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Antimicrobial Activity of Biosynthesized Silver Nanoparticles from *Cannabis Sativa* and Its Combination with Cefotaxime and Meropenem

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ABSTRACT

Multidrug resistance (MDR) is a major cause of human challenge that impairs the trust between doctors and their patients, concomitant with huge economic losses. The study determine antimicrobial activity of biosynthesized silver nanoparticles (AgNPs) from *Cannabis sativa* and its combination with cefotaxime and meropenem. The AgNPs were biosynthesized from *Cannabis sativa* extracts and characterized with Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy. The antimicrobial activity of AgNPs, cefotaxime and meropenem against the *Escherichia coli, Salmonella typhi* isolates from clinical sample were evaluated using the microbroth dilution method. The effect of combination of the AgNPs and cefotaxime or meropenem was evaluated using the checkerboard method. The size of Ag-NPs derived from *Cannabis sativa* particles was within the range of 179nm-269nm with an average of 224nm.The AgNPs biosynthesized from *Cannabis sativa* had activity against the isolates with minimum inhibitory concentrations (MICs) ranges from 62.5-31.25 mg/mL, cefotaxime MIC ranges from 16-8 (µg/mL) while meropenem 1-0.5(µg/mL). The combination of *Cannabis sativa* / cefotaxime, *Cannabis sativa* /meropenem had synergistic effect against *Salmonella typhi* and *Escherichia coli* with FICs ranges from 0.25-0.04 but had additive effect cefotaxime in combination with meropenem with FICs of 1.0-0.5. Finally, the combination of *Cannabis sativa*/cefotaxime had synergistic effect on *E. coli* and *Salmonella typhi* with FICs of 0.25. The biosynthesized AgNPs from *Cannabis sativa* had *E. coli* and *Salmonella typhi* activity with consistent effect in its combination with cefotaxime and meropenem against the *E. coli* and *Salmonella typhi* isolates.

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INTRODUCTION

Multidrug resistance (MDR) is a major cause of human challenge that impairs trust between doctors and their patients, concomitant with huge economic losses. In this world of microbe-man cohabitation, survival of the human race has been threatened and there will be no way to survive the emergence of MDR superbugs. Hence, from a public health perspective, antibiotics have been our best strategy. The impact of Antimicrobial resistance (AMR) is a huge concern, which results in the greatest loss to individual and social economy (1). By the year 2050, the death rate due to AMR is estimated to be 10 million lives per year, corresponding to one hundred trillion dollars of healthcare cost. The rapid development of MDR in microorganisms is increasing global health problems and presents a challenge for the treatment of infectious diseases that researchers claim could return to the pre-antibiotic era (2). Plants have played unique holistic roles for the provision of food, drugs, clothing, shelter, etc. Natural compounds have been extensively explored for new drug discoveries. Indeed, plants have been used as medicines for more than 5000 years, as a source of antibiotics, antineoplastic, analgesics, cardioprotective, among others. In the recent past, humans have been using natural compounds to ward off infections (3). About 70-90% of the population in developing countries rely on ancient medicines based on plant extracts (4). The most powerful and promising elements of plants are their secondary metabolites. Significantly, natural products and their derivatives contribute to more than half of the Food and Drug Administration (FDA) approved drugs (5). In the last two decades, most of the efforts have made attempts to discover novel therapeutics to combat MDR, especially with plants and deep-sea flora (5).

Cannabis sativa, a member of the Cannabaceae family (5), is known by a number of common names around the world. In South Africa, it is referred to as "dagga," while hemp and marijuana are well-known common names (The Plant List 2020). Although it originated in Western and Central Asia, *C. sativa* is a well-known plant that is grown all over the world (7, 9). This plant was primarily used for pharmacological and medicinal purposes (10). The antimicrobial activity of biosynthesized silver nanoparticles (Ag-NPs) from *Cannabis sativa* has been studied recently (4), but the effects of combining Ag-NPs from *C. sativa* have not been studied, which is why the current study is focused on this topic.

Globally, bacterial resistance to antibacterial therapy is a serious problem. Bacterial infections cause 17 million deaths annually, making infectious diseases the second most common cause of death. Therefore, the current study concentrated on the antimicrobial activity of biosynthesized AgNPs derived from *Cannabis sativa* on isolates of *Escherichia coli* and multidrug-resistant *Salmonella typhi* from clinical samples, both alone and in combination with meropenem or cefotaxime.

MATERIALS AND METHODS

Plant Collection

The Department of Plant Science at Nasarawa State University in Keffi received fresh *Cannabis sativa* leaves from the National Drug Law Enforcement Agency (NDLEA), Lafia command, for identification and authentication by a plant taxonomist.

Biosynthesis of Silver-Nanoparticles (AgNPs)

The biosynthesis of silver nanoparticles (AgNPs) from *Cannabis sativa* leaf extract was carried out with slight modifications to the method described by Asif *et al.* (39). Briefly, 20.0 g of powdered *C. sativa* leaves was suspended in 200 mL of sterile distilled water and filtered through Whatman filter paper to obtain the aqueous leaf extract. Subsequently, 100 mL of the filtrate was mixed with 900 mL of 2 mM silver nitrate ($AgNO_3$) solution. The reaction mixture was incubated at 60 °C for 1 hour and subsequently kept at room temperature in a dark cupboard for 24 hours to facilitate nanoparticle formation. The formation of AgNPs was preliminarily indicated by a colour change from dark brown to reddish-brown, suggesting the reduction of silver ions (Ag^+) to metallic silver nanoparticles. The synthesized nanoparticles were recovered by centrifugation at 3,500 rpm for 15 minutes, washed twice with sterile distilled water to remove residual plant metabolites and unreacted reagents, and dried in a water bath at 35 °C for subsequent analysis.

Characterization of Silver Nanoparticles

Fourier-transform infrared (FTIR) spectroscopy was used to characterize the biosynthesized silver nanoparticles (AgNPs) in order to determine the functional groups linked to nanoparticle formation and the possible phytochemical compounds involved in the reduction and stabilization of silver ions. To ensure accurate spectral measurements, the FTIR instrument was suitably calibrated before analysis, and the samples were prepared in accordance with the analytical requirements of the instrument. A scanning electron microscope (SEM; FEI Nova NanoSEM 450, USA) was used to analyze the surface morphology and structural characteristics of the produced AgNPs. The particle morphology, surface properties, and approximate particle size distribution were evaluated using the SEM micrographs. However, rather than using SEM alone, a suitable crystallographic technique, like X-ray diffraction (XRD), is needed to confirm the crystalline structure of the AgNPs.

Test Organisms.

The National Institute for Pharmaceutical Research and Development (NIPRD), located in Idu, Abuja, Nigeria,

provided the multidrug-resistant clinical isolates of *Salmonella typhi* and *Escherichia coli* used in this investigation.

Evaluation of Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of biosynthesized silver nanoparticles (AgNPs) derived from *Cannabis sativa*, cefotaxime, and meropenem. In short, a sterile 96-well microtitre plate was filled with 100 µL of Mueller–Hinton broth (MHB; Oxoid Ltd., UK) that contained serially diluted concentrations of AgNPs (50.0-1.5625 mg/mL) and the antibiotics cefotaxime and meropenem (512–1.5625 µg/mL). 5 µL of the standardized bacterial suspension (roughly 10^5) CFU) was added to each well, and the mixture was incubated for 24 hours at 37°C. The lowest concentration of each test agent that totally prevented visible bacterial growth after incubation, as shown by the lack of turbidity, was determined to be the minimum inhibitory concentration (MIC).

Evaluation of the Effect of Combinations of Silver-Nanoparticles and Some Antibiotics

The checkerboard microdilution assay was used to assess the combined antibacterial activity of biosynthesized silver nanoparticles (AgNPs) derived from *Cannabis sativa* and the antibiotics cefotaxime and meropenem. In short, 100 µL of double-strength Mueller-Hinton broth (MHB) in sterile 96-well microtitre plates were used to prepare serial dilutions of AgNPs and each antibiotic in combination at concentrations ranging from -2\times\) MIC to −1/128\times\) MIC.

After inoculating each well with 5µL of the standardized bacterial suspension (roughly 10^5) CFU/mL, the wells were incubated for 24 hours at 37°C. The lowest concentrations of AgNPs and the corresponding antibiotic that stopped visible bacterial growth, as shown by the lack of turbidity, were identified as the combined minimum inhibitory concentrations (MICs) after incubation.

The following formula was used to determine the fractional inhibitory concentration (FIC) indices for the AgNP–antibiotic combinations:

$$FICI = \frac{MIC_A \text{ in combination}}{MIC_A \text{ alone}} + \frac{MIC_B \text{ in combination}}{MIC_B \text{ alone}}$$

The synergistic, additive, indifference and antagonistic effect of combination were interpreted as follows; Synergistic effect: FIC ≤ 0.5, Additive effect: FIC≥ 0.5 and ≤ 1.0, indifference effect FIC≥ 1.0 and <4.0, Antagonistic effect: ≥ 4.

RESULTS

Fourier Transform Infrared Spectroscopy (FTIR) Characteristics of Crude Leaf extracts of *Cannabis sativa*

The FTIR spectrum of the crude *Cannabis sativa* extract, illustrating the characteristic absorption peaks, corresponding functional groups, and their intensities, is presented in Figure 4.1.

Scanning Electron Microscopy Characteristics of *C. sativa* Leaf Extracts

Scanning electron microscopy (SEM) was used to analyze the morphological features and particle-size distribution of silver nanoparticles (AgNPs) biosynthesized using the crude aqueous extract of *Cannabis sativa*, as shown in Plates 4.1 and 4.2. Plates 4.1 and 4.2 show that the synthesized AgNPs had a mean particle size of 224 nm and ranged in size from 179 to 269 nm. Figure 4.1 displays the peak, functional group, and intensity of the *C. sativa* crude extract.

Minimum Inhibitory Concentration (MICs) of Silver Nanoparticles and Some Selected Antibiotics

Table 1 shows the minimum inhibitory concentrations (MICs) of silver nanoparticles (AgNPs) biosynthesized from crude *Cannabis sativa* extract against isolates of bacteria resistant to multiple drugs. Against both test organisms, the AgNPs demonstrated antibacterial activity. The MICs for AgNPs, cefotaxime, and meropenem for *Escherichia coli* were 31.25 mg/mL, 8 µg/mL, and 0.5 µg/mL, respectively. According to Table 1, the corresponding MIC values for *Salmonella typhi* were 31.25 mg/mL for AgNPs, 16 µg/mL for cefotaxime, and 1 µg/mL for meropenem.

Effect of Combination of Silver-Nanoparticles in Combination with some Antimicrobials

Table 4.2 illustrates how Ag-NPs made from *C. sativa* work against *Salmonella typhi* and multidrug-resistant *Escherichia coli* when combined with cefotaxime and meropenem. Most notably, the combination of meropenem and Ag-NPs derived from *C. sativa* showed synergistic effects on *Salmonella typhi*, with FICs ranging from 0.14 to 0.5, but indifference and synergistic effects on *Escherichia coli*, with FICs ranging from 0.06 to 1.0. Lastly, as Table 2 illustrates, the combination of Ag-NPs derived from *C. sativa* and cefotaxime had a synergistic effect on isolates of *Salmonella typhi.*

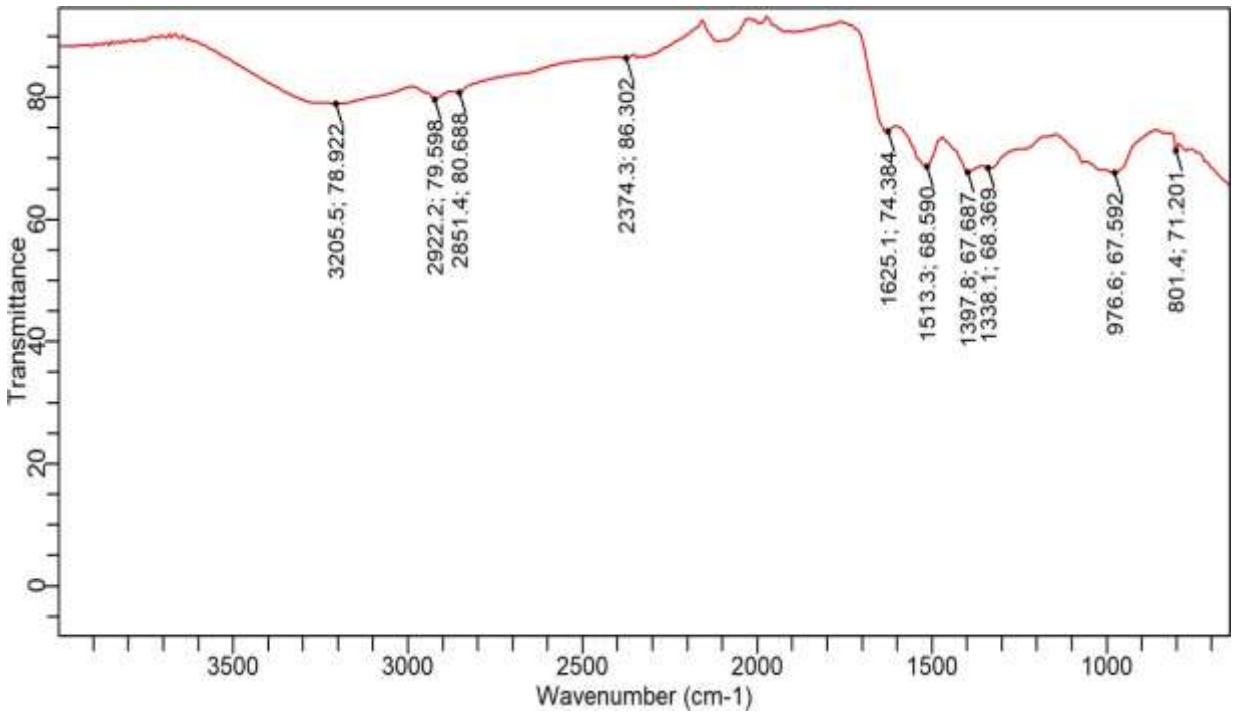

Figure 4.1: FTIR Spectra of the Silver Nanoparticles synthesized from Crude Aqueous Extracts of *Cannabis sativa*

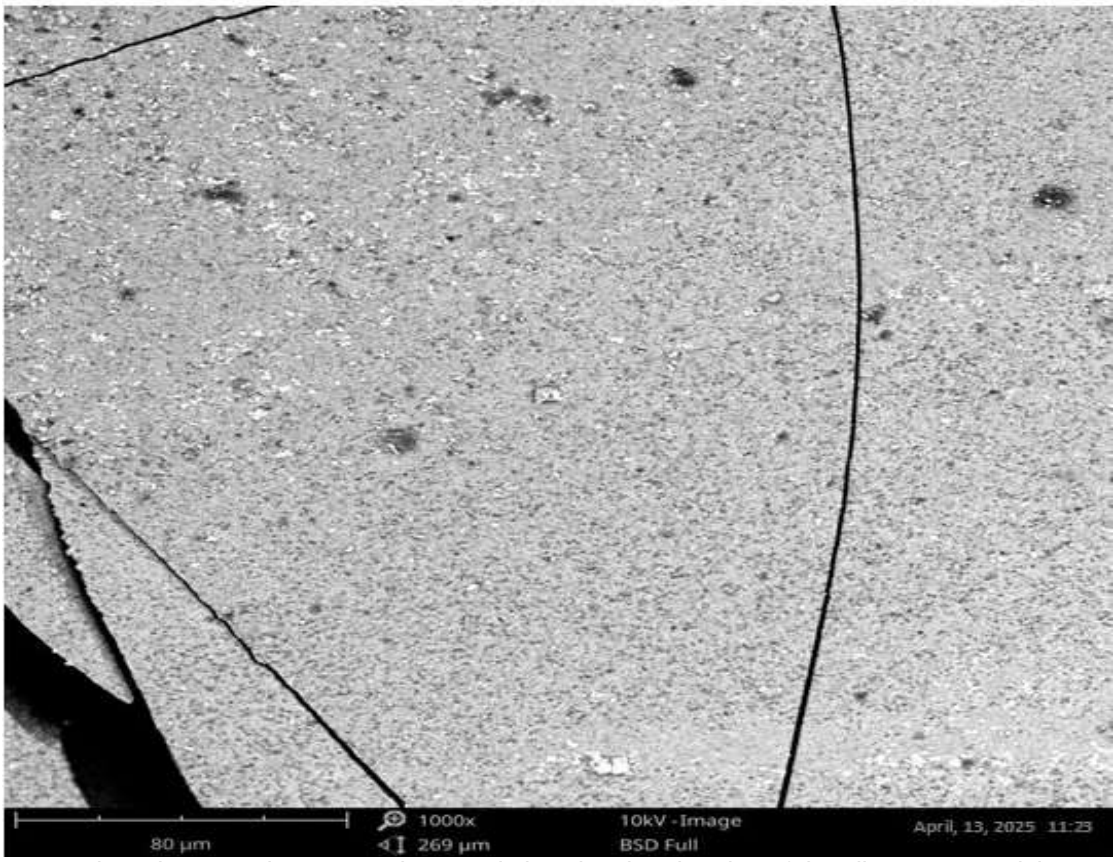

Plate 4.1: Scanning Electron Microscopy characteristics showing the size of the silver nanoparticles synthesized from the leaf extracts of *C. sativa* in magnification 1000x

1: Minimum Inhibitory Concentration of Silver nanoparticles synthesize from crude leaf extract of *Cannabis sativa* against test organisms

Test Organisms	MICs		
	***C. sativa* (mg/mL)**	**Cefotaxime (µg/mL)**	**Meropenem (µg/mL)**
Escherichia coli	31.25	8	0.5
Salmonella typhi	62.5	16	1

Table 2: Fractional Inhibitory Concentration of Silver nanoparticles synthesize from crude leaf extract of *Cannabis sativa* against test organisms

Test Organisms	FICs		
	AgNPs + Cefotaxime	**AgNPs + Meropenem**	**Cefotaxime +Meropenem**
Escherichia coli	0.25	0.04	1.0
Salmonella typhi	0.25	0.13	0.5

DISCUSSION

Because of their possible antimicrobial uses, silver nanoparticles (AgNPs) made from *Cannabis sativa* extract have garnered a lot of interest. Particle size, morphology, and surface-area-to-volume ratio all have an impact on their physicochemical characteristics, which may have an impact on how they interact with biological molecules and microbial cells (19). The unique physicochemical properties of nanomaterials enable their use in a variety of fields, especially biomedical and pharmaceutical research (11). It's crucial to remember, though, that nanoparticles are typically described as having sizes between 1 and 100 nm. Based on the reported SEM measurements, the AgNPs described in this study fall outside of this typical nanoscale size range, with sizes ranging from 179 to 269 nm and a mean diameter of 224 nm.

Although the biological effects of silver-based materials vary depending on concentration, particle size, surface chemistry, and the release of silver ions, they have been thoroughly studied for their antimicrobial qualities. Interactions between silver ions and cellular macromolecules, such as proteins and nucleic acids, can compromise the integrity of membranes, interfere with enzymatic processes, hinder cellular metabolism, and ultimately prevent bacterial growth (11). However, the possible cytotoxicity of silver-based materials emphasizes how crucial it is to assess their safety prior to therapeutic applications.

The functional groups that may be involved in silver-ion reduction and nanoparticle stabilization were revealed by the Fourier-transform infrared spectroscopy (FTIR) analysis of the AgNPs derived from *C. sativa*. Differences in absorption-peak positions and intensities were found when the FTIR spectra of the synthesized AgNPs and the crude aqueous plant extract were compared. According to Awadelkareem *et al.* findings, these spectral changes imply that specific phytochemical constituents might have taken part in the biosynthesis process (20). The produced AgNPs' FTIR spectrum showed absorption bands between 801.4 and 3205.5 cm^{-1}, suggesting the existence of various functional groups connected to biomolecules derived from plants.

The N-H, O-H, and C-O vibrational modes were mainly linked to the detected absorption bands. These functional groups could come from the aqueous extract's proteins, phenolic compounds, alcohols, aldehydes, ketones, and carboxylic acids. Through surface interactions, these phytochemicals may help reduce silver ions (Ag^{+}) to metallic silver (Ag^{+}) and help stabilize the resulting particles. Yamal *et al.* (21) and Selvi *et al.* (22) have reported similar findings. Nevertheless, FTIR analysis by itself is unable to definitively identify the specific biomolecules in charge of reduction and stabilization or determine the exact mechanism of nanoparticle formation.

The broth microdilution method was used to assess the antibacterial activity of the biosynthesized AgNPs against *Salmonella typhi* and multidrug-resistant (MDR) *Escherichia coli*. The AgNPs' minimum inhibitory concentration (MIC) against *E. coli* in this investigation was 31.25 mg/mL, while their reported MIC against *S. typhi* was 62.5 mg/mL. Under the experimental conditions, the MIC values of the conventional antibiotics cefotaxime and meropenem were lower than those of the AgNPs. These results show that, in comparison to the tested antibiotics, higher concentrations of the synthesized AgNPs were needed to prevent visible bacterial growth. However, because the nanoparticles and antibiotics were tested at different concentration ranges and expressed in different units, direct comparisons should be interpreted with caution.

AgNPs made with *C. sativa* extract showed MIC values of 6.25-12.5 mg/L against clinical bacterial isolates, such as *E. coli* and *S. typhi,* according to Moodley *et al.* (2018). Differences in nanoparticle properties, biosynthesis conditions, bacterial susceptibility profiles, or experimental techniques could account for the relatively higher MIC values found in this study. Although resistance to traditional antibiotics does not always

translate into resistance to silver nanoparticles due to possible differences in their antimicrobial mechanisms, the isolates' multidrug resistance may also be significant. According to reports from other studies by Ali *et al.* (24) and Michailidu *et al.* (25), MIC values were found in a range similar to the current study. Additionally, research by Reda *et al.* (26) and Vanlalveni *et al.* (28) has shown that plant-mediated AgNPs have antibacterial activity against Gram-negative bacteria. Gram-negative bacteria's outer membrane, which is made up of proteins, phospholipids, and lipopolysaccharides, is a crucial permeability barrier that affects the bacteria's susceptibility to antimicrobial agents. As a result, the interaction between the physicochemical characteristics of the AgNPs and the structural and physiological traits of the test organisms may determine the observed antibacterial effects.

A promising strategy for looking into ways to increase antimicrobial efficacy against resistant bacterial pathogens is combination therapy, which combines conventional antibiotics with nanoparticles. Possible synergistic interactions could improve the inhibition of bacterial growth and, in certain situations, lower the concentrations of individual agents needed to have an inhibitory effect. While the interaction against *E. coli* varied between indifferent and synergistic effects, the combination of *C. sativa*-derived AgNPs and meropenem showed synergistic activity against the tested *S. typhi* isolate. AgNPs and cefotaxime also demonstrated synergistic activity against the tested isolate of *S. typhi.* These results imply that the bacterial species or isolate and the particular antimicrobial combination assessed determine the antimicrobial interactions between AgNPs and antibiotics.

Complementary mechanisms of antibacterial action may be linked to the observed interactions. While cefotaxime and meropenem inhibit bacterial cell-wall synthesis by targeting penicillin-binding proteins, silver nanoparticles and released silver ions may damage bacterial membrane integrity, interfere with cellular proteins, and impair vital metabolic processes. In susceptible combinations, the combined effects of these agents may increase the inhibition of bacterial growth. However, additional experimental validation is needed for the suggested mechanisms, and rather than relying solely on MIC values, synergistic activity should be determined using the computed fractional inhibitory concentration index (FICI).

The results are generally in line with the study conducted by Suman *et al.* (27), which documented variations in AgNPs' antibacterial efficacy against *E. coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Such variations highlight the importance of bacterial species, strain characteristics, and nanoparticle properties in determining antimicrobial susceptibility. The antibacterial, anti-yeast, and α-amylase-inhibitory properties of AgNPs made with plant extracts, such as *C. sativa*, have also been reported in other studies (21).

The current study demonstrates the in vitro antibacterial activity of AgNPs derived from *C. sativa* when combined with cefotaxime and meropenem against the particular isolates of MDR *E. coli* and *S. typhi* that were investigated. However, since prior research may have examined comparable nanoparticle-antibiotic combinations, the novelty of this discovery should be verified by a thorough literature search. Overall, the results encourage more research into plant-mediated AgNPs as possible supplements to traditional antibiotics for the treatment of infections brought on by bacteria that are resistant to multiple drugs. Before considering any clinical application, more research should evaluate reproducibility, bactericidal activity, cytotoxicity, nanoparticle stability, and efficacy in suitable biological models.

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Conflicts of Interest

The author declared that the research was conducted in the absence of any commercial or financial relationship that could appeared as potential influence to the work reported in this paper.

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