advantageous as it known to transmit solubility and stability in aqueous solution and is more effective when used against gram-positive bacteria. So far, *M. oleifera sp.* seed extract have not been used in synthesizing silver nanoparticles as a reducing and capping agent.

In this study, we will be utilizing *M. oleifera sp.* seeds because of the presence biomolecules such as alkaloids, flavonoids, phenolic compounds, and proteins which are favorable in synthesizing silver nanoparticles. This study aims to use a green method of synthesizing silver nanoparticles by using extract of *M. oleifera sp.* seed as both a reducing and capping agent and if it exhibits antibacterial activity.

**Methods.** – The conduct of the research experiment was divided in two phases: first, the initial state where the silver nanoparticles were synthesized with extract obtained from *Moringa oleifera sp.* seeds. The samples of silver nanoparticles were characterized using Ultraviolet-visible Spectrophotometer (Shimadzu 1800) and transmission electron microscope while the second phase was the testing of the silver nanoparticles antibacterial activity against *Staphylococcus aureus* culture.

In the first phase, the silver nanoparticles were synthesized using extract from *M. oleifera sp.* seeds which acted as both a reducing and a capping agent. The synthesis was done through three concentrations of *M. oleifera sp.* seed extract: a) 5 g b) 10 g c) 15 g in a 100 mL distilled water. The Ultraviolet-visible Spectrophotometer was used to monitor the formation of the silver nanoparticles during synthesis by measuring the absorbance levels. It was then characterized using a transmission electron microscope to know its average diameter and shape.

In the second phase, the silver nanoparticles antibacterial activity was checked against *Staphylococcus aureus.* This phase utilized the disk diffusion method through the four treatments, namely: a) water b) silver nitrate c) *M. oleifera sp.* seed extract solution and d) synthesized silver nanoparticles. The zone of inhibition in each treatment was then measured using a vernier caliper.

*Seed extraction. M. oleifera sp.* seeds were washed and dried to get rid of dirt. The seeds were then crushed and blended. The powdered seeds were weighed (5 g, 10 g, 15 g), and placed into three different 250 mL beakers containing 100 mL of distilled water, and were then heated at 250°C using a hotplate. The seed extracts were then filtered onto three different Erlenmeyer flasks using Whatman number six filter papers. The seed extracts were then stored in a refrigerator.

*Synthesis of silver nanoparticles.* 40 mL of 0.03 molar concentration (M) silver nitrate was measured. The silver nitrate was stirred at 400 rotations per minute (rpm). Then, ten mL of *M. oleifera sp.* seed extract was slowly dropped into the silver nitrate using a pipette. The same procedure was also done with the synthesis of the 10-g and 15-g *M. oleifera sp.* seed extract.

*UV-visible absorbance spectroscopy.* To monitor the synthesis of the silver nanoparticles quantitatively. Samples from the solution were taken at intervals 0, 15, 30, 45, 60 minutes, and 24 hours for Ultraviolet-visible Spectrophotometer analysis. One mL of the silver nanoparticles solution was taken using a micropipette and was transferred into a cuvette glass. The solution was then diluted with three mL of distilled water to allow the light to pass through during the UV-Vis spectrophotometer analysis. Two samples of cuvette were then placed inside the machine: one containing the silver nanoparticles solution and another one containing distilled water as the blank solution. The absorption spectra were then recorded from 300 to 700 nm.

*Transmission electron microscopy.* The 15-g solution of silver nanoparticles was brought and a small amount of it was placed in a 10 mL Erlenmeyer flask. The sample was then placed in a sonicator for 10 minutes in order for the aggregates to separate from one another. After the sample was sonicated, a small amount of the sample was transferred on the copper grid in a container. The container was then placed in a vacuum concentrator for 20 minutes in order to remove all moisture from the copper grid. The samples were then viewed on a transmission electron microscope at 25 000x, 40 000x, 200 000x, 400 000x, and 600 000x the original size.

*Disk diffusion assay.* Disk diffusion method was done to test the antimicrobial activity of the silver nanoparticles against *S. aureus.* The first agar plates, were divided into four quadrants each quadrant containing silver nanoparticles, silver nitrate, 15-g *M. oleifera sp.* seed extract, and, distilled water. Filter disks was then placed in each of the quadrant. The petri dish was then incubated for 18 hours to let the bacteria culture grow. After the incubation period, the standard zone of inhibition (ZOI) was measured using a vernier caliper.

**Results and Discussion.** – In order to monitor the stability and formation of the silver nanoparticles being synthesized, their absorption spectra were recorded using a UV-visible spectrophotometer while using distilled water as blank. Figures 1 to 3 show the UV-visible spectra of the silver nanoparticles formation using silver nitrate (0.03 M) as the precursor material with different *M. oleifera sp.* seed extract concentrations. All three silver nanoparticles solutions turned from colorless to pale yellow to brown while being synthesized which indicates the formation of the silver nanoparticles (AgNPs). The absorbance levels of the AgNPs synthesized by the 5-g seed extract as shown in Figure 1 increased with each passing time interval. Figure 2 of the AgNPs synthesized by the 10-g *M. oleifera sp.*

seed extract also showed the same trend with the exception of the decline of the samples absorbance levels at its 45th minute. The samples absorbance levels, however, increased during its 60th minute and after 24 hours. The AgNP synthesized by the 15-g *M. oleifera sp.* seed extract declined during its 60th minute otherwise its absorbance levels to increases over time. Overall, the absorbance levels of all the samples were observed to have increased particularly after 24 hours. Out of all the samples the AgNP synthesized by the 15-g *M. oleifera sp.* seed extract had the highest absorbance levels. This indicates great concentration of synthesized AgNPs which make the sample the better extract in order to produce AgNPs.

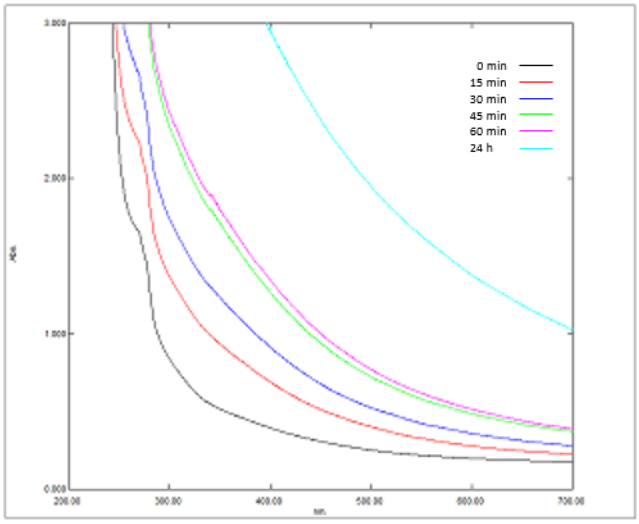


Fig. 1: Absorbance level of AgNPs solution using 5-g *Moringa oleifera sp.* seed extract.

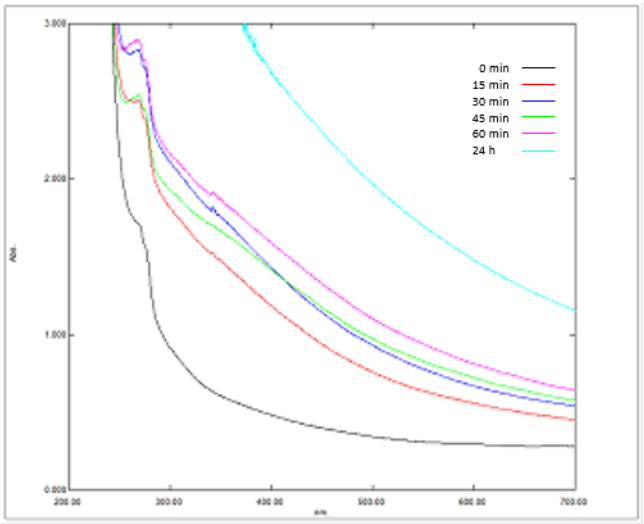


Fig. 2: Absorbance level of AgNPs solution using 10-g *Moringa oleifera sp.* seed extract.

Transmission electron microscopy indicated that the AgNPs synthesized with 15-g *M. oleifera sp.* seed extract

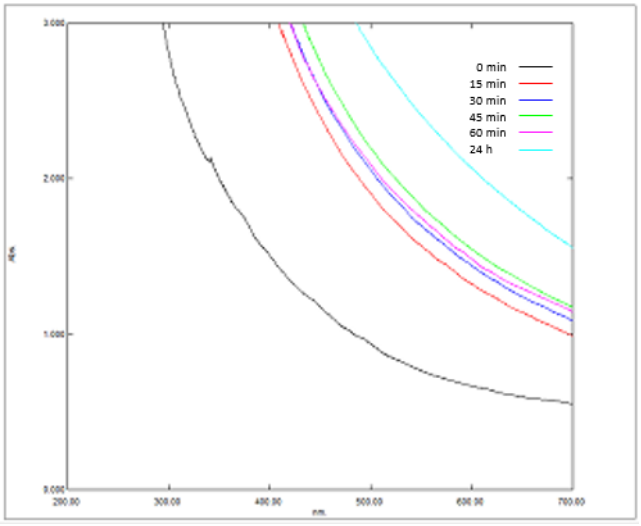


Fig. 3: Absorbance level of AgNPs solution using 15-g *Moringa oleifera sp.* seed extract.

were at a nanolevel, as shown in figure 4. Ultimately, the nanoparticles were mostly well dispersed and had a spherical shape while there were some to be found that were irregularly shaped. The diameter of the AgNPs had a mean size of 12 nm. Compared to the study of Sathyavathi et al. (2011)[9] which synthesized AgNPs with the mean size of 46 nm utilizing *Moringa oleifera sp.* leaf extract. This may be due to the fact that *Moringa oleifera sp.* seeds have more protein, that aids in the capping of AgNPs, content than the leaves[7]. It should be noted, however, that different concentrations of silver nitrate and plant extracts were used between these two studies which may have affected the size of the AgNPs. Typically AgNPs at smaller sizes exhibit more antibacterial property. In the study of Agnihotri et al. (2013)[10], it was concluded that AgNPs with the range sizes between 5-10 nm produced significant enhancement in killing bacteria. The study, however, used sodium borohydride and trisodium citrate as a reducing and capping agent respectively and did not utilize any biological compound. The shape of the synthesized nanoparticles was spherical which was very similar to studies that also synthesized using plant extracts. Shapes of nanoparticles usually have an effect on its performances including its antibacterial activity. Nanoparticles tend to usually be spherical since it minimizes energy in stable structures.

As shown in table 1, the AgNPs and silver nitrate exhibited antibacterial activity. The *M. oleifera sp.* seed extract and the distilled water treatments failed to inhibit the growth of the bacteria. The data from the disk diffusion assay were analyzed using Social Package for the Social Sciences Statistics software (SPSS). An independent samples t-test was conducted to compare the zone of inhibition in AgNPs and silver nitrate. There was no significant difference in the scores for AgNPs (M=0.90, SD=0.27) and silver nitrate (M=0.76, SD=0.27) condi-

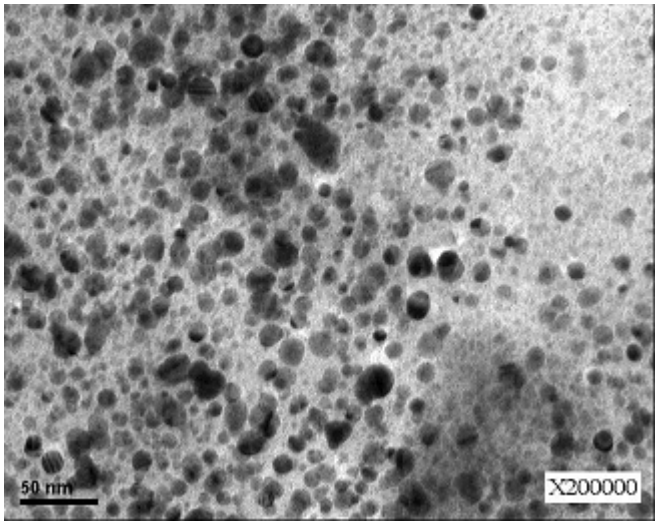


Fig. 4: Transmission electron microscope image of AgNPs synthesized with 15-g *Moringa oleifera sp.* seed extract.

tions; t (4)=0.622, p=0.568. The results suggest that both AgNPs and silver nitrate are comparable and can be both used as an agent against *S. aureus*.

**Conclusion and Recommendation.** – Silver nanoparticles were synthesized from the extract of the *M. oleifera sp.* seeds. The formation of the AgNPs was monitored via UV-Vis spectroscopy. Results showed that the AgNPs synthesized with 15-g *M. oleifera sp.* extract had greater absorbance levels than the AgNPs synthesized with the 5-g, and 10-g extracts. The synthesized silver nanoparticles were viewed under a transmission electron microscope and had a mean size of 12 nm and spherical shape. The silver nanoparticles also showed antibacterial activity against the *S. aureus* bacterial culture. It is still unknown whether this seed extract is only effective in synthesizing AgNPs; therefore, it is recommended in future studies to investigate the effectiveness of the *M. oleifera sp.* when used to synthesize other types of metal nanoparticles.

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Table 1: Susceptibility testing of *S. aureus* against AgNPs, silver nitrate, *M. oleifera sp.* seed extract, and distilled water.

| **Treatment** | **Average ZOI** (mm) |
|---|---|
| Silver nanoparticles | 9.0 |
| Silver nitrate | 7.6 |
| *M. oleifera sp.* seed extract | 0.0 |
| Distilled water | 0.0 |

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