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Original Research Article

Green Synthesis of Silver Nanoparticles Using *Cola nitida* Nut Extract (Vent.) Schott & Endl. (Malvaceae), Characterization and the Determination of their Antimicrobial Activity

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ABSTRACT

Pathogenic microorganisms have developed resistance to existing antibiotics at an alarming rate; this has encouraged researchers to make a shift to the development of new drugs. One of the new developments used is the synthesis of silver nanoparticles (AgNPs). The aim of this study was to characterize and evaluate the antimicrobial activity of silver nanoparticle synthesized using *Cola nitida* nut extract. The AgNPs were synthesized by mixing 150 mL of silver nitrate solution (1 mM) with 750 mL of aqueous extract of *Cola nitida* nut at room temperature. Characterization of the synthesized AgNPs was done by UV-VIS, FTIR and particle size analysis. AgNPs were screened against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans and Aspergillus niger*. The result of the UV-VIS analysis showed the maximum absorbance wavelength of 439 nm at 4.5 h while FTIR showed the presence of functional groups such as alcohols and amides in the AgNPs. The particle size analysis showed that the average particle size was 227.2 nm. The antimicrobial screening showed that the AgNPs have activity against *P. aeruginosa* and *B. subtilis* with inhibition zone diameter of 8 mm and 6 mm respectively. The minimum inhibitory concentration (MIC) against *S. typhi, S. aureus* and *C. albicans* was 9 µg/mL for each of the organisms. The MIC for each of *E. coli* and *A. niger* was 10 µg/mL while that of *B. subtilis* was 7 µg/mL. The synthesized silver nanoparticles were found to be stable and effective against the microorganisms.

Keywords: Silver nanoparticles, Green synthesis, *Cola nitida*, Ultraviolet-Visible spectrophotometer, FTIR.

Introduction

Nanoparticles are generally described as small particles that measures around 1 nm to 100 nm in size. Silver nanoparticles are the most extensively studied nanomaterial.[1] The large surface area of silver nanoparticles allows them to be in better contact with microorganisms, thereby impacting good antimicrobial activity.[2] Biological means of nanoparticle synthesis using plants (also known as green synthesis) is the most widely acknowledged way of nanoparticle synthesis because of the plentiful and diversified secondary metabolites present in plant extracts. These metabolites function as bioactive compounds and are capable of acting both as reducing and capping agents, thus eliminating the need to add any further chemical agent to synthesize the nanoparticles.[2-5]

Cola nitida is the nut of a tropical tree of African origin and belongs to the family Malvaceae with the common name kola nut.[6] Each fruit contains between two and five kola nuts. About the size of a chestnut, kola nut contains about 2-4 % of caffeine and theobromine, as well as tannins, alkaloids, saponins and flavonoids.[7]

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The plant is important because of its nut that has important pharmacological properties.[8] With the advent of modern medicine used in the treatment of infections, abusive and often uncontrolled use of antibiotics brings up a phenomenon of resistance in most bacteria and fungi. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases and one approach is to screen local medicinal plants for possible antimicrobial properties. This research is therefore essential to determine if silver nanoparticles synthesized using *Cola nitida* nut extract will affect microbial activities. The aim of this study was to synthesize silver nanoparticles using *Cola nitida* nut extract and to characterize the silver nanoparticles and evaluate their antimicrobial activity.

Materials and Methods

Plant materials

The nuts of *Cola nitida* plant were collected from Ukana in Udi Local Government Area of Enugu State, Nigeria in September, 2020 and were authenticated by Mr. Felix Nwafor, a botanist of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka. The voucher number PCG/UNN/0040 was deposited in the herbarium for further reference.

Extraction

The nut of *Cola nitida* was collected and dried for 7 days, and then milled using mechanical grinder. A certain quantity (20 g) of the powdered plant material was weighed using an analytical balance and added to 400 mL of distilled water in a beaker and boiled in a water bath for 30 min at 100ºC. The mixture was allowed to cool and then filtered using a filter paper. The final concentration of the aqueous extract was determined to be 5% w/v.

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Green Synthesis of Silver Nanoparticles (AgNPs)
Silver nitrate (0.1698 g) was weighed and dissolved with distilled water in a 50 mL beaker, it was then transferred to a 1000 mL volumetric flask where the volume of the solution was made up to the 1000 ml mark of the volumetric flask to produce a final concentration of 1 mM. Then, 150 mL (5% w/v) of the plant extract was mixed with 750 mL of silver nitrate solution. The reaction was incubated at 25ºC. The reactions were carried out in the dark. The obtained solution was centrifuged for 30 min at 5000 rpm. After centrifuging, the supernatant was discarded and the nanoparticles were rinsed with distilled water.

Characterization of the Silver Nanoparticles (AgNPs)
Ultraviolet – Visible Spectrophotometer (UV-Vis)
The UV-Vis spectral analysis of the AgNPs was done using UV-VIS spectrophotometer (JENWAY 6705) at different times at 30 min interval for 6 h. The UV-Vis analysis was recorded at wavelength ranging from 300-800 nm. This analysis was done to monitor the reaction mixture for the synthesis of the silver nanoparticles.

Fourier Transform Infrared (FTIR)
The FTIR analysis was carried out using Shimadzu FTIR Spectrophotometer (FTIR-8400S) to characterize the synthesized silver nanoparticles. The FTIR spectrum was generated at the wave number ranging from 4000-500 cm^{-1}.

Particle Size Analysis
The particle size analysis was carried out using dynamic light scattering (DLS) with Malvern machine (version 2.2).

Antimicrobial Evaluation
The antimicrobial activity was evaluated against the following five (5) micro-organisms (*Escherichia coli, Aspergillus niger, Staphylococcus aureus, Bacillus subtilis, and Candida albicans)*. Agar dilution was employed for the analysis. Briefly, 10 concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/mL) of the AgNPs were prepared and poured into the hot agar in the plates. These were allowed to cool. The surface of each agar was marked out in five quadrants and numbered as 1 to 5. Each of the five microorganisms was streaked on a separate quadrant in each plate. The plates were incubated for 24 h. The minimum inhibitory concentration (MIC) was determined for each microorganism as the least concentration that inhibited the growth of the particular organism.

Results and Discussion

The various figures (Fig. 1 to Fig. 6) show the results of UV-Vis analysis of the synthesized silver nanoparticles for various hours (1 h to 4.5 h) after mixing the $AgNO_3$ and the *Cola nitida* nut extract. These results were combined in Fig. 7. The maximum wavelength ranges from 424 nm to 439 nm at various time intervals (1 h to 4.5 h). From this combined absorbance spectra, it was observed that, the maximum absorbance wavelength was 439 nm; the wavelength of absorption increased as time increased (from 1 h to 4.5 h). The UV-Vis spectral study was used to monitor the formation and the stability of the silver nanoparticles. It was observed that the mixture of the silver nitrate ($AgNO_3$) solution and *Cola nitida* nut extract turned from light brown to dark brown and then to dark grey colour after 1 h. This is due to surface plasmon vibration,[9] which indicates the reduction of Ag^+ to Ag^0 and the presence of silver nanoparticles (AgNPs). Different absorbance wavelength was observed at different time intervals (Fig. 7). An absorbance wavelength of 431 nm was observed at 1 h, 3 h and 4 h (Fig.1, 3 and 4); this absorbance wavelength is similar to previous studies where AgNPs synthesized using *Tithonia diversifolia* had a maximum wavelength of absorption of 430 nm at 90 min.[10] Other literature where *Agaricus spp* of wild mushrooms was used to synthesize AgNPs had its absorbance of 432 nm at 60 min.[11] A study by Soares *et al.,* stated that the variations in the value of the absorbance are an indication of the changes in particle size.[12]

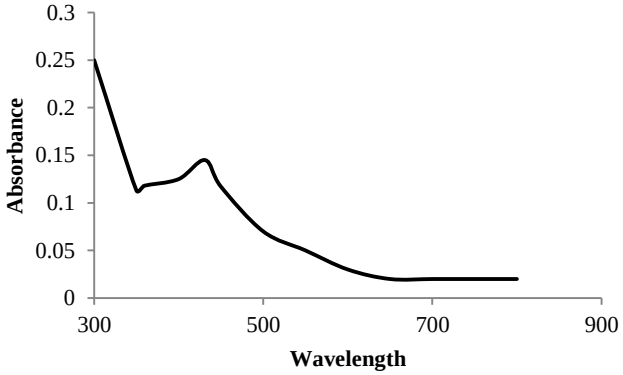

Figure 1: UV - VIS Spectrum at 1 h

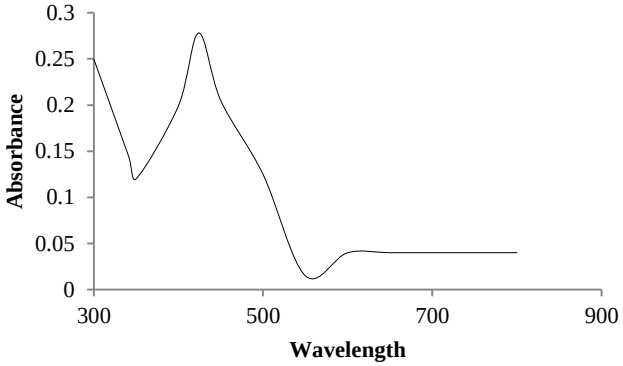

Figure 2: UV - VIS Spectrum at 1.5 h

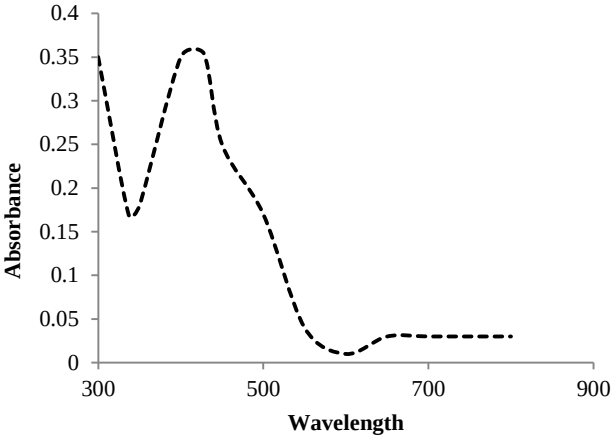

Figure 3: UV - VIS Spectrum at 2 h

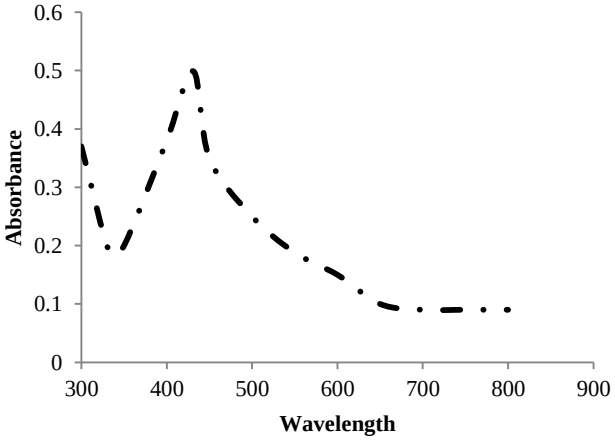

Figure 4: UV - VIS Spectrum at 3 h

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This confirms that the constant wavelength of 431 nm at 1 h, 3 h and 4 h is indicative of the same AgNPs being produced at these times. An absorbance wavelength of 424 nm which was observed at 1.5 h (Fig. 2) was also seen in previous studies where synthesized AgNPs (using *Caesalpinia ferrea* extract) had a maximum absorbance wavelength of 425 at 24 h.[12] Also, an absorbance wavelength of 428 nm was observed at 4 h (Fig.5) which was similarly observed in previous study (AgNPs synthesized from *Rhazya stricta* leaf extract) at 2 h.[13] The decrease in absorption spectra which was observed as time increased from 431 nm at 1 h – 424 nm at 1.5 h (Fig.7) was as a result of the blue shift of the absorbance resulting in the reduction of larger particle size to smaller particle size.[14, 15] The maximum absorbance which was 439 nm at 4.5 h (Fig. 6) without any observable increase in absorbance is an indication that all the Ag^+ has been reduced to Ag^0 and that the UV-VIS spectral peak had become stable suggesting the completion of the *Cola nitida* silver nanoparticles synthesis. It was observed that the reaction between the $AgNO_3$ and the *Cola nitida* nut extract was not instantaneous because there was no observable peak 30 min after mixing the $AgNO_3$ and the *Cola nitida* nut extract.

The FTIR analysis (Table 1) was used to confirm the dual role of the *Cola nitida* nut extract as a reducing and capping agent and the presence of the functional groups that help in performing these roles in the synthesis of the silver nanoparticles. The peak shifting in the AgNPs spectral profile could be attributed to the interactions between those chemical functional groups and the AgNPs.[16] The absorption peak at 3441.12 cm^{-1}, 3626.2 cm^{-1}, 3695.73 cm^{-1} and 3857.76 cm^{-1} was due to the OH functional groups of flavonoids, polyphenols and alcohols which are the phytochemicals acting as reducing and capping agents. This was seen in similar studies[17,18] having similar peaks. The absorption peak at 1041.6 cm^{-1}, 1126.47 cm^{-1} was due to C – O bond of functional groups which were esters, alcohols and carboxylic acid contained in flavonoids and terpenoids. This corresponds with previous studies by Banjeer *et al.*[19] and Elangovan *et al.*[20] having similar results. The absorption peak at 1735.99 cm^{-1} was due to C = O bond with functional groups ketones and aldehydes and this corresponds with previous studies having similar results.[21] The absorption peak at 1671.25 cm^{-1}, 1627.99 cm^{-1} was due to C = C bond, the absorption peak at 1465.95 cm^{-1} was due to C – H bond and the absorption peak at 3857.76 cm^{-1} was due to C ≡ C and they all revealed the presence of aromatic compounds, alkenes, alkanes and alkynes respectively, which were in line with previous studies by Satyavani *et al.*[22] The absorption peak at 1373.36 cm^{-1}, 1512.24 cm^{-1} and 1627.97 cm^{-1} are indicative of C – N stretching in amides.[23] The occurrence of these absorption peaks in the FTIR spectrum evidently indicates the dual role of *Cola nitida* nut extract as a reducing and capping agent on the synthesized silver nanoparticles.

The particle size analysis was used to determine the size distribution of the silver nanoparticles. From the results it was observed that some of the silver nanoparticles were within the nano range (1 – 100) while majority were out of the range (Fig. 8). The Z-average which is the intensity weighed mean of the hydrodynamic size of collection of particles measured by dynamic light scattering was 227.2 nm. The large size of the silver nanoparticle could be due to the agglomeration of the silver nanoparticle with time. The poly dispersity index of the silver nanoparticle was 0.412. The poly dispersity index was used to measure the homogeneity of the silver nanoparticle which ranges from 0.0 – 1.0.[24] These results are similar with those of previous studies by Ezealisiji *et al.* on *Annona muricata* root bark having an average particle size of 392.10 nm and poly dispersity index of 0.44.

Table 2 shows the results of the antimicrobial studies. Silver nitrate was used as positive control because of its well documented antibacterial effects.[25] The silver nitrate solution was effective against both Gram positive bacteria (*Staphylococcus aeureus* with 7 mm inhibition zone diameter) and Gram-negative bacteria (*Escherichia coli* – 7 mm; *Pseudomonas aeruginosa* – 7 mm) (Table 2). The synthesized AgNPs inhibited the growth of the microorganism at a minimum concentration of; 9 µg/ml (*S. typhi*), 10 µg/ml (*E.coli*), 7 µg/ml (*B. subtilis*), 9 µg/ml (*S. aureus*), 9 µg/ml (*C. albicans*), 10 µg/ml (*A. niger*). *B. subtilis* was the microorganism that was inhibited with the least minimum inhibitory concentration out of the microorganisms used.

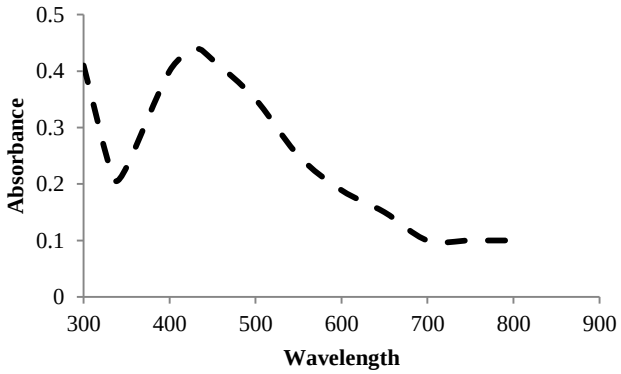

Figure 5: UV - VIS Spectrum at 4 h

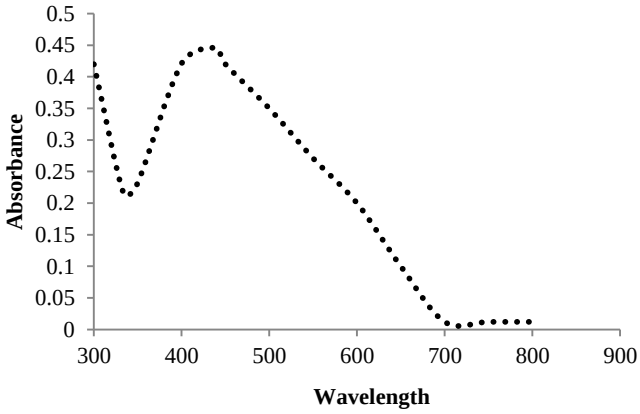

Figure 6: UV - VIS Spectrum at 4.5h

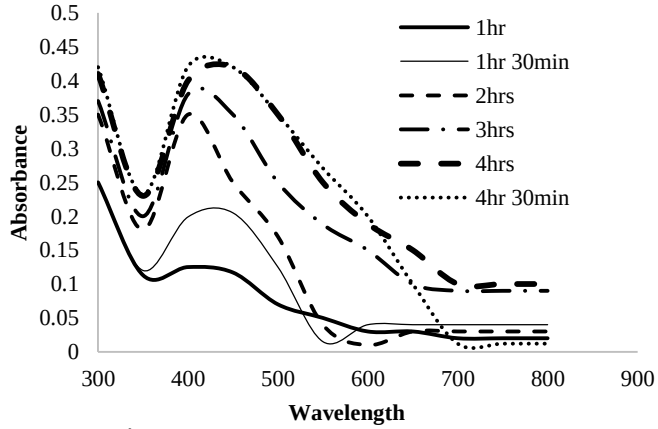

Figure 7: Combined UV -VIS Spectra at Different Times

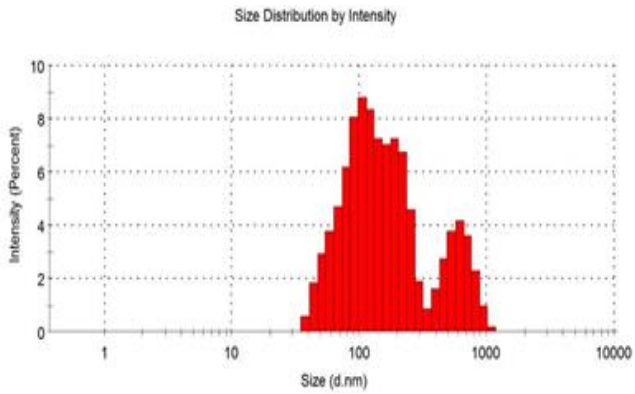

Figure 8: Particle size analysis of the synthesized silver nanoparticle of *Cola nitida*.

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Table 1: Results of FTIR analysis of the synthesized silver nanoparticle of *Cola nitida*

S/N	Absorption Frequency (cm^{-1})	Area	Intensity	Strength of intensity	Possible bonds	Possible functional groups
1	1671.25	0.217	0.894	Weak	C = C	Alkene
2	748.41	0.18	0.933	Weak	C – Cl	Halo compound
3	1041.6	7.041	18.432	Strong	C – O	Alcohol, ester carboxylic acid
4	1126.47	0.028	0.278	Weak	C – O	Alcohol, ester, carboxylic acid
5	1373.36	1.301	3.119	Medium	C – N	Amide
6	1465.95	0.005	0.152	Weak	C – H	Alkane
7	1512.24	0.064	0.271	Weak S	C – N	Amide
8	1627.97	0.298	1.274	Medium	C – N	Amide
9	1735.99	0.067	0.702	Weak	C = O	Ketone, aldehyde
10	2067.76	0.154	0.49	Weak	C ≡ C	Alkyne
11	2399.53	0.000	0.033	Weak	C – H	Alkane
12	2947.33	3.333	7.353	Strong	C – H	Alkane
13	3441.12	2.297	2.219	Medium	O – H	Alcohol
14	3626.29	0.002	0.035	Weak	O – H	Alcohol
15	3695.73	0.331	1.634	Medium	O – H	Alcohol
16	3857.76	0.403	1.402	Medium	O – H	Alcohol

Table 2: Results of the MIC of AgNPs against the various organisms

S/N	Microorganisms	Minimum Inhibitory Concentration (µg/mL)
1.	*Salmonella typhi*	9.0
2.	*Escherichia coli*	10.0
3.	*Bacillus subtilis*	7.0
4.	*Staphylococcus aureus*	9.0
5.	*Candida albicans*	9.0
6.	*Aspergillus niger*	10.0

This could be as a result of thin layer of the peptidoglycan cell wall of *B. subtilis* that allows the easy penetration of the silver nanoparticles (AgNPs).[26] The activity of the AgNPs was suggested to be more effective against the Gram-negative bacteria than the Gram positive, due to the difference in the structure of their cell wall.[27] *P. aeruginosa* is composed of a thin layer of peptidoglycan cell wall making it easier for the AgNPs to penetrate the cell wall but *B. subtilis* possesses a thick peptidoglycan cell wall. This explains why *P. aeruginosa* had a wider inhibition zone diameter than *B. subtilis*.

Conclusion

The synthesized silver nanoparticles of *Cola nitida* nut extract showed an effective antimicrobial property. The FTIR analysis showed the presence of the functional groups from *Cola nitida* nut extract which played a dual role as a reducing and stabilizing agent. The particle size analysis showed that the silver nanoparticles were within the nano range and that it was fairly homogenous which made it a suitable pharmaceutical product.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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