

Green-synthesized silver nanoparticles using *Syzygium jambos* leaf extract: antioxidant, antibacterial, and potential therapeutic applications in functional foods

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Abstract

Food nanotechnology offers novel strategies for enhancing functional foods through bioactive delivery and antimicrobial protection. In this study, silver nanoparticles (AgNPs) were synthesized using *Syzygium jambos* leaf extract, which was confirmed by visual examination and ultraviolet-visible spectroscopy. Further characterization using Fourier transform-infrared spectroscopy, X-ray diffraction, scanning electron microscopy, energy dispersive X-ray spectroscopy, and transmission electron microscopy analysis identified the physicochemical properties of synthesized AgNPs, which displayed strong antioxidant potential, inhibiting 2,2-diphenyl-1-picrylhydrazyl and nitric oxide radicals in a dose-dependent manner. AgNPs also exhibited antibacterial activity against *Bacillus cereus* and *Shigella flexneri*, with zones of inhibition comparable to that of antibiotic control. However, zebrafish embryo toxicity studies revealed dose-responsive effects. These findings highlight *S. jambos*-mediated AgNPs as promising functional food additives with antimicrobial and antioxidant benefits, suitable for food preservation, disease sensing, and therapeutic delivery. Further safety evaluations are necessary for their integration into food and biomedical applications.

Keywords: silver nanoparticles; *Syzygium jambos*; antioxidant and antibacterial activity; zebrafish

Introduction

Nanotechnology considers nanoparticles (NPs) as advanced core material derived through the controlled atomic or molecular modification of bulk matter. Nanoparticles, typically ranging from 1 to 100 nanometers (nm), possess a high surface-to-volume ratio and enhanced chemical reactivity, which confer superior physicochemical and biological properties not observed in their bulk counterparts (Anbumani *et al.*, 2022). Recently, metal and metal-based nanoparticles—such as selenium, iron oxide, silver, gold, and platinum nanoparticles—have been extensively studied in the field

of nanoscience because of their significant contributions to the diagnosis and treatment of life-threatening human diseases (Patel *et al.*, 2024; Priya *et al.*, 2017).

Silver nanoparticles (AgNPs) are gaining increasing attention because of their exceptional stability and superior biological, electrical, and optoelectronic properties, enabling their application across a wide range of sectors (Zhang *et al.*, 2016). Their antimicrobial and anti-inflammatory properties at varying dosages support their use in commercially available surgical bandages and dressings, where they help to accelerate wound healing. Additionally, the anti-infective quality of AgNPs makes

them suitable for use in biomedical implants, surgical instruments, and catheters (Jamkhande *et al.*, 2019). AgNPs also serve as active components in personal hygiene products such as toothpaste, bath gels, creams, ointments, and shampoos, functioning as biocidal agents (Pulit-Prociak *et al.*, 2019). Their unique characteristics enable a broad range of biomedical applications, including the development of novel therapeutics, biosensors, drug delivery systems, nutraceuticals, diagnostic tools, and bioimaging platforms (Jeevanandam *et al.*, 2022). Beyond biomedicine, AgNPs are also employed in environmental remediation, textiles, optoelectronics, energy generation, food packaging, and in the paint and ink industries. AgNPs exhibit diverse pharmacological properties, including antimicrobial, antiproliferative, virucidal, antiplatelet, anticoagulant, antifungal, antidiabetic, antibiofilm, and antioxidant activities (Dakshayani *et al.*, 2019; Khan *et al.*, 2023; Noppradit *et al.*, 2023; Tarannum and Gautam, 2019).

Owing to their versatile applications, various protocols have been developed to meet the demand for AgNPs. Physicochemical methods, such as spray pyrolysis, electrochemical techniques, ball milling, arc discharge, and laser ablation, are commonly employed for AgNP fabrication. Because these physical methods do not require chemical reducing agents (e.g., sodium citrate, sodium borohydride, or hydrazine), the resulting nanoparticles are typically pure and well-structured (Wei *et al.*, 2015). However, the requirement of high pressure and temperature, along with the associated costs, makes these methods less suitable for large-scale AgNP production. Additionally, the use of toxic chemical-reducing agents in chemical synthesis may pose risks in biomedical applications, as residual chemicals may adhere to nanoparticles and cause adverse health effects (Wang *et al.*, 2018).

The abundance, biodegradability, nontoxicity, and cost-effectiveness of biogenic protocols—using biological or native resources as bio-reducing agents or reaction media for synthesizing metal-based nanoparticles—have recently attracted significant interest from researchers as a sustainable alternative to conventional physicochemical methods (Kora and Rastogi, 2018). Green synthesis effectively addresses the issue of hazardous by-product generation commonly associated with traditional methods. Biological extracts derived from various plant parts (including stem bark, seeds, flowers, roots, leaves, and peels), as well as bacterial supernatants, fungi, macroalgae, and cyanobacteria, are widely utilized in the green synthesis of nanoparticles (Sonbol *et al.*, 2021).

Globally, antimicrobial resistance (AMR) is a rapidly escalating concern that urgently requires innovative strategies. The uncontrolled and excessive use of anti-infective drugs and antibiotics is a major contributor to

the emergence of drug-resistant pathogenic bacterial strains (Alex *et al.*, 2024). Phyto-engineered nanoparticles have shown promising potential in combating multidrug-resistant (MDR) microbes through various mechanisms or via synergistic action when combined with conventional antibiotics, offering improved biocompatibility and biodegradability (Keshari *et al.*, 2020). Currently, nano-antibiotics—non-traditional antimicrobial agents synthesized from plant extracts—are widely explored as alternatives to conventional antibiotics and have garnered increasing attention from research groups globally (Huh and Kwon, 2011).

Extensive research is underway to design metallic nanoparticles using phytoextracts. Among these, plant extract-mediated synthesis of platinum, silver, and gold nanoparticles has demonstrated potent microbicidal activity against a broad range of microorganisms (Bordiwala, 2023; Chinnaraj *et al.*, 2023). The green synthesis of AgNPs using phytoextracts is particularly advantageous, as it is simple, rapid, cost-effective, and environmentally benign. Additionally, the biosynthesized AgNPs often exhibit defined morphology and size, achieved under optimized environmental conditions. These nanoparticles are well suited for biomedical applications because of their minimal or nontoxic disposition.

Green-synthesized AgNPs also demonstrate high yield, solubility, and stability (Jain *et al.*, 2021). Phytoconstituents, such as phenolics, ketones, tannins, flavonoids, ascorbic acid, phenols, amides, saponins, and terpenoids, contribute to both reduction of silver precursors and stabilization of the resulting nanoparticles (Kajani *et al.*, 2014). These phyto-derived compounds enhance the functional efficiency, stability, biocompatibility, and half-life of AgNPs (Arshad *et al.*, 2024). Various medicinal plant extracts are successfully employed in AgNP synthesis, including *Adhatoda vasica* leaf extract (Chaudhari *et al.*, 2023), *Calendula officinalis* seed extract (Baghizadeh *et al.*, 2015), *Zingiber officinale* root extract (Hu *et al.*, 2022), *Malva sylvestris* flower extract (Mahmoodi Esfanddarani *et al.*, 2018), *Trapa bispinosa* peel extract (Pandey *et al.*, 2013), *Phyllanthus emblica* (amla) fruit extract (Masum *et al.*, 2019), and stem extracts of *Entada spiralis* and *Piper chaba* (Mahiuddin *et al.*, 2020). AgNPs synthesized using these phytoextracts have shown multiple bioactivities, such as antibiofilm, antioxidant, antidiabetic, antifungal, anti-inflammatory, and antiproliferative effects (Ghojavand *et al.*, 2020).

Free radicals are generated as secondary products by living cells during essential aerobic metabolic processes. While low levels of free radicals play a vital role in maintaining immune function, excessive accumulation can lead to oxidative stress. Antioxidative compounds

enhance the activity of enzymes, such as glutathione peroxidase, superoxide dismutase, and catalase, or inhibit the expression of free radical-producing enzymes, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase (Chaudhary *et al.*, 2023). When the production of free radicals outweighs the body's antioxidant defenses, oxidative stress damages key macromolecules, including nucleic acids, lipids, and proteins. This imbalance contributes to the onset and progression of chronic degenerative diseases, such as rheumatoid arthritis, Parkinson's disease, diabetes mellitus, liver disorders, and cardiovascular conditions (Baskaran *et al.*, 2025; Dhandapani *et al.*, 2020). Natural compounds are a primary source of antioxidants. Phytochemicals—secondary metabolites produced by plants—play a beneficial role in regulating various cellular processes in the human body (Priya *et al.*, 2022). Nanoparticle formulations of plant-derived medications, often referred to as phyto nano-antioxidants, have shown enhanced therapeutic efficacy through improved antioxidant activity (Han *et al.*, 2022). Nanoparticles synthesized using plant extracts or active polyphenolic ingredients have demonstrated strong antioxidant properties, along with improved solubility, bioavailability, and targeted delivery (Balkrishna *et al.*, 2021). Among *in vivo* animal models, *Danio rerio* (zebrafish) is widely recognized as a cost-effective and reliable system for nanoparticle toxicity studies. Zebrafish share significant anatomical, genetic, and physiological similarities with humans. Their rapid development, small size, ease of maintenance, and transparent embryos make them ideal for real-time observation in toxicological research (Wehmas *et al.*, 2015).

Medicinal plants have long been used as natural healing agents in traditional medicine. The phytochemicals they contain possess both preventive and therapeutic potential against a variety of health conditions (Priya *et al.*, 2022; Uddin *et al.*, 2022).

The *Syzygium* genus, classified under the Myrtaceae family, includes nearly 3,000 species, all of which exhibit notable health benefits (Avila-Peña *et al.*, 2007). *Syzygium jambos* (commonly known as rose apple) is a small-to-medium size tree or shrub found in tropical regions globally. In traditional medicinal systems, various parts of the plant have been used to treat ulcers, hemorrhages, dental problems, respiratory disorders, leprosy, syphilis, and wounds. Leaf decoctions are traditionally used for treating eye infections and rheumatic conditions. Scientific studies have confirmed that the leaves and other plant parts of *S. jambos* exhibit antiulcerogenic, antibacterial, anti-inflammatory, anticancer, antioxidant, anticholinesterase, and antidiabetic properties (Rawa *et al.*, 2022). High flavonoid content found in most parts of the plant is largely responsible for its anti-inflammatory and antioxidant activities. Gallic acid, the principal phenolic acid in

S. jambos, is distributed throughout the plant (Ochieng *et al.*, 2022) and may play a crucial role in reducing silver nitrate to form AgNPs. Previous studies have reported the successful synthesis of gold and AgNPs using leaf and bark extracts of *S. jambos*, primarily for antimalarial applications (Dutta *et al.*, 2017). Additionally, aqueous extracts from other *Syzygium* species, including *S. cumini*, *S. alternifolium*, and *S. aromaticum*, have been used for nanoparticle synthesis because of their strong reduction potential (Dhanislas *et al.*, 2023; Dutta *et al.*, 2017). Given its abundance and rich phytochemical profile, *S. jambos* presents itself as a promising candidate for green nanoparticle synthesis.

In this study, we focus on the green synthesis of AgNPs using *S. jambos* leaf extract and provide evidence of its reduction potential to produce uniform AgNPs. We further characterize the basic physicochemical properties of synthesized nanoparticles. Additionally, antibacterial and toxicity assessments offer foundational insights into their potential applications across biomedical and environmental domains.

Materials and Methods

Collection and preparation of the sample

The mature, healthy, and infection-free leaves of *S. jambos*, acquired from the local market in University of Bisha, Bisha, Saudi Arabia, were identified by a taxonomist, and specimen (voucher No. 09/2024) were stored at the University of Bisha, Bisha, Saudi Arabia. Three times washing of the leaves with tap water was executed to rule out detritus materials, and then the leaves were air-dried. Once the leaves were dried, these were finely powdered in a home mixer. To procure the aqueous leaf extract of *S. jambos*, exactly 5 g of leaf powder was mixed with 100 mL of distilled water in a sterile Erlenmeyer flask (200-mL capacity) placed on a hot plate with magnetic stirrer and boiled for 20 min at 60°C. The synthesized solution was filtered using a filter paper (Whatman No. 1) and light yellowish solution was carefully collected and retained for refrigeration (4°C) and used when needed for investigations.

Synthesis of AgNPs

The aqueous leaf extract of *S. jambos* was applied in the genesis of AgNPs. Briefly, in a sterile conical flask, exactly 10 mL of leaf aqueous extract was poured slowly into silver nitrate solution (1 mM, 100 mL), and stirred steadily for 30 min with a magnetic stirrer. After 24 h, a dark-brownish colored solution emerged, which was centrifuged (10,000 rpm) for 15 min. A dark brownish

pellet that appeared at the bottom of centrifuge tube was scrapped and redispersed thrice in distilled water to remove impurities. Purified AgNPs were air-dried and retained at 4°C for further analysis.

Spectroscopic and microscopic characterization

Ultraviolet-visible (UV-vis) spectral analysis

The UV-vis spectroscopic absorption investigation is a primary tool to characterize metallic nanoparticle's creation. The phyto-based reduction of silver ions by the secondary metabolic ingredients of *S. jambos* was checked. Exactly, 1 mL of the sample was poured in a sterile cuvette (quartz) and then the optical density from 200 nm to 800 nm (wavelength) was recorded.

Fourier transform-infrared spectroscopy (FTIR)

The specific functional groups of the leaf aqueous extract of *S. jambos* were identified using FTIR instrument. The vibrational frequencies developed in the spectra of FTIR discloses the phytoingredient's dynamic functional groups participation in the biological reduction, stabilization, and capping of metallic nanoparticles. The sample (2 mg) was amalgamated with potassium bromide (200 mg) and the pellet was designed. The pellet was scanned in the range of 500–4,000 cm^{-1} and the spectra were studied under ambient environment with specific resolution (4 cm^{-1}).

X-ray diffraction (XRD) analysis of AgNPs

To identify the crystalline trait and particle size of the AgNPs designed using *S. jambos* leaf aqueous extract, powder XRD was executed. The fabricated AgNPs were evenly layered on a microscopic sterile glass slide and the diffraction spectrum developed was captured in X-ray diffractometer. The diffractometer is functioned using 30 mA (current) and 40-kV voltage at 2θ range (20°–80° scanning mode) with $\lambda = 1.54056 \text{ \AA}$ ("copper k alpha" [Cu $\text{k}\alpha$] radiation). To compute the crystallite size of the designed AgNPs, Debye–Scherrer's equation was applied:

$$D = 0.94 \lambda \div \beta \cos \theta,$$

where D is the mean crystallite size of nanoparticles, λ is the X-ray radiation wavelength, β is full width at half maximum (FWHM) measure, and θ is the diffraction angle.

Scanning electron microscopy (SEM) with Energy dispersive X-ray (EDX)

The exterior features of the fabricated nanoparticles, such as morphology and dimension, were identified through SEM tool. The designed AgNPs were sonicated using distilled water and placed as a thin layer on a grid having carbon coating. The synthesized AgNPs were chemically

analyzed for their elemental ingredient by using EDX analysis fitted with SEM tool.

Transmission electron microscopy (TEM)

Morphology along with the dimensional information of the designed AgNPs was achieved through TEM analysis. Sonication of AgNPs was conducted for 5 min in distilled water. On a copper-grid layered with carbon, a drop of nanoparticles was coated as a thin layer. With the aid of blotting paper, excess solution was wiped out and grid was air-dried for 10 min. Scanning was performed at different areas and the micrographic images was photographed.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging methodology was applied to scrutinize the free radical quenching efficacy of the AgNPs synthesized applying *S. jambos* leaf aqueous extract. For the assay, DPPH (100 μM) was diluted in methanol (99.9%). Experiment was executed by amalgamating DPPH methanolic solution (3 mL) with 1-mL varying doses (25 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$, 75 $\mu\text{g}/\text{mL}$, 100 $\mu\text{g}/\text{mL}$, and 125 $\mu\text{g}/\text{mL}$) of AgNPs. The synthesized mixture was retained to react for 30 min in a dark environment at ambient conditions (37°C) and optical density (OD) was observed spectrophotometrically (517 nm). Ascorbic acid (as a standard positive control) and methanol (as a blank solution) were used. The percentage DPPH inhibition was computed by applying the following equation:

$$\% \text{ Inhibition of DPPH} = \frac{AB_C - AB_S}{AB_C} \times 100,$$

where AB_C is the control absorbance, and AB_S is the AgNPs absorbance.

Nitric oxide (NO) assay

To evaluate the NO scavenging efficacy of the AgNPs designed using aqueous leaf extract of *S. jambos*, a published protocol with mild amendment was employed (Borquaye *et al.*, 2020). Precisely, 2 mL of 10-mM sodium nitroprusside, 0.5 mL of phosphate buffer solution (PBS), and 0.5 mL of test samples of varying doses (25 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$, 75 $\mu\text{g}/\text{mL}$, 100 $\mu\text{g}/\text{mL}$, and 125 $\mu\text{g}/\text{mL}$) were blended in a sterile test tube. Incubation of the reacting mixture was performed for 150 min at 25°C. To the incubated reaction solution (0.5 mL), 30% of 1-mL sulphanilamide, made using glacial acetic acid, was poured and retained for 5 min. Re-incubation of the reaction solution was performed for 30 min at 25°C, after the addition of 1 mL of 0.1% w/v naphthylethylenediamine dihydrochloride (NED). A pinkish color appeared from the diazotization reaction between nitrite ions and sulfanilamide; conjugation with NED was observed at 540 nm through spectrophotometer. In a similar manner, standard solution (ascorbic acid) was prepared. For blank solution, the

S. jambos leaf aqueous extract was replaced by ethanol. The inhibitory percentage of NO radicals was computed by using the following equation:

$$\% \text{ of NO inhibition} = \frac{AB_C - AB_S}{AB_C} \times 100,$$

where AB_C is the control absorbance, and AB_S is the AgNPs absorbance.

Well-diffusion methodology for antibacterial assay

As per the published methodology of Ragunathan *et al.* (2022), with milder alterations, the antibacterial performance of the AgNPs designed from the *S. jambos* aqueous leaf extract was checked for two infective bacterial strains: *Bacillus cereus*/*B. cereus* and *Shigella flexneri*/*S. flexneri*. Agar-well diffusion methodology using Mueller–Hinton (MH) agar media was performed. The freshly made MH agar media was gently poured into petri-plates and waited for solidification in a laminar airflow (LAF) chamber to restrict contamination. Using 100 μ L of bacterial culture, each petri-plate was swabbed individually. The wells were designed using steel well borer (3 mm). Then, 10 μ L of AgNPs of varying doses (20 μ g/mL, 30 μ g/mL, 40 μ g/mL, and 50 μ g/mL) were poured into the agar-well and the plated AgNPs were retained for a day at ambient conditions (37°C) in a bacterial incubator. Exactly, 10 μ g/mL of ciprofloxacin (antibiotic) was applied as a standard/positive reference drug. Inhibition zones that emerged around all the wells in each petri-plate were measured in millimetres (mm).

Zebrafish embryo toxicity assay

To assess the toxicity impact of the fabricated AgNPs on the embryonic zebrafish, the protocol experimented by Ganeshkumar *et al.* (2012) with slight amendment was performed. Through spawning, the egg of the zebrafish was collected and retained for embryo formation. Healthier embryos (eight-cell stage) of zebrafish were collected, washed thrice with distilled water, and preserved in a petri-plate. In a 24-well microtiter plate, 20 zebrafish embryos were placed in each well. The embryos were reacted with varying doses (100 μ g/mL, 200 μ g/mL, and 300 μ g/mL) of *S. jambos* aqueous leaf extract-fabricated AgNPs and incubated at three varying intervals (24, 48, and 72 h). All the procedures were carried out strictly with institutional and national guidelines for the care and use of fisheries. This study was approved by the Animal Ethics Committee at University of Bisha (UB-H-03/23).

Statistical analysis

All the protocols were performed for three times ($n = 3$) and the data were collected as means \pm SD. Paired test

statistical analysis was carried out wherever required. The GraphPad Prism software (version 6) was employed for determining the half-maximal inhibitory concentrations (IC_{50}), and the graphs were illustrated.

Results

Visual investigation and UV analysis

The yellow color *S. jambos* leaf aqueous extract when amalgamated with NO aqueous solution swiftly turned to dark-brownish color within 10 min. The dark-brownish color intensity deepens with increase in retention period, reflecting direct proportionality. The appeared dark-brownish color was the basic hallmark for the genesis of AgNPs after 24 h of retention at ambient conditions. The fabricated AgNPs evinced a specific intensive peak at 420 nm when assessed through UV-vis spectrophotometer. This was due to surface plasmon resonance (SPR) of AgNPs and ranged almost from 350 nm to 480 nm (Figure 1). The purity of the synthesized nanoparticles was observed from the result. Despite the washing steps, there was a peak at 250 nm, which signified the presence of phenolic acid and flavonoids present in the plant extract.

FTIR

Applying the FTIR tool, the bioactive ingredients of the aqueous leaf extract of *S. jambos*, engaged in the

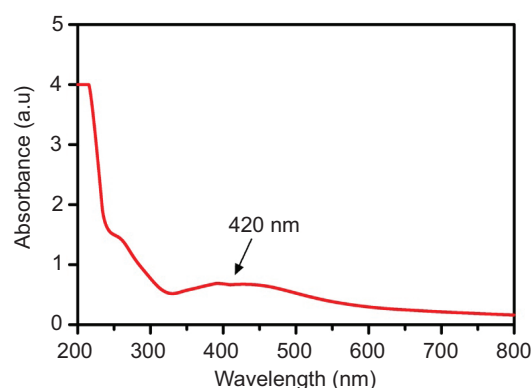


Figure 1. The UV-visible absorption spectrum of AgNPs synthesized using *S. jambos* leaf aqueous extract. A prominent surface plasmon resonance peak was observed at 420 nm, confirming AgNP formation. The absorption band between 350 nm and 480 nm supports the colloidal nature of the nanoparticles, while a minor peak near 250 nm indicates the presence of phytochemicals, such as phenolic acids and flavonoids, adsorbed on surface of the nanoparticles. Spectra were recorded between 200 nm and 800 nm using quartz cuvettes after 24-h incubation at room temperature.

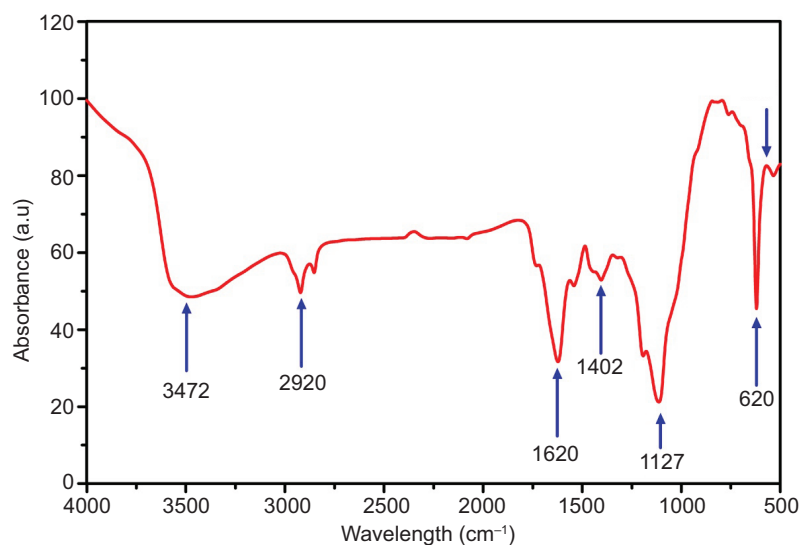


Figure 2. The FTIR spectrum of AgNPs synthesized using *S. jambos* leaf aqueous extract recorded in the range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1} under ambient conditions. Distinct peaks observed at 3,472 cm^{-1} (O-H stretching of alcohols/phenols), 2,920 cm^{-1} (C-H stretching of methyl groups), and 1,620 cm^{-1} (C=O or C-O stretching of flavonoids) indicate the involvement of phytochemicals in the reduction, stabilization, and capping of AgNPs.

bio-based reduction, stabilization as well as capping of AgNPs, were revealed (Figure 2). A peak formed at 3,472 cm^{-1} demonstrated the O-H stretch of the alcoholic/phenolic ingredients of *S. jambos* leaf extract. For flavonoid's C-O stretch, a peak was discovered at 1,620 cm^{-1} . A peak for the methyl group's C-H stretch was formed at 2,920 cm^{-1} .

XRD spectral pattern of AgNPs designed from *S. jambos* leaf aqueous extract

The XRD was conducted to discover the crystalline quality as well as crystallite size of fabricated AgNPs. The diffraction pattern of peak developed at 2θ with the hkl plane values (Miller indices) as 38.90° (111), 44.91° (200), 64.34° (220), and 77.29° (311), as shown in Figure 3. This diffraction spectrum dictated the face-centered cubic (fcc) of AgNPs with crystallinity. Moreover, the spectra matched with the Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783. The AgNPs were spheroidal in morphology. The mean grain size (particle size) of the AgNPs, calculated using Debye–Scherrer equation, was 17.80 nm.

SEM analysis

The exterior morphology of the fabricated AgNPs was projected through the SEM micrographic images presenting spheroidal nanoparticle's shape along with aggregation (Figure 4). Similitude to the present report, the

AgNPs acquired using *Salvia officinalis* also dictated spheroidal morphology, in addition to agglomeration.

EDX analysis

The EDX spectra that appeared from 2.5 kiloelectron volt (keV) to 4 keV was specific for AgNPs (Figure 5). The spectra showed that the fabricated AgNPs owned 45.95% silver, 22.80% oxygen, and 33.27% carbon. Carbon existence in the spectra might be from the SEM grid, and the oxygen atom was contributed by the phytometabolites leaf aqueous extract of *S. jambos*. Similar form of outcomes is found in the literature.

TEM analysis

The micrographic image of TEM provided spheroidal shape and smooth edged nanoparticles having a dimensional range of 12–25 nm and a mean size of 18.9 nm (Figure 6). The synthesized AgNPs also revealed a layer of phytocomponents at the edge of nanoparticles. This prescribed the major activity of the aqueous leaf extract of *S. jambos* in the various phases of nanoparticle's synthesis.

DPPH assay

The DPPH radical quenching impact of *S. jambos* leaf aqueous extract-fabricated AgNPs was assessed. The AgNPs showed suppression in DPPH radical with the

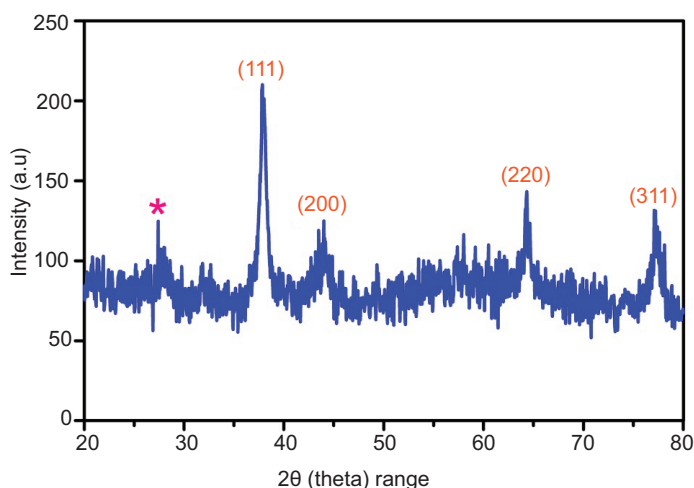


Figure 3. The XRD pattern of AgNPs synthesized using *S. jambos* leaf aqueous extract, scanned over a 2θ range of 20° – 80° using Cu $k\alpha$ radiation ($\lambda = 1.54056 \text{ \AA}$). The diffraction peaks observed at 38.90° , 44.91° , 64.34° , and 77.29° matched with the (111), (200), (220), and (311) planes of fcc silver, respectively, consistent with the JCPDS file No. 04-0783. The average crystallite size, calculated using the Debye–Scherrer equation, was approximately 17.8 nm.

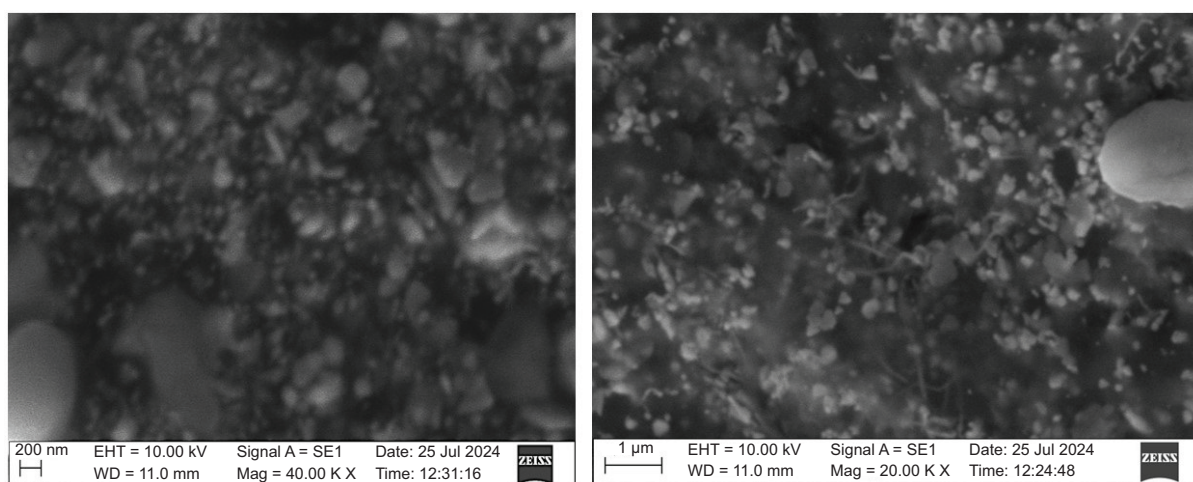


Figure 4. The SEM images of AgNPs synthesized using *S. jambos* leaf aqueous extract captured at two different magnifications. The images reveal predominantly spherical morphology with moderate agglomeration and relatively uniform particle distribution. Samples were sonicated in distilled water and deposited on a carbon-coated grid prior to imaging.

inhibitory proportion of 19.29–79.02% when screened from $25 \mu\text{g/mL}$ to $125 \mu\text{g/mL}$. The inhibitory activity escalated with an upsurge in the concentration of AgNPs. Similarly, the standard positive control (ascorbic acid) displayed escalated percentage inhibition of DPPH radical (26.02–82.82%) (Figure 7). The IC_{50} doses for AgNPs and ascorbic acid were computed as $88.76 \mu\text{g/mL}$ and $86.37 \mu\text{g/mL}$, respectively.

Nitric oxide scavenging assay

The NO scavenging assay was conducted to display the anti-radical efficacy of the AgNPs acquired from *S. jambos* aqueous leaf extract. At the lowest experimented dose

of AgNPs ($25 \mu\text{g/mL}$), the inhibition of NO was 23.82%, and at maximum dose ($125 \mu\text{g/mL}$), 65.38% was the NO inhibition. The inhibitory performance of AgNPs toward the NO radical was entirely dose-based. As the concentration escalated, the inhibitory percentage also increased with an IC_{50} of $68.34 \mu\text{g/mL}$. Similarly, ascorbic acid also showed an escalated inhibitory proportion of 27.87–75.69% with an IC_{50} dose of $66.67 \mu\text{g/mL}$ (Figure 8).

Antibacterial activity

The antibacterial efficacy of the AgNPs designed by applying *S. jambos* aqueous leaf extract was analyzed. Gram-positive and Gram-negative strains of bacteria

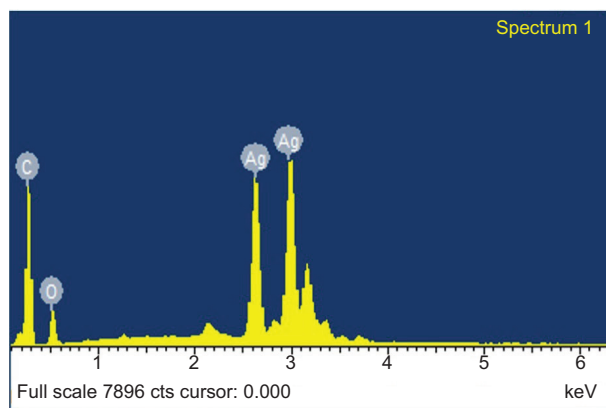


Figure 5. The EDX signal of AgNPs synthesized using *S. jambos* leaf aqueous extract. A strong characteristic silver signal is observed between 2.5 keV and 4 keV, confirming the elemental composition of AgNPs. The spectrum indicated 45.95% silver, 33.27% carbon (attributed to the carbon-coated SEM grid), and 22.80% oxygen, probably originating from the phytochemicals capping the surface of nanoparticles.

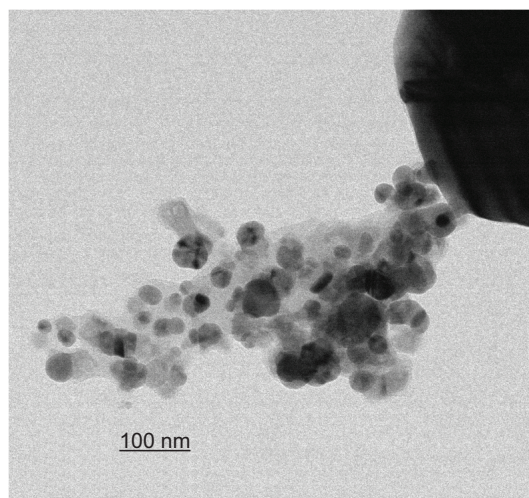


Figure 6. The TEM micrographs of AgNPs, synthesized using *S. jambos* leaf aqueous extract, show spherical nanoparticles. The micrographs reveal predominantly spherical nanoparticles with smooth edges, exhibiting size ranging from 12 nm to 25 nm and an average diameter of approximately 18.9 nm. A thin layer of phytochemical capping is visible around nanoparticles, indicating the role of plant metabolites in stabilization. Scale bar represents 100 nm.

were used. The AgNPs demonstrated strong antibacterial activity against each bacterium with visible inhibitory zone. The diameter of inhibitory zone increased with increased dose of AgNPs. The antibacterial activity was observed to be dose-dependent. At a maximum dose (50 $\mu\text{g/mL}$) of AgNPs, the inhibitory zone of *Bacillus cereus* was 10.3 ± 0.62 mm, whereas for *Shigella flexneri*, the

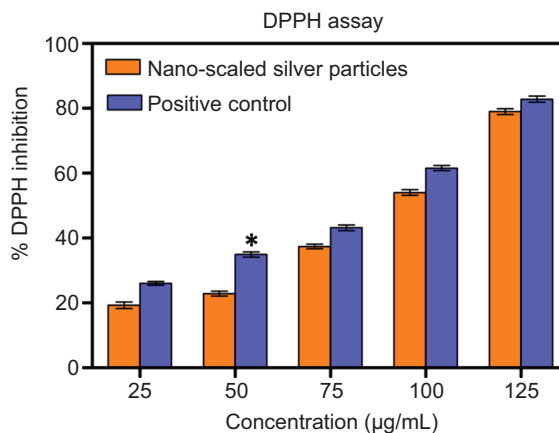


Figure 7. The dose-dependent DPPH radical scavenging activity of AgNPs synthesized using *S. jambos* leaf aqueous extract, compared to ascorbic acid as a positive control. The inhibitory effect increased with AgNP concentration from 25 $\mu\text{g/mL}$ to 125 $\mu\text{g/mL}$, reaching a maximum inhibition of 79.02%, close to ascorbic acid's 82.82%. IC_{50} values were 88.76 $\mu\text{g/mL}$ for AgNPs and 86.37 $\mu\text{g/mL}$ for ascorbic acid. Data represent mean \pm SD; and * indicates statistical significance versus control ($P < 0.05$).

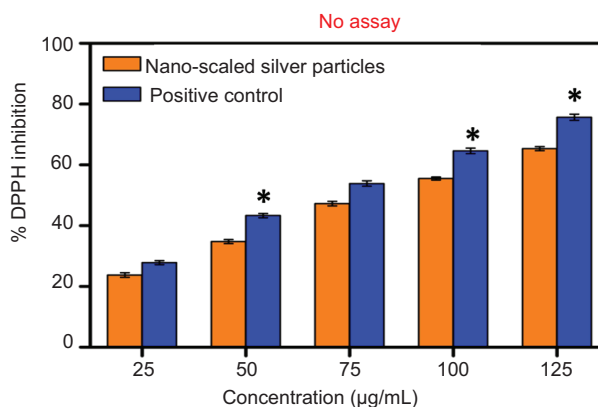


Figure 8. The dose-dependent NO radical scavenging activity of AgNPs synthesized using *S. jambos* leaf aqueous extract, compared to ascorbic acid as a positive control. The AgNPs showed increasing NO inhibition from 23.82% to 65.38% across concentration of 25–125 $\mu\text{g/mL}$, with an IC_{50} of 68.34 $\mu\text{g/mL}$. Ascorbic acid exhibited inhibition from 27.87% to 75.69%, with an IC_{50} of 66.67 $\mu\text{g/mL}$. Data represent mean \pm SD; and * indicates statistical significance versus control ($P < 0.05$).

inhibitory zone was 8.0 ± 0.40 mm. The acquired outcomes are displayed in Table 1 and Figure 9.

Zebrafish embryo cytotoxicity study

The toxic nature of AgNPs synthesized using *S. jambos* leaf aqueous extract was assessed on the formation and

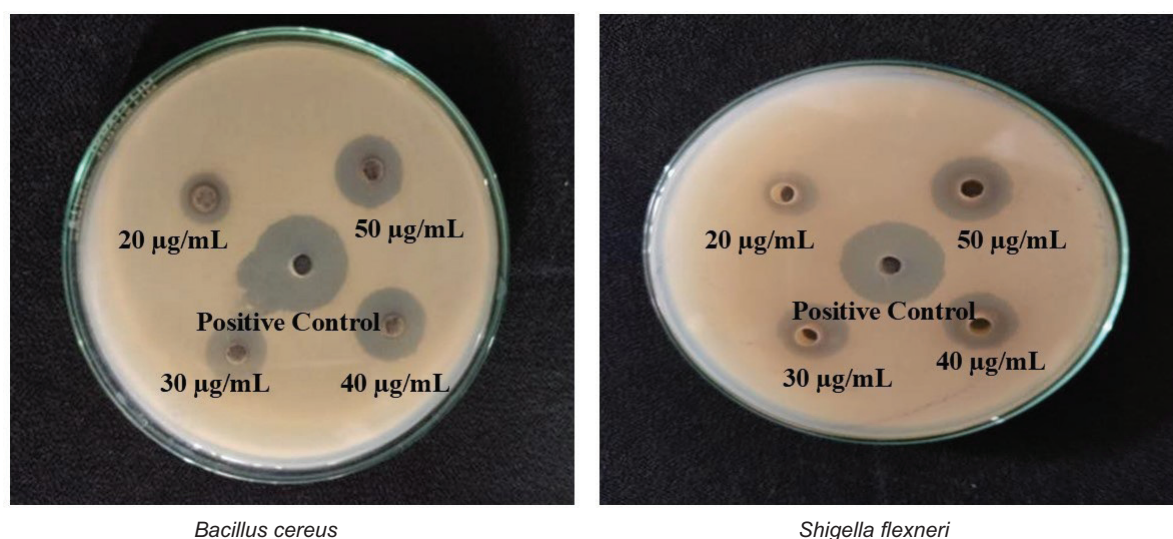


Figure 9. The agar well-diffusion assay showing the dose-dependent antibacterial activity of AgNPs synthesized using *S. jambos* leaf aqueous extract against *Bacillus cereus* (Gram-positive) and *Shigella flexneri* (Gram-negative). Clear inhibition zones increased with AgNP concentration from 20 µg/mL to 50 µg/mL. Ciprofloxacin (10 µg/mL) was used as a positive control.

Table 1. Inhibitory zones developed for two infective bacterial strains (Gram-positive and Gram-negative) showing dose-dependant antibacterial activity.

Doses (µg/mL)	Inhibitory zone (mm)	
	<i>Bacillus cereus</i>	<i>Shigella flexneri</i>
20	5.2 ± 0.70	4.0 ± 0.20
30	7.0 ± 0.54	5.1 ± 0.90
40	8.1 ± 0.31	6.3 ± 0.36
50	10.3 ± 0.62	8.0 ± 0.40
Antibiotic (ciprofloxacin, 10 µg/mL)	13.0 ± 0.20 ^{a-c}	10.0 ± 0.82 ^{d-f}

Notes: ^{a-c}Significance ($P < 0.05$), compared to 20 µg/mL, 30 µg/mL, and 40 µg/mL against *Bacillus cereus*, respectively.
^{d-f}Significance ($P < 0.05$), compared to 20 µg/mL, 30 µg/mL and 40 µg/mL against *Shigella flexneri*, respectively.

growth of zebrafish embryo. The AgNPs were exposed to zebrafish embryo for three different periods. With an upsurge in the doses of AgNPs and exposure intervals, toxicity occurred toward the morphology of zebrafish embryo. At 100 µg/mL, the toxicity was small. AgNPs revealed toxicity at 200 µg/mL and 300 µg/mL toward zebrafish embryo. The notochord of zebrafish appeared bent, with visualized edema of the pericardial, abnormalities of the eyes and craniofacial and yolk sac edema deformations (Figure 10). With increase in the dose of AgNPs, the exterior of fertilized eggs turned rough and the yolk sac was distorted, thus impacting the survival and development of the embryo. No such alteration of morphology was observed in untreated zebrafish larvae.

Discussion

The AgNPs synthesized utilizing the aqueous extracts of *Petalium murex* (leaf extract), *Piper nigrum*, *Saraca indica* (leaf extract), and *Capsicum annum L.* (juice) also manifested dark-brownish coloration (Anandalakshmi *et al.*, 2016; Li *et al.*, 2007; Paulkumar *et al.*, 2014). Our results were in concordance with other investigations. The functional groups (carboxylic and hydroxyl) of secondary metabolites after losing hydrogen ions achieve negative charge and acquire electrostatic form of interaction with silver ions, which efficaciously facilitate the biological reduction of silver ions to zero valent silver ions (Ag⁰), aiding in the creation of AgNPs (Safa and Koohestani, 2024). The electromagnetic field that stimulates electron oscillation in the conductive band region of AgNPs is the pivotal contributor of SPR (Mollick *et al.*, 2019). AgNPs synthesized using *Moringa oleifera* aqueous leaf extract presented peak at 419 nm, which was very close to our outcome (Asif *et al.*, 2022). *Berberis asiatica* root aqueous extract-designed AgNPs formed peak at 427 nm. Additionally, the SPR of AgNPs is governed by dielectric trait, dimension, morphology, and the elemental composition of fabricated AgNPs (Dangi *et al.*, 2020). The leaf aqueous extract of *Cucumis prophetarum* and silver nitrate-blended AgNPs had a defined peak at 420 nm (Hemlata *et al.*, 2020). Also, an aqueous leaf extract of *Morinda lucida*-fabricated AgNPs showed peak at 420 nm. The phytometabolites (proteins, steroids, tannins, phenols, saponins, and flavonoids) aid in the bio-based reduction of silver ions to AgNPs (Labulo *et al.*, 2022).

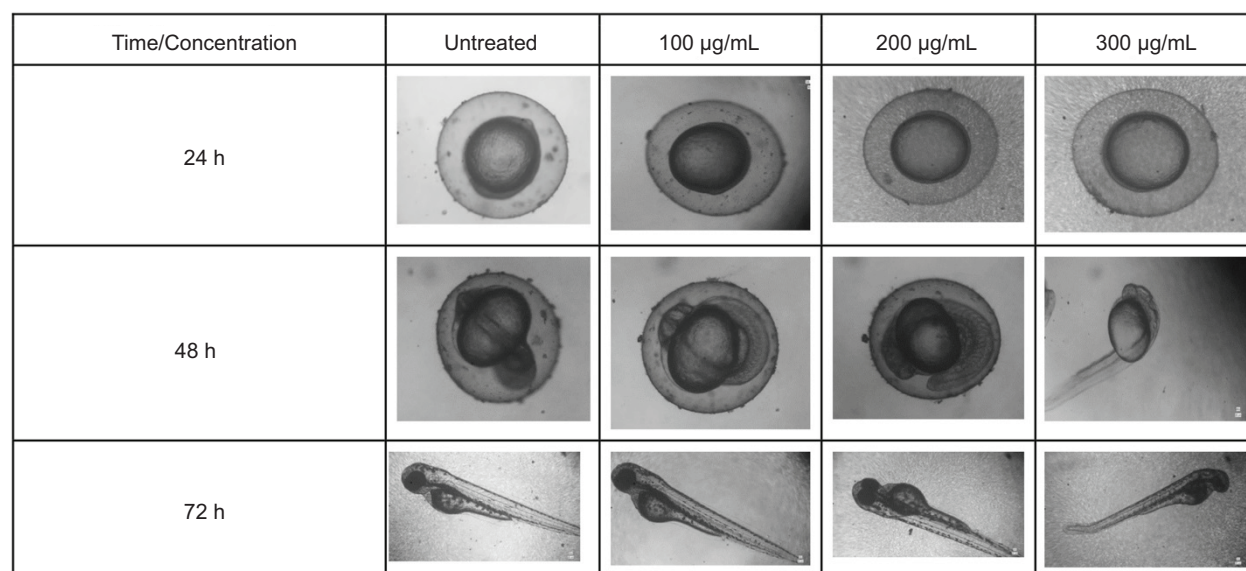


Figure 10. Toxicity effects of AgNPs synthesized using *S. jambos* leaf aqueous extract on zebrafish embryo at 24, 48, and 72 h post-exposure. Increased concentrations (100, 200, and 300 µg/mL) induced morphological abnormalities, including bent notochord, pericardial edema, craniofacial deformities, eye abnormalities, and yolk sac edema. Control embryos showed normal development with no morphological alterations.

The functional group attributed to M-O (metal-oxygen) bonding, specifically Ag-O bonding, appeared at 583 cm^{-1} (Patra and Baek, 2017). The C-C stretching vibrational peak that appeared at 1,402 cm^{-1} dictated aromatic structural compound's existence. Peak that emerged at 1,112 cm^{-1} signified amine group's C-N vibrational stretching. An intensive peak formed at 618 cm^{-1} revealed alkyl group's C-Cl vibrational stretching. Glycosides, phenolic acids, saponins, tannins, and flavonoids aided in the phyto-based formation of AgNPs (Dobrucka *et al.*, 2019). Usually, the interaction of silver ions with the plant's bioactive molecules encourages the formation of zero valent silver ions from silver ions via reduction. Precisely, from the enolic group of phytophenolic compound, electron is released through the breaking of hydroxyl bond. This electron is utilized in the genesis of AgNPs. The broader peak (OH group peak) that appeared in the spectra of FTIR, justified its involvement in the phyto-designed development of AgNPs (Sukweenadhi *et al.*, 2021).

The AgNPs designed by applying the aqueous extract of *Moringa oleifera* and *Phlomis* leaf provided XRD diffraction spectra similar to our result (Haris and Ahmad, 2024). Additional peak presented the participation of phyto-organic molecules in the fabrication of AgNPs (Karuppiyah and Rajmohan, 2013). Even the *Momordica cymbalaria* (fruit extract)- and *Cydonia oblong* (seed extract)-utilized AgNPs also manifested similar sort of diffraction pattern through XRD study (Zia *et al.*, 2016). Higher surface area and greater energy of the fabricated

AgNPs were attributable to their aggregation facilitated by physical force (Vander Waal's force). The AgNPs using *Salvia spinosa* (seed extract) and *Strychnos potatorum* Linn F (leaf extract) also demonstrated spheroidal shape (Kagithoju *et al.*, 2015). Spheroidal AgNPs with 45-nm dimension were formed when Flax seed extract was used for procurement (Alzubaidi *et al.*, 2023). The AgNPs designed by applying the aqueous leaf extract of *Perilla* also had spheroidal shape with less than 50-nm dimension (Sharma *et al.*, 2024). Even, the outcome was in exact parallelism to the previous data. AgNPs synthesized using the extract of *Camellia sinensis* var. (Chinese green tea) had spheroidal shape coated with phytomolecules with a mean dimension of 35.76 nm (Al-Otibi *et al.*, 2023). Similarly, the AgNPs acquired by applying the *Ctenolepis garcinia* L. aqueous leaf extract also had spheroidal shape, with dimensional range of 10–25 nm (Narayanan *et al.*, 2021). Phytochemicals could also act as capping agents for the synthesis of nanoparticles (Siddique *et al.*, 2024).

The AgNPs created from the water fruit extract of *Prosopis farcta* showed dose-mannered increment in the percentage of DPPH inhibition. At 0.2 mg/mL of AgNPs, the DPPH inhibition was 43%, and at 1 mg/mL of AgNPs, the inhibition was 63%, with 0.70 mg/mL as IC_{50} value. Nanosilver had superior anti-radical function, compared to the fruit extract. AgNPs synthesized using the aqueous leaf extract of *Erythrina suberosa* displayed marvellous anti-radical potency with 30.04 µg/mL as IC_{50} value (Mohanta *et al.*, 2017). Similarly, AgNPs fabricated

by utilizing the aqueous leaf extract of *Brassica oleracea* provided dose-responsive (50–200 µg/mL) form of anti-radical activity through DPPH assay, with an IC₅₀ of 50.37 µg/mL. The biological potential of extracts of *Kadsua coccinea* leaves evaluated by *in vitro* antioxidant assay showed promising DPPH scavenging activity (Shi *et al.*, 2022). The phytoingredient's phenolic and hydroxyl (OH) function groups layer on the AgNPs would have donated the electron/hydrogen and aided in the anti-radical function of the nanoparticles (Ansar *et al.*, 2020). The aqueous root extract of *Helicteres isora*-applied phyto-formulated AgNPs also showed dose-basis (10–100 µg/mL) anti-radical efficacy with 90% DPPH radical inhibition (Bhakya *et al.*, 2016). In accordance with these reports, the DPPH inhibition would be due to a synergistic action of AgNPs and the phenolic content present on the surface of nanoparticles, both of which portray anti-radical effect by donating electrons/hydrogen.

Amid free radicals, NO is a highly diffusible bioregulatory form and has indispensable functions in the human body, such as anticancer, relaxation of smooth muscles, neurological messenger, hindering platelet's aggregation, microbicidal, and controlling cellular-influenced toxicity (Andrabi *et al.*, 2023). Chronic generation of NO upsurges the issue of carcinoma, inflammation, diabetes mellitus, ulcerative colitis, Alzheimer's disease, heart diseases, and autoimmune conditions (rheumatoid arthritis and multiple sclerosis) (Roy *et al.*, 2013). Phytophenolics also confer NO scavenging ability. As appeared in our investigations, AgNPs wrapped with the fruit extract of *Piper longum* delivered increased percentage inhibition of NO ions in a dose-dependent manner (Shi *et al.*, 2022). At a maximum dose of 500 µg/mL, the NO percentage inhibition shown by AgNPs was 70%, which was greater, compared to fruit extract.

Usually, the lower stabilized NO ion acquires electron from AgNPs and develops formazan on blending with Griess reagent and monitored spectrophotometrically (Reddy *et al.*, 2014). AgNPs designed by adopting the aqueous leaf extract of *Decaschistia crotonifolia* showed 64.98% of NO inhibition, whereas 52.64% of NO inhibition was shown by *Decaschistia crotonifolia* leaf extract at 100-µg/mL dose. Both samples projected dose-based NO scavenging function (Palithya *et al.*, 2021). Similarly, NO scavenging efficacy of the AgNPs designed from the aqueous pod extract of *Pisum sativum* L. was entirely dose-related when screened from 10 µg/mL to 50 µg/mL. Phytophenolics, in addition to nanoparticle's stabilization, also render antioxidative function toward free radicals (Alarjani *et al.*, 2022).

Our antibacterial results confirmed the previously reported outcomes. Nanosilver acquired through the application of leaf extract of *Plumeria alba* (frangipani)

when screened offered excellent antibacterial performance toward both classes of bacteria (Gram +ve and Gram -ve), such as *Bacillus subtilis*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, and *Enterobacter aerogenes*, in a dose-dependent manner at a concentration of 25–100 µg/mL, with pronounced inhibitory zone at a higher dose (100 µg/mL) (Rudrappa *et al.*, 2022). Bharadwaj *et al.* (2021) showed that the spheroidal-shaped AgNPs using the fruit extract of *Diospyros malabarica* appeared to be bactericidal toward *Staphylococcus aureus* and *Escherichia coli* at two varying doses (500 µg/mL and 1,000 µg/mL).

Numerous mechanistic ways have been suggested for the bactericidal functionalities of AgNPs. The amino, phosphate, and carboxylic groups are attributed to the negative charge of bacterial cellular membrane (Mortazavi-Derazkola *et al.*, 2021). The positive charge of AgNPs and the bacterial cell negative charge create electrostatic force between the two components enduring efficacious binding. AgNPs trigger the genesis of pit on bacterial cellular surface, which aid in the feasible penetrability of silver ions (Firdhouse and Lalitha, 2016). AgNPs strongly bind to the S-H (thiol) group of the enzymes containing sulphur and develop sulphur-silver (S-Ag) bond, thereby hindering the transport of crucial ions by those enzymes. The reactive oxygen species (ROS) molecules encourage the hydrogen bonding interruption between the anti-parallel polynucleotide strands of DNA and facilitate its denaturation by intercalating nitrogenous bases (purine/pyrimidine) (Yassin *et al.*, 2022).

Macromolecular component (protein/reducing sugar) leakage from the bacterial cell was intended to damage cellular membrane permeability, thus leading to bacterial lysis (Sharma *et al.*, 2009). The adherence of AgNPs to bacterial cell wall renders denaturation of proteins, loss of proton motive force through the precursor protein built-up eventually enhancing cellular killing (Jemilugba *et al.*, 2019). Even lipids and lipopolysaccharides (LPS) of bacterial cellular membranes were also tremendously damaged by AgNPs. The exterior charge, surface nature, and concentration of nanoparticles have marvellous impact on bactericidal activity (Abdelsattar *et al.*, 2022). Smaller dimension of AgNPs enriches its bactericidal function by offering easy penetration and huge-surface space for better interaction, facilitating the release of silver (Ag⁰) ions (Kathiravan *et al.*, 2015). Superoxide anion, a form of ROS, stimulates oxidative-type of stress within the bacterial cells, promoting the interruption of electron transport chain (ETC), mutation, and damage to genome content (Nishanthi *et al.*, 2019). A study done by Zeng *et al.* 2020, explained anti-nociceptive and bioactive phytoingredients present in the extracts of *Typha angustifolia* L that could offer therapeutic outcome. Similarly,

the plant extracts of *S. jambos* also showed therapeutic potential, and the phenolic ingredient layered on AgNPs also contributed to efficacious bactericidal potential (Esmale *et al.*, 2020). Similarly, the present study also showed antibacterial effect against both organisms due to the positive charge present on the surface of the nanoparticles. This was further enhanced by the phenolic content present on the surface of plant extract.

A study done by Xia *et al.* (2016) reported that at 10 mg/L of AgNPs dose, the surviving embryo projected malformations and degeneration of body after 48 h. The nano spheroidal silver particles synthesized using the green tea extract also showed similar forms of morphological alterations as reported in our study, thus demonstrating the toxicity of nanoparticles (Girigoswami *et al.*, 2024). Similarly, AgNPs synthesized by utilizing seed extract of *Syzygium aromaticum* showed moderate level of toxicity toward the growth and formation of zebrafish embryo at a dose of 50 µg/mL. Greater toxicity occurred at 75 µg/mL and 100 µg/mL of the applied AgNPs on the embryo of zebrafish (Dhanislas *et al.*, 2023).

Multifunctional nanoparticles portray significant applications in various fields. A study done by Jabbar *et al.* (2023) showed the ability to detect chemical ions using plant extract functionalized AgNPs as well as antimicrobial activity. Similarly, the AgNPs developed using extracts of *S. jambos* could provide multifunctional capability as evidenced through antioxidant and antibacterial properties. The safe use of nanoparticles in multifarious applications is studied. Regulations demand thorough physicochemical characterization, *in vitro* and *in vivo* toxicity evaluations, and other biochemical interactions prior to being implemented on humans (Wang and Tang, 2021).

Conclusions

In the executed study, a cost-effective and environment-friendly phyto-based methodology was followed to procure AgNPs from the aqueous leaf extract of *S. jambos*. The transformation of Ag⁰ to AgNPs was considered by the formation of dark-brownish color by mixing the leaf aqueous extract of *S. jambos* with silver nitrate solution. The physicochemical and microscopic characterization was done for the created AgNPs. A peak at 420 nm justified the formation of AgNPs via UV-vis spectroscopy. FTIR spectra showed the role of glycosides, phenolic acids, saponins, tannins, and flavonoids in the reduction, stabilization, and capping of nanoparticles. XRD demonstrated crystallinity and grain size (17.8 nm) of the designed nanoparticles. In SEM and TEM micrographic images, the fabricated nanoparticles conveyed spheroidal morphology with agglomeration

and had an average dimension of 18.9 nm. The EDX spectra revealed silver, oxygen, and carbon. Through DPPH and NO assays, it was conveyed that the fabricated AgNPs quelled both free radicals in a dose-dependent manner. AgNPs showed effective inhibitory performance toward the screened *Bacillus cereus* and *Shigella flexneri*. Moreover, AgNPs were toxic toward zebrafish embryo by creating phenotypic deformities and also impacting its growth. This zebrafish embryo toxicity study aids in screening the beneficial concentration of AgNPs, so that it could be practised in the biomedical field for versatile applications. Overall, more experimental trials have to be conducted on various bioactivities of AgNPs to enhance their applications. In-depth molecular mechanistic studies must be performed to acquire the detailed mode of AgNPs functioning for therapeutic usage.

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Data Availability Statement

All data generated and analyzed in this study are included in this article.

Author Contributions

Mosleh Mohammad Abomughaid: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—original draft, review and editing, and project administration.

Conflicts of Interest

The author declared no conflict of interest.

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Supplementary

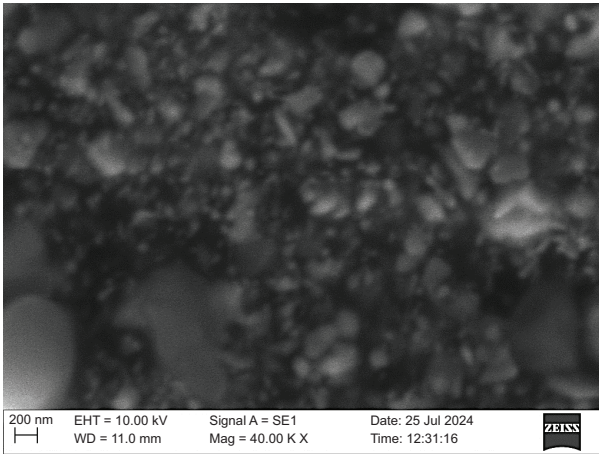


Figure S1. SEM image.

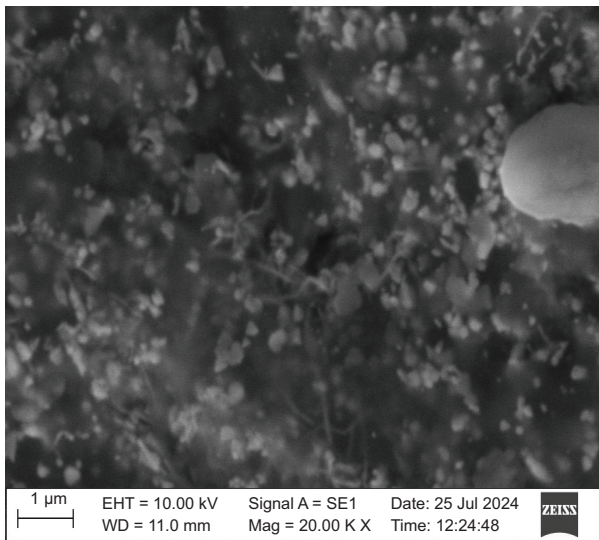


Figure S2. SEM image.

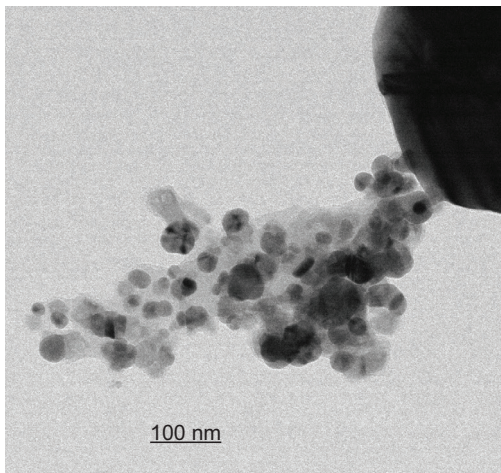


Figure S3. TEM image.



Figure S4. *Bacillus cereus*.



Figure S5. *Shigella flexneri*.