

## GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PROPOLIS EXTRACT: EVALUATING ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL

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### Abstract

The development of eco-friendly and sustainable nanomaterials has gained significant attention due to increasing concerns regarding the environmental and biological toxicity of chemically synthesized nanoparticles. In the present study, silver nanoparticles (AgNPs) were synthesized via a green synthesis approach using propolis extract as a natural reducing and stabilizing agent. Propolis, a resinous product collected by honeybees, is rich in polyphenols, flavonoids, and bioactive compounds that facilitate nanoparticle formation while imparting biological functionality. The synthesized AgNPs were characterized using UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X ray Diffraction (XRD), and Scanning Electron Microscopy (SEM). UV-Vis analysis confirmed AgNP formation through the appearance of a characteristic surface plasmon resonance band. Structural and morphological analyses revealed crystalline, predominantly spherical nanoparticles with nanoscale dimensions. The antimicrobial activity of the propolis mediated AgNPs was evaluated against selected Gram positive and Gram-negative bacterial strains using the agar well diffusion method, demonstrating enhanced inhibitory effects compared to crude propolis extract. Antioxidant potential was assessed using DPPH and ABTS radical scavenging assays, showing concentration dependent antioxidant activity. The results suggest that propolis derived AgNPs possess significant antimicrobial and antioxidant properties, highlighting their potential application in biomedical, pharmaceutical, and environmental fields.

### Keywords:

*Green synthesis, silver nanoparticles, Propolis extract, antimicrobial activity, Antioxidant activity.*

## Introduction

Nanotechnology has emerged as one of the most rapidly advancing areas of modern science due to its ability to manipulate materials at the nanoscale and thereby impart novel physicochemical and biological properties [1]. Nanoparticles, typically ranging from 1 to 100 nm in size, exhibit unique optical, electrical, catalytic, and biological characteristics that differ significantly from their bulk counterparts. These properties have enabled extensive applications of nanomaterials in diverse fields such as medicine, pharmaceuticals, food preservation, agriculture, environmental remediation, and energy technologies. Among various metallic nanoparticles, silver nanoparticles (AgNPs) have received exceptional attention owing to their well-documented antimicrobial, antioxidant, anticancer, and anti-inflammatory activities [2].

Silver has been used for centuries as an antimicrobial agent in wound care, water disinfection, and food storage. With the advent of nanotechnology, silver in its nanoscale form has demonstrated enhanced surface area, reactivity, and biological efficiency, making AgNPs superior to bulk silver compounds [3, 4]. AgNPs exhibit strong antimicrobial activity against a broad spectrum of microorganisms, including Gram-positive bacteria, Gram-negative bacteria, fungi, and certain viruses. The mechanism of action of AgNPs is attributed to multiple pathways, such as disruption of microbial cell membranes, generation of reactive oxygen species (ROS), interaction with thiol groups of proteins, and interference with DNA replication. In addition to antimicrobial activity, AgNPs also demonstrate significant antioxidant potential, which is crucial in combating oxidative stress-related disorders [5].

Conventionally, AgNPs are synthesized using physical and chemical methods such as thermal decomposition, chemical reduction, electrochemical techniques, and photochemical synthesis. Although these methods allow precise control over particle size and shape, they often involve hazardous chemicals, high energy consumption, toxic solvents, and generate environmentally harmful by-products [6,7]. Furthermore, chemically synthesized nanoparticles may pose risks to human health and ecosystems due to residual toxic reagents. These limitations have necessitated the development of alternative, eco-friendly synthesis routes that align with the principles of green chemistry.

Green synthesis of nanoparticles has emerged as a sustainable and environmentally benign approach that utilizes biological entities such as plant extracts, microorganisms, enzymes, and natural products for the reduction and stabilization of metal ions. This method offers several advantages, including simplicity, cost-effectiveness, scalability, reduced toxicity, and enhanced biocompatibility [8]. Plant- and natural-product-mediated synthesis is particularly attractive because plant extracts are rich in secondary metabolites such as phenolic acids, flavonoids, terpenoids, alkaloids, proteins, and polysaccharides. These biomolecules act simultaneously as reducing agents, capping agents, and stabilizers, eliminating the need for external chemicals.

Among natural products, propolis has gained considerable interest due to its remarkable biological properties and rich chemical composition. Propolis is a resinous substance collected by honeybees from buds, bark, and exudates of plants and mixed with beeswax and enzymes [9]. It has been used extensively in traditional medicine across various cultures for its antimicrobial, antioxidant, anti-inflammatory, antiviral, antifungal, and wound-healing properties. The chemical composition of propolis is complex and varies depending on geographical origin, botanical source, and climatic conditions. However, it is generally rich in polyphenols, flavonoids, phenolic acids, aromatic esters, and terpenoids, which are primarily responsible for its bioactivity [10-14].

The high phenolic and flavonoid content of propolis makes it an excellent candidate for green nanoparticle synthesis. These compounds possess strong reducing power and can effectively convert silver ions ( $\text{Ag}^+$ ) into metallic silver ( $\text{Ag}^0$ ) nanoparticles while simultaneously stabilizing them through surface adsorption. Moreover, the presence of bioactive compounds on the surface of nanoparticles can enhance their biological functionality through synergistic effects [15]. Propolis-mediated AgNPs are therefore expected to exhibit superior antimicrobial and antioxidant activities compared to chemically synthesized nanoparticles or propolis extract alone.

Oxidative stress, caused by an imbalance between free radicals and antioxidants, plays a crucial role in the pathogenesis of various chronic diseases, including cancer, cardiovascular disorders, neurodegenerative diseases, and inflammatory conditions. Antioxidants neutralize free radicals by donating electrons or hydrogen atoms, thereby preventing cellular damage. Natural antioxidants derived from plant and bee products are increasingly preferred over synthetic antioxidants due to safety concerns [16, 17]. The integration of antioxidant-rich propolis with silver nanoparticles

offers a promising strategy to develop multifunctional nanomaterials with enhanced free radical scavenging capacity.

Similarly, the increasing prevalence of antibiotic-resistant microorganisms has become a global public health concern. The overuse and misuse of conventional antibiotics have led to the emergence of multidrug-resistant strains, necessitating the search for alternative antimicrobial agents. Silver nanoparticles synthesized via green routes have demonstrated strong antimicrobial activity even against resistant strains, making them potential candidates for next-generation antimicrobial formulations [18]. The incorporation of propolis further enhances antimicrobial efficacy due to its inherent antibacterial and antifungal constituents.

Despite the growing interest in green-synthesized AgNPs, studies focusing on the use of propolis as a reducing and stabilizing agent remain relatively limited compared to plant extracts. Moreover, systematic evaluation of both antimicrobial and antioxidant properties of propolis-mediated AgNPs is essential to establish their potential biomedical and pharmaceutical applications [19]. Therefore, the present study aims to synthesize silver nanoparticles using propolis extract through a green and sustainable approach and to comprehensively evaluate their physicochemical characteristics, antimicrobial activity, and antioxidant potential.

This work highlights the dual functionality of propolis as a natural nano factory and a bioactive agent, contributing to the development of eco-friendly nanomaterials with enhanced biological performance. The findings of this study may provide valuable insights into the design of propolis-based nanocomposites for applications in medicine, pharmaceuticals, food packaging, and environmental protection.

## **Materials and Methods**

### **3.1 Materials**

Raw propolis was obtained from a local apiary and stored in airtight containers at room temperature until use. Silver nitrate ( $\text{AgNO}_3$ , analytical grade,  $\geq 99\%$  purity) was used as the silver precursor. Distilled/deionized water was used throughout the experimental work. All glassware was thoroughly washed, rinsed with distilled water, and dried prior to use to avoid contamination.

No chemical reducing or stabilizing agents were used in order to maintain a completely green synthesis route.

### **3.2 Preparation of Propolis Extract**

Raw propolis was first cleaned to remove dust and foreign materials and then air-dried at room temperature. The dried propolis was ground into a fine powder using a mortar and pestle. Approximately 5–10 g of propolis powder was added to 100 mL of distilled water and heated at 60–70 °C under continuous magnetic stirring for 30–45 minutes to extract bioactive compounds. The mixture was then allowed to cool to room temperature and filtered using Whatman No. 1 filter paper to obtain a clear aqueous propolis extract. The extract was stored at 4 °C and used within 48 hours for nanoparticle synthesis.

### **3.3 Green Synthesis of Silver Nanoparticles**

An aqueous solution of silver nitrate (1 mM) was prepared using distilled water. The propolis extract was added dropwise to the AgNO<sub>3</sub> solution in a ratio of 1:9 (extract: AgNO<sub>3</sub>) under constant stirring. The reaction mixture was maintained at room temperature and protected from direct light. The formation of silver nanoparticles was visually confirmed by a gradual color change from pale yellow to dark brown, indicating the reduction of Ag<sup>+</sup> ions to metallic Ag<sup>0</sup> nanoparticles.

The reaction was allowed to proceed for 24 hours to ensure complete reduction. The synthesized AgNPs were separated by centrifugation at 10,000 rpm for 15 minutes. The pellet was washed three times with distilled water to remove excess phytochemicals and unreacted ions. The purified nanoparticles were dried in a hot air oven at 50–60 °C and stored in a desiccator for further characterization and biological evaluation.

### **3.4 Characterization of Synthesized Silver Nanoparticles**

#### **3.4.1 UV–Visible Spectroscopy**

UV–Visible spectroscopy was used to confirm the formation of silver nanoparticles by detecting surface plasmon resonance (SPR). The absorbance spectrum of the colloidal AgNP solution was recorded in the range of 300–700 nm using distilled water as a blank.

### 3.4.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was carried out to identify functional groups involved in the reduction and stabilization of AgNPs. Dried nanoparticle samples were mixed with potassium bromide (KBr) and scanned in the range of 4000–400  $\text{cm}^{-1}$ .

### 3.4.3 X-ray Diffraction (XRD) Analysis

XRD analysis was performed to determine the crystalline nature and phase purity of the synthesized AgNPs. Diffraction patterns were recorded using Cu  $K\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) over a  $2\theta$  range of 20–80°. The average crystallite size was calculated using the Debye–Scherrer equation.

### 3.4.4 Scanning Electron Microscopy (SEM)

SEM was used to examine the surface morphology and approximate particle size of the synthesized AgNPs. Dried samples were mounted on carbon-coated stubs and sputter-coated with a thin gold layer before imaging.

## 3.5 Antimicrobial Activity

The antimicrobial activity of propolis extract and synthesized AgNPs was evaluated using the agar well diffusion method. Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) were used as test organisms. Fresh bacterial cultures were adjusted to 0.5 McFarland standard and inoculated onto nutrient agar plates. Wells were punched into the agar, and different concentrations of AgNP suspension were added. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimetres.

### **3.6 Antioxidant Activity**

#### **3.6.1 DPPH Radical Scavenging Assay**

The antioxidant activity of AgNPs was assessed using the DPPH free radical scavenging assay. Various concentrations of AgNPs were mixed with DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm, and percentage inhibition was calculated.

#### **3.6.2 ABTS Radical Scavenging Assay**

The ABTS assay was performed to further evaluate antioxidant potential. ABTS radicals were generated and reacted with different concentrations of AgNPs. Absorbance was recorded at 734 nm, and radical scavenging activity was expressed as percentage inhibition.

### **Results and Discussion**

#### **4.1 Visual Observation and Formation of Silver Nanoparticles**

The formation of silver nanoparticles synthesized using propolis extract was initially confirmed through visual observation. Upon addition of the propolis extract to the aqueous silver nitrate solution, the reaction mixture gradually changed color from pale yellow to dark brown. This color transformation is a well-known indicator of silver nanoparticle formation and is attributed to the excitation of surface plasmon resonance (SPR) arising from collective oscillation of conduction electrons on the nanoparticle surface. The absence of precipitation and the stability of the brown coloration over time indicated successful reduction and stabilization of AgNPs by bioactive compounds present in propolis.

#### **4.2 UV–Visible Spectroscopic Analysis**

UV–Visible spectroscopy was employed to confirm the synthesis and optical properties of the silver nanoparticles. The UV–Vis absorption spectrum of the synthesized AgNPs showed a characteristic surface plasmon resonance band in the range of 420–450 nm, which is typical for silver nanoparticles. The presence of a single, well-defined absorption peak suggests the formation of predominantly spherical nanoparticles with a relatively narrow size distribution. The intensity

of the SPR peak increased with reaction time, confirming the progressive reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$  nanoparticles [20, 21].

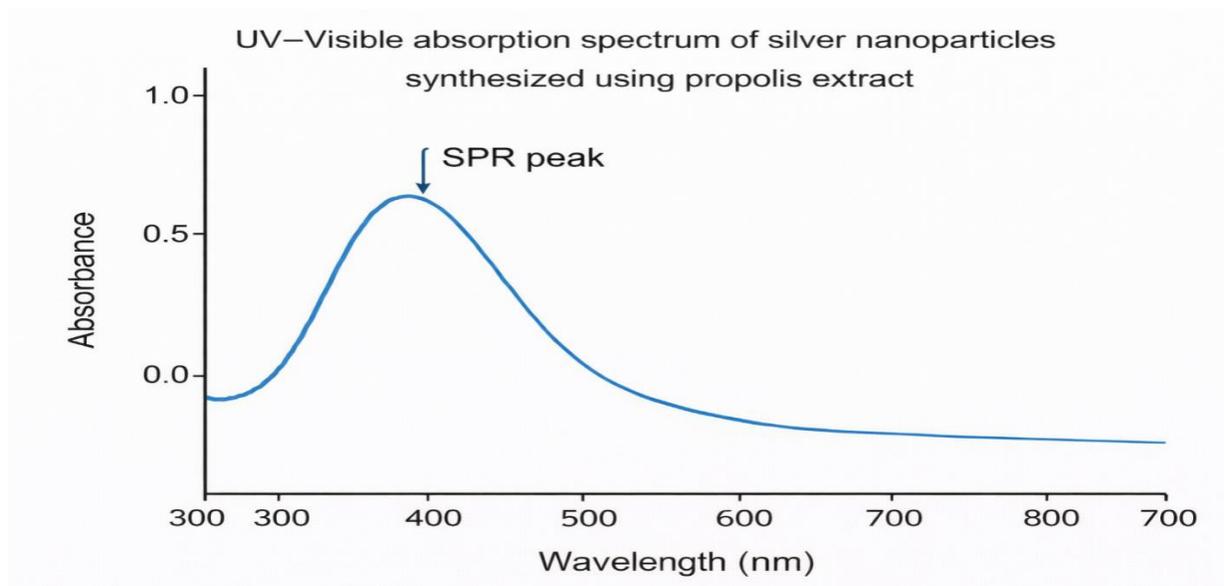


Figure 4.1 UV-Visible absorption spectrum of propolis-mediated silver nanoparticles

Table 4.1 UV-Visible absorption peak of synthesized AgNPs

Sample	SPR Peak (nm)	Observation
Propolis extract	—	No SPR peak
AgNPs	$430 \pm 10$	Characteristic surface plasmon resonance

### 4.3 FTIR Analysis

FTIR spectroscopy was used to identify the functional groups involved in the reduction and stabilization of silver nanoparticles. The FTIR spectrum of propolis extract displayed characteristic absorption bands corresponding to hydroxyl ( $-\text{OH}$ ), carbonyl ( $\text{C}=\text{O}$ ), and aromatic ( $\text{C}=\text{C}$ ) functional groups, indicating the presence of polyphenols and flavonoids [22]. Similar peaks were observed in the FTIR spectrum of the synthesized AgNPs, with slight shifts in peak positions, suggesting the interaction of these functional groups with the nanoparticle surface. These results confirm that phenolic and flavonoid compounds present in propolis act as both reducing and capping agents during nanoparticle synthesis.

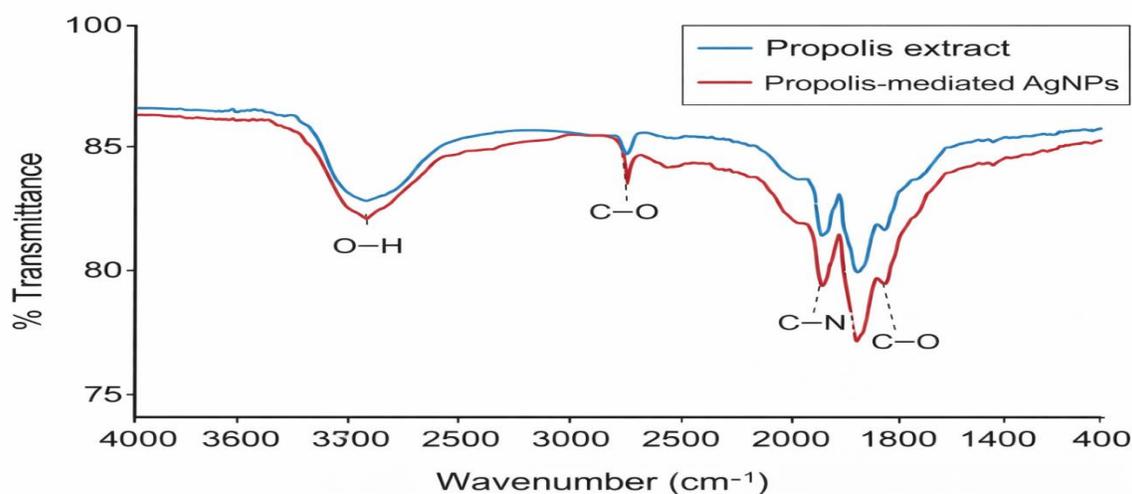


Figure 4.2. FTIR spectra of (a) propolis extract and (b) synthesized AgNPs

Table 4.2. FTIR functional groups involved in AgNP synthesis

Wavenumber (cm <sup>-1</sup> )	Functional Group	Role
~3400	O-H stretching	Polyphenols / flavonoids
~1630	C=O stretching	Protein/phenolic compounds
~1380	C-N stretching	Stabilization
~1050	C-O stretching	Capping of nanoparticles

#### 4.4 X-ray Diffraction (XRD) Analysis

XRD analysis was performed to determine the crystalline nature of the synthesized silver nanoparticles. The XRD pattern exhibited distinct diffraction peaks corresponding to the (111), (200), (220), and (311) planes of face-centered cubic (fcc) silver [23]. These peaks confirm the crystalline structure and phase purity of the synthesized AgNPs. The average crystallite size, calculated using the Debye–Scherrer equation, was found to be in the nanometre range, further validating the successful synthesis of nanoscale silver particles.

Table 4.3 XRD diffraction peaks of silver nanoparticles

<b>2θ (degrees)</b>	<b>Plane (hkl)</b>	<b>Crystal Structure</b>
~38.1	(111)	FCC silver
~44.3	(200)	FCC silver
~64.4	(220)	FCC silver
~77.5	(311)	FCC silver

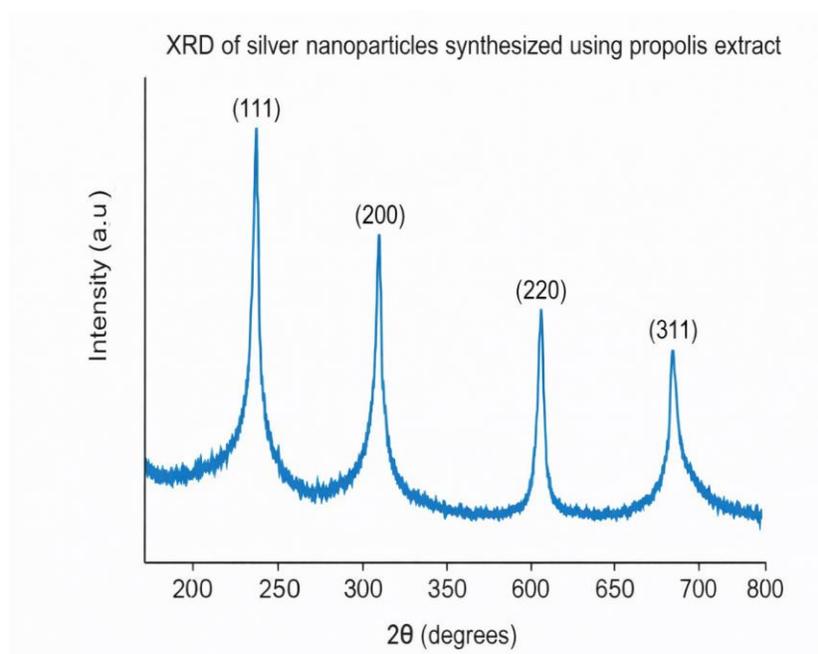


Figure 4.3 XRD pattern of propolis-mediated silver nanoparticles showing FCC structure

#### 4.5 Scanning Electron Microscopy (SEM) Analysis

SEM analysis provided insight into the surface morphology and size distribution of the synthesized AgNPs. The SEM images revealed predominantly spherical nanoparticles with relatively uniform distribution. Some degree of aggregation was observed, which is commonly reported in biologically synthesized nanoparticles due to the presence of organic capping agents [24, 25].

Nevertheless, the particles remained within the nanoscale range, confirming the effectiveness of propolis extract as a stabilizing agent.

Table 4.4SEM particle size analysis

Parameter	Observation
Shape	Predominantly spherical
Particle size range	20–50 nm
Aggregation	Slight
Distribution	Fairly uniform

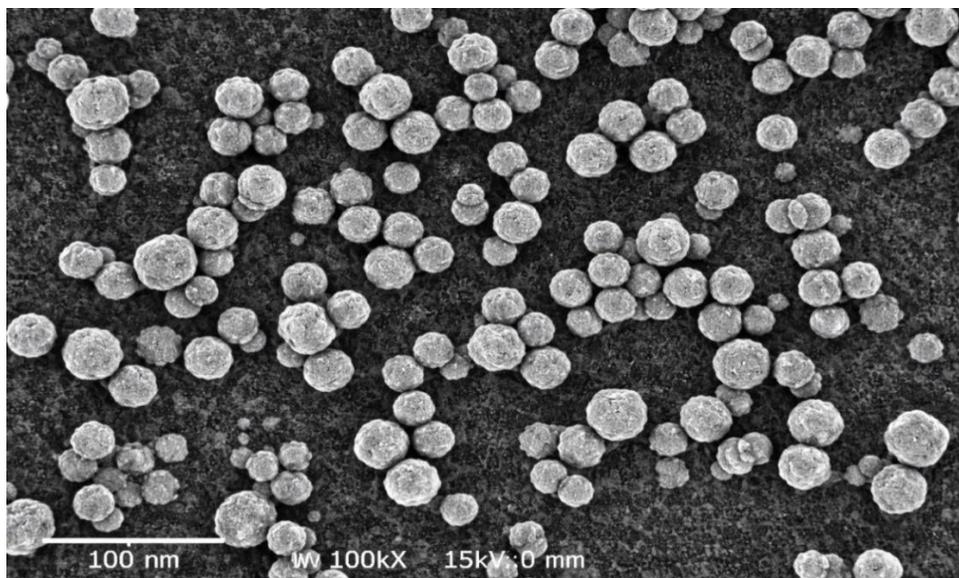


Figure 4.4SEM micrographs of synthesized silver nanoparticles

#### 4.6 Antimicrobial Activity

The antimicrobial activity of propolis extract and propolis-mediated silver nanoparticles was evaluated against selected Gram-positive and Gram-negative bacterial strains. The synthesized

AgNPs exhibited significantly larger zones of inhibition compared to the crude propolis extract, indicating enhanced antimicrobial efficacy [26]. The increased activity can be attributed to the combined effects of silver ions and bioactive compounds adsorbed on the nanoparticle surface. The nanoparticles likely disrupt bacterial cell membranes, increase membrane permeability, generate reactive oxygen species, and interfere with essential cellular processes, leading to microbial cell death [27].

Table 4.5 DPPH radical scavenging activity of AgNPs

Concentration ( $\mu\text{g/mL}$ )	% Inhibition
20	$32 \pm 1.2$
40	$48 \pm 1.5$
60	$63 \pm 1.8$
80	$75 \pm 2.0$
100	$86 \pm 2.3$

#### 4.7 Antioxidant Activity

The antioxidant potential of the synthesized AgNPs was assessed using DPPH and ABTS radical scavenging assays. The results demonstrated concentration-dependent radical scavenging activity, with higher concentrations of AgNPs showing greater antioxidant potential [28-30]. The enhanced antioxidant activity is attributed to surface-bound polyphenols and flavonoids derived from propolis, which retain their electron- or hydrogen-donating ability after nanoparticle formation. These findings suggest that propolis-mediated AgNPs possess strong free radical scavenging properties and may be useful in combating oxidative stress-related damage.

Table 4.6 Antimicrobial activity (Zone of inhibition in mm)

Microorganism	Propolis Extract (mm)	AgNPs (mm)
<i>Staphylococcus aureus</i>	10 ± 0.5	18 ± 0.6
<i>Bacillus subtilis</i>	9 ± 0.4	17 ± 0.5
<i>Escherichia coli</i>	8 ± 0.3	16 ± 0.4
<i>Pseudomonas aeruginosa</i>	7 ± 0.3	15 ± 0.5

#### 4.8 Discussion Summary

Overall, the results confirm that propolis extract is an efficient reducing and stabilizing agent for the green synthesis of silver nanoparticles. The synthesized AgNPs exhibited desirable physicochemical characteristics along with enhanced antimicrobial and antioxidant activities. The synergistic interaction between silver nanoparticles and propolis-derived bioactive compounds significantly contributes to their multifunctional biological performance.

### Conclusion and Future Perspectives

#### 5.1 Conclusion

In the present study, an eco-friendly and sustainable approach was successfully employed for the green synthesis of silver nanoparticles using propolis extract. The bioactive compounds present in propolis effectively reduced silver ions and stabilized the resulting nanoparticles without the use of toxic chemicals. Characterization studies confirmed the formation of crystalline, predominantly spherical silver nanoparticles in the nanoscale range. The synthesized propolis-mediated silver nanoparticles exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, outperforming crude propolis extract. Additionally, the nanoparticles demonstrated strong, concentration-dependent antioxidant activity in DPPH and ABTS assays. The enhanced biological performance is attributed to the synergistic effects of silver and surface-bound propolis phytochemicals. These findings highlight the potential of propolis-derived silver

nanoparticles as multifunctional nanomaterials for biomedical, pharmaceutical, and antimicrobial applications.

## **5.2 Future Perspectives**

Despite promising results, further studies are required to fully explore the potential applications of propolis-mediated silver nanoparticles. Future research should focus on *in vitro* and *in vivo* cytotoxicity studies to evaluate their safety and biocompatibility. Mechanistic studies investigating silver ion release and long-term stability are also recommended. Additionally, scaling up the synthesis process and standardizing propolis extraction methods will be essential for commercial applications. The incorporation of propolis-mediated AgNPs into wound dressings, coatings, food packaging materials, and drug delivery systems represents promising future directions. Overall, green-synthesized silver nanoparticles using propolis extract offer a sustainable and effective platform for the development of advanced nanomaterials with enhanced biological functionality.

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