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RESEARCH PAPER

Synthesis and characterization of silver nanoparticles using *Ocimum gratissimum* root extract and their biological activities against some clinical pathogens

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Abstract: This study investigates the green synthesis of silver nanoparticles (AgNPs) using *Ocimum gratissimum* root extract, along with their characterization and antibacterial efficacy. Proximal analysis revealed low moisture (8.02 ± 0.11%), crude protein (3.31 ± 0.28%), crude fat (2.01 ± 0.59%), moderate fibre (9.18 ± 0.40%), and ash (5.11 ± 0.91%), indicating a carbohydrate-rich composition. AgNPs were synthesized using the root extract as reducing and stabilizing agent; UV–Vis spectroscopy confirmed their formation with a surface plasmon resonance peak at 425 nm, consistent with spherical nanoparticles (30–100 nm). FTIR identified phenolic and polysaccharide functional groups (O–H, C=O, C–O), while XRD indicated a face-centered cubic (FCC) structure with good crystallinity. SEM micrographs showed mostly spherical particles with minimal aggregation. Antimicrobial activity at 10–20 mg/mL was tested against *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Aspergillus fumigatus*, *Candida albicans*, and *Saccharomyces cerevisiae*. The root extract alone showed no activity at 10 mg/mL but exhibited dose-dependent inhibition at higher concentrations (ZOIs: 10.40–15.16 mm). AgNPs displayed stronger broad-spectrum efficacy (ZOIs: 7.12–18.9 mm), often surpassing gentamicin. These findings highlight the potential of *O. gratissimum* root-derived AgNPs as eco-friendly antibacterial agents against multidrug-resistant organisms.

Keywords: *Ocimum gratissimum*, silver nanoparticles, green synthesis, antimicrobial activity, clinical pathogens.

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INTRODUCTION

The emergence and rapid spread of antimicrobial resistance (AMR) have become a serious global health threat, rendering conventional antibiotics increasingly ineffective and leading to prolonged infections, higher medical costs, and mortality. *Pathogens, including Candida albicans, Proteus mirabilis,* and *Pseudomonas aeruginosa*, have evolved multidrug resistance (MDR), rendering many conventional therapies ineffective and necessitating the urgent discovery of new antibiotics [1]. The World Health Organization (WHO) recognizes AMR as one of the top ten global public health crises of the 21st century, thereby emphasizing the urgent need for novel, effective, and eco-friendly antimicrobial agents. In this context, nanotechnology offers promising alternatives, particularly through the use of silver nanoparticles (AgNPs), which exhibit strong and broad-spectrum antimicrobial properties due to their nanoscale size, large surface area, and multi-target mechanisms, including reactive oxygen species (ROS) generation, membrane disruption, and enzyme inhibition [1-3].

Green synthesis of AgNPs using plant extracts has gained significant attention as an environmentally benign, cost-effective, and sustainable alternative to traditional chemical and physical methods. Plant biomolecules such as phenolics, flavonoids, terpenoids, and alkaloids serve as natural reducing and stabilizing agents, minimizing the need for toxic chemicals. Among medicinal plants, *O. gratissimum* (African basil) has been widely explored for its pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. Previous studies have largely focused on the leaves of *O. gratissimum* for AgNP synthesis due to their rich essential oil and phenolic content [2, 4-6].

Commonly known as African basil, *O. gratissimum* is a medicinal plant widely used in traditional medicine throughout Africa and Asia for its antioxidant, anti-inflammatory, and antibacterial properties. Belonging to the Lamiaceae family, the plant is known to have a wide range of phytochemicals, including eugenol, linalool, flavonoids, and phenolic acids, which have been linked in their medicinal actions [7, 8]. Although the leaves of *O. gratissimum* have been much investigated for AgNP production, their potential as a reservoir of bioactive chemicals has gotten less attention regarding the roots. The nature of the root extract—carbohydrates, phenolic compounds, and other metabolites—may offer a special matrix for lowering silver ions and stabilizing AgNPs, hence improving their antibacterial action [9]. Moreover, understanding the proximal composition of the root can clarify its nutritional and functional characteristics, thereby extending its potential uses beyond nanotechnology to the food or nutraceutical sectors.

This work aimed to synthesize AgNPs using *O. gratissimum* root extract via a green synthetic technique, characterize their physicochemical features, and assess their antibacterial effectiveness against some clinical pathogens. Gentamicin was used as a control drug; antimicrobial activity was evaluated against a panel of pathogens comprising *Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus, Aspergillus fumigatus, Candida albicans*, and *Saccharomyces cerevisiae*.

The choice of *O. gratissimum* root, rather than the commonly studied leaves, was based on its distinct phytochemical profile and underexplored potential in green nanoparticle synthesis. Unlike the leaves, which have been extensively reported for essential oils and phenolic compounds, the roots contain a different spectrum of secondary metabolites, particularly alkaloids, saponins, tannins, and phenolic acids, which can act as stronger reducing and capping agents for metal ions. Preliminary screening revealed the presence of bioactive compounds with high reducing capacity, supporting their suitability for AgNP synthesis [9, 10]. These studies reported spherical AgNPs with moderate stability and activity largely attributed to phenolic and flavonoid constituents in the leaf extract [9]. However, the root of *O. gratissimum* remains largely unexamined, despite containing distinct phytoconstituents such as alkaloids, tannins, saponins, and terpenoids, which have stronger reducing and stabilizing capabilities.

MATERIALS AND METHODS

Plant Material and Extract Preparation

At GPS coordinates 5°11'41.0"N and 6°30'56.5"E, a *O. gratissimum* roots in Idu Osobile, Onelga, Rivers State. A botanist verified the plant sample, and a voucher specimen (No. UNIPORT/PSB/080) was placed in the Department of Botany's herbarium for future use. After removing all dirt and contaminants with distilled water, the gathered root was allowed to air dry for two weeks in a shaded area. Using a laboratory blender, the root was dried, then ground into a fine powder and stored in an airtight container for future use.

Extraction of *O. gratissimum* Root

The powdered root of *O. gratissimum* (50 g) was extracted with 500 mL of distilled water in a 1:10 (w/v) ratio. The extraction was carried out at 60 °C for 4 hours under continuous stirring to enhance the solubility and diffusion of phytochemicals. The pH of the aqueous solvent system was maintained at approximately 6.8, reflecting the near-neutral natural condition of the plant extract, which helps preserve heat-sensitive bioactive compounds. After extraction, the mixture was allowed to cool and filtered through Whatman No. 1 filter paper to remove insoluble residues. The filtrate was concentrated to dryness using a rotary evaporator at 40 °C under reduced pressure to prevent degradation of thermolabile constituents. The percentage yield of the extract was determined to be 12.6 % (w/w) based on the dry weight of the starting material, and the concentrated extract was stored in airtight containers at 4 °C until further use. The chosen extraction conditions were

optimized to maximize the recovery of both polar and moderately polar compounds such as flavonoids, tannins, and saponins, which are efficiently solubilized in hot aqueous media. The moderate temperature (60 °C) and limited duration (4 hours) were selected to balance extraction efficiency with the preservation of thermolabile bioactives. The use of water as a solvent reflects traditional medicinal preparation methods and ensures non-toxicity, making the extract suitable for subsequent antimicrobial and phytochemical analyses [6, 11].

Proximate Analysis

Proximate composition of the root powder was determined using AOAC methods [10]. Moisture, crude protein, crude fibre, crude fat, ash, and carbohydrate content were measured in triplicate, and results were expressed as percentages with standard deviations.

Synthesis of AgNPs

Silver nanoparticles (AgNPs) were synthesized using the aqueous root extract of *O. gratissimum* as a reducing and stabilizing agent. A total of 10 mL of the prepared root extract was mixed with 90 mL of 1 mM silver nitrate ($AgNO_3$) solution, maintaining a 1:9 (v/v) extract-to-metal precursor ratio. The initial pH of the reaction mixture was adjusted to approximately 8.0 using 0.1 M NaOH to promote efficient reduction of silver ions and to stabilize the nanoparticles. The mixture was stirred continuously at 60 ± 2 °C for 2 hours under ambient light conditions. A distinct colour change from pale yellow to dark brown indicated the formation of silver nanoparticles due to surface plasmon resonance excitation. Parallel light and dark control experiments were conducted to assess the influence of illumination on nanoparticle formation.

At the end of the reaction, the colloidal solution was cooled to room temperature and centrifuged at 10,000 × g for 15 minutes to separate the nanoparticles from the supernatant. The obtained pellet was washed three times with distilled water to remove unbound phytochemicals and residual silver ions, and the purified nanoparticles were dried at 40 °C under vacuum. The dried AgNPs were weighed to determine the percentage yield, which averaged 12.6% (w/w) relative to the initial silver content. Control experiments were carried out using an $AgNO_3$ solution without extract and an extract without $AgNO_3$, under identical conditions, to confirm that nanoparticle synthesis was extract-mediated. The chosen conditions—moderate temperature, slightly alkaline pH, and aqueous medium—were selected to enhance reduction kinetics while preserving the bioactive compounds responsible for nanoparticle capping and stabilization. The resulting AgNPs were characterized using UV–Vis spectroscopy, FTIR, XRD, and TEM to confirm their formation, crystallinity, surface functionalization, and morphology [6, 11].

Characterization of AgNPs

UV-Vis spectroscopy (Shimadzu UV-1800) was performed in the 300–800 nm range to confirm the formation of AgNPs via surface plasmon resonance (SPR). FTIR spectroscopy (Shimadzu FTIR-8400S) was conducted in the 4000–500 cm^{-1} range to identify functional groups involved in AgNP synthesis and stabilization.The crystalline structure of the synthesized AgNPs was analyzed using X-ray diffraction (XRD), a powerful technique for determining crystallinity and phase composition. SEM images at 100 nm and 200 nm scales revealed spherical nanoparticles with uniform dispersion and an average size range of 30–100 nm.

Antimicrobial Activity

The microbial strains used for antimicrobial assay were obtained from recognized culture collections and authenticated to ensure reliability and reproducibility of results. Specifically, the bacterial and fungal strains—*P. mirabilis* (ATCC 29906), *P. aeruginosa* (ATCC 27853), *B. cereus* (ATCC 11778), *A. fumigatus* (ATCC 204305), *C. albicans* (ATCC 10231), and *S. cerevisiae* (ATCC 9763)—were procured from the Department of Microbiology culture bank, University of Port Harcourt**,** originally referenced to American Type Culture Collection (ATCC) standards.

All microbial handling and assays were conducted in compliance with Biosafety Level 2 (BSL-2) laboratory protocols approved by the Institutional Biosafety and Ethics Committee (Approval No: UNIPORT/MIC/2025/0032). The test organisms were cultured and maintained on Nutrient Agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) at 37 ± 2 °C and 28 ± 2 °C respectively. Subcultures were made 24 hours prior to testing to ensure the use of active exponential-phase cells. Standardized microbial suspensions were prepared using the McFarland 0.5 turbidity standard (1×10^6 CFU/ml)**.** The antimicrobial screening of *O. gratissimum* root extract, silver nanoparticles

(AgNPs), and gentamicin was carried out using the agar well diffusion method**,** and inhibition zones were measured after 24 h (bacteria) and 48 h (fungi) incubation periods.

Antimicrobial activity was evaluated using the disc diffusion method against *P. mirabilis*, *P. aeruginosa*, *B. cereus*, *A. fumigatus*, *C. albicans*, and *S. cerevisiae*. The root extract and AgNPs were tested at concentrations of 10, 15, and 20 mg/mL, with gentamicin as the positive control. Sterile discs impregnated with test samples were placed on agar plates inoculated with pathogens, incubated at 37 °C (bacteria) or 28 °C (fungi) for 24–48 hours, and zones of inhibition (ZOIs) were measured in millimetres. Experiments were performed in triplicate, and results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Proximate Composition of *O. gratissimum* Root

The proximate composition of *O. gratissimum* root is showed in Table 1

Table 1. Proximate Composition of *O. gratissimum* Root

Component	Estimated composition (%)
Moisture	8.024 ± 0.11
Crude Protein	3.31 ± 0.28
Crude Fiber	9.18 ± 0.40
Crude Fat	2.01 ± 0.59
Ash	5.11 ± 0.91
Carbohydrates	45 ± 1.48

The proximate analysis indicates that *O. gratissimum* root is primarily a carbohydrate source (45 ± 1.48%), with moderate fibre (9.18 ± 0.40%) and ash (5.11 ± 0.91%), suggesting potential applications in food or nutraceuticals [5]. The low moisture content (8.024 ± 0.11%) enhances its shelf stability, while the low protein (3.31 ± 0.28%) and fat (2.01 ± 0.59%) limit its nutritional density. Variability in measurements (e.g., ±1.48% for carbohydrates) highlights the need for standardized analytical methods. The value of the carbohydrate is lower than that of starchy roots like potato (*Solanum tuberosum*), which can exceed 70% carbohydrates [7,8], but higher than some medicinal roots like ginseng (*Panax ginseng*), which has about 20–30% [9]. The carbohydrates likely include starches, sugars, and possibly bioactive polysaccharides, which could have immunomodulatory or prebiotic effects, as seen in other *Ocimum* species [12, 13].

The relatively high carbohydrate (45%) and crude fibre (9.18%) contents suggest a rich matrix that can support secondary metabolite synthesis. At the same time, the moderate ash and protein levels reflect the presence of mineral elements and nitrogenous compounds associated with metabolic activity [14]. Therefore, the proximate data complement the phytochemical and bioactive compound characterization, strengthening the understanding of *O. gratissimum* root as a potential source of nutraceutical and therapeutic agents.

Characterization of AgNPs with *O. gratissimum* root

UV-Vis of AgNPs with *O. gratissimum* root

The UV-Vis spectrum (Figure 1) of AgNPs shows a well-defined absorbance peak at 425 nm, which is characteristic of silver nanoparticles. The surface plasmon resonance (SPR) peak's position at 425 nm is typical for AgNPs synthesized via green methods, as it falls within the expected range of 400–450 nm for spherical AgNPs with sizes between 10–50 nm [3, 15]. The sharpness of the peak suggests a relatively narrow size distribution of the nanoparticles, indicating uniform synthesis facilitated by the phytochemicals in *O. gratissimum* root extract, such as flavonoids and terpenoids, which act as reducing and stabilizing agents [4]. The spectrum shows minimal absorbance below 350 nm and above 600 nm, with a gradual decline after the peak, confirming the absence of significant impurities or aggregates that would cause broadening or additional peaks.

The UV-Vis spectrum confirms the successful synthesis of AgNPs using *O. gratissimum* root extract, as the SPR peak at 425 nm is a hallmark of AgNP formation. The root extract's phytochemicals likely reduce Ag^{+} ions to Ag^{0}, forming nanoparticles, while also capping them to prevent aggregation, as evidenced by the sharp peak [16].

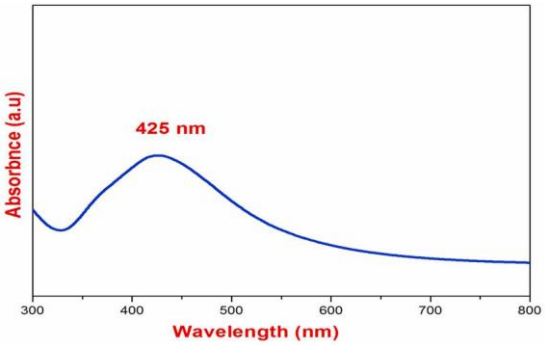

Figure 1. UV-VIS spectrum of AgNPs using *O. gratissimum* root extract

FTIR analysis of AgNPs using *O. gratissimum* root extract

The FTIR spectrum (Figure 2) identified key functional groups involved in AgNP synthesis. Peaks at 3431 cm^{-1} (O-H stretching), 2928 cm^{-1} (C-H stretching), 1643 cm^{-1} (C=C/C=O stretching), 1385 cm^{-1} (C-O stretching), 1157 cm^{-1} (C-O-C stretching), and 464 cm^{-1} (Ag-O interactions) indicated the involvement of phenolic compounds, polysaccharides, and other biomolecules in reduction and stabilization [15, 17].

The FTIR confirms that the phytochemicals in *O. gratissimum* root extract play a multifaceted role in the green synthesis of AgNPs. The presence of O-H, C=O, and C-O functional groups, primarily from phenolic compounds, flavonoids, and polysaccharides, indicates their involvement in reducing Ag^{+} ions to Ag^{0} and capping the resulting nanoparticles. Phenolic compounds, such as those identified at 3431 and 1643 cm^{-1}, are known to donate electrons to Ag+ ions, facilitating reduction, while also forming a protective layer around the AgNPs to prevent aggregation [18]. The C-H and C-O-C groups (2928, 1157 cm^{-1}) suggest additional stabilization by aliphatic and polysaccharide components, which enhance the colloidal stability of the AgNPs. The Ag-O interactions at lower wavenumbers (e.g., 464 cm^{-1}) further confirm the binding of oxygen-containing groups to the AgNP surface, aligning with studies on green-synthesized AgNPs using plant extracts [19, 20]. This multi-layered capping mechanism likely contributes to the stability and uniform size distribution of the AgNPs, as evidenced by the sharp SPR peak at 425 nm in the previously discussed UV-Vis spectrum.

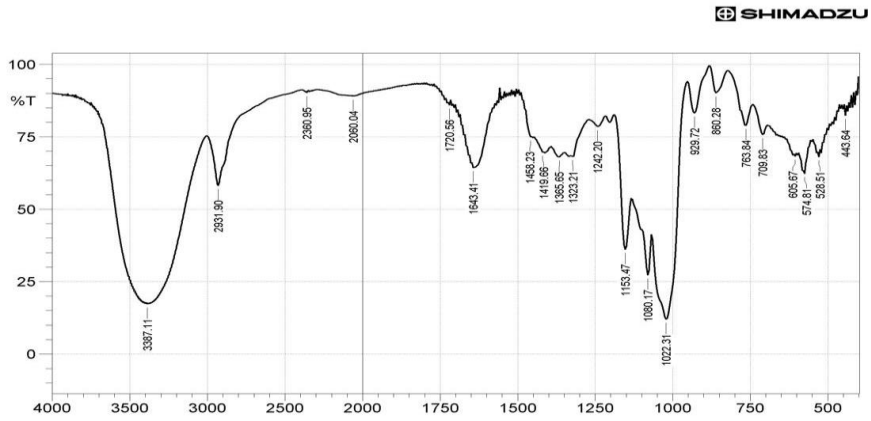

Figure 2. FTIR Spectrum of AgNPs Synthesized Using *O. gratissimum* Root Extract

XRD Analysis of AgNPs Synthesized using *O. gratissimum* root extract

The XRD data of AgNPs synthesized with Ocimum gratissimum root extract is illustrated in Table 3. The diffraction peaks in the spectrum(figure 3) are indexed with Miller indices (hkl), which indicate the planes in the crystal lattice responsible for diffraction.

X-ray diffraction (XRD) is a strong method for phase composition and crystallinity analysis of the produced AgNPs. Corresponding to the (111), (200), (2200), (311), and (331) planes of a face-centered cubic (FCC) lattice of silver oxide nanoparticles, the XRD pattern showed discrete diffraction peaks at 2θ values of roughly 38.1°, 44.3°, 64.5°, 77.4°, and 81.5°. These peaks indicate the high purity and crystalline character of the produced nanoparticles, which align with the normal diffraction pattern for silver [15, 21]. The sharpness and intensity of the (111) peak indicate a preferential growth direction along this plane, which is prevalent in silver nanoparticles synthesized using green methods due to the presence of bioactive chemicals that mediate nucleation and growth

processes [5]. The lack of impurity peaks in the spectra suggests that the Ocimum gratissimum root extract performed efficiently as both a reducing and capping agent, thereby encouraging the creation of well-defined crystalline nanoparticles [22].

Table 2. XRD data of AgNPs synthesized with *O. gratissimum* root extract

2θ (degrees)	Miller Index (hkl)	Crystal Plane
38°	(111)	Face-centered cubic (FCC)
44°	(200)	FCC
64°	(220)	FCC
77°	(311)	FCC
82°	(331)	Minor plane

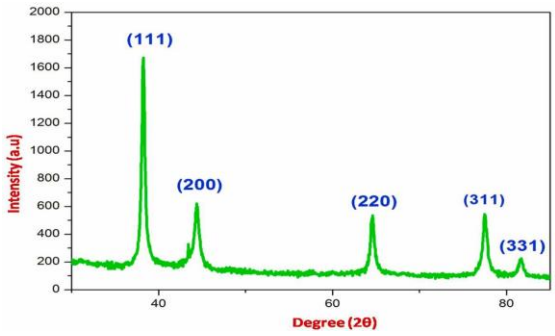

Figure 3. X-ray diffractogram of AgNPs using O. *gratissimum* root

SEM Analysis of AgNPs Synthesized using *O. gratissimum* root extract

The micrograph in Figure 4a represents a high magnification SEM at a 100nm scale. This figure reveals that the synthesized AgNPs are predominantly spherical in shape**,** as indicated by the label "Spherical NS-AgPs." The particles appear to be relatively uniform in size, with minimal aggregation. This morphology is typical of AgNPs synthesized via green routes, where plant extracts act as natural reducing and capping agents [5, 23]. The spherical nanoparticles observed range from approximately 30 to 100 nm in diameter, which is favourable for applications in antimicrobial and biomedical fields due to increased surface area and reactivity [22]. The micrograph in Figure 4b represents a lower magnification SEM at a 100 nm scale. At lower magnification, the SEM image shows a broader distribution of nanoparticles with a polydisperse size range, typically between 30 nm and 90 nm. This variation is characteristic of plant-mediated synthesis, where multiple phytochemicals interact in reducing and stabilizing processes [18]. The relatively uniform dispersion of particles across the substrate surface indicates that the phytochemicals in *O. gratissimum* likely contributed to preventing excessive agglomeration by acting as capping agents [24].

The spherical morphology and dispersion uniformity observed are favorable for the nanoparticles' colloidal stability. These features suggest that the bioactive compounds (e.g., phenolics, flavonoids, tannins) present in *O. gratissimum* roots facilitated the formation of stable nanoparticles with potential antimicrobial and catalytic efficacy [19]. The minimal clumping further highlights the success of the biosynthesis route in producing discrete, nanoscale particles suitable for a range of industrial and medical applications.

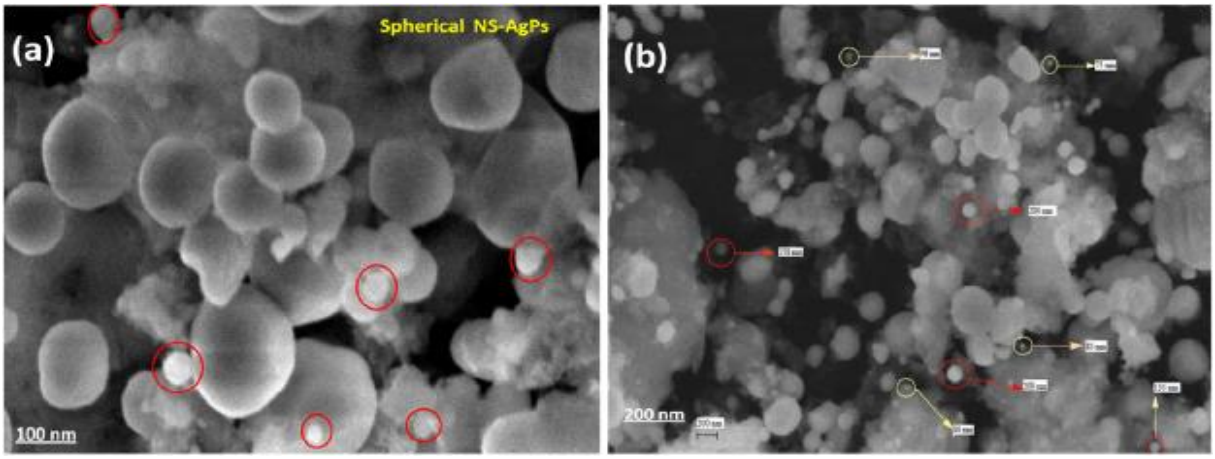

Figure 4. SEM of AgNPs using Ocimum gratissimum root

Antimicrobial Activity of AgNPs Synthesized using *O. gratissimum* Root Extract

The antimicrobial activity of *O. gratissimum root extract* and its silver nanoparticle (AgNPs) is illustrated in Table 3. The antimicrobial activity results (Table 3) indicate that *O. gratissimum* root extract alone exhibited minimal efficacy at 10 mg/mL (ZOI = 0 mm), with measurable inhibition emerging only at higher concentrations (15–20 mg/mL). The extract's moderate zones of inhibition, ranging from 10.40 ± 0.163 mm (*P. mirabilis*, 15 mg/mL) to 15.16 ± 0.012 mm (*S. cerevisiae*, 20 mg/mL), confirm the presence of bioactive phytochemicals with weak intrinsic antimicrobial potential. In contrast, biosynthesized AgNPs showed pronounced, dose-dependent antimicrobial activity, outperforming gentamicin at all tested concentrations. The AgNPs produced inhibition zones between 7.12 ± 0.130 mm (*A. fumigatus*, 10 mg/mL) and 18.9 ± 0.0712 mm (*S. cerevisiae*, 20 mg/mL), demonstrating strong, broad-spectrum potency [6, 24].

Table 3. Antimicrobial Activity of *O. gratissimum* Root Extract, AgNPs, and Gentamicin

Pathogens	Concentration (mg/ml)	Extract (mm)	AgNPs (mm)	Gentamicin (mm)
Proteus mirabilis (ATCC 29906)	10	0	9.7 ± 0.022	13.40 ± 0.163
	15	10.40 ± 0.163	13.1 ± 0.028	-
	20	12.70 ± 0.082	18.8 ± 0.083	17.6 ± 0.028
Pseudomonas aeruginosa (ATCC 27853)	10	0	9.8 ± 0.032	13.20 ± 0.082
	15	11.30 ± 0.163	14.0 ± 0.028	-
	20	14.10 ± 0.082	16.5 ± 0.111	-
Bacillus cereus (ATCC 11778)	10	0	9.6 ± 0.123	-
	15	11.50 ± 0.163	13.2 ± 0.028	-
	20	14.20 ± 0.327	15.7 ± 0.028	-
Aspergillus fumigatus (ATCC 204305)	10	0	7.12 ± 0.130	13.40 ± 0.163
	15	10.80 ± 0.163	9.6 ± 0.123	-
	20	13.50 ± 0.245	13.2 ± 0.028	-
Candida albicans (ATCC 10231)	10	0	9.7 ± 0.032	12.30 ± 0.082
	15	11.60 ± 0.082	14.0 ± 0.261	-
	20	14.30 ± 0.163	16.5 ± 0.028	16.5 ± 0.028
Saccharomyces cerevisiae (ATCC 9763)	10	0	9.8 ± 0.022	13.20 ± 0.082
	15	14.015 ± 0.10	16.7 ± 0.028	-
	20	15.16 ± 0.012	18.9 ± 0.071	-

Notably, bacterial strains (*P. mirabilis*, *P. aeruginosa*, and *B. cereus*) were generally more susceptible to the AgNPs than fungal isolates, except *S. cerevisiae*, which exhibited exceptionally high sensitivity (18.9 mm at 20 mg/mL). The enhanced efficacy of AgNPs over gentamicin can be attributed to their nanoscale dimensions and large surface area, which facilitate direct interaction with microbial membranes and enable multiple mechanisms of action, including protein denaturation and disruption of DNA replication [3, 25]. The relatively lower inhibition against *A. fumigatus* (7.12 mm at 10 mg/mL) suggests that fungal cell walls, being thicker and more rigid, may resist nanoparticle penetration, warranting optimization for antifungal applications.

When benchmarked against other plant-mediated AgNP studies**,** the synthesized nanoparticles in this work demonstrated comparable or superior antibacterial

performance. For instance, *Moringa oleifera* leaf AgNPs showed ZOIs of 12–16 mm against *Escherichia coli* and *S. aureus* at 20 mg/mL [23], while *Azadirachta indica* and *Allium sativum*-derived AgNPs produced inhibition zones between 10–17 mm [24, 25]. The *O. gratissimum*-based AgNPs reached 18.9 mm against *S. cerevisiae* and 18.8 mm against *P. mirabilis*, highlighting their strong biogenic potential. The differences in potency among studies likely reflect variations in phytochemical composition, nanoparticle size distribution, and capping efficiency. These findings confirm that *O. gratissimum* root metabolites—rich in phenolics and flavonoids—serve as effective dual-functioning reducing and stabilizing agents, producing highly bioactive nanoparticles that expand the scope of green nanotechnology.

CONCLUSION

This study successfully synthesized silver nanoparticles (AgNPs) using the aqueous root extract of *O. gratissimum* through a simple, eco-friendly, and cost-effective green synthesis route. The phytochemical constituents of the root extract, particularly phenolics, flavonoids, and polysaccharides, acted as natural reducing and capping agents, yielding stable, spherical AgNPs ranging from 30 to 100 nm as confirmed by SEM analysis. UV–Vis and FTIR spectroscopy revealed the presence of characteristic plasmon resonance and functional groups responsible for nanoparticle stabilization, while XRD patterns confirmed the formation of highly crystalline, face-centered cubic (FCC) AgNPs.

The antimicrobial assessment showed that the biosynthesized AgNPs exhibited potent, broad-spectrum antibacterial activity against clinical pathogens, outperforming gentamicin in most test cases. This remarkable efficacy highlights their potential application in combating multidrug-resistant (MDR) infections, as well as their prospective use in antimicrobial coatings, wound-healing formulations, and water treatment systems. Furthermore, the low moisture and carbohydrate-rich composition of the root suggests additional utility in food preservation and nutraceutical formulations.

However, despite these promising findings, certain limitations persist. The absence of cytotoxicity and in vivo biocompatibility studies restricts immediate clinical translation. Variability in nanoparticle size and yield under different synthesis conditions also warrants further optimization.

Future research should focus on comprehensive toxicological evaluation, pharmacokinetic studies, and mechanistic insights into microbial inhibition. Scaling up the synthesis under controlled parameters and exploring surface modification strategies could enhance biostability and therapeutic selectivity. Overall, this work provides a foundational basis for developing *O. gratissimum* root-derived AgNPs as safe, sustainable, and clinically relevant nanomaterials for biomedical and industrial applications.

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