RESEARCH PAPER



Green synthesis of silver nanoparticles using *Annona squamosa* L. seed extract: characterization, photocatalytic and biological activity assay

Vimala Jose¹ · Lidiya Raphel¹ · K. S. Aiswariya¹ · Paulson Mathew²

Received: 28 February 2021 / Accepted: 23 March 2021

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

The aqueous seed extract of *Annona squamosa* L. was used as a reducing and stabilizing agent for the synthesis of silver nanoparticles (AgNPs). The formation of AgNPs in aqueous silver nitrate solution after the addition of the extract was indicated by a colour change from pale yellow to dark brown corresponding to a λ_{max} at 430 nm. The phytochemicals in the extract, responsible for efficient capping and stabilization of the nanoparticles, were identified by FTIR. Powder XRD pattern demonstrated the polycrystalline nature of the AgNPs. TEM image confirmed that AgNPs were spherical in shape and the average particle size was found to be 22 nm. Further, the nanoparticles exhibited good catalytic activity towards the degradation of coomassie brilliant blue dye and demonstrated significant antibacterial activity. Their larvicidal activity against mosquito larvae showed a LC₅₀ value 22.44 µg/mL against III instars. In addition, AgNPs positively influenced the germination of chickpea seeds.

Graphic abstract



Keywords Silver nanoparticles · Annona squamosa L. · Photocatalysis · Bioactivity

Introduction

Nanoparticles are made up of atomic aggregations which are spherical or quasi-spherical structures with 1–100 nm in diameter. When compared to conventional physical or

Paulson Mathew paulson.org@gmail.com

¹ Department of Botany, Centre for Biotechnology, St. Thomas College (Autonomous), University of Calicut, Thrissur, Kerala 680001, India

² Department of Chemistry, Centre for Sustainability Science, St. Thomas College (Autonomous), University of Calicut, Thrissur, Kerala 680001, India chemical processes for the preparation of nanoparticles, green methods have a greater number of benefits. Their main advantage is the lack of impact on the environment, as the methods are in line with the principles of green chemistry and the process is cost-effective. Another advantage is the easy transfer of the process to an industrial scale. Due to their medicinal and antimicrobial properties [1], silver nanoparticles (AgNPs) are incorporated in many consumer products and have become one of the most commercialised nanoparticles [2].

Various plant species had been investigated for the synthesis of AgNPs [3] and such nanoparticles possess promising antibacterial properties [4]. For instance, nanoparticles of silver are effective in inhibiting the growth of both grampositive and gram-negative bacteria [5–7]. With the rise in antibiotic resistance and the development of new antibiotics, research has begun to focus on these antibacterial nanoparticles as potential new medical tools. Silver is generally used in the nitrate form to induce an antimicrobial effect, but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbe to be exposed to. Bactericidal effects of silver nanoparticles have been documented against bacterial strains such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Listeria innocua, Salmonella Choleraesuis* [8–10]. Silver nanoparticles have also been used as optical sensors for the formation of small-molecule adsorbates [11].

Excessive usage of conventional chemicals and pesticides in land and water resources causes many risks to people and the environment. Silver nanoparticles and their composites show improved catalytic activities in dye reduction and removal [12] as documented for organic dyes using *Trigonella foenum-graecum* seeds [13], methyl orange using *Ulva lactuca* [14]. The colloidal solution of silver nanoparticles was found to exhibit mosquito larvicidal activity against dengue and filariasis vector [15]. Larvicidal activity of AgNPs has been studied using *Leucas aspera* [16], *Belosynapsis kewensis* [17], *Excoecaria agalloch* [18] and *Ficus racemosa* [19]. Among the different metal nanoparticles, AgNPs are known to be positively influencing seed-germinaton process. To date, there are only a few reports on the impact of AgNPs to promote the growth and seed germination [20].

Annona squamosa L., the sugar apple which belongs to the Annonaceae family was selected for the present study. Annonaceae, plant family commonly named as custard apple family is marked for many medicinal properties such as antiulcer, anti-convulsant and antibacterial activity. The plant also possesses analgesic, anti-inflammatory, anti-microbial, cytotoxic, anti-oxidant, anti-lipidimic, molluscicidal, genotoxic, vasorelaxant, anti-tumour, hepatoprotective, larvicidal, insecticidal and anthelmintic properties [21]. The roots, leaves and seeds of A. squamosa have several medicinal properties [22]. The active fraction isolated from A. squamosa seed extract has strong antibacterial, antioxidant and antitumor activities due to the presence of annonaceous acetogenins, the expanding class of potential long-chain fatty acid which were initially noticed only in this species [23]. Significant interest in the studies of its derivatives present in the seeds of the plant also points to their remarkable antitumour and pesticidal activities [24]. Biosynthesis of AgNPs using the seed extract of A. squamosa, its characterization and biological activity assay has been done in this study. Present approach for the synthesis of AgNPs using a waste part of the plant material has economic and environmental benefits compared to conventional chemical or physical methods of nanoparticle synthesis. As reported in other plant-mediated synthesis of silver nanopartilcles, the phytochemicals act as capping and stabilizing agent, can enhance the biological activity of the AgNPs [8].

Materials and methods

Sample preparation

The fruits of *Annona squamosa* L. were procured from Thrissur, Kerala, India (Fig. 1). The plant was identified with the help of Flora of the Presidency of Madras [25]. The seeds were separated from the fruit and oven dried. Finely ground seeds of *A. squamosa* (20 g) were boiled with 200 mL of distilled water to prepare the aqueous extract. The filtrate of the extract was further centrifuged at 2000 rpm for 10 min and the extract was stored in amber coloured reagent bottles at 4 °C for further use.

Synthesis of silver nanoparticles

Silver nitrate solution (100 mL, 1 mmol) was added to a beaker containing 10 mL of the seed extract and mixed well. The reaction mixture was kept under different physical conditions such as at room temperature, at 60 °C, sunlight and ultraviolet lamp (30 W, 253 nm, Philips Holland). When the solution was kept under sunlight for 30 min, the colour of the mixture changed to yellowish-brown and later to reddish-brown.

Characterization of AgNPs

UV–Visible analysis

To monitor the complete bioreduction of $AgNO_3$ to silver nanoparticles, 1 mL of the sample suspension was diluted with 2 mL of distilled water and the spectrum of this sample was recorded using UV-Visible



Fig. 1 Fruit and seed of Annona squamosa L.

spectrophotometer (Shimadzu UV probe 1800) in the scanning range 200–700 nm having a resolution of 1 nm.

FT-IR analysis

The seed extract (10 mL) was added to 100 mL $AgNO_3$ (1 mmol) and kept under sunlight with continuous stirring. After 30 min exposure, it was centrifuged at 14,000 rpm (High speed-refrigerated-centrifuge, Thermo Electron LED, Germany) for 20 min. The pellet was suspended in distilled water, centrifuged twice and allowed to dry in a hot air oven. The AgNPs thus obtained were used for FT-IR and XRD analysis.

FT-IR analysis of the dried AgNPs was carried out using potassium bromide (KBr) pellet method. The spectrum was recorded using Fourier transform infrared spectrometer (Par-kin-Elmer Pvt Ltd.) equipped with JASCO IRT-7000 Intron Infrared Microscope in transmittance mode operating at a resolution of 4 cm⁻¹.

XRD analysis

PANalytical X-ray diffractometer at a scanning rate of 20 min⁻¹ with an operating voltage of 40 kV, monochromatic filter in the 2 θ range 10–80, was used in the present study. It was used to examine phase identification and characterization of the crystal structure of the nanoparticles.

TEM analysis

Transmission electron microscopy (TEM) technique was used to visualize the morphology and size of the synthesized AgNPs. The 200 kV ultra-high resolution transmission electron microscope (JEOL, JEM 2100 h with EELS) was used. TEM grids were prepared by placing 5μ L of the assynthesized AgNP solutions on carbon-coated copper grids and dried under the lamp.

Biological activity of AgNPs

Photocatalytic degradation of toxic dye

Coomassie Brilliant Blue (CBB) is a member of triphenylmethane dyes that were developed for use in the textile industry but are now commonly used for staining proteins in analytical biochemistry. A freshly prepared solution (1 mL) containing silver nitrate and the plant extract (ratio10:1) was added to 5 mL of 1% CBB solution with stirring and kept under sunlight. At specific time intervals (10, 20, 30 min), 2 mL of the solution was taken and the absorbance (480–680 nm) was recorded.

Antibacterial assay

Nutrient agar media (25 mL, Hi-Media, Mumbai) was poured into petriplates under sterile conditions and left to solidify at room temperature. The culture suspensions from pure cultures of Gram-positive bacteria Staphylococcus aureus (MTCC96) and Gram-negative bacteria Klebsiella pnuemoniae (MTCC109) were chosen based on their clinical and pharmacological importance [26]. The bacterial strains were obtained from the Institute of Microbial Technology, Chandigarh, India. Antibacterial activities of AgNPs against these two bacterial strains were investigated by agar disc diffusion method [27]. The sterile filter paper discs (6 mm diameter) were saturated with 30 µL each of 100 µg/mL of AgNPs, 0.1 g/mL of the plant extract (negative control) and tetracycline disc (positive control) with deminerised water as solvent. The filter paper discs were placed equidistantly on the inoculated media and diffusion of the solution was allowed to occur for 30 min at room temperature. Plates were then inverted and incubated at 37 °C for 24 h. Triplicates were employed per treatment and the average zone of inhibition was recorded. Significance levels of standard and treatments were compared with one way ANOVA test using SPSS 20.0 software.

Larvicidal bioassay

Anopheles stephensi mosquitos were reared in the vector control laboratory, Kerala Veterinary and Animal Sciences University, Thrissur, Kerala, India. The larvae were fed on dog biscuits and yeast powder in 3:1 ratio. Adults were fed blood through a parafilm. Mosquitoes were held at 28 ± 2 °C temperature and 70–85% relative humidity with a 12 h light/12 h dark photoperiod. Larvicidal activity of the biogenic AgNPs was evaluated according to WHO protocol [28]. Five replicates having 100 larvae each of late III and early IV instar stages were used for bioassays. Five concentrations of AgNPs (12, 24, 36, 48 and 60 µg/mL) and water control replicates were run simultaneously. The number of dead and alive larvae in the replicates was recorded after 24 h and the results were expressed as percent mortality. The lethal concentrations that kill 50 percent of the treated larvae (LC 50) and 90 percent of the treated larvae (LC 90) were calculated [29]. The average larval mortality data were subjected to probit analysis for calculating LC 50 and LC 90 at 95% of upper confidence limit and lower confidence limit. Chi-squared values were calculated using SPSS 20.0 software.

Phytotoxicity assay

Germination test was performed using chickpea (*Cicer arietinum*) seeds. 50 surface-sterilized seeds were kept on

moist filter papers soaked in the respective treatment solution (25%, 50%, 75% and 100% v/v of AgNP dispersions) in sterilized petriplates and were incubated in dark at 25 °C. Seeds with root tip 1 mm and higher were considered as germinated. The length of root and shoot (in mm) obtained following 72 h after the germination of seeds was observed and percent germination was calculated thereafter.

Results and discussion

Synthesis of silver nanoparticles

Reduction of $AgNO_3$ to silver nanoparticles took place when the aqueous seed extract of *A. squamosa* was added to 1 mmol silver nitrate solution. Formation of AgNPs was indicated by the colour change of the solution to deep reddish-brown when the solution was exposed to sunlight for 30 min (Fig. 2a). The solution kept at 30 °C (rt), 60 °C or under UV lamp showed only light yellow colour even after 24 h of exposure. AgNPs appeared to be reddish-brown in the aqueous medium as a result of surface plasmon vibrations.

Characterization of AgNPs

Characterization of the AgNPs has been done using UV–Visible spectroscopy, Fourier transform infrared spectrophotometer, X-ray diffractometer, and Transmission electron microscope. These techniques help to monitor the shape, size, surface area, and crystalline structure of nanoparticles.

UV–Visible analysis

Absorption spectra of AgNPs formed in the reaction media after 30 min from the initiation of the reaction have an absorption maximum in the range 420–450 nm due to surface plasmon resonance (SPR) of AgNPs (Fig. 2b) [30]. Secondary metabolites such as fatty acids and polyphenols present in the seed extract is responsible for the reduction of silver nitrate to silver nanoparticles. Previous studies have reported that the seeds of *A. squamosa* can be used as a potential candidate in pharmacological preparations due to their bioactive secondary metabolites [31]. The broadening of the peak also indicated the formation of polydispersed nanoparticles [32].

FT-IR analysis

Prominent bands of absorbance in FT-IR spectra (Fig. 3) were observed at 1096, 1232, 1319, 1386, 1636, 2208, 3400 cm^{-1} . These peaks represent respectively ether linkage, C–N, O–H bending, aliphatic and aromatic –C=C–, amide,

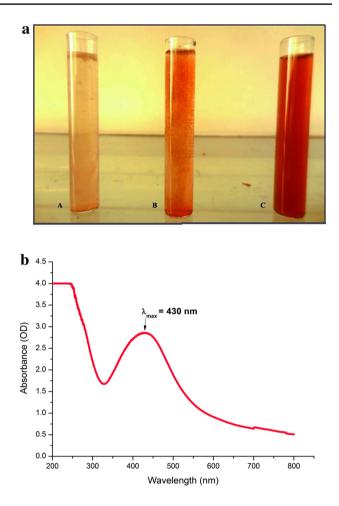


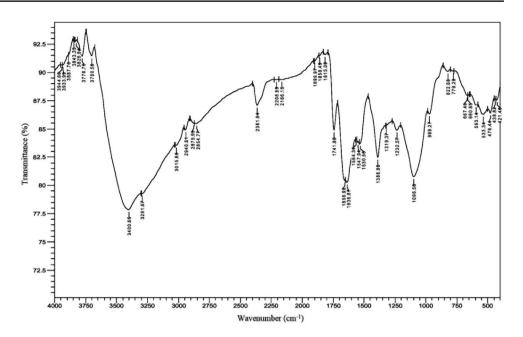
Fig.2 a Colour change during the formation of AgNPs. b UV–Vis spectrum

alkynic and phenolic or alcoholic groups present in the phytochemicals. The FT-IR analysis confirms the capping over AgNPs with plant-derived secondary metabolites. These capped AgNPs helped to enhance the stability of AgNPs in colloidal solution by preventing aggregation of particles. Since they are capped by biomolecules they may serve as a better candidate for the drug delivery systems [33]. Synthesis of nanoparticles using plant extracts can potentially eliminate the problem of chemical agents for nanoparticle capping, which may have adverse effects in its application, thus making plant-derived nanoparticles more compatible.

XRD analysis

XRD pattern of the biosynthesized AgNPs showed two intense peaks in the spectrum with 2θ values ranging from 20 to 80 (Fig. 4). XRD spectra of pure crystalline silver structures have been published by the Joint Committee on Powder Diffraction Standards (File no.04-0783). A comparison of the XRD spectrum with the standard confirmed

Fig. 3 IR spectrum of AgNPs synthesised using *Annona squamosa* L. seed extract



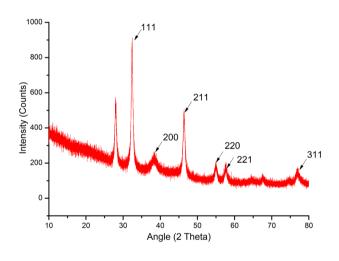


Fig. 4 XRD pattern of the silver nanoparticles

that the silver particles formed in our experiments were in the crystalline phase. The peaks at 2θ values 31.55° and 45.63° can be indexed respectively as 111, 200 planes of face-centred cubic silver. The Bragg reflections corresponding to (111) sets of lattice planes were observed which may be indexed based on the face-centred cubic structure of silver. The XRD pattern thus clearly indicated that the silver nanoparticles were crystalline. The other peaks having 2θ values at 27.17° , 35.36° , 53.65° and 56.81° are due to the coexistence of organic compounds accompanying crystalline AgNPs. Such unidentified crystalline peaks are apparent in many works in which the XRD pattern includes the relevant 2θ range [34].

TEM analysis

Nanoparticle composition and size distribution could be visualized through the Transmission electron microscope. The TEM images of AgNPs obtained in the present study are shown in Fig. 5. TEM image explained the size and shape of the silver nanoparticles. The particle size of AgNPs showed a size range 14-28 nm. The average particle size observed is 22 ± 5 nm. Furthermore, the analysis demonstrated that the AgNPs were polycrystalline with irregular spherical shapes and narrow size distribution. Energy-dispersive X-ray spectroscopy (EDAX) analysis of the AgNPs showed a highpitched absorption peak at 2.5 keV inferring the existence of metallic silver (Fig. 5c). Elements from the plant residue as well as residual copper from copper grid account for the rest of the peaks in the spectrum. AgNPs prepared using plant extracts are stable in solution up to 4 weeks after its synthesis due to the presence of a thin layer of organic material surrounding the nanoparticles. This is one of the advantages of nanoparticles synthesised using plant extract than the ones which were synthesised using chemical methods.

Biological activity assay of AgNPs

The unique properties of silver nanoparticles have been investigated for dye degradation, antimicrobial applications, larvicidal activities and phytotoxicity assay.

Photocatalytic degradation of toxic dye

Silver nanoparticles and their composites showed greater catalytic activity towards dye degradation and its removal.

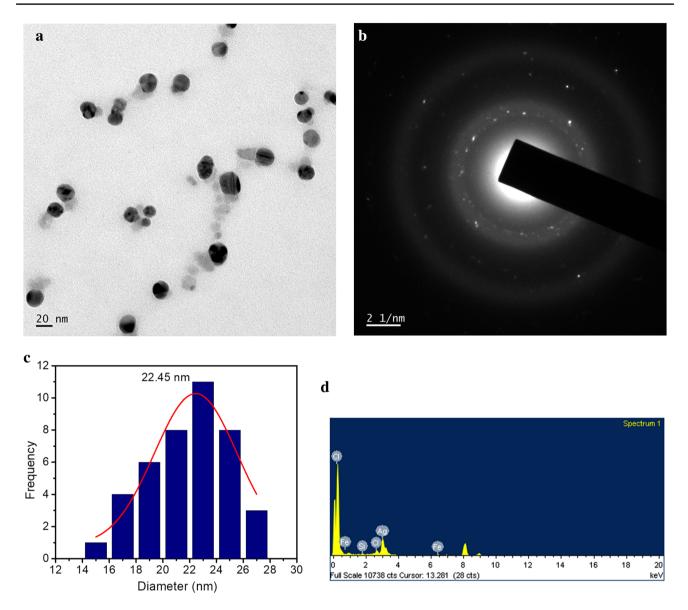
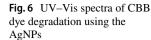


Fig. 5 a TEM image b SAED pattern and c particle size distribution histogram and d EDAX image of AgNPs

In the present study, the surface plasmon resonance (SPR) band of CBB–AgNPs solution was observed at 430 nm which became apparent after 10 min of mixing CBB with silver nitrate and the plant extract (ratio10:1). The absorption at 430 nm is due to the formation of metallic silver nanoclusters and its intensity increases with time. This hyperchromic shift is due to the increase in the number of nanoparticles formed by the reduction of silver ions present in the aqueous solution. The peak corresponding to CBB observed around 580 nm disappeared completely within 30 min indicating the photocatalytic degradation of Coomassie Brilliant Blue (Fig. 6).

Antibacterial assay

Silver nanoparticles have been extensively studied in recent years due to their antibacterial and therapeutic potential. They can create reactive oxygen species which cause irreversible damage to bacteria and also have a strong affinity in binding to DNA or RNA which interferes with the microbial replication process [35]. In this study, the antimicrobial property of AgNPs was investigated against *Klebsiella pneumonia* (Gram negative) and *Staphylococcus aureus* (Gram positive) by disc diffusion method under sterilized conditions using Tetracyclin as positive control (Fig. 7).



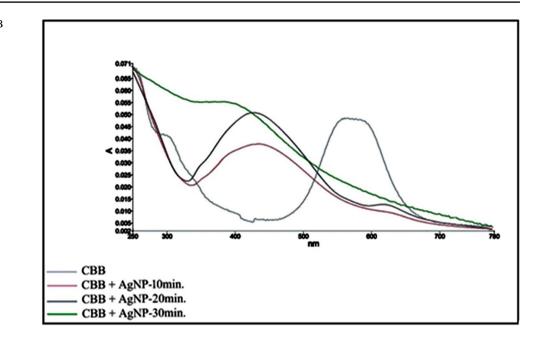
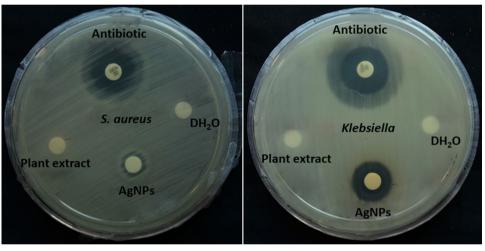


Fig. 7 Antibacterial study of AgNPs



Triplicates were employed per treatment and the average zone of inhibition was recorded. Significance levels of standard and treatments were compared by independent sample t test using SPSS 20.0 software (Table 1).

From the table, it is clear that, in the case of *S.aureus*, the area of the inhibition zone corresponding to AgNPs is not significantly different from that of Tetracyclin; but it is significantly different from that of the plant extract. Positive value of the test statistic (9.35) indicates that the area corresponding to AgNPs is significantly larger than that of the plant extract. Similarly, the area of the inhibition zone corresponding to plant extract is significantly smaller than that of Tetracyclin. In the case of *K.pneumoniae*, area of the inhibition zone corresponding to AgNPs is significantly different from that of Tetracyclin and the plant extract. Positive values of the test statistics (63.25 and 49.68) indicate that the area corresponding to AgNPs is significantly larger than that of

Table 1	Inhibitory	effects	of	AgNs	against	pathogenic	microorgan-
isms							

Bacterial isolate	Treatments	Mean*	SD	Test statistic
S.aureus	AgNPs	13	1.62	1.56
	Tetracyclin (control)	11	0.81	
	AgNPs	18	0.12	9.35*
	Plant extract	4	0.38	
	Plant extract	2	0.38	-14.23*
	Tetracyclin (control)	11	0.81	
K.pneumoniae	AgNPs	18	0.12	63.25*
	Tetracyclin (control)	12	0.06	
	AgNPs	18	0.12	49.68*
	Plant extract	4	0.38	
	Plant extract	4	0.38	-29.41*
	Tetracyclin (control)	12	0.06	

*Values corresponding to three replicates

Test statistic in bold indicate that the values are significant

Tetracyclin and the plant extract. As in the case of *S.aureus*, area of the inhibition zone corresponding to plant extract is significantly smaller than that of Tetracyclin. AgNPs produced a maximum zone of inhibition against both bacterial strains when compared with positive control. Among the gram strains, the highest zone of inhibition occurred in gram-negative bacteria. This might be due to the presence of a thin peptidoglycan layer in gram-negative strain, allowing the sulphur and phosphorous atom of deoxyribonucleic acid [36, 37]. The antibacterial activity of AgNPs was found to be efficient compared to commercial antibiotic tetracycline.

Larvicidal bioassay

Silver nanoparticles synthesized using seed extract of *A*. *squamosa* were also tested for their activity against mosquito larvae. Larvicidal bioassays were performed against III and IV instars of *Anopheles stephensi* (Fig. 8).

Considerable mortality was evident after the treatment of silver nanoparticle solution for the two important larval stages of the vector mosquito, Anopheles stephensi. Mosquito larvae at III instar stage showed 100 percent mortality in bioassays with AgNPs at 60 µg/mL. The LC50 and LC90 of III instars were the lowest (LC50 22.44 µg/ mL and LC90 40.65 µg/mL), while that of IV instars was highest (LC50 27.83 µg/mL and LC90 48.92 µg/ mL). The control showed nil mortality in the concurrent assay. χ^2 value was significant at $p \le 0.05$ level (Table 2). Mechanism of the larvicidal activity of AgNPs can be attributed to the penetration of AgNPs into the insect gut wall followed by binding to phosphorous and sulfur group of deoxyribonucleic acid which eventually affect cellular function leading to cell death [38]. Routine use of synthetic insecticidal products for mosquito controlling disturbs the biological system and cause resurgences in mosquito populations. The prospect of utilising plants for synthesizing silver nanoparticles and testing their efficacy in controlling mosquito larvae is an approach facilitating

Fig. 8 Effect of AgNPs against III and IV instars of *Anopheles stephensi*

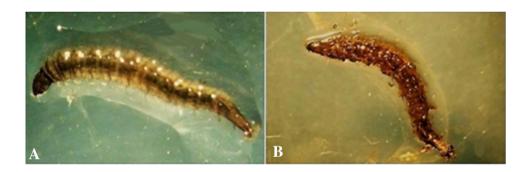


Table 2 Dose-dependent larvicidal activity of silver nanoparticles synthesized from A. squamosa seed extract against 3rd and 4th instars of mosquito larvae

Larval stage	Concentration (µg/mL)	Mortality (%)±SD	LC ₅₀ (µg/mL)	LCL-UCL	LC ₉₀ (µg/mL)	LCL-UCL	χ^2
3rd Instar	60	100 ± 0.0	22.44	16.25-28.30	40.65	33.69–54.73	18.881*
	48	84.2 ± 0.6					
	36	66.7 ± 1.5					
	24	45.2 ± 2.2					
	12	29.3 ± 1.2					
	Control	0.0 ± 0.0					
4th Instar	60	95.3 ± 1.2	27.83	21.67–34.46	48.92	40.73-66.06	17.428*
	48	71.4 ± 0.8					
	36	50.2 ± 1.4					
	24	32.7 ± 0.2					
	12	23.5 ± 1.5					
	Control	0.0 ± 0.0					

SD standard deviation, LCL lower confidence limit, UCL upper confidence limit, χ^2 chi-square test

p < 0.05, level of significance

Values are mean \pm SD of five replicates

Table 3 Impact of AgNPs on shoot length of chickpea seeds

Concentration of AgNPs (%)	Length of shoot (Mean \pm SE) (mm)			
	After 48 h	After 72 h		
100	1.94 ± 0.02	6.92 ± 0.03		
75	1.41 ± 0.00	2.94 ± 0.02		
50	1.40 ± 0.00	2.95 ± 0.01		
25	1.39 ± 0.01	4.92 ± 0.03		
0	2.92 ± 0.03	4.97 ± 0.00		

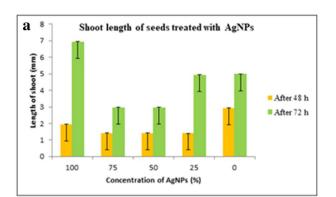
Table 4 Impact of AgNPs on root length of chickpea seeds

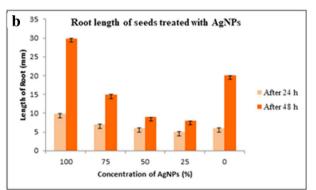
Concentration of AgNPs (%)	Length of root (Mean \pm SE) (mm)		
	After 24 h	After 48 h	
100	9.82 ± 0.04	29.84 ± 0.07	
75	6.99 ± 0.00	14.91 ± 0.03	
50	6.01 ± 0.01	8.92 ± 0.01	
25	5.02 ± 0.02	7.94 ± 0.02	
0	5.97 ± 0.04	20.02 ± 0.02	

the development of a more potent and environmentally safe biopesticide.

Phytotoxicity assay

Toxicity analysis of the AgNPs was carried out on Chickpea (Cissus arietinum) seeds and their resultant root and shoot lengths were recorded. Seeds were considered to have germinated by observing the emergence of radicles. Results obtained varied significantly with each treatment. Shoot length and root length of seedlings (Tables 3, 4; Fig. 9b, c) increased significantly in a dose-dependent manner, with a marked increase in seeds treated with 100% AgNP solution. The germination of chickpea seeds treated with 100% AgNP solution was significantly higher than those treated with a low concentration of AgNP solution and control (Table 5; Fig. 9a). The surface coating of AgNPs, their aggregation state and the release of dissolved silver are related to the toxicity of AgNPs. Studies have reported that compared to PVP-coated AgNPs, citrate-coated AgNPs were toxic to freshwater organisms [39]. Hence the increased growth and germination of chickpea seeds could be attributed to the biomolecules of Annona squamosa seed extract coated over the synthesized AgNPs. Therefore the biosynthesized AgNPs showed low toxicity and improved plant growth.





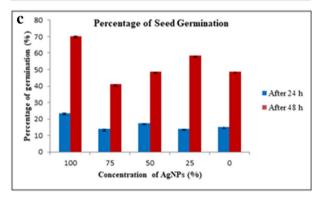


Fig.9 Impact of AgNPs on chickpea, a seed germination, b root length and c shoot length

Table 5 Imapct of AgNPs on chickpea seed germination

Concentration of AgNPs (%)	Percentage of seed germination (Mean±SE) (%)			
	After 24 h	After 48 h		
100	23.6 ± 0.02	70.18 ± 0.04		
75	13.9 ± 0.00	41.00 ± 0.01		
50	17.37 ± 0.01	48.62 ± 0.03		
25	13.91 ± 0.00	58.4 ± 0.01		
0	15.28 ± 0.01	48.67 ± 0.01		

Conclusion

Green methods for nanoparticle synthesis are attractive due to their importance in human health and environment. In the present study, we demonstrated that Annona squamosa L. seed extract can act as a reducing agent to generate AgNPs via a green approach. The synthesis of AgNPs was confirmed using UV-Visible spectroscopy with an absorption maximum at 430 nm. FT-IR spectrum revealed the presence of phytochemicals which is responsible for efficient capping and stabilization of the nanoparticles. XRD pattern demonstrated the polycrystalline nature of AgNPs. HR-TEM image showed its size distribution in the range 14-28 nm and spherical morphology. Moreover, biological assay indicated that AgNPs could be used as antibacterial, larvicidal and seed growth-promoting agent. The nanoparticles exhibited good photocatalytic activity towards the degradation of coomassie brilliant blue (CBB) dye ultimately formulating a bioremediation protocol. Formulation of optimal dosage of AgNP based herbal nanolarvicide for the control of vector borne diseases is a need of the hour. The present study act as a baseline for the preparation and application of valuable nanomedicines in future.

Declarations

Conflict of Interest The authors declare no conflict of interest.

References

- Korkmaz N, Ceylan Y, Karadağ A, Bülbül AS, Aftab MN, Saygili S, Şen F (2020) Biogenic silver nanoparticles synthesized from Rhododendron ponticum and their antibacterial, antibiofilm and cytotoxic activities. J Pharm Biomed Anal 179:112993
- Pourzahedi L, Vance M, Eckelman M (2017) Life cycle assessment and release studies for 15 nanosilver-enabled consumer products: investigating hotspots and patterns of contribution. Environ Sci Technol 51:7148–7158
- 3. Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res 7:17–28
- Chung I-M, Park I, Seung-Hyun K, Thiruvengadam M, Rajakumar G (2016) Plant-mediated synthesis of silver nanoparticles: their characteristic properties and therapeutic applications. Nanoscale Res Lett 11:40
- Guzman M, Dille J, Godet S (2012) Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gramnegative bacteria. Nanomed Nanotechnol Biol Med 8:37–45
- Korkmaz N, Ceylan Y, Hamid A, Karadağ A, Bülbül AS, Aftab MN, Cevik O, Sen F (2020) Biogenic silver nanoparticles synthesized via Mimusops elengi fruit extract, a study on antibiofilm, antibacterial, and anticancer activities. J Drug Deliv Sci Technol 59:101864

- Korkmaz N, Ceylan Y, Karadağ A, Bülbül AS, Aftab MN, Saygılı S, Sen F (2020) Biogenic silver nanoparticles synthesized from Rhododendron ponticum and their antibacterial, antibiofilm and cytotoxic activities. J Pharm Biomed Anal 179:112993
- Gopinath SM, Saha NS, John J, Khanum NS, Ganesh S, Patil A (2013) Biological synthesis characterization and application of silver nano particles a review. Int J Pharm Appl 4:19–28
- Korkmaz N, Ceylan Y, Taslimi P, Karadag A, Bülbül AS, Sen F (2020) Biogenic nano silver: synthesis, characterization, antibacterial, antibiofilms, and enzymatic activity. Adv Powder Technol 31:2942–2950
- Korkmaz N (2020) Bioreduction, the biological activity, characterization and synthesis of silver nanoparticles. Turkish J Chem 44:325–334
- McFarland AD, Van Duyne RP (2003) Single silver nanoparticles as real-time optical sensors with zeptomole sensitivity. Nano Lett 3:1057–1062
- Marimuthu S, Antonisamy AJ, Malayandi S, Rajendran K, Tsai PC, Pugazhendhi A, Ponnusamy VK (2020) Silver nanoparticles in dye effluent treatment: a review on synthesis, treatment methods, mechanisms, photocatalytic degradation, toxic effects and mitigation of toxicity. J Photochem Photobiol B Biol 205:111823–111826
- Radinia IA, Hasan N, Malik MA, Khan Z (2018) Bio-based synthesis of magnetic nanoparticles and their applications. J Photochem Photobiol B 183:154–163
- Kumar P, Govindaraju M, Senthamilselvi S, Premkumar K (2013) Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from *Ulva lactuca*. Colloids Surf B 103:658–661
- 15. Marimuthu S, Rahuman AA, Rajakumar G, Santhoshkumar T, Kirthi AV, Jayaseelan C, Bagavan A, Zahir AA, Elango G, Kamaraj C (2011) Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res 108:1541–1549
- 16. Sivapriyajothi S, Kumar PM, Kovendan K, Subramaniam J, Murugan K (2014) Larvicidal and pupicidal activity of synthesized silver nanoparticles using *Leucas aspera* leaf extract against mosquito vectors, *Aedes aegypti* and *Anopheles stephensi*. J Entomol Acarol Res 46:77–84
- Bhuvaneswari R, Xavier RJ, Arumugam M (2016) Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (*Anopheles stephensi* and *Aedes aegypti*). J King Saud Univ Sci 28:318–323
- Anil Kumar V, Ammani K, Jobina R, Parasuraman P, Siddhardha B (2016) Larvicidal activity of green synthesized silver nanoparticles using *Excoecaria agallocha* L. (Euphorbiaceae) leaf extract against *Aedes aegypti*. IET Nanobiotechnol 10:382–388
- Velayutham K, Rahuman AA, Rajakumar G, Roopan SM, Elango G, Kamaraj C, Siva C (2013) Larvicidal activity of green synthesized silver nanoparticles using bark aqueous extract of *Ficus racemosa* against *Culex quinquefasciatus* and *Culex gelidus*. Asian Pac J Trop Med 6:95–101
- Gupta SD, Agarwal A, Pradhan S (2018) Phytostimulatory effect of silver nanoparticles (AgNPs) on rice seedling growth: an insight from antioxidative enzyme activities and gene expression patterns. Ecotoxicol Environ Saf 161:624–633
- Gajalakshmi S, Divya R, Deepika VD, MythiliSathiavelu SA (2011) Pharmacological activities of Annona squamosa; a review. Int J Pharm Sci Rev Res 10:24–29
- Shirwaikar A, Rajendran K, Kumar CD, Bodla R (2004) Antidiabetic activities of aqueous leaf extract of *Annona squamosa* in streptozotocin–nicotinamide type 2 diabetic rats. J Ethnopharmcol 91:171–175
- Alali FQ, Liu X-X, McLaughlin JL (1999) Annonaceous acetogenins: recent progress. J Nat Prod 62:504–540

- 24. Biba VS, Jeba MPW, Remani P (2013) Differential effects of *Annona Squamosa* seed extracts: antioxidant, antibacterial, cytotoxic and apoptotic study. Int J Pharm Biol Sci 4:899–907
- 25. Gamble JS (1935) The flora of the presidency of Madras. Adlard & Son Ltd, London
- 26. McCracken WA, Cowsan RA (1983) Clinical and oral microbiology. Hemispher Publishing Corporation, New York, p 512
- Rios JL, Recio MC, Villar A (1988) Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmacol 23:127–149
- WHO (1992) Lymphatic filariasis: the disease and its control. 5th report. Who expert committee on filariasis. Technical Report Series p 821
- 29. Finney DJ (1971) Probit analysis, vol 551. Cambridge University Press, London, pp 68–72
- Amendola V, Bakr OM, Stellacci F (2010) A study of the surface plasmon resonance of silver nanoparticles by the discrete dipole approximation method: effect of shape, size, structure, and assembly. Plasmonics 5:85–97
- Zahid M, Arif M, Rahman MA, Singh K, Mujahid M (2018) Solvent extraction and gas chromatography-mass spectrometry analysis of *Annona squamosa* L. seeds for determination of bioactives, fatty acid/fatty oil composition, and antioxidant activity. J Diet suppl 15:613–623
- Fatimah I (2016) Green synthesis of silver nanoparticles using extract of Parkia speciosa Hassk pods assisted by microwave irradiation. J Adv Res 7:961–969
- 33. Barman SR, Nain A, Jain S, Punjabi N, Mukherji S, Satija J (2018) Dendrimer as a multifunctional capping agent for metal

nanoparticles for use in bioimaging, drug delivery and sensor applications. J Mater Chem B 6:2368–2384

- 34. Sands DE (1993) Introduction to crystallography. Dover, New York, p 51
- Harrison JJ, Tremaroli V, Stan MA, Chan CS, Vacchi-Suzzi C, Heyne BJ, Parsek MR, Ceri H, Turner RJ (2009) Chromosomal antioxidant genes have metal ion-specific roles as determinants of bacterial metal tolerance. Environ Microbiol 11:2491–2509
- Dakal TC, Kumar A, Majumdar RS, Yadav V (2016) Mechanistic basis of antimicrobial actions of silver nanoparticles. Front Microbiol 7:1831
- 37. Qing Y, Cheng L, Li R, Liu G, Zhang Y, Tang X, Wang J, Liu H, Qin Y (2018) Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. Int J Nanomed 13:3311–3327
- Kumar D, Kumar P, Singh H, Agrawal V (2020) Biocontrol of mosquito vectors through herbal-derived silver nanoparticles: Prospects and challenges. Environ Sci Pollut Res 27:25987–26024
- Rani PU, Yasur J, Loke KS, Dutta D (2016) Effect of synthetic and biosynthesized silver nanoparticles on growth, physiology and oxidative stress of water hyacinth: *Eichhornia* crassipes (Mart) Solms. Acta Physiol Plant 38:1–9

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.