

Characterization, Antibacterial and Antioxidant Properties of Silver Nanoparticles Synthesized from Aqueous Extracts of *Allium sativum*, *Zingiber officinale*, and *Capsicum frutescens*

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ABSTRACT

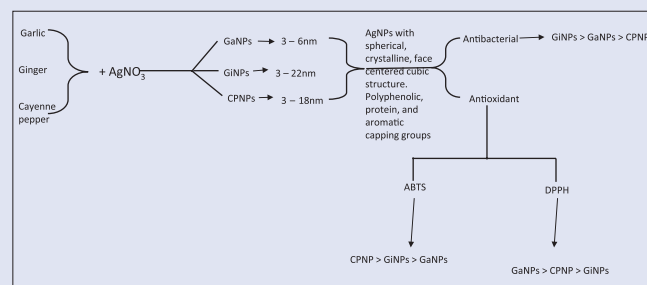
Background: Herbal drug delivery is limited by poor solubility and bioavailability which can be overcome with suitable nanomaterials that will enhance their pharmacokinetics and performance. **Objective:** This study aimed to analyze the synthesis, characterization, and biological activities of silver nanoparticles (AgNPs) from three spices. **Materials and Methods:** AgNPs were prepared using 0.1 M silver nitrate and aqueous extracts of *Allium sativum* L. (garlic), *Zingiber officinale* Rosc. (ginger), and *Capsicum frutescens* L. (cayenne pepper). The AgNPs were characterized using ultraviolet-visible (UV-Vis) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray, X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. **Results:** The AgNPs were formed within an hour of the reaction and showed maximum UV-Vis absorption in the 375–480 nm range. SEM and TEM revealed well-dispersed spherical particles with little agglomeration, average sizes of 3–6 nm, 3–22 nm, and 3–18 nm for garlic, ginger, and cayenne pepper, respectively. FTIR showed that amine, protein, phenolic, aromatic, and alkynes groups contributed to AgNP synthesis and XRD confirmed their crystalline and face-centered cubic nature. Antibacterial action of the AgNPs was in the following order: ginger (minimum inhibitory concentration [MIC] <25 µg/mL) > garlic > cayenne pepper (MIC 125 µg/mL). Antioxidant action showed cayenne pepper > ginger > garlic (inhibitory concentration 50% [IC50]: 40, 240, and 250 µg/mL, respectively) against 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and garlic > cayenne pepper > ginger (IC50: <31.25, 40, and 120 µg/mL, respectively) against 1,1-diphenyl-2-picrylhydrazyl. **Conclusion:** Optimization of this green synthesis would support the production of AgNPs with great therapeutic potentials.

Key words: Antimicrobial, antioxidant, characterization, silver nanoparticles, spices

SUMMARY

- The synthesis, characterization, and biological activities of silver nanoparticles (AgNPs) from garlic, ginger and cayenne pepper were evaluated
- The AgNPs formed were characterized using UV-Vis spectroscopy, SEM and TEM microscopy, as well as EDX, XRD and FTIR spectroscopy

- AgNPs were well dispersed with spherical shapes and average sizes of 3–6nm, 3–22nm and 3–18 nm for garlic, ginger and cayenne pepper respectively
- Amine, protein, phenolic and alkyne groups were revealed as the capping agents for the nanoparticles
- The silver nanoparticles were confirmed to be crystalline with characteristic face centred cubic nature
- The antibacterial and antioxidant activities of the AgNPs confirmed the therapeutic potential of the AgNPs.



Abbreviations used: AgNPs: Silver nanoparticles; UV-Vis: ultraviolet-visible; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; EDX: Energy dispersive X-ray; XRD: X-ray diffraction; FTIR: Fourier transform infrared; GaNPs: Garlic nanoparticles; GiNPs: Ginger nanoparticles; C.PeNPs: Cayenne pepper nanoparticles; FCC: Face centred cubic; SPR: Surface Plasmon resonance; ABTS-2: 2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); DPPH-1: 1-diphenyl-2-picrylhydrazyl.

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INTRODUCTION

Nanotechnology is the synthesis, characterization, fabrication, and manipulation of structures, devices, or materials that have at least one dimension (or contain components with at least one dimension) that is approximately 1–100 nm in length. Particle size below this threshold results in materials with physical and chemical properties that are significantly different from the properties of macroscale materials composed of the same substance.^[1]

In the last decade, biosynthesis of nanoparticles has received increasing attention due to a growing need to develop environmentally friendly technologies in material synthesis. The biosynthetic method employing plant extracts has received some attention as a simple and viable

alternative to chemical procedures and physical methods synthesizing metal nanoparticles only in recent years. Nanotechnology is becoming

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increasingly important for the food and health sectors. Promising results and applications are already being developed in the areas of nutrient and drug delivery systems through bioactive nanoencapsulation, biosensors to detect and quantify pathogens, as well as novel resources for the evaluation and development of newer, safer, and effective drug formulations.

Recently, the use of biological molecules as templates for “green nanotechnology” is increasing and plants, plant wastes, bacteria, and fungi are frequently been used for the synthesis of nanoparticles.^[2,3] According to Krithiga *et al.*,^[4] plants are better options for nanoparticle synthesis because they are mostly not toxic, provide natural capping agents, and reduce the cost of microorganism isolation and culture media.

Silver nanoparticles (AgNPs) are of current importance because of easy preparation process and unique optical, electrical, and thermal properties which enhances electrical conductivity, near infrared absorption, and effective charge separation.^[5]

Plant extracts are preferred over other biological sources due to their ample availability and wide array of reducing metabolites. Plant secondary products provide rich resources as potential drugs, nutraceuticals, and food additives. Plant polyphenols are one of the largest groups of natural antioxidant secondary metabolites.^[6] The reduction properties of these antioxidant metabolites have been linked with the higher potential ability of plant extracts to synthesize nanoparticles with improved characteristics.^[7] Biosynthesis of AgNPs using plant sources offers several advantages such as cost-effectiveness, eco-friendliness, and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods.^[8] Among the various inorganic metal nanoparticles, AgNPs have received substantial attention as preservatives, effective antimicrobial and anticancer agents, and biomedical sensors and detectors that exhibit low toxicity for *in vitro* and *in vivo* applications.^[6-8] Other researchers have reported the larvicidal,^[9,10] anticoagulant, thrombolytic,^[11,12] and anti-inflammatory^[13,14] applications of AgNPs.

Medicinal plants with known therapeutic properties and no side effects have now occupied lead positions in the pharmacopoeia. However, the delivery of plant/herbal therapeutic molecules as drugs is problematic due to poor solubility, poor permeability, low bioavailability, instability in biological milieu, etc. These limitations of herbal drugs can be overcome by attaching or encapsulating them with suitable nanomaterials which can significantly enhance the pharmacokinetics and greatly improve their performance.^[15] Medicinal plants are of special concern since they control the size and shape of nanoparticles by providing capping layers.^[16] Several medicinal plants including spices have been utilized for the production of AgNPs.^[8,17-19]

Allium sativum (garlic), *Zingiber officinale* (ginger), and *Capsicum frutescens* (cayenne pepper) are important spices with medicinal properties common to most cuisines all over the world. These spices are individually considered as general remedies for many diseases. Various biological activities attributed to these spices include antimicrobial, anticancer, antioxidant, antidiabetic, antiemetic, antihypertensive, hypoglycemic, hypolipidemic, and immunomodulatory.^[20-25] Phytochemical studies of these spices have shown that they are rich in alkaloids, tannins, carotenoids, saponins, phenols, and flavonoids, and they have been reported to exhibit high antioxidant activities^[26,27] and may be considered as potential factors for reducing silver to silver nanoparticles.

In continuation of our research on the medicinal, nutraceutical, and economic usage of these spices, the present work is aimed at the synthesis, characterization, and evaluation of antibacterial and antioxidant potentials of nanoparticles from aqueous silver nitrate (AgNO₃) and extracts of *A. sativum*, *Z. officinale*, and *C. frutescens*.

MATERIALS AND METHODS

Materials

Fresh garlic, ginger, and cayenne pepper were purchased from the local fruit and vegetable outlet in Alice, South Africa. The spices were identified and authenticated with the previously collected herbarium specimens available. AgNO₃ (Sigma-Aldrich, USA) and all other reagents used in this study were of analytical grades. The individual spices were dried (60°C, 72 h), pulverized into powder with a coffee grinder, and stored in air-tight bottles at 4°C till needed. Deionized water was used throughout the experiment.

Preparation of silver nanoparticles

Twenty grams each of *A. sativum*, *Z. officinale*, and *C. frutescens* was weighed into individual 250 mL conical flasks and was extracted with 100 mL of deionized water at 60°C for 10 min in a water bath. The extracts were cooled and filtered using Whatman filter paper no. 1 under vacuum. Exactly 15 mL of the prepared extracts was added to 45 mL of aqueous AgNO₃ (0.1 M solution) at room temperature and stirred continuously with a magnetic stirrer for 15 min. The solution obtained was kept in dark to prevent auto-oxidation of silver.

Characterization of silver nanoparticles

After 24 h, the solution containing AgNPs was centrifuged at 3000 rpm for 10 min, the resulting pellets were dried in an oven at 100°C for 24 h. The purified AgNPs were characterized using the following techniques.

The formation of AgNPs was monitored by visual assessment of the color changes of the solutions. The reduction of silver was measured periodically at a wavelength range of 300–700 nm using a UV-Vis spectrophotometer (UV-3000 PC, UK). The UV-Vis spectra of AgNPs produced were plotted and recorded as a function of bioreduction time (15 min intervals) at room temperature at a resolution of 0.5 nm. Size, shape, and morphology of the nanoparticles were determined by scanning electron microscopy (SEM) (JEOL JSM-6390 LV) and transmission electron microscopy (TEM) (Zeiss LIBRA® 120), while the presence of silver was confirmed by energy dispersive X-ray (EDX) spectrometry analysis: the samples for SEM and EDX assays were sonicated for 5 min to make a suspension of AgNPs in distilled water. A drop of the suspension was then placed on double-sided-coated carbon stubs, allowed to dry, and observed using SEM at a voltage of 15–20 kV at different magnifications. EDX analysis was also performed for the confirmation of elemental silver. Both SEM and EDX analyses were carried out on the same instrument (JEOL JSM-6490A). The samples for TEM were prepared by sonicating the pellet of centrifuged AgNPs in methanol. A drop of the suspension was placed on a carbon-coated copper grid and allowed to dry at room temperature.

TEM analysis was performed using a TEM operating at 80 kV. X-ray diffraction (XRD) measurement of the nanoparticles was recorded on an X-ray diffractometer (Bruker D8 Advanced) operated at a voltage of 45 kV and a current of 30 mA equipped with a proportional counter Cu K α radiation ($\lambda = 1.5405 \text{ \AA}$, nickel filter). Fourier transform infrared (FTIR) analysis was used to determine the possible biomolecules responsible for the reduction of silver ions to AgNPs. The samples were analyzed using a JASCO FT-IR 4100 spectrometer. Spectra were collected from 50 scans at a resolution of 4 cm⁻¹ in the transmission mode of 4000–440 cm⁻¹.

Antimicrobial assay

The comparative antibacterial activities of the AgNPs from the respective spices were assessed against two Gram-negative and two Gram-positive bacterial strains. The organisms *Streptococcus faecalis* (ATCC: 29212), *Bacillus cereus* (ATCC: 10702), *Escherichia coli* (ATCC: 25922), and

Shigella flexneri (KZN) were chosen primarily on the basis of their importance as opportunistic pathogens of humans and food deterioration and were obtained from the Microbiology Unit of the Medicinal Plants and Economic Development Research Centre, Botany Department, University of Fort Hare.

Agar dilution method was used for evaluating the minimum inhibitory concentration (MIC) of the AgNPs against the bacterial strains. In brief, Mueller–Hinton agar was prepared by autoclaving and allowed to cool at 55°C. A stock solution of each synthesized AgNPs was prepared in dimethyl sulfoxide (Sigma). The agar medium containing the nanoparticles at final concentrations of 0.015625–0.25 mg/mL was poured into Petri dishes, swirled gently until the agar began to set, and left over night for solvent evaporation. Organisms were streaked in radial pattern on the agar plates. The inoculum of each test strain was standardized at 5×10^5 cfu/ml using McFarland Standard as described by the National Committee for Clinical Laboratory Standards.^[28] The plates were incubated under aerobic conditions at 37°C and examined after 24 h. Each treatment was performed in triplicate, and complete suppression of growth at a specific concentration of the nanoparticles was taken as the MIC. Ciprofloxacin was used as positive control in this experiment.

Antioxidant assay

The antioxidant activities of the spices and AgNPs were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays.

2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging assay

The modified method described by Re *et al.*^[29] using microtiter well plates was adopted for the determination of ABTS scavenging activity. In brief, the stock solutions consisting of 7 mM ABTS solution and 2.4 mM potassium persulfate solution were prepared. The working solution was prepared by mixing the two stock solutions in equal proportions (1:1 v/v) and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 mL ABTS + solution with 60 mL of methanol to obtain an absorbance of 0.708 ± 0.001 units at 734 nm using the spectrophotometer. A volume of 100 μ L of methanol was added into all the wells with the exception of second (B) and third (C) rows. Exactly 200 μ L of the nanoparticles (0.5 mg/ml) or standards (Rutin) prepared in methanol were added in triplicates to the third row (C). A 2-fold serial dilution was done by mixing the contents in each well of the third row (starting from the first column) and transferring 100 μ L into the second well of the same column, and the procedure was repeated up to the 7th well of the same column, and the last 100 μ L from the 7th well was discarded. A 2-fold dilution yielding concentrations of the plant extracts and standards ranging from 0.01 to 0.5 μ g/mL was thus prepared in the wells. The AgNPs (100 μ L) and the control were allowed to react with 100 μ L of the ABTS + solution, and the absorbance was measured at 734 nm after 7 min using the spectrophotometer. The ABTS + scavenging capacity of the extract was then compared with that of the standards, and the percentage inhibition was calculated as follows:

$$\text{ABTS scavenging activity (\%)} = [1 - (\text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}})] \times 100$$

where, $\text{Abs}_{\text{control}}$ is the absorbance of ABTS radical + methanol and $\text{Abs}_{\text{sample}}$ is the absorbance of ABTS radical + sample (nanoparticles/standard drugs).

1,1-diphenyl-2-picrylhydrazyl radical scavenging assay

To assess the scavenging ability on DPPH radicals, the method described by Zou *et al.*^[29] using micro-well titer plates was used. In brief, a stock solution

of 0.135 mM DPPH radical was prepared in methanol. A volume of 100 μ L of methanol was added into all the wells with the exception of second (B) and third (C) rows. Exactly 200 μ L of the nanoparticles (0.5 mg/ml) or standards (rutin) prepared in methanol were added in triplicates to the third row (C). A 2-fold serial dilution was done by mixing the contents in each well of the third row (starting from the first column) and transferring 100 μ L into the second well of the same column, and the procedure was repeated up to the 7th well of the same column, and the last 100 μ L from the 7th well was discarded. A 2-fold dilution yielding concentrations of the plant extracts and standards ranging from 0.01 to 0.5 mg/mL was thus prepared in the wells. The reaction mixture was then vortexed thoroughly and left in dark at room temperature for 30 min, after which the absorbance was measured in a spectrophotometer at 517 nm. The DPPH radical scavenging ability of the nanoparticles was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / (\text{Abs}_{\text{control}})] \times 100$$

Where, $\text{Abs}_{\text{control}}$ is the absorbance of DPPH + methanol and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH + sample (nanoparticles/standards).

Statistical analysis

Results were expressed as mean \pm standard deviation of three replicates and were subjected to analysis of variance using Minitab release version 12, Windows 95. Significant levels were tested at $P < 0.05$.

RESULTS

Synthesis and characterization of silver nanoparticles

Addition of the spice extracts to aqueous AgNO_3 solution resulted in changes of color of the mixtures from faint yellow to colloidal brown indicating AgNP formation [Figure 1].

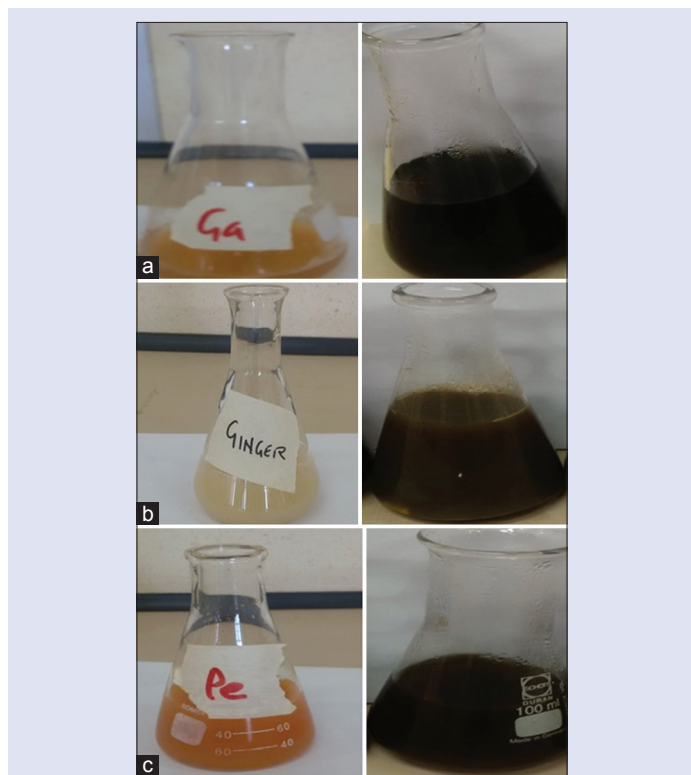


Figure 1: Spice extracts before and after reaction with silver nitrate. (a) *Allium sativum* (garlic), (b) *Zingiber officinale* (ginger), and (c) *Capsicum frutescens* (cayenne pepper)

The ultraviolet-visible (UV-Vis) spectrum of AgNPs [Figure 2] was recorded from the reaction medium as a function of time intervals of 15, 30, 45, and 60 min. The AgNPs from the spices gave absorbance peaks ranging between 375 and 480 nm, with a strong resonance centered at 375, 400, and 480 nm at 60, 15, and 60 min for garlic, ginger, and cayenne pepper, respectively.

The UV-Vis spectra also revealed that the AgNPs formed rapidly within 60 min and remained stable even after 24 h. The most intense Plasmon bands were observed for ginger after 15 min between 400 and 435 nm, while for cayenne pepper, it was at 480 nm, an indication of high-reducing ability. The UV-Vis absorption of AgNPs for garlic was at 375 nm. In all the cases, the peak due to silver ion at 300 nm was found missing.

Scanning electron microscopy analysis of silver nanoparticles

SEM analysis of the AgNPs revealed that well-dispersed, highly crystalline AgNPs were formed [Figure 3]. Morphological examination also revealed that the nanoparticle crystals were cuboidal and not agglomerated.

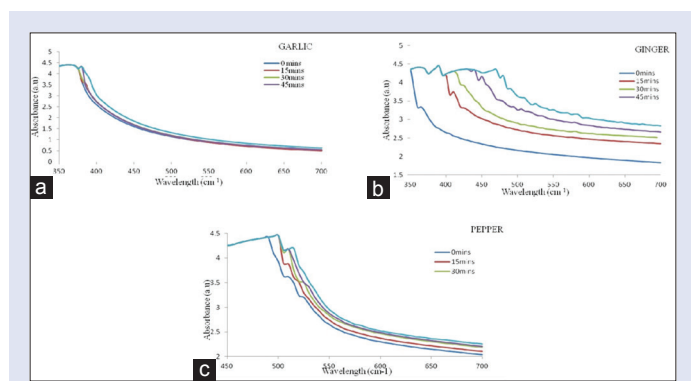


Figure 2: Ultraviolet-visible spectra of silver nanoparticles as a function of time intervals of 15, 30, 45, and 60 min

Energy dispersive X-ray analysis of silver nanoparticles

EDX further confirmed the formation of AgNPs from the individual spices as there was a strong signal in the silver region confirming the formation of AgNPs [Figure 3]. From the EDX spectra, AgNPs reduced by the spices have the weight percentage of silver as 45.84%, 79.27%, and 57.16% for garlic, ginger, and cayenne pepper, respectively.

Transmission electron microscopy analysis of silver nanoparticles

Representative TEM images of AgNPs of the three spices are shown in Figure 4. The TEM imaging of AgNPs synthesized from the spice extracts revealed spherical shapes with uniform particle size distribution and average sizes of 5.28, 12.97, and 10.86 nm for garlic, ginger, and cayenne pepper, respectively.

Fourier transform infrared microscopic analysis of silver nanoparticles

FTIR spectral measurements were carried out at a resolution of 4 cm^{-1} to identify the potential functional groups of biomolecules in the spice extracts responsible for reducing and capping the bio-reduced AgNPs. The FTIR analysis revealed different stretches of bonds at different peaks for each of the spices [Figure 5].

The nanoparticles from garlic showed peaks at 1048, 1331, 1583, 2946, and at 3317 characteristic of C-O (acid and ester stretch), C-N (amine), and O-H stretch/bends, respectively; ginger showed the sharp, less intense peaks at 1238, 1386, 1439, 1475, 2970, and 3443 of C-O (acid and ester stretch), C-N (nitrile), C-H, C=C (aromatic), and the C-H/O-H stretch. The FTIR of nanoparticles from cayenne pepper was characterized by peaks at 1232, 1377, 1443, 1761, 2141, 3019, and 3468 assigned to the C-N (amine), C-H, C=O, C≡C/CN (alkyne/nitrile), C=O (carbonyl), O-H, and N-H stretches, respectively.

X-ray diffraction analysis of silver nanoparticles

The dry powder XRD pattern of the AgNPs synthesized from the spices is shown in Figure 6. XRD provides information about the

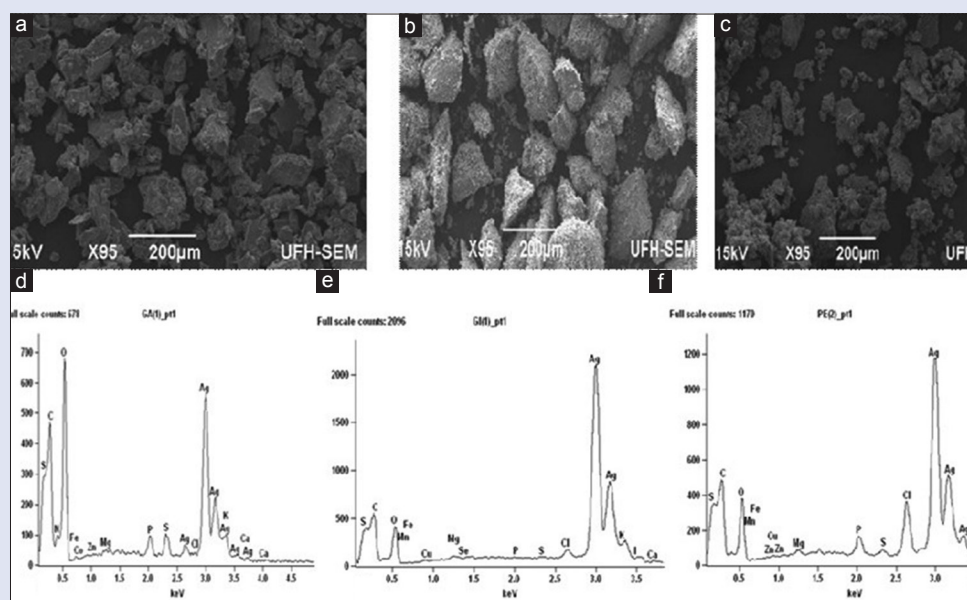


Figure 3: Scanning electron micrographs of nanoparticles derived from (a) garlic, (b) ginger, and (c) cayenne pepper; (d-f) Energy dispersive absorption spectra of silver nanoparticles derived from (d) garlic, (e) ginger, and (f) cayenne pepper

arrangement of atoms within a crystalline material. All diffraction peaks corresponded to the characteristic crystalline face-centered cubic (FCC) silver lines compared with the standard powder diffraction card of the Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04-0783.

Strong Bragg reflections which correspond to the reflections of silver are shown for each of the spice nanoparticles. The powder diffraction pattern and Miller Indices (hkl) to each peak are assigned and shown in Table 1. Three peaks at 2θ values of (63.23, 77.53, 99.90), (61.63, 80.02, 101.38) corresponding to (220), (311) planes of silver were observed for garlic and ginger, respectively, while four peaks at 2θ values of (63.23, 76.74, 86.07, 92.80) corresponding to (220) and 311 planes of silver were observed for cayenne pepper.

Antibacterial activities of silver nanoparticles

Antibacterial activities of the AgNPs were evaluated by Agar dilution method using Mueller–Hinton agar. Agar dilution is one of the most commonly used techniques to determine the MIC of antimicrobial agents, including antibiotics and other substances with bactericidal or bacteriostatic activities. The MIC for each spice nanoparticle is shown in Figure 7.

Antioxidant activity of silver nanoparticles

The antioxidant activity of synthesized AgNPs was evaluated by DPPH and ABTS radical scavenging assay, and Rutin was used as a positive control. The AgNPs exhibited potential free radical scavenging activity against both radicals as shown in Figure 8. The maximum scavenging activity against ABTS was exhibited by nanoparticles from cayenne pepper (inhibitory concentration 50% [IC₅₀] = 31.25 $\mu\text{g}/\text{mL}$), while ginger and garlic nanoparticles exhibited a concentration-dependent scavenging activity with IC₅₀ of 240 and 250 $\mu\text{g}/\text{mL}$, respectively. Garlic nanoparticles exhibited the highest scavenging activity against DPPH

(IC₅₀: <31.25 $\mu\text{g}/\text{mL}$), followed by cayenne pepper (IC₅₀: 40 $\mu\text{g}/\text{mL}$) and ginger (IC₅₀: 120 $\mu\text{g}/\text{mL}$).

DISCUSSION

The chemical reduction of aqueous solution of AgNO₃ is one of the most widely used methods for the synthesis of AgNPs. According to reports by

Table 1: Powder diffraction pattern and Miller Indices (h k l) from d-spacing

2θ	d	1000/d ²	(1000/d ²)/60.62	h, k, l
Garlic				
63.23	1.47	463	8	2 2 0
77.53	1.23	660.5	11	3 1 1
99.90	1.01	987.2	16	- - -
Ginger				
61.63	1.51	438.58	7	- - -
80.02	1.20	696.62	11	3 1 1
101.38	0.99	1012	17	- - -
Cayenne Pepper				
63.23	1.47	463.0	8	2 2 0
76.74	1.24	647.9	11	3 1 1
86.07	1.13	784.9	13	- - -
92.80	1.06	883	15	- - -

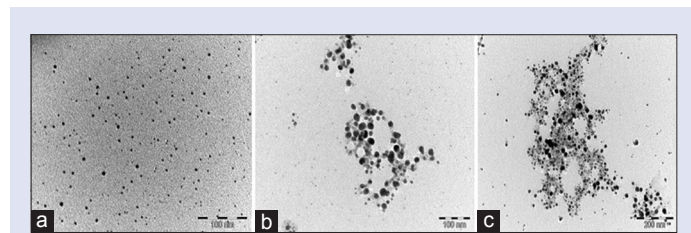


Figure 4: Transmission electron microscopy micrographs of silver nanoparticles from (a) garlic, (b) ginger, and (c) cayenne pepper

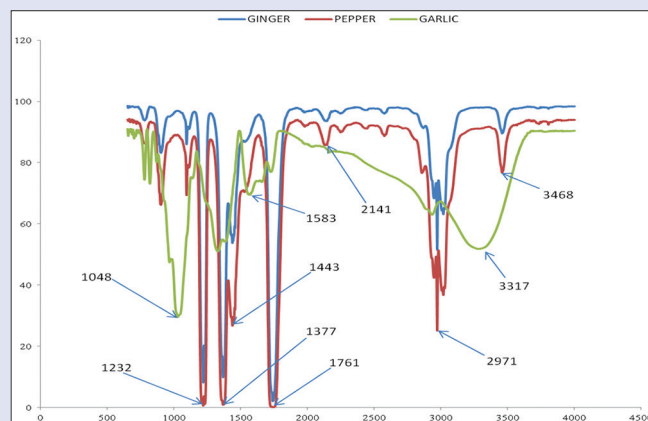


Figure 5: Fourier transform infrared spectra of silver nanoparticles from garlic, ginger, and cayenne pepper

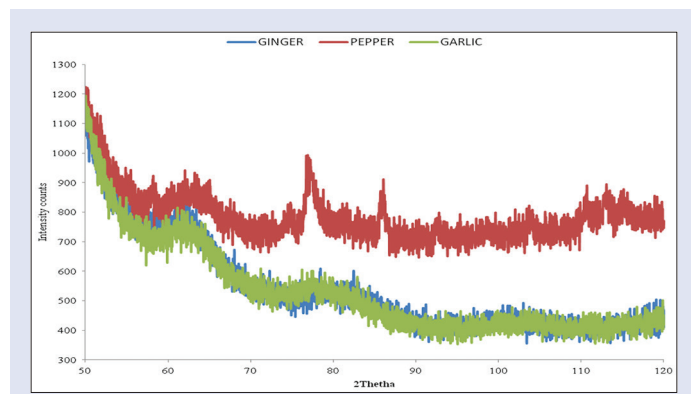


Figure 6: X-ray diffraction pattern of silver nanoparticles from garlic, ginger, and cayenne pepper

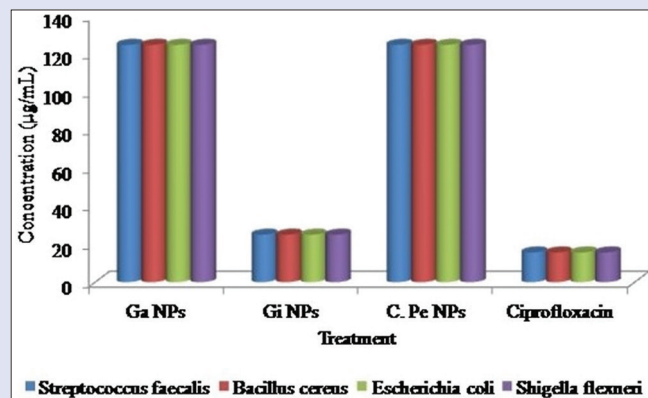


Figure 7: Minimum inhibitory concentration (MIC, $\mu\text{g}/\text{mL}$) of silver nanoparticles against bacterial isolates. Note–GaNPs: Garlic nanoparticles, GiNPs: Ginger nanoparticles, C.PeNPs: Cayenne pepper nanoparticles

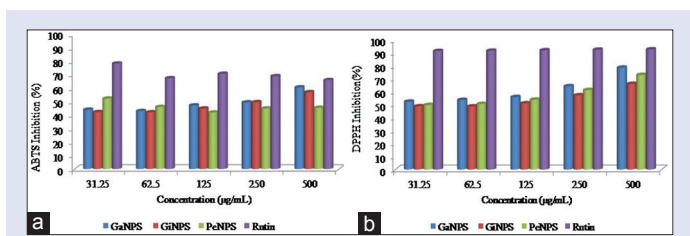


Figure 8: Inhibition of (a) 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and (b) 1,1-diphenyl-2-picrylhydrazyl radicals by synthesized silver nanoparticles

Lalitha *et al.*^[30] and Hyllested *et al.*,^[31] color change is an important factor for the synthesis of AgNPs. AgNPs appear brown in aqueous medium as a result of surface Plasmon vibrations.^[32] Other studies have reported similar color changes which confirm the formation of AgNPs.^[33,34] Several authors have reported color formations ranging from light yellow, yellowish brown, to dark brown for colloidal AgNPs.^[4,35,36] The nanoparticles were primarily characterized by UV-Vis spectroscopy, which has proved to be a very useful technique for the analysis of nanoparticles. Reduction of Ag⁺ ions in the aqueous solution of silver complex during the reaction with the ingredients present in the spice extracts observed by the UV-Vis spectroscopy revealed that AgNPs in the solution may be correlated with the UV-Vis spectra. This has been attributed to excitation of Plasmon vibrations of AgNPs in the solution which indicates the reduction of silver ions to AgNPs. Researchers have reported surface Plasmon resonance for colloidal silver ranging from 320 to 390 nm when sorghum bran was used,^[37] while others^[10,35] have reported 420 nm when soluble starch and *Annona squamosa* leaves were used, respectively. These tend to suggest that the nature of the capping agent could affect the SPR of colloidal AgNPs.

The UV-Vis absorption for garlic implies that AgNPs were coated and stabilized by bioactives such as proteins which prevented the transition of electrons from the surface of AgNPs. All these results cumulatively suggest that the colloidal silver system so obtained was ideally monodispersed. This clearly shows that only AgNPs are present in the colloidal dispersion.^[38] SEM is commonly used to provide images about the morphology, size, shape, and organization of the surface topography of specimens.^[39] According to Theivasanthi and Alagar,^[40] most metals tend to nucleate and grow into twinned and multiply twinned particles with their surfaces bounded by the lowest energy facets. The observation of some larger nanoparticles may be attributed to the fact that AgNPs have the tendency to agglomerate due to their high surface energy and high surface tension of the ultrafine nanoparticles. The fine particle size results in a large surface area that, in turn, enhances the nanoparticle activity.

EDX micro-analysis is performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a specimen. EDX spectra recorded from the AgNPs confirmed the synthesis of AgNPs by the extracts. According to previous reports, AgNPs typically absorb in the region of 3 keV owing to surface Plasmon resonance.^[41] Other weak peaks observed in the EDX spectra may have originated from biomolecules bound to the surface of the AgNPs.^[34,42]

TEM is the most suitable method for providing detailed information about very fine structures in nanoparticles.^[43] The magnification factor for TEM is typically in the range of about 10²–10⁶ times, which enables very fine structures to be observed.^[44] There were no obvious particle aggregations in the TEM imaging. The identical shapes observed for the three NPs could be attributed to similarity in the reductive agents present in the three spices.^[45] AgNPs of various sizes (ranging from 5 to 100 nm) and morphologies (spherical, nanorods, hexagonal, etc.) have been

synthesized using various plant extracts such as soluble starch, coffee, green tea, fungal, spices, and other biological extracts.^[46-49]

FTIR spectral measurements are used to identify the potential functional groups of biomolecules responsible for reducing and capping the bio-reduced AgNPs. According to Sasidharan *et al.*,^[50] FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups, and the spectra of pure compounds are usually so unique that they are like a molecular “fingerprint.” In this study, the bands observed denote stretching vibrations responsible for compounds such as flavonoids, phenols, terpenoids, and proteins,^[17] and could confirm that these biomolecules in the spice extracts were responsible for reducing, capping, and stabilizing of the AgNPs.^[34,51] The reduction and capping of silver ion into AgNPs in the present study could be attributed to flavonoids, phenols, and proteins, which abound in the three spices. Several studies have reported similar observations from FTIR analysis of AgNPs synthesized from plant extracts such as *Cycas circinalis*, *Ficus amplissima*, *Commelina benghalensis* and *Lippia nodiflora*,^[52] *Pedaliium murex*,^[53] *Urtica dioica*,^[49] and *Azadirachta indica*.^[54]

XRD provides information about the chemical composition and arrangement of atoms within a crystalline material. The observed arrangement in this study confirmed that the main composition of the nanoparticles was silver.^[55] All the peaks in XRD pattern were readily indexed to a FCC structure of silver as per the available literature (JCPDS, File No. 4-0783), and the particles are crystalline in nature. The intensity of peaks reflected the high degree of crystalline nature of the AgNPs. However, the diffraction peaks which are broad indicate that the crystal sizes are very small.^[56]

Further, the Bragg reflections revealed that both ginger and garlic were assigned to the same space group. Some unassigned peaks have also been observed, suggesting the crystallization of other biomolecules responsible for silver ion reduction and stabilization of resultant nanoparticles.^[57-59] This is in agreement with the results obtained by other researchers on *Morganella* spp. and *Elaeagnus latifolia*.^[60,61]

The AgNPs showed strong antibacterial activity against all the tested bacterial strains. Ginger AgNPs exhibited the maximum inhibition for both Gram-positive and Gram-negative bacteria, while garlic and cayenne pepper nanoparticles exhibited equipotent antibacterial property. This implies that the ginger nanoparticles had the smallest diameter compared to those from garlic and cayenne pepper. The biocidal activity of the spice nanoparticles could be due to the high affinity of Ag⁺ for thiols, leading to disruption of enzyme function responsible for nutrient uptake and cellular energy production/storage processes, thereby killing the microbes.^[49,62] Most AgNPs obtained from medicinal plants have been credited with high antimicrobial properties, especially against drug-resistant human pathogenic and food spoilage microbes.^[63,64] The results from this study correlate with other reports that most plant extract AgNPs exhibit enhanced broad-spectrum antibacterial activities.^[65] This suggests that nanoparticles from spice extracts could yield valuable alternative as antibacterial drugs.

DPPH and ABTS are widely used for testing preliminary radical scavenging activity of compounds or nanoparticles and provide easy and rapid evaluation. In the present study, the synthesized AgNPs exhibited potential free radical scavenging activity against both radicals. Polyphenolic compounds such as flavonoids, flavonols, proanthocyanidin, and phenolics in plants have been reported to have strong antioxidant activities which help to protect cells against oxidative damage by free radicals. The antioxidant activities were enhanced by conversion into AgNPs. The enhanced antioxidant activities of AgNPs from plant sources such as *Chenopodium murale*,^[66] *Piper longum*^[67] as well as zinc nanoparticles from *A. sativum*, *Rosmarinus officinalis*, and *Ocimum basilicum*^[66] have been reported.

CONCLUSION

AgNPs were successfully synthesized and characterized from AgNO₃ and aqueous extracts of garlic, ginger, and cayenne pepper. Due to the varying properties of the three spices, variations in size of the synthesized AgNPs were observed. The synthesis of AgNPs using garlic, ginger, and cayenne pepper was a rapid, large-scale, size- and shape-controlled process. All the nanoparticles exhibited potent antibacterial and antioxidant activities, with ginger nanoparticles being the most active. Further optimization of the current green-synthesis method would help in the production of monodispersed AgNPs having great potential for the treatment of several diseases and food preservation. Thus, the AgNPs synthesized from these spices could be promising candidates for use in nanomedicine and other areas where such applications are needed, especially with drug-resistant bacteria.

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Conflict of Interest

There are no conflicts of interests.

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