

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *Lagenaria breviflora* FRUIT AND ITS ANTIMICROBIAL APPLICATIONS

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ABSTRACT

Green synthesis of nanoparticles is a synthetic method that utilizes biological sources for their production. It is gaining attention due to its cost-effectiveness, eco-friendly nature, and compatibility with large-scale production. Herein is a detailed report on the green synthesis of AgNPs and evaluation of their antimicrobial activity. Aqueous extract of *Lagenaria breviflora* fruit was obtained via maceration and then screened to determine the presence of important phytochemicals. The synthesis of AgNPs was carried out in the dark to avoid photodegradation of silver nitrate solution. 0.005M AgNO₃ solution was added to aqueous extract of *Lagenaria breviflora* fruit in a ratio of 80:20. The mixture was maintained at 60°C for 6 hours under constant stirring. A color change was observed, indicating the formation of AgNPs; which was confirmed by a visual color change from light yellow to dark brown due to the phenomenon of surface plasmon resonance. The biosynthesized AgNPs were characterized using UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). SEM revealed the surface morphology and the average particle size of 73.20 nm, X-ray diffraction showed an average crystallite size of 12.83 nm in the range of 10-70°, FTIR revealed the presence of important functional groups responsible for reduction of silver ions and UV-Visible spectrophotometer showed maximum absorbance peak of 435 nm in the range of 300-700nm. The antibacterial activity of the synthesized AgNPs were tested against clinical pathogens including gram negative (*Escherichia coli* and *Salmonella typhi*) and gram positive (*Staphylococcus aureus*) using agar well diffusion method. The result showed that the green synthesized silver nanoparticles (AgNPs) have a potential to inhibit the growth of bacterial with inhibition zones diameter of 12mm, 11mm and 11mm observed for the tested organisms compare to the 19mm of the control (Ciprofloxacin). Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, terpenoids, saponins and carotenoids.

Keywords: Green synthesis, silver nanoparticles, *Lagenaria breviflora*, antimicrobial activity.

INTRODUCTION

The increasing awareness of environmental degradation and health risks associated with traditional chemical processes has enhanced the development of 'green' chemistry, a discipline dedicated to creating more sustainable and environmentally friendly chemical practices [1].

Green chemistry encompasses a set of principles aimed at minimizing the use and generation of hazardous substances. This breakthrough is not merely a reaction to environmental concerns; it represents a proactive approach that incorporates ecological considerations into the focus of

chemical research and development [2]; like the green synthesis of nanoparticles, which represents a transformative approach in the field of nanotechnology, emphasizing environmentally sustainable practices that leverage natural biological processes [3]. This methodology utilizes various biological entities, particularly plant extracts, to facilitate the synthesis of nanoparticles, offering a better alternative to conventional chemical methods that often involve hazardous reagents and complex procedures [4]. The inherent reducing and stabilizing properties of phytochemicals such as phenolic and terpenoids, in plants play a crucial role in the reduction of metal ions to nanoparticles, thereby enhancing the safety and efficacy of the synthesis process [5].

Recent advancements in green synthesis have highlighted its potential for producing a wide array of nanomaterials. Silver is one of the most widely used nano-materials in everyday applications; due to its unique role in bimolecular detection, catalysis, medicine, agriculture, biosensor and environmental remediation; it is been known to possess antimicrobial and anti-inflammatory properties making them suitable candidates for drug delivery and infection control [6]. There are various techniques available for the synthesis of silver nanoparticles, including physical, chemical and biological methods. Unfortunately, many of the physical and chemical methods are time and high energy demanding, expensive and involve the use of

hazardous chemicals making them challenging and often involving contamination of the environment [7]. Therefore, it is increasingly important to prioritize an alternative synthetic route that is not only cost-effective but also environmentally friendly. Considering these, the biological green synthesis methods that involve the use of natural resources such as plant extracts, micro-organisms and enzymes as reductants and capping agent for the preparation of silver nanoparticles are attracting more interest in nanotechnology [8]. Moreover, the green synthesis method is cheap, renewable and produces more stable nanomaterials. Microorganisms can be used to produce nanoparticles, but the synthesis process tends to be slower compared to methods that involve plant-mediated synthesis [9].

Plants provide many benefits, whether used whole or in part. Active ingredients derived from plants, possess the capability to cap and reduce metal ions to stable metal nanoparticles [10]. The ability to control the size, shape, and surface properties of nanoparticles through careful selection of plant materials and extraction methods further underscores the versatility of this approach [11-12]. The process typically involves mixing a metal salt solution with an aqueous plant extract under mild conditions, which not only reduces energy consumption but also minimizes waste generation. In this regard, fruit extract of *Lagenaria breviflora* (commonly known as wild colocynth) a perennial vine plant from the family

of Cucurbitaceae [13]; was used for bio-reduction of silver ions to nanoparticles. This plant is widely found in west tropical Africa such as Nigeria, Cameroon and Congo. However, part of

the plant has been used as a traditional remedy for various health issues. It has been particularly valued for its effectiveness in treating viral, bacterial, and fungal infections [14].



figure 1: *Lagenaria breviflora* fruits

MATERIALS AND METHODS

The plant extract-mediated bio-reduction involves mixing the aqueous extract with a solution of the appropriate metal salt. The synthesis of nanoparticles takes place at 60°C in dark condition and finishes within 6h.

Preparation of plant extract

Lagenaria breviflora fruit extract was used to prepare silver nanoparticles due to its cost-effectiveness, easy availability, and well-known medicinal properties. Fresh fruits were obtained from the botanical garden Joseph Sarwua Tarka University, Makurdi, Benue State, Nigeria. They were washed with tap and double distilled water to remove dust particles, then shade-dried to a

constant weight. The dried fruits were pulverized. 20g of the sample was introduced into a 250cm³ glass beaker containing 200cm³ of double distilled water and allowed to stay for 48hrs with intermittent shaking. The mixture was filtered with Whatman No.1 filter paper and stored in the refrigerator for further usage.

Phytochemical screening

Phytochemical screening of *Lagenaria breviflora* fruits aqueous extract was conducted following the method prescribed by [15]. Bioactive compounds, such as alkaloids, saponins, flavonoids, tannins, terpenoids, and carotenoids were screened for.

Green synthesis of silver nanoparticles

Silver nitrate (AgNO_3) was sourced from Sigma Aldrich. 240cm³ of 0.005M solution of silver nitrate was prepared and added to 60 cm³ of *lagenaira breviflora* fruit extract. Under constant stirring, the mixture was heated on a magnetic hot plate at 60°C for about 6h until the colour of the solution changes from yellow to dark brown. The observed colour change confirmed the formation of silver nanoparticles and the solution was taken from the hot plate. The formation was also monitored using UV-Visible spectroscopy ranging from 300-700 nm. The synthesis was conducted in the dark to prevent photo-degradation of silver nitrate. The synthesized silver nanoparticles were centrifuge at 3500rpm for 30 minutes. The crystals obtained were washed with double distilled water, oven dried at 40°C and stored for further use.

Characterization of synthesized silver nanoparticles

UV-Vis spectral analysis was conducted using a scan Jen-way (Model 7415) Single beam UV-visible Spectrophotometer at a wavelength ranging from 300-700 nm. For the analysis, 1cm³ of the sample was pipetted into a cuvette and examined at room temperature. FTIR spectra were recorded using an Agilent Cary 630 FTIR spectrophotometer. The average crystallite size was determined using X-ray diffraction (XRD) Thermo scientific model: ARL'XTRA X-ray and serial number 197492086. The particle size and surface morphology were examined using

Scanning Electron Microscopy (SEM) model Phenom ProX, by PhenomWorld Eindhoven; The Netherlands.

Assessment of antimicrobial assay

The antibacterial assays were evaluated against; a (gram-negative pathogens *Escherichia coli* and *Salmonella typhi*) and gram positive pathogen *Staphylococcus aureus*, by using Agar well diffusion method. Nutrient Agar (N.A) plates were swabbed with 24h old broth culture of respective micro-organisms. After which, wells of about 2cm apart were bored on the surface of inoculated agar plates using 10mm cork borer. About 0.2 mL of different concentrations (500mg/mL, 250mg/mL and 125mg/mL) of plant extracts (P-E) and silver nanoparticles were added into the well using Pasteur pipette; using Ciprofloxacin as control. The plates were incubated at 37°C for 24hr. The experiment was performed in triplicate and the antibacterial activity was measured based on the inhibition zone around the well impregnated with plant extract and synthesized silver nanoparticles.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical screening tests were conducted to identify bioactive compounds present in the extract that are responsible for the reduction of silver ion to nanoparticles. The results of this

screening for aqueous extracts are summarized in Table 1.

Table 1: Phytochemical screening of aqueous extract of *lagenaira breviflora* fruit

Phytochemical constituents	Test performed	Aqueous extract
Alkaloids	Mayer's and Dragendorffs tests	+
Tannis	Ferric chloride test	+
Flavonoids	Ammonia Test	+
Saponins	Frothing test	++
Terpenoids	Salkowski Test	+
Carotenoids	Carr-price reaction test	+

Key: (+, present) (++, very present)

Visual observation and UV-Vis spectroscopy

When the plant extract of *Lagenaira breviflora* was added to the beaker containing aqueous solution of silver nitrate and stirred at 60°C for 6h, the color of the solution changed from yellow to dark-brown indicating the formation of the silver nanoparticles (as shown in Figure 2). This colour change is attributed to the excitation of surface plasmon vibrations in the silver nanoparticles [16]. Several parameters were optimized, including the concentrations of silver nitrate and *Lagenaira breviflora* fruit extract, temperature, as well as the reaction time, all of which have been identified as key factors influencing the yield of silver nanoparticles.

Silver nanoparticles synthesized at concentrations of fruit extract of 60cm³ using 240cm³ of silver nitrate was analyzed by UV spectrum of Plasmon resonance band observed at 350 nm, indicates the peak of the plant extract while the peak observed at 435 nm was that of the synthesized silver nanoparticles similar to the finding of [17]. The recorded UV-vis spectrum indicated that bio-reduction was successfully achieved using the fruit extract of *Lagenaria breviflora* as a reducing agent. Both the UV-vis spectrum and visual observations showed that silver nanoparticles were formed within a reaction time of 6 hours.

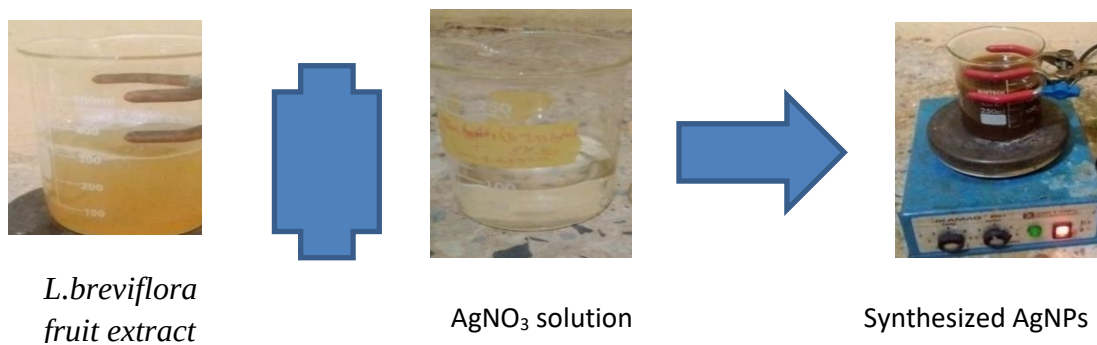


Figure 2: Image of the formation of silver nanoparticles

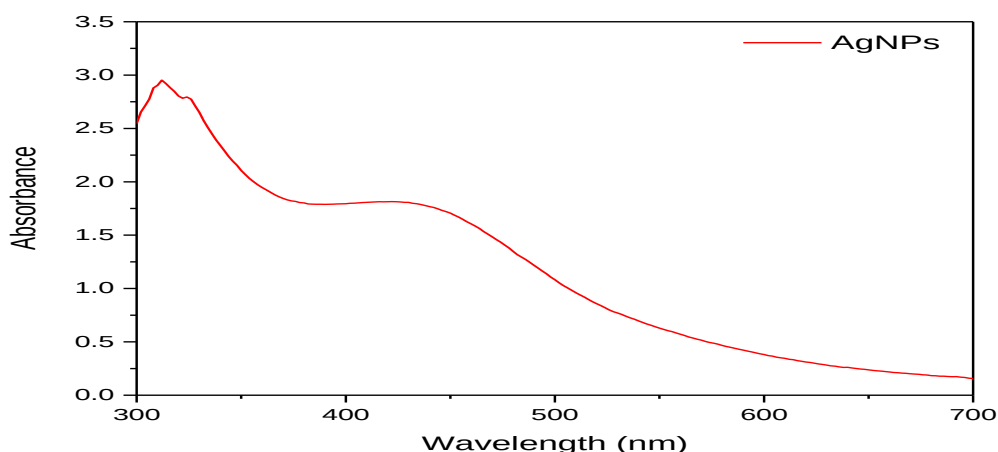


Figure 3: UV-vis absorption spectrum of the synthesized AgNPs

FTIR analysis

FTIR analysis revealed the presence of specific functional groups responsible for reduction and capping of silver ion (Ag^+) to silver nanoparticles (AgNPs). The peak at 3400 cm^{-1} indicates the presence of O-H stretching attributed for water and *lagenaira breviflora* fruit extracts molecule. The band at 3200 cm^{-1} is due to N-H stretching of amine (NH_2) and the peak at 2800 cm^{-1} is typical of aliphatic C-H bonds. The peak at 1700

cm^{-1} , corresponding to the C=O stretching of carbonyl groups. The peak at 1600 cm^{-1} is related to the C=C stretching of alkene groups present in the phytoconstituents of the extract and the peak between ($1000\text{-}1200\text{cm}^{-1}$) is assigned to aldehydes hydrogen stretch of ester, or alcohol as showed in figure 4. The observed peaks are primarily associated with the flavonoids and terpenoids found in the plant extract. This agrees with the findings of [18].

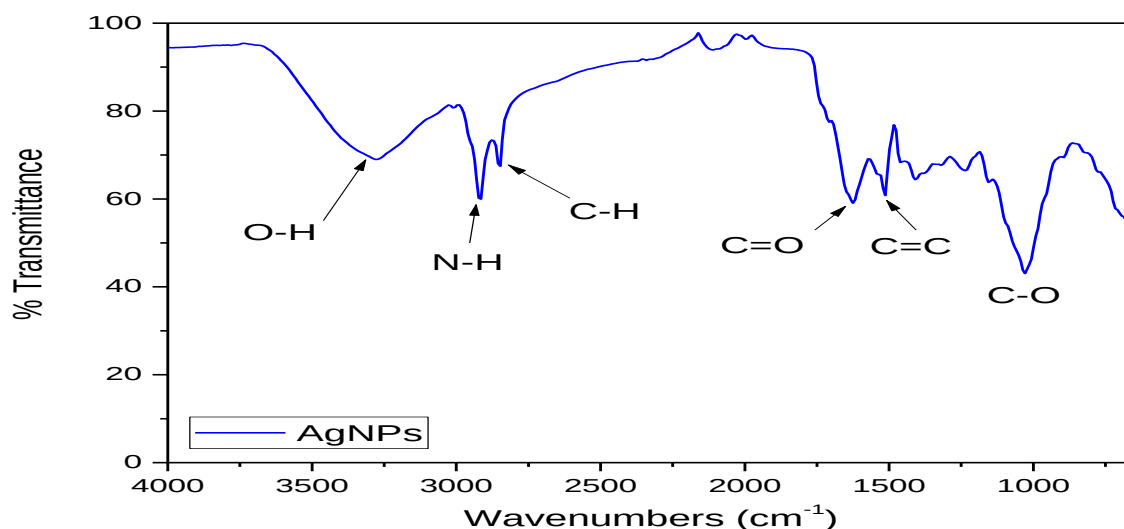


Figure 4. FTIR Spectra of the synthesized AgNPs

X-ray Diffraction analysis

The X-ray diffraction (XRD) pattern of the synthesized AgNPs is shown in figure 5. The XRD pattern showed three peaks for 2θ values ranging from 27- 50°; indexed as (111), (200) and (220). These peaks indicate crystalline structure, with the prominent peak at 32.20° (200) with a d -spacing of 0.28 nm; with an average crystallite size of 12.83 nm. The intensity of these peaks suggests high crystallinity. The crystalline silver nanoparticle

was calculated using Debye-Scherrer equation as reported by [19-20].

$$\text{Debye-Scherrer equation: } D = k \lambda / \beta \cos \theta \dots (1)$$

Where D= size of the nano-crystal,

k = Scherrer constant (0.9),

λ = x-ray wavelength ($1.5406 \text{ \AA} = 1.5406 \times 10^{-10}$),

β = full width at half maxima of (200) reflection at Bragg's angle 2θ ,

θ = Bragg angle (32.20°).

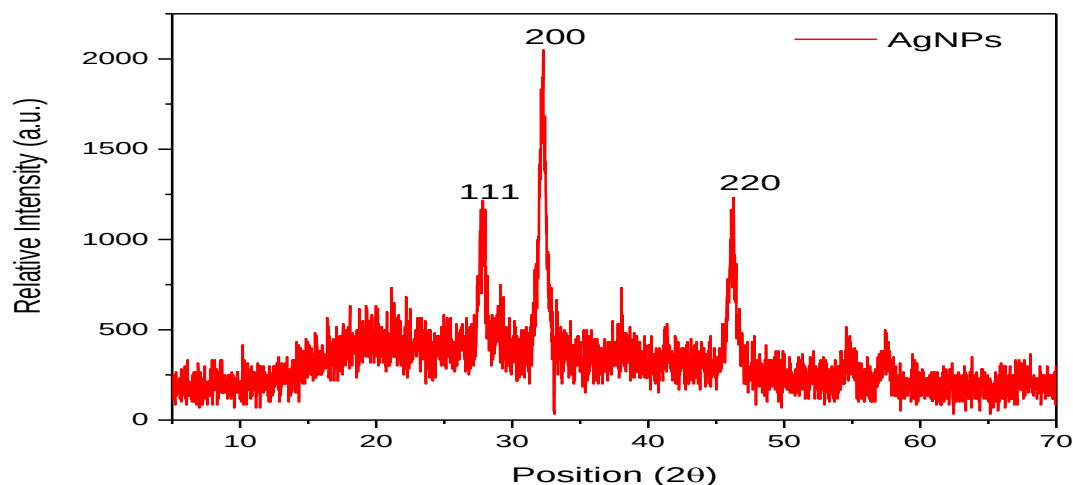


Figure 5. X-ray diffractogram of the synthesized LB-AgNPs

Scanning Electron Microscopy (SEM) analysis

Scanning electron microscopy was used to determine the size and morphology of the nanoparticles. The analysis showed that the silver nanoparticles are well-dispersed and spherical in shape. The resulting histogram displays the size distribution while the micrographs depict the surface morphology of the synthesized silver

nanoparticles (AgNPs) attributed to the reaction mode of the active phytochemicals present in the plant extracts as shown in figure 6. The average particle size of the AgNPs was observed to be 73.20 nm while the average area was 38.80 nm². This result falls within the nanomaterials range of 1-100 nm reported by [21].

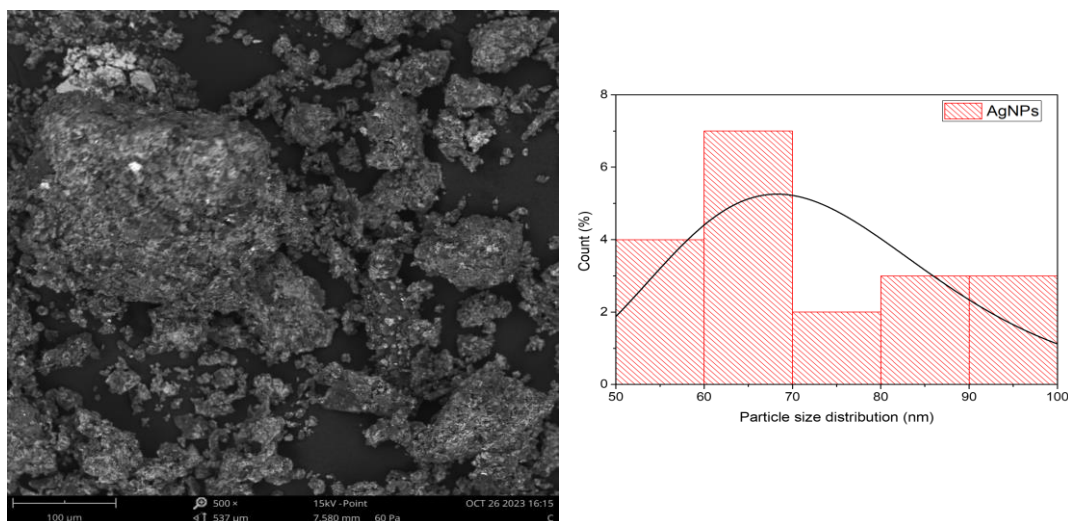


Figure 6. SEM image of synthesized silver nanoparticles (AgNPs)

Antimicrobial activity

Silver nanoparticles as well as elemental silver and its compounds have been used across various fields, especially in the health industry, because of their antimicrobial properties [22]. Silver nanoparticles mediated by *lagenaria breviflora* extract were examined as effective antibacterial agents. The plant extract and the mediated silver nanoparticles were tested for their antimicrobial activities against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Salmonella typhi*) bacterial strains showing the zones of inhibition for different concentration (500, 250, and 125 mg/mL). Based on the zone of inhibition produced, synthesized silver nanoparticles proved to exhibit good antibacterial activity

against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* than the fruit extract of the plant that shows low activity. On the other hand, control (Ciprofloxacin) demonstrates the highest zones of inhibition at the lowest concentration (125 mg/mL), showing high effectiveness. The results of antibacterial activities of the plant extract and the silver nanoparticles showed varying degree of inhibition against the organisms as presented in Table 2. The synthesized silver nanoparticles showed better antimicrobial properties compared to the plant extract. This effectiveness is attributed to their larger surface area, which enhances their contact with the cell walls of microorganisms. The plant extract as well as the synthesized AgNPs.

Table 2: Zone of inhibition (mm) obtained by agar well diffusion method.

Samples	Concentration mg/mL	Zone of inhibition (mm)		
		Gram negative		Gram positive
		<i>E. coli</i>	<i>S.typhi</i>	<i>S.aureus</i>
Plant extract	500	11	10	10
	250	8	7	8
	125	-	-	-
AgNPs	500	15	13	14
	250	12	11	11
	125	9	8	9
Ciprofloxacin	500	21	22	21
	250	19	20	18
	125	17	19	16

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CONCLUSION

This study reports a simple, eco-friendly, one-spot method for synthesizing stable silver nanoparticles using *Lagenaria breviflora* fruit extract at 60°C. The process was found to be

efficient in terms of reaction time, and does not require external stabilizers or reducing agents. The synthesized silver nanoparticles showed efficient antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The use of plant extracts for nanoparticle synthesis is energy-efficient, cost-effective, and environmentally friendly, producing less waste and safer products. This green method is a promising alternative to conventional physical and chemical techniques and thus has potential applications in biomedicine, optoelectronics, and medical devices.

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