



## Ginger (*Zingiber officinale*) Extract Mediated Green Synthesis of Silver Nanoparticles and Evaluation of Their Antimicrobial Activity

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### ABSTRACT

Silver nanoparticles (AgNPs) are potent for fighting antimicrobial resistance independently. This study aimed to prepare AgNPs using ginger (*Z. officinale*) rhizome extracts and to evaluate the antibacterial efficacy of these AgNPs against multidrug-resistant (MDR) *Staphylococcus aureus*, and *E. coli*. AgNPs were synthesized using ginger rhizome extracts and further characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy analysis (EDAX). Disc and agar well diffusion techniques were utilized to determine the in vitro antibacterial activity of plant extracts and AgNPs. The crystalline structure of ZO-Ag-NPs was displayed in the XRD analysis. SEM analysis revealed the surface morphology. The EDAX analysis also confirmed the element of silver. It was revealed that AgNPs were seemingly spherical in morphology. The biosynthesized AgNPs exhibited complete antibacterial activity against the tested pathogenic bacterial strains. This study indicates that AgNPs of ginger extracts exhibit potent, antibacterial, antifungal activity and antibiotic resistance against bacterial strains.

**Keywords:** Biosynthesized silver nanoparticles, AgNPs, MDR pathogens, ginger rhizome extract, antibacterial activity.

### INTRODUCTION

The field of nanotechnology is one of the most active fields of research nowadays. It is a science that works at the nanoscale and gives different focal points to the diverse fields of science like dentistry, Pharmaceuticals, and bio-engineering. Nanocrystalline silver properties have found tremendous applications in the fields of biomolecular detection, diagnostics, antimicrobials, and therapy and catalysis<sup>1</sup>

The scientist gives special attention to the production of nanoparticles because of their unique features and technical uses that have a favourable impact on various aspects of the economy, including energy, pharmaceutical industry, agriculture, and cosmetics. Nanoparticles have unique Physicochemical characteristics that aren't present in bulk material. Nanoparticles are attractive because they're easy to make, environmentally friendly, and cost-effective, which makes them great options for many applications. Silver nanoparticles are well-established and commonly utilized in a variety of nanomedicine, anti-microbial products, drug delivery, Nano fertilizers, Nano pesticides, and biomedical applications. The production of bacterial biofilms was decreased by silver nanoparticles. The utilization of plants for the synthesis of NPs has an advantage as compared to other natural strategies (for example, microorganisms) because it does not require a long cultivation time to reduce the metal particles. Plant-made NPs are cost-effective and have nontoxic impact<sup>2</sup>The World Health Organization (WHO) believes that 80% population of the world still uses traditional medicine, including plant-based medications.<sup>3</sup>One of the most utilized herbs in folk medicine is ginger. It is used as a spice and flavoring agent worldwide, and it is said to have a

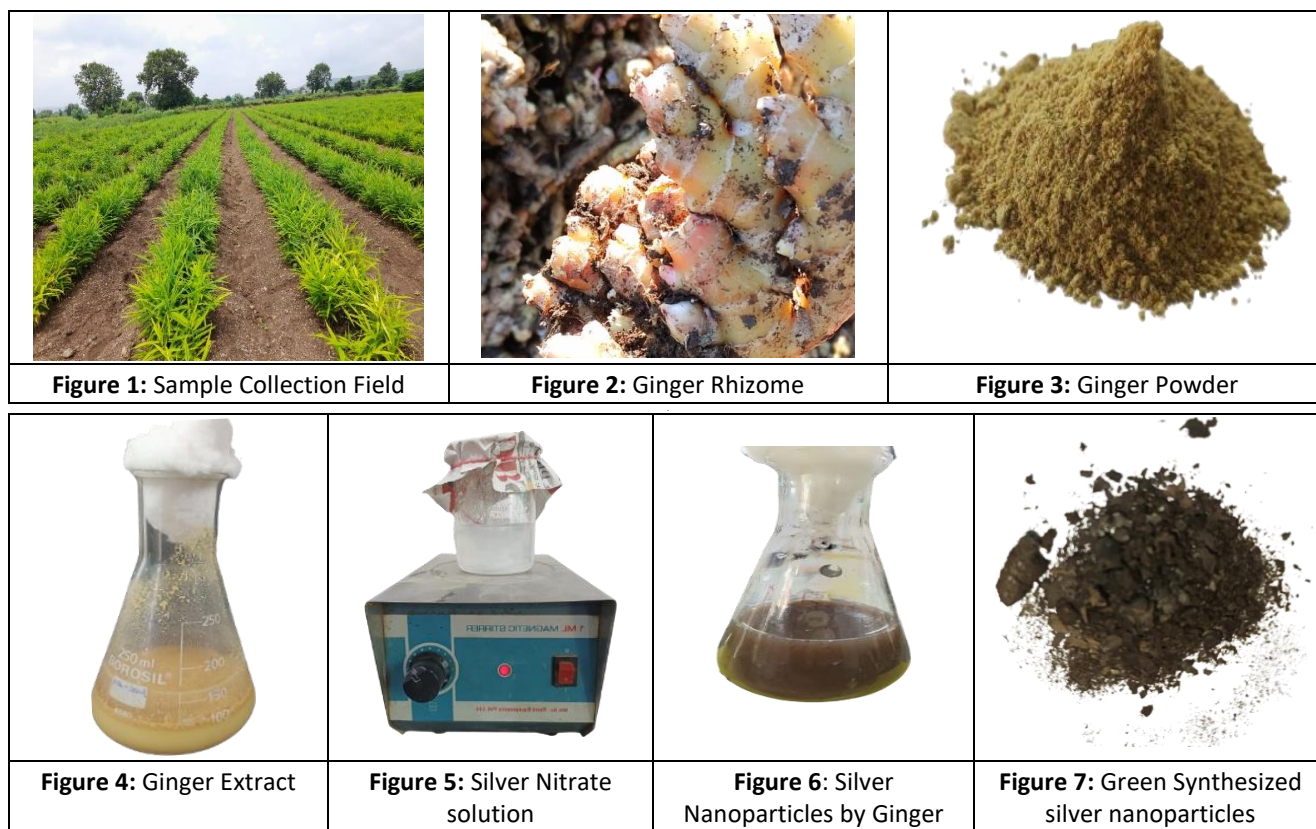
variety of medicinal Properties<sup>4</sup>. Many scientific studies have discovered its many pharmacological effects, including antioxidant, antibacterial, anti-inflammatory, antinociceptive, antimutagenic, and hepatoprotective activities<sup>5</sup>. *Z. officinale*, commonly known as ginger. It has many bioactive compounds which play critical role as reducing and capping agents. Therefore, it would be of high interest to take advantage of them for the green synthesis of AgNPs. Currently, various antimicrobial agents are being developed for use on bacterial infections that are resistant to antibiotics. In addition to the development of new antibiotics, there is a need for alternate ways of treating various bacterial infections to overcome the high mortality rates. Keeping in mind the situation, the current study provides data on the synthesis of the AgNPs using ginger extracts. In the current study, rhizome extracts were initially utilized for the biosynthesis of NPs. The green biosynthesis of AgNPs was characterized by various physical procedures such as UV/Vis, followed by XRD, FTIR, and SEM-EDAX. The antimicrobial activity of aqueous ginger extracts was determined against *S. aureus*, *Streptococcus*, and *E. coli*.

### MATERIALS AND METHODS

#### Plant material collection

Fresh rhizomes of *Z. officinale* were collected in December 2023 from the Takli Antur, ch. Sambhaji nagar, Maharashtra India. Ginger rhizome was properly cleaned with Tap water many times followed by deionized water, Fresh ginger was cleaned, and then it was finely chopped to dry. The sun-dried plant material was coarsely powdered in a grinder and passed through the muslin cloth before storing in an airtight container for further extraction.





### Preparation of Aqueous Extract of *Z. officinale*

Twenty-five grams of rhizome powder of *Z. officinale* was taken in a glass bottle (size:250 mL), and 100 mL of distilled water (1:10 w/v) was added and then mixed thoroughly. After mixing, the mixture was boiled at 35°C for 30 min using a water bath and then allowed to cool at room temperature. After cooling, the mixture was filtered using Whatman filter paper and stored at 4°C to be further used for the synthesis of AgNPs while some filtrates were evaporated to dryness at 40°C in a vacuum using a rotary evaporator. The extract was stored in an airtight container and kept at 4°C in a refrigerator until further experiments for the crude extract's biological activity and phytochemical analysis.

### Green Synthesis of silver nanoparticles

The first precursor stock solution (2.5 mM) was created by dissolving of silver nitrate (AgNO<sub>3</sub>) into 100 ml of distilled water to produce AgNPs utilizing extracts. Drop by drop, 10 mL of ginger extract was added to 100 mL of 2.5 mM silver nitrate solution, and the mixture was then left to react at room temperature without being disturbed. The initiation of the reaction results in the production of yellowish-brown color in the aqueous solution of silver nitrate, which shows the synthesis of Ag nanoparticles. As the reaction progresses, the color of the Ag nanoparticles gradually changes to dark brown (Fig. 6). The reduction of Ag<sup>+</sup> to Ag was followed by FTIR spectroscopy. (Fig.8) presents a schematic drawing of AgNPs synthesis.

### Characterization of AgNPs

#### Scanning Electron Microscopy EDAX Detector Analysis (SEM-EDAX)

SEM (JEOL JSM-6510) was used to examine the morphology of AgNPs synthesized using *Z. officinale* aqueous and organic extracts, which were operated at a 12 keV accelerating voltage. The sample was prepared by simply dropping a minimal sample on a carbon-coated copper grid. After drying for 5 min under a mercury lamp, SEM scans at different magnifications were obtained. The dried powdered AgNP sample was drop-coated on a carbon sheet for elemental analysis. After that, an EDAX detector connected to the SEM was used to perform.

#### Estimation of antibacterial activity

The comparative antibacterial activities of the ginger extract and the AgNPs synthesized from the respective extracts were effectively assessed against Gram (-)ve *Escherichia Coli (E.coli)* bacteria and the well diffusion method was followed for testing of ginger extract and their respective Ag NPs containing solution. The nutrient agar is poured on the plate. In the procedure used in the disk diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, the hole with a diameter of 6-8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (40-60µL) of the antimicrobial agent or extract solution at the desired concentration is introduced in the wells. The plates were incubated at 37°C for 24 to 48

hr. Then the maximum zone of inhibition was observed and measured for analysis against the test microorganism.<sup>6</sup>

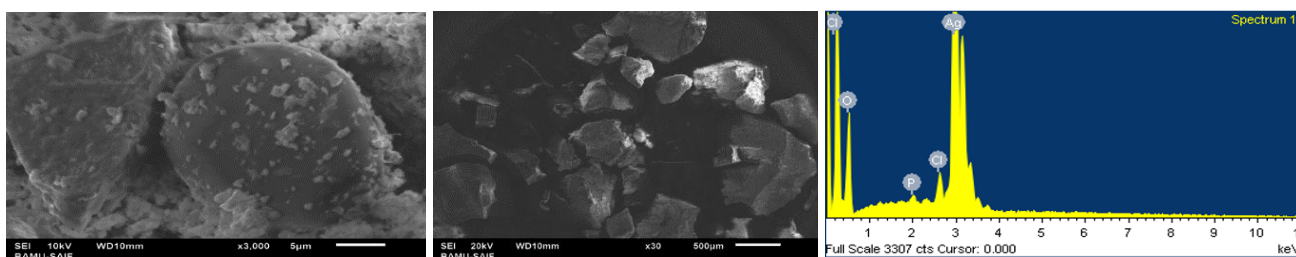
### Estimation of antifungal activity

The comparative antifungal activities of the ginger extract and the AgNPs synthesized from the extract were effectively assessed against *Penicillium notatum* and the well diffusion method was followed for testing of ginger extract and their respective AgNPs containing solution. The czeppadox agar pours onto the plate. In the procedure used in the disk diffusion method, the agar plate surface is inoculated by spreading a volume of the fungal inoculum over the entire agar surface. Then, the hole with a diameter of 6-8 mm is punched aseptically with a sterile cork borer or tips, and a volume (60,75 $\mu$ L) of the antifungal agent or extract solution at the desired concentration is introduced in the wells. The plates were incubated at 37°C for 48 to 78

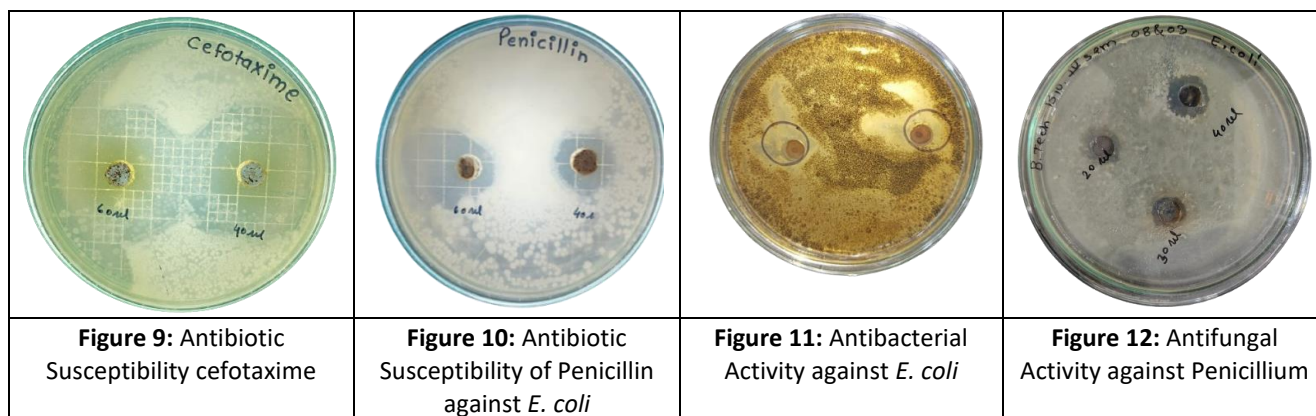
hr. Then the maximum zone of inhibition was observed and measured for analysis against the test microorganism.<sup>7</sup>

### Estimation of Antibiotic susceptibility

The antibacterial sensitivity of bacterial strains was determined by the well diffusion method. Using a sterile cotton swab, nutrient agar plates were swabbed with a fresh culture of bacterial isolates. The surface is allowed to dry for about 5-7min. Then the hole with a diameter of 6-8mm is punched aseptically with a sterile cork borer or tips and a volume of commercial antibiotics including cefotaxime (75 $\mu$ L) and penicillin (60 $\mu$ L) of the antibiotic agent or extract solution at the desired concentration is introduced in the wells. The plates were incubated at 37°C for 24 h. After 18 to 24 hr of incubation, the zone of inhibition was measured in mm using the measuring scale, and the three measurements were determined.<sup>8</sup>



**Figure 8:** Scanning Electron Microscopy analysis of Ginger AgNPs(L) EDAX AgNPs



## RESULTS AND DISCUSSION

The color changes of the synthesized solution are shown in Fig.6 in which the colour of the mixture of AgNO<sub>3</sub> and extract solution is changed from yellowish to brown and there is no more change in the colour after the reaction goes to completion. This is evidence for the catalytic role of light and temperature on the synthesis of Ag NPs via plant extracts. The formation of silver nanoparticles was confirmed by the change in color of the silver nitrate solution. According to the addition of extract drop by drop of volume 5 mL in the reaction solution for 30 minutes, the reaction mixture's color changed to yellowish brown, brown, and deep brown. Silver ions are converted to silver nanoparticles at a faster rate as ginger extract volume increases. The instantaneous color variation that occurred after combining the silver nitrate and aqueous extract solution showed that the reaction occurred. This color

change shows the activity of a redox reaction, in which extract ingredients, which are oxidized to various species, decrease Ag<sup>+</sup> ions to Ag<sup>0</sup>. Our findings are similar to previously reported<sup>9</sup>.

### Scanning electron microscopy (SEM -EDAX) Analysis

Fig.8 and 9 show the SEM images of synthesized Ag NPs via rhizome extracts. Ag NPs are spherical with a mean size of 30 nm for ginger extract. Here, it should be noted that the ginger extract concentration (5 gm of ginger in 100 mL of DI water) is 25 times more than the extract. At this condition, Ag ions are reduced more and the final Ag NPs' size is increased more in the ginger extract which again confirms the higher reduction power of the rhizome extract.

A scanning electron microscope was used to assess the size and morphology of silver nanoparticles. As a result, SEM images are shown in figure 8 and the ginger extract was

used to produce the silver nanoparticles, and the silver nanoparticles were spherical and ranged in size from 5 to 35 nm.<sup>10</sup> reported that silver nanoparticles mediated by ginger extract were spherical.

The elemental compositions of the as-prepared samples of Ag NPs were also determined by EDX (energy dispersive X-ray spectrometry). The EDAX spectra shown in Figure 8 reveal the clear elemental composition profile of the green synthesized Ag NPs using both ginger rhizome extract. Figure 9 shows the EDAX signals of silver nanoparticles prepared from 2.5 mM of silver precursor salt. In spectra, the intense signal at 3 keV strongly suggests that Ag was the major element, which has an optical absorption in this range due to the surface Plasmon resonance (SPR)<sup>11</sup> The other signals in the range of 0.0–0.5 keV typically represent the absorption of carbon and oxygen and thus indicate the presence of the extract (as a capping ligand) on the surfaces of the NPs.

The SEM–EDAX result confirmed that the AgNPs were spherical in shape. The aggregation of NPs indicates that they were in direct contact but stabilized by a capping agent. Functional groups had a stable size and were responsible for the capping of AgNPs. The presence of elemental silver was verified by the EDAX signal at 3 keV. The emission energy of 3 keV indicated the reduction of silver ions to elemental silver. Metallic silver nanocrystals usually show strong absorption spectra in the range of 2.5–3.5 keV. Similar findings were reported in many previous studies.<sup>12,13</sup>

### Antifungal activity

The antifungal activity of Ag NPs, pure extracts, silver nitrate, and Ginger rhizome extract against *C. albicans* was investigated by the MIC method, and the results are shown in Table 1. The extract of ginger rhizome demonstrated MIC at 40, 60, and 80 ppm, respectively. Silver nitrate demonstrated MIC of 40 ppm. The results showed that the MIC of silver nitrate and Ag NPs synthesized by ginger were 40 and 60, µg/ml, respectively. It can be inferred that the Ag NPs prepared via ginger extract higher inhibitory effect against *Penicillium notatum* in comparison to silver nitrate. with MIC of 40 ppm. Indeed, Ag NPs synthesized ginger extract demonstrated higher MIC which can be attributed to the synergetic effect of Ag NPs and plant extract. It has been shown that the antifungal activity of the extract of ginger may be involved due to the hydrophobic properties of compounds which can attach to the fungal plasma membrane and interfere with fungal proliferation by increasing the membrane permeability or inhibit spore germination and cell respiration<sup>14</sup> Therefore, prepared Ag NPs attach more to the cell membrane and destruct fungi plasma membrane, and finally prohibit the growth of *Penicillium notatum* It observed that the combination of the natural extract with Ag NPs may open diverse antifungal mechanisms.

### Antibacterial activity

In Figure 11, the antibacterial effects of silver nanoparticles synthesized from ginger rhizome on specific bacterial species are presented. Silver nanoparticle concentrations of 20 µg/ml, 30 µg/ml, and 40µg/ ml were prepared from ginger rhizome. Better zones of inhibition were found against *E. coli* (15 mm) at 40µg/ml concentration (Table 1). The growth of all the examined bacterial isolates was stopped by silver nanoparticles that were isolated from ginger rhizomes. It is necessary to find new antimicrobial medications using environmentally friendly and green methods due to the rise in antibiotic resistance among human infections. Plant phytochemical extract may have the potential for application in allopathic medicine as a source of antiviral, antitumoral, and antibacterial drugs.

The rapid development of bio nano technology stimulates the antibacterial action of silver nanoparticles among synthetic metal nanoparticles and has been thoroughly studied and experimentally verified<sup>15</sup>. The pathogen's sensitivity to silver nanoparticles is strongly affected by the structure of the bacterial cell surface<sup>16,17,18</sup>

**Table 1:** Evaluation of Antibacterial activity of *Zingiber officinale*

Bacterial culture	Concentrations	Zone of Inhibition
<i>E.coli</i>	20µl	7 mm
<i>E.coli</i>	30µl	9mm
<i>E.coli</i>	40µl	15mm

### Antibiotic susceptibility

In the current study, three clinically isolated strains of bacteria *E. coli* were tested for AMR using the disc diffusion method, and their susceptibility to the biosynthesized AgNPs and ginger extracts was evaluated, as summarized in Table 2. These bacteria showed resistance to the tested antibiotics using the disc diffusion method. However, in the presence of the synthesized AgNPs and ginger extracts, the antibacterial activity was seen in the form of clear zones of inhibition. The antibacterial susceptibilities of MDR strains of different bacteria using ginger extracts were previously reported in different studies<sup>19,20</sup> including It could be possible that the Ag component of AgNPs confers antimicrobial properties. A strong reaction takes place between the silver ions and thiol groups of vital enzymes, ultimately inactivating them. Similar observations were reported for *Bosewillia ovalifliolata* and *Shoeratumbuggai*.<sup>21</sup>

**Table 2:** Antibiotic Susceptibility of *Zingiber officinale*

Bacterial Culture	Antibiotic	Zone of Inhibition
<i>E.coli</i>	Penicillin	7 mm (40 µl) 19 mm (60 µl)
<i>E.coli</i>	Cefatoxime	9 mm (40 µl) 10 mm (60 µl)
<i>E.coli</i>	Control	7 mm (40 µl) 14 mm (60 µl)



## CONCLUSION

The green synthesis of AgNPs was carried out in this investigation utilizing ginger rhizome extracts. SEM–EDAX techniques were used to characterize the produced AgNPs. Using SEM, it was discovered that the AgNPs generated by wild ginger extracts had a spherical configuration. Green synthesis is a low-cost, straightforward, and environmentally friendly process, and the biosynthesis of AgNPs described here is an alternative to chemical synthesis methods. On antibiotic susceptibility bacterial strains, the biosynthesized AgNPs had a good antibacterial and antifungal impact. Green-synthesized AgNPs will open up a new pharmaceutical sector for the manufacture of pharmaceutical, and biomedical goods.

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