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Biosynthesis, optimization of process parameters and antimicrobial activity of silver nanoparticles from *Moringa oleifera* leaf extract

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Abstract

The present study was explored for biosynthesis, optimization of process parameters and antimicrobial activity of silver nanoparticles from *Moringa oleifera* leaf extract**.** When factors affecting the synthesis were optimized, the optimum conditions were temperature of 60^0C at time t, of 20 min, pH of 8.5, silver nitrate ($AgNO_3$) concentration of 2m M and 20 mL leaf extract. Formation of Ag NPs was primarily confirmed by change in color of reaction mixture from pale yellow to dark brown. UV-Vis spectrophotometer showed maximum absorbance peak at 400nm at different time intervals indicating formation of Ag NPs. The surface morphology of Ag NPs investigated using SEM was found to be spherical with homogeneous morphology. The average diameter of the primary particles was found to be 27 nm with a standard deviation and 4.85% as a coefficient of variation. The bio synthesized Ag NPs demonstrated effective antibacterial activity against both grams positive and gram negative pathogens investigated. It revealed the highest zone of inhibition at 16.2 mm for *E. coli* and 15.9 for *S. typhimurium* which were followed by *S. aureus* at 15.2 mm and *B. cereus* at 13.5 mm. On the other hand, the deionized water (negative control) and the leaf extract exhibited no any zone of inhibition. The $AgNO_3$ being the positive control displayed antimicrobial efficacy against all tested pathogens, with their activities around 11.4 mm and 9.6 mm for gram negative bacteria, *E. coli* and *S. typhimurium* respectively. In the same vein, gram positive bacteria, *S. aureus* and *B. cereus* both have their zone of inhibition at 9.5 mm. With the foregoing, one may easily suggest that, Ag NPs fabricated from *Moringa oleifera* leaf extract be used in the treatment of diseases caused by the pathogens investigated in this research as it demonstrated high antibacterial potentials.

Keywords: Biosynthesis; Optimization; Process Parameters; Antimicrobial Activity; Silver Nanoparticles; *Moringa oleifera*

1. Introduction

Nano science is concerned with the fabrication and manipulation of materials and miracles at nanometer scale in which materials properties behave significantly different as opposed to bulk material entities. Silver nanoparticles (Ag NPs), in particular , are among the most promising materials that have attracted much attentions and have been extensively studied in different fields including materials science and engineering, biomedical, antimicrobial and catalytic applications simply because they have unique properties due to their surface area and particle size [1-2]. In recent times, the green route for synthesis of silver nanoparticles using plants leaf extract as a reducing and capping agents is an emerging research area in nanotechnology. Several research studies conducted upon silver nanoparticles reported improved properties, including good electrical and thermal properties, chemical stability as well as catalytic and antimicrobial properties [3-4]. These nanoparticles could be biofabricated using different plant extract such as *Laurus*

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nobilis, Crocus Haussknechtii Bois, Parkia biglobosa aquous stem extract, among others[5-7]. Furthermore, Because of electrostatic interactions between silver ions and proteins in plant material extract, the bioreduction of Ag was considered to involve capturing Ag^+ ions on the protein surface. Proteins reduce the Ag^+ ions, resulting in a change in secondary structure and the formation of silver nuclei. Silver nuclei are formed by further reducing Ag^+ ions and their build- up at the nucleus, resulting in the production of AgNPs as reported by previous studies[8-9]. To give fairness a chance, nanoparticles are nurtured as building blocks of the next generation of optoelectronics, electronics and various chemical and biochemical sensors[10]. Although there abound several methods for the synthesis of metal and their bimetallic hybrid nanoparticles, biosynthetic processes have received much attention as a viable alternative for the development of metal nanoparticles where plant extract is used without any chemical ingredients[11-12]. In this research work, a meticulous study of antimicrobial potential of green synthesized Ag NPs as well as optimization of process parameters (synthesis conditions) of silver nanoparticles were the main thrust of this study.

2. Material and methods

2.1 Materials

Some of the materials employed during this work were but not limited to; *Moringa oleifera* leaves, deionized water, $AgNO_3$, magnetic stirrer, hot plate, whatman no. 1 filter paper, crucible, beaker. All the reagents used during this work were of analytical grade.

2.2 Methods

2.2.1 Sample Collection

The *Moringa oleifera* leaves were collected in Billiri Local Government Area of Gombe State and were transported to Federal University of Kashere for further identification. The collected *Moringa oleifera* leaves were thoroughly washed under running tap water and rinsed severally with distilled water followed by sun drying to remove residual moisture. The dried materials were cut into smaller sizes and ground with the aid of motar and pistle.

2.2.2 *Preparation of the Plant* Extract

Here, Yuet *et al.,*2018 method[13]was adopted with slight modifications as follows:Ten gram of moringa leaf powder was weighed and dispersed in 100 ml of deionized water in a 250 ml glass beaker. The mixture was heated on hot plate at 60^0C for 20 min. After 20 min, the extract was filtered using 0.45 mm Millipore membrane filter and followed by 0.2 mm Millipore membrane filter and the filtrates collected(figure 1D). This was used immediately for the synthesis of Silver nanoparticles.

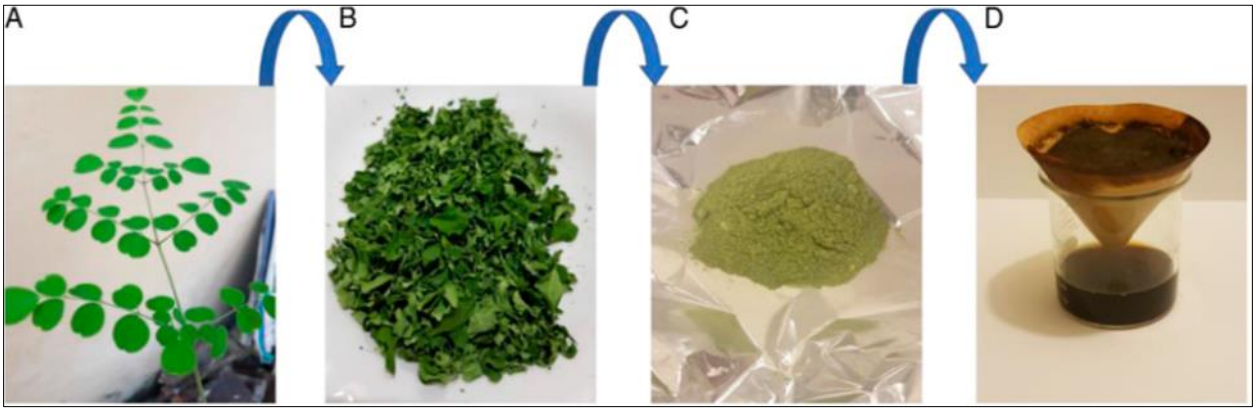

Figure 1 Fresh(a) Dried(b) Powdered(c) and Extract from *Moringa oleifera* leaves (d)

2.2.3 Green Synthesis of Silver Nanoparticles

Here, an approach described by the previous research was adopted with little modifications as follows: Silver nanoparticles were synthesized by adding 10 mL of a 0.01 M aqueous solution of silver nitrate into different volume (1.0, 2.0, 3.0, 4.0 and 5.0 mL) of *Moringa oleifera* extract taken into five beakers separately at room temperature. The color of the solution started changing within 5 min of vigorous stirring indicating the formation of nanoparticles. After 20 min (figure 2), there was no further change in color observed, and at this point, the observation was recorded. The

separation of silver nanoparticles from the dispersion was carried out by centrifugation and after that, Ag NPs were washed 4 times with distilled water and acetone to remove water soluble impurities and then nanoparticles were lyophilized and stored in dry bottles for further use [14].

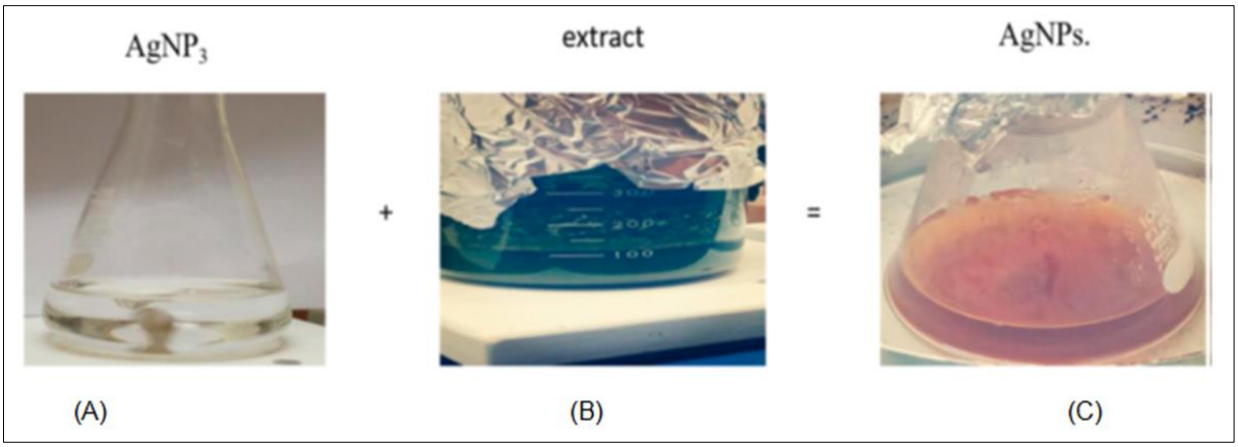

Figure 2 Synthesis of silver nanoparticles using *Moringa oleifera* leaves extract

2.3 Characterization of the Sample Synthesized

2.3.1 UV-visible spectral analysis

The silver nanoparticles were confirmed by measuring the wavelength of reaction mixture in the UV-Vis spectrum at a resolution of 1 nm from 200 to 800 nm.

2.3.2 SEM Analysis

The surface morphology of the nanomaterial (Ag NPs) was characterized by scanning electron microscope (SEM).

2.4 Antibacterial Assay

Here, a method outlined by a previous research [15] was used as follows: The antibacterial activity of green synthesized Ag NPs was tested against various gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) bacteria by the standard agar well diffusion method. To examine the antibacterial activity of biosynthesized Ag NPs, Muller-Hinton agar plates were sterilized and allowed to solidify. After solidification, 30µl of each bacterial suspension was inoculated on the petri plates by a sterile glass rod. Then, 0.1 g of synthesized Ag NPs powder were dissolved in 1.0 ml (100 ppm) autoclaved distillated water to provide the suspension of Ag NPs. Sterilized paper disks about 6.4 mm in diameter were impregnated with 30µl of suspension and placed on Muller Hinton agar plates. The negative (deionized water) and positive ($AgNO_3$) controls, and the *Moringa oleifera* leaf extract were also employed for the antibacterial assay. The plates were incubated at 37^0C for 24 h. After the incubation period, the zones of inhibitions were observed around the discs. Antibacterial activity was investigated by measuring the diameter of the zones of inhibition after using the plant extract.

3. Results and discussion

3.1 Spectroscopic, UV Visible and SEM Analysis

3.1.1 Spectroscopic Observation

Spectroscopic study revealed color change of the solution from pale yellow to brown within 5 min of addition of the $AgNO_3$ against aqueous extract. A dark brown color of the solution was noticed after another 15 min of constant stirring and beyond this time, no further change in color. Color changes of the solution was due to some phytoconstituents including flavonoids, saponins, steroids, alkaloids, and color present in plant extract. These act as reducing agent that reduced Ag^+ (silver ion) to Ag^0 (silver atom). This finding agrees with some literature reports on silver nanoparticles synthesized from different plants [16-17]. It is important however, to note that, detection of silver nanoparticles was predicted by its dark brown color formation, a phenomenon that could be attributed to excitation by surface Plasmon vibrations [18-19]. More so, it was observed that complete color transition took 20 min at room temperature.

3.1.2 UV- Visible Analysis

The formation of Ag NPs in the medium was confirmed by a surface Plasmon resonance (SPR) band and a UV-vis absorption peak corresponding to Ag NPs at approximately 400 nm (Figure 2e). Beyond 400nm, the absorption maxima slightly shifted to longer wavelengths, which probably originated from the aggregation of Ag NPs in uncontrolled reactions. This result suggests that 400nm is the optimal wavelength to obtain high quality Ag NPs. This finding is no doubt, in consonance with the previous research conducted on the Ag NPs bio fabricated from *Moringa oleifera* root extract with its UV – visible absorption peak maxima within the range of 300 to 500nm [20].

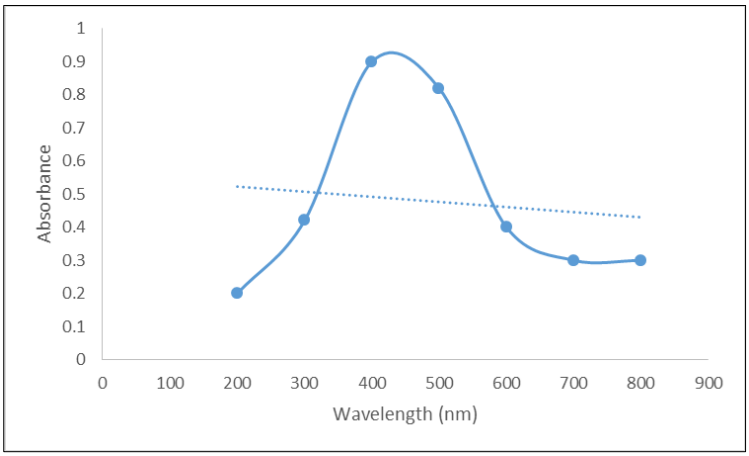

Figure 2e UV- Visible Spectrum

3.1.3 SEM Interpretation

The morphology and size of Ag NPs were evaluated using SEM. Figure 2f depicts the SEM image of the Ag NPs. The surface morphology of Ag NPs investigated were found to be spherical with homogeneous morphology. The average diameter of the primary particles was found to be 27 nm with a standard deviation and 4.85% as a coefficient of variation, a research outcome that conforms relatively to the one reported by a separate researcher [21]. It follows then that; the Ag NPs have actually been synthesized since the result of the SEM analysis does not only agree with the UV result of the sample investigated, but also in concord with the SEM result of a different researcher carried out under different working conditions [21].

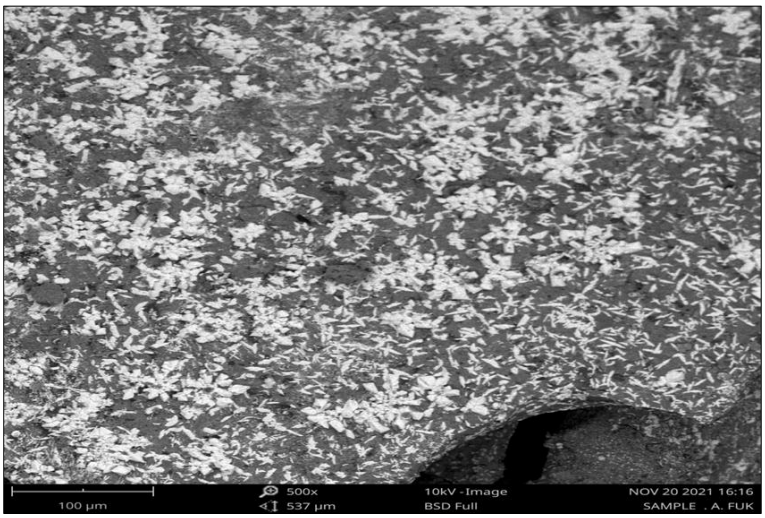

Figure 2f SEM Image of Ag NPs from *M. oleifera* Leaf extract

3.2 Effect of Process Parameters (Synthesis Conditions)

3.2.1 Effect of concentration of leaf extract on Ag NPs sizes and concentration

Above a particular limit, silver ions could be toxic to protein present in plant extract and they can cause precipitation. To prevent this, plant extracts are used to transform ionic forms to nanoparticle. UV- spectrophotometer and Zeta sizer are used here to account for the effect of leaf extract concentration on the size and concentration of Ag NPs in the reaction mixture by varying the concentration of the leaf extract. Size and shape of Ag NPs were controlled by changing the concentration of extract from 1 to 5 mL in 2m M $AgNO_3$ of 20 mL solution. With the increase in the leaves extract concentration, the absorbance of the sample increased. Therefore, it follows that, increasing concentration of the silver nitrate led to particle size reduction till the optimum concentration beyond that particle size increase. Optimum particles size was observed at 2m M $AgNO_3$ (Figures 3a and 3b). It can hence be deduced that, at higher concentration, the possibility of particles size formation might increase. Similar trends were reported by the previous literature [21].

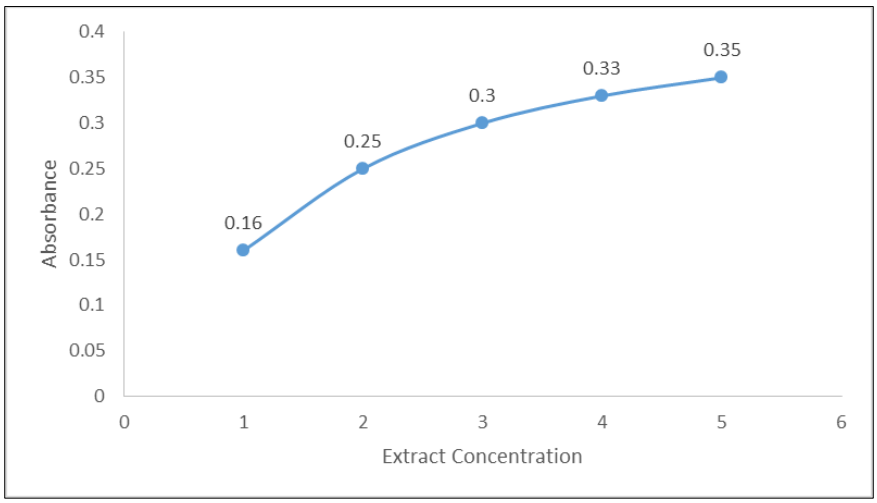

Figure 3a Effect of concentration of leaf extract on Ag NPs concentration in solution

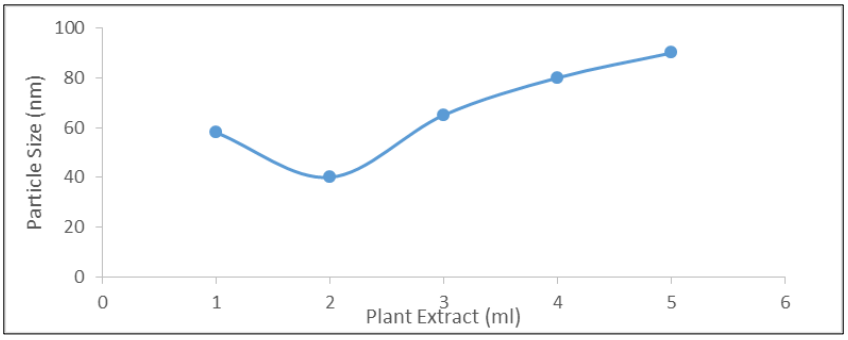

Figure 3b Effect of varying concentration of leaf extract on Ag NPs sizes

3.2.2 Effect of concentration of $AgNO_3$ on size and concentration of Ag NPs

The reduction and capping of Ag^+ to Ag^0 is a function of the ratio of reducing agent to substrate. Higher ratio of reducing agent to substrate accelerates the process. Changes in absorption peak of the reaction mixture were observed at varying salt concentrations from 1m M to 5m M. Dark brown colors and maximum peak intensity were observed at salt concentrations 2m M. Thereafter absorption peak intensity was decreased from 2m M to 3m M and then remained constant from 3m M to 5m M. This is because Ag^+ concentration was increased in the solution by adding more $AgNO_3$. It could be observed that, the maximum peak intensity was recorded at 2m M of $AgNO_3$ as seen in Figures 3c and 3d. Furthermore, variation in silver nitrate concentration also influenced nanoparticles size. Because of increase in the concentration of $AgNO_3$, Ag NPs size was observed to decrease from 1m M to 2m M and then increased from 2m M $AgNO_3$ to 5m M. This result agrees to a larger extent, with the one reported by another researcher [21- 22].

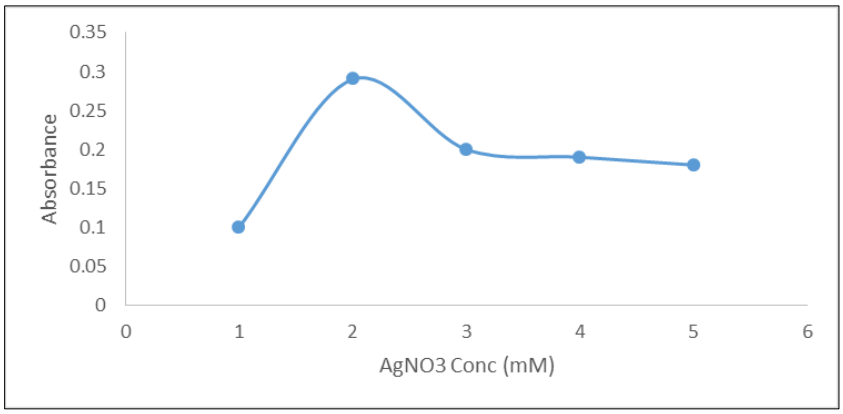

Figure 3c Effect of concentration of $AgNO_3$ on Ag NPs concentration

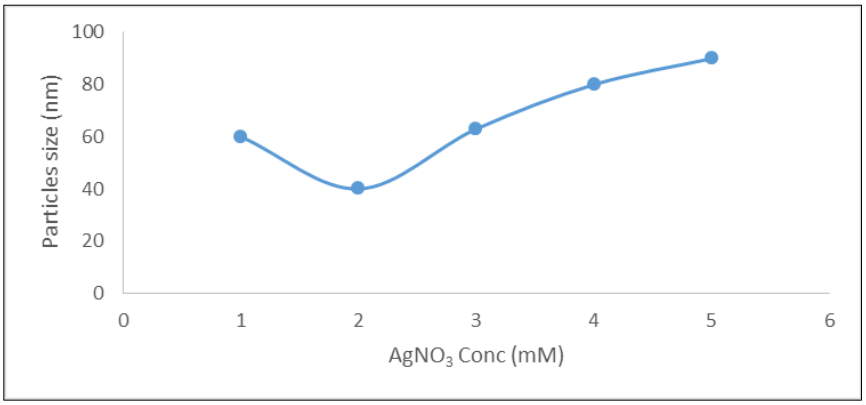

Figure 3d Effect of concentration on Ag NPs size

3.2.3 Effect of pH on the Formation of Ag NPs

It should be noted that, solution pH performed an essential role in the formation of nanoparticles. At pH between 2-5 (low pH), the formation of Ag NPs in the solution was relatively slow and large size nanoparticles were obtained as was shown by color change during the reaction. pH of the solution affects the shape and size of the particles and equally have the ability to change their capping as well as stabilizing abilities. Absorption peak intensity increased when pH of the solution was increased from acidic to basic (pH 5-13). Due to increase in reduction rate of Ag^+ in the solution, concentration of Ag NPs in solution increased. It was observed that, at pH 13, Ag NPs were very unstable in solution and agglomeration of silver nanoparticles was noticed.

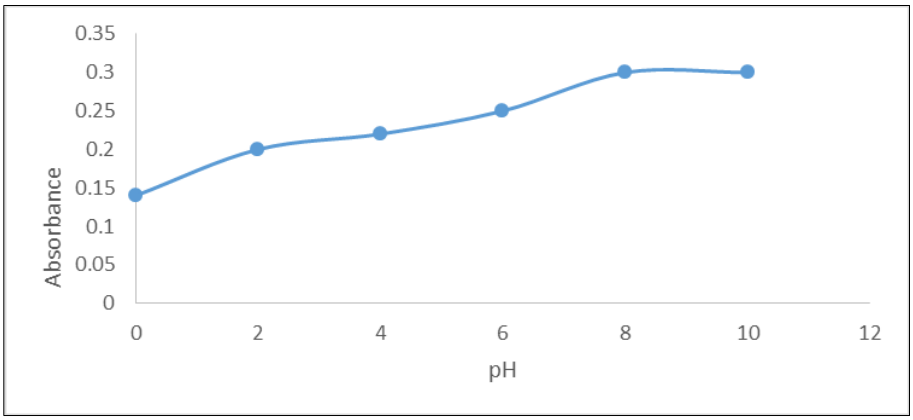

Figure 3e Effect of pH on Ag NPs concentration

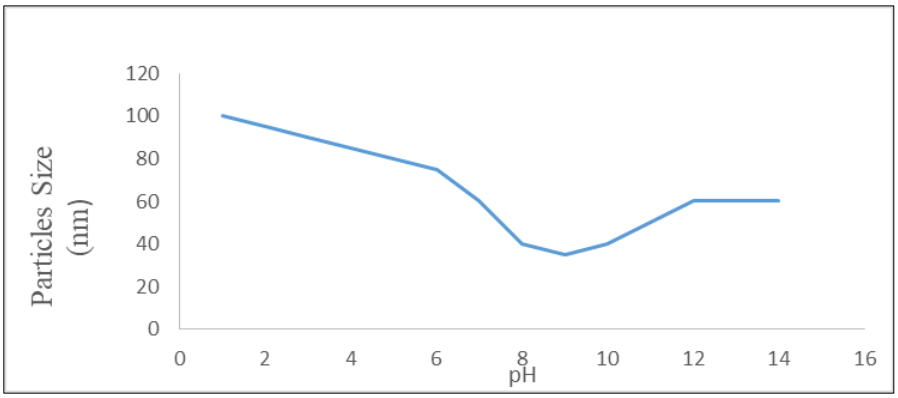

Figure 3f Effect of pH on Ag NPs size

3.2.4 Effect of Temperature on Ag NPs size and concentration

The research revealed that, the absorption of SPR increased with temperature as the change in the color of the solutions was accelerated at higher temperatures, indicating the rapid reduction of Ag^+ to form Ag NPs. Optimum temperature was obtained for this experiment at 60°C (see figure 3h). Beyond 60^0C, the absorption maxima slightly shifted to longer wavelengths, which probably originated from the aggregation of Ag NPs in uncontrolled reactions. This result suggests that 60^0C is the optimum temperature to obtain high quality Ag NPs as the case may be, a report that is similar to the recent research findings [21 -22].

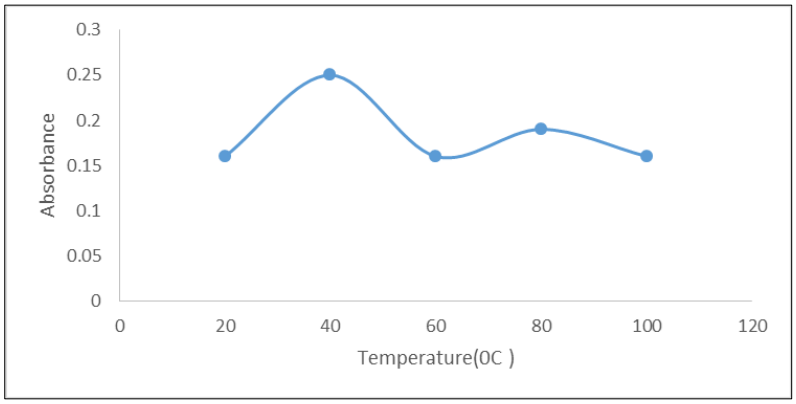

Figure 3g Effect of Temperature on Ag NPs concentration

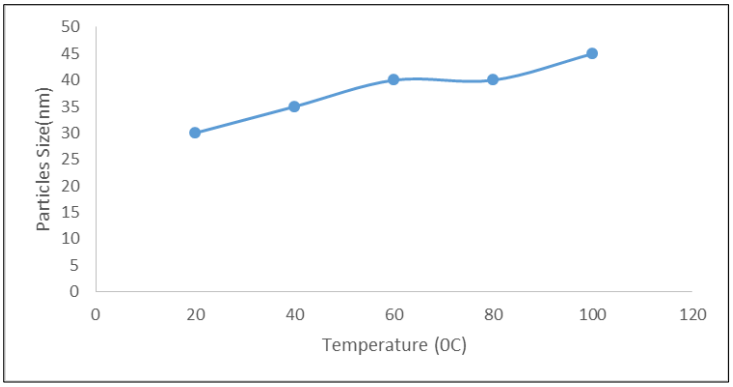

Figure 3h Effect of Temperature on Ag NPs size

3.2.5 Effect of contact time on Ag NPs

Figure 3i and 3j revealed the time course of the absorption spectra of the reaction mixtures consisting of the *M. oleifera* leaf extract (2 mL) and $AgNO_3$ solution (20 mL, 2m M) at 60^0C and pH = 8.5. The intensity of the absorption increased with the reaction time, indicating the gradual formation of Ag NPs. On the other hand, the absorption maxima gradually shifted toward longer wavelengths after 20 min, which shows the agglomeration of Ag NPs to form larger particles. Hence a reaction time range from 20 to 30 min can be a good time range for the synthesis of high quality Ag NPs for this work, a report that differ from 1 to 6h time frame given by the previous research [21].

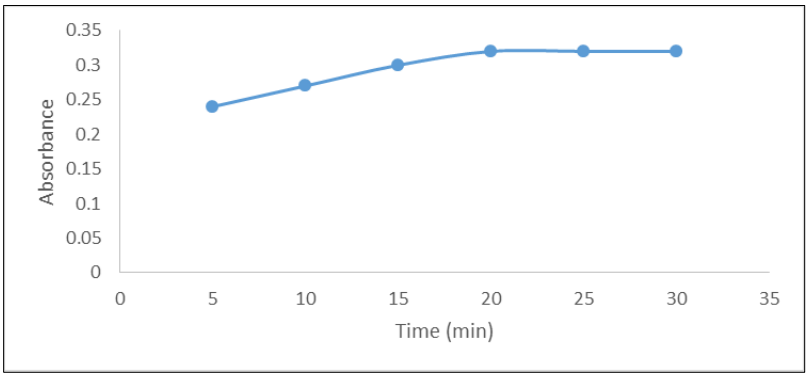

Figure 3i Effect of Time on Ag NPs concentration

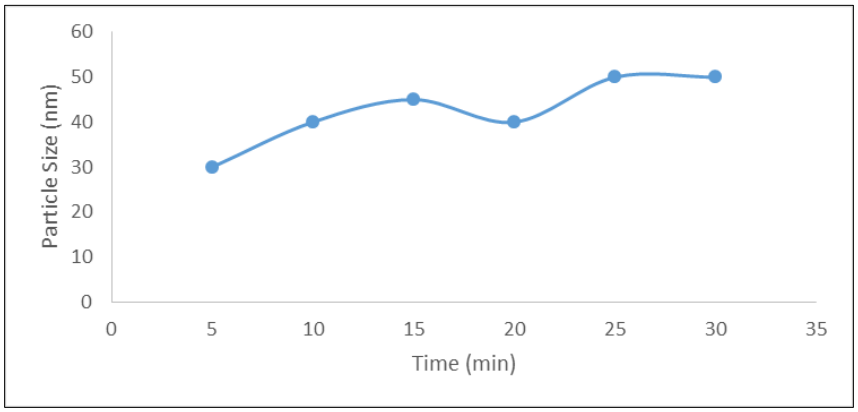

Figure 3j Effect of Time on Ag NPs Size

3.3 Antibacterial activity

The antibacterial potency of green synthesized Ag NPs, $AgNO_3$, *M. oleifera* leaf extract and distilled water were investigated against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) bacteria using the agar well diffusion assay, and the zone of inhibition was tabulated as appeared in Table 1. The bio synthesized Ag NPs demonstrated effective antibacterial activity against both Gram-positive and Gram- negative pathogens. The silver nanoparticles synthesized by *M. oleifera* leaf extracts revealed the highest zone of inhibition at 16.2 mm for E. coli and 15.9 for *S. typhimurium* which were followed by *S. aureus* (15.2 mm) and *B. cereus* (13.5 mm). On the other hand, the deionized water (negative control) and the leaf extract exhibited no any zone of inhibition. The $AgNO_3$ being the positive control displayed antimicrobial efficacy against all tested pathogens, with their activities around 11.4 mm and 9.6 mm for gram negative bacteria, *E. coli* and *S. typhimurium* respectively. In the same vein, gram positive bacteria, *S. aureus* and *B. cereus* both have their zone of exhibition at 9.5 mm. The same trend was reported by the previous research [23]. Although the mechanisms behind the antibacterial potency of Ag NPs is not quite clear, Ag NPs might have been attached to the surface of the cell membrane of microorganisms, leading to the disturbance of its functions like permeability and respiration. It is obvious, therefore, that the binding of particles to the microorganism depends on the surface area available for interaction. Generally, small nanoparticles have a larger surface area for interaction with bacteria, as opposed to that of bigger particles [23-24]. The cell walls of gram positive bacteria composed of a rigid thicker multiple layer of peptidoglycan, which prevented the nanoparticles from entering

into cell wall, hence, explains why the gram positive bacteria exhibited the lower zone of inhibition, as compared to gram negative bacteria in this study.

Table 1 Zone of inhibition (mm) of Ag NPs, *M. oleifera* leaf extract, $AgNO_3$, and deionized water against tested organisms

Components	Zone of inhibition (mm)			
	S. aureus	***B. cereus***	*E. coli*	***S. typhimurium***
Ag NPs	15.1	13.5	16.2	15.9
Leaf Extract	NA	NA	NA	NA
$AgNO_3$	9.5	9.5	11.4	9.6
deionized water	NA	NA	NA	NA

NA = No Activity

4. Conclusion

Silver nanoparticles were synthesized using eco-friendly approach from *Moringa oleifera* leaf extract and the same were characterized using UV and SEM. The synthesized Ag NPs were found to exhibit dark brown color as a primary confirmation of the synthesis of Ag NPs. The maximum absorption peaks revealed 400nm when UV was conducted while a spherical shape was identified when SEM analysis was carried out. The particles size could be affected by a number of process parameters including temperature, time, concentrations of $AgNO_3$ etc. The biosynthesized nanoparticles presented strong antimicrobial activity against gram positive (*S. aureus* and *B. cereus*) and gram negative (*S. typhimurium* and *E. coli*) bacteria.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

Authors' Contributions

This work was carried out in collaboration among all authors. Author MY conceived and designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Authors JDS, JWKJ, PDB, DGA and NU managed literature searches. All authors read and approved the final manuscript.

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