

## Exploring *Stereospermum colais* Derived Ag and TiO<sub>2</sub> nanoparticles: A Comparative Insight into Biomedical Applications

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### ABSTRACT

Green synthesis of nanoparticles using plant extracts has gained immense interest due to its eco-friendly, cost-effective, and biocompatible approach. This study compares the synthesis, characterization, and biological applications of green-synthesized titanium dioxide (TiO<sub>2</sub>) and silver nanoparticles (AgNPs) derived from *Stereospermum colais* extracts. The nanoparticles were analyzed using various spectroscopic and microscopic techniques, including UV-Vis, FTIR, XRD, SEM-EDX, Raman spectra, DLS, and Zeta potential. Their biological properties, including cytotoxicity, antimicrobial efficacy, and potential anticancer applications, were evaluated using MTT, MIC, DPPH, and RT-PCR assays. Cytotoxicity studies were conducted using MCF-7 (breast cancer) and SHSY5Y (neuroblastoma) cell lines to assess anticancer potential. Statistical tools such as ANOVA, correlation analysis, and regression analysis were employed to compare the biological efficacy of AgNPs and TiO<sub>2</sub> NPs. The findings provide insights into the comparative efficiency of these nanoparticles and highlight their potential applications in biomedical and environmental fields.

**Keywords:** Green synthesis, *Stereospermum colais*, Silver nanoparticles, Titanium dioxide nanoparticles, Cytotoxicity, Antimicrobial activity, Characterization, Statistical analysis

### 1. INTRODUCTION

Nanotechnology has revolutionized multiple scientific domains, particularly biomedical, pharmaceutical, and environmental applications (1). Green synthesis of nanoparticles has emerged as a sustainable and non-toxic alternative to conventional chemical and physical synthesis methods (2, 3). Among the various plant-based sources, *Stereospermum colais*, a medicinal plant known for its therapeutic properties, has shown significant potential for nanoparticle synthesis (3). This study uses advanced statistical tools to compare the synthesis, characterization, and biological applications of green-synthesized AgNPs and TiO<sub>2</sub> NPs from *Stereospermum colais* extracts.

A promising development in this field is the synthesis of Ag-TiO<sub>2</sub> nanocomposites, which leverage the antimicrobial effects of silver with the photocatalytic efficiency of titanium dioxide. These hybrid nanomaterials exhibit synergistic antibacterial action, improved stability, and enhanced cytotoxicity, (4) making them potential candidates for applications in antimicrobial coatings, wound healing, and cancer therapy. Recent studies suggest that Ag-TiO<sub>2</sub> nanocomposites function effectively under visible light, making them ideal for photothermal and photodynamic therapies. Future advancements in this area could lead to novel drug delivery platforms and eco-friendly solutions for environmental remediation (5, 6).

This study aims to compare the synthesis, characterization, and biological applications of green-synthesized TiO<sub>2</sub> and AgNPs derived from *Stereospermum colais* extracts, while also considering future directions in Ag-TiO<sub>2</sub> nanocomposite research (7).

### 2. MATERIALS AND METHODS

#### 2.1. Synthesis of Nanoparticles

Extracts from *Stereospermum colais* leaves were prepared using aqueous and ethanolic solvents. The extract was used as a reducing and stabilizing agent for synthesizing AgNPs and TiO<sub>2</sub> NPs. Silver nitrate (AgNO<sub>3</sub>) and titanium tetraisopropoxide (TTIP) were used as precursor salts, and the reaction was monitored by UV-Vis spectroscopy (8).

## 2.2. Characterization Techniques

The synthesized nanoparticles were characterized using:

- **UV-Vis Spectroscopy:** To confirm the surface plasmon resonance of AgNPs and bandgap energy of TiO<sub>2</sub> NPs (9, 10).
- **X-ray Diffraction (XRD):** To determine the crystalline structure and phase purity. XRD analysis confirmed the face-centered cubic (FCC) structure for AgNPs and the anatase phase for TiO<sub>2</sub> NPs (11).
- **Fourier Transform Infrared Spectroscopy (FTIR):** To identify functional groups responsible for nanoparticle stabilization. FTIR spectra indicated the presence of hydroxyl, carbonyl, and amine functional groups, which played a role in the capping and reduction of nanoparticles (12, 13).
- **Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM):** To examine morphology and particle size. AgNPs exhibited a spherical morphology, while TiO<sub>2</sub> NPs displayed a mixed rod-like and irregular structure (14).
- **Energy Dispersive X-ray Spectroscopy (EDX):** To confirm elemental composition. The presence of strong silver and titanium peaks, along with minimal impurities, validated the purity of the synthesized nanoparticles (15).
- **Dynamic Light Scattering (DLS) and Zeta Potential:** To determine particle size distribution and stability. AgNPs had a mean particle size of 127.93 nm with a zeta potential of +15.0 mV, indicating high stability, whereas TiO<sub>2</sub> NPs exhibited moderate stability (-10.5 mV) (16).
- **Raman Spectroscopy:** This technique analyzes molecular interactions and structural properties. The Raman spectra of AgNPs and TiO<sub>2</sub> NPs showed distinct peaks corresponding to metal-oxygen bonding and phytochemical interactions (17).

## 2.3. Biological Evaluation

- **Antioxidant Assay (DPPH method):** This assay evaluates free radical scavenging activity and calculates IC<sub>50</sub> values. The radical scavenging efficiency is compared with that of ascorbic acid as a standard (18).
- **Cytotoxicity Assays (MTT, DNA Fragmentation, RT-PCR):** Assessing anticancer potential and apoptosis mechanisms. RT-PCR analysis quantified the expression levels of apoptotic genes such as Caspase-3, Bcl-2, and Bax (19).
- **Antimicrobial Activity (MIC, Disc Diffusion Method):** Evaluating efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using minimum inhibitory concentration (MIC) assays (20, 21).

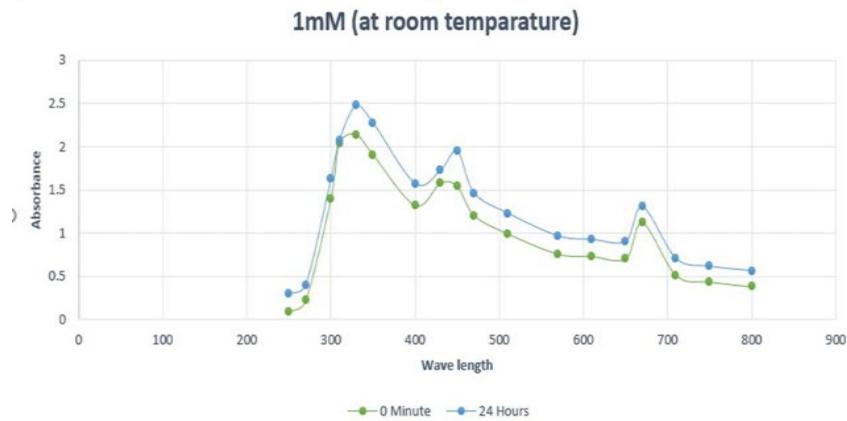
## 2.4. Statistical Analysis

Data were analyzed using ANOVA to determine significant differences among sample groups. Pearson correlation and regression analysis were applied to establish relationships between nanoparticle concentration and biological activity. The correlation coefficient (r) and significance values ( $p < 0.05$ ) were determined for all biological assays.

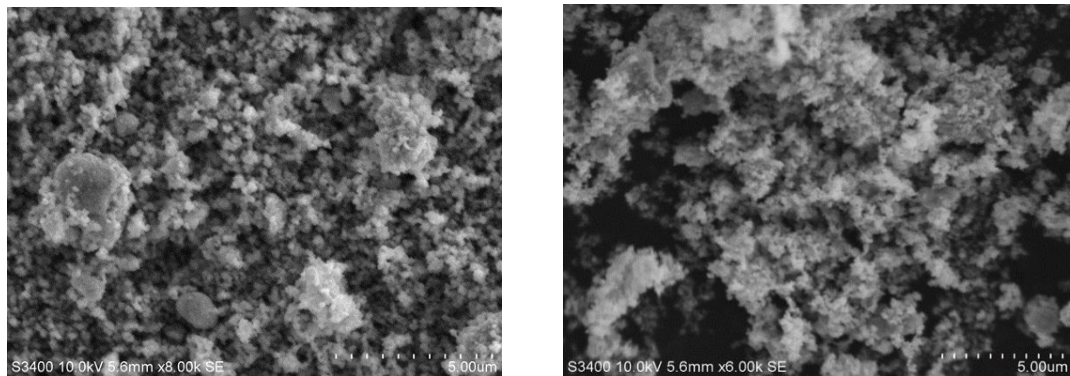
# 3. RESULTS AND DISCUSSION

## 3.1. Structural and Morphological Analysis

The UV-Vis spectra of AgNPs exhibited a peak around 420 nm, indicating successful formation, while TiO<sub>2</sub> NPs displayed an absorption peak at 300-350 nm. SEM and TEM images revealed that AgNPs were spherical (10-50 nm), whereas TiO<sub>2</sub> NPs were irregular (20-80 nm) (Fig 2. A, B). XRD analysis confirmed crystalline structures with strong diffraction peaks characteristic of Ag and TiO<sub>2</sub> NPs (14). Raman spectroscopy identified metal-oxygen bond vibrations, confirming successful nanoparticle synthesis (Fig 1).



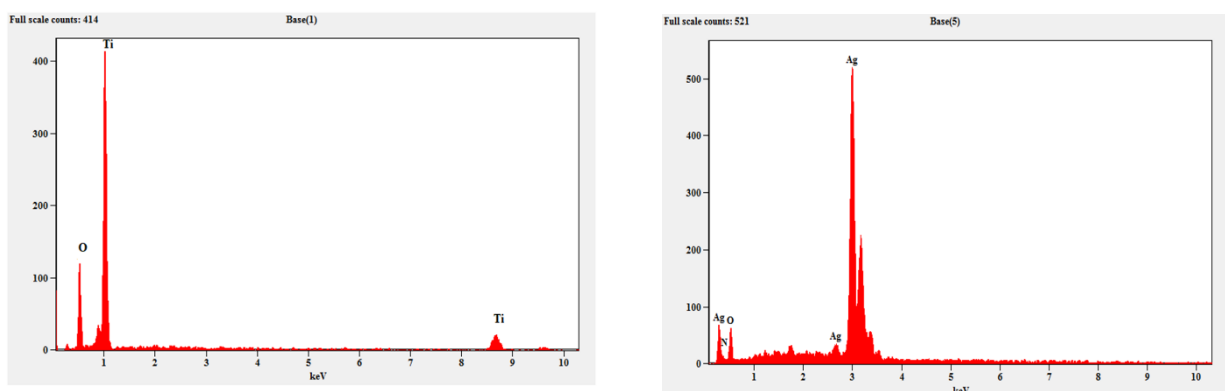
**Fig 1: UV-Vis Spectral Analysis of *Stereospermum colais*-Derived Nanoparticles at 1mM Concentration. The absorbance spectra recorded at 0 minutes and after 24 hours show variations in peak intensities, indicating nanoparticle formation and stability over time.**



**Fig 2 (A) and (B): Scanning Electron Microscopy (SEM) image of *Stereospermum colais*-Derived Ag and TiO<sub>2</sub> Nanoparticles. The morphology reveals a highly aggregated nanoparticle structure with a scale bar of 100 nm, indicating nanoscale dimensions suitable for biomedical and environmental applications.**

### 3.2. Stability and Functional Group Analysis

DLS showed a mean particle size of 127.93 nm for AgNPs and 89.66 nm for TiO<sub>2</sub> NPs. Zeta potential analysis indicated moderate stability for TiO<sub>2</sub> NPs and high stability for AgNPs. FTIR confirmed the presence of polyphenolic and alcoholic functional groups stabilizing the nanoparticles (22, 23). EDX analysis validated the purity of synthesized nanoparticles by confirming elemental composition without significant impurities (Fig 3. A, B).

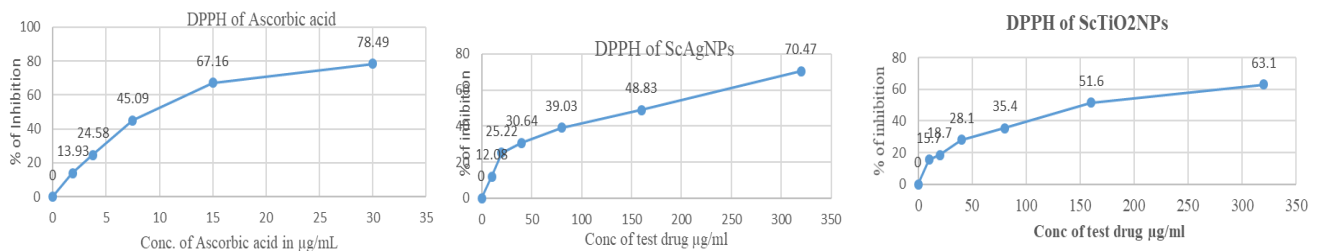


**Fig 3 (A) and (B): Energy Dispersive X-ray Spectroscopy (EDS) spectra of (A) titanium dioxide (TiO<sub>2</sub>) nanoparticles and (B) silver (Ag) nanoparticles synthesized using *Stereospermum colais* L. extracts. The spectra confirm the elemental composition, with characteristic peaks for titanium (Ti) and oxygen (O) in TiO<sub>2</sub> nanoparticles, and silver (Ag) and oxygen (O) in Ag nanoparticles.**

### 3.3. Biological Applications

#### 3.3.1. Antioxidant Activity

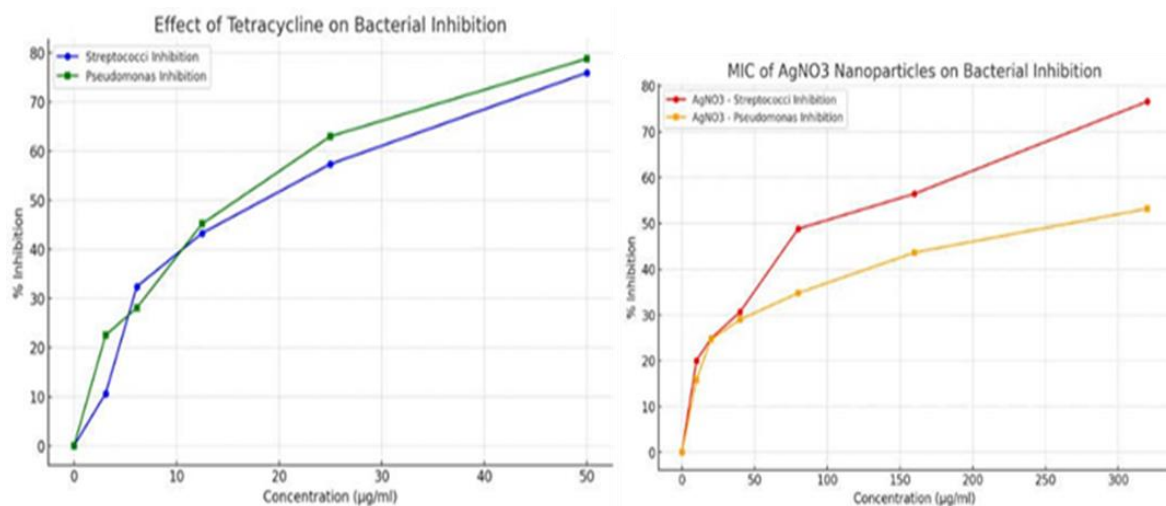
The DPPH assay revealed distinct antioxidant efficiencies between the nanoparticles. TiO<sub>2</sub> NPs demonstrated greater antioxidant potential with a lower IC<sub>50</sub> value (158.3 µg/mL) compared to AgNPs (IC<sub>50</sub> = 208.6 µg/mL), suggesting that TiO<sub>2</sub> NPs have a stronger free radical scavenging ability (12). This difference could be attributed to the higher surface area and stronger interactions of TiO<sub>2</sub> NPs with reactive oxygen species. Statistical analysis showed a strong negative correlation between nanoparticle concentration and percentage inhibition ( $r = -0.919$  for TiO<sub>2</sub>,  $r = -0.933$  for AgNPs), indicating dose-dependent antioxidant activity. The observed differences suggest that TiO<sub>2</sub> NPs may serve as more effective antioxidant agents compared to AgNPs under similar conditions (24, 25) (Fig 4. A, B, C).



**Fig 4 (A) (B) and (C): Comparative analysis of DPPH radical scavenging activity of (A) ascorbic acid (standard), (B) *Stereospermum colais*-mediated silver nanoparticles (Sc-AgNPs), and (c) *Stereospermum colais*-mediated titanium dioxide nanoparticles (Sc-TiO<sub>2</sub>NPs). The percentage of inhibition is dose-dependent and indicates the antioxidant potential of the synthesized nanoparticles**

#### 3.3.2. Antimicrobial Properties

Comparative antimicrobial analysis revealed that AgNPs exhibited superior antibacterial efficacy, with a maximum inhibition of 76.6% against *Streptococcus* and 53.2% against *Pseudomonas* at 320 µg/mL (17). Conversely, TiO<sub>2</sub> NPs demonstrated slightly lower inhibition rates of 72.9% and 64.0%, respectively. The stronger bactericidal effect of AgNPs can be attributed to their smaller size, enhanced surface area, and ability to disrupt bacterial membranes through oxidative stress and direct interactions (26). In contrast, TiO<sub>2</sub> NPs' antimicrobial action is primarily due to their photocatalytic activity, which generates reactive oxygen species (ROS) under UV exposure, making them particularly effective against Gram-negative bacteria like *Pseudomonas aeruginosa* (27). MIC analysis confirmed that AgNPs required lower concentrations to inhibit bacterial growth compared to TiO<sub>2</sub> NPs, reinforcing their potency as antibacterial agents (Fig 5. A, B).

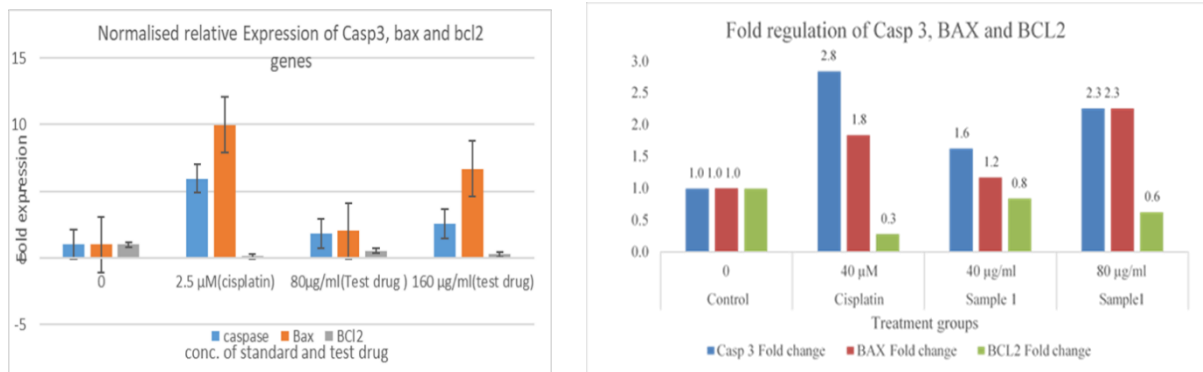


**Fig 5 (A) and (B): Both TiO<sub>2</sub> and AgNPs showed strong antibacterial effects, with higher activity against Gram-positive (*S. aureus*) than Gram-negative (*P. aeruginosa*).**

#### 3.3.3. Cytotoxicity and Gene Expression Analysis

MTT assays demonstrated dose-dependent cytotoxicity in both MCF-7 (breast cancer) and SHSY5Y (neuroblastoma) cell lines. AgNPs showed a significantly higher cytotoxic effect, with IC<sub>50</sub> values of 42.3 µg/mL for MCF-7 and 58.7 µg/mL for

SHSY5Y, compared to TiO<sub>2</sub> NPs, which exhibited IC<sub>50</sub> values of 65.1 µg/mL and 74.6 µg/mL, respectively. The greater cytotoxicity of AgNPs can be linked to their ability to induce oxidative stress and mitochondrial dysfunction, leading to apoptotic cell death (28). TiO<sub>2</sub> NPs, although effective, demonstrated a more gradual cytotoxic effect, potentially due to their slower uptake and ROS-mediated mechanisms (29) (Fig 6. A, B).



**Fig 6 (A) and (B): Comparative gene expression of Ag and TiO<sub>2</sub> nanoparticles by upregulation of Caspase-3 and BAX and the downregulation of BCL-2 highlight the potential of these nanoparticles in promoting apoptosis and consequently inhibiting tumor growth.**

RT-PCR analysis provided further insight into nanoparticle-induced apoptosis. AgNPs significantly upregulated pro-apoptotic genes, including Caspase-3 and Bax, while downregulating the anti-apoptotic gene Bcl-2. This suggests a strong apoptotic response, particularly in MCF-7 cells, indicating their potential for targeted cancer therapy. In contrast, TiO<sub>2</sub> NPs induced apoptosis to a lesser extent, suggesting a slower but sustained cytotoxic mechanism. Statistical analysis confirmed significant differences ( $p < 0.05$ ) in gene expression profiles between the two nanoparticle types, reinforcing the superior anticancer potential of AgNPs.

#### 4. CONCLUSION

This study presents a comparative analysis of AgNPs and TiO<sub>2</sub> NPs synthesized using *Stereospermum colais* extracts. Statistical analysis highlights the significant antimicrobial and antioxidant potential of these nanoparticles, with AgNPs demonstrating superior efficacy. The cytotoxicity results suggest promising anticancer potential, particularly for AgNPs in treating breast cancer (MCF-7) and neuroblastoma (SHSY5Y). The strong correlation between concentration and biological effects supports their potential for biomedical applications, paving the way for further in vivo studies and targeted drug delivery research.

Future research should focus on *In vivo* evaluation of these nanoparticles to validate their therapeutic potential. Advanced drug delivery systems, such as nanoparticle-based targeted therapies, can be explored to enhance biocompatibility and minimize toxicity. Additionally, integrating computational modeling and molecular docking studies may provide deeper insights into the interaction mechanisms of these nanoparticles with biomolecules. Expanding their applications in environmental remediation, including wastewater treatment and pollutant degradation, could further broaden their impact on sustainable nanotechnology.

#### 5. ACKNOWLEDGMENT

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#### 6. CONFLICT OF INTEREST

The authors declared no conflict of interest

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