

Synthesis and Characterization of Silver Nanoparticles from Ethanolic Extracts of Leaves of *Annona muricata*: A Green Nanobiotechnology Approach

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Authors' contributions

This work was collaboratively carried out among all authors. Authors YG, HAES, FW and GM participated in the conceptualization. Authors YG, ENM, FW and GM participated in the funding acquisition. Authors YG, ENM, AMM, ESM, HAES, FW and GM participated in the methodology, investigations, data curation, validation and formal analysis. All authors participated in the writing of the original draft, review, editing and approval of the submitted manuscript.

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ABSTRACT

Introduction: The biological green synthesis of nanoparticles via nanobiotechnology processes have a significant potential to boost nanoparticles production without the use of harsh, toxic, and expensive chemicals commonly used in conventional physical and chemical processes. *Annona muricata*, a tropical plant belonging to family Annonaceae is one of the most used plants in folk medicine because of its many medicinal uses and therefore presents a strong candidate for use in green synthesis.

Aims: The aim of this study was to optimize a method for the synthesis of Silver Nanoparticles (AgNPs) from ethanolic extracts of leaves of *Annona muricata* as well as to characterize the green synthesized AgNPs.

Methodology: AgNPs were synthesized from *Annona muricata* leaves using AgNO₃ solution. The AgNPs were characterized using spectroscopy and microscopy techniques.

Results: The formed AgNPs had an absorption maximum at 429 nm using UV–Visible spectroscopy and were stable under different pH, temperature, and storage conditions. Fourier transform infrared analysis revealed the different functional groups responsible for the synthesis and stabilization of the AgNPs. Scanning electron microscopy analysis revealed a spherical nature of the synthesized AgNPs. Energy dispersive x-ray spectroscopy analysis showed presence of Ag, O, and Cl with Ag having the highest composition at 60%. X-Ray Diffraction and Dynamic Light Scattering revealed a crystalline nature of AgNPs with an average size of 87.36 nm and a polydispersity index of 0.16 respectively. Transmission Electron Microscopy analysis further confirmed the crystalline and spherical nature of the AgNPs.

Conclusion: In this article, an efficient, eco-friendly and low-cost method for the synthesis and recovery of stable AgNPs using *Annona muricata* leaves ethanolic extracts as both a reducing and capping agent has been reported for the first time. The synthesized AgNPs could be promising candidates for many biomedical, clinical, engineering, and polymer applications.

Keywords: *Annona muricata*; silver nanoparticles (AgNPs); UV/VIS; FTIR; XRD; SEM; DLS; TEM.

ABBREVIATIONS

AgNPs	: Silver Nanoparticles
AgNPs-L	: Silver Nanoparticles from ethanolic extracts of <i>Annona muricata</i> leaves
DLS	: Dynamic Light Scattering
EDX	: Energy Dispersive X-ray Spectrometer
EEAM-L	: Ethanolic Extracts of <i>Annona muricata</i> Leaves
FTIR	: Fourier Transform Infrared Resorption
PDI	: Polydispersity Index
SEM	: Scanning Electron Microscopy
SPR	: Surface Plasmon Resonance
TEM	: Transmission Electron Microscopy
UV/VIS	: Ultraviolet Visible Spectrum
XRD	: X-Ray Diffraction Analysis

1. INTRODUCTION

Plant use in treating diseases is as old as civilization and traditional medicines are still a major part of habitual treatments of different conditions. In recent times, folk medicine has taken an important place especially in developing countries where limited health services are

available [1,2]. *Annona muricata* L. is a species of the Annonaceae family that has been widely studied in the last decades due to its therapeutic potential. *Annona muricata* is known as Soursop (English), Graviola (Portuguese), Guanábana (Latin American Spanish), Omusitafeli / Ekitafeli (Uganda), and other local indigenous names as has been enlisted [3,4]. This plant is a species of the genus *Annona* with the following taxonomic classification. Kingdom: Plantae, Division: Angiosperms (Magnoliophyta), Class: Magnolids, Order: Magnoliales, Family: Annonaceae, Genus: *Annona*, Species: *Annona muricata* L. [5]. The *Annona muricata* tree is about 5–10 m tall and 15–83 cm in diameter with low branches [6–8]. It is widely distributed in the tropical regions of Central and South America, Western Africa, Central and Eastern Africa and Southeast Asia [5,9,10] at altitudes below 1200 m above sea level, with temperatures between 25 and 28°C, relative humidity between 60 and 80%, and annual rainfall above 1500 mm. The fruit is an edible collective ovoid berry, dark green in color.

Various medicinal uses have been reported across the globe ranging from the use of leaves,

bark, roots, fruits and seeds of *Annona muricata* [11]. The most widely used preparation in traditional medicine is the decoction of bark, root, seed or leaf but applications are varied. Ethnobotanical studies have indicated that *Annona muricata* has been used as insecticide [12] and parasiticide [13]. Fruit juice and infusions of leaves or branches have been used to treat fever [14,15], sedative [16,17], respiratory illness [18–20], malaria [21–25], gastrointestinal problems [15,26,27], liver, heart and kidney affections [11,28]. In recent years it has become widely used for hypoglycemic [29], hypotensive [27,29,30] and cancer [2,14,24,31–37].

It is important to note that the effectiveness of many species of medicinal plants depends on the supply of active compounds. It has been reported that most of the biologically active constituents of extracts, such as flavonoids, tannins, and terpenoids, are highly soluble in water, but have low absorption, because they are unable to cross the lipid membranes of the cells, have excessively high molecular size, or are poorly absorbed, resulting in loss of bioavailability and efficacy. Therefore some extracts despite having very good activity *in vitro* which are not reproducible *in vivo*, are not used clinically because of these obstacles [38]. It has therefore been widely proposed to combine herbal medicine with nanotechnology, because nanosystems can deliver the bioactive components at a sufficient concentration during the entire treatment period, directing them to the desired sites of action, and hence potentiating the action of the compounds, an aspect that conventional herbal treatments do not meet [38,39].

Nanoparticles are materials that are small enough to fall within the nanometric range, with at least one of their dimensions being less than a few hundred nanometers. This reduction in size brings about significant changes in their physical properties with respect to those observed in bulk materials. A very interesting application of nanoparticles in the scope of life sciences is their use as 'smart' delivery systems where they are usually loaded with a drug or therapeutic agent [40]. The various developed chemical and mechanical methods of producing nanoparticles include ball milling, thermal quenching, precipitation techniques, vapor deposition. However, these methods are often costly, and may result in toxic byproducts [41–45]. The commercial significance of nanoparticles therefore remains limited by the nanoparticle

synthesis process, which is generally energy intensive or requires toxic chemical solvents and is costly.

Biological approaches, including use of microorganisms or plant extracts to synthesize metal nanoparticles, have been suggested. An emerging field in nanotechnology is the synthesis of metal nanoparticles using herbal plants. Metal nanoparticles display improved and / or novel properties compared to their source materials. These properties may be derived from their size, morphology, or distribution. This method is referred to as the green approach and is environmentally friendly. Thus, the advancement of green syntheses of nanoparticles is progressing as a key branch of nanotechnology; where the use of biological entities like microorganisms, plant extract or plant biomass for the production of nanoparticles could be an alternative to chemical and physical methods in an ecofriendly manner[46].

Among several noble metal nanoparticles, silver nanoparticles have attained a special focus [46]. Silver nanoparticles (AgNPs) are of particular interest because of their antimicrobial, anticancer and cytotoxic activities. We previously reported about the successful synthesis of silver nanoparticles from ethanolic extracts of fruits of *Annona muricata* [47], nevertheless, there had been no reported method or publication on the use of ethanolic extracts of *Annona muricata* to prepare nanoparticles from leaves of this plant, despite their known therapeutic potential. The aim of this study was therefore to optimize a method for the synthesis of AgNPs from ethanolic extracts of leaves of *Annona muricata* as well as to characterise the green synthesised AgNPs.

2. MATERIALS AND METHODS

2.1 Samples Collection and Authentication

Fresh leaves of *Annona muricata* were collected from the wild in Eastern Uganda in the districts of Kaliro, Iganga and Mbale. A sample of the plant was collected, pressed, dried and the plant was identified and authenticated in the Makerere University Botanical Herbarium (MHU) by Dr Namaganda Mary and a voucher specimen was deposited in the herbarium with the accession number MHU50860. The study was registered by the Uganda National Council for Science and Technology (Reg no: NS 43ES).

2.2 Samples Preparation and Extraction

The Leaves of *Annona muricata* were washed with distilled water and then chopped into small pieces and allowed to air dry for a period of 2 weeks. The dried leaves were then milled into a powder using an electric grater. 50 g of powdered leaves were extracted using 250 ml of absolute ethanol for three days by the plant tissue homogenization method as previously described [2]. The dark green Ethanolic Extracts of *Annona muricata* leaves was then filtered and kept at 4°C until use. Fig. 1 shows the samples collection, drying and extraction process.

2.3 Chemicals and Reagents

All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard. AgNO₃ (99.7% purity), Ethanol (Absolute), KBr, NaOH, HCl, were all was obtained from Sigma and Aldrich (Germany).

2.4 Preparation of the 1 mM AgNO₃ Solution

Extra pure AgNO₃ at a percentage purity of 99.7% was used for the preparation of the AgNO₃ solution. 0.1699 g of AgNO₃ were weighed on an ultrasensitive measuring balance and transferred to 1000 ml volumetric flask. Then distilled water was added to the volumetric flask

with continuous shaking until the 1000 ml mark was reached. The solution was then left to completely dissolve the salt.

2.5 Synthesis of Silver Nanoparticles

AgNPs were synthesized by the following method. About 50 ml of the filtered leaves extract was mixed with about 450 ml of 1 mM AgNO₃ solution in a 500 ml flask and mixed thoroughly, forming a uniform mixture. The mixture was then rested at room temperature in the dark storage cabinets for up to about 72 hours, with continuous monitoring. After about 3 hours, the mixture was observed to start changing from dark green to yellowish brown. After about 72 hours, the mixture had completely changed colour to dark brown. This color change is visual evidence of formation of AgNPs or reduction of silver ions into AgNPs due to the excitation of surface plasmon resonance vibration (SPR) [48–53].

2.6 Characterization of the AgNPs

2.6.1 UV/VIS measurements to confirm formation of AgNPs

The synthesis of AgNPs from the ethanolic extract of leaves of *Annona muricata* was further confirmed by ultraviolet - visible spectroscopy (UV/VIS) in the range of between 300 nm to 650 nm [48–51] and ethanol was used as a blank.



Fig. 1. Plate showing the leaves collection, drying and extraction process

2.6.2 pH stability of the synthesized AgNPs

About 15 ml of the formed AgNPs suspension was aliquoted into 5 test tubes each containing about 3 ml of the AgNPs suspension. The suspensions in the test tubes were then adjusted to and subjected to different pH conditions ranging from about pH 2 to about pH 11. The suspension in each test tube was subjected to a different pH condition. The specific pH conditions tested were pH 2, 4, 7, 9, and 11. The pH were adjusted by either adding drops of 1 N NaOH or 1 N HCl until the desired pH was achieved as observed on the pH meter [54,55]. The absorbance spectra of the suspensions were then measured on the UV/VIS in a scan range of 300 nm to 650 nm.

2.6.3 Temperature stability of the synthesized AgNPs

About 10 ml of the formed AgNPs suspension in boiling tubes were subjected to different temperature conditions by heating in a digital water bath for about 3 minutes each and measuring the absorbance spectra on the UV/VIS in a scan range of 350 nm to 650 nm [55]. The temperature tested included room temperature (25°C), 35°C, 45°C, 55°C, 65°C, 75°C, and 85°C.

2.6.4 Storage stability of the AgNPs

About 20 ml of the formed AgNPs suspension was aliquoted into four 15 ml universal tubes each containing about 5 ml of the AgNPs suspension. The suspensions in the tubes were then stored at different temperature conditions for a period of 3 months. The temperatures at which the storage was done included room temperature (which varied between at about 20°C to 30°C during the experimental period), 4°C, -20°C and -80°C. At the end of the 3 months, the samples were retrieved from the different storage facilities allowed to thaw at room temperature and then their absorbance spectra were measured on the UV/VIS in a scan range of 300 nm to 650 nm.

2.6.5 Recovery of the synthesized AgNPs

About 400 ml of the AgNPs suspension were transferred into different plastic bottles of about 250 ml capacity each and frozen in freezer at -80°C for a period of about 12 hours. The frozen suspension was then removed from the freezer

and allowed to completely thaw at room temperature. Upon thawing, the AgNPs were visibly observed spread throughout the now much clear suspension. The suspension with the dispersed AgNPs were then recovered by transferring them into 50 ml universal centrifuge tubes and centrifuging them at a speed of about 6000 RPM for a period of between about 20 minutes to about 45 minutes. After centrifugation, the supernatant in each of the tubes was poured off and the silver nanoparticles were retained as pellets at the bottom of the tubes. The pellets were then washed several times with distilled water (about 10 ml of distilled water were added to each tube and then centrifuged afresh for about 5 minutes to wash and dissolve any water-soluble impurities). The now clean AgNPs were then lyophilized and kept in airtight tubes at 4°C until further use. A total of 1.0 g of AgNPs were recovered following lyophilization.

2.6.6 Functional groups analysis using FTIR

FTIR measurements were carried out to identify the promising biomolecules in the *Annona muricata* ethanolic extract accountable for the reduction of the silver ions and also the capping agents liable for the stability of the bio-reduced AgNPs. The functional groups present in the AgNPs were analyzed by a Bruker Tensor II FT-IR spectrophotometer model (Bruker, Ettlingen, Germany). The KBr pellets of samples were prepared by grinding 10 mg of samples, with 250 mg KBr (FT-IR grade). The 13 mm KBr pellets were prepared in a standard device under a pressure of 75 kN cm⁻² for 3 min. The spectral resolution was set at 4 cm⁻¹ and the scanning range from 400 to 4000 cm⁻¹ [56]. The representative FTIR spectra of the recovered and dried AgNPs synthesized from ethanolic extracts of fruits of *Annona muricata* were recorded and the major and minor peaks were manifested and identified accordingly.

2.6.7 SEM and EDX Measurements

Scanning electron morphological analysis of Silver nanoparticles was performed using Scanning electron microscope FEI XL30 Sirion FEG (Oxford Instruments Plc, Abingdon, United Kingdom) operated at an accelerating voltage of 6 kV. The system was equipped with an Energy Dispersive X-ray Spectrometer (EDX) system from EDAX having a lithium doped silicon detector.

2.6.8 Crystalline size determination using XRD

XRD analysis was employed to determine the average crystalline size of the AgNPs formed. The XRD (D8 Advance; Bruker Optik, Ettlingen, Germany) with CuK α radiation ($\lambda=1.5406 \text{ \AA}$) and working at 40 kV/40 mA in the range of 10°–80° with a 2°-per-minute scanning rate was used. The XRD diffraction data was analyzed using the Match! Software (Crystal Impact, Bonn, Germany) and the average crystalline size of the AgNPs formed in the bio-reduction was determined using the Scherrer equation, with a constant of 0.94.

2.6.9 TEM analysis

TEM was employed to characterize the size, shape and morphologies of formed biogenic synthesized AgNPs. A drop of AgNPs suspension was deposited on carbon coated copper grids and the film on grid was then dried. The TEM was operated and the measurements were performed at accelerating voltage of 100 KV.

2.6.10 Dynamic light scattering (DLS)

The hydrodynamic size distributions and polydispersity index (PDI) of the silver nanoparticles were analyzed by using dynamic light scattering (DLS) instrumentation. The average particle size, size distribution by intensity as well as PDI were determined by injecting 1:20 dilution of silver nanoparticle resuspension into the U-shaped glass cuvette of

the photon correlation microscope as previously reported [47,50,53,57].

3. RESULTS AND DISCUSSION

Fig. 2 shows the colour of the green synthesized AgNPs relative to the Ethanolic extract of *Annona muricata* Leaves (EEAM-L) and Silver Nitrate solution (AgNO₃). The color change in the flask with the AgNPs is visual evidence of formation of AgNPs or reduction of silver ions into AgNPs due to the excitation of SPR. On the other hand, the crude extract remained dark green while the AgNO₃ solution remained colourless.

The spectrum shown in Fig. 3 has a maximum absorption peak at a wavelength of about 429 nm, which is in the range of the SPR for AgNPs which is reported to have an absorption maximum of between about 400nm to about 450nm. The presence of the maximum peak absorption peak at 429 nm is therefore an indication and confirmation that the AgNPs were present.

Silver nanoparticles exhibit a yellowish/ dark brown color in solution due to excitation of surface plasmon vibrations in AgNPs, and therefore reduction of the silver ion to AgNPs during exposure to the plant extracts could be followed by color change and thus UV/VIS spectroscopy [51,58,59]. In the current study, the AgNPs formation was confirmed by the change in colour of the mixture from dark green to dark brown indicating the successful green synthesis process. The UV/VIS maximum absorption

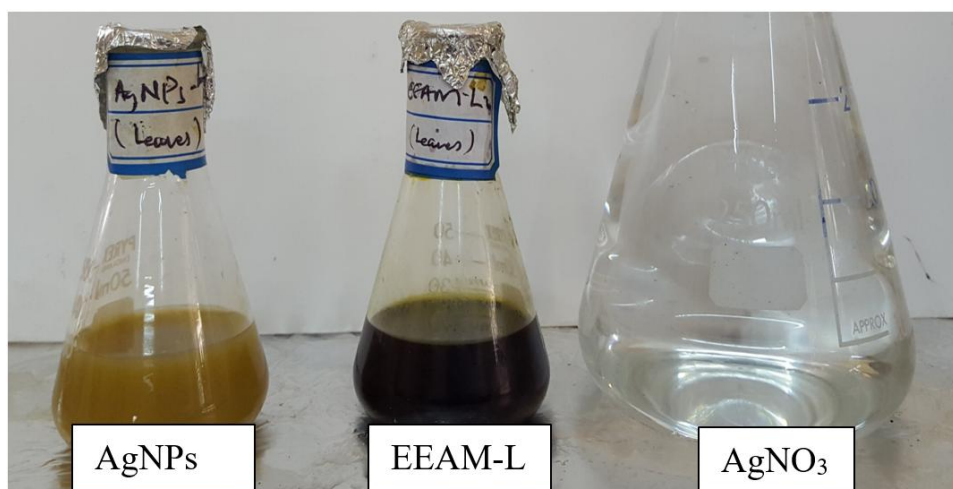


Fig. 2. Photo showing colour of the green synthesized AgNPs relative to the ethanolic extract of *Annona muricata* leaves (EEAM-L) and Silver Nitrate solution (AgNO₃)

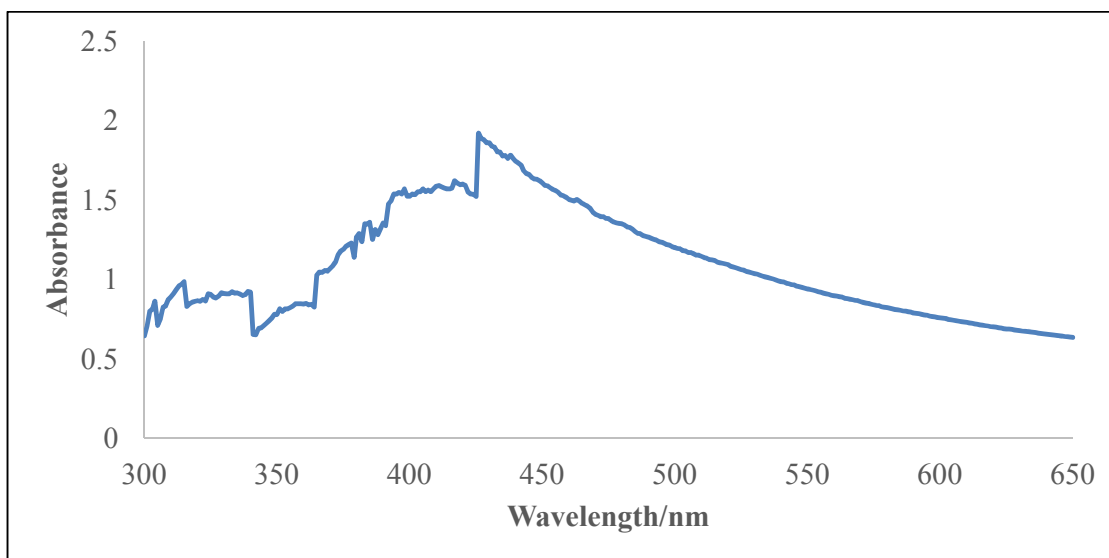


Fig. 3. UV/VIS spectrum of leaves derived AgNPs at 72 hours of incubation

spectra of the synthesized AgNPs was recorded at 429 nm which is in range with previously reported studies on synthesis on AgNPs from plant extracts. Various studies have reported synthesis of AgNPs with UV/VIS absorption maxima at 410 nm [60], 420 nm [53,61], 430 nm [59], 435 nm [50], among others. The current results further provide, for the first time, a confirmation on the use of the *Annona muricata* leaves extracts in the green synthesis of AgNPs with the use of ethanol as a cheap and eco-friendly approach.

From Fig. 4, the AgNPs remained stable at all pH conditions tested maintaining a characteristic absorption maximum of between 410 nm to 420 nm which is within the AgNPs range. There notable relationship between absorption spectra of the AgNPs at extreme acidic and alkaline pH conditions of 2 and 11.

The importance and use of any substances greatly depend on its stability under different conditions. In the current study, the pH, temperature and heat stability, and storage stability of the biosynthesized AgNPs was studied and results have been presented. In relation to pH stability, it is evident that at all pH conditions tested, the AgNPs remained stable maintaining a characteristic absorption maximum of about between 410 nm to about 420 nm which is within the AgNPs range [49,50]. This is very important implying that the AgNPs can be stable under various pH conditions without losing their effectiveness. This property is very important especially of the AgNPs are going to be delivered

via the gastrointestinal tract which has gradients of pH conditions. Similarly, the strong relationship between AgNPs absorption spectra at extreme acidic and alkaline pH conditions of 2 and 11 could be attributed to these conditions have nearly similar effects on the AgNPs. This behavior is in line with earlier studies that showed that extreme changes in pH affect the shape and size of the particles because of the pH's ability to alter the charge of biomolecules by having the potential to affect their capping as well as stabilizing abilities leading to a shift in the peak wavelength thereby indicating a slight increase in size of the particles [47,54,55]. Generally, the reported stability plays a critical role in ensuring maintenance of effectiveness of the AgNPs and thus helps overcome one of the obstacles encountered by many conventional crude extracts from plants which lose effectiveness *in vivo* due to the changing pH gradients as previously reported [38].

Fig. 5 shows the UV/VIS spectra for the temperature stability of the AgNPs synthesized from leaves extracts of *Annona muricata*.

From the results on temperature stability, it is evident that at all temperatures tested, the AgNPs remained stable maintaining a characteristic absorption maximum of about between 420 nm to about 430 nm which is within the AgNPs range [49,50]. This is very important implying that the AgNPs can be stable under various temperature/ heating conditions without losing their effectiveness.

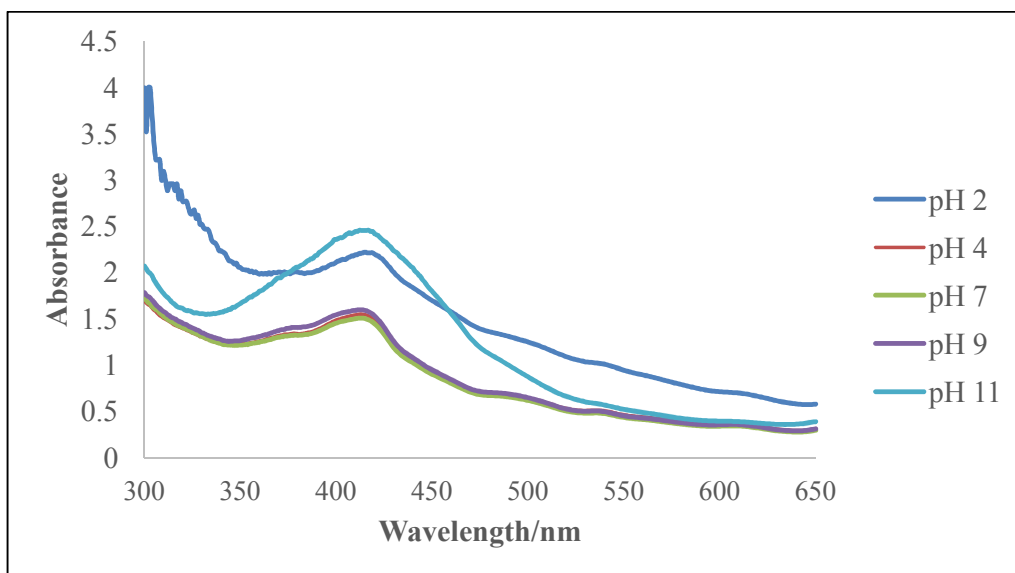


Fig. 4. UV/VIS spectra showing pH stability of AgNPs synthesized from leaves extract

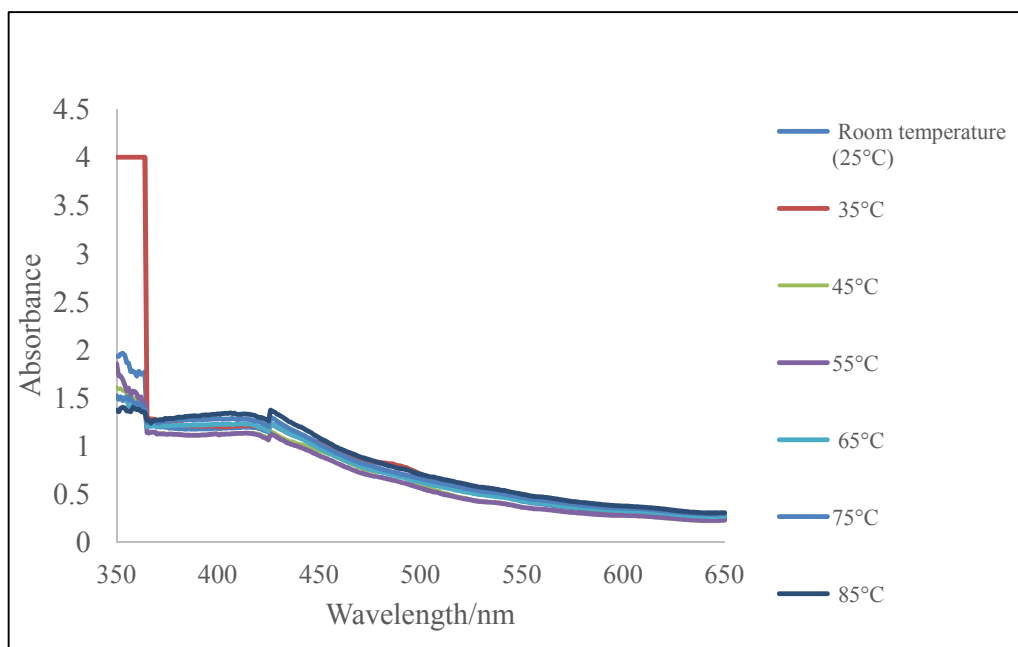


Fig. 5. UV/VIS spectra showing temperature stability of AgNPs synthesized from leaves extract

From Fig. 6, it is evident that at all storage temperatures tested, the AgNPs remained stable maintaining a characteristic absorption maximum of about between 410 nm to about 430 nm which is within the AgNPs range. There was a notable increase in the absorption of the AgNPs at room temperature compared to other storage conditions, nevertheless, the absorption maximum was maintained in the AgNPs range.

As far as storage stability is concerned, it is evident that at all storage temperatures tested for the 3 months, the AgNPs remained stable maintaining a characteristic absorption maximum of about between 410 nm to about 430 nm which is within the AgNPs range. This is very important implying that the AgNPs can be stable under different storage temperature conditions without losing their effectiveness for long periods of time.

The notable increase in the absorption of the AgNPs at room temperature compared to other storage conditions, could probably be attributed to the continuous exposure to the same conditions as those used in the synthesis process thereby allowing the process of formation of the AgNPs to continue throughout the storage period, but at very low rates.

Recovery of the biosynthesized AgNPs is of critical importance in the synthetic process. Various methods have been reported about the recovery of AgNPs [49]. These however are not optimal for all plants. In the current study, we came up with a blended method for quick and

fast recovery of the AgNPs. We introduced a step where the AgNPs suspension is frozen for a period of 12-48 Hours followed by thawing, centrifugation, washing and then drying. The freezing step allows for the particles to aggregate and thus easy sedimentation when the centrifugation step is conducted. This is the first study to report on such an optimization in the recovery of AgNPs.

As shown in Fig. 7 and Table 1, the functional groups responsible for the formation of the AgNPs included; Alkanes and alkyls, ether, esters, nitro groups, amines, alkenes and alkyl halides.

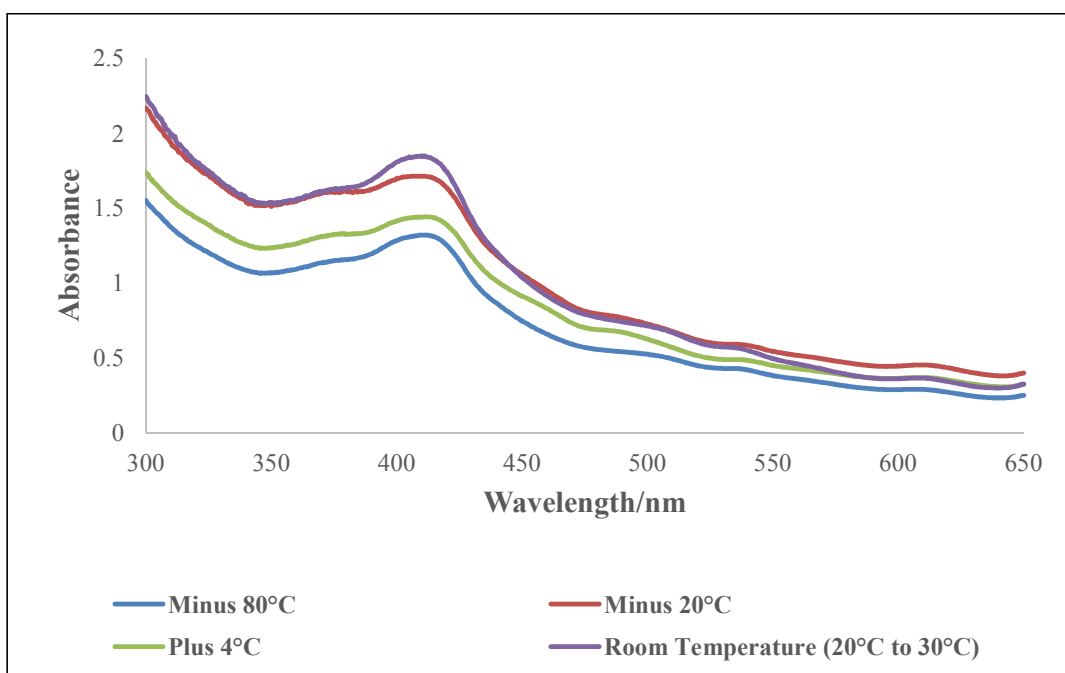


Fig. 6. UV/VIS spectra showing storage stability of AgNPs synthesized from leaves extract

Table 1. FTIR functional group analysis of biosynthesized AgNPs from ethanolic extracts of leaves of *Annona muricata*

Type of peak	Frequency (cm ⁻¹)	Bond	Functional groups
Major	2950	C-H stretch	Alkanes and alkyls
	2850	C-H stretch	Alkanes and alkyls
	1750	C=O stretch	Ester
	1550	N-O Stretch	Nitro group
	1100	C-O Stretch	Ether
Minor	3400	N-H Stretch	Amines
	1600	N-H Bending	Amines
	1400	-C-H Bend	Alkanes
	900	=C-H bend	Alkenes
	700	C-Cl Stretch	Alkyl halide
	550	C-Br Stretch	Alkyl halide

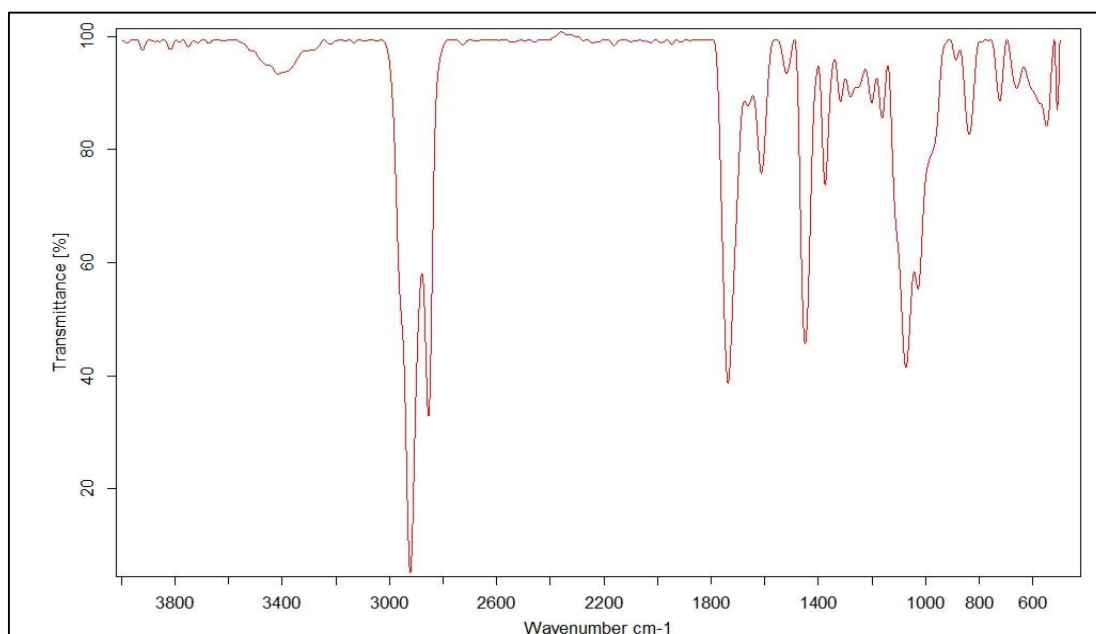


Fig. 7. FTIR spectra of functional groups from the AgNPs synthesized from leaves extract

FTIR results showed that the functional groups responsible for the formation of the AgNPs from ethanolic extracts of leaves of *Annona muricata* included; Alkanes and alkyls, ether, esters, nitro groups, amines, alkenes and alkyl halides. These are probably due to the presence of most of the secondary metabolites reported much earlier in the plant [4,62–64]. Notably, the narrow band at 1600 cm^{-1} can be attributed to N–H Bending probably due to the presence of amines which may be accountable for the reduction of Ag^+ ions to AgNPs.

Fig. 8 shows the typical XRD pattern of biosynthesized AgNPs derived from ethanolic extracts of leaves of *Annona muricata*. Eight characteristic diffraction peaks were observed at 28.07° , 32.47° , 38.31° , 38.41° , and 46.56° , 55.50° , 57.50° , and 77.50° . The average size of the AgNPs formed in the bio-reduction was determined using the Scherrer equation and is estimated as 87.36 nm.

From the XRD diffraction patterns, the 2θ peak observed at 38.31° , 38.41° and 77.50° corresponds to (111), (111) and (311) reflection planes representing the face centered spherical structure of silver respectively [50,65]. The extra peaks near to 28.07° , 32.47° , 46.56° , 55.50° , and 57.50° are due to the presence of bio-organic phase on the surface of particles. Generally, the broadening of peaks in the XRD patterns of solids signifies smaller particle size and reflects

the effects of the experimental conditions on the nucleation and growth of the crystal nuclei [50,66]. In comparison to the other seven peaks, the strong reflection at 32.47° , may perhaps signify the growth path of the nanocrystals. The average size of the AgNPs formed in the bio-reduction was estimated as 87.36 nm.

As shown in Fig. 9 (A) and (B), the AgNPs were approximately spherical in shape with smooth surface and heavily scattered throughout. These results are in agreement with the shape of SPR band recognized from the UV- visible spectrum with absorption maximum at 429 nm.

From Fig.10, the EDX spectra showed the presence of elements such as Ag, O, and Cl. EDX quantitative analysis demonstrated that the highest concentration of a single element in the *Annona muricata* derived AgNPs was silver (Ag), at about 60 %. The high elemental composition of Ag in the samples is an indication of the activity of the same element in the formation of the AgNPs.

The AgNPs were approximately spherical in shape with smooth surface. These results are in agreement with the shape of SPR band recognized from the UV- visible spectrum with absorption maximum at 429 nm. Many previous studies reported different shapes of AgNPs including spherical, conical, cuboidal, hexagonal, pentagonal among others [46,49–51,67]. The

spherical AgNPs synthesized in the current study are therefore in line with the expected shapes for AgNPs. Similarly, EDX elemental analysis revealed that the AgNPs were composed of various elements as reported much earlier, with Ag taking the highest percentage composition at 60%. These results indicate the high purity of the AgNPs albeit with a few contaminants at the different subtle concentration which are probably due to the environmental conditions used during the synthesis process. Earlier studies on had also reported elemental compositions of AgNPs having Ag as the principle component [67–69].

Fig. 11 shows the TEM micrographs of the AgNPs at different resolutions. The Micrographs reveal a spherical nature of the monodispersed AgNPs as well as their crystalline structure. Particle size analysis using the Image-J software further revealed the AgNPs having an average particle size of about 55 nm. TEM analysis further confirmed the crystalline and spherical nature of the monodispersed AgNPs.

Table 2 shows the DLS analysis revealing the average particle size for the AgNPs as 68.7 nm with a polydispersity index of 0.16. The PDI further gives an indication on the homogeneity and monodispersed nature of the AgNPs.

Dynamic light scattering is a method that depends on the interaction of light with particles and the method can be used for measurements of narrow particle size distributions especially in the range of 2–500 nm [70]. The AgNPs size was on average smaller as presented by DLS (68.7 nm) as compared to XRD (87.36 nm), but higher than that as calculated by TEM (55 nm). This difference could be explained by the fact that the size measured by DLS is based on a combination of the particles as well as the hydrodynamic radius which is not a true size of the AgNPs due to the hydration layer around the particles as well as the presence of capping and stabilizing agents as previously explained [47,53,57].

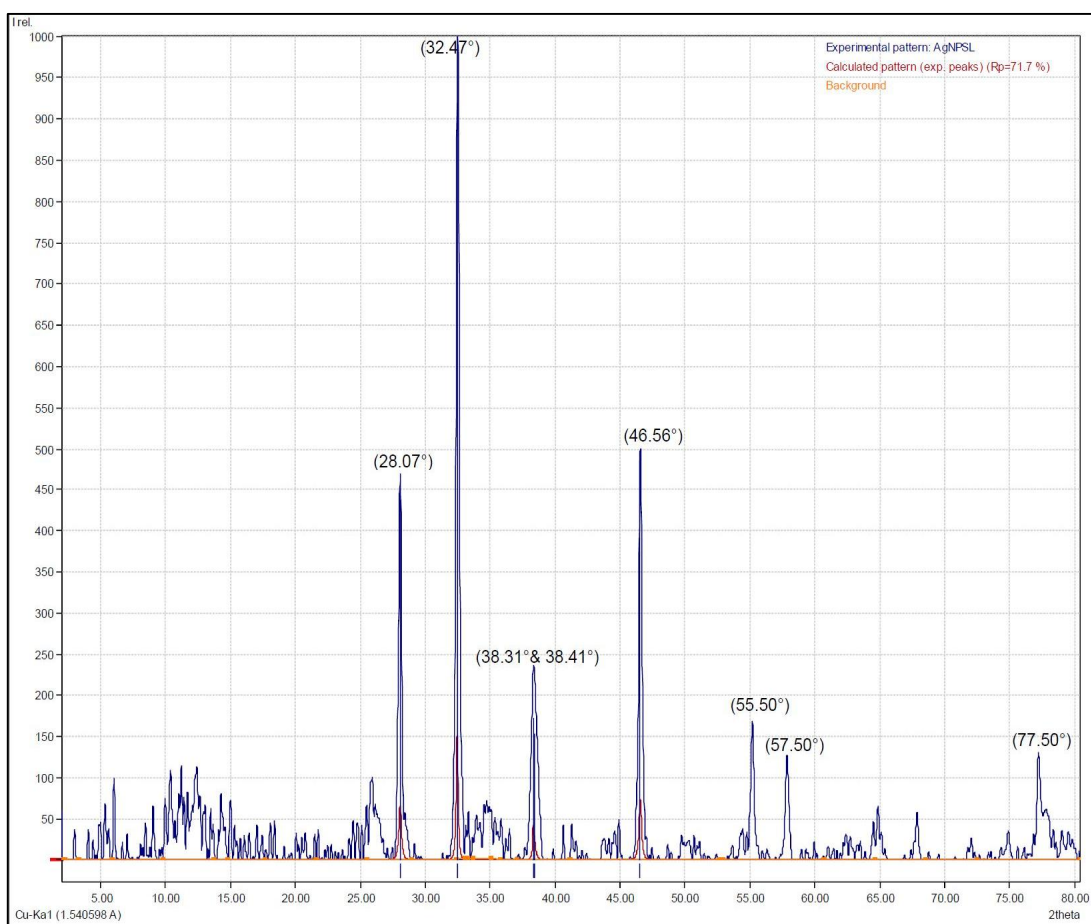


Fig. 8. XRD diffraction pattern spectra of AgNPs synthesized from leaves extract

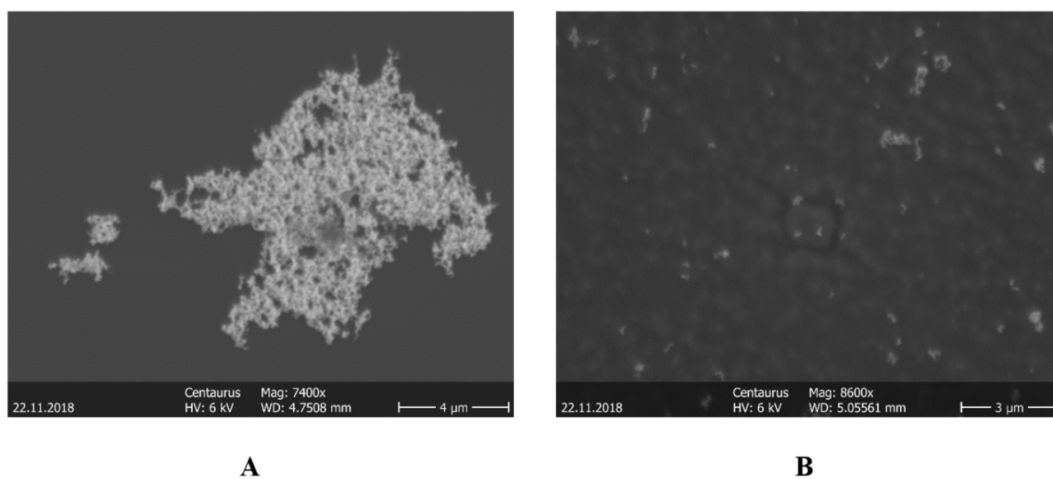


Fig. 9. SEM micrograph showing the shape of AgNPs synthesized from leaves extract at different magnifications

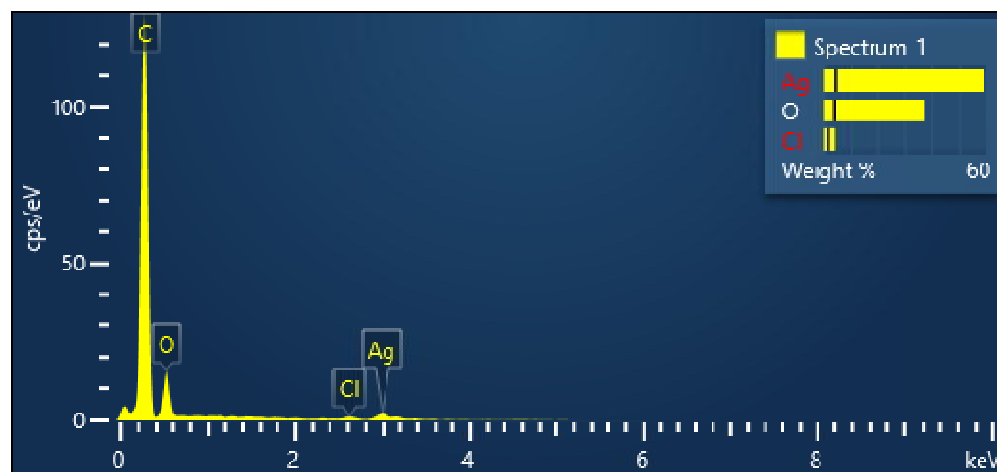


Fig. 10. EDX spectra demonstrating the quantitative amounts of different elements present in the AgNPs synthesized from the leaves extract

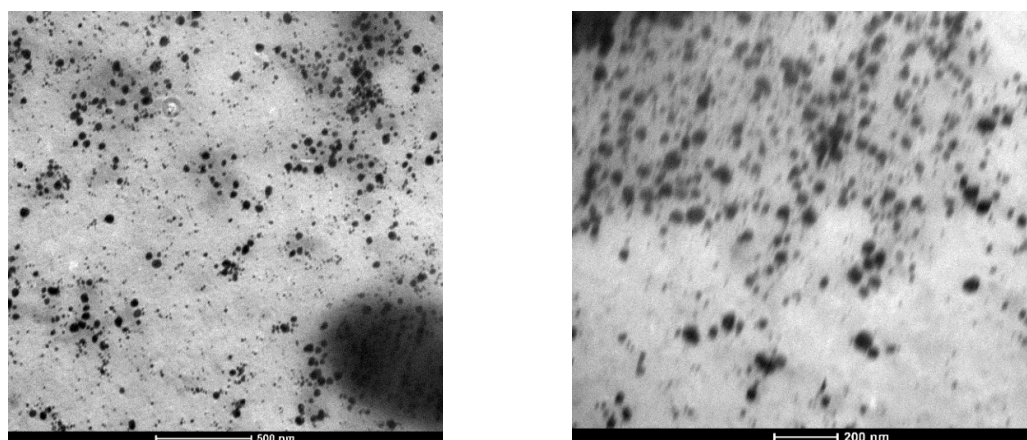


Fig. 11. TEM Micrographs of the AgNPs at different resolutions

Table 2. DLS Analysis results for the AgNPs

Counts	Intensity (kCnt/s)	Attenuation level (%)	Diameter (nm)	PD index
1	1144	90.6	68.2	0.1343
2	1157	90.6	68.0	0.1522
3	1173	90.6	69.5	0.1865
4	1191	90.6	69.0	0.1766
5	1035	90.6	68.6	0.1503
Mean	1140	90.6	68.7	0.1600

Finally, PDI measures the homogeneous nature of nanoparticles, whereby the smaller the PDI the more homogeneous nanoparticles. The numerical value of PDI ranges from 0.0 (for a perfectly uniform sample with respect to the particle size) to 1.0 (for a highly polydisperse sample with multiple particle size populations). Values of 0.2 and below are most commonly deemed acceptable in practice for polymer-based nanoparticle materials, while nanoparticles with PDI smaller than 0.3 is considered acceptable for drug delivery [57,71]. The synthesized AgNPs had an average PDI of 0.16, which is a great indication that they are highly homogenous and would be effectively used in various applications.

4. CONCLUSION

We have reported and optimized for the first time an efficient, eco-friendly and low-cost method for the synthesis and recovery of AgNPs using ethanolic extracts of leaves of *Annona muricata*. The synthesized AgNPs are stable under different temperature, pH and storage conditions. The method used resulted into formation and recovery of spherical crystalline AgNPs with an average size of about 87.36 nm and a PDI of 0.16. With the successful synthesis of AgNPs in the current study, we do recommend further studies aimed at testing the synthesized AgNPs from this method for different biomedical and clinical bioactivities, such as Antimicrobial, Anticancer, Anti-inflammatory, Antimalarial, Antidiabetic, Toxicities among others as a step towards the pharmaceutical utilization of these green synthesized AgNPs. Engineering applications may as well be explored.

DISCLAIMER

Part of the work reported in this manuscript has been filed for a grant of patent at the African Regional Intellectual Property Organization (ARIPO) under the title: "Synthesis of Silver Nanoparticles from Extracts of *Annona muricata*

and Use Thereof". ARIPO Patent Application number: AP/P/2019/011514. The above information notwithstanding, we further declare that the patent application cannot in any way affect the outcome of this manuscript submission.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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