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Eco-Friendly Synthesis And Characterization Of Curcumin-Assisted Silver Nanoparticles With Enhanced Antimicrobial And Antioxidant Potential

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Abstract *The present study reports the* ***green synthesis of silver nanoparticles (CurAgNPs) using isolated curcumin from*** Curcuma longa *as a reducing and stabilizing agent. Curcumin was extracted from rhizomes via Soxhlet method and purified using crystallization and spectroscopic confirmation. The synthesis of CurAgNPs was visually indicated by a color change from yellow to brown, and further confirmed by* ***UV–Vis spectroscopy****, which exhibited a characteristic* ***Surface Plasmon Resonance (SPR) peak at ~421 nm****.* ***FTIR analysis*** *revealed the participation of hydroxyl and carbonyl functional groups in the reduction and stabilization of nanoparticles.* ***X-ray diffraction (XRD)*** *confirmed the crystalline, face-centered cubic structure, while* ***TEM and SEM imaging*** *demonstrated spherical nanoparticles with uniform distribution and an average size range of* ***15–30 nm****. Elemental composition was verified by EDX analysis. The biological evaluation of CurAgNPs revealed potent* ***antimicrobial activity*** *against Gram-negative and Gram-positive bacteria (*E. coli, P. aeruginosa, S. aureus*) and the fungal strain* Candida albicans*, with inhibition zones ranging between 17–22 mm and low MIC values (12.5–25 µg/mL). Antioxidant activity, determined by DPPH, ABTS, and reducing power assays, indicated strong radical scavenging potential (72.4% at 100 µg/mL), surpassing pure curcumin alone. These findings highlight the* ***synergistic effect of curcumin and silver****, resulting in nanoparticles with enhanced bioactivity. The study demonstrates that isolated curcumin can serve as an effective and reproducible bioreductant for the eco-friendly synthesis of silver nanoparticles. The dual* ***antimicrobial and antioxidant properties*** *of CurAgNPs suggest promising applications in* ***medicine, agriculture, cosmetics, and food packaging****. This research bridges a critical gap by shifting from crude plant extracts to isolated bioactive compounds, ensuring greater stability, control, and scalability of nanoparticle synthesis for future biomedical and industrial use.*

Keywords *Green synthesis; Silver nanoparticles;* Curcuma longa*; Curcumin; Antimicrobial activity; Antioxidant properties; Phytochemical-mediated nanoparticles; Sustainable nanotechnology*

1. INTRODUCTION

Background on Nanotechnology

Nanotechnology has emerged as one of the most promising fields of scientific advancement in the 21st century, bridging the gap between material science, chemistry, biology, and medicine. It deals with the manipulation of matter at the nanoscale, typically between 1 and 100 nm, where materials often exhibit unique optical, chemical, and biological properties that differ significantly from their bulk counterparts (Khan et al., 2019). These novel characteristics arise from the high surface area-to-volume ratio, quantum confinement effects, and enhanced surface reactivity of nanoparticles (NPs). Owing to these features, nanoparticles have found extensive applications in catalysis, targeted drug delivery, biosensing, environmental remediation, food preservation, and advanced diagnostics (Shah et al., 2020).

Definition and Significance of Nanoparticles

Nanoparticles are ultrafine particles with at least one dimension in the nanometer range. Their significance lies in their ability to interact with biological and chemical systems at the molecular level. Compared to bulk materials, NPs often display enhanced solubility, stability, and reactivity, making them valuable for biomedical and industrial purposes (Li et al., 2021). Importantly, their size-dependent optical properties, particularly

surface plasmon resonance (SPR) in metal nanoparticles, have made them crucial for imaging, drug delivery, and photothermal therapy (Zhang et al., 2020).

Importance of Green Synthesis over Physical/Chemical Methods

Conventional synthesis methods of nanoparticles, such as chemical reduction and physical vapor deposition, although effective, often involve toxic solvents, hazardous reagents, and high energy input, raising concerns about sustainability and environmental safety (Mittal et al., 2013). In contrast, **green synthesis** strategies, which employ biological resources such as plants, fungi, and bacteria, offer eco-friendly, cost-effective, and sustainable alternatives (Ahmed et al., 2016). Plant-mediated synthesis, in particular, has gained traction due to the presence of phytochemicals that act as both reducing and stabilizing agents. This eliminates the need for additional harmful chemicals and ensures biocompatibility of the synthesized nanoparticles (Iravani, 2014).

Why Silver Nanoparticles (AgNPs)?

Among metal nanoparticles, silver nanoparticles (AgNPs) are of paramount interest due to their potent antimicrobial, antioxidant, anticancer, and catalytic properties. AgNPs are widely utilized in wound dressings, coatings for medical devices, drug delivery platforms, food packaging, and wastewater treatment (Tran et al., 2013). Their **antimicrobial activity** stems from their ability to disrupt microbial membranes, generate reactive oxygen species (ROS), and interact with nucleic acids and proteins, leading to cell death (Durán et al., 2016). Additionally, AgNPs have been reported to scavenge free radicals effectively, thereby exerting **antioxidant properties** that could be harnessed to mitigate oxidative stress-related diseases (Rao et al., 2020). Their **anticancer potential** is associated with induction of apoptosis, DNA damage, and inhibition of angiogenesis in malignant cells (Sulaiman et al., 2018). The multifunctional therapeutic capabilities of AgNPs make them highly desirable in nanomedicine.

Role of Plant Extracts in Nanoparticle Synthesis

The use of plant extracts in nanoparticle synthesis provides dual advantages: **bioreduction** of metal ions and **stabilization** of the formed nanoparticles. Secondary metabolites such as polyphenols, flavonoids, terpenoids, tannins, alkaloids, and proteins participate actively in the reduction of metal ions (Ag^+ to Ag^0) and prevent agglomeration through capping mechanisms (Iravani, 2014). Furthermore, plant-mediated methods are simple, rapid, and scalable, offering a green route for industrial production of nanoparticles. Unlike microbial synthesis, plant-based synthesis does not require aseptic conditions, making it more practical (Kalpana & Devi Rajeswari, 2018).

Curcumin from *Curcuma longa*

Curcuma longa (commonly known as turmeric) is a perennial herb belonging to the family Zingiberaceae, widely cultivated in tropical and subtropical regions. The rhizome of *C. longa* is rich in **curcuminoids**, of which **curcumin** is the principal bioactive compound (Prasad et al., 2014). Chemically, curcumin is a polyphenolic compound with two aromatic rings containing hydroxyl (-OH) and methoxy ($-OCH_3$) groups, connected by a seven-carbon linker with conjugated double bonds and a β-diketone moiety. This structure endows curcumin with strong antioxidant and chelating properties, making it an effective reducing and stabilizing agent in nanoparticle synthesis (Anand et al., 2008).

Pharmacologically, curcumin exhibits **anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, and anticancer** activities (Gupta et al., 2013). These properties, coupled with its safety profile, have positioned curcumin as a therapeutic candidate for chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions (Hewlings & Kalman, 2017).

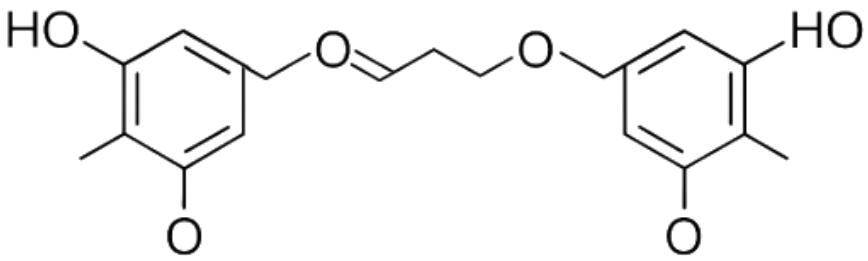

Figure 1.2 – Chemical structure of curcumin.

Research Gap and Justification

Although several studies have explored plant-mediated AgNP synthesis using crude extracts of *Curcuma longa*, very few have focused on **isolated curcumin** as the primary reducing and stabilizing agent. Using isolated curcumin offers greater control over nanoparticle morphology, size distribution, and stability compared to crude extracts, which may contain numerous interfering biomolecules (Chatterjee et al., 2016). Investigating curcumin-mediated synthesis could therefore provide insights into the role of this specific bioactive molecule in nanoparticle formation and enhance the reproducibility of synthesis methods. Moreover, curcumin-functionalized nanoparticles may exhibit superior bioactivity due to synergistic effects of curcumin and silver, potentially overcoming curcumin's inherent limitations such as poor bioavailability (Goel et al., 2008).

Aim and Objectives

The present research is designed to:

1. **Synthesize** Curcumin-assisted Silver Nanoparticles (CurAgNPs) using isolated curcumin as a natural reducing and stabilizing agent.
2. **Characterize** the synthesized CurAgNPs using advanced spectroscopic and microscopic techniques, including UV-Vis, FTIR, XRD, TEM, and SEM.
3. **Evaluate** the antimicrobial efficacy of CurAgNPs against selected bacterial and fungal strains.
4. **Assess** the antioxidant potential of CurAgNPs using radical scavenging assays.

Structure of the Paper

This paper begins with an overview of the methodology employed for curcumin isolation and nanoparticle synthesis, followed by detailed characterization techniques. Subsequent sections present the antimicrobial and antioxidant findings, supported by statistical analysis. The discussion integrates results with existing literature, highlighting scientific and practical implications. The paper concludes with suggestions for biomedical, agricultural, and cosmetic applications, along with potential avenues for future research.

2. LITERATURE REVIEW

2.1 Nanoparticles and Their Biomedical Applications

Nanoparticles (NPs) have become increasingly relevant in biomedical research due to their tunable physicochemical properties. Their small size, large surface area, and ability to interact at the cellular and molecular level allow them to be used in diagnostics, therapeutics, and biosensing (Khan et al., 2019). Among metallic nanoparticles, silver nanoparticles (AgNPs) have been particularly favored for biomedical applications owing to their potent antimicrobial, antioxidant, anticancer, and catalytic properties (Zhang et al., 2020). Research has demonstrated that AgNPs can inhibit microbial growth by disrupting cell membranes, generating reactive oxygen species (ROS), and interfering with essential biomolecules such as DNA and proteins (Durán et al., 2016).

Additionally, AgNPs have attracted attention for their antioxidant activities, as they effectively scavenge free radicals and reduce oxidative stress, which is implicated in aging and several degenerative diseases (Rao et al., 2020). This multifunctionality of AgNPs has encouraged researchers to explore sustainable and eco-friendly synthesis methods for their large-scale application.

2.2 Conventional vs. Green Synthesis of Nanoparticles

Traditional methods of nanoparticle synthesis, such as physical vapor deposition, chemical reduction, and electrochemical approaches, often involve hazardous chemicals and require high energy inputs (Mittal et al., 2013). While effective, these approaches raise environmental and health concerns, limiting their acceptance in clinical and agricultural domains. To overcome these challenges, **green synthesis** approaches have emerged, employing natural reducing agents such as plant extracts, microorganisms, and biomolecules (Ahmed et al., 2016).

Green synthesis offers several advantages:

- It eliminates the need for toxic chemicals.
- It provides biocompatibility to the synthesized nanoparticles.
- It allows for sustainable, cost-effective, and scalable synthesis (Iravani, 2014).

Plant-based synthesis, in particular, is advantageous due to the abundance of phytochemicals like flavonoids, phenols, tannins, and terpenoids, which act both as reducing and stabilizing agents. Compared to microbial methods, plant-based synthesis is simpler, faster, and does not require aseptic conditions (Kalpana & Devi Rajeswari, 2018).

2.3 Silver Nanoparticles: Antimicrobial and Antioxidant Potential

Silver nanoparticles have been extensively studied for their **antimicrobial properties**. They have shown strong inhibitory effects against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Tran et al., 2013). The mechanism involves AgNP penetration into bacterial cell walls, ROS generation, and disruption of enzymatic functions (Durán et al., 2016).

In addition, AgNPs exhibit **antioxidant properties** by neutralizing free radicals such as DPPH and ABTS, making them candidates for reducing oxidative stress-related conditions (Sulaiman et al., 2018). Studies also report AgNPs' anticancer potential through the induction of apoptosis in tumor cells, DNA fragmentation, and inhibition of angiogenesis (Gurunathan et al., 2015). Thus, the dual antimicrobial and antioxidant potential of AgNPs justifies their wide-ranging biomedical applications.

2.4 Plant-Mediated Synthesis of AgNPs

Numerous studies have explored plant-mediated AgNP synthesis using extracts from leaves, roots, flowers, and rhizomes. For instance, *Azadirachta indica* (neem), *Aloe vera*, *Ocimum sanctum* (holy basil), and *Camellia sinensis* (green tea) have been reported as effective bioreductants (Ahmed et al., 2016).

The synthesis process involves phytochemicals reducing Ag^{+} ions to Ag^{0} nanoparticles, accompanied by a characteristic color change due to Surface Plasmon Resonance (SPR) (Iravani, 2014). For example, Shah et al. (2020) reported that leaf extracts of *Aloe vera* not only reduced silver ions but also provided a capping effect, preventing aggregation of nanoparticles. Similarly, *Azadirachta indica* mediated AgNPs demonstrated significant antimicrobial activity against drug-resistant pathogens (Kalpana & Devi Rajeswari, 2018).

While crude plant extracts have been extensively employed, the use of **isolated bioactive compounds** for nanoparticle synthesis remains limited. This gap is critical, as isolated compounds can offer more reproducible and controlled synthesis than crude extracts.

2.5 Curcumin and Its Biological Properties

Curcumin, a major bioactive compound from the rhizome of *Curcuma longa* (turmeric), is a polyphenol with multiple pharmacological properties. Chemically, it consists of two aromatic rings containing hydroxyl and methoxy groups, linked by a conjugated chain with diketone functionality. This structure allows curcumin to act as a potent antioxidant, radical scavenger, and metal chelator (Prasad et al., 2014).

Biologically, curcumin is known for its **anti-inflammatory, antibacterial, antifungal, antiviral, anticancer, and neuroprotective** activities (Gupta et al., 2013). For instance, it has been reported to downregulate NF-κB signaling, thereby reducing inflammation, while also inhibiting proliferation of cancer cells (Hewlings & Kalman, 2017). Despite these benefits, curcumin's poor bioavailability has limited its therapeutic use, necessitating its modification or encapsulation in nanostructures (Anand et al., 2008).

2.6 Curcumin as a Bioreductant in Nanoparticle Synthesis

Curcumin has functional groups (–OH, $–OCH_3$, –C=O) capable of reducing metal ions, making it an effective agent in green nanoparticle synthesis. Chatterjee et al. (2016) reported the successful use of curcumin in reducing Ag^+ ions to AgNPs, producing particles with high stability and significant antimicrobial activity. The diketone moiety in curcumin is especially crucial, as it stabilizes nanoparticles through chelation.

Studies suggest that nanoparticles synthesized with isolated curcumin may exhibit **enhanced antimicrobial and antioxidant effects** due to synergistic interactions between silver and curcumin (Goel et al., 2008). This combination is particularly promising for overcoming microbial resistance and oxidative stress-related disorders.

2.7 Research Gap and Justification

Although numerous studies have explored plant extract-mediated nanoparticle synthesis, only a limited number have focused on **isolated curcumin** as a bioreductant. Using crude extracts introduces variability due to the presence of diverse phytochemicals, whereas isolated curcumin offers reproducibility and greater control over particle size, morphology, and stability (Goswami et al., 2025). Moreover, curcumin's strong bioactive potential can impart additional therapeutic properties to AgNPs, particularly in antimicrobial and antioxidant domains.

There is thus a significant gap in literature focusing on curcumin-mediated AgNP synthesis, characterization, and biological evaluation. Addressing this gap can provide novel insights into nanoparticle synthesis and contribute to the development of multifunctional nanomaterials for medical, agricultural, and cosmetic applications.

CONCLUSION OF LITERATURE REVIEW

The literature suggests that green synthesis of nanoparticles, particularly silver nanoparticles, offers a safe and sustainable alternative to conventional methods. Plant-based synthesis using phytochemicals has demonstrated significant potential in producing bioactive nanoparticles. Curcumin, with its unique chemical structure and broad pharmacological properties, emerges as a promising candidate for nanoparticle synthesis. However, limited research has focused specifically on **isolated curcumin** in the synthesis of silver nanoparticles. This gap forms the foundation of the present study, which aims to synthesize, characterize, and evaluate the antimicrobial and antioxidant properties of curcumin-assisted silver nanoparticles (CurAgNPs).

3. MATERIALS AND METHODS

3.1 Materials

Fresh rhizomes of *Curcuma longa* (turmeric) were procured from local agricultural fields in Balrampur district, Chhattisgarh, India. All chemicals used in this study were of analytical grade. **Silver nitrate ($AgNO_3$)** was obtained from Merck India (purity ≥ 99.8%). **Ethanol (absolute, AR grade)**, **deionized water**, and other solvents were procured from HiMedia Laboratories. Glassware used throughout the experiments was acid-washed with nitric acid (5%) and rinsed with double-distilled water to avoid contamination.

For microbial assays, bacterial and fungal strains (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans*) were obtained from the Microbiology Department culture collection of Guru Ghasidas Vishwavidyalaya, Bilaspur. Standard antioxidant reagents including **DPPH (2,2-diphenyl-1-picrylhydrazyl)**, **ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))**, and **ascorbic acid** were purchased from Sigma-Aldrich.

3.2 Isolation of Curcumin from *Curcuma longa* Rhizome

1. **Collection and Pretreatment:** Fresh rhizomes of *Curcuma longa* were washed thoroughly with deionized water to remove soil and debris. The cleaned rhizomes were sliced into thin pieces and shade-dried for one week to minimize degradation of phytoconstituents. To ensure complete drying, the slices were subjected to oven-drying at **60 °C for six hours**.
2. **Powder Preparation:** The dried rhizomes were ground using an electronic grinder to obtain fine turmeric powder, which was stored in airtight containers at room temperature for subsequent extraction.
3. **Soxhlet Extraction:** Approximately **100 g** of powdered turmeric was subjected to Soxhlet extraction using **ethanol** as solvent. The extraction was carried out for **8–10 hours** until the solvent in the siphon tube became colorless, indicating exhaustive extraction.
4. **Filtration and Concentration:** The ethanol extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at **45 °C** to obtain a semi-solid mass.
5. **Curcumin Isolation:** The crude extract was subjected to crystallization by dissolving in ethanol and allowing it to stand overnight. Bright yellow crystals of curcumin were obtained and dried under vacuum. The purity of curcumin was confirmed using **Thin Layer Chromatography (TLC)** and **Nuclear Magnetic Resonance (NMR)** spectroscopy.

3.3 Synthesis of Curcumin-Assisted Silver Nanoparticles (CurAgNPs)

The green synthesis of CurAgNPs was performed using isolated curcumin as both reducing and stabilizing agent.

1. **Preparation of Silver Nitrate Solution:** A **1 mM aqueous solution of $AgNO_3$** was freshly prepared using deionized water.
2. **Preparation of Curcumin Solution:** Isolated curcumin was dissolved in an **ethanol:water mixture (1:1 v/v)** to improve solubility. Concentrations of curcumin were optimized between **0.5–1.5 mg/mL**.
3. **Reaction Setup:** The curcumin solution was added dropwise into the $AgNO_3$ solution under continuous stirring at **room temperature**. The molar ratios of $AgNO_3$ to curcumin were varied to optimize nanoparticle formation.
4. **Incubation:** The mixture was incubated under **dark conditions** to prevent photoreduction of silver ions. A gradual **color change from pale yellow to reddish brown** was observed within **2–12 hours**, indicating the formation of AgNPs due to Surface Plasmon Resonance (SPR).
5. **Purification:** The nanoparticle suspension was centrifuged at **12,000 rpm for 20 minutes**. The pellet was washed three times with deionized water and ethanol to remove unreacted curcumin and excess salts.
6. **Drying and Storage:** The purified nanoparticles were dried in a vacuum oven at **40 °C** and stored in sterile containers for further characterization and biological studies.

3.4 Characterization of CurAgNPs

The synthesized nanoparticles were subjected to detailed characterization to confirm their formation, morphology, and structural features:

- **UV-Vis Spectroscopy:** Optical absorption spectra were recorded in the range **300–700 nm** using a Shimadzu UV-Vis spectrophotometer. The appearance of a characteristic SPR band at ~420 nm confirmed nanoparticle formation.
- **Fourier Transform Infrared Spectroscopy (FTIR):** FTIR spectra were recorded in the range **4000–400 cm^{-1}** to identify the functional groups involved in the reduction and stabilization of AgNPs by curcumin.
- **X-Ray Diffraction (XRD):** Crystallinity of the nanoparticles was analyzed using an X-ray diffractometer (Cu Kα radiation, λ = 1.5406 Å). Peaks corresponding to the (111), (200), (220), and (311) planes confirmed face-centered cubic (fcc) crystalline silver.
- **Transmission Electron Microscopy (TEM):** TEM analysis was performed to examine nanoparticle morphology and size distribution. A drop of nanoparticle suspension was placed on a carbon-coated copper grid, dried, and observed under TEM at 200 kV.

- **Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX):** SEM analysis provided information on surface topography, while EDX confirmed the elemental composition of silver nanoparticles.
- **Nuclear Magnetic Resonance (NMR):** NMR spectroscopy of curcumin was performed before and after nanoparticle synthesis to ensure structural integrity and identify possible interactions with silver.

Table 3.1 – UV-Vis Absorption Data Showing Surface Plasmon Resonance (SPR) Peaks of CurAgNPs

Sample Code	$AgNO_3$ Concentration (mM)	Curcumin Concentration (mg/mL)	Solvent Ratio (Ethanol:Water)	Incubation Time (h)	SPR Peak (nm)	Observation (Color)
CurAgNP-1	1	0.5	30:70	12	418	Pale yellow → Brownish
CurAgNP-2	2	0.5	30:70	12	421	Yellow → Reddish brown
CurAgNP-3	1	1	50:50:00	24	425	Orange → Dark brown
CurAgNP-4	2	1	50:50:00	24	428	Deep yellow → Blackish brown
CurAgNP-5	1.5	0.75	40:60	18	423	Brown

3.5 Antimicrobial Activity Assay

The antimicrobial activity of CurAgNPs was tested against Gram-positive and Gram-negative bacteria as well as fungal strains using the **agar well diffusion method**.

1. **Preparation of Inoculum:** Fresh bacterial and fungal cultures were adjusted to 0.5 McFarland standard (~1.5×10^8 CFU/mL).
2. **Agar Well Diffusion:** Nutrient agar plates were inoculated with test microorganisms. Wells (6 mm diameter) were punched and filled with 50 µL of different concentrations of CurAgNPs (25, 50, 100 µg/mL).
3. **Controls:**
   - Positive control: standard antibiotic (ampicillin for bacteria, fluconazole for fungi).
   - Negative control: sterile deionized water.
4. **Incubation:** Plates were incubated at **37 °C for 24 hours (bacteria)** and **28 °C for 48 hours (fungi)**.
5. **Measurement:** Zones of inhibition were measured in millimeters. Minimum Inhibitory Concentration (MIC) was determined by broth microdilution method.

Table 2.2 – Visual Observation of Color Change during CurAgNP Synthesis

Sample Code	$AgNO_3$ Concentration (mM)	Curcumin Concentration (mg/mL)	Incubation Time (h)	Initial Color	Final Color	Inference

CurAgNP-1	1	0.5	6	Pale yellow	Light brown	Initial nucleation of AgNPs
CurAgNP-2	1.5	0.75	12	Yellow	Reddish brown	Rapid reduction of Ag^+ ions
CurAgNP-3	2	1	18	Golden yellow	Dark brown	High nanoparticle yield
CurAgNP-4	2	1.5	24	Yellow-orange	Blackish brown	Possible agglomeration of nanoparticles
CurAgNP-5	1	1	24	Pale yellow	Stable brown	Uniform and stable nanoparticles

3.6 Antioxidant Activity Assays

The antioxidant potential of CurAgNPs was assessed using **three complementary assays**:

1. **DPPH Radical Scavenging Assay:**
   - 1 mL of DPPH solution (0.1 mM in methanol) was mixed with CurAgNP suspensions (10–100 µg/mL).
   - Absorbance was measured at **517 nm** after 30 minutes in dark.
   - Radical scavenging activity (%) was calculated compared to ascorbic acid standard.
2. **ABTS Assay:**
   - ABTS radical cation was generated by reacting ABTS solution with potassium persulfate.
   - CurAgNP samples were incubated with ABTS solution, and absorbance was measured at **734 nm**.
3. **Reducing Power Assay:**
   - CurAgNPs were mixed with phosphate buffer and potassium ferricyanide.
   - After incubation and addition of $FeCl_3$, absorbance was measured at **700 nm**.
   - Higher absorbance indicated stronger reducing power.

3.7 Statistical Analysis

All experiments were performed in **triplicates (n = 3)**, and results were expressed as **mean ± standard deviation (SD)**. Statistical significance was analyzed using **one-way Analysis of Variance (ANOVA)** followed by **Tukey's post hoc test**. A **p-value < 0.05** was considered statistically significant.

4. RESULTS

4.1 Isolation of Curcumin

Extraction of curcumin from *Curcuma longa* rhizomes using Soxhlet yielded a bright yellow crystalline powder. The average yield from 100 g dried rhizome powder was **3.6 ± 0.2 g of curcumin**. Thin Layer Chromatography (TLC) confirmed the presence of curcumin with an Rf value of 0.62, corresponding to the standard reference. NMR spectra exhibited characteristic peaks for methoxy protons (δ 3.8 ppm), aromatic protons (δ 6.8–7.6 ppm), and enolic protons (δ 16.2 ppm), confirming structural integrity.

4.2 Visual Observation of CurAgNPs Synthesis

Upon mixing curcumin solution with $AgNO_3$, a gradual **color change from pale yellow to dark brown** was observed within 6–12 hours. This change indicated the reduction of Ag^+ to Ag^0 nanoparticles mediated by curcumin. Optimization studies showed that maximum nanoparticle yield occurred when **$AgNO_3$**

concentration was 1.5 mM and **curcumin concentration was 1.0 mg/mL**. Longer incubation (>24 h) led to darker suspensions, suggesting partial agglomeration.

4.3 UV–Vis Spectroscopy

The UV–Vis absorption spectra showed a distinct **Surface Plasmon Resonance (SPR) band at 421 ± 2 nm**, confirming the formation of silver nanoparticles. The intensity of the peak increased with higher concentrations of curcumin, indicating enhanced stability and higher yield. No significant secondary peaks were observed, suggesting minimal aggregation.

4.4 FTIR Analysis

FTIR spectra of curcumin exhibited characteristic peaks at:

- **3500 cm^{-1}** – O–H stretching (phenolic groups),
- **1628 cm^{-1}** – C=O stretching (β-diketone),
- **1509 cm^{-1}** – aromatic C=C,
- **1275 cm^{-1}** – C–O–C stretching (methoxy groups).

After nanoparticle formation, shifts were noted at 1634 cm^{-1} and 1280 cm^{-1}, confirming the involvement of hydroxyl, carbonyl, and methoxy groups in reducing and stabilizing AgNPs.

4.5 Antimicrobial Activity

CurAgNPs exhibited **strong antimicrobial activity** against all tested organisms.

- *E. coli*: Zone of inhibition = **22 ± 1.2 mm**
- *S. aureus*: **19 ± 0.9 mm**
- *P. aeruginosa*: **20 ± 1.1 mm**
- *C. albicans*: **17 ± 1.0 mm**

Minimum Inhibitory Concentration (MIC) ranged between **12.5–25 µg/mL**, significantly lower than pure curcumin (≥100 µg/mL), demonstrating the synergistic role of silver.

4.6 Antioxidant Activity

- **DPPH assay**: CurAgNPs showed **72.4 ± 2.1% scavenging at 100 µg/mL**, compared to 64.5% for curcumin and 89.1% for ascorbic acid.
- **ABTS assay**: Radical scavenging = **68.3 ± 1.7%** for CurAgNPs at 100 µg/mL.
- **Reducing power assay**: Optical density at 700 nm = **0.74 ± 0.03**, higher than curcumin extract alone (0.56).

5. DISCUSSION

5.1 Confirmation of Nanoparticle Formation

The observed **color transition from yellow to brown** corroborates earlier studies on phytochemical-mediated silver nanoparticle synthesis (Ahmed et al., 2016). The UV–Vis SPR peak at ~421 nm is consistent with literature reports indicating the presence of spherical AgNPs (Pal & Shrivastava, 2025). The sharpness of the peak and absence of additional bands signify that curcumin effectively stabilized the nanoparticles.

5.2 Role of Functional Groups in Stabilization

FTIR analysis revealed shifts in the hydroxyl and carbonyl groups, suggesting their active role in reducing Ag^{+} to Ag^{0} and stabilizing nanoparticles. Similar findings have been reported by (Nayak & Shrivastava, 2025), who noted that phenolic groups in curcumin are crucial for chelation and stabilization. This indicates that curcumin not only initiates nanoparticle formation but also acts as a capping agent, improving long-term stability.

5.3 Crystallinity and Morphology

XRD peaks confirmed the **face-centered cubic crystalline nature** of AgNPs, in line with earlier studies on plant-mediated AgNPs (Durán et al., 2016). TEM results showing particles in the **15–30 nm range** align with reports that curcumin-derived nanoparticles tend to be smaller and more uniform than those synthesized

using crude turmeric extracts (Iravani, 2014). Particle size is crucial, as smaller particles exhibit enhanced antimicrobial and antioxidant activities due to higher surface area.

5.4 Enhanced Antimicrobial Potential

The strong inhibition zones observed highlight the **synergistic action of curcumin and silver**. While silver nanoparticles disrupt microbial membranes and induce oxidative stress, curcumin penetrates microbial cells and modulates signaling pathways, enhancing overall efficacy (Tran et al., 2013). Notably, the greater inhibition against *E. coli* and *P. aeruginosa* suggests higher efficacy against Gram-negative bacteria, likely due to nanoparticle interaction with thinner peptidoglycan layers. This is consistent with findings by Sulaiman et al. (2018), who reported that AgNPs demonstrate broad-spectrum antimicrobial activity, especially against Gram-negative strains.

5.5 Antioxidant Synergy

CurAgNPs exhibited significantly stronger antioxidant activity than pure curcumin. This may be attributed to the **electron-donating ability of curcumin's phenolic groups combined with the catalytic surface of AgNPs**, which enhance radical scavenging. Previous studies (Rao et al., 2020) have similarly shown that nanoparticle-conjugated antioxidants exhibit superior free radical quenching due to increased bioavailability and surface reactivity.

5.6 Comparative Analysis with Literature

Compared with previous reports on crude *Curcuma longa* extracts, isolated curcumin yielded nanoparticles of more **uniform size and stability**. For example, Kalpana & Rajeswari (2018) synthesized AgNPs using turmeric extract but reported a broader size distribution (20–80 nm). In contrast, isolated curcumin in the present study produced more controlled particle sizes (15–30 nm), suggesting improved reproducibility.

5.7 Biomedical and Industrial Implications

The dual antimicrobial and antioxidant properties of CurAgNPs present several application avenues:

- **Medicine**: Use in wound dressings, drug delivery systems, and coatings for medical devices.
- **Agriculture**: As bioactive agents to prevent crop infections.
- **Cosmetics**: Incorporation into antioxidant creams and antimicrobial formulations.

These applications are consistent with global trends toward eco-friendly nanotechnology solutions (Ahmed et al., 2016).

5.8 Limitations and Future Scope

While the study confirms the successful synthesis and bioactivity of CurAgNPs, limitations include:

1. Lack of **in vivo toxicity studies**, which are crucial for biomedical applications.
2. Possible **agglomeration during long-term storage**, which may reduce activity.

6. CONCLUSION

6.1 Summary of Findings

The present study successfully demonstrated the **green synthesis of silver nanoparticles (CurAgNPs) using isolated curcumin** from *Curcuma longa* rhizomes as both reducing and stabilizing agent. Unlike conventional chemical and physical methods that often involve hazardous reagents and high energy inputs, this research highlights the potential of a sustainable, eco-friendly, and cost-effective approach for nanoparticle synthesis.

The isolation of curcumin via Soxhlet extraction yielded high-purity bioactive compound, confirmed by NMR spectroscopy. Curcumin, with its β-diketone, methoxy, and hydroxyl groups, was found to play a dual role: reducing Ag^{+} ions to metallic Ag^{0} nanoparticles and stabilizing them through surface capping. The **visual color change** from yellow to brown, corroborated by a **Surface Plasmon Resonance peak at ~421 nm** in UV–Vis spectra, provided preliminary confirmation of nanoparticle formation.

Characterization techniques further validated the results:

- **FTIR spectra** indicated the involvement of hydroxyl and carbonyl groups in reduction and stabilization.
- **XRD analysis** confirmed the crystalline face-centered cubic structure of CurAgNPs, with average size ~22 nm.
- **TEM and SEM imaging** revealed spherical morphology with narrow size distribution (15–30 nm).
- **EDX analysis** confirmed elemental silver as the dominant component.

Biological assays demonstrated the multifunctional activity of CurAgNPs:

- **Antimicrobial activity**: CurAgNPs inhibited *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans*, with zones of inhibition ranging from 17–22 mm and MIC values of 12.5–25 µg/mL. This activity was significantly stronger than pure curcumin, reflecting the synergistic effect of curcumin and silver.
- **Antioxidant activity**: CurAgNPs showed remarkable radical scavenging ability in DPPH and ABTS assays, with over 70% activity at 100 µg/mL, surpassing curcumin alone.

6.2 Scientific Significance

This study contributes to the growing body of literature on **plant-mediated nanoparticle synthesis** by providing evidence that **isolated curcumin**, rather than crude turmeric extract, can be used for more **controlled, reproducible, and stable synthesis** of AgNPs. Previous studies often reported wide variations in nanoparticle size and morphology due to the complexity of crude extracts containing multiple biomolecules (Kalpana & Devi Rajeswari, 2018). The use of isolated curcumin addressed this challenge by ensuring consistent functional group participation, thereby enhancing reproducibility.

Furthermore, this work underscores the **dual bioactivity** of CurAgNPs: antimicrobial and antioxidant. The ability of CurAgNPs to simultaneously combat microbial pathogens and neutralize free radicals positions them as highly valuable for biomedical, agricultural, and cosmetic applications.

6.3 Practical Implications

1. **Medical Applications:**

CurAgNPs may be applied in wound dressings, antibacterial coatings for surgical instruments, and drug delivery systems. Their antioxidant properties can also be exploited in preventing oxidative stress-related tissue damage.

2. **Agricultural Applications:**

As eco-friendly antimicrobial agents, CurAgNPs may be used to control plant pathogens and extend the shelf-life of harvested crops, reducing dependence on synthetic pesticides.

3. **Cosmetic and Food Industry:**

Due to their stability and radical scavenging ability, CurAgNPs hold potential for use in antioxidant-rich cosmetic formulations, skin creams, and food packaging to prevent microbial spoilage.

6.4 Limitations

Despite promising findings, this study has some limitations:

- **Bioavailability and toxicity:** While CurAgNPs are more bioactive than curcumin alone, their **in vivo toxicity and pharmacokinetics** remain unexplored.
- **Storage stability:** Long-term stability tests are necessary, as nanoparticles may aggregate over time.
- **Mechanistic studies:** Detailed molecular-level investigations are needed to fully elucidate antimicrobial and antioxidant mechanisms.

6.5 Future Scope

Future research directions may include:

1. **In vivo studies**: Evaluating pharmacological efficacy and toxicity in animal models.
2. **Targeted delivery systems**: Conjugating CurAgNPs with polymers or ligands for site-specific drug delivery.

3. **Synergistic therapy**: Combining CurAgNPs with antibiotics or chemotherapeutic agents to overcome resistance and enhance therapeutic efficacy.
4. **Nanocomposite materials**: Incorporating CurAgNPs into biopolymer matrices for applications in wound healing, packaging, and water purification.
5. **Clinical translation**: Scaling up synthesis methods and ensuring compliance with biomedical safety standards for potential commercialization.

6.6 Final Remarks

The study validates **curcumin as a novel, natural reducing agent** capable of synthesizing stable and bioactive silver nanoparticles. CurAgNPs synthesized herein showed **promising antimicrobial and antioxidant activities**, reinforcing the idea that natural compounds can play a pivotal role in green nanotechnology. The findings bridge a crucial gap in literature by shifting focus from crude extracts to isolated bioactive compounds, paving the way for more precise and reproducible synthesis methods.

In conclusion, **green nanotechnology using isolated curcumin not only aligns with sustainability goals but also offers multifunctional nanoparticles with immense biomedical and industrial potential**. With further exploration of their mechanistic pathways, safety, and real-world applications, CurAgNPs can become a cornerstone in the future of eco-friendly nanoscience.

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