

Ginger-Derived Nanoparticles: A Study on Phytochemical Content, Antioxidant Activity, Antimicrobial Properties, and Toxicity

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ABSTRACT

To synthesis silver nanoparticles (AgNP's), the benefits of employing plants and their metabolic pathways over other biotic processes have motivated researchers to investigate the mechanism of Ag⁺ ion reduction to Ag⁰. Here, we present a straightforward aqueous method that uses ginger (*Zingiber officinale*) extract as a capping and reducing agent. Considering the synthesis of metal and semiconductor nanoparticles has the potential to be used in creating new technologies, this field of study is growing. To produce silver (AgNPs), *Z. officinale* root extract was combined with silver nitrate (AgNO₃). AgNPs' surface plasmon absorbance spectra were measured between 436 and 531 nm. Ginger rhizome extract biomolecules that reduce metal ions have the right peaks and artificially generated silver nanoparticles are revealed by analysis utilising Fourier transform infrared spectroscopy.

INTRODUCTION:

Plants provide the basic needs of humans by giving them food, clothing, and shelter. Ayurvedic medicine uses both fresh and dry plant parts. Plant components, either fresh or dried, are typically used in herbal preparations (Kavitha *et al.*, 2017). India has a pitiful share of the global market because of the export of crude extracts and pharmaceuticals, despite its wealth of traditional knowledge, history of using herbal remedies, and abundant biodiversity. One of the most popular members of the ginger family (Zingiberaceae) is ginger, which is the rhizome of *Zingiber officinale*. It is frequently used as a condiment for a variety of dishes and drinks (Bhargava *et al.*, 2012).

Ginger root, often known as the flowering plant, has several uses as a spice and in traditional medicine. There is strong evidence of a rise in demand for medicinal plants as a result of global tendencies toward an enhanced "quality of life" (Kotnis *et al.*, 2004). These compounds are produced by plants as a defence mechanism, but new studies show that many phytochemicals can shield people from conditions including cancer, heart disease, diabetes, arthritis, and ageing (Velavan, 2011). Almost 4,000 phytochemicals have been categorized and arranged according to their chemical, physical, and defensive properties (Meagher and Thomson, 1999).

Ginger is a complex substance consisting of more than 60 compounds. The essential oil and resin found in ginger rhizomes are combined to form oleoresin (Neeru Bhatt, *et al.*, 2013). Jolad *et al.* 2004 investigated fresh ginger produced organically and found 63 chemicals, of which 20 were previously unidentified and 31 have been recognized as components of ginger in the past. The components that were detected included the following:

shogaols, gingerols, paradols, 1-dehydrogingerdiones, 3-dihydroshogaols, gingerdiols, dihydroparadols,

diarylheptanoids, Gingerdiols' mono- and di-acetyl derivatives and several of these chemicals in methyl ether.

Studies have proven the antiviral effects of ginger. Numerous noteworthy non-volatile, bioactive phenolic compounds, such as paradols, zingerones, gingerols and shogaols, are found in the rhizome of ginger (Rathinavel *et al.*, 2020). Natural compounds and plant metabolites are employed in environmentally friendly methods to organise NPs for therapeutic and other purposes. Some characterization methodologies and applications of NPs are discussed (Gour, A., & Jain, N. K. 2019). Because they are created using a biological matrix and have excellent photostability, bioavailability and biostability, these NPs also demonstrate noteworthy chemical reactivity and superior photocatalytic activity during degradation reactions (Koe *et al.*, 2020, Deng *et al.*, 2021, Shivakumar *et al.*, 2020, Kaur, 2019, Kulkarni & Muddapur, 2014, Shivakumar *et al.*, 2020, Batool *et al.*, 2021, Emmanuel & Adesibikan, 2021, Sarkar *et al.*, 2014).

Reducing chemicals used in the reduction of stabilizing agents and metal ions used to stop the generated nanoparticles from clumping together unintentionally are hazardous to the environment and the cell when used in conventional physical and chemical processes. Moreover, the shape, size, and surface chemistry of the generated nanoparticles are believed to be hazardous. Biocompatible nanoparticles are created using a green synthesis approach that uses naturally occurring substances found in the biological organisms used (Hussain I *et al.*, 2016).

MATERIALS AND METHODS:

Herbarium specimen, *Zingiber officinale* (Authentication number - GU/BOT/Z/Z3) is authenticated by Dr Hitesh Solanki, Professor Department of Botany, Bioinformatics &

Sample collection:

Ginger (*Zingiber officinale*) was purchased from the local market of Ahmedabad, Gujarat. Ginger was washed with distilled water and the peels were removed, cut into small pieces and allowed to air dry in the sun for 4 to 5 days. A grinder a mortar and a pestle were used to thoroughly grind the dried ginger into powder, which was then kept at room temperature in an airtight container for further use.

For Carbohydrates:

Test	Formulation of solution	Observation
Molisch test (Chaitanya <i>et al.</i> , 2017)	0.5 ml Molisch reagent + 0.5 ml extract + 1 or 2 ml H ₂ SO ₄	Junction two liquid
Fehling's test (K. Santhi <i>et al.</i> , 2016)	0.5 ml Fehling A + 0.5 ml Fehling B + 0.5 ml extract + heat.	Brick red ppt
Benedict's test (J.S Jaya <i>et al.</i> , 2019)	0.5 ml extract + few drops of Benedict + heat.	Green yellow colour

For Flavonoids:

For protein Test	Formulation of solution	Observation
Biuret test (Suresh Patil <i>et al.</i> , 2017)	0.5 ml biuret reagent + 0.5 ml extract	Violet colour appears

For Amino acid:

Test	Formulation of solution	Observation
Ninhydrin test (J.S Jaya <i>et al.</i> , 2019)	1 ml of extract + 5 drop of ninhydrin reagent + heated for two minutes in a bath of boiling water.	Purple or violet Colour appear
Xenotho protein's test (D. Sujin <i>et al.</i> , 2019)	3 ml of sample + 1 ml of conc. Nitric acid, then heated for 3 min then cool and add 0.5ml NaOH	Reddish orange colour
Folin test (Chaitanya <i>et al.</i> , 2017)	1 ml sample + 1 ml Folin reagent + 1 N sodium carbonate	Blue colour

Secondary metabolite extraction by the soxhlation method:

Zingiber officinale roots were ground into a fine powder and thimble into the extractor. In this instance, root powder was concurrently passed through distilled water and methanol in the ratio of 70:30 (Sivalingam *et al.*, 2023).

Qualitative analysis of phytochemicals from ginger extract:

The presence or lack of specific chemical compounds in a plant is ascertained using the power source of tests.

For Flavonoids:

Test	Formulation of solution	Observation
Alkaline test (K. Nagalakshmi <i>et al.</i> , 2012)	0.5 ml aqueous NaOH + 0.5ml extract + 0.5 ml HCl	Yellow-orange colour
Aluminium chloride test (Ranjani Yadav <i>et al.</i> , 2016)	0.5 ml aluminium chloride + 0.5 ml extract	Colour formation
Ammonia test (K. Santhi <i>et al.</i> 2016)	1 ml extract + 2 ml of 10% ammonia solution + 1 ml conc. Sulphuric acid	Yellow colour indicates

Test	Formulation of solution	Observation
Lieberman's Burchard's test (D. Sujin <i>et al.</i> , 2019)	0.5 ml extract + 2 drops of conc. H ₂ SO ₄ + 2 ml chloroform + 2 ml acetic anhydride	Reddish to green colour
Salkowski test (K. Santhi <i>et al.</i> , 2016)	2 ml chloroform + 0.5 ml extract + 2 ml conc. H ₂ SO ₄ was added from the side of the test tube.	Red colour in chloroform layer

For Terpenoids:

Test	Formulation of solution	Observation
Terpenoids test (Madhan Kumar <i>et al.</i> , 2019)	1 ml alcoholic extract dissolved in 1 ml chloroform evaporated to dryness + 1 ml conc. H ₂ SO ₄ + heat	Greenish colour appears
Lieberman Burchard test (Ranjani Yadav <i>et al.</i> , 2016)	2 ml acetic acid + 2ml extract + 2 ml chloroform + 1 ml conc. H ₂ SO ₄ Observation	Blue-green colour/reddish ring indicates

For Terpenoids:

Test	Formulation of solution	Observation
Terpenoids test (Madhan Kumar <i>et al.</i> , 2019)	1 ml alcoholic extract dissolved in 1 ml chloroform evaporated to dryness + 1 ml conc. H ₂ SO ₄ + heat	Greenish colour appears
Lieberman Burchard test (Ranjani Yadav <i>et al.</i> , 2016)	2 ml acetic acid + 2ml extract + 2 ml chloroform + 1 ml conc. H ₂ SO ₄ Observation	Blue-green colour/reddish ring indicates

For Coumarin:

Test	Formulation of solution	Observation
Sodium hydroxide test (Chaitanya <i>et al.</i> , 2017)	1 ml extract + 1 ml sodium hydroxide	Yellow colour

For Anthocyanin:

Test	Formulation of solution	Observation
Kellar Killiani test (Madhan Kumar <i>et al.</i> , 2019)	2ml extract + few drops of 5% Feels and conc. H ₂ SO ₄ +2 ml glacial acetic acid	Blue-green/reddish brown colour
Test for glycoside (Madhan Kumar <i>et al.</i> , 2019)	3 ml of chloroform + 2 ml extract + 1 ml of 10% ammonia solution	Pink colour

Test	Formulation of solution	Observation
Sulphuric acid test (Madhan Kumar <i>et al.</i> , 2019)	1 ml extract + 1 ml conc. H ₂ SO ₄	Yellowish orange

For Phenol:

Test	Formulation of solution	Observation
FeCl ₃ : test (M.A. Farook <i>et al.</i> , 2019)	1 ml extract + 1 ml 5% ferric chloride	The formation of a darkgreen, reddish-brown, blue-violet, or purple color
		indicates the presence of phenol.

For Coumarin:

Test	Formulation of solution	Observation
Sodium hydroxide test (Chaitanya <i>et al.</i> , 2017)	1 ml extract + 1 ml sodium hydroxide	Yellow colour

Ginger extract-based green synthesis of silver nanoparticles:

After acquiring fresh *Z. officinale* rhizome at the neighbourhood market in Ahmedabad, Gujarat, India. To remove any dirt, it was properly cleansed with water many times. A mortar and pestle were used to finely grind twenty grammes of the rhizome after it had been chopped into tiny bits. To separate the extract, the ground material was squeezed into a fresh muslin cloth. Whatman No. 1 filter paper was used to filter the extract, which was then kept for later use at 4°C. AgNPs were synthesised using the AgNO₃. The experiments that followed used distilled water. In order to create AgNPs, 45 mL of distilled water, 5 mL of *Z. officinale* root extract, and either 1 mM AgNO₃ were added to one 100-mL Erlenmeyer flask. The *Z. officinale* root extract was utilised exactly as it was. With the addition of AgNPs, the metal ion reaction and *Z. officinale* root extract mixture gradually took on a brown-yellow hue. This was the initial method used to identify AgNP production (Palanivel Velmurugan *et al.*, 2013).

PHYTOCHEMICALS QUANTITATIVE ANALYSIS:

Computing the TPC: The TPC of the samples was determined using, with a few minor modifications, the Folin-Ciocalteu colourimetric method as outlined by S. Ojha *et al.*, 2018. The Folin-Ciocalteu technique was used to calculate the total phenolic content. One millilitre of clear water, one milligramme of extract (10 mg/10 ml), and ten, twenty, thirty, forty, and fifty µg/ml of standard gallic acid were added. 500 µl of Folin-Ciocalteu's reagent (2N) ad 500 µl of the sample were added, combined, and agitated. The mixture was let to stand at room temperature for forty-five minutes following the addition of one millilitre of 1% sodium carbonate. Deep blue was created as a result. After incubation, absorbance at 760 nm was determined using spectrophotometry. A reagent and solvent blank were used for the blank test.

TFC calculation: The aluminium chloride colourimetric method was used to calculate the TFC (Ojha *et al.*, 2018). Standard quercetin (50, 40, 30, 20, and 10 µg/ml) was made by mixing one milligram of extract with one milliliter of distilled water (10 mg/10 ml) in test tubes. Retrieve 250µl of the sample. After that, fill a 2.5 ml container with distilled water and thoroughly swirl. It turned out to be an orange-yellow colour. At 510 nm, the absorbance was determined using spectrophotometry. A reagent and solvent blank were used for the blank test. The calibration curve was created using standard quercetin. The amount of quercetin equivalents (mg) per 100g dry mass of total flavonoids was reported.

Calculation of TTC: S. Ojha *et al.* (2018) state that their approach was used to compute TTC. Ten minutes were spent settling the mixture at ambient temperature (272°C) after gradually adding 0.5 ml of 0.1M FeCl₃, 0.5 ml of 8 mM potassium ferricyanide, distilled water (8 ml), and the extracts (500 µl; 1 mg/ml stock solution). The absorbance at 720 nanometers was measured by me using a spectrophotometer. For each solvent, sufficient reagent blanks were made without the need for a sample.

Evaluation of antioxidant activity: Activity for DPPH scavenging: Using the procedure described by (Shalaby *et al.*, 2012), the DPPH was eliminated. Prepare a methanol solution containing 0.1 mM DPPH. Create extracts with 50, 40, 30, 20, and 10 µg/ml concentrations. Prepare different quantities of ascorbic acid as a standard. Each test tube needs to contain one milliliter of both of the test specimen and the DPPH solution. Add 200 µl of Tris HCl to each test tube.

Brine Shrimp Lethality Test (BSLT) toxicity test: To increase sample concentration, concentrations of 50, 40, 30, 20, and 10 µg/ml were computed. One millilitre of the test sample was obtained using the concentration calculation, and it was then put in a test tube with one millilitre of seawater. For every

SILVER NANOPARTICLES SYNTHESIS:

concentration, there are two tubes. The test sample was mixed after 200 µl of saltwater and 10 nauplii were added. Following a 24-hour incubation period, the number of dead and living larvae was counted, and the LC50 values were determined using probit analysis (Wu C., 2014).

Caenorhabditis elegans Death Assay (C. elegans):

Synchronisation and culture conditions for *C. elegans*: *Meledogyne* sp. was utilised for *C. elegans* species. According to Schnabel and Schnabel (1990), *C. elegans* develops normally at 15°C but does not reproduce over 25°C. Similarly, in our lab, where the studies were incubated, we did not see nematode growth over 22°C. This characteristic stopped new progeny from disturbing the incubating organism for up to 96 hours. According to Stiernagle (2006), the nematodes were kept on NGM agar plates and were seeded with *Escherichia coli* OP50 as a food source at 15°C. Nematode cultures were age-synchronized by washing NGM plates in sterile MilliQ water, which eliminated all nematodes and left only bacteria and eggs adhered to the plates. The eggs were incubated at 15°C for 72 hours. The plates were examined under a microscope to make sure that any worm stages older than hatching had been eliminated. In fact, it was quite simple to identify and remove these worm stages from the plates through washing. In our lab testing, we discovered that this approach was just as dependable and required less time than the conventional synchronisation procedure that involved bleach and NaOH. Worms in the L2 stage were collected in sterile tubes and cleaned from the plates using K-medium prior to testing. To rid the worms of bacteria, the K-medium was switched out twice for new K-medium (Lea Ellegaard-Jensen *et al.*, 2012).

Transfer 10 nematodes per well of 12 well plates. Record the number of nematodes per plate. 5 wells for different concentrations one for standard and one for control were taken. After that, we added the different conc. of AgNPs and DW. Then, incubate these plates at room temperature for 24 hours. Second day check and count the live and dead. Plot the graph as a box plot to see data distribution. We isolated the bacteria from the soil and we used Amoxicillin (Antibacterial medicine) as a standard.

ANTIMICROBIAL ACTIVITY (Muzamil *et al.*, 2020):

Prepare suspension of gram-negative bacteria and gram-positive bacteria. Take one N. agar plate and allow it to solidify. Take the suspension and spread it all over the plate with the help of a spreader. Bore four cups or gel puncher into seeded agar medium with the help of a standard cup borer having a diameter of 0.5mm equal distance. Label them as Standard (5mg/ml), Extract (5mg/ml), GAgNPs (10mg/ml) and control. Fill up the respective cups using Standard, Extract, and GAgNPs (we don't fill up any solution in the well of control) up to the brim of the cups accurately to deliver an equal amount in all the cups. Keep the plates in the refrigerator for 10 minutes otherwise test organism will start growing before the diffusion of the solution has taken place and a standard load of the organism will not be maintained. Take out the plates from the refrigerator and incubate them in an upright position at 37°C for 24 hours.

After the incubation period observe the plate for zone of inhibition around the respective cups and measure and express their sizes in mm.

RESULTS

Characterization of Synthesized Silver Nanoparticle:

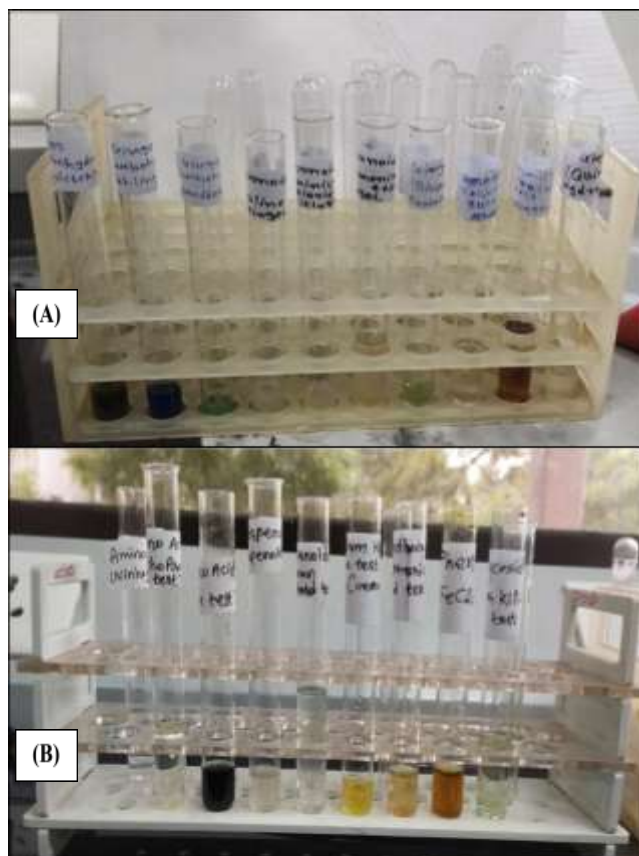
Qualitative analysis of phytochemical from ginger extract:

Sr. No.	Phytoconstituents	Test	Observation	Result
1	Carbohydrates	Molisch test (Chaitanya <i>et al.</i> , 2017)	Junction two liquid	+
		Fehling's test (K.Santhi <i>et al.</i> , 2016)	Brick red ppt	+
		Benedict's test (J.S.Jaya <i>et al.</i> , 2019)	Green yellow colour	+
2	Flavonoids	Alkaline test (K. Nagalakshmi <i>et al.</i> , 2012)	Yellow-orange colour	+
		Aluminium Chloride test (Ranjani Yadav <i>et al.</i> , 2016)	Colour formation	+
		Ammonia test (K.Santhi <i>et al.</i> , 2016)	Yellow colour indicates	+
3	Proteins	Biuret test (SureshPatil <i>et al.</i> , 2017)	Violet colour appears	+
4	Amino acid	Ninhydrin test (J.S.Jaya <i>et al.</i> , 2019)	Purple or violet colour appear	+
		Xantho protein's test (D. Sujin <i>et al.</i> , 2019)	Reddish-orange colour	+
		Folin test (Chaitanya <i>et al.</i> , 2017)	Blue colour	+

Table 1: Qualitative analysis result of phytochemical from ginger extract

Generally, qualitative analysis is used to determine whether phytochemicals are present or absent. Each sort of secondary metabolite has a specific test.

Sr. No.	Phytoconstituents	Test	Observation	Result
5	Terpenoids	Terpenoids test (MadhanKumar <i>et al.</i> ,2019)	Greenish colour appears	-
		Lieberman Burchard test (Ranjani Yadav <i>et al.</i> ,2016)	Blue-green colour/reddish hring indicates	-
6	Steroids	Lieberman's Burchard'stest (D. Sujin <i>et al.</i> ,2019)	Reddish to green colour	+
		Salkowski test (K. Santhi <i>et al.</i> ,2016)	Red colour in chloroform layer	+
7	Quinones	Hydrochloride test (D. Sujin <i>et al.</i> ,2019)	Yellow colour indicates	-
8	Coumarin	Sodium hydroxide test (Chaitanya <i>et al.</i> ,2017)	Yellow colour	+
9	Anthocyanin	Sulphuric acid test (Madhan Kumar <i>et al.</i> ,2019)	Yellowish orange	+
10	Phenol	FeCl ₃ test (M.A. Farook <i>et al.</i> ,2019)	The presence of phenol is indicated by the appearance of reddish brown, dark green, blue-violet, or purple colours.	+
11	Glycoside	Kellar Killiani test (Madhan Kumar <i>et al.</i> ,2019)	Reddish brown/ blue-green colour	+
		Test for glycoside(Madhan Kumar <i>et al.</i> ,2019)	Pink colour	+
12	Oxalate	Glacial acetic acid test (S. Subash <i>et al.</i> ,2019)	Green colour	-



Figures 1(A) and 1 (B): Figure Represents Qualitative Analysis of Ginger Extract

Synthesis of Silver Nanoparticles:

Green synthesis of silver nanoparticles using Ginger extract:
When ginger extract was added to 1mM AgNO₃ solution. With the addition of AgNPs, the Z. officinale root extract and metal ion reaction mixture gradually took on a brown-yellow hue. This served as the initial method of AgNP emergence detection (Palanivel Velmurugan *et al.*, 2013).

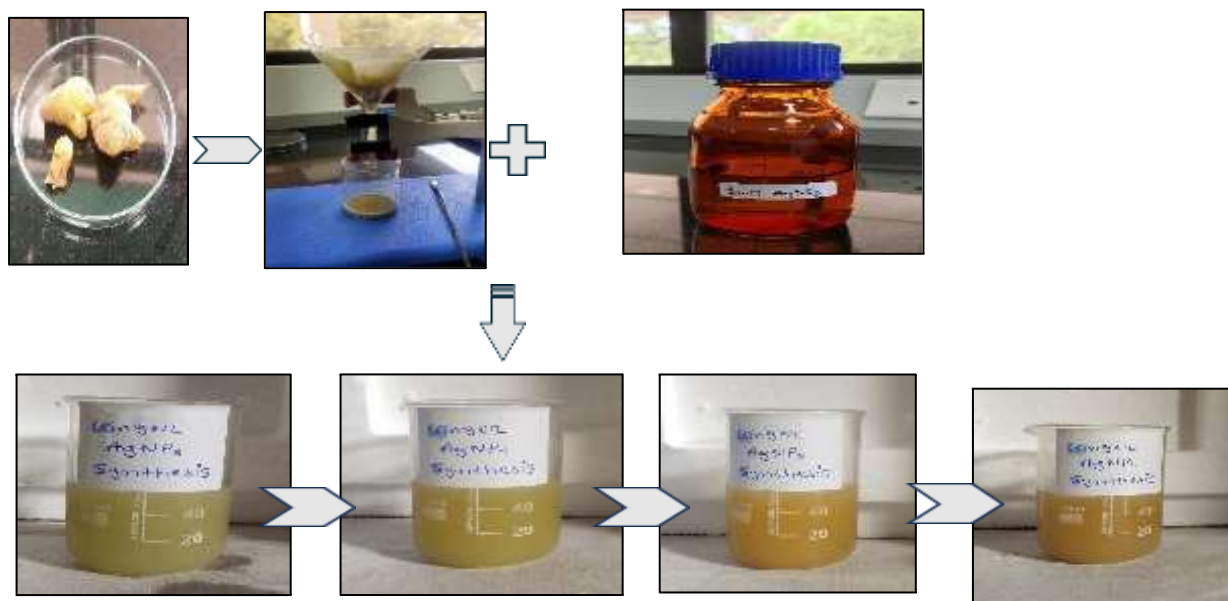


Figure 2: Synthesis of Silver Nanoparticles

Quantitative phytochemical screening:

Quantitative tests are run to determine the number of phytochemicals in the extract. Our objective was to determine antioxidant activity, thus we also assessed the total flavonoid and phenolic contents, as they might be connected to antioxidant activity (Hiram Saiyed et al., 2023).

Estimating Total Phenolic Content:

Plotting the graph of gallic acid, which was used as the standard, reveals that the extract of *Z. officinale* contains TPC at a concentration of -140.6 mg GAE/g.

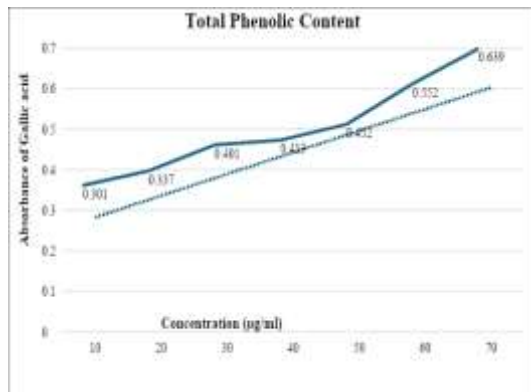


Figure 3: Calibration Curve for Gallic acid Determining Total Flavonoid Content (TFC):

Total Flavonoid Content (TFC) was calculated using quercetin as the endorsement. The curve of this compound was plotted,

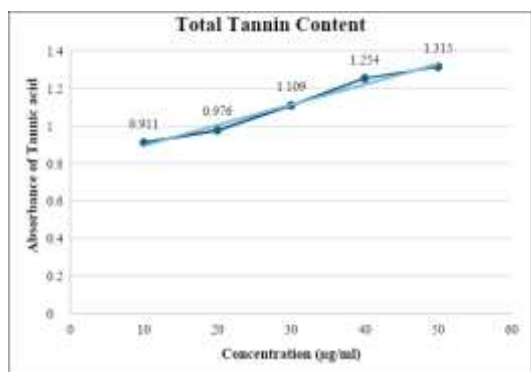


Figure 5: Calibration Curve for Tannic Acid Characterization of Synthesized Silver Nanoparticle: UV-VIS Spectrophotometer:

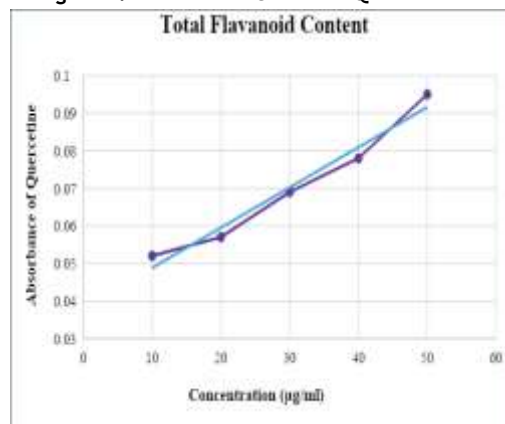
UV-VIS spectral analysis, which recorded data from the 200 to 800 nm wavelength range, further confirmed the brown-yellow shade observed during AgNP synthesis. The creation of silver nanoparticles using ginger extract under green synthesis was validated by the distinctive absorption band detected at 425 nm (Palanivel Velmurugan et al., 2013).

FTIR Spectroscopy: FTIR spectroscopy was used to further characterize the produced AgNPs. It shows how a secondary metabolite found in ginger extract interacts with silver nanoparticles. The creation and stability of nanoparticles may be attributed to the secondary metabolite that is present.

To determine which possible biomolecules in the ginger rhizome were responsible for the reduction and subsequent stabilization of the bio-reduced AgNPs, FTIR studies were carried out. Figure 5.4.2 displays the FTIR spectra of AgNPs where the peak at 2924 cm^{-1} indicated the presence of C-H stretching (alkane), N-H stretching (amine salt) and O-H stretching (carboxylic acid). The peak at 1645 cm^{-1} corresponds to N-H bend primary amines. The assignment at 1515 cm^{-1} corresponds to N-O stretching (nitro compound) and the bands observed at 1078 cm^{-1} C-N stretching (amine), C-O stretching (primary alcohol), and C-F stretching (fluoro compound). These groups are water soluble and primarily formed from heterocyclic compounds found in ginger extract. Based on this, it may be inferred that various water-soluble heterocyclic

and the results indicate that the extract of *Z. officinale* contains - 802 mg QE/g of TFC.

Figure 4: Absorbance Curve for Quercetin

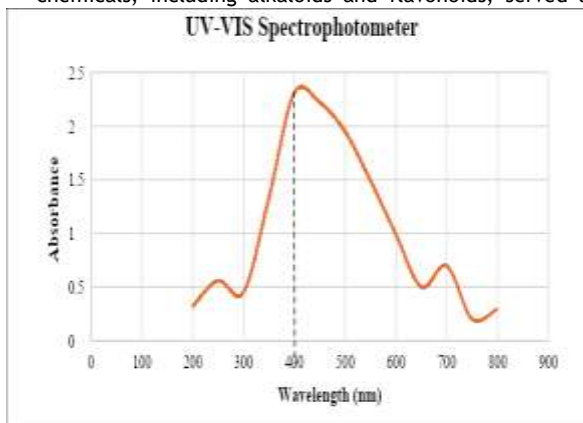


Estimation of Total Tannin Content:

Tannic acid was used as the standard, and a graph of it was generated. Based on calculations, *Z. officinale* extract contains TTC at - 940.8mg TAE/g.

Figure 6: UV-VIS analysis for Ginger silver nanoparticles

chemicals, including alkaloids and flavonoids, served as the



capping ligand during the creation of silver nanoparticles (Thanaa I. Shalaby, et al., 2015).

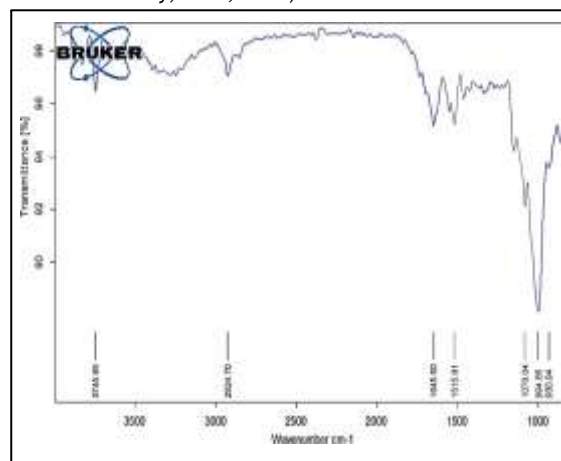


Figure 7: FTIR analysis for ginger silver nanoparticles

Zeta sizer analysis: The dispersion and stability of AgNPs were examined using the zetasize measurement, which was carried out at 25 °C.

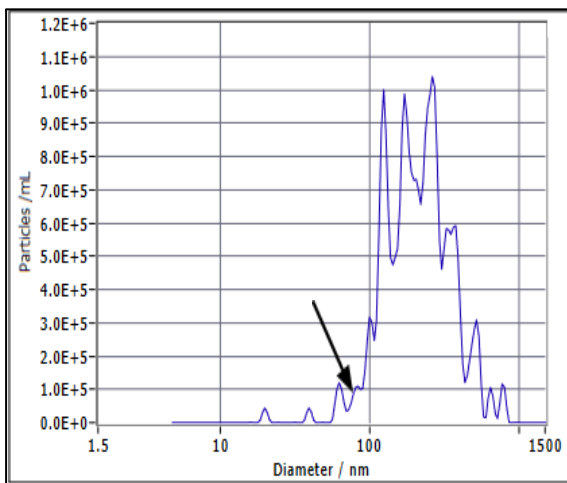


Figure 8: Size analysis of NPs by zeta sizer

Pick analysis shows that we find out that on the 62 nm diameter, the present particle/ml was $9.892E+4$, and on 10nm the particle/ml was $4.00E+05$.

Brine Shrimp Lethality Assay (BSLA)

The 50% lethal concentration (LC_{50}) of the plant-mediated AgNPs was determined by treating brine shrimp with varying concentrations of AgNPs; the results, as shown in Graph 3, were then subjected to Probit analysis, which yielded an LC_{50}

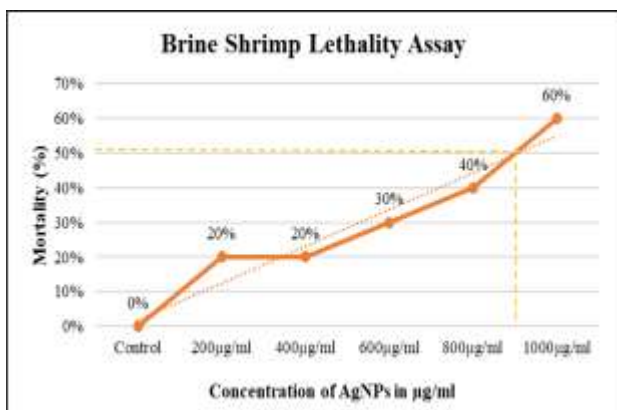


Figure 10: %Mortality of brine shrimp of the biosynthesized AgNPs at various concentrations

C. Elegans Lethality Assay (CELA): The result was found to be in such a way that in the control the mortality was about 0%. We find out that LD_{50} at $50\mu\text{g/ml}$. At a minimum Concentration of $20\mu\text{g/ml}$, the mortality was 30% and as the concentration increased to $40\mu\text{g/ml}$, $60\mu\text{g/ml}$, $80\mu\text{g/ml}$, and $100\mu\text{g/ml}$ the mortality was increased to 40%, 50%, 80%, 100% respectively.

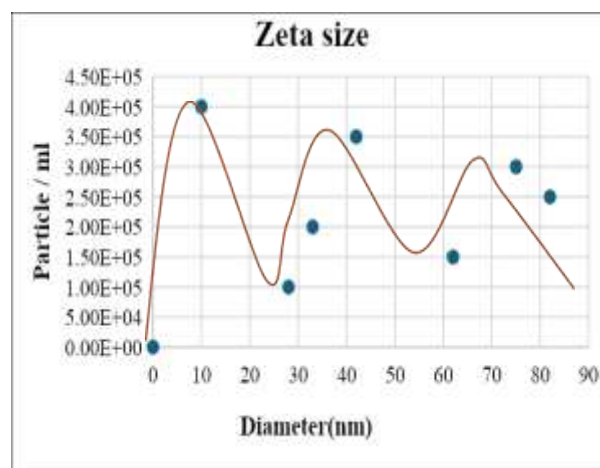


Figure 9: In detail size analysis of NPs by zeta sizer

of $900\mu\text{g}\cdot\text{mL}^{-1}$ of the biosynthesized AgNPs. Previous literature reviews have mentioned that $LC_{50} > 500\mu\text{g}\cdot\text{mL}^{-1}$ indicates non-toxic of the desired products (Lydia M. Clemen-Pascual *et al.*, 2022). As a result, it's important to remember that the predicted synthetic AgNPs were only preliminary safe for use in biomedicine.

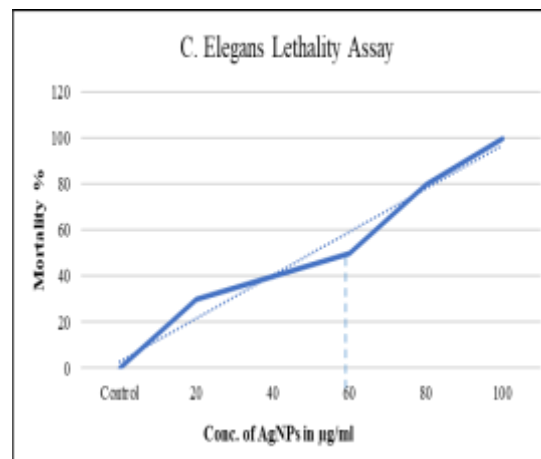


Figure 11: %Mortality of C. elegans of the biosynthesized AgNPs at various concentrations

Antimicrobial activity (Agar well diffusion method): Using the agar well diffusion assay, the antibacterial activity of ginger AgNPs with plant extract and standard was examined against gram-negative and gram-positive bacteria. The means of the zone of inhibition recorded for each organism and are shown in figure 12.



Gram (+) Bacteria



Gram (-) Bacteria

Figure 12: Zone of inhibition from Agar well diffusion method

Sr no.	Species	Standard (5mg/ml)	Extract (5mg/ml)	AgNPs (10mg/ml)
1	Gram positive bacteria	35mm	13mm	17mm
2	Gram negative bacteria	25mm	5mm	15mm

CONCLUSION:

We conclude by presenting a quick and easy method for synthesizing AgNPs using root extract from *Z. officinale*. AgNP antibacterial efficacy against both gram-negative and gram-positive bacteria has also been shown. AgNPs that had been produced exhibited remarkable stability and antibacterial efficacy. Growing health consciousness and microorganism-induced cross-infection have sparked interest in AgNP's potent antimicrobial properties. Numerous items will be significantly impacted by using AgNP nanotechnology in the food sector. Several industries, including agriculture, food manufacturing, and antimicrobial coatings for medical equipment, have seen an increase in the use of antimicrobial materials. Gram-positive bacteria were shown to exhibit potent antibacterial action. To identify the atoms in the functional groups responsible for the binding and stability of AgNPs synthesised from *Z. officinale* root extract, more research is required. The capacity to use *Z. officinale* root extract to synthesise AgNPs as possible antibacterial agents holds great promise for the environmentally friendly, sustainable manufacturing of nanoparticles with a wide range of industrial uses. In the current work, *Z. officinale* root extract was used as a reducing and capping agent during the green synthesis of silver nanoparticles, which have a particle size of 62 nm. Alkaloids, steroids, proteins, amino acids, phenol, and carbohydrates were all found in the extract. In summary, compared to chemical and physical synthesis, the biosynthesis of AgNPs from plant material is a traditional and environmentally benign process. The fact that the plants are readily available, widely dispersed, and safe to handle makes this method quite popular.

FUTURE PROSPECTIVE: As biosensors and catalysts, medicinal applications, antimicrobial surfaces, and so on, biogenic nanoparticles can theoretically be used in any application that currently makes use of chemically manufactured silver nanoparticles. Many investigations are currently being conducted to examine the broad antibacterial action of biogenic silver. Nevertheless, the application of biogenic silver in real-world applications aside from water disinfection treatments has not received much research, leaving a vast array of uncharted territory. In addition to their anti-inflammatory and wound-healing properties, silver nanoparticles have antibacterial activity, which makes them intriguing for use in biomedical applications like burn dressings (Rodriguez-Carmona and Villaverde, 2010). Therefore, a superior substitute is biogenic silver, which is made with probiotic plants or bacteria. Additionally, some plants contain therapeutic qualities that might enhance the value of the nanoparticles. However, for each form of biogenic silver, the biocompatibility and toxicity need to be thoroughly investigated. Biogenic silver has a wide range of antimicrobial uses outside of the biomedical and water treatment industries. Food packaging, textiles, and self-cleaning computer keyboard and smartphone coatings are just a few examples. In these situations, viruses shouldn't be the primary choice for producing biogenic silver, just like in the case of biological applications. Probiotics or food-related biosystems, like edible mushrooms, are better sources of protein (Philip, 2009).

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Table 4: Zone of inhibition size in mm

The outcomes demonstrated that artificially generated AgNPs exhibited stronger antibacterial activity than those of pure plant extracts.

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