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Biological Synthesis and Characterisation of Silver Nanoparticles of *Zingiber officinalis* and Evaluation of its Biological Activity

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ABSTRACT

Ginger *(Zingiber officinale)* belongs to Zingiberaceae family is one of the famous spices all over the world. It is a perennial creeping plant with long leaves, yellow green flowers and thick tuberous rhizome. Silver nanoparticles' potential uses in Green Chemistry have received attention. The current study focuses on the rapid biological production of silver nanoparticles using different plant materials and characterizations using UV-visible spectrophotometry, IR, SEM, and HPLC investigations. Within 15 minutes of adding sodium carbonate, an aqueous extract of dried Zingiber officinalis rhizome reduces silver nitrate. No further reduction and stabilizing chemicals are required for the entire process, showing a green synthesis. Escherichia coli and *Staphylococcus aureus* use biosynthesized "Zinger-AgNps" as a competent antibacterial agent. Zinger-AgNps were evaluated as its catalytic capability to reduce the model pollutant methylene blue. Both of them are displayed 2, 2-diphenyl-1-picrylhydrazyl-specific free radical scavenging activity (DPPH). Silver biological material nanoparticles are created by utilizing less hazardous and nontoxic reduction agents, such as ascorbic acid and sodium citrate. The antioxidant and lipid peroxidation inhibition properties of ginger prevent peroxidative damage, indicating the benefits of ginger in the prevention of microbial food spoilage, free-radical-induced damage and rancidity. The sodium citrate aggregation for silver nanoparticles, firm surface contact, and synthesized silver nanoparticles are used to study antibacterial efficacy and antioxidant activity.

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INTRODUCTION

Zingiber officinale is a typical ingredient in Indian cuisine. It is known as Adrak in numerous regions of the nation. This perennial herb contains a system of underground branches. When the plant's leaves and rhizomes are chopped, they emit a distinct scent. Before being utilized, the Rhizomes of the plant are extracted and dried. Ginger, or *Zingiber officinale*, is known to have originated in India, and the sun-dried Adrak plant has been recorded numerous times in the medical histories of India and China. The plant's Sanskrit name is Singabera. During the Vedic Era,

this plant was known as *Maha aushadhi*, or a great remedy. This medicinal herb was employed by the great physician Galen to treat faulty humour senses in the body. There are numerous applications for silver nanoparticles in biomolecular detection, diagnostics, antimicrobials, and therapies [1].

He also utilised this to treat individuals who are suffering from paralysis and phlegmatic abnormalities in their bodies. Other physicians throughout the world employed this plant as an aphrodisiac and carminative. Ginger, a perennial herb of the Zingiberaceae family claimed to have originated in Southeast Asia, is utilised in spices, flavourings, cuisines, and medications. The generic name Zingiber is derived from the Greek Zingiberis, which is derived from the Sanskrit word Singabera. Nanoparticles have numerous medical applications, including antigen delivery during immunizations and topical wound healing aids in addition, a plethora of additional applications motivate academics to study novel methodologies for synthesis of green nanomaterials.

Scientific Classification

Kingdom : Plantae

Division : Mangoliophyta

Class : Liliopsida

Order : Zingiberales

Family : Zingibaraceae

Genus : *Zingiber*

Species : *Z. officinale*

Figure 1: Dried Rhizome of Zingiber officinalis

This present method is focused on synthesizing AgNp using plant extract in a green nano technique. The preparation method is also cost-effective and time-consuming. The antibacterial properties of silver are boosted when it is transformed into a nanoparticle, making it more efficient at eradicating microorganisms. The biological processes are disrupted by silver and its nanoparticles, which prevent microbial growth. The harmful effect on microorganisms is targeted, and it may prevent microbial resistance from creating. Microbial resistance develops because of the overuse and misuse of antibiotics. The factor that has become a significant issue. We must discover alternative chemotherapeutic drugs for illness therapy. It required plant sources to produce medicines that are easily accessible and have fewer side effects [2]. The herbs and extracts to treat us have been used for infectious illnesses in various areas of the globe for many years [3]. We may utilize medicinal plant products in many forms, including powder, and fluid mixes that can be crude (or) boiling, such as liniments, ointments, and incisions [4]. "Ginger is a medicinal herb used worldwide since ancient times for a broad range of unrelated illnesses, including arthritis, rheumatism, sprains, sore necks, aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever, and infection." [5]. Ginger has direct antibacterial action and may thus treat bacterial illness [6]. Ginger is part of the family of Zingiberaceae [7]. Non-tuberous rhizomes define Zinger plants, which are noted for their strong fragrance and medicinal properties (Figure 1).

Materials and Methods

Material of the Plant

We got Rotted ginger from an Ayurvedic medicine store and silver nitrate (silver salt) of Analytical grade 0. We utilized 01mm as supplied. We utilized analytical grade 10 to 20 sodium carbonate as received. Deionized water has nearly all its mineral ions removed, resulting in bacteria-free pure water. E. coli [ATCC 10798] and S. aureus [ATCC 6538] pathogenic bacterial strains were used.

Preparation of Ginger Extract

We made ginger extract by combining the dried ginger powder with distilled water to produce a 20% solution. We put this on a magnetic stirrer for 10 minutes to ensure good mixing, then heated it in a water bath for 10 minutes and filtered it through filter paper. The filtrate was the aqueous extract utilized in nanoparticle production.

Synthesis of Silver Nanoparticles

At room temperature, an equal quantity of 20% aqueous extract of dried ginger was added dropwise to a conical flask containing 1mM silver nitrate solution while constantly mixing. To avoid silver auto-oxidation, we maintained the solution in the dark. 20ml of 0.1M sodium carbonate was added, and we centrifuged the resulting solution combination at 10000rpm for 15 minutes before we removed

the supernatant to produce Ag-Nps. UV-Vis spectroscopy and scanning electron microscopy were used to examine the nanoparticles that were created (SEM). We performed HPLC analysis on nanoparticle solutions.

Characterization of Zingiber – AgNps

"Characterization of Zingiber-AgNps was conducted according to our previous studies" [8, 9].

In Vitro Biological Evaluation of Zingiber-AGNPS

Antimicrobial Activity of Zingiber-AgNps

We tested the biogenic AgNPs' antibacterial efficacy against gram-negative bacteria. The Escherichia I bacteria mostly chose coli organisms based on their human infections. The inhibitory effects of silver nanoparticles and TMC on bacterial growth were first assessed using turbidity measurements. We must do dilution in a certain order. We mixed 1 ml of bacterial broth with 9 ml of different diluted silver nanoparticle suspensions for antimicrobial testing and kept them gently agitated at room temperature. Turbidity is measured by adding pH 7.4 to a mixture of cultured media bacteria and PBS (phosphate-buffered saline). We used ciprofloxacin as a positive control in this experiment. I regulated the bacteria using a tube that crosses antibiotics. After three days of aerobic incubation at 37 degrees Celsius, we checked tubes for turbidity and silt. I determined the minimal inhibitory concentration of the tube with no visible growth and the lowest antibiotic content (MIC) [9, 10].

Catalytic Reduction of Methylene Blue During Zingiber-AgNps

The absorption value of methylene blue is used for the effective catalytic reduction of chemical dyes. It is demonstrated using 10.2 mL of aqueous Zingiber root extract containing "x" and 1.8 mL of water. After 30 minutes and 60 minutes of incubation, they monitored the response at room temperature. In addition, 1mL methylene blue (1 x 10-4M) was added to the 0.3mL generated Zingiber-AgNps, and they saw the response after 30 and 60 minutes of incubation. When compared to methylene blue, the absorption values at 670nm were the greatest." [11].

The Antioxidant Action of DPPH Free Radicals

Around "6" different concentrations of nanoparticles (100, 250, 500, 1000, 1500, and 2000ug/mL) were combined with 1mL DPPH 0.1 mM and incubated for 30 minutes in the dark. Using ascorbic acid as a positive control, we assessed the absorbance at 517 nm. The Scavenging activity of free radical was determined using the following formula: Percent inhibition = [[control absorption - (sample absorption - blank absorption)] / 100Blank absorbance was 99.5% ethanol replacement for DPPH solution when an equivalent amount of distilled water replaced control absorption. We carried three replications out to enhance the reliability of the analyses.

RESULTS AND DISCUSSION

Green Synthesis of Zi-Ag [Nps]

Dried ginger was procured from a local shop for Ayurvedic medicines, Silver nitrate (silver salt) of Analytical grade 0.01mm was used as received. Sodium carbonate of Analytical grade 10 to 20 was used as received. Deionized water is water that has had almost all its mineral ions removed, resulting in bacterial–free purified water. The AgNPs were formed within an hour of the reaction marked by a prominent change of color from yellow to dark greenish black indicating that silver has been reduced. The blackening of the previously white reaction mixture may be attributable to the surface Plasmon resonance of the silver nanoparticles, which is regarded as the key characteristic of nanoparticle production. Most silver-synthesized nanoparticles exhibited the same colour change as anthoceros [12], a mushroom (Figure 2).

Figure 2: Formation of Ag-Nps of Aqueous Extract of Zingiber Over a 1-hour Time Period

Characterization of Synthesized AgNps from *Zingiber officinalis* Rhizome Extract

UV-Vis Spectroscopy

The surface Plasmon band in the silver nanoparticles solution is around 410nm for ginger extract at ambient temperature (Figure 3) and 450nm for ginger extract at 600c (Figure 4). The Absorption maxima of synthesised Ag-Nps were evaluated in a Double beam UV –VIS spectrophotometer and the Ag-Nps displayed maximum UV-Vis absorption in the 410–450 nm region. Similar readings were obtained from the leaf extract of *Eucalyptus hybrida*, which were centred at 412nm [13].

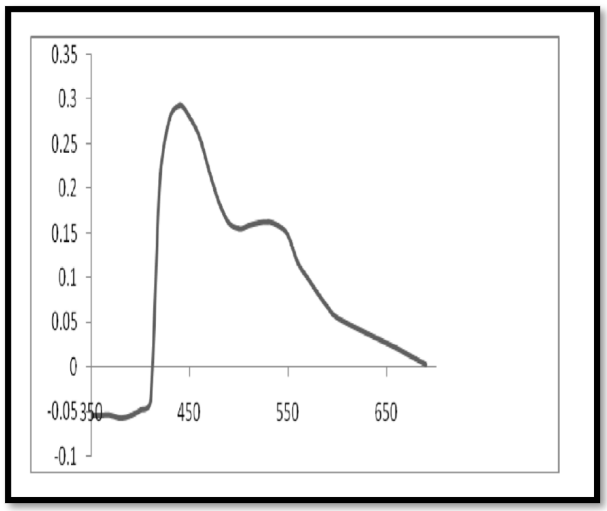


Figure 3: UV–VIS Spectrum of Zi AG-Nps

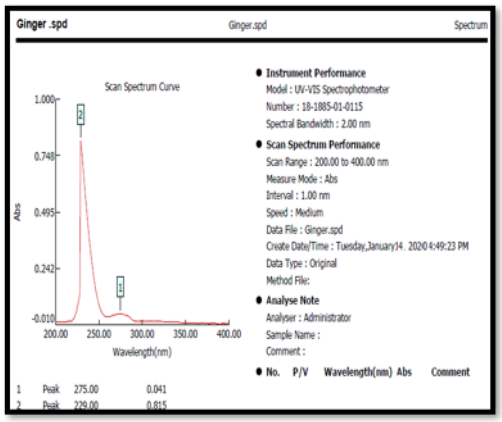


Figure 4: UV–VIS Spectrum of *Zingiber* Aqueous Extract

Fourier Transforms Infrared Spectroscopy (FT-IR)

The FT-IR transmittance of AgNps and Zingiber rhizome extracts reveals the probable bio-molecules involved in nanomaterial capping and efficient stability. Strong bands are observed in the FT-IR spectra of synthesised AgNps at 3470, 1609, 1345, 1082, and 750 cm^{-1}, which correspond to the stretching of the free hydroxyl O-H of alcohols and phenols, respectively. C=C-alkenes, C-H rock alkanes, C-O long stretching ethers, and C-H strong bending alkenes are found in Rhizome extract, followed by strong bands at 3438, 2144, 1630, 1289, 1011, and 548 cm^{-1}.

These influential bands correspond to the stretching free hydroxyl O-H of alcohols and phenols. C-H strong stretching alkanes, C=C strong stretching alkenes, C-N variable stretching amines, C-O weak stretching ethers, and C-Br stretching alkyl halides, respectively. The variation in transmittance level shows unequivocally that it functionalized the metal nanoparticles with plant biomolecules [14].

These significant bands correlate to the stretching of alcohols and phenols' free hydroxyl O-H. C-H stretchy alkanes, C=C strong stretched alkenes, C-N strong stretchy amines, C-O stretchy ethers, and C-Br stretchy strong stretched alkyl halides, in that order. The difference in transmittance demonstrates conclusively that the metal nanoparticles were designed and synthesized with plant biomolecules [14] (Figure 5).

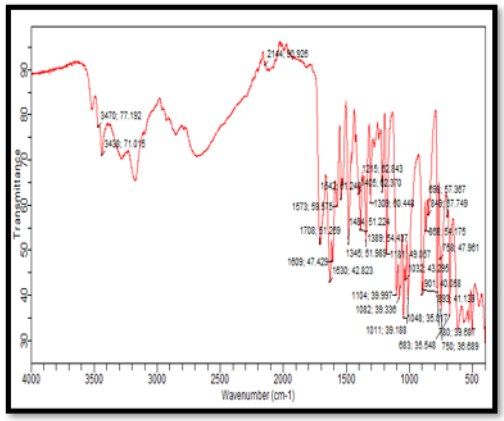


Figure 5: FTIR Analysis of Zi Ag-Nps

Scanning Electron Microscopy (SEM)

"SEM micrographs of synthesized AgNps at various magnifications, such as 500nm, 5 and 10 um, show evenly dispersed spherical AgNps with sizes less than 100nm. Although there was a significant aggregation of nanoparticles, we saw no direct fusion, owing to capping agents. All diffraction peaks correspond to the characteristic of functional groups silver lines [15]. This finding is consistent with several previous studies in which biosynthesized AgNps utilizing various plant entities produced spherical-shaped particles" [16, 17] (Figure 6).

High-Performance Liquid Chromatography (HPLC)

The test shows a wide retention peak at 2,096 minutes when dissolved in acetone. At 2.626 min and 4.148 min, we saw a modest intensity peak (Figure 7).

Evaluation of Biological Activity of Zi-Ag [Nps]

Antimicrobial Activity

The antimicrobial activity of silver nanoparticles was tested by standard agar well diffusion method [18]. Many pathogens, including Ecoli and S.aureus, have been evaluated for antibacterial activity in the biosynthesized Ag-NPs. We relate better antimicrobial effectiveness to bacterial wall features, given that the gram cell wall is thinner

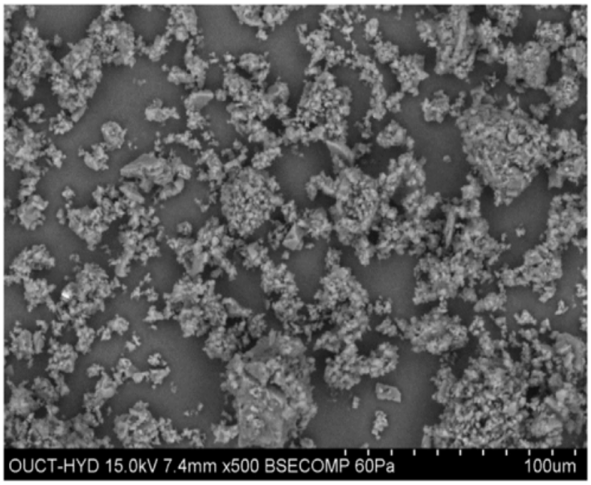
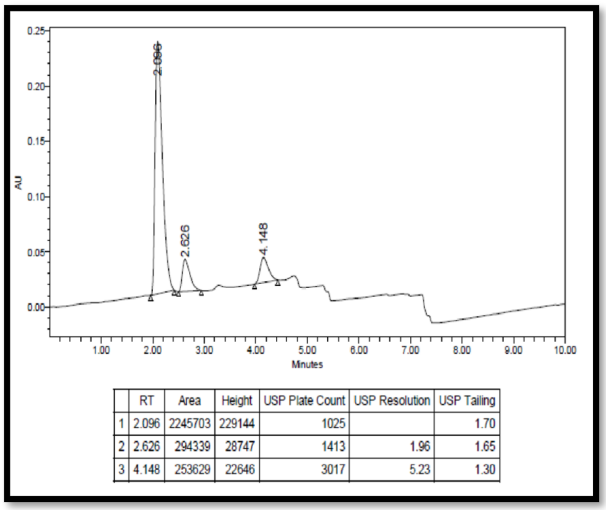


Figure 6: SEM Analysis of Zi Ag-Nps



	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
1	2.096	2245703	229144	1025		1.70
2	2.626	294339	28747	1413	1.96	1.65
3	4.148	253629	22646	3017	5.23	1.30

Figure 7: HPLC Analysis of Zi Ag-Nps

and thus more susceptible. We ascribe the primary antibacterial mechanism to the electrostatic interaction between positively charged silver ions and negatively charged spots in the microbial cell. The cell wall is 7-8nm thick which helps cell lysis.

Compared to prior research utilising garlic extract (26.30nm), the diameters of the deposits in our results are so small that they may be the product of distinct metal incubation conditions [19]. Results verified silver nanoparticle formation. Similar outcomes were reported using 3-12nm silver nanoparticles extracted from Citrus sinesis peels [20]. The widths of the inhibition zones of silver nanoparticles against various pathogenic cultures revealed that ginger extract exhibited a smaller inhibition zone than the manufactured nanoparticles. Measuring the widths of silver nanoparticle inhibition zones against distinct pathogenic cultures (Figure 8). The inhibitory zone of the control was significantly smaller than that of the produced nanoparticles. The antibacterial properties of E. coli and Klebsiella were comparable.

Figure 8: Antimicrobial Activity of the Produced Silver Nanoparticles Against (a) Klebsiella pneumonia and (b) Escherichia coli, as Displayed on Microtiter Plates

Catalytic Activity of ZI-AG [NPS]

Methylene blue decrease by nanoparticles mediated by plants is widely established. The maximum absorption is 670nm of Pure Methylene Blue. We added the Zi Rhizome extracts thirty minutes after the teal; the absorption reduced progressively and increased to a higher wavelength. The decrease in absorption shows that the ginger rhizome extract can destroy the methylene blue. The dye-containing reaction combination of Zi Ag[Nps] and the extract exhibited a greater absorbance rate after 30 minutes than Ginger rhizome extract. However, a significant reduction in the absorption of Methylene blue occurred during the 60% reaction mix.

Antioxidant Activity of ZI-AG [NPS]

DPPH reducing the ability of Zi-AG[NPS] was determined by evaluating the colour change spectrophotometrically from purple to yellow colour at 517nm.

DISCUSSION

The ginger extract may produce silver nanoparticles in this research. These silver-produced extract nanoparticles have been verified using UV-visible spectroscopy. Silver nanoparticles also have a powerful antibacterial impact. The antibacterial impact against pathogens was considerable. The mechanism of action of silver ions against bacteria may lead to this. These silver ions may destroy the cell wall and the cell membrane lysis. Silver ions attach to the basis of DNA, causing the DNA to lose its capacity to replicate, preventing replication through

binary fission. It also stimulates the production of ROS (Reactive Oxygen Species), producing highly reactive radicals that kill cells. Further research should be conducted to evaluate silver nanoparticles with gram-positive bacteria and fungus and toxicity tests.

Zingiber-AgNps synthesis by decreasing aqueous metal ions after exposure to Zingiber leaves was measured using a "UV-visible specimen" at various time intervals. We observed the highest absorption at 450nm, which clearly shows 'AgNps' production (Figure 3). We have also established that it directly related the incubation period to "AgNps size", form dispersion, and synthesis. "Similarly, a previously described phenomenon in which 'AgNps' produced under optimum reaction circumstances produces a 450nm intensive absorbing spectrum owing to collectively fluctuating surface electrons" [21].

CONCLUSION

In this research, "AgNps" were effectively produced utilizing the Z.officinale rhizome extract as a new bio-reduction. We may utilize the plant rhizome for large-scale "AgNps" synthesis in the nanotechnology processing sectors as being environmentally friendly and cost-efficient. The current research produced "AgNps" from the Z.officinale rhizome extract seems promising and efficient bacterial and fungal antibacterial agents. It also has a tremendous antioxidant activity which may help develop a novel medication for biomedical use. Because of its many benefits, this biological chemistry method to manufacture "AgNps" is a very vital endeavour in nanomedicine.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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