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GREEN NANOALCHEMY WITH *ZINGIBER OFFICINALE*: PHYTOCRAFTED GOLD NANOPARTICLES AS ANTIFUNGAL AGENTS

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ABSTRACT

Biogenically synthesized metal nanoparticles have attracted significant attention due to their diverse biomedical applications in the rapid advancement of nanotechnology. In the present study, gold nanoparticles were synthesized using an eco-friendly method, where *Zingiber officinale* (ginger) rhizome extract served as a natural reducing agent for Gold (III) chloride hydrate ($HAuCl_4$). The resulting nanoparticles were thoroughly characterized using UV–Visible spectroscopy, FTIR, EDX, and SEM analyses. Their antifungal activity was evaluated against *Aspergillus niger* and *Saccharomyces cerevisiae* through the agar well diffusion method, which revealed distinct zones of inhibition, confirming the remarkable antimicrobial potential of the *Zingiber officinale mediated* gold nanoparticles (ZGNPs).

Keywords : Gold nanoparticles, *Zingiber officinale*, SEM, UV-Visible, FTIR, Zone of inhibition

1. INTRODUCTION

Nanomedicine is a rapidly evolving and dynamic area of research within the modern pharmaceutical industry. In particular, nanomaterials with unique nanoscale properties such as size, distribution, and morphology exhibit significant potential in biomolecular detection, diagnostics, antimicrobial development, and therapeutic applications **[1]**. Although nanomaterials have numerous applications, there is growing concern over environmental contamination caused by conventional chemical synthesis methods. This highlights the need for green synthesis approaches that are robust, non-toxic, and environmentally friendly, while also being commercially sustainable **[2]**. Green synthesis utilizes eco-friendly and safer materials such as plant extracts, bacteria, fungi, and enzymes for the production of nanoparticles **[3]**. These methods offer several advantages, including environmental compatibility and suitability for pharmaceutical and other biomedical applications **[4]**.

Gold nanoparticles are characterized by their small size (typically between 1-100 nm), which provides a high surface-to-volume ratio, and by their unique physical and chemical properties that can be tuned by modifying their size, composition, and shape. Gold nanostructured materials display exceptional resilience and stability under diverse conditions, making them suitable for demanding applications **[5]**. In addition, gold nanoparticles possess strong quantitative and qualitative target-binding capabilities, enhancing their effectiveness in precision-based uses. These properties make them highly attractive for a wide range of applications, including drug delivery, imaging, diagnostics, and biosensing. Furthermore, their excellent biocompatibility and ease of functionalization contribute to their growing use in targeted therapies and other biomedical fields **[6]**.

Zingiber officinale, commonly known as ginger, is a flowering plant whose rhizome is widely utilized both as a culinary spice and in traditional medicine **[7]**. Ginger is recognized for its broad spectrum of medicinal properties, including its use in the treatment of skin disorders, colorectal cancer, arthritis, and cardiovascular conditions, as well as its well-documented antibacterial activity **[8]**. Beyond its therapeutic applications, ginger remains a globally valued spice in culinary practices. In the present study, the use of ginger extract for the biosynthesis of gold nanoparticles offers a cost-effective and entirely biogenic alternative to conventional chemical synthesis methods.

2. MATERIALS AND METHODS

2.1 Collection and Extract Preparation

Fresh rhizomes of *Zingiber officinale* **(Figure 1)**, belonging to the family Zingiberaceae, were collected from the fields of Anakapalle district of Andhra Pradesh state in India for the present study. The rhizomes were thoroughly washed with sterile distilled water to remove surface contaminants and particulate matter. The cleaned rhizomes were then finely chopped and homogenized using a pestle and mortar, with 20 g of this material suspended in 100 mL of distilled water to prepare the extract.The resulting mixture was first filtered through Whatman No. 1 filter paper to remove solid residues. The filtrate was then subjected to centrifugation at 5000 rpm for 15 minutes. The clear supernatant was carefully collected and stored at 4°C for further use in nanoparticle synthesis.

Figure 1: Rhizomes of *Zingiber officinale*

2.2 Synthesis of Gold Nanoparticles

An aqueous solution of Gold (III) chloride hydrate ($HAuCl_4 \cdot 3H_2O$) was prepared **(Figure 2)**. To initiate the synthesis, 5 mL of the prepared ginger extract was added to 50 mL of 1.0×10^{-3} M $HAuCl_4$ solution at room temperature. The reaction mixture was then placed in a shaker incubator at 120 rpm and maintained at 37 °C for 48 hours to facilitate the biosynthesis of gold nanoparticles. Following the reaction, the remaining plant extract was dried into powder using a vacuum evaporator and stored for future use.

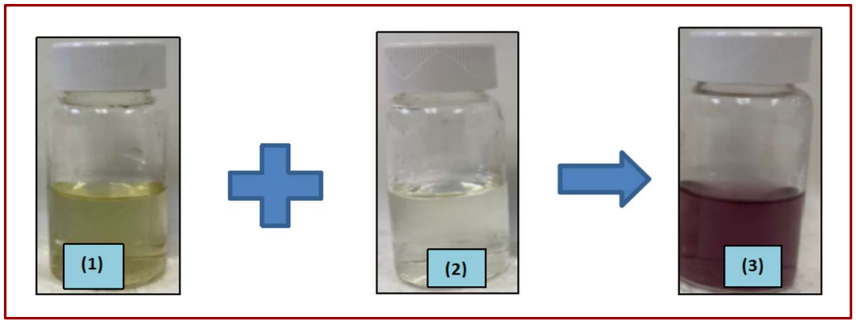

Figure 2: Synthesis of gold nanoparticles: (1) ginger extract, (2) $HAuCl_4 \cdot 3H_2O$ and (3) ZGNPs

2.3 UV-Visible Spectral analysis

The reduction of Au^{3+} ions to gold nanoparticles was monitored using UV–Visible spectroscopy. A small aliquot of the reaction mixture was diluted by adding distilled water, and the absorbance spectrum was recorded using a UV–Visible spectrophotometer in the range of 190–1100 nm **(Figure 3)**.

2.4 FTIR analysis

FTIR spectral analysis of ginger extract and the synthesized ZGNPs was performed using Shimadzu FT-IR spectrophotometer. A few drops of the sample was placed directly onto the ATR (Attenuated Total Reflectance) crystal of the FTIR spectrometer. The spectrum was recorded in the range of 4000–400 cm^{-1} to analyze the functional groups present in the extract and also for formed ZGNPs **(Figure 4)**.

2.5 SEM and EDX Characterization

Surface morphology of the synthesized ZGNPs was examined using a Thermo Scientific Scanning Electron Microscope (SEM). For SEM imaging, a minute volume of the nanoparticle suspension was carefully placed onto a carbon-coated copper grid. Excess fluid was gently blotted using filter paper, and the sample was subsequently air-dried under a mercury lamp for approximately 5 minutes **(Figure 5)**. Elemental composition analysis was carried out using Energy Dispersive X-ray Spectroscopy (EDX) to validate the presence of gold in the nanoparticle samples.

2.6 Determination of antifungal activity

Antifungal activities of the ZGNPs were assessed by agar-well diffusion method. *Aspergillus Niger* (MTCC281) and *Saccharomyces cerevisiae* (MTCC170) (Source of strains- IMTECH, Chandigarh, India) were the two fungi tested in the present study.The standardized cultures of two fungi were spread by means of sterile cotton swabs on Sabouraud's Dextrose Agar plates **[9]**. Five wells (6 mm diameter) were built in each plate with sterile cork borer. Twenty microliters of of the nanocompound and positive control were applied in wells. The drug Fluconazole (30 µg/mL) was used as reference antifungal agent. Diffusion of nanocompounds, reference drug and DMSO were allowed for 60 minutes at 25 ^{o}C (room temperature). All of the plates were then closed with lids and kept for incubation at 37 ^{o}C for 24 hours.After incubation, plates were examined for zone of inhibition of fungal growth.

3. RESULTS AND DISCUSSION

3.1 Visual confirmation of nanoparticle formation

The initial indication of nanoparticle synthesis was a visible color change to ruby-golden **(Figure 2)**, which appeared within minutes of introducing the plant extract to the gold salt solution. This color transition signified the reduction of gold ions and the

formation of gold nanoparticles **[10]**. After 24 hours of incubation, the solution exhibited a deeper color intensity along with the formation of a fine particulate suspension, suggesting the continued growth and stabilization of ZGNPs.

3.2 UV-Vis Spectral Characteristics of ZGNPs

A distinct absorption peak was observed at 509 nm **(Figure 3)**, indicating the successful synthesis of gold nanoparticles. The surface plasmon resonance (SPR) band, typically located between 520–530 nm **[11]**, confirms the presence of nanoparticles within the colloidal solution. This spectral peak arises due to a quantum size-dependent optical phenomenon known as Surface Plasmon Resonance (SPR), which becomes prominent when the De Broglie wavelength of conduction electrons is comparable to or smaller than the nanoparticle dimensions generally below 50 nm **[12]**.

3.3 FTIR Analysis of Gold Nanoparticles

Fourier Transform Infrared (FTIR) spectroscopy was employed to detect the bioactive compounds present in ginger rhizome extract that contributed to the reduction of gold ions and stabilization of the synthesized ZGNPs **(Figure 4)**. The FTIR spectra of both the ginger extract and the synthesized gold nanoparticles displayed prominent peaks at 3494, 1623, 1381, and 1019 cm^{-1}, corresponding to the presence of NH, C=N, and N=O functional groups in the extract **[13]**. In the case of the gold nanoparticles, these peaks shifted toward higher frequencies, indicating the involvement of these functional groups in the reduction and stabilization processes during nanoparticle synthesis **[14]**.

These functional moieties are likely derived from water-soluble heterocyclic phytochemicals such as alkaloids, flavonoids, and related compounds found in the ginger extract. It is therefore proposed that these organic molecules served as capping agents, while the oxygen-containing groups enhanced nanoparticle stability through surface interaction with the gold nanostructures.

3.4 SEM and EDX

Characterization of Synthesized ZGNPs

Scanning Electron Microscopy (SEM) analysis demonstrated that the ZGNPs were uniformly distributed without significant aggregation. The morphological assessment indicated that the nanoparticles possessed both spherical and cubic geometries with an approximate size of 100 nm. These observations are consistent with the particle dimensions. Furthermore, the Energy-Dispersive X-ray (EDX) spectrum **(Figure 5)** exhibited a prominent elemental gold signal, validating the successful fabrication of ZGNPs. The EDX data also supported the structural insights obtained from SEM, confirming the purity and formation of gold nanoparticles.

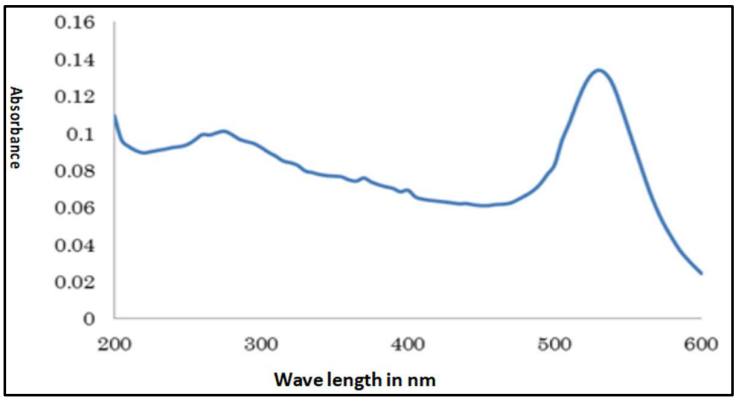

Figure 3: UV-Visible spectral analysis

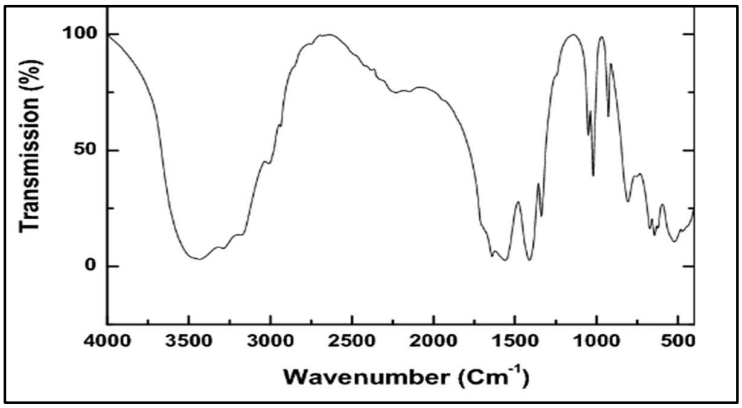

Figure 4: FTIR spectrum of ZGNPs

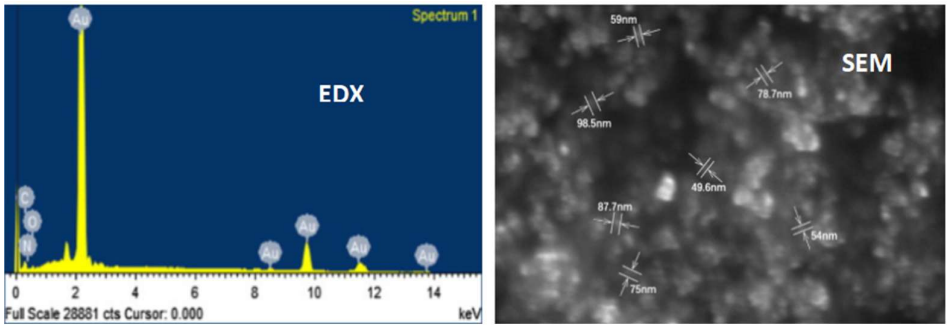

Figure 5: EDX and SEM analysis of ZGNPs

3.5 Antifungal activity of ZGNPs

The diameter of inhibition zones was determined and antimicrobial activity of the compounds was stated with regard to the average diameter of inhibition zone in millimeters **[15]**. Compounds that did not show an inhibition zone (with a diameter less than 6 mm) were classified as poorly active or inactive. Each compound was examined in triplicate with two individualistic experiments and average values of diameters of inhibition zone were considered. The formed ZGNPs demonstrated significant fungicidal activity against the two selected fungi, 12 mm against *A. niger* and 16 mm against *S.*

cerevisiae at 1mg concentration **(Table 1, Figure 6)**. The activity showed a 7 mm inhibition zone in *A. niger* and a 2 mm inhibition zone in *S. cerevisiae* at 0.25mg concentration. The synthesized nanoparticles demonstrated a marginally higher antifungal activity against *Aspergillus niger* than against *Saccharomyces cerevisiae*, indicating a differential susceptibility between the two fungal species.

Table 1: Zone of inhibition values of ZGNPs against selected fungal species

S.No.	Compound Name	Zone of inhibition (mm)							
		Aspergillus Niger				*Saccharomyces cerevisiae*			
		1mg	0.75 mg	0.5 mg	0.25 mg	1 mg	0.75 mg	0.5 mg	0.25 mg
1	ZGNPs	12	10	9	7	16	12	9	2
2	Flucanazole	16				21			

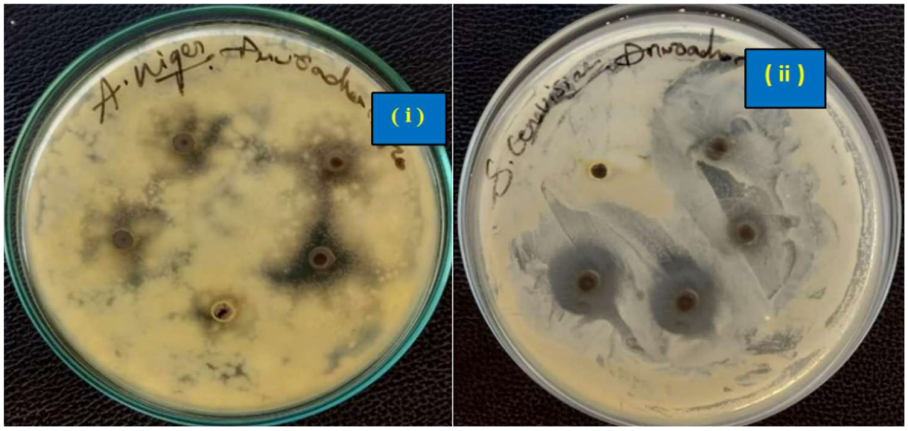

Figure 6 : Antifungal activity of ZGNPs against (i) *A.niger* (ii) *S. cerevisiae*

CONCLUSIONS

An environmentally benign approach has been employed for the green synthesis of gold nanoparticles using ginger (*Zingiber officinale*) rhizome extract. UV-Visible spectroscopy confirmed the nanoscale nature of the particles through characteristic Surface Plasmon Resonance (SPR) bands. FTIR analysis revealed the involvement of phytochemical secondary metabolites as both reducing and capping agents during nanoparticle formation. EDX and SEM analyses further validated the effective synthesis of gold nanoparticles, exhibiting predominantly spherical morphology with sizes ranging from 30 to 100 nm. Antifungal assays through the well diffusion technique demonstrated that the biosynthesized nanoparticles exhibit notable antifungal activity against *Aspergillus niger* and *Saccharomyces cerevisiae*. This antimicrobial efficacy can be ascribed to the presence of bioactive plant-derived compounds capping the outer surface of the nanoparticles.

Building upon the findings of this study, future research should aim to explore the detailed mechanisms underlying the antifungal activity of the biosynthesized gold nanoparticles. Broadening the antimicrobial spectrum to include other pathogenic microorganisms will provide deeper insights into their potential applications. Additionally, comprehensive toxicological and biocompatibility assessments are necessary to evaluate their suitability for biomedical use. The formulation of nanoparticle-based antifungal products and investigation of possible synergistic effects with conventional drugs may further enhance their efficacy. Lastly, efforts toward large-scale synthesis and stability analysis will be vital for transitioning this green nanotechnology approach into practical and commercial domains.

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