Journal of Inorganic and Organometallic Polymers and Materials
https://doi.org/10.1007/s10904-019-01262-5

Green Synthesis and Characterization of Highly Stable Silver Nanoparticles from Ethanolic Extracts of Fruits of *Annona muricata*

Yahaya Gavamukulya[1,2] · Esther N. Maina[1,3] · Amos M. Meroka[3,4] · Edwin S. Madivoli[1,5] · Hany A. El-Shemy[1,6] · Fred Wamunyokoli[1,7] · Gabriel Magoma[1]

Received: 15 May 2019 / Accepted: 15 July 2019

Abstract

Green synthesis of nanoparticles from plant materials opens a new scope in nanobiotechnology and discourages the use of expensive toxic chemicals. The aim of this study was to develop and optimize a method for the synthesis of Silver Nanoparticles (AgNPs) from ethanolic extracts of fruits of *Annona muricata* as well as to characterize the green synthesized AgNPs. AgNPs were synthesized via $AgNO_3$ solution. The AgNPs were characterized using spectroscopy and microscopy techniques. The formed AgNPs had an absorption maximum of 427 nm and were stable under different temperature, pH and storage conditions. Fourier Transform Infrared Resorption spectroscopy revealed the functional groups responsible for the synthesis and stabilization of the AgNPs. Scanning Electron Microscopy analysis revealed a spherical nature of the AgNPs. Energy Dispersive X-Ray spectroscopy showed presence of Ag, Cl, Ca, and Si with Ag having the highest composition at 80%. X-ray diffraction and dynamic light scattering revealed a crystalline nature of AgNPs with an average size of 60.12 nm and a polydispersity index of 0.1235 respectively. Transmission Electron Microscopy analysis further confirmed the crystalline and spherical nature of the AgNPs. In this article, an efficient, eco-friendly and low-cost method for the synthesis and recovery of stable AgNPs using ethanolic extracts of *Annona muricata* fruits as both reducing and capping agents has been reported. The synthesized AgNPs could have many biomedical and clinical applications.

Keywords *Annona muricata* · Silver nanoparticles (AgNPs) · UV/VIS · FTIR · XRD · Fruit extracts

Abbreviations

AgNPs	Silver nanoparticles
DLS	Dynamic light scattering
EDX	Energy Dispersive X-ray Spectrometer
EEAM	Ethanolic extracts of *Annona muricata*
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

✉ Yahaya Gavamukulya
gavayahya@yahoo.com

[1] Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI), P. O. Box 62000-00200, Nairobi, Kenya

[2] Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, Busitema University, P. O. Box 1460, Mbale, Uganda

[3] Department of Biochemistry, College of Health Sciences, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya

[4] Department of Biochemistry, School of Medicine and Health Sciences, Kenya Methodist University, P. O. Box 267-60200, Meru, Kenya

[5] Department of Chemistry, College of Pure and Applied Sciences, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya

[6] Department of Biochemistry, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt

[7] Department of Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya

Published online: 19 July 2019

1 Introduction

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 nanometres. 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 [1]. 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. Generally, nanoparticles are synthesized in three ways: physically by crushing larger particles, chemically by precipitation, and through gas condensation [2–6]. The commercial significance of nanoparticles is 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. However, synthesis of nanoparticles using microorganisms involves an expensive process requiring cell culture and multistep purification. 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 [7].

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 [8, 9]. 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. [10]. The *Annona muricata* tree is about 5–10 m tall and 15–83 cm in diameter with low branches [11–13]. It is widely distributed in the tropical regions of Central and South America, Western Africa, Central and Eastern Africa and Southeast Asia [10, 14] 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* [15]. From the studies reported, the most widely used preparation in traditional medicine is the decoction of bark, root, seed or leaf, each having unique bioactive compounds [8]. On the other hand, the least studied plant part are the fruits, despite their being the most widely used and eaten as a food, unlike the leaves, barks and seeds. Because of their use as a food, fruits can provide an easier and more acceptable way of delivering the requisite bioactive compounds to the human consumers. Nevertheless, a number of unique bioactive compounds have been reported in the fruits extracts including alkaloids such as anonaine, asimilobine, nornuciferine [8]; acetogenins such as muricin, montanacin [8]; phenols such as kaempferol 3-O-rutinoside, myricetin [8]; and a number of other compounds such as vitamin C, carotenes, tocopherol [8], 1,3-dimethylthiourea, (4-chlorophenyl)-[4-(3-chlorophenyl)-2-[(Z)-3-(dimethylamino)prop-1-enyl]quinolin-6-yl]-(3-methylimidazol-4-yl)methanol [16], among others. These bioactive compounds are reported as having antioxidant, cytotoxic, antidiabetic, anti-hypotensive, antimalarial, and anticancer properties among others [8, 9, 16].

The effectiveness of many species of medicinal plants depends on the supply of active compounds. 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 [17, 18]. Among several noble metal nanoparticles, silver nanoparticles have attained a special focus [7]. Silver nanoparticles are of particular interest because of their antimicrobial, anticancer and cytotoxic activities. Studies involving the use of *Annona muricata* leaves [19, 20], bark [21], and fruits [22] utilizing aqueous extracts in the synthesis of nanoparticles have previously been reported [19–22]. Nevertheless, there had been no reported method or publication on the use of ethanolic extracts of *Annona muricata* to prepare nanoparticles from fruits, despite the known advantages of use of ethanolic extracts as an extraction solvent, which include high recovery rate [8, 9, 23]. The aim of this study was therefore to develop and optimize a method for the synthesis of AgNPs from ethanolic extracts of fruits of *Annona muricata* as well as to characterize the green synthesized AgNPs.

2 Materials and Methods

2.1 Samples Collection and Authentication

Ripe fruits of *Annona muricata* were collected from the wild in Eastern Uganda in the districts of Kaliro, Iganga and Mbale during the month of January 2018. 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) as well as the PAUSTI Board of Examiners (MB400-0007/17).

2.2 Samples Preparation and Extraction

The Fruits of *Annona muricata* were washed with clean water and then peeled to remove the fresh pulp. The pulp was then cut into small pieces and placed in a hot air oven to dry at 50 °C for a week. The dried pulp was then milled into a powder using an electric grater. 50 g of powdered fruits were extracted using 250 ml of absolute ethanol for three days by the plant tissue homogenization method as previously described [23]. The light brown Ethanolic Extracts of *Annona muricata* fruits was then filtered and kept at 4 °C until use. Figure 1 shows the samples collection, drying and extraction process.

3 Chemicals and Reagents

All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard.

3.1 Preparation of the 1 mM $AgNO_3$ Solution

Extra pure $AgNO_3$ at a percentage purity of 99.7% was used for the preparation of the $AgNO_3$ solution. 0.1699 g of $AgNO_3$ 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. The 1 mM $AgNO_3$ solution had been successfully prepared.

3.2 Synthesis of Silver Nanoparticles

AgNPs were synthesized by the following method. About 50 ml of the filtered fruits extract was mixed with about 450 ml of 1 mM $AgNO_3$ 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 h, with continuous monitoring. After about 3 h, the mixture was observed to start changing from light brown to yellowish brown. After about 72 h, the mixture had completely changed colour to dark brown. This color change is visual evidence of formation of AgNPs or

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

reduction of silver ions into AgNPs due to the excitation of surface plasmon vibration [21, 24–28].

3.3 Characterization of the AgNPs

3.3.1 UV/VIS Measurements to Confirm Formation of AgNPs

The synthesis of AgNPs from the ethanolic extract of fruits of *Annona muricata* was further confirmed by ultraviolet–visible spectroscopy (UV/VIS) in the range of between 300 and 650 nm [24–27] and ethanol was used as a blank.

3.3.2 Temperature/Heat 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 min each and measuring the absorbance spectra on the UV/VIS in a scan range of 350 nm to 650 nm [29]. The temperature tested included room temperature (25 °C), 35 °C, 45 °C, 55 °C, 65 °C, 75 °C, and 85 °C.

3.3.3 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 [29, 30]. The absorbance spectra of the suspensions were then measured on the UV/VIS in a scan range of 300 nm to 650 nm.

3.3.4 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.

3.3.5 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 h. 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 min to about 45 min. 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 min 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 1.2 g of AgNPs were recovered following lyophilization.

3.3.6 Functional Groups Analysis

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} [31]. 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.

3.3.7 SEM and EDX Measurements

Scanning electron morphological analysis of Silver nanoparticles were performed using Scanning Electron Microscope FEI XL30 Sirion FEG (Oxford Instruments Plc, Abingdon, UK) 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.

3.3.8 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.

3.3.9 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 CuKα radiation (λ = 1.5406 Å) 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.

3.3.10 Dynamic Light Scattering (DLS) Analysis

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 [21, 26, 32].

4 Results

Figure 2 indicates the color change which is a visual evidence of the formation of AgNPs or reduction of silver ions into AgNPs due to the excitation of surface plasmon vibration.

The spectrum shown in Fig. 3 has a maximum absorption peak at a wavelength of about 427 nm, which is in the range of the surface plasmon resonance for AgNPs which is reported to have an absorption maximum of between about 400 nm to about 450 nm.

From Fig. 4 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.

From Fig. 5, 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. There was a notable and strong relationship between AgNPs absorption spectra at extreme acidic and alkaline pH conditions of 2 and 11.

From Fig. 6, it is evident that at all storage temperatures tested for the 3 months, the AgNPs remained stable

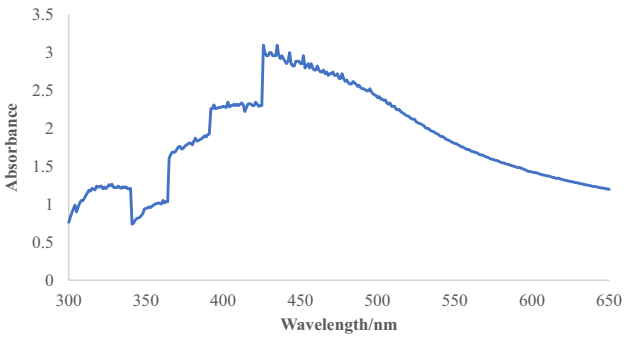

Fig. 3 UV/VIS spectrum of fruits derived AgNPs at 72 h of incubation

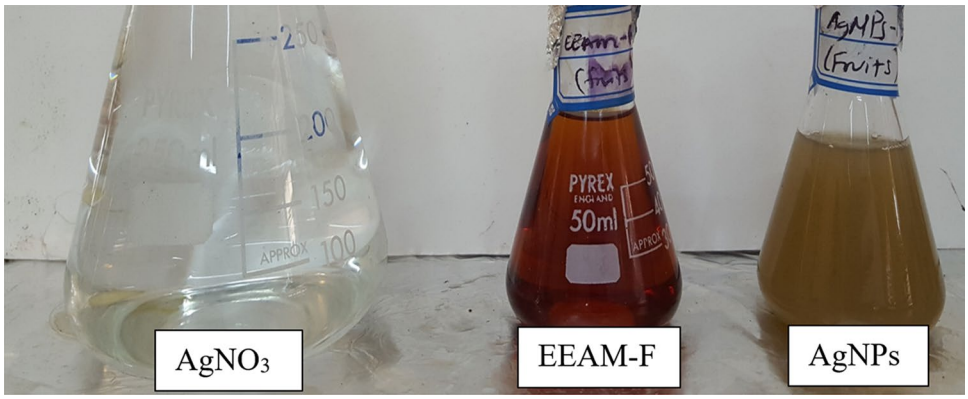

Fig. 2 Photo showing colour of the green synthesized AgNPs relative to the ethanolic extract of *Annona muricata* fruits (EEAM-F) and Silver Nitrate solution ($AgNO_3$) (Color figure online)

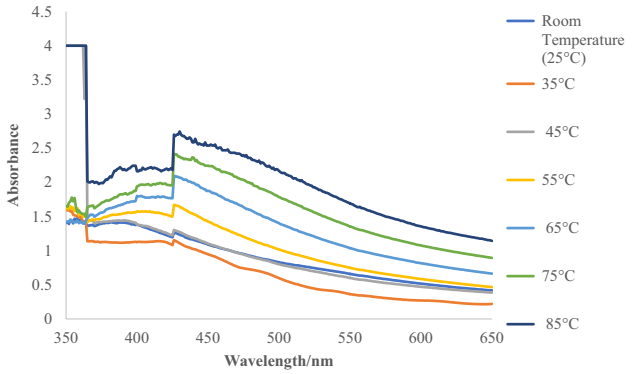

Fig. 4 UV/VIS spectra showing temperature stability of AgNPs synthesized from fruits extract

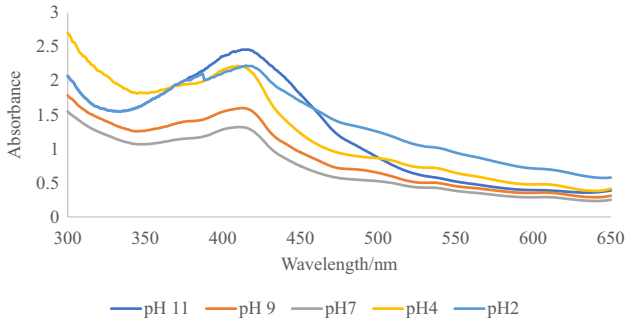

Fig. 5 UV/VIS spectra showing pH stability of AgNPs synthesized from fruits extract

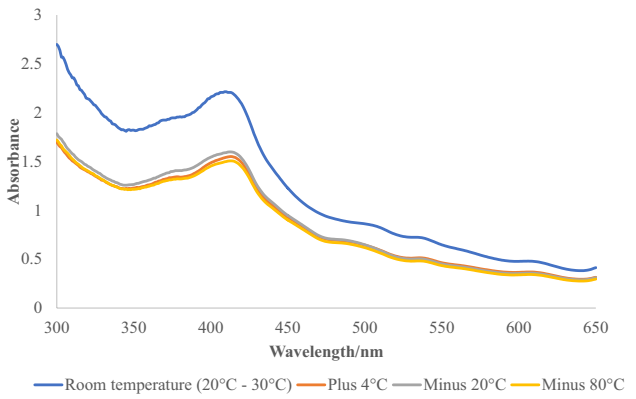

Fig. 6 UV/VIS spectra showing storage stability of AgNPs synthesized from fruits extract

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.

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

Type of Peak	Frequency (cm^{-1})	Bond	Functional groups
Major	2922.58	C–H stretch	Alkanes and alkyls
	2850	C–H stretch	Alkanes and alkyls
	1739.48	C=O stretch	Aldehyde and esters
	1500	N–O stretch	Nitro group
	1068.44	C–O stretch	Alcohol group
Minor	3400	O–H stretch	Carboxylic acids
	1650	C=O STRETCH	Amide
	1400	–C–H bend	Alkane
	1200	C–O stretch	Acid
	900	=C–H bend	Alkenes
	700	C–Cl stretch	Alkyl halide
	550	C–Br stretch	Alkyl halide

Table 1 shows the functional group analysis of the FTIR spectrum of the biosynthesized AgNPs from ethanolic extracts of fruits of *Annona muricata*.

As shown in Fig. 7 and Table 1 the functional groups responsible for the formation of the AgNPs included; alkanes and alkyls, aldehydes and esters, nitro groups, alcohol groups, carboxylic acids, amides, alkenes, acids and alkyl halides.

As shown in Fig. 8, 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 427 nm.

From Fig. 9, the EDX spectra showed the presence of elements such as Ag, Cl, Ca, and Si. EDX quantitative analysis demonstrated that the highest concentration of a single element in the *Annona muricata* derived AgNPs was silver (Ag), at about 80%.

Figure 10 shows the TEM micrographs of the AgNPs at different resolutions. The micrographs reveal a spherical nature of the monodispersed AgNPs as well as a crystalline structure. Particle size analysis using the Image-J software further revealed the AgNPs having an average particle size of about 51 nm.

Figure 11 shows the typical XRD pattern of biosynthesized AgNPs derived from ethanolic extracts of fruits of *Annona muricata*. Nine prominent diffraction peaks were observed at 28.07°, 32.50°, 38.41°, 44.61°, 46.56°, 55.18°, 57.85°, 64.86°, and 67.85°. The average size of the AgNPs formed in the bio-reduction was determined using the Scherrer equation and is estimated as 60.12 nm.

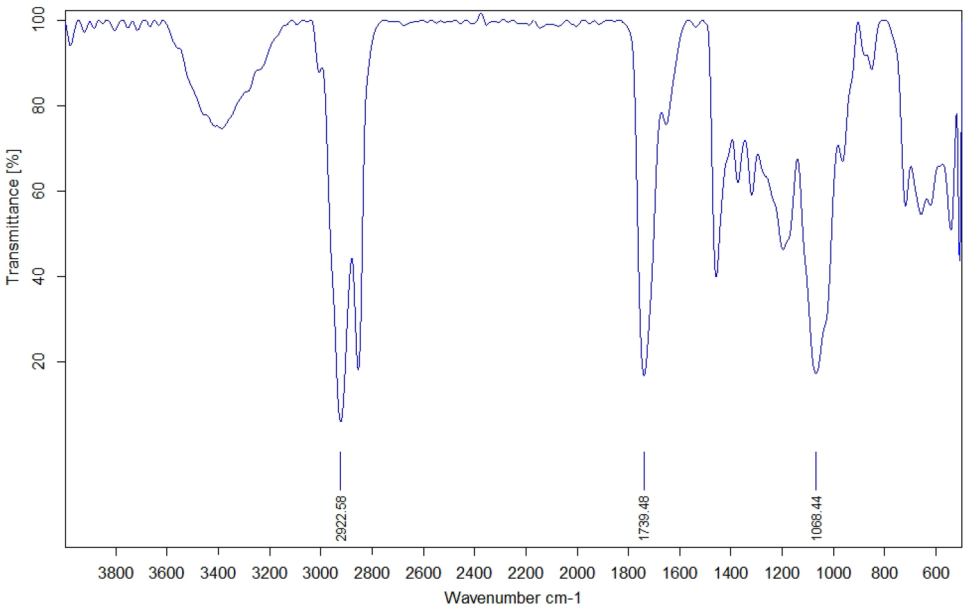

Fig. 7 FTIR spectra of functional groups from the AgNPs synthesized from fruits extract

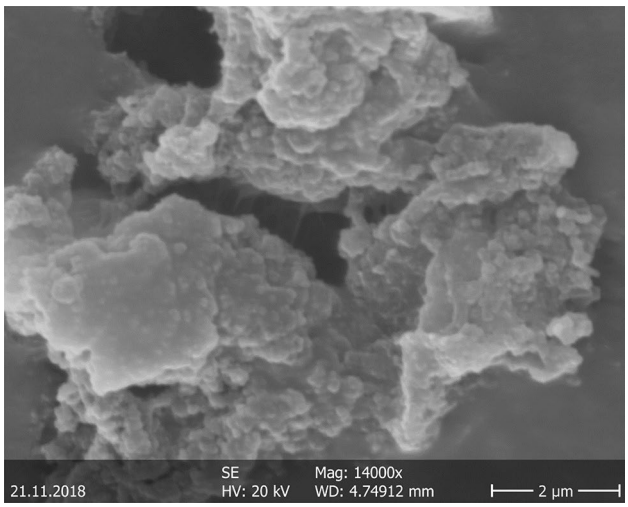

Fig. 8 SEM micrograph showing the shape of AgNPs synthesized from fruits extract

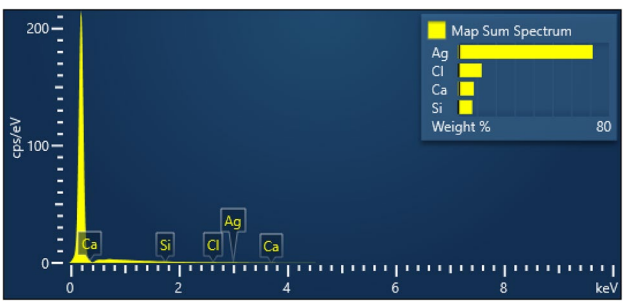

Fig. 9 Energy Dispersive X-ray Spectrometer (EDX) spectra demonstrating the quantitative amounts of different elements present in the AgNPs synthesized from the fruits extract

Table 2 shows the DLS analysis revealing the average particle size for the AgNPs as 103.5 nm with a polydispersity index of 0.1235. The bold values in the table indicate the mean values upon which quick reference maybe made in relation to the DLS and PDI results.

5 Discussion

It has been known for a long time that 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 [27, 33, 34]. In the current study, the AgNPs formation was confirmed by the change in colour of the mixture from light brown to dark brown indicating the successful green synthesis process. The UV/VIS maximum absorption spectra of the synthesized AgNPs was recorded at 427 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 435 nm [26], 430 nm [34], 420 nm [19, 21], 410 nm [35] among others. The current results further provide, for the first time, a confirmation on the use of the *Annona muricata* fruits extracts in the green synthesis of AgNPs as a cheap and eco-friendly approach.

The importance and use of any substances greatly depend on its stability under different conditions. In the current study, the temperature and heat stability, pH and storage stability of the biosynthesized AgNPs was studied and results have been presented. From the results on temperature stability, it is evident that at all temperatures tested, the AgNPs

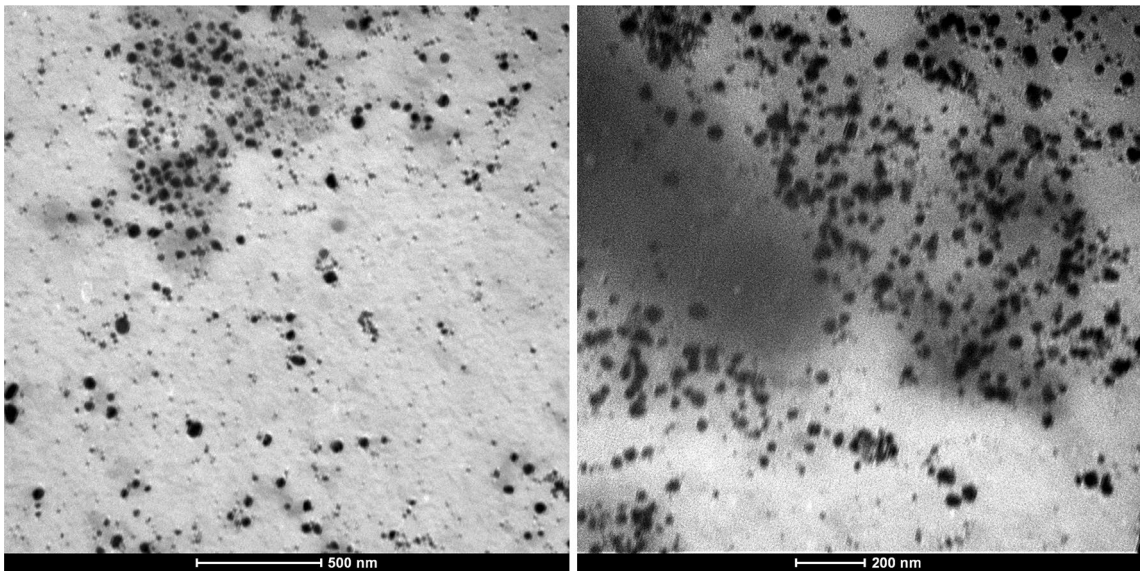

Fig. 10 TEM micrographs of the AgNPs at different resolutions

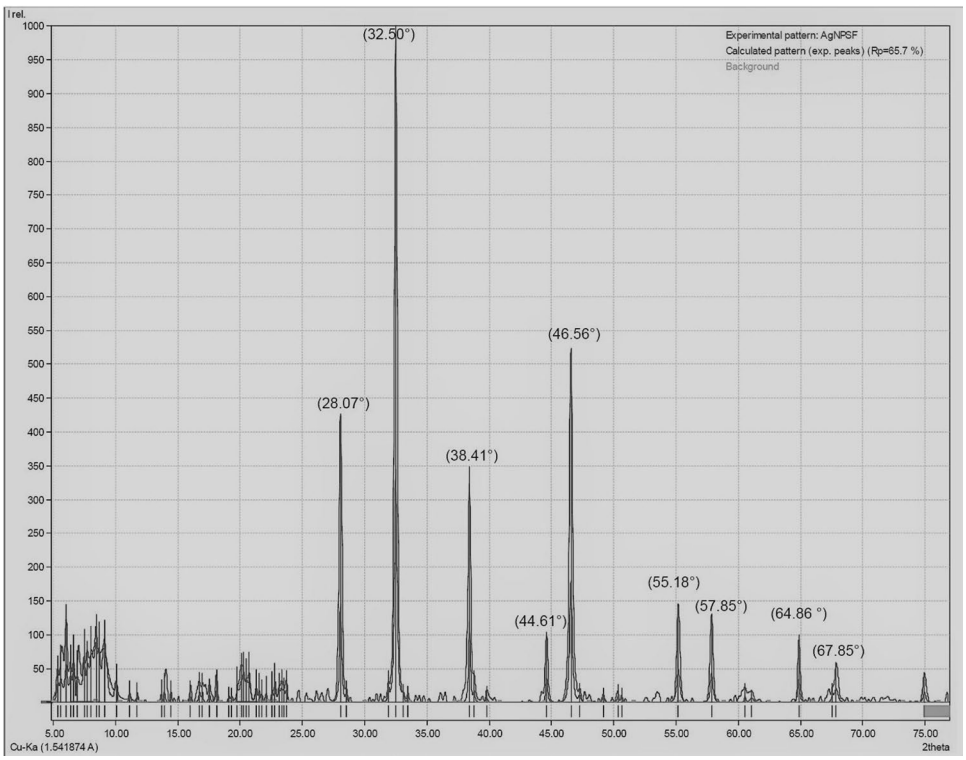

Fig. 11 XRD diffraction pattern spectra of AgNPs synthesized from fruits extract

remained stable maintaining a characteristic absorption maximum of about between 420 nm to about 430 nm which is within the AgNPs range [25, 26]. This is very important implying that the AgNPs can be stable under various temperature/heating conditions without losing their effectiveness. It is further important to note that there was an observed general spike in absorbance in the 350–370 nm region, but later stabilized and followed the normal trend for AgNPs. The possible explanation for this behavior is that these spikes would be due to the conditions of the synthesis process being slightly altered with the initial change in temperatures, even when they not affect the overall stability.

In relation to pH stability, it is evident that at all pH conditions tested, the AgNPs remained stable maintaining

Table 2 DLS analysis results

Counts	Intensity (kCnt/s)	Attenuation level (%)	Diameter (nm)	PD index
1	1361	95.1	103.3	1.268e−01
2	1331	95.1	103.9	1.047e−01
3	1378	95.1	103.7	1.105e−01
4	1360	95.1	103.3	1.349e−01
5	1321	95.1	103.1	1.406e−01
Mean	**1350**	**95.1**	**103.5**	**1.235e−01**
S	24	0.0	0.3	1.547e−02
S^2	559	0.0	0.1	2.394e−04

a characteristic absorption maximum of about between 410 nm to about 420 nm which is within the AgNPs range [25, 26]. 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. 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 [17]. Accordingly, 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. Generally, extreme changes in pH affect the shape and size of the particles because of the pH's ability to alter the charge of biomolecules, which might affect their capping as well as stabilizing abilities. These observations are in line with earlier studies that showed that extreme pH conditions lead to a shift in the peak wavelength indicating a slight increase in size of the particles [29, 30].

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, albeit 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 [25]. These however are not optimal for all plants. In the current study, we developed 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 h 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.

FTIR measurements are used to elucidate the functional groups responsible for the biosynthesis as well as stabilization and capping of the AgNPs. It is important to note that peaks in FTIR spectra can be divided into two regions: 4000–1500 cm^{-1} (the functional group region) and the 1500–400 cm^{-1} (the fingerprint region). Peaks in the functional group region arise from complex deformations of the molecule and they may be characteristic of molecular symmetry, or combination bands arising from multiple bonds deforming simultaneously. On the other hand, peaks in the fingerprint region are characteristic of specific kinds of bonds, and therefore can be used to identify whether a specific functional group is present. FTIR results showed that the functional groups responsible for the formation of the AgNPs from ethanolic extracts of fruits of *Annona muricata* included; Alkanes and alkyls, aldehydes and esters, nitro groups, alcohol groups, carboxylic acids, amides, alkenes, acids and alkyl halides. These are probably due to the presence of most of the secondary metabolites reported much earlier in the plant [9, 21, 23, 36, 37]. Notably, the narrow band at 1650 cm^{-1} can be attributed to C=O stretching probably due to the presence of amides which may be accountable for the reduction of Ag^+ ions to 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 427 nm. Many previous studies reported different shapes of AgNPs including spherical, conical, cuboidal, hexagonal, pentagonal among others [7, 25–27, 38]. 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 80%. 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 [38–40].

From the XRD diffraction patterns, the 2θ peaks observed at 38.41°, 44.61°, and 64.86° corresponds to (111), (200), and (220) reflection planes representing the face centered spherical structure of silver respectively [26, 41]. The extra

peaks near to 28.07°, 32.50°, 46.56°, 55.18°, 57.85°, and 67.85° 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 [26, 39, 42]. In comparison to the other eight peaks, the strong reflection at 32.50°, may perhaps signify the growth path of the nanocrystals or presence of other related intermediate compounds. The average size of the AgNPs formed in the bioreduction was estimated as 60.12 nm. TEM analysis further confirmed the crystalline and spherical nature of the monodispersed AgNPs. The average particle size as determined by TEM analysis was on average 51 nm, which is within range with that calculated using XRD.

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 [43]. The AgNPs size was larger as presented by DLS (103.5 nm) as compared to XRD (60.12 nm) and TEM (51 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 [21, 32].

Polydispersity Index measures the homogeneous nature of nanoparticles, the smaller the PDI the more homogeneous nanoparticles. It is basically a representation of the distribution of size populations within a given sample. 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 [32, 44]. The synthesized AgNPs had an average PDI of 0.1235, which is a great indication that they are highly homogenous and would be effectively used in various applications.

6 Conclusions

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 fruits 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 monodispersed AgNPs with an average size of about 60.12 nm and a polydispersity index of 0.1235. 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.

Acknowledgments The Authors thank The Sino-Africa Joint Research Centre (SAJOREC), the Uganda Natural Chemotherapeutics Research Institute and The Uganda National Crops Resources Research Institute (NaCRRI) for the support that enabled part of the work to be conducted in their respective Institutions. We also wish to thank Mr Atwijukire Evans, Wembabazi Enoch, Mr Mukasa Yusuf, and Dr Nuwamanya Ephraim of NaCRRI for their professional and technical support rendered during the time of the study. Last but not least, we thank the management of the Microanalytical System at the Earth Science Department, at the University of Fribourg, Switzerland for the additional support offered in the characterization of the AgNPs. Finally, we thank everyone that supported the process of samples collection in the different districts of Eastern Uganda, especially Naigaga Maureen, Mukwantampola George and Gaati Joweria (Kaliro District); Naikoba Macklyn and Lulenzi Jalia (Iganga District); as well as Kasajja Anthony, Salya Fred and Kirenzi Juma (Mbale District).

Author Contributions All Authors contributed substantively towards the work. "Conceptualization, YG, HAE, FW and GM; Data curation, YG, ENM, AMM and ESM; Formal analysis, YG and ESM; Funding acquisition, YG, ENM, FW and GM; Investigation, YG and ESM; Methodology, YG, AMM, ESM, HAE, FW and GM; Project administration, YG, ENM, FW and GM; Resources, YG, ENM, ESM, FW and GM; Software, YG and ESM; Supervision, ENM, HAE, FW and GM; Validation, YG, ENM, AMM, ESM, HAE, FW and GM; Writing – original draft, YG; Writing – review & editing, YG, ENM, AMM, ESM, HAE, FW and GM."

Funding This research was funded by the Pan African University Institute for Basic Sciences, Technology and Innovation Doctoral grant to YG, MB400-0007/17.

Data availability Sufficient data associated with this research and enough to draw the results and conclusions has been provided within the manuscript. However, all datasets have deposited in the public repository, Mendeley Data and is accessible via the link http://dx.doi.org/10.17632/jkj2x782wh.1 [45].

Compliance with Ethical Standards

Conflicts of interest 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.

References

1. P. Gonzalez-Melendi, R. Fernandez-Pacheco, M.J. Coronado, E. Corredor, P.S. Testillano, M.C. Risueo, C. Marquina, M.R. Ibarra, D. Rubiales, A. Perez-de-Luque, Nanoparticles as smart treatment-delivery systems in plants: assessment of different techniques of microscopy for their visualization in plant tissues. Ann. Bot. **101**, 187–195 (2008). https://doi.org/10.1093/aob/mcm283
2. C.J. Murphy, A.M. Gole, S.E. Hunyadi, C.J. Orendorff, One-dimensional colloidal gold and silver nanostructures. Inorg. Chem. **45**, 7544–7554 (2006). https://doi.org/10.1021/ic0519382
3. B.J. Wiley, Y. Chen, J.M. McLellan, Y. Xiong, Z.-Y. Li, D. Ginger, Y. Xia, Synthesis and optical properties of silver nanobars and nanorice. Nano Lett. **7**, 1032–1036 (2007). https://doi.org/10.1021/nl070214f
4. Y. Xiong, H. Cai, B.J. Wiley, J. Wang, M.J. Kim, Y. Xia, Synthesis and mechanistic study of palladium nanobars and nanorods. J. Am. Chem. Soc. **129**, 3665–3675 (2007). https://doi.org/10.1021/ja0688023
5. J. Fang, H. You, P. Kong, Y. Yi, X. Song, B. Ding, Dendritic silver nanostructure growth and evolution in replacement reaction. Cryst. Growth Des. **7**, 864–867 (2007). https://doi.org/10.1021/cg0604879
6. A. Narayan, L. Landström, M. Boman, Laser-assisted synthesis of ultra small metal nanoparticles. Appl. Surf. Sci. **208–209**, 137–141 (2003). https://doi.org/10.1016/S0169-4332(02)01352-1
7. S. Ahmed, M. Ahmad, B.L. Swami, S. Ikram, A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J. Adv. Res. **7**, 17–28 (2016). https://doi.org/10.1016/j.jare.2015.02.007
8. A.V. Coria-Téllez, E. Montalvo-Gónzalez, E.M. Yahia, E.N. Obledo-Vázquez, *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab. J. Chem. **11**, 662–691 (2018). https://doi.org/10.1016/j.arabjc.2016.01.004
9. Y. Gavamukulya, F. Wamunyokoli, H.A. El-Shemy, *Annona muricata*: is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. Asian Pac. J. Trop. Med. **10**, 835–848 (2017). https://doi.org/10.1016/J.APJTM.2017.08.009
10. A. Pinto, Q. De, M. Cordeiro, F. De Andrade, S.R.M. Ferreira, H. Filgueiras, C. De, R. Alves, D. Kinpara, *Annona muricata*, in *Annona species*, ed. by J.T. Williams (Taxon. Bot. International Centre of Underutilized Crops, University of Southampton, Southampton, 2005), pp. 3–16
11. A. Benavides, A. González, J. Cisne-Contreras, Numerical characterization of Guanabana (*Annona muricata* L.) germplasm Sampling in situ in the Pacific and northern Nicaragua. La Calera **10**, 46–52 (2004)
12. S. Evangelista-Lozano, J. Cruz-Castillo, S. Pérez-González, E. Mercado-Silva, G. Dávila-Ortiz, Production and fruit quality of guanabanos (*Annona muricata* L.) from Jiutepec seed, Morelos, Mexico. Hortic. Ser. **9**, 69–79 (2003)
13. C. Orwa, A. Mutua, R. Kindt, Agroforestree Database: A Tree Species Reference and Selection Guide Version 4.0. ICRAF, Nairobi (2009)
14. Y. Gavamukulya, F. Abou-Elella, F. Wamunyokoli, H.A. El-Shemy, GC-MS analysis of bioactive phytochemicals present in ethanolic extracts of leaves of *Annona muricata*: a further evidence for its medicinal diversity. Pharmacogn. J. **7**, 300–304 (2015). https://doi.org/10.5530/pj.2015.5.9
15. N. Badrie, A. Schauss, Soursop (*Annona muricata* L.): composition, nutritional value, medicinal uses, and toxicology, in *Bioactive Foods in Promoting Health*, ed. by R. Watson, V. Preedy (Academic Press, Oxford, 2010), pp. 621–643
16. Y. Gavamukulya, E.N. Maina, A.M. Meroka, E.S. Madivoli, H.A. El-Shemy, G. Magoma, F. Wamunyokoli, Liquid Chromatography single quadrupole mass spectrometry (LC/SQ MS) analysis reveals presence of novel antineoplastic metabolites in ethanolic extracts of fruits and leaves of *Annona muricata*. Pharmacogn. J. **11**(4), 660–668 (2019)
17. B.V. Bonifácio, P.B. da Silva, M.A.D.S. Ramos, K.M.S. Negri, T.M. Bauab, M. Chorilli, Nanotechnology-based drug delivery systems and herbal medicines: a review. Int. J. Nanomed. **9**, 1–15 (2014). https://doi.org/10.2147/IJN.S52634
18. S.H. Ansari, F. Islam, M. Sameem, Influence of nanotechnology on herbal drugs: a review. J. Adv. Pharm. Technol. Res. **3**, 142–146 (2012). https://doi.org/10.4103/2231-4040.101006
19. S.B. Santhosh, R. Yuvarajan, D. Natarajan, *Annona muricata* leaf extract-mediated silver nanoparticles synthesis and its larvicidal potential against dengue, malaria and filariasis vector. Parasitol. Res. **114**, 3087–3096 (2015). https://doi.org/10.1007/s00436-015-4511-2
20. S.A. Akintelu, A.S. Folorunso, Characterization and antimicrobial investigation of synthesized silver nanoparticles from *Annona muricata* leaf extracts. HSOA J. Nanotechnol. Nanomed. Nanobiotechnol. **6**, 22 (2019). https://doi.org/10.24966/ntmb-2044/100022
21. K.M. Ezealisiji, X.S. Noundou, S.E. Ukwueze, Green synthesis and characterization of monodispersed silver nanoparticles using root bark aqueous extract of *Annona muricata* Linn and their antimicrobial activity. Appl Nanosci. **7**, 905–911 (2017). https://doi.org/10.1007/s13204-017-0632-5
22. V.S. Shaniba, A. Abdul-Aziz, P.R.M. Kumar, Phyto-mediated synthesis of silver nanoparticles from *Annona muricata* fruit extract, assessment of their biomedical and photocatalytic potential. Int. J. Pharm. Sci. Res. **8**, 170–181 (2017). https://doi.org/10.13040/ijpsr.0975-8232.8(1).170-81
23. Y. Gavamukulya, F. Abou-Elella, F. Wamunyokoli, H.A. El-Shemy, Phytochemical screening, anti-oxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). Asian Pac. J. Trop. Med. **7**, S355–S363 (2014). https://doi.org/10.1016/S1995-7645(14)60258-3
24. S.B. Santhosh, C. Ragavendran, D. Natarajan, Spectral and HRTEM analyses of *Annona muricata* leaf extract mediated silver nanoparticles and its larvicidal efficacy against three mosquito vectors *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. J. Photochem. Photobiol. B Biol. **153**, 184–190 (2015). https://doi.org/10.1016/j.jphotobiol.2015.09.018
25. M. Shah, D. Fawcett, S. Sharma, S. Tripathy, G. Poinern, Green synthesis of metallic nanoparticles via biological entities. Materials (Basel). **8**, 7278–7308 (2015). https://doi.org/10.3390/MA8115377
26. B. Kumar, K. Smita, L. Cumbal, A. Debut, Green synthesis of silver nanoparticles using Andean blackberry fruit extract. Saudi J. Biol. Sci. **24**, 45–50 (2017). https://doi.org/10.1016/J.SJBS.2015.09.006
27. S. Ahmed, M. Saifullah, B.L. Ahmad, S. Swami, Ikram, Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J. Radiat. Res. Appl. Sci. **9**, 1–7 (2016). https://doi.org/10.1016/j.jrras.2015.06.006
28. P. Patil Shriniwas, T. Kumbhar Subhash, Antioxidant, antibacterial and cytotoxic potential of silver nanoparticles synthesized using terpenes rich extract of *Lantana camara* L. leaves. Biochem. Biophys. Rep. **10**, 76–81 (2017). https://doi.org/10.1016/j.bbrep.2017.03.002
29. G. Ghoshal, S. Bhatnagar, Rapid green synthesis of silver nanoparticles (AgNPs) using (*Prunus persica*) plants extract: exploring its antimicrobial and catalytic activities. J. Nanomed. Nanotechnol. (2017). https://doi.org/10.4172/2157-7439.1000452

30. A. Verma, M.S. Mehata, Controllable synthesis of silver nanoparticles using neem leaves and their antimicrobial activity. J. Radiat. Res. Appl. Sci. **9**, 109–115 (2016). https://doi.org/10.1016/j.jrras.2015.11.001
31. E.S. Madivoli, E.G. Maina, P.K. Kairigo, M.K. Murigi, J.K. Ogilo, J.O. Nyangau, P.K. Kimani, C. Kipyegon, In vitro antioxidant and antimicrobial activity of *Prunus africana* (Hook. f.) Kalkman (bark extracts) and *Harrisonia abyssinica* Oliv. extracts (bark extracts): a comparative study. J. Med. Plants Econ. Dev. **2**, 1–9 (2018). https://doi.org/10.4102/jomped.v2i1.39
32. M. Danaei, M. Dehghankhold, S. Ataei, F. Hasanzadeh Davarani, R. Javanmard, A. Dokhani, S. Khorasani, M.R. Mozafari, Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics **10**, 1–17 (2018). https://doi.org/10.3390/pharmaceutics10020057
33. S. Shankar, A. Rai, A. Ahmad, A. Sastry, Rapid synthesis of Au, Ag, and bimetallic Au core Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. J. Colloid Interface Sci. **275**, 496–502 (2004)
34. J.Y. Song, S.K. Beom, Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng. **32**, 79–84 (2009). https://doi.org/10.1007/s00449-008-0224-6
35. S.V. Otari, S.H. Pawar, S.K.S. Patel, R.K. Singh, S.Y. Kim, J.H. Lee, L. Zhang, J.K. Lee, *Canna edulis* leaf extract-mediated preparation of stabilized silver nanoparticles: characterization, antimicrobial activity, and toxicity studies. J. Microbiol. Biotechnol. **27**, 731–738 (2017). https://doi.org/10.4014/jmb.1610.10019
36. A.V. Coria-Téllez, E. Montalvo-Gónzalez, E.M. Yahia, E.N. Obledo-Vázquez, *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab. J. Chem. (2016). https://doi.org/10.1016/j.arabjc.2016.01.004
37. S.Z. Moghadamtousi, M. Fadaeinasab, S. Nikzad, G. Mohan, H. Ali, H. Kadir, *Annona muricata* (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. Int. J. Mol. Sci. **16**, 15625–15658 (2015). https://doi.org/10.3390/ijms160715625
38. M. Goudarzi, N. Mir, M. Mousavi-Kamazani, S. Bagheri, M. Salavati-Niasari, Biosynthesis and characterization of silver nanoparticles prepared from two novel natural precursors by facile thermal decomposition methods. Sci. Rep. **6**, 1–13 (2016). https://doi.org/10.1038/srep32539
39. G.A. Otunola, A.J. Afolayan, In vitro antibacterial, antioxidant and toxicity profile of silver nanoparticles green-synthesized and characterized from aqueous extract of a spice blend formulation. Biotechnol. Biotechnol. Equip. **32**, 724–733 (2018). https://doi.org/10.1080/13102818.2018.1448301
40. A. Sudha, J. Jeyakanthan, P. Srinivasan, Green synthesis of silver nanoparticles using *Lippia nodiflora* aerial extract and evaluation of their antioxidant, antibacterial and cytotoxic effects. Resour. Technol. **3**, 506–515 (2017). https://doi.org/10.1016/J.REFFIT.2017.07.002
41. B. Kumar, Y. Angulo, K. Smita, L. Cumbal, A. Debut, Capuli cherry-mediated green synthesis of silver nanoparticles under white solar and blue LED light. Particuology. **24**, 123–128 (2016). https://doi.org/10.1016/j.partic.2015.05.005
42. M. Umadevi, S. Shalini, M.R. Bindhu, Synthesis of silver nanoparticle using *D. carota* extract. Adv. Nat. Sci. Nanosci. Nanotechnol. **3**, 025008 (2012). https://doi.org/10.1088/2043-6262/3/2/025008
43. E. Tomaszewska, K. Soliwoda, K. Kadziola, B. Tkacz-Szczesna, G. Celichowski, M. Cichomski, W. Szmaja, J. Grobelny, Detection limits of DLS and UV–Vis spectroscopy in characterization of polydisperse nanoparticles colloids. J. Nanomater. (2013). https://doi.org/10.1155/2013/313081
44. S.P. Clarke, Development of hierarchical magnetic nanocomposite materials for biomedical applications. Ph.D. Thesis, Dublin City University, Northside, Dublin, 2013
45. Y. Gavamukulya, E.N. Maina, A.M. Meroka, E.S. Madivoli, H.A. El-Shemy, F. Wamunyokoli, G. Magoma, Data for green synthesis and characterization of silver nanoparticles from ethanolic extracts of fruits of *Annona muricata*. Mendeley Data. v1 (2019). https://doi.org/10.17632/jkj2x782wh.1