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Silver Nanoparticles Synthesized by Bacteria and Their Antimicrobial Activity

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Abstract
Soil microbial communities play a crucial role in biotechnological applications, particularly in the process of making nanoparticles. Soil samples were obtained for this investigation from two different areas in Benha (SO1 and SO2) to isolate bacterial strains capable of synthesizing silver nanoparticles (AgNPs). There were ten of bacterial isolates collected, with six from SO1 and four from SO2. The ability of these isolates to synthesize AgNPs was assessed based on color change, indicating nanoparticle formation. Among them, SDO6 exhibited the highest biosynthetic activity (+++), suggesting a strong potential for silver ion reduction and nanoparticle stabilization.

The antibacterial ability of biosynthesized AgNPs from SDO6 was tested against ***Escherichia coli*** and ***Staphylococcus aureus***, using ciprofloxacin (5 µg/mL) as a standard antibiotic. The results showed significant antibacterial effects, with an inhibition rate of 89.025% against *E. coli*, though slightly lower than ciprofloxacin (99.05%). In contrast, AgNPs exhibited a weaker inhibitory effect against ***S. aures*** (40.12%), compared to ciprofloxacin (98.25%). The higher susceptibility of ***E. coli*** suggests that AgNPs interact more effectively with Gram-negative bacterial membranes, leading to oxidative stress and cell disruption. The lower efficacy against ***S. aureus*** may be attributed to its thick peptidoglycan layer, this layer serves as a barrier against nanoparticle penetration.

Key Words: Silver Nanoparticles; *Staphylococcus aureus, E. coli*. And antimicrobial activity

Introduction
Nanoparticles, with a dimension ranging between 1 and 100 nm, are considered to be one of the most promising materials in nanotechnology. Silver nanoparticles (AgNPs) have attracted substantial interest as one of the nanomaterials because of their unique physical and chemical properties. Recently, AgNPs have a broad application in commercial, medicinal, agricultural, and industrial purposes. The above applications are mainly owing to their high surface area, stability, larg surface volume ratio, biocompatibility, and the power to release silver ions. In addition, AgNPs have found extensive research interest for use in biomedical applications such as antibiotic drug carriers, anticancer agents, enzyme biosensors, DNA biosensors, DNA detection, and antibacterial agents. Currently, the use of AgNPs in the form an additive in vaccines has been an interesting biomedical application. [1]

Nanoparticles can be fabricated through various methods, which are generally categorized into two main groups. The first group involves top-down techniques, which begin with bulk material and break it down to nanosized particles. The second group refers to emerging and eco-friendly methods, which are the bottom-up techniques. In this category, nanoparticles are synthesized from atomic or molecular sources. Generally, nanoparticles fabricated from bottom-up techniques have high solubility, stability, and bioavailability compared to those from top-down techniques. Several bottom-up techniques include thermal, chemical, photochemical reduction, sol gel preparation, electrochemical, and biological methods. Biological synthesis approaches are emerging as a well-recognized and eco-friendly method [2]. There is a growing concern on the robustness and reliability of chemical and physical techniques used for nanoparticle synthesis. There is also an awareness of the toxicity of chemicals used in the synthesis of nanoparticles. In light of these concerns, attention has turned to alternative biological methods for nanoparticle synthesis. Understanding the method of synthesis and characteristics of these nanoparticles is important for researchers and commercial applications. [3]

Nanoparticles exhibit significant physical and chemical properties when their size is reduced to the nanometer level. Nanotechnology has become an active area of research in the fields of physics, chemistry, biology, medicine, and environmental science due to its potential applications in various sectors. Metal nanoparticles are of tremendous interest because of their unique chemical, optical, catalytic, electrical, and magnetic properties compared to bulk metals. Silver nanoparticles (AgNPs) have received special interest in recent years due to their potential uses in electronics, optics, catalysis, biosensors, and nanomedicine. AgNPs have demonstrated effectiveness against a variety of pathogens and have been used in textiles,

coatings, plastics, cosmetics, and food packaging [4].

Traditionally, metal nanoparticles have been synthesized using physical or chemical methods. However, These synthetic methods frequently require the use of poisonous chemicals, posing environmental hazards, as well as complex post-synthesis processing steps. Therefore, there is a growing demand for the development of "green" synthetic methods that are eco-friendly and can produce stable metal nanoparticles. Biological systems, particularly plants and microbes, are being explored as an alternative to chemically synthesized nanoparticles. Bacteria have demonstrated the ability to reduce metallic ions to nanoparticles of metal, and biosynthesis of nanoparticles via bacterial processes presents a green alternative to chemical methods. [5]

Microbial pathogens seriously threaten human health and the stability of the food supply. They are capable of evolving resistance to antibiotic agents. It is critical to develop new antimicrobial agents with novel mechanisms to counteract microbial resistance. Silver nanoparticles (AgNPs) are efficient against a wide range of pathogens, including both Gram-positive and Gram - negative bacteria as well as viruses and fungi. The antimicrobial properties of silver nanoparticles have several interconnected mechanisms of action on microbial cells [8]. Silver nanoparticles can emit silver ions (Ag+), disrupting cellular functions and interacting with microbial cell membranes. Free Ag+ can permeate the cell membrane and attach to essential biological components, such as DNA and protein molecules, resulting in diminished viability and proliferation of bacteria. Silver ions also inhibit the function of respiratory enzymes and produce reactive oxygen species (ROS), which causes stress from oxidation. Nanoparticles without silver ion release cannot generate ROS, indicating that ROS generation is secondary to microbial cell interaction. Oxidative stress increases the effectiveness of silver nanoparticles. [9]

Materials and Methods

Bacterial Sample Collection

Bacterial samples were collected from soil,in Banha on December 2024. The samples were transported under sterile conditions and stored at appropriate temperatures for further microbiological analysis.

Isolation of Bacterial Strains

Bacterial strains were isolated using the serial dilution technique, as per established microbiological methods. The obtained sample was diluted 10× in a sterile diluent solution (e.g., saline solution, peptone water, or nutrient broth) by placing 25 g (solid sample) or 25 mL (liquid sample) into a flask with 225 mL of diluent solution. constituting the first dilution (10^{-1}). The sample was homogenized using a vortex mixer, peristaltic agitator, or orbital shaker for 1–5 minutes,To create a 100× dilution (10^{-2}), 1 mL of the 10^{-1} dilution was aseptically transferred to a tube with 9 mL of diluent solution. This serial dilution process was repeated as needed, typically up to 10^{-7}, depending on the bacterial population in the samples. The prepared solutions were distributed on plates of nutrient-agar and incubated at 37°C for 24 to 48 hours. Following incubation, different colonies of bacteria selected and sub cultured on new agar plates for further purification and identification.

Fermentation of Isolated Bacteria in Nutrient Broth for Filtrate Preparation

The purified bacterial isolates were cultured in sterile nutrient broth to obtain bacterial filtrate for nanoparticle biosynthesis. The broth medium, composed of peptone, yeast extract, and sodium chloride, was prepared to support optimal bacterial growth. Each bacterial isolate was injected into the broth and incubated at 37 degrees Celsius. under constant agitation (150–200 rpm) to enhance metabolic activity. After the fermentation period (typically 24–48 hours), the culture of bacterial was centrifuged at 10,000 rpm for 15 minutes for separating the bacterial biomass. The supernatant (bacterial filtrate) was collected and filtered through a 0.22 µm membrane filter to eliminate leftover bacterial cells, ensuring a cell-free filtrate. This filtrate was then utilized as a bioreducing agent during the biosynthesis of silver nanoparticles.

Biosynthesis of Silver Nanoparticles with Bacterial Filtrate

Silver nanoparticles (AgNPs) were synthesized utilizing bacterial filtrate as a reducing and stabilizing agent. A precursor solution of silver nitrate ($AgNO_3$) was prepared at an optimized concentration (e.g., 1–5 mM). The bacterial filtrate was mixed with the silver nitrate solution in a 1:1 ratio and incubated under controlled conditions (pH, temperature, and light exposure). The reaction mixture was stirred continuously at room temperature, and the formation of silver nanoparticles was monitored by the appearance of a color change, typically from light yellow to brown, indicating nanoparticle synthesis. The synthesized silver nanoparticles were collected by centrifugation (12,000 rpm, 15 minutes), washed with sterile distilled water, and dried for further characterization.

Antimicrobial Activity of Biosynthesized Silver Nanoparticles

The antimicrobial activity of the biosynthesized silver nanoparticles was evaluated against two bacterial pathogens: *Escherichia coli* (Gram-negative) and ***Staphylococcus aureus*** (Gram-positive). The broth microdilution method using a polystyrene 96-well plate was employed to assess bacterial growth inhibition. A bacterial suspension was prepared at a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and inoculated into nutrient broth. The biosynthesized silver nanoparticles were added at different concentrations, and the plates were incubated at 37°C for 24 hours. After incubation, bacterial growth was assessed by measuring optical density (OD) at 600 nm using a microplate reader. Control wells included a negative control (without nanoparticles) and a positive control (containing a standard antibiotic, e.g., ciprofloxacin).

Results

Soil sample collection

Soil sample collection plays a crucial role in microbial isolation and nanobiotechnology studies, as the diversity of microbial communities in different environments directly influences the biosynthesis potential of nanoparticles. In this study, two soil samples were collected from distinct locations in Benha, designated as SO1 and SO2 (Table 1). These locations were selected based on environmental variability, including potential differences in microbial diversity, organic matter content, and metal ion availability, all of which are known to impact bacterial metabolic activities.

The choice of soil as a source for bacterial isolation is supported by its rich microbial biodiversity, particularly in regions with industrial, agricultural, or naturally enriched ecosystems. Various bacterial genera found in soil, such as ***Streptomyces, Bacillus***, and ***Pseudomonas***, have been reported for their ability to produce extracellular metabolites that facilitate the biosynthesis of nanoparticles. The collected samples are expected to harbor diverse bacterial strains capable of reducing metal ions, including silver, into stable nanoparticles through enzymatic and biochemical pathways. Comparing the bacterial isolates obtained from SO1 and SO2 will help determine whether geographical variations influence microbial-mediated nanoparticle synthesis. Factors such as pH, moisture content, and the presence of organic compounds may contribute to differences in the biosynthetic efficiency of bacterial filtrates from these locations. Future studies could expand the sampling sites and analyze physicochemical parameters of the soil to establish correlations between microbial diversity and nanoparticle synthesis efficiency. By systematically exploring soil microbial communities from different locations, this research aims to identify potent bacterial strains with high nanoparticle biosynthesis potential, contributing to the production of environmentally sustainable and economical nanoparticles for antibacterial application.

Table 1. Soil sample collection

Serial	Sample code	Location
1	**SO1**	**Benha location 1**
2	**SO2**	**Benha location 2**

Isolation of bacteria from soil samples

The isolation of bacteria from soil samples is a fundamental step in exploring microbial diversity and their potential applications in biotechnology, particularly in the biosynthesis of nanoparticles. In this investigation, bacterial strains were effectively recovered from two different soil samples (SO1 and SO2). yielding a total of ten isolates (Table 2). The variation in the number of isolates per sample suggests potential differences in microbial composition between the two soil sources, which may be attributed to factors such as soil type, nutrient availability, moisture content, and environmental conditions.

From sample SO1, six bacterial isolates (SDO1–SDO6) were obtained, indicating a rich microbial population in this location. In contrast, sample SO2 yielded four isolates (XSDO1–XSDO4), suggesting a relatively lower bacterial density or diversity. This variation may be influenced by site-specific factors such as organic matter content, pH levels, exposure to pollutants, or agricultural practices that affect bacterial growth and survival.

The diversity of bacterial isolates is critical in identifying strains with unique metabolic capabilities, including their ability to biosynthesize metal nanoparticles. Bacteria-mediated nanoparticle synthesis is largely dependent on extracellular biomolecules, enzymes, and reducing agents produced by different microbial strains. Therefore, the isolates obtained from these soil samples will undergo further characterization to determine their taxonomic classification, metabolic potential, and suitability for applications such as silver nanoparticle biosynthesis.

Comparing the biosynthetic abilities of isolates from SO1 and SO2 will provide insights into the influence of environmental factors on microbial-driven nanoparticle formation. Future studies should focus on optimizing growth conditions, analyzing the biochemical properties of these isolates, and investigating their efficiency in biosynthesis processes. Additionally, molecular identification techniques such as 16S rRNA sequencing can help classify these isolates and

assess their genetic potential for industrial and biomedical applications.

Table 2. Isolated of bacteria from soil samples

Serial	Sample code	Isolate code
1	**SO1**	**SDO1**
		SDO2
		SDO3
		SDO4
		SDO5
		SDO6
2	**XSO2**	**XSDO1**
		XSDO2
		XSDO3
		XSDO4

Fig. 1. Isolation of bacteria

screening of silver nanoparticles biosynthesis ability of isolated bacteria

The screening of isolated bacteria for their ability to biosynthesize silver nanoparticles (AgNPs) revealed varying levels of nanoparticle production, as indicated by the observed color change (Table 4). Color change is a qualitative indicator of nanoparticle formation, with higher intensity suggesting enhanced biosynthetic activity caused by bacterial metabolites reducing silver ions.

Among the tested isolates, *SDO6* exhibited the highest biosynthesis potential (+++), indicating a strong reducing capacity and efficient nanoparticle formation. This suggests that *SDO6* may possess highly active extracellular biomolecules, such as proteins, enzymes, or secondary metabolites, that help reduce silver ions and stabilize nanoparticles. Similarly, *SDO1* showed a notable biosynthetic activity (++), suggesting a moderate but significant potential for silver nanoparticle production.

The remaining isolates, including *SDO1, SDO2, SDO3, SDO4, SDO5, XSDO1, XSDO2, XSDO3, and XSDO4*, exhibited a weak but detectable biosynthesis ability (+). While these strains demonstrated some capacity for AgNP synthesis, their lower intensity indicates that they may require optimization of culture conditions, such as pH, temperature, incubation time, or silver ion concentration, to enhance their biosynthetic efficiency.

The observed differences in biosynthesis ability among bacterial isolates may be attributed to variations in their metabolic pathways, enzyme activity, and extracellular secretions. Certain bacterial strains have been reported to produce

biomolecules that act as reducing and stabilizing agents in nanoparticle formation, making them more efficient in biosynthesis. The presence of at least one highly efficient strain (*SDO6*) suggests potential for further research into its mechanisms of nanoparticle synthesis and optimization for large-scale production.

These findings highlight the importance of bacterial selection for biosynthetic applications in nanotechnology. Future studies should focus on characterizing the physicochemical properties of the synthesized AgNPs, evaluating their antimicrobial efficacy, and identifying the genetic and biochemical factors responsible for enhanced nanoparticle formation. Additionally, optimizing culture parameters for the most promising isolates could improve nanoparticle yield and stability, paving the way for their use in medical, environmental, and industrial applications.

Table 3. screening of silver nanoparticles biosynthesis ability of isolated bacteria

Serial	Isolate code	Color change due to formation
1	**SDO1**	+
2	**SDO2**	+
3	**SDO3**	+
4	**SDO4**	+
5	**SDO5**	+
6	**SDO6**	+++
7	**XSDO1**	+
8	**XSDO2**	+
9	**XSDO3**	+
10	**XSDO4**	+
11	**SDO1**	++

Antibacterial activity of biosynthesized silver using bacteria SDO6

The antibacterial activity of biosynthesized silver nanoparticles (AgNPs) using the bacterial isolate *SDO6* was tested against ***Escherichia coli*** and ***Staphylococcus aureus***, with ciprofloxacin (5 µg/mL) used as a standard antibiotic control (Table 5). The results indicate that the AgNPs demonstrated notable antibacterial effects, though their efficacy varied between the two tested bacterial strains.

Against ***E. coli***, the biosynthesized AgNPs exhibited an inhibition rate of **89.025%**, demonstrating a strong antimicrobial effect. However, ciprofloxacin, a broad-spectrum antibiotic, showed a slightly higher inhibition rate of **99.05%**, indicating its superior bactericidal activity. The high effectiveness of AgNPs against ***E. coli*** can be attributed to their capacity to break bacterial membranes, generate reactive oxygen species (ROS), and interfere with vital intracellular processes. The Gram-negative nature of ***E. coli***, which includes an outer membrane with negatively charged lipopolysaccharides, may facilitate stronger interactions with AgNPs, leading to increased permeability and bacterial cell death.

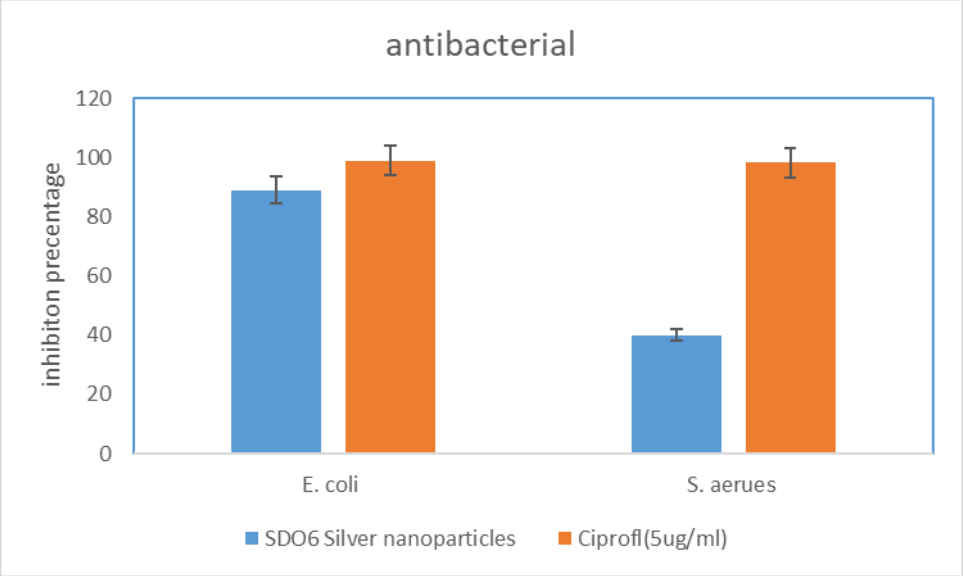
In contrast, the AgNPs exhibited a lower antibacterial effect against *S. aureus*, with an inhibition? l/Gram-positive cell wall structure, which consists of a thick peptidoglycan layer that acts as a protective barrier, potentially limiting AgNP penetration. Additionally, *S. aureus* is known to possess various defense mechanisms, such as efflux pumps and enzyme-mediated resistance, which may contribute to its lower susceptibility to silver nanoparticles.

Although the biosynthesized AgNPs did not match the antibacterial potency of ciprofloxacin, their significant inhibitory effects, particularly against *E. coli*, suggest their potential as alternative antimicrobial agents. The selective activity observed between Gram-negative and Gram-positive bacteria highlights the need for further optimization of AgNP synthesis parameters, Size, shape, and surface charge are all factors that contribute to their broad-spectrum antibacterial activity.

Future research should focus on investigating the mechanism of action of the biosynthesized AgNPs, optimizing their synthesis conditions to improve efficacy, and evaluating their potential for synergistic effects when combined with conventional antibiotics. These findings underscore the promise of bacterial-mediated silver nanoparticle synthesis as a sustainable and effective approach to developing novel antimicrobial agents, particularly in the fight against antibiotic-resistant bacterial strains.

Table 4. Antibacterial activity of biosynthesized silver using bacteria SDO6

	E. coli	S. aureus
SDO6 Silver nanoparticles	**89.025**	**٤٠.١٢**
Ciprofl(5ug/ml)	**99.05**	**98.25**



Antioxidant activity

The antioxidant activity of the biosynthesized silver nanoparticles (AgNPs) using *Bacteria SDO6* was evaluated through DPPH scavenging activity, as shown in Table 5. The results indicate that the SDO6 silver nanoparticles exhibited a scavenging activity of **52.35%**, which, while lower than the standard antioxidant **ascorbic acid** (**69.01%**), demonstrates significant free radical scavenging potential. The ability of SDO6 silver nanoparticles to neutralize DPPH radicals suggests that they possess electron-donating properties, contributing to their antioxidant effect. This activity could be attributed to the presence of biomolecules from *Bacteria SDO6* that may have acted as capping and stabilizing agents during nanoparticle synthesis, influencing their redox potential. The antioxidant capacity of AgNPs is often associated with their surface reactivity, size, and the functional groups from bacterial metabolites present on their surface, which may enhance their electron transfer ability. Although the antioxidant activity of SDO6 silver nanoparticles is lower than that of ascorbic acid, it remains within a promising range for potential biomedical and industrial applications. The radical scavenging potential of these nanoparticles suggests their possible use in oxidative stress-related applications, such as pharmaceutical formulations, food preservation, and cosmetic products. Further studies, including mechanistic evaluations and in vivo assessments, are necessary to explore their full potential and optimize their antioxidant efficacy.

Table 5. Antioxidant activity of the biosynthesized silver nanoparticles using Bacteria SDO6

Antioxidant activity DPPH scavenging activity	
SDO6 silver nanoparticles	**52.35**
Ascorbic acid	**69.01**

ADME Properties of Silver

Silver has a molecular weight of 107.87 Da, placing it in the lower percentile (4.54%) compared to other compounds in Drug Bank. The LogP value of -2.50e-03 suggests very low lipophilicity, which may affect its ability to permeate lipid membranes. Notably, silver lacks hydrogen bond acceptors and donors, indicating limited interaction with biological molecules via hydrogen bonding. The Lipinski Rule of 5 compliance (4/4) suggests potential oral bioavailability. However, the low Quantitative Estimate of Drug likeness (QED) score of 0.38 (31.68% percentile) indicates suboptimal drug-likeness. Additionally, the TPSA value of 0.00 Å² (1.80% percentile) suggests poor polarity, which could impact its solubility and permeability.

Silver exhibits high predicted human intestinal absorption (0.99, 47.69% percentile) and oral bioavailability (0.89, 69.25% percentile), making it a potentially good candidate for oral administration. However, its aqueous solubility is extremely low (-10.49 log(mol/L), 0.04% percentile), which could limit its dissolution and absorption. The high lipophilicity (3.39, 88.25% percentile) enhances membrane permeability but may contribute to bioaccumulation. Hydration free energy (-3.02 kcal/mol, 95.81% percentile) suggests a strong tendency to interact with water molecules, which could impact its distribution in the body. Additionally, the cell effective permeability (-3.73 log(10^{-6} cm/s), 99.73% percentile) indicates very poor passive diffusion across cell membranes.

Silver demonstrates moderate blood-brain barrier (BBB) penetration (0.93, 72.35% percentile), suggesting potential neuroactive effects. However, plasma protein binding is extremely high (100.00%, 97.56% percentile), which may restrict its free drug availability and affect distribution. The volume of distribution (Vd) at steady state is 0.00 L/kg (9.46% percentile), indicating limited tissue penetration. Silver shows significant interactions with cytochrome P450 enzymes, particularly CYP2C19 (0.81, 94.61% percentile) and CYP2C9 (0.80, 96.59% percentile), suggesting a high likelihood of metabolic transformations affecting its pharmacokinetics. CYP3A4 inhibition (0.36, 74.56% percentile) implies that silver could influence the metabolism of other drugs. However, its role as a substrate for these enzymes is relatively low (CYP2C9: 0.08, CYP2D6: 0.11, CYP3A4: 0.29), indicating limited metabolism via these pathways.

Silver exhibits a long half-life (33.15 hours, 78.01% percentile), suggesting prolonged systemic retention. Hepatocyte drug clearance (54.77 µL/min/10^6 cells, 66.15% percentile) is moderate, but microsomal clearance (0.00 µL/min/mg, 10.82% percentile) is very low, indicating inefficient hepatic elimination. This suggests that silver might accumulate in tissues, requiring alternative elimination pathways such as renal or fecal excretion.

Toxicity predictions indicate a moderate risk for clinical toxicity (0.37, 80.65% percentile), mutagenicity (0.67, 91.04% percentile), and carcinogenicity (0.60, 92.59% percentile).These values suggest that silver has potential long-term toxicity concerns. The hERG blocking potential (0.25, 46.80% percentile) indicates a moderate risk of cardiac side effects. Acute toxicity LD50 (1.44 log(1/(mol/kg), 5.82% percentile)) suggests relatively low acute toxicity. Skin reaction risk (0.89, 92.28% percentile) is high, indicating potential dermatological concerns. Silver also interacts with various nuclear receptors, including the Aryl Hydrocarbon Receptor (0.27, 85.96%), Peroxisome Proliferator-Activated Receptor Gamma (0.02, 72.66%), and Nuclear Factor (Erythroid-Derived 2)-Like 2/ARE (0.57, 86.74%), which may influence oxidative stress and inflammation responses. Heat Shock Factor Response Element (0.46, 96.67%) suggests possible cellular stress-related effects.

Table 6: ADME Properties of Silver

Category	Property	Value	DrugBank Percentile	Units
Physicochemical	Molecular Weight	107.87	4.54%	Dalton
	Logp	-2.50e-03	22.92%	log-ratio
	Hydrogen Bond Acceptors	0.00	1.94%	#
	Hydrogen Bond Donors	0.00	11.77%	#
	Lipinski Rule of 5	4.00	63.80%	# of 4
	Quantitative Estimate of Drug likeness (QED)	0.38	31.68%	-
	Stereo Centers	0.00	22.49%	#
	Topological Polar Surface Area (TPSA)	0.00	1.80%	Å²
Absorption	Human Intestinal Absorption	0.99	47.69%	-
	Oral Bioavailability	0.89	69.25%	-

	Aqueous Solubility	-10.49	0.04%	log(mol/L)
	Lipophilicity	3.39	88.25%	log-ratio
	Hydration Free Energy	-3.02	95.81%	kcal/mol
	Cell Effective Permeability	-3.73	99.73%	log(10-6 cm/s)
	PAMPA Permeability	0.86	61.03%	-
	P-glycoprotein Inhibition	0.06	44.67%	-
Distribution	Blood-Brain Barrier Penetration	0.93	72.35%	-
	Plasma Protein Binding Rate	100.00	97.56%	%
	Volume of Distribution at Steady State	0.00	9.46%	L/kg
Metabolism	CYP1A2 Inhibition	0.44	81.62%	-
	CYP2C19 Inhibition	0.81	94.61%	-
	CYP2C9 Inhibition	0.80	96.59%	-
	CYP2D6 Inhibition	0.03	43.51%	-
	CYP3A4 Inhibition	0.36	74.56%	-
	CYP2C9 Substrate	0.08	29.12%	-
	CYP2D6 Substrate	0.11	51.49%	-
	CYP3A4 Substrate	0.29	31.68%	-
Excretion	Half Life	33.15	78.01%	hr
	Drug Clearance (Hepatocyte)	54.77	66.15%	uL/min/106 cells
	Drug Clearance (Microsome)	0.00	10.82%	uL/min/mg
Toxicity	hERG Blocking	0.25	46.80%	-
	Clinical Toxicity	0.37	80.65%	-
	Mutagenicity	0.67	91.04%	-
	Drug Induced Liver Injury	0.50	54.28%	-
	Carcinogenicity	0.60	92.59%	-
	Acute Toxicity LD50	1.44	5.82%	log(1/(mol/kg))
	Skin Reaction	0.89	92.28%	-
	Androgen Receptor (Full Length)	0.01	20.05%	-
	Androgen Receptor (Ligand Binding Domain)	0.04	82.12%	-
	Aryl Hydrocarbon Receptor	0.27	85.96%	-
	Aromatase	0.18	83.56%	-
	Estrogen Receptor (Full Length)	0.02	8.14%	-
	Estrogen Receptor (Ligand Binding Domain)	0.03	62.97%	-
	Peroxisome Proliferator-Activated Receptor Gamma	0.02	72.66%	-
	Nuclear Factor (Erythroid-Derived 2)-Like 2/ARE	0.57	86.74%	-
	ATPase Family AAA Domain-Containing Protein 5 (ATAD5)	0.11	89.61%	-
	Heat Shock Factor Response Element	0.46	96.67%	-
	Mitochondrial Membrane Potential	0.03	46.49%	-
	Tumor Protein p53	0.02	50.37%	-

Discussion

The soil serves as an abundant reservoir of diverse microbial communities, making it an ideal source for isolating bacteria with unique metabolic potentials. In this study, soil samples were taken from two distinct locations in Benha (SO1 and SO2) to explore the influence of environmental variations on bacterial diversity and their potential for biosynthesis applications. Variations in soil composition, including organic matter content, metal ion availability, and pH, could contribute to differences in microbial populations across these locations

This research intended to establish a green approach for the production of silver nanoparticles by various Egyptian bacterial strains. The presence of diverse bacterial strains is crucial for identifying potent candidates for nanoparticle biosynthesis, particularly those capable of reducing metal ions into stable nanoparticles. The successful isolation of ten bacterial strains from these soil samples (six from SO1 and four from SO2) highlights the microbial richness of the selected sites, setting the foundation for subsequent screening and application in nanotechnology.

The ability of isolated bacterial strains to synthesize silver nanoparticles (AgNPs) was assessed based on color change, an established qualitative indicator of nanoparticle formation. The biosynthesized AgNPs were clearly validated by the creation of a dark brown color in the mixture as well as the presence of a silver surface plasmon resonance band by UV-Visible spectroscopy. The AgNPs were further characterized using SEM, EDX, and TEM. The antibacterial activity of the AgNPs was tested using Salmonella typhi, Escherichia coli, Staphylococcus epidermis, and Staphylococcus aureus utilizing the disk diffusion technique. (12) (13)(14).The UV-visible spectra of the aqueous medium containing silver ions revealed a peak at 425 nm, which corresponds to the plasmon absorbance of the silver nanoparticles. The biosynthesized AgNPs ranged in size from 7 to 31 nm and had a spherical form. Studies on the antibacterial action of the particles revealed the best inhibitory impact against ; *E. coli(12).*

The extracellular synthesis of silver nanoparticles by positive isolates was studied using UV-Vis spectroscopy. The results indicated that the UV-visible spectrum of an aqueous solution with silver ions had a peak at 420 nm, which corresponded to the plasmonic absorption of silver nanoparticles. This validates the presence of silver nanoparticles (AgNPs) in each of the positive isolates of bacteria. Among the tested isolates, SDO6 exhibited the highest potential (+++), suggesting a strong capability to reduce silver ions and form stable nanoparticles. This strain likely produces extracellular biomolecules, such as enzymes or metabolites, that enhance nanoparticle synthesis. Another isolate, SDO1, showed moderate biosynthetic activity (++), indicating a promising, though less efficient, ability to facilitate silver ion reduction. The remaining isolates displayed weak but detectable biosynthesis activity (+), suggesting that they may require optimization in growth conditions or silver ion concentration to improve their biosynthetic efficiency. The observed differences in biosynthetic ability among the bacterial strains can be attributed to variations in their metabolic pathways, enzyme secretion, and genetic makeup. Identifying the most potent bacterial isolates and optimizing their nanoparticle synthesis parameters could enhance their applicability in industrial and biomedical fields. (15).

The antibacterial activity of biosynthesized nanoparticles of silver (AgNPs) was assessed against ***Escherichia coli*** and ***Staphylococcus aureus***, two clinically significant bacterial pathogens. The results demonstrated that AgNPs synthesized by SDO6 exhibited a strong inhibitory effect against *E. coli* (89.025% inhibition), though slightly lower than the inhibition achieved by ciprofloxacin (99.05%). The high susceptibility of ***E. coli*** to AgNPs can be attributed to its Gram-negative cell wall structure, which contains an outer membrane that enhances interactions with silver ions, leading to membrane disruption, oxidative damage, and bacterial death.

In contrast, the biosynthesized AgNPs exhibited a lower inhibitory effect against *S. aureus* (40.12%), which was significantly less than ciprofloxacin (98.25%). The reduced susceptibility of ***S. aureus*** may be due to its Gram-positive cell wall, which consists of a thick peptidoglycan layer that acts as a protective barrier against nanoparticle penetration. Additionally, ***S. aureus*** possesses various resistance mechanisms, such as efflux pumps and enzyme-mediated detoxification, which may further limit the effectiveness of AgNPs. Despite this, the moderate antibacterial activity observed against ***S. aureus*** suggests that optimizing nanoparticle size, shape, and surface modifications could enhance their efficacy.

Although the biosynthesized AgNPs did not achieve the same antibacterial potency as ciprofloxacin, their significant inhibitory effects, ` The selective activity between Gram-negative and Gram-positive bacteria underscores the need for further studies to optimize synthesis conditions and improve nanoparticle stability. Future research should focus on elucidating the mechanisms of bacterial resistance to AgNPs, exploring synergistic effects with conventional antibiotics, and evaluating their applications in combating multidrug-resistant bacterial infections.

Various investigations have focused on the antibacterial properties of AgNPs. This research looked at the influence of AgNPs on the growth of both Gram-positive and Gram-negative organisms. Chudasama et al. (16) and Ramgopal et al. (2011) (17). found that S. aureus (Gram positive) exhibited more antibacterial activity than ***E. coli*** (Gram negative). Prakash et al. (2011) (18). found similar results for the antibacterial activities of AgNPs on Gram-negative (***E. coli***) and Gram-positive bacteria (Streptococous pyogene). Dipak and Sankar et al. in 2014 (19) and Priyadarshini et al. in 2013 (20) found the largest and lowest zones of inhibition against ***E. coli*** and ***Staphylococcus***, respectively. Our research has revealed a stronger antibacterial activity against Gram negative organisms, which might be attributed to the thick peptidoglycan layer in the bacterial cell wall, since the peptidoglycan layer of Gram positive bacteria is thicker than that of Gram negative bacteria. The peptidoglycan layers are made up of linear polysaccharide chains that are cross-linked with short peptides, resulting in a more rigid structure that creates a strong barrier against the entry of AgNPs (21).

Silver nanoparticles (AgNPs) have recently gained popularity in a variety of applications, including antibacterial agents, anticancer, diagnostics,

biomarkers, cell labels, and drug delivery systems for the treatment of various illnesses.Microorganisms usually develop antibiotic resistance throughout the course of antibacterial therapy. Multi-drug resistance (MDR) is becoming an increasingly serious issue in the treatment of infectious illnesses, and the widespread use of broad-spectrum antibiotics has led in the development of antibiotic resistance in a variety of human and animal bacterial pathogens. We assessed the efficiency of manufactured AgNPs against MDR pathogenic microorganisms, specifically ***Staphylococcus aureus*** (22) (23). The minimum inhibitory concentrations (MICs) of AgNPs against Staphylococcus aureus were reported to be 1 and 2 µg/mL. AgNPs' antibacterial action is due to the formation of reactive oxygen species (ROS), malondialdehyde (MDA), and leakage of proteins and carbohydrates in bacterial cells. Yuan et al. investigated the effects of silver nanoparticles on many drug-resistant strains of ***Staphylococcus aureus*** (24)

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