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Keywords: Nanoparticles, Biosynthesis, Absorbance Spectrum

Green Synthesis of Silver Nanoparticles Using Alagaw (*Premna odorata*) Leaf Extract

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Abstract. There is a worldwide interest in silver nanoparticles (AgNPs) synthesized by various chemical reactions for use in applications. Silver nanoparticles have gained significant interest due to their unique optical, antimicrobial, electrical, physical properties and their possible application. However, it is necessary to develop environmental friendly methods for their syntheses. To avoid chemical toxicity, biosynthesis of metal silver nanoparticles is proposed as a cost-effective and environmental friendly alternative. This study aimed to find out whether Alagaw plant can potentially act as a reducing agent for the biosynthesis of silver nanoparticles and whether the concentration of the leaf extract can affect the absorbance spectrum, size and shape of the synthesized silver nanoparticles. The synthesized silver nanoparticles were characterized using the UV-vis spectroscopy for its absorbance spectrum and Transmission Electron Microscope Analysis for its morphology and size. The experimental method of research was used using three treatments and replicates of the different concentrations of Alagaw leaf extract: Treatment A (0.2 g/mL), Treatment B (0.4 g/mL) and Treatment C (0.6 g/mL) with 10 minutes and 60 minutes interval of observation under UV-vis spectrophotometer. Based on the findings of the study, Alagaw plant can potentially act as a good reducing agent for the biosynthesis of silver nanoparticles. The results recorded from UV-vis spectrophotometer support the biosynthesis and characterization of silver nanoparticles that as the concentration of the leaf extract increases it significantly affect the wavelength peaks and absorbance peaks of the synthesized silver nanoparticles. Using the high-resolution Transmission Electron Microscopy, the size of silver nanoparticles measured 50 nm – 100 nm having near-spherical in shape.

Keywords: Nanoparticles, Biosynthesis, Spectroscopy, Absorbance Spectrum

A. Introduction

Silver nanoparticles have been the subject of this research due to their unique physical, chemical, and biological properties that is very beneficial to various fields including medical, food, health care, consumer, electricity, and industrial purposes. Its optical, antimicrobial, electrical and thermal properties are usually incorporated into products that range from photovoltaic to biological and chemical sensor. Silver nanoparticles' high electrical conductivity, stability, and low sintering temperature are utilized into conductive inks, pastes and filters (Gurunathan, et al., 2015).

There are various chemical and physical methods for the synthesis of metallic nanoparticles for example, one can use reduction of solutions, photochemical reactions in reverse micelles, electrochemical reduction, heat evaporation and radiation assisted methods, among others. These methods have usually been successful in the synthesis of nanomaterial in large quantities in short period of time, as well as for specific size and shape (Kuppusamy, et al., 2015).

The problem is, most of these methods are extremely expensive and involves the use of toxic, hazardous chemicals as the stabilizers which may pose potential environmental and biological risks. This challenge gives rise to the idea in using the green synthesis method for the biosynthesis of silver nanoparticles since it is cost-effective and biocompatible alternative to chemical and physical methods. Plant-mediated synthesis or simply the green synthesis of nanoparticles is green chemistry approach that highly associates nanotechnology with plants.

Thus, this study uses *Premna odorata* B. which is a Philippine native plant, and is locally known as Alagaw or Argaw since its family is known to have an antimicrobial, anticancer, and antioxidant property which can potentially act as a reducing agent to synthesize metallic nanoparticles. This also leads to the question whether Alagaw leaf extract can potentially act as reducing agent for the biosynthesis of silver nanoparticles.

B. Statement of the Problem

This study aimed to find out whether Alagaw plant can potentially act as reducing agent for the biosynthesis of silver nanoparticles. Specifically, this study sought to find out the following:

1. To determine the effect of the concentration of Alagaw leaf extract on the biosynthesis of silver nanoparticles in terms of its absorbance spectrum in 10 minutes and 60 minutes interval, size, and shape.
2. To determine the absorbance spectrum of each Treatments in 10 minutes vs. 60 minutes interval.
3. To determine the absorbance spectrum of all the Treatments in 10 minutes and 60 minutes interval.
4. To determine the average wavelength peaks and average absorbance peaks of silver nanoparticles in terms of the concentration of Alagaw leaf extract in 10 minutes and 60 minutes
5. To determine the speed and stability of the reaction of the solution in terms of its average wavelength and absorbance peaks.
6. To determine the best concentration of Alagaw leaf extract on the biosynthesis of silver nanoparticles in terms of the speed and stability of reaction.
7. To determine if there is a significant difference on the effect of the concentration of Alagaw leaf extract in terms on the wavelength peaks of silver nanoparticles in 10 minutes interval, 60 minutes interval and between 10 minutes and 60 minutes interval.
8. To determine if there is a significant difference on the effect of the concentration of Alagaw leaf extract in terms on the absorbance peaks of silver nanoparticles in 10 minutes interval, 60 minutes interval and between 10 minutes and 60 minutes interval.
9. To determine the morphology and size distribution of silver nanoparticles.

C. Significance of the Study

The result of this study would benefit various fields including biology, chemistry, electricity, and even medicine. Also, it is beneficial to other researchers, professionals, and society.

Biology. The result of this study would give insights in the field of biology in terms of the kinds of plant that have the quality of good reducing agents.

Chemistry. This is for the chemical applications since silver nanoparticles can be utilized to enhance the efficiency and efficacy of chemical reactions. The same factors also make them of use in chemical vapor sensors and other devices.

Electricity. Silver nanoparticles are utilized in a number of conductive products, including conductive adhesives, LCD and LED screens, touch screens, and conductive slurries used in microelectronics.

Medicine. The silver nanoparticles in this study can highly benefit the field of medicine because of the application of silver nanoparticles on biomedical applications, including diagnosis, treatment, drug delivery, medical device coating, and for personal health care.

Other researchers. The result of this study would highly benefit other researchers since they can use this as their bases for future needs. The methods of synthesizing the silver nanoparticles can be used as one of the basis in synthesizing silver nanoparticles completely.

Professionals. The results of this study would also benefit other professionals who are in the field of Nano science, physics, chemistry, biology, and etc. They can use the methods to prepare the exact size and shape of the silver nanoparticles for their specific purpose.

Society. The society will also benefit the result of this study since the application of silver nanoparticles is broad that it can be commercialized and then directed to the society.

D. Review of Related Literature

Synthesized nanomaterial has a wide variety of applications in the field of electronics, photonics, catalysis, medicine, etc. in that case many scientist became interested in the search of the different methods in synthesizing nanomaterial. Green synthesis or the use of plant in synthesizing nanomaterial offers a wide range of benefits over other biological method. According to Ramya and Sylvia (2012) the use of green synthesis method to biosynthesize nanoparticles can avoid the presence of toxic chemical and proven to be better methods over chemical and physical method because of its slower kinetics and is also cost effective, environment friendly, and easily scaled up for large scale synthesis.

Silver nanoparticles, an example of a nanomaterial, have gained significant interest due to their unique optical, antimicrobial, electrical, physical properties and their possible application used in commercial products such as paints and coatings and in medicine. Green synthesis is the best way to synthesized silver nanoparticles, according to Aromal, et al., (2012), plant crude extracts contain novel secondary metabolites such as phenolic acid, flavonoids, alkaloids, and terpenoids, which are mainly responsible for the reduction of ionic metal into bulk metallic nanoparticles and are constantly involved in redox reactions required to synthesize eco-friendly nanoparticles.

According to Ankana, et al., (2010) silver disassembles into particles following the addition of plant extract, which may lead to a color change. Solutions of silver nanoparticles appear dark, yellow-brown in color because of the surface plasmon resonance phenomenon.

Alagaw plant (*Premna odorata*) is a small hairy tree commonly seen in the forests of the Philippines and its neighboring countries are known to have anti- inflammatory activity and are concluded to have long chain fatty acids in free ester forms (57.18%) (El- Mudomy, et al., 2105)

which is a good characteristic of a reducing agent and thus applicable for the synthesis of silver nanoparticles.

Silver nitrate (AgNO_3) is an organic compound that is an adaptable progenitor to various other compounds of silver. The silver nitrate reacts with copper to produce silver crystals along with a blue copper nitrate solution.

UV-visible absorbance spectroscopy is very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the stability of silver nanoparticles, it is fast, easy, simple, sensitive, selective for different type of nanoparticles, needs only of a short period of time for measurement, and finally a calibration is not required for particle characterization of colloidal suspensions (Huang, et al., 2007). For the morphology, sized and shape of silver nanoparticles Transmission Electron Microscope is used (Anandaksmi, et al., 2015).

E. Research Design & Methodology

Experimental Design and Treatments

This study utilized the Complete Randomized Design (CRD). A single factor experiment with three (3) treatments done in three (3) replications shown in Table 1.

Table 1. Concentration of Alagaw leaf extract for the biosynthesis of Silver Nanoparticles.

Materials	Treatments		
	A	B	C
Alagaw Leaf	20g	40g	60g
Water	100mL	100mL	100mL

Experimental Procedure

Collection of Plant Materials

The Alagaw leaves were collected from Surongon Subdivision, Arnaldo Boulevard, Roxas City, Capiz, Philippines and authenticated by the Department of Agriculture.

Extraction – Decoction Method

A 20g of finely cut leaves, for Treatment A, 40g of finely cut leaves, for Treatment B, and 60g of finely cut leaves, for Treatment C, were thoroughly washed with running tap water to remove dirt and soil respectively. The washed leaves were boiled in 100mL water for 15 minutes to get the extract. The cooled extract was filtered using Whatman filter paper No. 1. The filtrate was collected and stored at 4 °C for further experiments.

Biosynthesis of Silver Nanoparticles

Aqueous solution of 225 mL of 1 mM silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. To prepare 225 mL of the solution, 0.038 g of silver nitrate was dissolved in 225 mL water.

5 mL of leaf extract with 20g of Alagaw leaf for treatment A, 40g of Alagaw leaf for treatment B, and 60g of Alagaw leaf for treatment C during extraction was added and vigorously

stirred with 25 mL of aqueous solution of 1 mM silver nitrate in a 100 mL Erlenmeyer flask for reduction into silver ions and kept at room temperature. The overall reaction process was carried out in a dark place to avoid unnecessary photochemical reactions. After 10 min, the change of the color was noted indicating the formation of silver nanoparticles (Jain, et al., 2009).

Research Instrument

Characterization of Silver Nanoparticles

UV-vis Spectrophotometry

The reduction of pure silver ions was monitored by measuring the UV–visible spectrum of the reaction mixture at a given time intervals of 10min and 60 min. The biosynthesized silver nanoparticle solution was then subjected to UV–Vis spectral analysis using a UV-Vis Spectrophotometer. The peak shifted in the absorption spectrum from 340 to 620 nm with increasing reaction time was observed.

Transmission Electron Microscopy (TEM) Analysis

TEM analysis was done using TEM machine. Thin film of the sample was prepared on a carbon-coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the TEM grid was allowed to dry by putting it under a mercury lamp for 5 min.

F. Results and Discussion

In terms of the polynomial trend line of the UV-vis absorbance peak of Treatment A within 10 minutes interval, the maximum absorption of the silver nanoparticles solution is at 440 nm corresponding to the surface Plasmon resonance of silver nanoparticles.

In terms of the polynomial trend line of the UV-vis absorbance peak of Treatment B within 10 minutes interval, the maximum absorption of the silver nanoparticles solution is approximately at 460 nm corresponding to the surface Plasmon resonance of silver nanoparticles.

In terms of the polynomial trend line of the UV-vis absorbance peak of Treatment C within 10 minutes interval, the maximum absorption of the silver nanoparticles solution is approximately at 530 nm.

In terms of the polynomial trend line of the UV-vis absorbance peak of Treatment A within 60 minutes interval, the maximum absorption of the silver nanoparticles solution is approximately at 430 nm.

In terms of the polynomial trend line of the UV-vis absorbance peak of Treatment B within 60 minutes interval, the maximum absorption of the silver nanoparticles solution is approximately at 465 nm.

In terms of the polynomial trend line of the UV-vis absorbance peak of Treatment C within 60 minutes interval, the maximum absorption of the silver nanoparticles solution is approximately at 470 nm.

In terms of UV-vis absorbance spectrum of silver nanoparticles in 10 minutes vs. 60 minutes within 620.0 nm – 340.0 nm for Treatment A, red-shift was observed during the reaction. The absorbance increases with the influence of time. Isosbestic points are also seen in the graph. The appearance of these points is used as reference in the study of reaction rates, as the absorbance at those wavelength remains constant throughout the whole reaction.

In terms of UV-vis absorbance spectrum of silver nanoparticles in 10 minutes vs. 60 minutes within 620.0 nm – 340.0 nm for Treatment B, red-shift was observed during the reaction. The absorbance after 10 minutes of interval increases with time.

In terms of UV-vis absorbance spectrum of silver nanoparticles in 10 minutes vs. 60 minutes within 620.0 nm – 340.0 nm for Treatment C, blue-shift was observed during the reaction. The absorbance after 10 minutes of interval increases yet the intensity shortens that suggests to the decelerating rate of reaction.

In terms of the effect of the concentration of Alagaw leaf extract on the formation of silver nanoparticles within 10 minutes. The SPR peak observed at around 440 nm, 455 nm, and 530 nm for 20 g, 40 g, and 60 g respectively. With increasing ratio of leaf extract, the concentration of SPR band increased yet particles were not stable and agglomeration was observed.

In terms of the effect of the concentration of Alagaw leaf extract on the formation of silver nanoparticles within 60 minutes. The SPR peak observed at around 430 nm, 465 nm, and 475 nm for 20 g, 40 g, and 60 g respectively. Treatment B and Treatment C gradual red shift from 400 nm to 460 nm suggests the formation of smaller monodispersed silver nanoparticles as the concentration of leaf extract increases over time. Both the Treatment shows fast reaction rate than Treatment A as their SPR band width increases.

All the results are in agreement with the earlier investigations conducted by Kantrao (2014) et al., Tripathy (2010) et al., and Jha (2010) et al.

In terms of the average wavelength peaks of replicates in 10 minutes and 60 minutes interval, replicate B3 of Treatment B (40 g) got the highest average wavelength in 10 minutes interval with 597.6 nm. It also acquired the highest average wavelength peaks among all the replicates with 545.8 nm. The stability of the reaction of Treatment A based on the average wavelength shows a slow reaction rate. Treatment B has faster reaction rate than Treatment A. Treatment C has the fastest reaction rate among the three treatments.

In terms of the average absorbance peaks of replicates in 10 minutes and 60 minutes interval, replicate A1, C2, and C3 of Treatment A (20 g) and Treatment C (60 g) got the highest average absorbance in 10 minutes and 60 minutes interval with 4. The stated replicates also acquired the highest average absorbance peaks among all the replicates with 4.

Treatment C turns out to be more stable and monodispersed than treatment B and treatment A based on the average wavelength and absorbance. Replicate C3 shows the best result among the replicates based on reaction rate and stability. Thus, it was the sample sent for TEM analysis.

In terms of the difference on the effect of the concentration of Alagaw leaf extract on the wavelength peaks of silver nanoparticles in 10 minutes and 60 minutes interval, result shows that there was a significant difference on the wavelength peaks of the synthesized silver nanoparticles treated with different concentration in 10 minutes and 60 minutes interval.

In terms of the difference on the effect of the concentration of Alagaw leaf extract on the absorbance peaks of silver nanoparticles in 10 minutes and 60 minutes interval, result shows that there was no significant difference on the absorbance peaks of the synthesized silver nanoparticles treated with different concentration in 10 minutes and 60 minutes interval.

The TEM analysis of the silver nanoparticles showed that the size of the synthesized silver nanoparticles ranges from 50 to 100 nm with near spherical in shape.

According to Khodashenas (2015) in his study, nanoparticles having polyhedral, spherical and near spherical shapes are close to equilibrium. This strengthens the latter analysis that Treatment C is almost in equilibrium due to its wavelength and absorbance peaks which in turn became the basis for the sample to undergo TEM analysis.

G. Conclusions and Recommendations

Based on the findings of the study, the following conclusions were formulated:

Alagaw plant can be a good reducing agent for the biosynthesis of silver nanoparticles.

In terms of the effect of the concentration of Alagaw leaf extract on the biosynthesis of silver nanoparticles on the absorbance spectrum in 10 minutes and 60 minutes interval, the SPR peaks increases as the concentration of Alagaw leaf extract increases. In terms of size and shape, higher concentrations show lesser variation of size and shape than lower concentration.

The intensity of the absorbance spectrum rises overtime regardless of the concentration.

With increasing ratio of leaf extract, the concentration of SPR band increased yet particles were not stable and agglomeration was observed. Higher concentration tends to have faster kinetics than lower concentration.

In terms of the average wavelength peaks of silver nanoparticles, higher concentrations of leaf extract tend to gain greater average wavelength. Higher concentrations also tend to undergo blue shift much faster than lower concentration of leaf extract.

In terms of the average absorbance peaks of silver nanoparticles, increase in concentration of leaf extract is also increase in the absorbance peaks of solution.

In terms of the reaction rate of the treatment based on the average wavelength peaks, the greater the concentration, the faster it reaches its equilibrium or the faster its reaction rate.

In terms of the stability of the reaction rate of the treatment based on the absorbance peaks, the greater the concentration, the stable the reaction rate of the solution. However, lesser concentration has the tendency to show stability because of the slow reaction that is taking place.

0.6 g/mL has a greater effect on the wavelength peaks of the solution.

Regardless of concentration, Alagaw leaf extract has the same absorbance effect on the biosynthesis of silver nanoparticles.

Based on the findings, the researchers highly recommend the following:

Use Alagaw leaf extract in the biosynthesis of silver nanoparticles with a concentration of 0.6 g/mL for effective result.

Determine the maximum Surface Plasmon Resonance concentration of the Alagaw leaf extract to standardize the stabilization of the reaction.

Increase the concentration of the extract to obtain monodispersed size nanoparticles.

Increase the time interval of the UV-vis spectroscopy to determine the stable absorbance spectrum of the solution.

Increase the concentration of the extract to have a faster and stable reaction rate towards equilibrium. Also, improve other parameters to control the reaction rate during synthesis.

It is recommended to use 0.6 g/mL of Alagaw leaf extract in the biosynthesis of silver nanoparticles since it is more effective in terms of wavelength peaks.

Use other characterization techniques to improve the physical and chemical properties of silver nanoparticles.

Undergo phytochemical analysis and FTIR scan to prove the role of biomolecules in the biosynthesis of silver nanoparticles.

Undergo microbial assay and inhibitory effect to prove the antibacterial property of the synthesized silver nanoparticles.

H. Outputs and Deliverable

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APPENDICES

A. UV-vis Absorbance Spectrum of AgNPs in 10 minutes interval within 620.0 nm - 340.0 nm

- a. Polynomial trend line of the UV-vis absorbance peak of Treatment A (0.2 g/mL) considering all the replicates within 10 minutes interval ranging from 620.0 nm – 340.0 nm

- b. Polynomial trend line of the UV-vis absorbance peak of Treatment B (0.4 g/mL) considering all the replicates within 10 minutes interval ranging from 620.0 nm– 340.0 nm

- c. Polynomial trend line of the UV-vis absorbance peak of Treatment C (0.6 g/mL) considering all the replicates within 10 minutes interval ranging from 620.0 nm– 340.0 nm

B. UV-vis Absorbance Spectrum of Silver Nanoparticles in 60 minutes interval within 620.0 nm – 340.0 nm

- a. Polynomial trend line of the UV-vis absorbance peak of Treatment A (0.2 g/mL) considering all the replicates within 60 minutes interval ranging from 620.0 nm– 340.0 nm

- b. Polynomial trend line of the UV-vis absorbance peak of Treatment B (0.4 g/mL) considering all the replicates within 60 minutes interval ranging from 620.0 nm– 340.0 nm

- c. Polynomial trend line of the UV-vis absorbance peak of Treatment C (0.6 g/mL) considering all the replicates within 60 minutes interval ranging from 620.0 nm– 340.0 nm

C. Comparison of Absorbance Spectrum in 10 minutes vs. 60 minutes within 620.0 nm - 340.0 nm of each Treatments

- a. Polynomial trend line of the UV-vis absorbance peak of Treatment A (0.2 g/mL) considering all the replicates within 10 minutes vs. 60 minutes interval

- b. Polynomial trend line of the UV-vis absorbance peak of Treatment B (0.4 g/mL) considering all the replicates within 10 minutes vs. 60 minutes interval

- c. Polynomial trend line of the UV-vis absorbance peak of Treatment C (0.6 g/mL) considering all the replicates within 10 minutes vs. 60 minutes interval

D. Comparison of Absorbance Spectrum in 10 minutes within 620.0 nm - 340.0 nm of the Treatments

- a. Polynomial trend line of the UV-vis absorbance peak recorded at different concentration after 10 minutes of reaction time

E. Comparison of Absorbance Spectrum in 60 minutes within 620.0 nm - 340.0 nm of the Treatments

a. Polynomial trend line of the UV-vis absorbance peak recorded at different concentration after 60 minutes of reaction time

F. Average Wavelength Peaks of replicates in 10 minutes and 60 minutes interval

a. Average wavelength peaks of replicates within 10 minutes and 60 minutes interval

Treatments and Replicates		10 minutes	60 minutes	Average Wavelength
Treatment A (0.2 g/mL)	A1	429.4 nm	462.0 nm	445.7 nm
	A2	415.8 nm	434.2 nm	425.0 nm
	A3	399.0 nm	430.3 nm	414.6 nm
Treatment B (0.4 g/mL)	B1	477.6 nm	492.1 nm	484.9 nm
	B2	503.8 nm	493.5 nm	498.6 nm
	B3	597.6 nm	494.0 nm	545.8 nm
Treatment C (0.6 g/mL)	C1	576.4 nm	495.8 nm	536.1 nm
	C2	513.0 nm	465.4 nm	489.2 nm
	C3	527.6 nm	463.4 nm	495.5 nm

b. Average wavelength peak of Treatment A replicates within 10 minutes and 60 minutes interval

c. Average wavelength peak of Treatment B replicates within 10 minutes and 60 minutes interval

d. Average wavelength peak of Treatment A replicates within 10 minutes and 60 minutes interval

G. Average Absorbance Peaks of replicates in 10 minutes and 60 minutes interval

a. Average absorbance peaks of replicates within 10 minutes and 60 minutes interval

Treatments and Replicates		10 minutes	60 minutes	Average Absorbance
Treatment A (0.2 g/mL)	A1	4	4	4
	A2	3.9588	3.9906	3.9747
	A3	3.9399	4	3.97
	B1	3.6975	4	3.8488
Treatment B (0.4 g/mL)	B2	3.9303	4	3.9652
	B3	2.9264	3.6611	3.2938
Treatment C (0.6 g/mL)	C1	3.6479	3.9432	3.7956
	C2	4	4	4
	C3	4	4	4

b. Average absorbance peak of Treatment A replicates within 10 minutes and 60 minutes interval

c. Average absorbance peak of Treatment B replicates within 10 minutes and 60 minutes interval

- d. Average absorbance peak of Treatment C replicates within 10 minutes and 60 minutes interval

H. Effect of the Concentration of Alagaw Leaf Extract on the Wavelength Peaks of Silver Nanoparticles in 10 minutes interval

- a. Difference on the effect of the concentration of Alagaw leaf extract on the wavelength peaks of silver nanoparticles in 10 minutes interval

	Sum of Squares	df	Mean Square	F	Sig.	Remarks
Between Groups	28057.209	2	14028.604	7.918	.021*	s
Within Groups	10630.133	6	1771.689			
Total	38687.342	8				

*Level of significance 0.05

b. Difference on the effect of the concentration of Alagaw leaf extract on the wavelength peaks of silver nanoparticles in 10 minutes interval between the treatments

(I) Treatment	(J) Treatment	Std. Error	Sig.	Remarks
.20 g/mL	.40 g/mL	34.36751	.040	S
	.60 g/mL	34.36751	.026	S
.40 g/mL	.20 g/mL	34.36751	.040	S
	.60 g/mL	34.36751	.929	ns
.60 g/mL	.20 g/mL	34.36751	.026	S
	.40 g/mL	34.36751	.929	ns

*. The mean difference is significant at the 0.05 level.

I. Effect of the Concentration of Alagaw Leaf Extract on the Wavelength Peaks of Silver Nanoparticles in 60 minutes interval

a. Difference on the effect of the concentration of Alagaw leaf extract on the wavelength peaks of silver nanoparticles in 60 minutes interval

	Sum of Squares	df	Mean Square	F	Sig.	Remark
Between Groups	4009.802	2	2004.901	9.556	.014	s
Within Groups	1258.893	6	209.816			
Total	5268.696	8				

*Level of significance 0.05

b. Difference on the effect of the concentration of Alagaw leaf extract on the wavelength peaks of silver nanoparticles in 60 minutes interval between the treatments

(I) Treatment	(J) Treatment	Std. Error	Sig.	Remarks
.20 g/mL	.40 g/mL	11.82696	.012	S
	.60 g/mL	11.82696	.073	ns
.40 g/mL	.20 g/mL	11.82696	.012	S
	.60 g/mL	11.82696	.335	ns
.60 g/mL	.20 g/mL	11.82696	.073	ns
	.40 g/mL	11.82696	.335	ns

*. The mean difference is significant at the 0.05 level.

J. Effect of the Concentration of Alagaw Leaf Extract on the Absorbance Peaks of Silver Nanoparticles in 10 minutes interval

a. Difference on the effect of the concentration of Alagaw leaf extract on the absorbance peaks of silver nanoparticles in 10 minutes interval

	Sum of Squares	Df	Mean Square	F	Sig.	Remarks
Between Groups	.341	2	.170	1.605	.276	ns
Within Groups	.637	6	.106			
Total	.977	8				

*Level of significance 0.05

b. Difference on the effect of the concentration of Alagaw leaf extract on the absorbance peaks of silver nanoparticles in 10 minutes interval between the treatments

(I) Treatment	(J) Treatment	Std. Error	Sig.	Remarks
.20 g/mL	.40 g/mL	.26599	.285	ns
	.60 g/mL	.26599	.947	ns
.40 g/mL	.20 g/mL	.26599	.285	ns
	.60 g/mL	.26599	.412	ns
.60 g/mL	.20 g/mL	.26599	.947	ns
	.40 g/mL	.26599	.412	ns

*. The mean difference is significant at the 0.05 level.

K. Effect of the Concentration of Alagaw Leaf Extract on the Absorbance Peaks of Silver Nanoparticles in 10 minutes interval

a. Difference on the effect of the concentration of Alagaw leaf extract on the absorbance peaks of silver nanoparticles in 60 minutes interval

	Sum of Squares	df	Mean Square	F	Sig.	Remarks
Between Groups	.021	2	.011	.806	.490	ns
Within Groups	.079	6	.013			
Total	.100	8				

*Level of significance 0.05

- b. Difference on the effect of the concentration of Alagaw leaf extract on the absorbance peaks of silver nanoparticles in 60 minutes interval between the treatments**

(I) Treatment	(J) Treatment	Std. Error	Sig.	Remarks
.20 g/mL	.40 g/mL	.09356	.509	ns
	.60 g/mL	.09356	.984	ns
.40 g/mL	.20 g/mL	.09356	.509	ns
	.60 g/mL	.09356	.601	ns
.60 g/mL	.20 g/mL	.09356	.984	ns
	.40 g/mL	.09356	.601	ns

L. Transmission Electron Microscopy Analysis of Silver Nanoparticles in terms of Size and Shape

- a. TEM images of synthesized silver nanoparticles in (a) 60000x magnification. (b) 100000x magnification. (c) 300000x magnification**

b. Size Distribution of the synthesized silver nanoparticles in 5000x magnification

c. Size Distribution of the synthesized silver nanoparticles in 15000x magnification