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## Green synthesis of silver nanoparticles using *Lagenaria breviflora* aqueous leaves extract

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The development of biologically inspired silver nanoparticles (AgNPs) has attracted considerable worldwide attention in the field of medical nanotechnology. This is because secondary metabolites possess the unique reducibility required in the green synthesis of nanoparticles. This study for the first time describes the use of an aqueous extract of *Lagenaria breviflora* leaves (LBAq) in the synthesis of AgNPs. The methanolic extract of *Lagenaria breviflora* leaves (LBme) was analyzed to determine the presence of some important phytochemicals. AgNPs were prepared at different concentrations, using the green-synthetic method. The synthesized AgNPs were characterized using UV-Visible Spectrophotometer (UV-Vis), Energy Dispersive X-ray Spectrometry (EDS), Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD). Qualitative phytochemical analysis of the methanolic extract of *Lagenaria breviflora* leaves confirmed the presence of flavonoids, terpenoids, saponins, alkaloids and cardiac glycosides. A colour change from light yellow to dark/reddish-brown colouration after 6 hours indicated the formation of AgNPs, which was confirmed by a maximum absorption band observed at almost 450 nm in the UV-Vis spectrum. The crystalline nature of AgNPs was confirmed by the XRD pattern. The SEM results revealed the morphology of the synthesized AgNPs including some oval and nano-sphere-shaped particles. The EDS analysis confirmed the presence of elemental silver in abundance. AgNPs were successfully obtained from the bioreduction of AgNO<sub>3</sub> solution using LBAq. The results obtained in this study prove that *Lagenaria breviflora* leaves possess the bioreducing ability required to synthesize silver nanoparticles at the nanoscale level.

**Keywords:** Silver nanoparticles, Green synthesis, *Lagenaria breviflora*, Phytochemicals.

**1. Introduction**

The study and application of nanomaterials are increasing day by day in recent years since nano-sized object shows differing properties of their bulk materials because of changes in their surface-to-volume ratio<sup>1,2</sup>. In many biochemical reactions and several applications in different industries, most nanomaterials have shown excellent quantum confinement with unique catalytic properties to improve the technique for electronic, environmental, and biomedical purposes<sup>3</sup>. The use of plants in the synthesis of nanoparticles is largely encouraged. This is because plant phytochemicals show greater reduction and stabilization ability. Biologically, nanomaterials are used in bio-imaging, diagnosis, bio-sensing, gene therapy, and antimicrobial and anticancer medications<sup>4,5</sup>. Silver nanoparticles (AgNPs) are intriguing nanomaterials that show particular promise in

fields such as colorimetric sensors, bactericidal materials, and electrochemical sensor components. Metallic nanoparticles (NPs) have received significant attention in the area of biomedical applications. Many chemical, physical and biological approaches exist for producing metallic NPs. Together with the widespread utilization, the exponentially growing need for nanomaterials and the industrial-scale production of these nanomaterials, some concerns have emerged mainly from environmentally conscious and eco-sensitive individuals, including numerous researchers<sup>5</sup>. These originate from the fact that nanoparticle production places an enormous burden on the environment since conventional synthetic approaches often require the administration of toxic chemical entities during the production process, which may cause

harmful reactions in the environment and possibly in animal and human health; moreover, such unpleasant chemicals might critically restrict the application possibilities and the biocompatibility of the generated particles. Thus, the pressing demand for metal nanoparticles must be accompanied by eco-friendly, cheap and novel synthesis approaches to minimize or completely avoid the administration of dangerous chemicals and at the same time diminish the accumulation of hazardous wastes. Safer production alternatives applying gentle solvents, environment-friendly reducing or stabilizing materials or mild experimental conditions, or even involving the application of biological materials, such as plant extracts or biomolecules of plants, bacteria, fungi or their lysates are called green approaches<sup>6,7</sup>. Physical and chemical methods are time and energy consuming, expensive and not eco-friendly. For the biological approach, various enzymes, algae, microbes and plants are used<sup>8</sup>. Silver NPs specifically have gained attention due to their unusual physiochemical properties. Recently, the green synthesis of NPs using plant extracts emerged as a promising methodology for the fabrication of metallic NPs because it involves a straightforward, low-cost, fast, environmentally friendly bioprocess, ease of scale-up, less biohazardous, and avoids the hideous procedure of maintaining the cell lines<sup>8,9</sup>. Since traditional physical or chemical methods of metal nanoparticle synthesis have obvious limitations and disadvantages, green chemical processes emerged as a new direction in the chemical industry about two decades ago. Ever since, these biologically inspired green syntheses have attracted considerable attention, offering a promising alternative for maintaining the economy while protecting the environment. Biological synthesis protocols offer a clean, highly tunable, and environmentally benign method for producing nanoparticles with a broad range of sizes, shapes, and unique physical, chemical and biological properties<sup>8,10</sup>.

The peaked interest given to AgNPs in the scientific community necessitated its novel plant-mediated green synthesis using *Lagenaria breviflora* (Figure 1) leaf extract for the first time in this study.



Figure 1: Image of *Lagenaria breviflora* plant.

## 2. Materials and Methods

### 2.1 Sample and reagent collections

Silver nitrate (99.9 % purity) was obtained from CDH chemicals. All other chemicals and reagents used in this study were of analytical grade. Fresh leaves of *L. breviflora* were locally sourced in an agricultural farm within the vicinity of Tai Solarin University of Education, Ijagun, Ijebu Ode, Ogun State, Nigeria. The collected leaves were authenticated by Esimekhuai, D.P.O in the Department of Botany, University of Ibadan, Oyo State, Nigeria.

### 2.2 Qualitative phytochemical analysis of *Lagenaria breviflora* leaves

*Lagenaria breviflora* leaves extract was qualitatively screened for its phytochemicals using methanolic extract (ME) of the leaves. Phytochemicals including tannins, flavonoids, alkaloids, terpenoids, cardiac glycosides, and saponins were analyzed. Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out for the extract as well as the powder samples using the standard procedures as described by Khan *et al.*, (2011)<sup>11</sup>.

#### 2.2.1 Test for Steroids

##### *Liebermann Burchard's test*

The acetic anhydride (2 mL) was added to 0.5 g of ME in a test tube, followed by the addition of 2 mL of sulphuric acid by the wall of the test tube. The formation of a reddish-brown ring at the interphase and a blue upper layer indicates the presence of steroids

#### 2.2.2 Test for alkaloids

##### *Mayer's and Dragendorff's tests*

Hydrochloric acid solution (8 mL of 1%) ) was added to 0.5 g of ME in a test tube. The resulting solution was warmed and filtered and 2 mL of the filtrate was treated with Mayer's and Dragendorff's reagents separately. The formation of a creamy precipitate with Mayer's

reagent and an orange precipitate with Dragendorff's reagent indicates the presence of alkaloids.

### 2.2.3 Test for saponins

#### Frothing test

In a test tube containing boiling water, 0.5 g of ME was added and the resulting hot aqueous solution was allowed to cool. After cooling, the solution was then shaken vigorously till a froth that lasted for 15 minutes appeared. The height of the froth was measured to ascertain the presence of saponin.

### 2.2.4 Test for Tannins

#### Ferric chloride test

In 10 mL of distilled water, 0.25 g of ME was carefully weighed and transferred. The resulting solution was then filtered and the resulting filtrate was added to 1% FeCl<sub>3</sub> (aq) solution. The appearance of an intense greenish, black, purple or blue colour was observed to indicate the presence of tannins.

### 2.2.5 Test for Flavonoids

#### KOH test

The 0.5 g of ME was defatted by shaking vigorously with petroleum ether. The defatted residue was dissolved in 20 mL of 80% ethanol. The mixture was filtered and 3 mL of the filtrate was mixed with 4 mL of 1 % potassium hydroxide in a test tube. A colour change to dark yellow, indicating the presence of flavonoids was observed.

### 2.2.6 Test for cardiac glycosides

#### Keller-Killani test

In a test tube containing 2 mL of glacial acetic acid, one drop of ferric chloride (FeCl<sub>3</sub>) and 1 mL of concentrated sulphuric acid, 5 mL of ME was added to the wall of the test tube. The formation of a brown ring at the interface, or the appearance of a violet ring below the brown ring indicates the presence of cardiac glycosides.

## 2.3 Preparation of plant material

The fresh leaves of *L. breviflora* were rinsed thrice with running tap water and finally with distilled water to remove dust particles. All working materials were cleaned including mortar and pestle. Clean leaves were transferred into the mortar and then chopped with a pestle to obtain tiny pieces of leaves. *L. breviflora* leaves extract was prepared by placing 10 g of pulverized leaves into a 250 mL glass beaker along with 200 mL of sterile distilled water. The chopped leaves were boiled for 30 minutes at 60 °C, while the hot leaves were further crushed and allowed to cool at room temperature for 15 minutes. The mixture was filtered with Whatman No. 1 filter paper and

the filtrate was centrifuged at 1500 rpm for 15 minutes to remove the heavy biomaterials. The extract was stored at room temperature for further use.

## 2.4 Green synthesis of silver nanoparticles

AgNPs were biologically synthesized by adding 240 mL of 0.001 M silver nitrate (AgNO<sub>3</sub>) to 60 mL *L. breviflora* aqueous leaf extract solution. Synthesis was carried out in the dark to avoid photodegradation of silver nitrate. Under constant stirring, the mixture was heated on a magnetic hot plate at 60 °C until the colour of the solution changes from light yellow to dark brown. Once a constant colour intensity was attained (within 6 h), the solution was removed from the hot plate and the synthesis of AgNPs was considered complete and successful because of the colour change. The resulting *L. breviflora* synthesized AgNPs were centrifuged at 3500 rpm for 30 minutes. Subsequently, obtained crystals were dispersed in sterile distilled water to get rid of any uncoordinated biological materials, dried and stored for further use. The rate of reduction of silver ions (Ag<sup>+</sup>) to nanosilver (Ag<sup>0</sup>) was monitored at varying wavelengths (200-700 nm) using UV-Visible Spectrophotometer. The same procedure was repeated at 0.003 M, 0.005 M and 0.007 M respectively.

## 2.5 Characterization of silver nanoparticles

The formation and stability of AgNPs in sterile distilled water are confirmed using a UV-vis spectrophotometer in a range of wavelengths from 200 to 700 nm. Then, the morphology of the NPs was determined using a scanning electron microscopy (SEM) assay. The presence of silver (Ag), including other components in the synthesized nanoparticles was detected using Energy-dispersive X-ray spectroscopy (EDX) analysis, JEM-2100F transmission electron microscope. The XRD of AgNPs was analyzed and determined the particle size by using the Debye-Scherrer equation.

## 2.6 Qualitative Phytochemical composition

Table 1 shows the result of the qualitative phytochemical investigation of methanolic leaf extracts of *L. breviflora*. From the results, the presence of important phytochemicals, including flavonoids, tannins, saponins, terpenoids, cardiac glycosides, steroids and alkaloids were revealed. The analysis revealed the strong presence of flavonoids, alkaloids, cardiac glycosides and saponins, meanwhile, terpenoids are moderately present and tannins are shown to be weakly

available in the extract. This is an indication of the presence of important phytochemicals that are not only responsible for the bioreduction of Ag ions to silver nanoparticles but also infer stability, less toxicity and reduced agglomeration<sup>10,12</sup>.

### 3. Results and Discussion

#### 3.1 Qualitative phytochemical composition

In Table 1 below, the qualitative phytochemical composition of the methanolic extract of the *L.breviflora* plant is shown. From the table, the presence of important phytochemicals, including flavonoids, tannins, saponins, terpenoids, cardiac glycosides, steroids and alkaloids were revealed. The analysis revealed the strong presence of alkaloids, saponins, flavonoids and steroids with a moderate presence of terpenoids and tannins. Meanwhile, alkaloids and cardiac glycosides are shown to be weakly available in the extract. This is an indication of the presence of important phytochemicals that are not only responsible for the bioreduction of Ag ions to silver nanoparticles but also infer stability, less toxicity and reduced agglomeration

**Table 1:** Qualitative phytochemical analysis of the methanolic leaf extract of *L.breviflora*

Class of compounds	Quality of <i>L. breviflora</i>
Alkaloids	+++
Terpenoids	++
Saponins	+++
Tannins	++
Flavonoids	+++
Steroids	+++
Cardiac glycosides	+

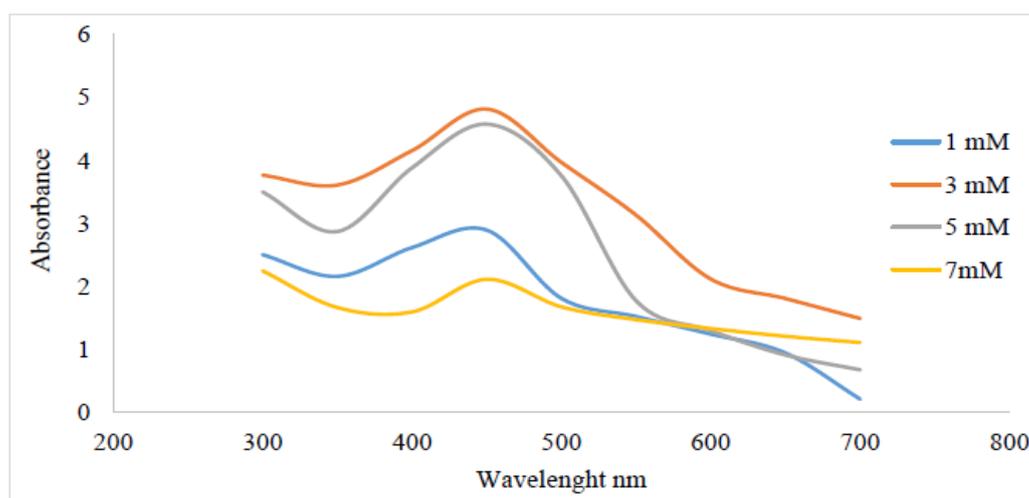
Key: +Weakly positive; ++ Moderately positive; +++ Strongly positive

#### 3.2 Green Synthesis of Silver nanoparticles

The green synthesis of silver nanoparticles was achieved using *L.breviflora* leaf extract as the natural bioreducing agent due to its phytoconstituents. After the addition of the plant extract to the AgNO<sub>3</sub> solution, the successful formation of AgNPs was evident from the appearance of a dark brown precipitate from the initial light yellow colouration. This can be related to the surface plasmon resonance (SPR) caused by the combined oscillation of electrons conducted when the electromagnetic field of the metal nanoparticles interacts<sup>13,14</sup>. Also, related literature suggests that a colour change to brown is an indication of the successful development of silver nanoparticles<sup>5,16</sup>.

#### 3.3 Characterization of AgNPs UV-Visible Spectroscopy

The UV-visible absorption spectra of different concentration of AgNO<sub>3</sub> synthesized with *L.breviflora* aqueous leaf extract is presented in Figure 1. From the spectra, the SPR peak appeared distinct with a broad and prominent band at around 440-460 nm while absorption intensity was found to increase with increased AgNO<sub>3</sub> concentration. The major peak intensity observed for *L.breviflora* synthesized AgNPs at different AgNO<sub>3</sub> concentrations can be because of their absorption within the visible region of the electromagnetic spectrum<sup>14,16</sup>. Also, AgNPs generally show absorption maximum in the range of 390-470 nm due to variations in shape and size distribution<sup>15</sup>.

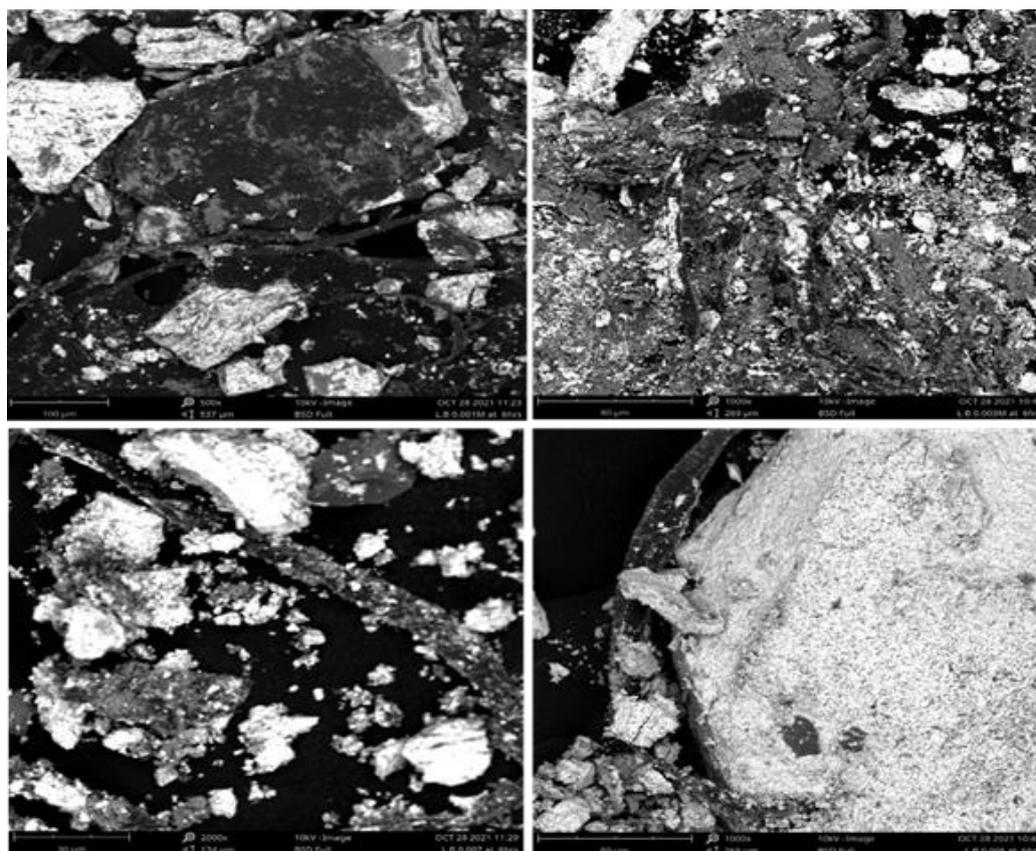


**Figure 2:** UV-vis absorption spectrum of the silver nanoparticles synthesised from *Lagenaria breviflora* aqueous leaves extract.

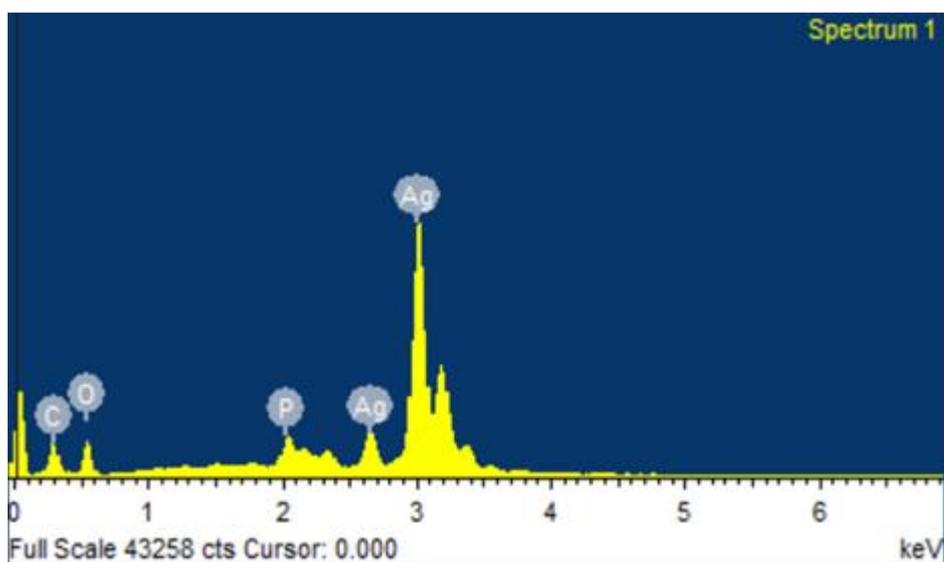
### 3.4 Scanning electron microscopic analysis and energy dispersive spectrometry

Surface imaging of particles with SEM gives information about the shape, surface morphology, size, and size distribution of materials<sup>17</sup>. The result of the SEM analysis of *L.breviflora* synthesized silver nanoparticles at 0.001 M, 0.003 M, 0.005 M and 0.007 M AgNO<sub>3</sub> concentration are presented in Figures 3a-3d. Particles in Figures 3a, 3b, 3c and 3d show sizes ranging from 30 to 60 nm, 35 to 85 nm, 17 to 40 nm and 15 to 62 nm respectively. Nanoparticles with larger dimensions (above 75 nm) were occasionally formed and similar results have

been previously reported<sup>18</sup>. The images in Fig 3a-3c occasionally demonstrated a nano-sphere and an almost oval morphological orientation, meanwhile, nanoparticles with slight aggregation were revealed in nanoparticles synthesized with 0.007 M AgNO<sub>3</sub>. This aggregation could be a result of the capping organic compounds that get adsorbed onto the surface of the nanoparticles<sup>19</sup>. The results of the EDX analysis (figure 3) confirmed the significant presence (61 %) of silver in *L.breviflora* synthesized AgNPs. Interestingly, the optical absorption peak in the EDX analysis of silver was recorded at 3 KeV, a characteristic surface plasmon resonance peak of metallic silver nanoparticle.



**Figure 3:** SEM micrograph of *L. brevisflora* synthesized AgNPs at 0.001 M (A), 0.003 (B), 0.005 M and 0.007 M (D) AgNO<sub>3</sub> solution respectively (100X).



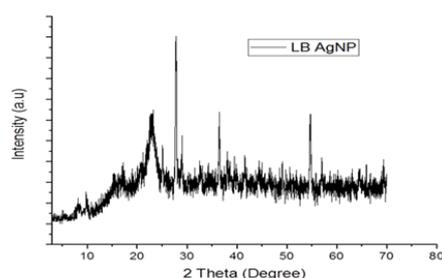
**Figure 4:** Energy Dispersive X ray analysis of *L. breviflora* synthesized AgNPs

### 3.5 X-ray Diffraction Analysis

The X-ray diffraction pattern of *L. breviflora* synthesized AgNPs is shown in Figure 4. From the result, characteristic peaks at 22.7°, 27.9°, 38.1° and 44.3° can be attributed to (111), (200), (220), and (311). This confirms that the phase formation of pure crystalline AgNPs has a face-centred cubic structure<sup>20</sup>. The broad peak in the XRD pattern indicates a smaller particle size. The crystallite size was calculated using Scherrer's formula:

$$d = 0.9\lambda/\beta\cos\theta$$

Where 0.9 is the shape factor, typically considered for a cubic system,  $\lambda$  is the x-ray wavelength, usually 1.54 Å,  $\beta$  is the full width at half the maximum intensity in radians, and  $\theta$  is the Bragg angle. Using the above formula, the crystallite size was calculated to be approximately 23 nm.



**Figure 5:** The X-ray diffraction pattern of *L. breviflora* synthesized AgNPs

## 4. Conclusion

The present study reports the simple and rapid synthesis of AgNPs with an aqueous extract of *L. breviflora* leaves extract. This bioreducing technique promises an efficient synthetic route that is cheap, environmentally friendly and safe, hence, an excellent alternative to chemical and

biological methods. Phytochemical investigation of *L. breviflora* leaves confirms the presence of important phytochemicals that are not only responsible for the bioreduction of Ag ions to silver nanoparticles but also infer stability. The *L. breviflora* reduced AgNPs were characterized using UV-Vis, SEM, EDS and XRD and the results obtained showed crystalline nanoparticles of particles size between 17 – 85 nm with an almost spherical shape, including a characteristic surface plasmon resonance peak of metallic silver nanoparticle. From the result of this study, it can be concluded that these simple, rapid and clean synthetic procedures possess several advantages including safety, cost-effectiveness, large-scale production and potential for biomedical applications.

## Conflict of Interest

The author declares that there is no conflict of interest.

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