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Characterization and antimicrobial evaluation of biomimetically synthesized silver nanoparticles using aqueous leaf extract of *Morinda lucida* Benth. (Rubiaceae)

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Abstract

Silver nanoparticles (AgNPs) are silver atom masses that are attracting widespread interest due to their diverse applications, particularly their activity as antimicrobial agents. Physico-chemical methods of AgNP synthesis are associated with high costs, high temperatures and toxic byproducts. Thus, the plant mediated pathway represents a better option. The indigenous medicinal plant *Morinda lucida* was employed in the fabrication of AgNPs. The nanoparticles were characterized using different analytical techniques and also evaluated for their antimicrobial potential. Phytochemical screening of the plant was also carried out. For the synthesis, 10 ml of aqueous *M. lucida* leaf extract was added to 90 ml of freshly prepared 3 mM silver nitrate ($AgNO_3$) solution in a flask. The mixture was allowed to stand at ambient temperature, in a dark cupboard for 48 hours. Positive AgNP synthesis, indicated by a colour change from red to brown was further validated by UV-vis spectroscopy wherein an absorption peak at 460.51 nm was recorded. The utilitarian aspects of the particles were further characterized using scanning electron microscopy (SEM), fourier transform infrared (FTIR) spectroscopy, dynamic light scattering (DLS), X-ray diffraction (XRD) and energy dispersive X-ray spectroscopy (EDX). The SEM images showed that particles were round to irregular in shape. Amide, amine, alkene and alkynes were the most occurring functional groups from the FTIR spectra. Quantitative phytochemical analysis revealed the presence of flavonoids, terpenoids, phenols, reducing sugars and alkaloids in varying amounts, which play a significant role in the synthesis and stabilization of the AgNPs. The XRD diffractogram of AgNPs showed two peaks at 45.53° and 77.17° that correspond to miller indices of (200) and (311) respectively and an average crystalline size of 62.60 nm obtained using the Debye-Scherrer's formula. The DLS result indicated a Z–average size of 235.1 nm and a polydispersity index (PDI) of 0.4. EDX analysis showed that elemental silver (Ag) had the highest atomic concentration of 64.50 %. Using the agar well diffusion assay, the nanoparticles exhibited antimicrobial activity against *P. aeruginosa* (bacteria) and *A. flavus* (fungi). It can be concluded that *M. lucida* is capable of synthesizing stable, small-sized AgNPs with antimicrobial potential.

Keywords: *M. lucida* aqueous leaf extract, biomimetic, antimicrobial, AgNPs.

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INTRODUCTION

Nanoparticles (NPs) refer to very small microscopic particles with at least one dimension under 100 nanometers (nm) (Alanazi *et al*., 2010). Metal nanoparticle research has of recent become the focus of intense scientific research and of the numerous metal nanoparticles, silver nanoparticles (AgNPs) particularly have drawn increasing attention because of their high conductivity, catalytic activity, localized surface plasmon resonance, antimicrobial properties and chemical stability (Zhang *et al*., 2016). Silver is known to induce antimicrobial effects. However, silver in its nano form exhibits a significant increase in antimicrobial activity due to increases in available surface area for microbes to be exposed to. Also, their diverse applications in the area of drug delivery, acting as pesticides, DNA detection and many more have been demonstrated (Netala *et al*., 2016; Entsar and Noha, 2016).

Traditionally, the synthesis of silver nanoparticles involves chemical and physical methods, some of which include; laser ablation, gamma irradiation, electron irradiation, chemical reduction, photochemical methods and microwave processing (Iravani *et al*., 2014). However, drawbacks associated with these methods necessitate improvements for which biological entities seems to be the best option providing an ecofriendly alternative, high yield, not requiring the use of pressure and toxic chemicals (Ge *et al*., 2014).

Plants in particular, are the best suited alternative as they are rich sources of phytochemicals whose functional groups play a significant role in the reduction of bulk silver to silver nanoparticles (Sumathi and Thomas, 2017; Tariq *et al*., 2022). These phytochemicals otherwise known as biomolecules also serve as capping/stabilizing agents (Joseph and Matthew, 2014). Properties of the nanoparticles such as shape, crystalline nature, size and antimicrobial efficacy are dependent upon the kind of phytochemical present in a given plant as well as concentration, conditions of synthesis such as reaction time and temperature (Patra and Baek, 2014). Phytochemicals such as terpenoids (Shriniwas and Subhash, 2017), alkaloids (Almadiy, 2018), carbohydrates (Panigrahi *et al*., 2004), flavonoids (Sahu *et al*., 2016) and proteins (Ondari *et al*., 2019) present in the plants have been reported to play major roles in the biomimetic synthesis of silver nanoparticles. The process of synthesis usually takes place by the reductive action of a reducing agent on a silver salt (metal precursor) in the presence of a stabilizer (Srikar *et al*., 2016; Zhang *et al*., 2016). Synthesis of nanoparticles is usually followed by their characterization which involves evaluation of functional aspects such as size, shape, size distribution, surface area, solubility and aggregation (Zhang *et al*., 2016). Characterization is carried out using a variety of analytical techniques such as UV-visible spectroscopy, X-ray diffractometry (XRD), Fourier transform infrared (FTIR) spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDX).

UV-visible spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles (Geiss *et al*., 2013). It allows detection of AgNPs through analysis of their characteristic surface plasmon resonance band, this is because AgNPs have distinct optical properties which make them strongly interact with particular wavelengths of light (between 400- 500 nm) (Nanocomposix, 2012). Scanning electron microscopy (SEM) is a visualization method capable of determining the exterior morphology of synthesized particles at the nano and microscales. It is a high-resolution microscopy technique that enables the examination of nanomaterials by making use of a stream of extremely energetic electrons to scrutinize objects (Yao *et al*., 2007). The use of EDX spectroscopy in elemental composition detection is mainly due to the fact that each element has a peculiar atomic structure that allows unique peaks to be generated on an electromagnetic spectrum. The peak generated by an element is determined when high energy beam of X-ray is targeted toward a given sample (Intertek, 2018). X- ray diffraction on the other hand is used in qualitative identification of various compounds, for measuring sizes and degree of crystallinity of particles. When X-ray light falls on any crystal, it leads to the formation of many diffraction patterns which reflect the physico-chemical characteristics of the crystal structures (Robin, 2009; Waseda *et al*., 2011). Fourier transform infrared (FTIR) spectroscopy is a simple, non-invasive and inexpensive technique for identifying the role of biological molecules in the reduction processes (Zhang *et al*., 2016). It is an important method in academic and industrial research for confirming

functional molecules grafted onto nanoparticles (Lin *et al*., 2014). To determine the capability of a nanomaterial, it is important to also characterize it in a solution and this is achieved using the dynamic light scattering (DLS) method which ascertains parameters like size distribution and average diameter of nanoparticles distributed in physiological or aqueous solutions (Pleus, 2012). The method depends on the interaction of light with particles by measuring the light scattered from a laser that passes through a colloid, relying mostly on Rayleigh scattering from the suspended nanoparticles (Fissan *et al*., 2014).

As far as the therapeutic application of AgNPs is concerned, their antimicrobial property is the most sought after (Prabu and Paulose, 2012). The rising threat of resistant microbes worldwide also calls for the production and introduction of new, more advanced platforms for the study and development of more efficacious antimicrobial agents against multidrug-resistant strains (Kapil, 2005; Dakal *et al*., 2016). Amongst the promising nanomaterials, the antimicrobial action of AgNPs is undisputed. The most commonly proposed mechanisms of action are firstly, uptake of free silver ions followed by disruption of ATP production and DNA replication. Secondly silver ion and AgNP generation of reactive oxygen species (ROS) and silver nanoparticle direct damage to cell membrane. In contrast to conventional antimicrobials and the resistance developed to them, AgNPs attack a broad range of targets in the organisms making it difficult and less likely for them to develop resistance. They would have to evolve a range of mutations simultaneously to fortify themselves (Pal *et al*., 2007).

Morinda lucida Benth. (Rubiaceae) is a native, small, average-sized tree commonly known as "brimstone tree". The various parts of the plant all have medicinal properties, the leaves are reported to have potential in the treatment of many types of fever, including yellow fever, also in the treatment of malaria, trypanosomiasis, and feverish conditions during childbirth**.** The extract of the stem bark has also been recommended for the prevention and treatment of hypertension and cerebral complications (Adeleye *et al*., 2018). Even though plants are widely used for the synthesis of AgNPs, they differ in their ability to produce these particles. Sometimes, particular phytochemicals may not have the potential to reduce bulk silver into its nanoform and this was demonstrated in an experiment carried out by Panigrahi *et al*. (2004). It is usual for experiments in which biosynthesis of AgNPs is carried out to be followed by evaluation of their antimicrobial activity due to the established role of AgNPs in serving as antimicrobials. Such results usually show significant or insignificant activity against test organisms.

The antimicrobial properties of *M. lucida* leaves against test organisms used in this study are known (Ogundare and Onifade, 2009; Addy *et al*., 2013; Olawuwo *et al*., 2022). However, it is also known that antimicrobial efficacy increases at the nanoscale due to larger surface area of AgNPs (Ahmed *et al*., 2021). The need to evaluate the capabilities of indigenous plants likewise cannot be overemphasized. To fill these gaps, the study was carried out to determine the ability of *M. lucida* leaves to synthesize AgNPs, characterize the particles and evaluate their potential/efficiency in serving as antimicrobial agents against test organisms. Screening of its leaves was also carried out to ascertain the phytochemicals present which serve as reducing and capping agents during the process of synthesis. The results of this study will extend the frontiers of *Morinda*-synthesized AgNP research to increase knowledge on their antimicrobial properties and also to serve as an academic resource for researchers who will engage in future research on species of *Morinda*.

MATERIALS AND METHODS

Collection of Plant and Preparation of Leaf Extract

Fresh leaves of *Morinda lucida* were collected from a farmland in Odoru, Nsukka (Enugu Sate – Nigeria), followed by thorough washing with running water and then left to air dry at room temperature. Afterwards, the plants were ground to obtain fine powder. Twenty grams was weighed and poured into a 500 ml Erlenmeyer flask**,** to which 400 ml of distilled water was added and boiled for 20 minutes. This was done in a water bath set at 80 degrees centigrade. The mixture was left to cool at ambient temperature, then filtered using cheese cloth followed by filtration using Whatman No.1 filter paper. The filtrate (extract) obtained was then used for the synthesis process.

Synthesis of Silver Nanoparticles

For the synthesis, 10 ml of *M. lucida* aqueous leaf extract was added to 90 ml of 3 mM $AgNO_3$ solution and the reaction mixture kept in a dark cupboard and monitored for visible colour change which is the first indication of the formation of silver nanoparticles (AgNPs). The samples were observed for a period of 48 h, before proceeding to characterization.

Figure 1: Showing *Morinda lucida* plant (foliage and fruits) in its natural habitat

Table 1: Experimental Outline (synthesis procedure)

Reducing and capping agent	Metal precursor	Temperature	Time
M. lucida aqueous leaf extract (10 ml)	Silver nitrate - $AgNO_3$ (3 mM, 90 ml)	room temperature (dark cupboard)	48hours

Characterization of the Synthesized Nanoparticles

UV spectrophotometer (UV- 1800 Shimadzu) was used to obtain absorption spectra of the AgNPs, followed by centrifugation and lyophilization. The morphologies of the particles were determined by scanning electron microscopy (SEM) (Phenom ProX). The combination of SEM with energy-dispersive X-ray spectroscopy (EDX) enabled the detection of the elemental composition of nanoparticles. X-ray diffractometer-XRD (Rigaku MiniFlex300) was used to determine the crystalline size and nature of the synthesized AgNPs.

The functional groups present in the phytochemicals were identified using fourier transform infrared (FTIR) spectroscopy (Cary 630 machine). The size distribution by intensity and Z-average size of AgNPs were determined using dynamic light scattering (DLS) (Zetasizer Nano ZS machine).

Phytochemical Analyses

Quantitative phytochemical screening of the plants was carried out to determine the presence of various phytochemicals including tannins, flavonoids, hydrogen cyanide, reducing sugars, steroids, soluble carbohydrates, terpenoids, phenolics, saponins and alkaloids. The

procedures used were in accordance with official analytical methods by Association of Official Analytical Chemists International (AOAC) (2005).

Antimicrobial activity of AgNPs

A total of six (6) pathogens; two Gram negative bacteria (*P. aeruginosa* and *E. coli*), two Gram positive bacteria (*B. cereus* and *S. aureus*) and 2 fungi (*A. flavus* and *A. niger*) were used for the antimicrobial study. Test pathogens for this assay were collected from the Department of Microbiology, University of Nigeria, Nsukka campus. The assay was done using modifications of the agar well diffusion assay described by Kora *et al*. (2010).

Agar Well Diffusion Assay

Mueller Hinton Agar (MHA) (manufactured by Oxoid, USA), a non-selective medium was prepared according to manufacturer's specification. Thirty-eight grams of the media was mixed with 1 litre of distilled water and sterilized at 121 ^{o}C for 15 min. The prepared media was allowed to cool to about 50 ^{o}C and then transferred into 90 mm sterile agar plates which were then left to solidify; after which the test organisms in suspension adjusted to 0.5 McFarland standard were inoculated. A sterile 6 mm cork borer was used to make six wells on each of the plates. A stock concentration of 25 mg/ml was prepared by dissolving 0.1 g of the *M. lucida* AgNP solution in 4 ml of sterile distilled water containing 10 % Dimethylsulfoxide (DMSO). Then a two-fold serial dilution was made to achieve the concentration gradients of 25, 12.5, 6.25, 3.125 and 1.56 mg/ml. Aliquots of 60 µl of each AgNP dilutions were added into each well in the culture plates already seeded with the test organisms. The cultures were incubated at 37 ^{o}C for 18 - 24 hours for the bacterial plates and 25 – 27 ^{o}C for 48 h for the fungal plates respectively. The antimicrobial potential of the synthesized AgNPs was determined by measuring the zone of inhibition (ZOI) around each well. The AgNPs synthesized from each plant species were tested against all the bacterial and fungal isolates. Stock solution of 3 mM $AgNO_3$ and *M. lucida* leaf extract served as positive controls.

Data Analysis

The data collected from phytochemical analyses and antimicrobial studies were analyzed using Analysis of Variance (ANOVA) and significant means separated using Duncan Multiple Range Test (DMRT) at P≤0.05.

RESULTS

Visual Examination of Synthesis and UV – visible Spectroscopy

On adding 10 ml of *M. lucida* aqueous leaf extract to 3 mM of $AgNO_3$ (90 ml), an instantaneous colour change was observed which deepened over time. Figures 2A and 2B showed the visual observations noted upon synthesis of the silver nanoparticles. The mixture was observed to change colour from red to brown for *M*. *lucida* AgNPs. The plant reduced AgNPs showed a characteristic absorption peak at 460.51 nm whereas no peak was observed in the case of the *M*. *lucida* aqueous leaf extract as seen in figure 3.

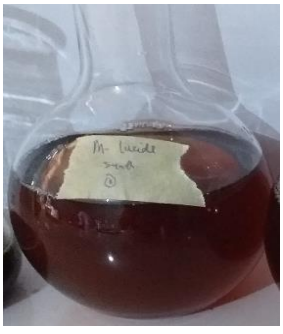

Figure 2A: showing *M. lucida* AgNP solution (0 hours after synthesis)

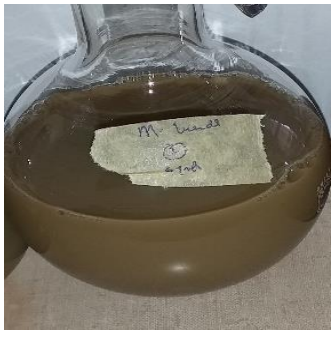

Figure 2B: showing *M. lucida* AgNP solution (48 hours after synthesis)

Characterization

The SEM micrograph suggests that the visible particles seen as white patches were mostly round and also irregular in shape. A few are indicated by red arrows in Figure 4**.** The elements present in the sample were detected using Energy Dispersive X-ray analytical technique. The spectrum shows a major peak was assigned to our element of interest, silver with a relatively high atomic concentration of 64.50 %, followed by carbon (24.78 %) and small percentages of magnesium, phosphorus, sulfur, silicon, calcium, potassium and sodium as seen in figure 5**.** The peaks indicate the positions assigned to each element present and the area underneath represents the number of elements existing in the X-ray irradiated portion of the given sample. The XRD diffractogram (figure 6) of silver nanoparticles synthesized using *M. lucida* aqueous leaf extract showed two distinct diffraction peaks at 2 theta angle values of 45.53° and 77.17° which correspond to miller indices of (200) and (311) respectively. The average crystalline size of 62.60 nm of the AgNPs was obtained using the Debye-Scherrer's formula.

From the DLS analysis, the size distribution by intensity of the AgNPs is presented in figure 7 indicating a broad scatter distribution and a Z average size of 235.1 nm. The polydispersity index (PDI) report for the *M. lucida* AgNPs was 0.4. The FTIR spectra of *Morinda lucida* leaf powder and its AgNPs shows different functional groups such as primary amines, alkane, alcohol, phenol, alkyne, alkene and amides (figure 8 and Table 2).

The quantitative phytochemical analysis of the *M. lucida* leaf is shown in table 3. The highest concentration of phytochemical recorded was flavonoids (126.56±0.01 mg/ml) and this was significantly different from phenolics (45.01±0.03 mg/ml) that followed. The phytochemical, soluble carbohydrate showed the least concentration (0.00±0.00 mg/ml).

Antimicrobial activity

Both Gram positive bacteria tested were resistant to the AgNPs. On the other hand, *P. aeruginosa* (figure 9) and *A. flavus* showed susceptibility as seen by a zone of inhibition (ZOI) indicated by red arrows. However, the susceptibility was only observed at the higher concentrations tested (25 mg/ml and 12.5mg/ml). All the test organisms were resistant to the *M. lucida* plant extract (control). Only *B. cereus* showed susceptibility to $AgNO_3$ (control).

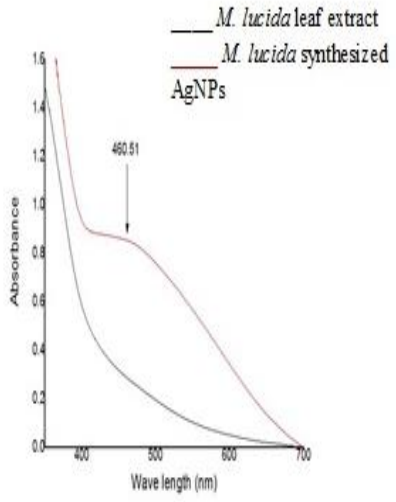

Figure 3: UV-vis Absorption Spectra of *M. lucida* AgNPs

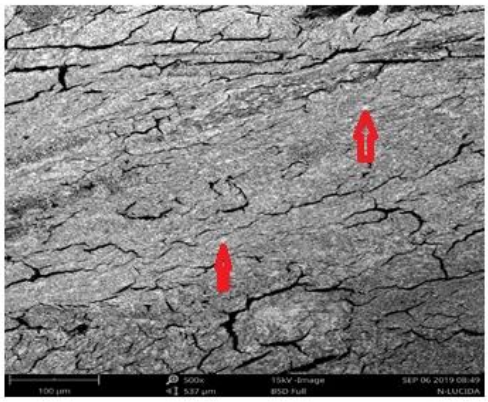

Figure 4: SEM micrograph of *M. lucida* AgNPs

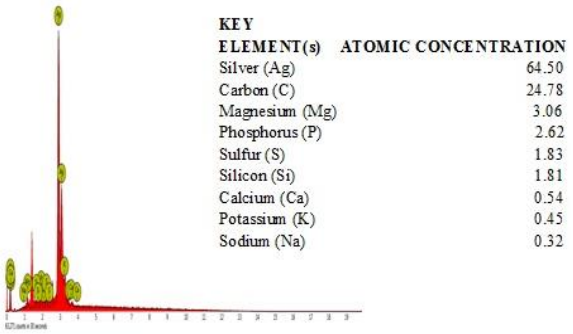

Figure 5: SEM-EDX representation of elements present in *M. lucida* AgNPs

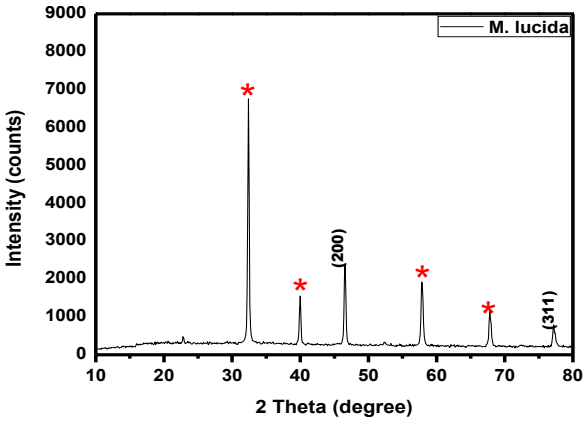

Figure 6: XRD diffractogram of *M. lucida* AgNPs

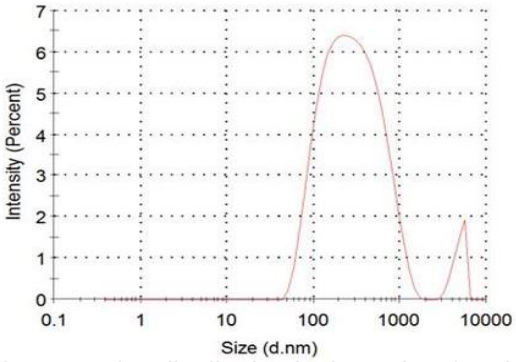

Figure 7: Size distribution by intensity of *M. lucida* AgNPs

Figure 8: FTIR spectra showing peaks representing various functional groups present in *M. lucida* AgNPs and *M. lucida* leaf powder

Table 2: FTIR Spectra of *M. lucida* leaf powder and its AgNPs with their Spectra

Type of bond	*M. lucida* leaf powder (cm^{-1})	*M. lucida* AgNPs (cm^{-1})	Compound name
Broad N-H stretch	3280.0	-	aliphatic primary amine
Sharp medium C-H stretch/Weak O-H stretch	2917.5, 2851.4	3137.9,2924.0	alkane/alcohol
Weak C≡C stretch	2125.5	2115.0	di and mono substituted alkyne
Medium C=C stretch/ medium N-H bending	1604.1	1649.3	cyclic and conjugated alkene/ amine and amide groups
Medium O-H bending	1313.7	1397.7	phenol
Medium C-N stretching	1237.0	1078.4	amine
Medium C=C bending	778.9	3137.9	alkene

Table 3: Quantitative Phytochemical Analysis of *M. lucida* leaves

S/no	Phytochemical(s)	Concentration in mg/100ml
1	phenolics	45.01 ± 0.03^{b}
2	tannins	0.03 ± 0.00^{h}
3	flavonoids	126.56 ± 0.01^{a}
4	hydrogen cyanide	0.004 ± 0.00^{i}
5	reducing sugars	20.23 ± 0.00^{c}
6	steroids	0.17 ± 0.00^{g}
7	saponins	0.34 ± 0.01^{f}
8	soluble carbohydrate	0.00 ± 0.00j
9	terpenoids	9.54 ± 0.01^{d}
10	alkaloids	5.74 ± 0.01^{e}

Means with different alphabets in each column are significantly different from each other by DMRT (P≤0.05)

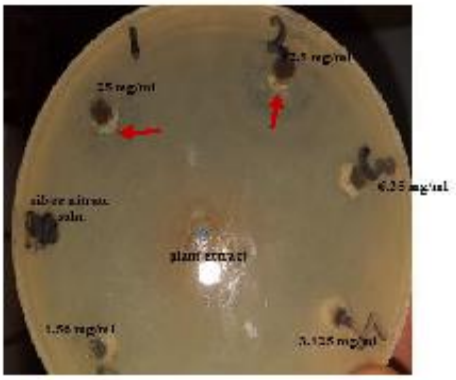

Figure 9: Effect of *M. lucida* AgNPs on *P. aeruginosa* (showing ZOI)

Table 4: Antimicrobial activity of *M. lucida* AgNPs

Treatments/Concentrations	Test Organisms					
	B. cereus	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. flavus*	*A. niger*
	Mean ZOI (mm)					
25 mg/ml	-	-	-	11.33±0.72[b]	7.33±0.78[a]	-
12.5 mg/ml	-	-	-	15.33±0.14[a]	-	-
6.25 mg/ml	-	-	-	-	-	-
3.125 mg/ml	-	-	-	-	-	-
1.56 mg/ml	-	-	-	-	-	-
Control ($AgNO_3$)	20.00±2.64[a]	-	-	-	-	-
Control (*M. lucida* leaf extract)	-	-	-	-	-	-

Data are presented with means ± standard error. Means with different alphabets in each column are significantly different from each other by DMRT (P≤0.05)

DISCUSSION

The visual colour change observed on mixing the *M. lucida* aqueous leaf extract with the $AgNO_3$ solution is similar to reports by Ajayi *et al*. (2017) who synthesized AgNPs with stem extract of *Sarcocephalus latifolus* also belonging to the family Rubiaceae. This colour change indicating AgNP synthesis was further validated using UV-vis spectroscopy and an absorption peak at 460 51 nm was observed. Similar absorption peaks were observed in AgNPs synthesized using plant extracts of *Saccharum officinarum* (Paulkumar *et al*., 2017) and *Acacia cyanophylla* (Jalab *et al*., 2021). The absorption peak usually arises due to surface plasmon resonance (SPR), wherein the free electrons of the metallic nanoparticles oscillate in the presence of an electromagnetic field of the light (Kelly *et al*., 2002; Reddy *et al*., 2020). The irregularly shaped AgNPs observed from the SEM micrographs are common occurrences with plant mediated synthesis and are consistent with reports by (Jagtap and Bapat, 2013; Tak *et al*., 2015; Jesse and Jasvin, 2018). The EDX analysis clearly identified a distinct silver peak showing that it was the predominant element, and a further confirmation of the formation of AgNPs. Nowadays, EDX detectors are integrated with SEM machines and enable users to detect both the type of elements present and the percentage concentration of each

element within the sample (Nanakoudis, 2019). The observance of distinct peaks from XRD analysis confirms the crystalline nature of the AgNPs with an FCC (face cubic centre) lattice structure. The asterisked peaks seen in the diffractogram are assumed to be due to the presence of phytochemical constituents on the surface of the AgNPs (Shaik *et al*., 2018).

Dynamic light scattering is an analytical technique applicable in nanoparticle studies for the measurement of particle size distribution, average particle diameter (Z average), zeta potential and polydispersity index (PDI), important parameters when considering nanoparticles for application as drug delivery systems (Zasshi, 2019). The polydispersity index obtained from DLS analysis of the *M. lucida* synthesized AgNPs indicate that they could be suitable for medical applications. Many factors have been attributed for the antimicrobial activity of AgNPs and nanoparticles in general. Some of which include; difference in bacterial cell wall composition in which Gram-negative bacteria have a thinner cell wall while Gram positive bacteria has a thicker cell wall. Concentration-dependency may be another factor to consider as most of the AgNPs that had minimal activity exhibited those activities at the highest concentrations tested. Also, ZOI increased in a concentration-dependent manner. This is in agreement with various works available in literature that ascribe concentration-dependency as a factor that influenced the activity of their plant-mediated AgNPs (Skandalis *et al*., 2017; Sharma *et al*., 2018; Shehzad *et al*., 2018; Masum *et al*., 2019). These factors are in agreement with the findings of this study because *P. aeruginosa*, a Gram-negative bacteria showed susceptibility only at the highest concentration/dose tested. Also, the reported crystalline size of the *M. lucida* AgNPs may have hindered its ability to act as the surface area to volume ratio of these particles are said to increase as the particles size decreases. The smaller sized particles are thought to have larger surfaces and therefore release more silver ions resulting in better penetration and toxicity (Wiley *et al*., 2005; Salomoni *et al*., 2015). The negligible antifungal activity could be ascribed to the deactivation of sulfhydryl group on the *A. flavus* cell wall, precipitation of insoluble cellular substances, interference with membrane enzymes which lead to cell lysis and death (Ajitha *et al*., 2015; Xia *et al*., 2016).

CONCLUSION

The study shows that the aqueous leaf extract of *Morinda lucida* can effectively be used for the eco-friendly synthesis of silver nanoparticles. Its recorded crystalline size of 62.20 nm is well within the prescribed range for nanoparticles. Its polydispersity index (PDI) of 0.4 indicates that it could be used in medical applications. The *M. lucida* AgNPs also showed antimicrobial activity against *P. aeruginosa* and *A. flavus* signifying that they could be used as antimicrobial agents. The possibility of obtaining differences in results due to variations in the phytochemical composition of extracts from plants of the same species from diverse regions is a limiting factor applicable to this study. Research involving individual phytochemicals is needed to evaluate whether synthesis of the AgNPs can be due to the action of a particular phytochemical or due to a synergy of them. *M. lucida* leaf extract can also be experimented with for the production of nanoparticles other than silver.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

ESN conceived the study, performed the experiment/validation and wrote the first draft
Of the manuscript. CCK provided resources and performed experiment and edited the draft. UOS was involved in conceiving and supervising the study. All authors approved the final draft of the manuscript.

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