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Synthesis, Characterization and Antibacterial Activity Studies of Eco-Friendly Silver Nanoparticles from the Leaf Extract of *Jatropha Curcas*

Ogbogo, I. O[1*], Ikyenge, B. A[1], Ishwah, B[1], Weor, T. T[1], Adoga, S. O[1]

[1]Department of Chemistry, Benue State University, Makurdi, Nigeria

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***Corresponding author:** Ogbogo, I. O
Department of Chemistry, Benue State University, Makurdi, Nigeria

Abstract

Production of environmentally amenable silver nanoparticles (AgNPs) has earned the interest of the scientific community owing to their broad applications, primarily in the field of optronics, sensing, and extensively in pharmaceuticals as promising antioxidant, antimicrobial and anticancer agents. Conventionally silver nanoparticles are synthesized by a chemical method using chemicals as reducing agents which later become accountable for various biological risks due to their general toxicity; engendering the serious concern to develop environment-friendly processes. This study explored the production of eco-friendly AgNPs and the investigation of their antibacterial activity using ethanolic leaf extract of *Jatropha curcas* (LEJC) as the reducing agent and aqueous silver nitrate as the precursor. The characteristics of the synthesized LEJC-AgNPs were studied by ultraviolet-visible spectroscopy (UV-Vis), Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM). The LEJC-AgNPs formation was observed from the colour change of the mixture from dark-yellow to colloidal brown. A distinctive absorption maximum with surface Plasmon resonance at 425 nm confirmed the formation of LEJC-AgNPs and data on SEM analysis have shown that the synthesized nanoparticles were in the nano range and predominantly irregular and spherical in shape. FTIR identified the functional groups present in the extract for the formation of the LEJC-AgNPs. This green synthesis provides an economic, eco-friendly, and clean synthetic route to AgNPs. The assessed antibacterial activity of the LEJC-AgNPs obtained depicts activity against *Staphylococcus aureus* (Gram-positive), *Escherichia coli* and *Klebsiella Pneumonia* (Gram-negative) at 20, 40, and 80 mg/mL respectively. This data is reflective of the role of LEJC-AgNPs as a potential and promising antimicrobial agent against bacterial infections.

Keywords: Green synthesis; Silver nanoparticles; Leave extract; Precursor; Antimicrobial.

1.0 INTRODUCTION

Nanotechnology today is regarded as a revolutionary technology that deals with matter at the nanoscale (1-100 nm). Within this size range, all the properties (chemical, physical and biological) change in fundamental ways of both individual atoms/molecules and their corresponding bulk. Novel applications of nanoparticles and nanomaterials are growing rapidly on various fronts due to their completely new or enhanced properties based on size, distribution, and morphology. Nanotechnology is emerging as the sixth revolutionary technology in the current era. It is now an emerging and fast-growing field of science that is being exploited over a wide range of disciplines such as physics, chemistry, biology, material science, electronics, medicine, energy, environment, and health sectors. The nanoparticles are used for all the aforesaid purposes; the metallic nanoparticles are considered the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio. Amongst the noble metal nanoparticles, silver nanoparticles are significant and have gained boundless interest because of their unique properties such as chemical stability, good conductivity, catalytic, and most important antimicrobial, and anti-inflammatory activities (Liu and Lin 2004; Vorobyova *et al*., 1999). Silver's mode of action is presumed to be dependent on Ag+ ions, which strongly inhibit bacterial growth through suppression of respiratory enzyme and electron transport components and through interference with DNA functions (Bae *et al*., 2002).

Because of their wide range of applications, the synthesis of silver nanoparticles is of much interest

to the researcher. Generally, nanoparticles are prepared by a variety of chemical and physical methods which are quite expensive and potentially hazardous (Mandal *et al.,* 2000; Basavaraja *et al.,* 2008). Eco-compatible pathways for nanoparticles, scientists used microorganisms (He *et al.,* 2007; Kowshik *et al.,* 2008) and plant extracts (Song and Kim 2009; Krishmaraj *et al.,* 2010). Green synthesis of nanoparticles has proven to be better method due to slower kinetics, offers better manipulation, control over crystal growth, and their stabilization. Greener synthesis provides advancement over traditionally used nanoparticles synthesis methods i.e. chemical (Dwivedi and Gopa, 2010; Deby *et al.,* 2010) and physical method as it is cost-effective, easily scaled up, environment-friendly (Geethalakshmi and Sarada, 2010) or large scale synthesis and in this method, there is no need to use toxic chemicals. Green synthesis of nanoparticles is a bottom-up approach where the main reaction occurring is reduction. Biogenic synthesis is useful not only because of its reduced environmental impact (Ahmad *et al.,* 2010; Nabikhan *et al.,* 2010) compared with some of the physicochemical production methods, but also because it can be used to produce large quantities of nanoparticles that are free of contamination and have a well-defined size and morphology [Vidhu *et al.,* 2011). Biosynthetic routes can actually provide nanoparticles of a better-defined size and morphology than some of the physicochemical methods of production (Singhal *et al.,* 2011). The methods for obtaining nanoparticles using naturally occurring reagents such as vitamins, sugars, plant extracts, biodegradable polymers, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology. But among the above-mentioned reagents, plant extract using leaf, root, stem, latex, resin, and seed seems to be the best candidates as they are suitable for large-scale "Green synthesis" of nanoparticles. The advancement of green syntheses over chemical and physical methods is environment-friendly, cost-effective and easily scaled up for large-scale syntheses of nanoparticles. Furthermore, there is no need to use high temperatures, pressure, energy, and toxic chemicals (Veerasamy *et al.,* 2011). Although, among the various biological methods of silver nanoparticle synthesis, microbe-mediated synthesis is not of industrial feasibility due to the requirements of highly aseptic conditions and their maintenance. Therefore, the use of plant extracts for this purpose is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard, and the elaborate process of maintaining cell cultures (Yilmaz *et al.,* 2011).

Jatropha curcas is a popular semi-evergreen shrub or small tree belonging to the family Euphorbiaceae and can grow up to 8 meters high or more in a favourable condition. It is considered one of the most important sources of medicine as it contains derived compounds (Phytochemicals) that have attracted much interest as natural alternatives to synthetic compounds (Richard *et al.,* 2016). It possesses effective biological and immunological activities and uses for the treatment of various human and veterinary ailments. Traditionally the plant is used for treating stomach ache, dysentery and diarrhea. It is also used for skin diseases, rheumatism, and for sores on domestic livestock (Asogwa *et al.,* 2016). The white latex serves as a disinfectant in mouth infections in children. The leaves have been reportedly used against malaria and muscular pains. The seed of *Jatropha curcas* or expressed oil have been used medicinally as a purgative and as remedy against syphilis. Previous works have shown that many *Jatropha* species possess antimicrobial and antifungal activity (Asogwa *et al.,* 2015). Hence, in the present study owing to the presence of phytochemicals, the NPs synthesized from *J. curcas* have been hypothesized to play role as an effective antimicrobial tool. Moreover, the production of AgNPs mediated from *J. curcas* need to be evaluated for antimicrobial activity to provide a green alternative to other chemically synthesized AgNPs. The study thus aimed to produce silver nanoparticles from *J. curcas* leaf extract by green chemistry process, an alternative eco-friendly approach for its potential applications.

2.0 MATERIALS AND METHODS

2.1 Materials

All reagents and solvents used were of analytical grade and were prepared according to standard methods.

Methods

2.2 Preparation of *Jatropha curcas* leaf extract

Fresh leaves, (Plate 1) were collected from *Jatropha curcas* tree at Adum-East Ito in Obi Local Government Area (LGA) of Benue State, Nigeria. The plant material was identified in the Herbarium unit of the Department of Biological Sciences, Benue State University, Makurdi. The leaves were washed several times with distilled water to remove unwanted substances and then air-dried at room temperature for 14 days to remove the residual moisture. The dried leaves were ground into a coarse powder using a mortar and pestle. 100 g of the ground sample was measured into a clean container after which 400 mL of ethanol was added, the mixture was macerated for 24 hours. The mixture was filtered, and the filtrate collected was concentrated using a rotary evaporator. The concentrate was heated to dryness over a water bath at 80 ºC to obtain a solvent-free extract. The extract obtained was preserved in a desiccator to keep it dried and prevent contamination prior to the biosynthesis of silver nanoparticles from silver nitrate and other analysis.

Plate 1: Picture of *Jatrapha curcas* leaves

2.3 Phytochemical Analysis

The phytochemical analysis of the extract was performed using standard methods (Igoli *et al.*, 2005; Sharma *et al.*, 2012). The plant extract was screened for the presence of alkaloids, glycosides, flavonoids, tannins, phenolic compounds, saponins, terpenoids and steroids.

2.4 Biosynthesis of AgNPs

The ethanolic leaf extract of *Jatopha curcas* was used for the synthesis of AgNPs. 5 g of the dried extract was first dissolved in 50 mL of ethanol. 5 mL of 10 % ammonium solution and 10 mL of 1 M of $AgNP_3$ were measured into a 250 mL Erlenmeyer flask and the resultant mixture was mixed together. 20 mL of the plant extract was added, and the final volume was adjusted to 50 mL with ethanol. The resultant mixture in the Erlenmeyer flask was incubated at 38 ºC under agitation for 24 – 48 hours. A change in color from dark yellow to colloidal brown indicates the synthesis of silver nanoparticles (Nester *et al.*, 2008).

The reaction was carried out in a fume cupboard to avoid photo-activation of $AgNO_3$ at room temperature. The stable colloidal AgNPs obtained were evaporated, sealed, and stored properly for characterization.

2.5 Characterization technique

UV–Vis absorption spectra were measured using a (PerkinElmer 1.00.00) spectrophotometer. Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was done using a (model) SEM machine. Fourier transform infrared (FT-IR) spectra for the synthesized silver nanoparticles were obtained in the range 4,000 to 500 cm^{-1} with an (Shimadzu 8400S) spectrophotometer, by KBr pellet method.

2.6 Antibacterial Assay

Test organisms

One gram-positive bacterium (Staphylooccous aureus) and two gram-negative bacteria (Escherichia coli and Klebsiella pneumonia) were used in the antibacterial activity studies. The bacteria were clinical isolates obtained from the Microbiology Laboratory of the Benue State University Teaching Hospital, Makurdi, Benue State. The micro-organisms were maintained at 4 ºC on Nutrient Agar slant in the department of Microbiology, Benue State University, Makurdi and fresh subcultures were made before use.

2.7 Preparation of extract impregnated paper discs

The paper discs were prepared using a modified method described by [Ekundayo and Ezeogu, 2006]. Whatman No. 1 filter paper was cut into discs of 6 mm diameter using an office perforator. The discs were placed in a glass petri dish and sterilized in a hot air oven at 160 ºC for 1 hour. Each disc was impregnated with a 2 mL portion of stock solution of the AgNPs (80 mg/mL) and lower concentrations of 40 mg/mL and 20 mg/mL were obtained by serial dilution. The discs were dried in an incubator at 35 – 37 ºC for 2 hours. Discs of Tetracycline (4 mg/mL) and $AgNO_3$ (1 M) used as positive and negative control were similarly prepared.

2.8 Antimicrobial activity by zone inhibition

The antimicrobial activity of the synthesized AgNPs was assessed using the disc diffusion and broth dilution methods. A cell suspension of the test bacteria strains was prepared by transferring 4-5 isolated colonies on a nutrient agar plate into sterile normal saline in a bijou bottle. The turbidity was adjusted to McFarland turbidity standard tube No. 0.5 by adding sterile normal saline. The surface of the Nutrient Agar plate was inoculated by swabbing the surface with a sterile swab stick dipped into the bottle containing the standardized cell suspension. The prepared disc was aseptically transferred unto the inoculated culture plate using a pair of flame-sterilized forceps. The plates were incubated aerobically at 37 ºC for 18–24 hours. The diameter zone of inhibition was measured using a transparent plastic ruler. The tests were carried out in triplicates.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

Table 1: Description of *Jatropha curcas* leaf extract

Extract	Description
Mass (g)	12.808
% Yield (g)	12.808
State (%)	Solid
Color	Dark green

Table 2: Phytochemical Screening result of the ethanolic leaf extract of *Jatrapha curcas*

Phytochemicals	Inference
Alkaloids	+
Glycosides	+
Flavonoids	+
Tannins	+
Phenolic	+

Phytochemicals	Inference
Saponins	-
Terpenoids	+
Steroids	+

Key: + = present, - = Absent

The phytochemicals, which are secondary metabolites present in *Jatropha curcas* which includes alkaloids, glycosides, flavonoids, tannins, phenolic, saponins, terpenoids and steroids causes the bio-reduction reaction during the nanoparticles synthesis because the act as reducing and stabilizing agents (Dubey *et al.,* 2009).

UV–vis absorbance study

The addition of *Jatropha curcas* leaf extract to silver nitrate ($AgNO_3$) solution resulted in colour change of the solution from transparent to brown due to the production of silver nanoparticles. The colour changes occurred from the excitation of surface plasmon vibrations with the silver nanoparticles. The surface plasmon resonance (SPR) of silver nanoparticles produced a peak centered near 425 nm (Figure 2) indicating the reduction of silver nitrate into silver nanoparticles. AgNPs absorb radiation intensely at this wavelength due to the transition of electrons (Noginov *et al.,* 2006).

Table 3: Antibacterial activity result of AgNPs synthesized from the leaf extract at different concentrations

Bacteria	Zone of Inhibition (mm)		
	80 mg/mL	40 mg/mL	20 mg/mL
E. coli	10.33 ± 0.58	7.67± 0.58	3.00 ±1.00
K. pneumonia	13.00 ± 1.41	12.33 ± 1.69	6.33 ±1.24
S. aureus	9.67 ± 0.58	7.00 ± 1.00	5.00 ± 1.00

Mean values ± SD indicates the replicates of three experiments

Antibacterial Assays

Biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against gram-positive bacterium (*S.aureus*) and gram-negative bacteria (*E.coli and K.pneumonia*) by disc diffusion and broth dilution methods. It was observed (Table 3) that the synthesized silver nanoparticles significantly inhibited the growth of all the test organisms but there was an appreciable reduction in the zone of inhibition of all the bacteria treated with the various concentrations obtained by serial dilution from the stock solution. The trend of inhibitory zone of inhibition (ZOI) produced by the synthesized AgNPs showed that the gram-negative bacteria (K-pneumonia and E.coli) were more sensitive than gram-positive bacterium stain *S.aureus*). The highest ZOI was obtained with *K.pneumonia* to be 13.00 mm at 80 mg/mL, followed by 10.33 mm for *E.coli*, making them more sensitive than the 9.67 mm obtained for *S.aureus* at the same concentration. Similar results were obtained for the lower concentrations [Nester *et al*, 2008]. From this it can be inferred that higher concentrations of AgNPs have more antibacterial activity than lower concentrations. Also, from the results, it can be observed that the antibacterial sensitivity of the gram-positive *Staphylococcus aureus* was greatly lower than that of the gram-negative *K.pneumonia* and *E.coli.* This is possibly due to the thickness of the peptidoglycan layer of *Staphylococcus aureus* which protects against toxins and chemicals. The result showed that the negative control media (ethanol) has no zone of inhibition as the growths observed were very high and unhindered. It can also be inferred that the positive control medium (tetracycline) significantly inhibited the growth of bacteria. This upholds its current use as a potent antibiotic for the treatment of bacterial infection (Geethalakshmi and Sarada, 2010).

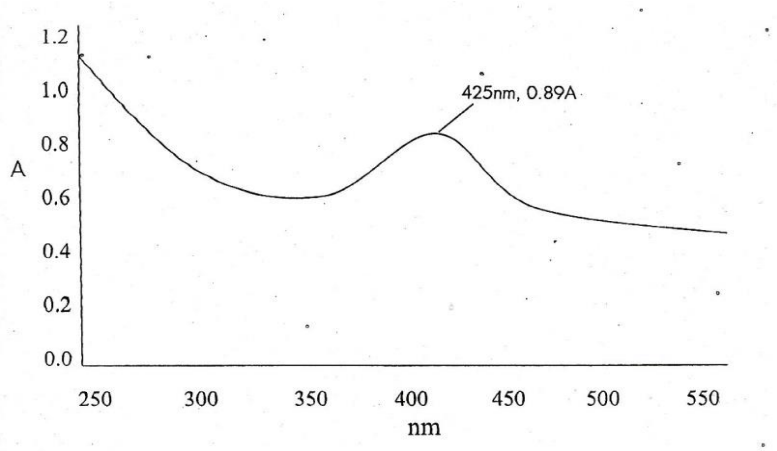

Figure 2: UV–Vis spectrum showing absorption of aqueous solution of silver nitrate with Jatropha leaf extract

FT-IR spectra of biosynthesized silver nanoparticles

Results of the FT-IR study of biosynthesized AgNPs showed sharp absorption peaks located at 3402.54, 2337.80, 2106.34, 1759.14, 1635.69, 1404.22, 1149.61 and 1049.31 cm^{-1} (Figure 3). The absorption peak at 3402.45 cm^{-1} is assigned to OH stretching in alcohols and *phenolic* compounds. The absorption peak at 2337.80 cm^{-1} C≡N medium strong stretching vibration, characteristics of nitriles and 2106.34 cm^{-1} corresponds to C≡C, characteristic alkynes. The peak at 1759.14 cm^{-1} corresponds to C=O stretch of esters. The absorption peak at 1635.69 cm^{-1} may be assigned to the amide I bond of proteins arising from carbonyl stretching in proteins, and the peak is close to that reported for native proteins, which suggests that proteins are interacting with biosynthesized silver nanoparticles and also their secondary structure was not affected during reaction with Ag^{+} ions or after binding with Ag^{0} nanoparticles. This FT-IR spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of a layer covering silver nanoparticles and acting as a capping agent to prevent agglomeration and provide stability to the medium. These results confirm the presence of possible proteins acting as reducing and stabilizing agents.

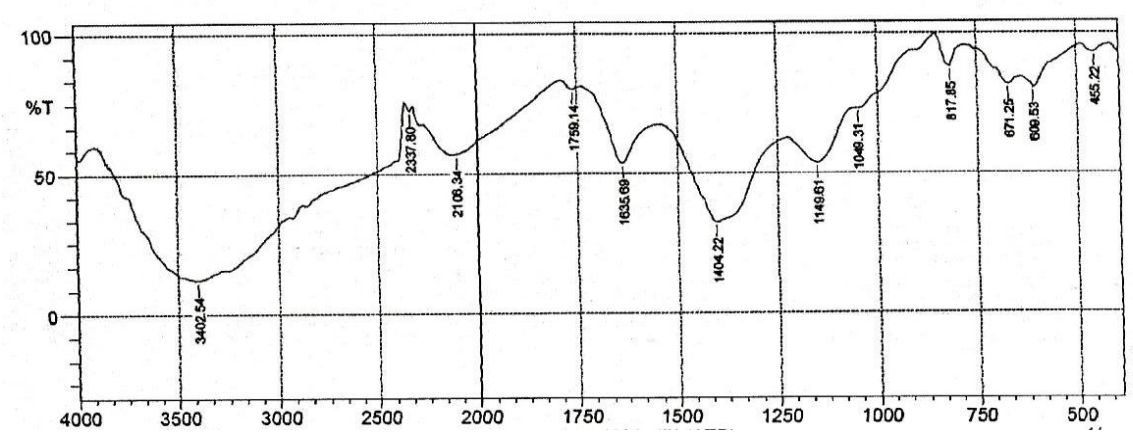

Figure 3: FT-IR of silver nanoparticles biosynthesized by Jatropha curcas leaf extract

SEM analysis of silver nanoparticles

The SEM images shows aggregation of crystalline morphology for the AgNPs with diameter range from 50 nm to 80 nm. This analysis of the bio reduced green synthesized AgNPs by SEM confirmed that they were in the nano range and of irregular and spherical shape.

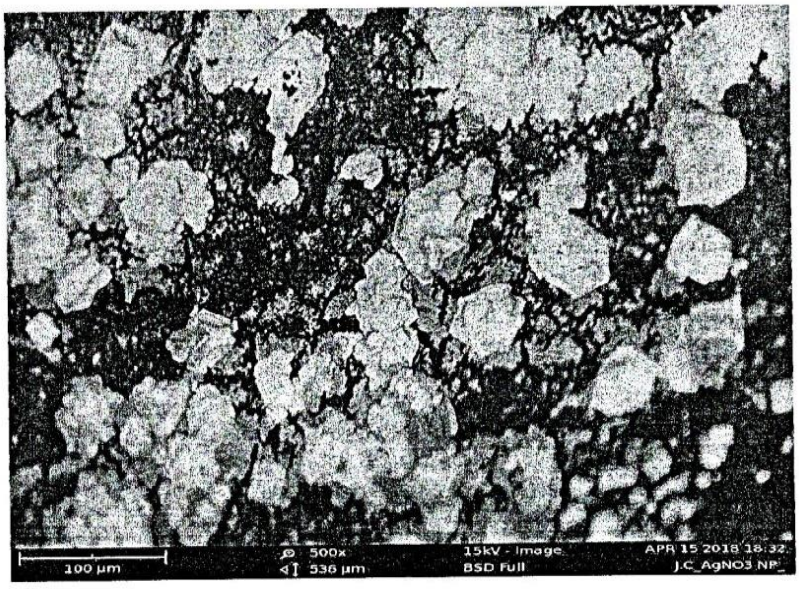

Figure 4: SEM image of silver nanoparticles synthesized by Jatropha curcas leaves extract

3.0 CONCLUSION

In this study, a fast, eco-friendly, and convenient green method for the synthesis of silver nanoparticles from silver nitrate using *Jatropha curcas* leaf extract was developed at ambient temperature. Spherical and irregular AgNPs of particle sizes ranging

from 50 to 80 nm with an average size of 65 nm were obtained. Colour changes occur due to surface plasmon resonance during the reaction with the ingredients present in the *Jatropha curcas* leaf extract resulting in the formation of silver nanoparticles, which is confirmed by UV–vis spectroscopy, FT-IR and SEM. FT-IR spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of a layer covering silver nanoparticles and acting as a capping agent to prevent agglomeration and provide stability to the medium, yet further research is needed in this area to explore the possible biomolecule responsible for the bio-reduction process. The antibacterial activity of biologically synthesized silver nanoparticles was potent against *S*.aureus, *E*.coli and *K*.pneumonia pathogen.

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