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Assess the application of Jojoba seed coating in the fabrication of silver nanoparticles and their subsequent utilization as antibacterial agents

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Abstract

Green synthesis is a method that uses plant waste to produce nanoparticles that is environmentally beneficial. It reduces plant waste and eschews the harsh chemicals frequently utilized in the typical manufacture of nanoparticles. These nanoparticles are generally thought to be less hazardous than those that are created chemically. green silver nanoparticles exhibit potential as antibacterial agents. They can potentially fight against numerous disease-causing bacteria, helping to public health. In this study, extract from jojoba seed coat (JSC) was used to create green silver nanoparticles thenThe potent chemical components were identified by identifying the active chemicals and groups in the generated materials and characterization of the nanoparticles using FTIR and a GC-Mass. After that, the nanoparticles were investigated and clarified utilizing TEM, Zeta potential, FTIR, and EDX equipment.

Key words: jojoba seed coat, aqueous extract, silver nanoparticles, antibacterial activity.

Introduction

Because of their special qualities, nanocomposites are a class of sophisticated materials that have transformed a number of industries, medical and biological fields (Hassan et al. 2021; Sim and Wong 2021). Research on nanomaterials is an exciting field that has great promise for treating critical illnesses (Huang et al. 2024). The nanoparticles' small size and capacity to interact with particular molecules allow them can be engineered to specifically target bacteria or cancer cells (Singh and Lillard 2009). However, medications can be protected by nanoparticles from being broken down by the body, which makes it easier for them to get to the source of the illness (Joseph et al. 2023).

A significant public health concern is the emergence of antibiotic resistance in bacteria (Muteeb et al. 2023)**.** Nanotechnology presents a viable solution to this problem, as nanoparticles can be engineered to kill bacteria through a variety of processes distinct from those of conventional antibiotics (Hetta et al. 2023). Because of this, they are effective against "superbugs," or bacteria that are resistant to many drugs.

Biofilms—sticky colonies made of bacteria—are a common way for them to evade the immune system and medications (Shree et al. 2023). These biofilms can be broken down by nanoparticles, which increases the susceptibility of bacteria to attack (Sahli et al. 2022). Nanoparticles can also be employed to create quick and extremely sensitive diagnostic tests for bacterial diseases (Thwala et al. 2023). This makes it possible to intervene earlier and provide more specialized care.In order to minimize the influence on healthy tissues, researchers are always looking for novel forms of nanoparticles with improved antibacterial characteristics. The objective is to

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transport the nanoparticles specifically to bacterial cells (Bruna et al. 2021).

Although there are still obstacles to be addressed, such as possible environmental effects and guaranteeing the safety of nanoparticles, the potential of nanotechnology in bacterial control cannot be denied (Ray et al. 2009). This is a fascinating field that could change the way we treat bacterial diseases in the future. there are significant challenges associated with manufacturing effective and safe nanoparticles in an environmentally friendly way (Pal et al. 2022; El-Sayyad et al. 2024).

In research labs, numerous efficient techniques for synthesizing nanoparticles are intricate, labor-intensive, and frequently entail hazardous chemicals (Altammar 2023). It is quite difficult to scale these techniques for large-scale production, so creating safe handling procedures is essential (Gupta et al. 2023).

On the one hand impact on the Environment, the creation and elimination of nanoparticles may have detrimental effects on the environment (Martínez et al. 2021). Certain nanoparticles might be hazardous to living things and might find their way into the food chain (Maharramov et al. 2019). Nanoparticles are very small and can be challenging to describe and regulate, thus developing environmentally friendly production methodologies practices is crucial and maintaining homogeneity in dimensions, form, and surface characteristics is essential to attaining the intended functioning (Allan et al. 2021; Osman et al. 2024).

The field of "green nanotechnology" is concerned with creating sustainable processes for the synthesis of nanoparticles from natural sources such as microbes or plant extracts and some waste (kazemi et al. 2023; Osman et al. 2024). Compared to conventional methods, these approaches may be less dangerous and more sustainable.

Plant extracts from a variety of plant parts, such as fruit, bark, leaves, and seed peels (Dhaka et al. 2023), mix with mineral salts such as zinc, iron, and silver to form environmentally safe green nanomaterials with antibacterial, antifungal, and other pathogenic properties (Castillo-Henríquez et al. 2020; Jadoun et al. 2021).

Egypt and the rest of the world are home to a multitude of plant wastes whose extracts contain a multitude of active ingredients that can play a major role in advancing the synthesis of promising nanocomposites (Antonio-Pérez et al. 2023). The plant extract's active ingredients function to lower metal salts like silver and produce the useful and eco-friendly nanocomposite (Dhaka et al. 2023). Egypt is a prospective country for jojoba cultivation, as the seeds are harvested for their oils, which are utilized in a variety of applications, including cosmetics (Abobatta et al. 2015).The seed peels that shield the seed are seen as trash and an environmental burden (Chaouch and Benvenuti 2020).

The objective of this study was to ascertain the fundamental constituents present in the jojoba seed shell, synthesize a nanocomposite utilizing the extracted material, and evaluate its efficacy against a variety of microorganisms.

Material and Methods

Collection of plant residues and extraction

The Upper Egyptian regions of Farafra are where the Jojoba seed coat (JSC) were harvested. The (JSC) were cleaned of dust and other undesirable materials by soaking them in running water for ten minutes and then keeping them out of the sun and any bacterial or insect pollutants for Fourteen days**.**

Over the course of 120 minutes, the temperature was progressively raised from 60 to 90 degrees during the aqueous extraction procedure in an inverter condenser then After filtering, gather the extract on filter paper and store it at 4°C until needed and with minor adjustments with minor adjustments (Elkobrosy et al. 2023)**.**

GC mass (scan) analysis

According to Medeiros (2018) and El Nemr et al. (2014), every fraction was concentrated before to GC-MS/MS analysis. Hexane, a nonpolar solvent, was used as the injection solvent to aid in fraction separation. Using a 1 µL injection volume, the samples were separated using a DB5 ms ultra-inert capillary column (0.25 mm× 30 m× 0.25 µm). The analyte was identified using a Thermo TRACETM 1300 gas chromatography fitted with the following parameters: temperature 75°C, splitless time 2 minutes, purge flow 5 mL/min, carrier gas saver flow 20 mL/min for 5 minutes, injection pressure 70 kPa for 0.1 minute, transfer pressure 210 kPa, transfer rate 2.5 °C/s, transfer temperature 280°C, transfer time 3.0 minutes, cleaning rate 2.5 °C/s, cleaning temperature 300°C, and cleaning time 5.0 minutes are all achieved with a PTV mode splitless injector. Data collection, reprocessing, and report preparation were done using Thermo Scientific Xcalibur.

Silver Nanoparticle (Ag-NPs) preparation

Silver nanoparticle preparation, as described by Elshaer et al. (2024), with a few modifications: 5 ml of aqueous extract was thoroughly and continuously combined with 0.1 M of AgNO3 solution in a dark environment. The reaction mixture's color shift confirmed that nanoparticles were formed. After that, the mixture was centrifuged for 30 minutes at 13,000 rpm. To get rid of impurities that were soluble in water, the pallet was again suspended in deionized water. Once more, the suspension was centrifuged for 20 minutes at 13,000 rpm. After that, the mixture was filtered via filter paper (Whatman no. 1) to eliminate any leftover impurities while maintaining the pallets' distribution. The pallet was exposed to 70°C for one day of drying. Pure Ag NPs powder was created after calcination and used for more research.

Characterization of Ag-NPs

Ag-NPs wase examined using energy dispersive X-ray (EDX) spectroscopy, Zeta Potential, Fourier transform

infrared (FT-IR) spectroscopy, and transmission electron microscopy (TEM).

Transmission electron microscope (TEM) analysis

Using a transmission electron microscope (TEM), the average size of NPs was ascertained. An electron beam is passed through a microscopic specimen in TEM to view the interior of a sample for three-dimensional imaging. Alduraihem et al. (2023) remarked that this strategy is thought to be among the most successful for gathering data on nanoparticles.

Zeta Potential

To look at the degree of charge attraction or electrostatic repulsion between the biosynthesized NPs and their stability. Using a PSS-NICOMP 380-ZLS, US apparatus, the dynamic light scattering (DLS) method was used to measure the zeta potential (Mohamed et al. 2022).

FTIR analysis

Ag-NPs and extracts from (JSC) were all subjected to a liquid phase chemical structure analysis. Many bioreducing functional groups in a material can be investigated using Fourier-transformed infrared spectroscopy (FT-IR). The spectra were obtained using a Vertex 70 Bruker Transform Infrared spectrophotometer, which has a resolution of 1 cm-1 in the 4000 to 400 cm-1 range (Schmitt and Flemming 1998; Hasanien et al. 2023).

EDX analysis

One of the most useful, efficient, and suitable analytical techniques for determining multiple elements is element-specific fingerprinting (EDX), which was employed to obtain qualitative data on the elements present in nanoparticles. Moreover, images of the nanostructure and morphology of NPs were taken with an FEI Quanta FEG 450 scanning electron microscopy (SEM) (Mohamed et al. 2022)**.**

Antibacterial efficacy test of Ag-NPs

Three pathogenic Gram +ve bacterial strains and three pathogenic Gram –ve bacterial strains were selected for antimicrobial evaluation. The *Bacillus subtilis* ATTC15442, *Staphylococcus aureus* ATTC6538, *Listeria monocytogenes* ATTC7644 represent the Gram +ve bacteria, whereas the *Escherichia coli* ATTC10536, *Pseudomonas aeruginosa* ATTC9027, *Klebsiella pneumonia* ATTC13883 represents the gram –ve bacteria. The tested bacteria stock cultures were sub-cultured onto Muller-Hinton agar plates and incubated overnight at 37 °C. The next day, three to four discrete bacterial colonies with similar morphology were inoculated into 10 ml sterile Mueller-Hinton broth (MHB) and incubated overnight at 37 °C. The overnight bacterial suspensions were adjusted to 0.5 McFarland Standard with sterile MHB broth. To aid comparison, the adjustment of bacterial suspensions to the density of the 0.5 McFarland Standard was done against a white background with contrasting black lines (Hasanien et al. 2024).

The agar well diffusion method was used to screen the antibacterial activities of green synthesised silver nanoparticles, aqueous extract of Jojoba seed coat (JSC) as a negative control, and some commercial antibiotics as a positive control (Balouiri et al. 2016). Log phase cells of tested pathogenic bacteria, 0.2 ml of the prepared bacterial cell suspension (10^8 CFU/ml) were regularly spread on petri plates containing Mueller–Hinton agar medium. Then, wells were made using a sterile cork-borer (6 mm in diameter) into agar plates containing inoculums. Finally, 100 µl of each tested sample was added to respective wells. The plates were placed in the refrigerator for 30 min to let the tested samples diffuse well into the agar. Then, the plates were incubated at 37°C for 18 h. Antimicrobial activity was detected by measuring the zone of inhibition (including the diameter of the well) that appeared after the incubation period (Zaki et al. 2022).

Results and Discussion

GC mass (scan) analysis

The components in **Table (1)** and **Fig**. **(1)** provide an explanation of the GC MASS analysis findings for (JSC) extract and The superabundant compounds were Decamethylcyclopentasiloxane , Pyrocatechol, Naphthalene, 2,7-di-tert-butyl, Pyridin-2(1H)-one, 3-amino, 4-Methylcatechol, Dodecamethylcyclohexasiloxane, Pyrogallol 1,3-dimethyl ether, 2,2,4,4,6,6,8,8,10,10,12,12,14,14 Tetradecamethylcycloheptasiloxane, Hexadecamethyl-cyclooctasioxane and octadeamthyl-cyclononasiloxane. These substances, which include polyphenolic compounds and have several active groups; as a result, they can be used to reduce silver salts and create nanoparticles utilizing (JSC) extract (Alowaiesh et al. 2023; Dhaka et al. 2023; Abd-Elraheem et al. 2024).

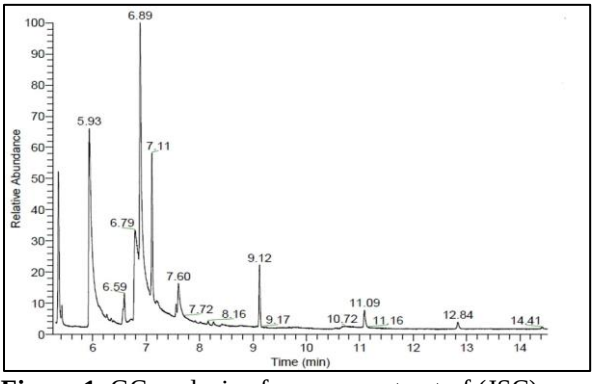

Figure 1. GC analysis of aqueous extract of (JSC).

Table 1. GC analysis of aqueous extract of (JSC).

R.T	M.F	M.W	Compound Name
5.35	$C_{10}H_{30}O_5Si_5$	370	Decamethylcyclopentasiloxane
5.93	$C_6H_6O_2$	110	Pyrocatechol
6.59	$C_{18}H_{24}$	240	Naphthalene, 2,7-di-tert-butyl
6.79	$C_5H_6N_2O$	110	Pyridin-2(1H)-one, 3-amino
6.89	$C_7H_8O_2$	124	4-Methylcatechol
7.11	$C_{12}H_{36}O_6Si_6$	444	Dodecamethylcyclohexasiloxane
7.61	$C_8H_{10}O_3$	154	Pyrogallol 1,3-dimethyl ether
9.12	$C_{14}H_{42}O_7Si_7$	518	2,2,4,4,6,6,8,8,10,10,12,12,14,14-Tetradecamethylcycloheptasiloxane
11.09	$C_{16}H_{48}O_8Si_8$	592	Hexadecamethyl-cyclooctasioxane
12.84	$C_{18}H_{54}O_9Si_9$	666	Octadeamthyl-cyclononasiloxane

R.T (Retention Time), **M.F** (Molecular Formula), **M.W** (Molecular weight)

Silver Nanoparticle (Ag-NPs) preparation

As the Fig. (2) shows and by seeing how the extract and silver nitrate salt mixed to create a solution whose color changed from white to dark brown after 25 minutes, it was possible to deduce the production of green silver particles (Ayad et al. 2019; Asif et al. 2022).

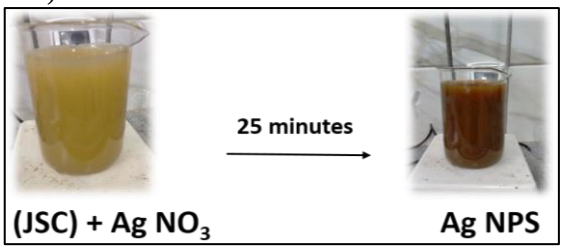

Figure 2. Synthesis of silver nanoparticles using (JSC) extract.

Transmission electron microscope (TEM analysis)

Using a transmission electron microscope, or TEM, the size and shape of the produced AgNPs were assessed. The findings of structural tests conducted using TEM revealed variations in the sizes of the nanoparticles that were generated. The maximum value for the nanoparticles formed was 20.43, while the smallest value was 7.53. These outcomes were Consistent to those of a preparatory experiment using a similar methodology conducted by Chand et al. (2020); Lite et al. (2022); Abdelaziz et al. (2023).

Fig. (3) displays the TEM picture and associated histogram. The sample Ag NPS's magnified TEM image revealed that the majority of the particles are spherical in form. This is comparable to what said in that spherical nanoparticles made with the green approach are produced (Rautela et al. 2019; Asif et al. 2022; Ali et al. 2023).

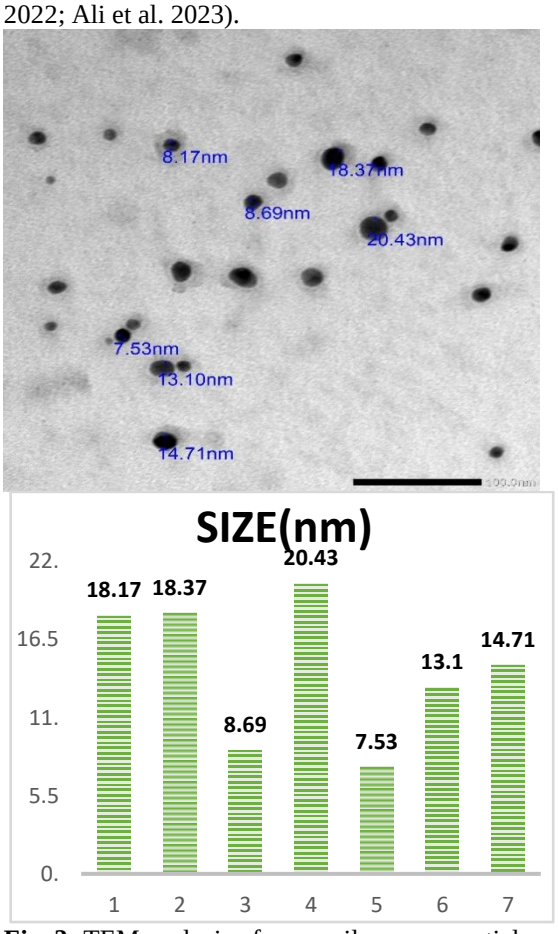

Fig. 3: TEM analysis of green silver nanoparticles using **(JSC)** extract

Zeta Potential

The primary metric used to assess the stability of the solution is the zeta potential. According to the result is in the **Fig. (4)** it the prepared zeta potential values (-23.0), both (Erenler et al. 2023; Sharifi-Rad et al. 2024) are in agreement as they fall within the typical stability range. As a result, the JSC extract's silver nanoparticles demonstrated stability, and the result's negative value suggests stability (Liaqat et al. 2022).

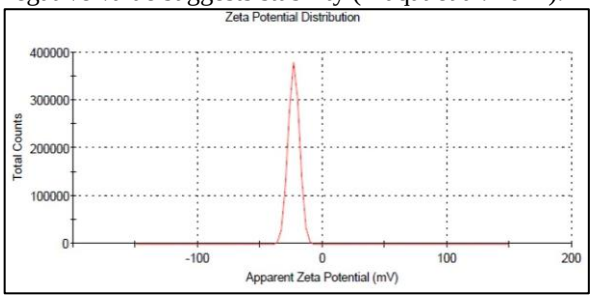

Figure 4. Zeta potential analysis of green silver nanoparticles using (JSC) extract.

EDX analysis

An estimate of the quantity and quality of elements that could be involved in the creation of silver nanoparticles is provided by an EDX examination.

According to the EDX results in **Fig. (5)**, percentages of C, O, S, Cl, K, and Cu are present in the liquid sample, and silver atoms make up 23.85% of its total components. It was seen that the atoms most likely came from the other elements, the SEM chamber, and the glass underneath (Ismail et al. 2018). The majority is made up of carbon and oxygen, which are derived from plant extracts that are used to make silver nanoparticles (Dada et al. 2017).

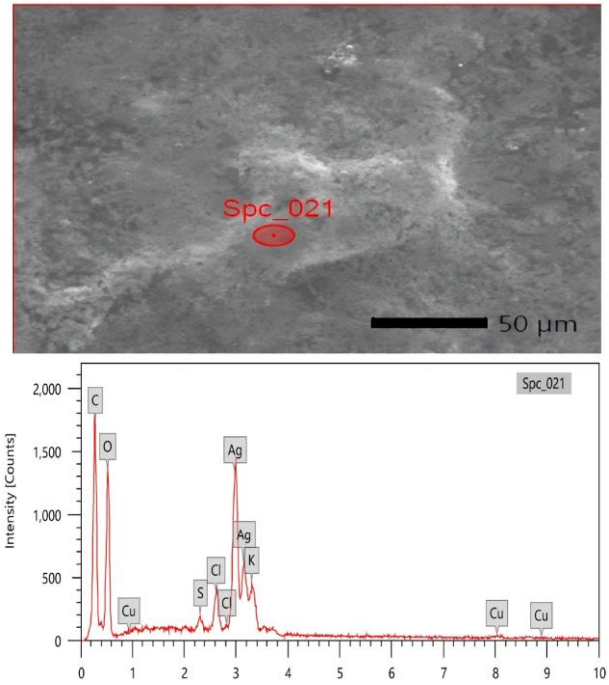

Figure 5. EDX analysis of green silver nanoparticles using (JSC) extract.

Antibacterial efficacy test

Human diseases' resistance to antibiotics and antimicrobials has become a significant problem that requires the development of new natural green alternatives (Sagar et al. 2019). Considerable progress has been made in the last several decades in the creation of medications based on nanotechnology to counteract bacteria' resistance to many drugs. Among these, silver nanoparticles (AgNPs) have a lot of potential for solving this problem because of their strong antibacterial capabilities and broad spectrum (Tang and Zheng 2018; Bruna et al. 2021). Therefor AgNPs were synthesized using green, effective and economic method for antibacterial applications.

The well diffusion method was used to examine the antibacterial activity of the produced AgNPs. After 24 hours, growth inhibition was noted on plates containing 100 µl of soluble AgNPs. Six bacteria were used to assess the antibacterial activity of the produced AgNPs: *E. coli, P. aeruginosa, K. pneumonia* as Gram negative and *S. aureus, B. subtilis, L. monocytogenes* as Gram positive bacteria. Two commercial antibiotics were used as positive control, while the extract of Jojoba seed coat extract used as negative control. Positive outcomes were indicated by the existence of an inhibitory zone or clear zone surrounding the wellbore in a petri dish. Table 1 displays the average values of the inhibition zone diameter.

Based on the findings, the extract from the jojoba seed coat showed no inhibition action against the tested bacteria (all isolates tested were resistant to the extract), whereas the extract-produced AgNPs showed different levels of inhibition against the tested bacteria.

E. coli was the most sensitive to AgNPs, followed by *P. aeruginosa*. As depicted in Table (2), B. subtilis showed the greatest resistance to AgNPs, followed by *L. monocytogenes*. Table (2) also shows that Gram-negative bacteria had a larger zone of inhibition than Gram-positive bacteria at the same AgNPs dose. The distinction between Gram-positive and Gram-negative bacteria could be attributed to differences in their cell wall composition. Gram-positive bacteria have a thick peptidoglycan coating made up of short, cross-linked linear polysaccharide chains. This causes the structure to become stiffer, making it difficult for silver nanoparticles to penetrate. Gram-negative bacteria have a cell wall made of a thinner peptidoglycan layer (Pazos-Ortiz et al. 2017).

Plant-based metal nanoparticle production has been thoroughly investigated and acknowledged as a non-toxic and effective technique in the biomedical sector in recent years (Sorbiun et al. 2018; Yadi et al. 2018; Bao et al. 2021). However, there are no publications on the utilization of the Jojoba seed coat seed extract in the synthesis of silver nanoparticles. Urnukhsaikhan et al. (2021) investigate the role of different parts of medical plant *Carduus crispus* on synthesizing silver

nanoparticles and characterize the produced nanoparticle with studying the antimicrobial activity. According to Premanathan et al. (2011) the antibacterial efficacy of silver nanoparticles against different bacteria depended on their form. When Khan et al. (2014) tested silver nanoparticles on various Gram-positive and Gram-negative bacterial strains, they found that the toxicity of the particles was as follows: *E. coli* > *P. aeruginosa* > *M. luteus* > *S. aureus*. The synthesized AgNPs are therefore more effective against Gram negative bacteria than Gram positive bacteria, as demonstrated by a number of experiments. Comparable to our findings, which show that *E. Coli* is more susceptible to produced AgNPs. Similar results were reported from the green manufacture of silver nanoparticles utilizing *Lysiloma acapulcensis* demonstrate strong antibacterial efficacy against *E. coli* and *P. aeruginosa* (Garibo et al. 2020).

Table (2): Antibacterial efficacy of green synthesized silver nanoparticles using aqueous extract of Jojoba seed coat.

Diameter of inhibition zone (mm) against tested pathogenic bacteria				
		Gram +ve bacteria		
		Bacillus subtilis ATTC15442	*Staphylococcus aureus* ATTC6538	*Listeria monocytogenes* ATTC7644
Ag-NPs	synthesized using aqueous extract of Jojoba seed coat (100 µl/well)	16	22	18
Negative control (Plant extract)	**Jojoba seed coat extract** (100 µl/well)	0.0	0.0	0.0
Positive control (Commercial antibiotics)	**Streptomycine** (10µg/disc)	0.0	18	0.0
	Amoxicilline (30µg/disc)	12	20	17
		Gram -ve bacteria		
		Escherichia coli ATTC10536	*Pseudomonas aeruginosa* ATTC9027	*Klebsiella pneumoniae* ATTC13883
Ag-NPs	synthesized using aqueous extract of Jojoba seed coat (100 µl/well)	25	23	20
Negative control (Plant extract)	**Jojoba seed coat extract** (100 µl/well)	0.0	0.0	0.0
Positive control (Commercial antibiotics)	**Streptomycine** (10µg/disc)	19	13	12
	Amoxicilline (30µg/disc)	22	16	19

Conclusion

In conclusion, silver nanoparticles were biosynthesized with simple, safe, and one-step process by using the Jojoba seed coat extract as a green reducing agent. The process does not require any chemical reagents or s surfactants, giving the bioprocess the advantage of being sustainable and ecofriendly.

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