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Research Article

Characterization and Pharmacological Efficacy of Silver Nanoparticles Biosynthesized Using the Bark Extract of *Garcinia Kola*

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The delinquent peril of bacterial infections affecting human kind is becoming unbearable. This study was embarked on to investigate the antimicrobial activity of biosynthesized silver nanoparticles (AgNPs) using *Garcinia kola* bark extract against some bacteria strains. Fresh barks of *Garcinia kola* were obtained from the "Gbeleju" farm land in Irele town in Ondo state region of Nigeria. Exactly 0.4 g of previously pulverized bark of *Garcinia kola* was immersed into 20 mL of distilled water and heated at 60–70°C for 10 minutes yielding the extract. The biosynthesized nanoparticle was characterized with UV spectroscope, Fourier infrared spectroscope (FTIR), transmission electron microscope (TEM), and energy dispersive X-Ray analyzer (EDX). Then, 0.2 g of the silver nanoparticles was dissolved in 2 ml of water to yield 100 mg/ml of the stock solution which was further diluted for the antibacterial analysis via the disc diffusion method. The result obtained from the analytical characterization of the biosynthesized silver nanoparticles revealed a spherical particle shape, particle size in the range of 12.23 to 27.90 nm with an average size of 20.07 nm via TEM analysis. The FTIR analysis confirmed the presence of -OH functional group for the stabilization of the silver nanoparticles formed due to the broad peak at wavelength 3324.52 and 3344.21 cm^{-1}. The EDX analysis revealed carbon, nitrogen, oxygen, aluminum, potassium, copper, and silver as the elements present in the nanoparticles. Results obtained from the antibacterial screening of the biosynthesized AgNPs showed inhibitory potential that are capable of obstructing the growth of the test bacteria. This investigation ascertained the biosynthesized AgNPs as a remedy for curing bacterial infections and also a promising source for novel antibacterial agent.

1. Introduction

Diverse physiochemical approaches have been adopted for AgNP synthesis among which we have the chemical reduction approach [1]. Despite the effectiveness and high yield associated with these approaches, limitations such as toxicity of chemicals used, relatively high cost, and energy requirement are of great concern. Considering the disadvantages linked with the use of these methods of synthesis, synthesis of AgNPs using microorganisms, enzymes, metabolites from arthropods [2–4], and plant extracts has been adopted due to their cost-effectiveness, eco-friendly, and energy efficient substitute [5, 6].

The global encumbrance of infectious illnesses initiated by bacterial agents has remained a severe menace especially in undeveloped nations [7]. Antibacterial agents are substances capable of either terminating bacterial life cycle or inhibiting their growth by obstructing the essential mechanism of the bacterial cell. These agents perform their function via surface contact with the bacterial cell wall [8]. Antibacterial agents could either be obtained from natural products or synthetic materials. The use of several synthetic antibacterial drugs had played crucial roles in reduction of the mortality rates emanating from bacterial infections over the years. However, the potency of many synthetic antibacterial drugs is limited and less effective due to the harms associated with its usage and bacterial resistance against it [9, 10]. The scientific search and isolation of bioactive compounds with effectiveness of overcoming the pathogenic resistant to synthetic antibiotics will be needful in eradicating bacterial infections [7]. Antibacterial agents made from plant have vast therapeutic efficiency due to their effectiveness in curing infections from bacterial attack with few or no side effects compared with synthetic antibacterial agents [11–13]. Prevention and cure of many ailments have been achieved via the use of plants or components derived from medicinal plants, thereby rendering natural products as reliable sources of novel antibacterial agents [14]. The readily availability, easy method of extraction, antibacterial effectiveness, nonallergenic reaction, shelf life, durability, and low cost are relevant importance of plant-based antibacterial drugs [15]. Biosynthesis involving the use of plant extracts has been established as an efficient technique for the synthesis of silver nanoparticles [16]. Silver nanoparticles has been reported to be the most commercialized nanoparticles used worldwide due to its usefulness in dressing of wound, coating of working surfaces, and their biological activities [16].The following plant extracts, namely, *Silybum marianum* [17], Amaranthus tricolor [18], *Saraca asoca* [19], *M. balbisiana*, *A. indica* and *O. tenuiflorum* [20], *Berberis vulgaris* [21], *Glycosmis mauritiana* [22], *Gleichenia Pectinata* [23], and *Salvia spinosa* [24] have been used for the biosynthesis of silver nanoparticles. Similarly, plants that produce stimulants such as *Theobroma cacao* (Cocoa), *Cola nitida* (Kolanut), and Wonderful kola (*Buchholzia coriacea*) have been used to synthesize silver nanoparticles [25–27].

Garcinia kola, also referred to as bitter kola, is a multipurpose indigenous plant in the western part of Africa whose medicinal value has not been totally utilized. The nutritional values and medicinal features of *Garcinia kola* has led to its pharmacological application in the treatment of some ailments in the Nigeria [28]. The leaves, stem, barks, and roots of *Garcinia kola* have been stated to have many ethnobotanical and pharmacological relevance. However, the bark of *Garcinia kola* remains the most crucial part used by herbal practitioners for the preparation of herbs and decoction [29]. Unfortunately, the potentials of *Garcinia kola* bark extract in the synthesis of silver nanoparticles against bacterial infection have not been explored. Thus, the aim of this study was to investigate the antibacterial activity of biosynthesized AgNPs using *Garcinia kola* bark extract.

2. Samples Collection and Preparation

Fresh and healthy barks of *Garcinia kola* were obtained from "Gbeleju" farm land in Irele town in Ondo state region of Nigeria. The barks of *Garcinia kola* were chopped and air-dried. The dried barks were pulverized and stored in a sample bottle prior to extraction. *Garcinia kola* bark was transferred into a clean beaker containing 20 ml of distilled water. This solution was boiled in a water bath for 10 min and cooled. The cooled solution was filtered using Whatman filter paper. The crude extract was then stored in the refrigerator till further analysis.

2.1. Biosynthesis of Silver Nanoparticles. Aqueous solution of silver nitrate (1 mM) was prepared and mixed with the bark extract of *Garcinia kola*. The solution obtained was kept on a magnetic shaker with constant stirring at room temperature for 5 hours to aid biosynthesis.

2.2. Characterization of the Silver Nanoparticles. UV spectrophotometer (UV-245 Shimadzu) was used for the assessment of the biosynthesized silver nanoparticles. FTIR analysis was carried out to determine the functional groups present in the plant extract that is responsible for silver ion reduction. The neat solution of the biosynthesized AgNPs was placed in the cuvette of spectrophotometer Nicolet iS50 (Thermo Fisher Scientific, Waltham, MA, USA) and scanned over the range of 4000–500 cm^{-1}.

Further characterization of the biosynthesized nanoparticles was conducted using transmission electron microscope (JEOL TEM instrument operated at 120 kV) for shape and particle size determination. Information on the elemental composition of the biosynthesized AgNPs was determined using energy dispersive X-ray analyzer (Oxford-Horiba Inca X-Max 50 instrument).

2.3. Preparation of Stock Solution of Synthesized AgNPs. The stock solution of the synthesized AgNPs was made by dissolving 200 mg of the biosynthesized AgNPs from *Garcinia kola* bark extract in 2 ml of water to yield 100 mg/ml of the stock solution. The stock solution was further diluted to yield concentrations of 75 and 50 mg/ml.

2.4. Antimicrobial Screening of Synthesized AgNPs. Clinical isolates of bacteria used are *Clostridium sporogenes, Bacillus cereus, Enterococcus faecalis*, and *Escherichia coli*. The bacterial strains were inoculated on agar slants. Cell suspension of each bacterium was made by conveying the colonies from the agar plates to the sterilized bottle filled with physiological saline. The turbidity was regulated to 0.5 McFarland's. Similarly, plates of Mueller Hinton Agar (MHA) were prepared, left to set, and incubated overnight at a temperature of 37°C. Disc diffusion test was used for the antibacterial investigation of the biosynthesized AgNPs against test bacteria. The Whatman filter paper discs used were sterilized at a temperature of 160°C in an oven for 2 hours. Each disc was impregnated with 20 μl of biosynthesized AgNPs solution of 50, 75, and l00 mg/ml concentrations and was properly labeled. The discs were left to dry in an incubator for 2 hours and were used immediately. The leftover was stored in a refrigerator. Disc containing 10 mg/disc of chloramphenicol was used as control. The discs containing various concentration of biosynthesized AgNPs and the control were placed on the MHA plates and were incubated for 24 hours at 37°C. The diameter of the zone of inhibition around each disc was measured with a transparent ruler. This experiment was carried out on the control and extract at given concentrations and replicated twice.

2.5. Statistical Analysis. The antibacterial investigation of the synthesized AgNPs was carried out in triplicate, and the result obtained from zones of inhibition was expressed in mean with standard deviation and statistical analysis via Excel$^{(R)}$ 2013 data analysis software.

3. Results and Discussion

3.1. UV Analysis. The peak at wavelength 445 nm on the UV spectrum of the biosynthesized AgNPs shown in Figure 1 corresponds to the surface plasmon resonance of silver which confirmed the synthesis of AgNPs from *Garcinia kola* bark. As stated by Mie theory, that spherical nanoparticles show only a single SPR band [30], and then it can be justified that the biosynthesized AgNPs are consistently spherical. The wavelength of silver in this study is similar to the wavelength obtained from the study of Krishna et al. [31].

3.2. Fourier Transform Infrared Spectroscopic Analysis. Responsible metabolites for the reduction of Ag ions were examined from the FTIR spectra of the aqueous plant extract and biosynthesized silver nanoparticles shown in Figures 2(a) and 2(b). The broad absorption bands at 3324.52 and 3344.21 cm^{-1} wavelength correspond to the free -OH group of phenols or alcohols [32]. The bands at wavelength 2928.45 and 2928.44 cm^{-1} suggest the presence of alkanes in the biosynthesized AgNPs [32]. The absorption peaks at 1630.32 and 1631.43 cm^{-1} are indication of C=C of aromatic compounds and -NH out amide of plane [33, 34]. The shifting of peaks and conspicuous variation that occurred in the absorption bands on the FTIR spectrum of the plant extract and biosynthesized AgNPs confirmed that some compounds are present in plant extract from which AgNPs are biosynthesized which performed a vital role in reducing and stabilizing AgNPs [35]. Our findings are similar to the study of Folorunso et al. [36].

3.3. TEM Analysis. The TEM micrograph of biosynthesized AgNPs is shown in Figure 3. Most of the AgNPs were spherical in shape, and there were evenly spread in the solution. The size of the biosynthesized AgNPs ranges from 12.23 nm to 27.90 with an average size of 20.07 nm in diameter according to TEM micrograph. As stated by Pirtarighat et al. [24], plant extract possess metabolites that play crucial role in the synthesis and stabilizing of the biosynthesized AgNPs. This confirms that the metabolites present in *G. kola* extract are responsible for the stability of the biosynthesized AgNPs. This finding agrees with earlier studies [5, 37–39].

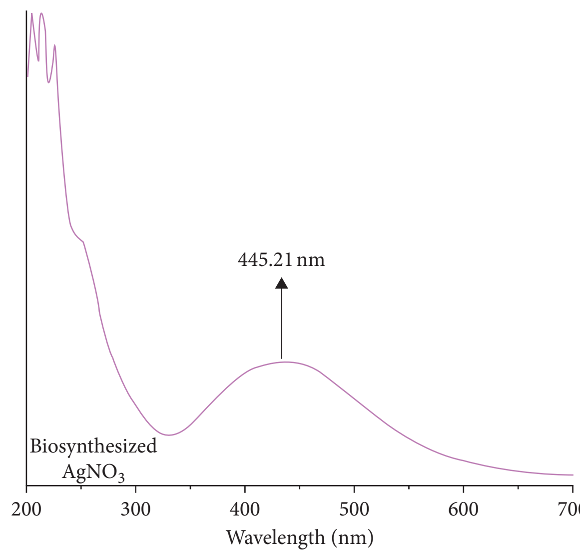


Figure 1: UV absorption spectrum of the biosynthesized AgNPs.

3.4. Energy Dispersive X-Ray (EDX) Analysis of the Biosynthesized AgNPs. The result obtained from the elemental composition of the biosynthesized AgNPs are shown in the EDX spectrum in Figure 4 which revealed that the synthesized AgNPs demonstrated signal corresponding to silver at 3 keV. Carbon, nitrogen, oxygen, aluminum, potassium, copper, and silver are the other elements present in the nanoparticle with a weight percentage of 1.90, 11.52, 28.45, 2.51, 12.56, 1.15, and 41.91(wt. %), respectively, where nitrogen, oxygen, and silver are detected as the major elements. The presence of nitrogen, oxygen, and silver as the major element might have resulted from the nanosalt ($AgNO_3$) used in the preparation and synthesis of the silver nanoparticle.

Silver nanocrystals has been reported to display an absorption peak around 3 keV because of its surface plasmon resonance [40]. This finding is in accordance with the findings of Abiola et al. [23].

3.5. Antibacterial Activity of Biosynthesized AgNPs. The zones of inhibition in diameter (mm) displayed by the biosynthesized AgNPs at various concentrations are listed in Figure 5. The biosynthesized AgNPs at a concentration of 50 mg/ml showed zones of inhibition of 6, 4, 2, and 10 mm against *Clostridium sporogenes, Bacillus cereus, Enterococcus faecalis*, and *Escherichia coli*, respectively. At 75 mg/ml, the zones of inhibition of the AgNPs against *Clostridium sporogenes, Bacillus cereus, Enterococcus faecalis*, and *Escherichia coli* were 11, 8, 8, and 12 mm, respectively, while at 100 mg/ml, zones of inhibition of 12, 10, 8, and 16 mm were demonstrated against *Clostridium sporogenes, Bacillus cereus, Enterococcus faecalis*, and *Escherichia coli*, respectively. Similarly chloramphenicol showed highest inhibition zones of 22, 18, 16, and 26 mm against *Clostridium sporogenes, Bacillus cereus, Enterococcus faecalis*, and *Escherichia coli*. The antibacterial screening of the biosynthesized AgNPs showed inhibitory

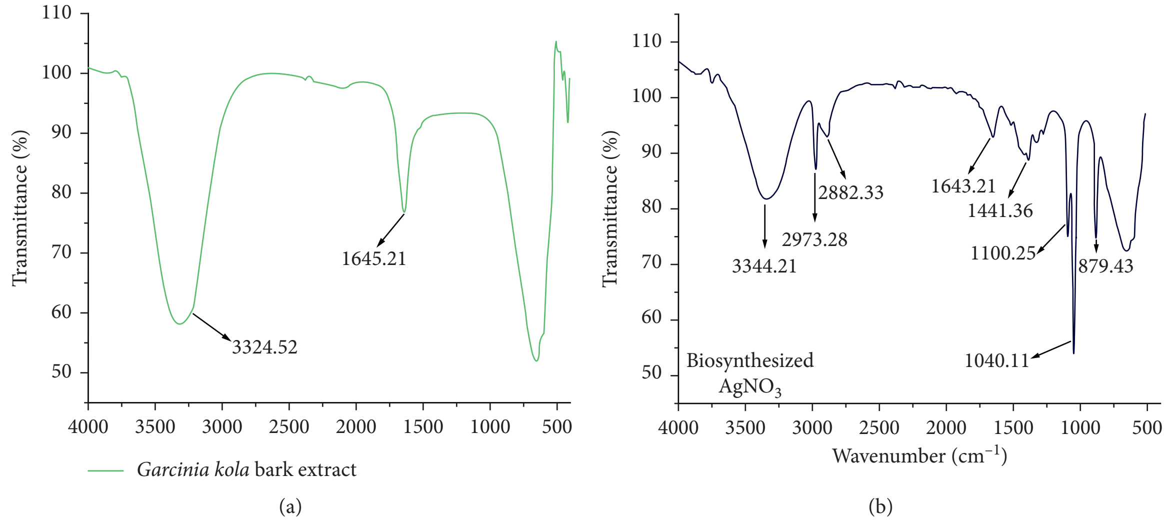


FIGURE 2: (a) FTIR spectrum of the aqueous plant extract. (b) FTIR spectrum of biosynthesized silver nanoparticles.

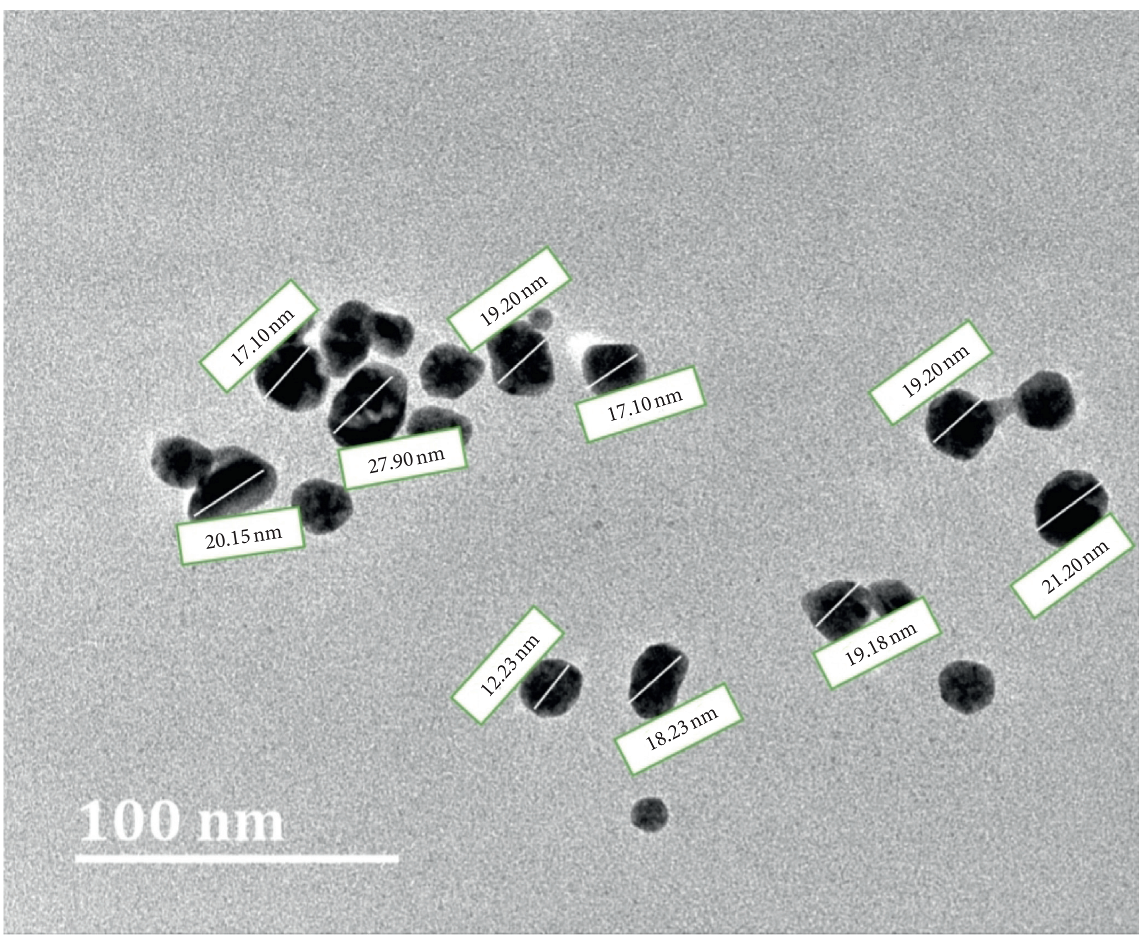
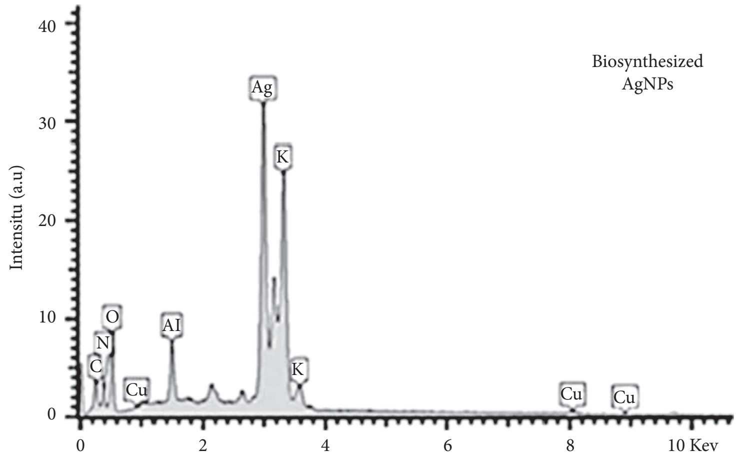


FIGURE 3: TEM micrograph of biosynthesized AgNPs.

potential on all test bacteria at various concentrations with least inhibitory zone of 2 mm against *Enterococcus faecalis* at 50 mg/ml and highest inhibitory effects against *Escherichia coli* at 100 mg/ml. The inhibitory potential of AgNPs increases with increase in concentration of AgNPs. This suggested that the biosynthesized AgNPs have good antibacterial activity against test bacteria strain. This finding agrees with previous studies [41–44].



Biosynthesized AgNPs	Wt.%	Wt.% sigma
C	1.90	065
N	11.25	1.09
O	28.15	0.47
AI	2.51	0.08
K	12.56	0.34
Cu	1.15	0.11
Ag	41.91	0.78
Total	100	

Figure 4: EDX spectrum of biosynthesized AgNPs.

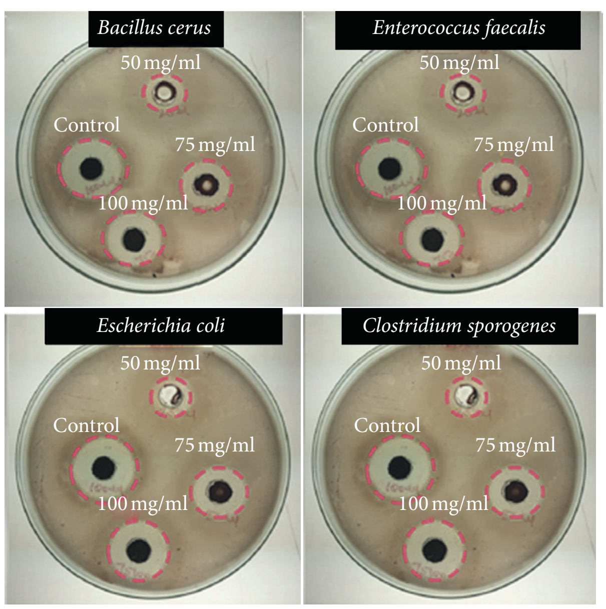


Figure 5: Antibacterial screening of biosynthesized AgNPs against test bacterial strains.

3.6. Possible Mechanisms of Antibacterial Activity of AgNPs. The antibacterial mechanism demonstrated by the synthesized AgNPs is subjected to the degree of susceptibility of microbes. When the synthesized AgNPs comes in contact with the bacteria, it binds with the bacterial surface through electrostatic interaction. The distinct smaller size and proton motive force of the synthesized AgNPs enable its penetration into the bacterial cell through the membrane proteins especially the sulfur groups present in proteins to form thiols due to its greater affinity towards sulfur groups and also on the phosphates forming complexes, thereby leading to their DNA damage [45]. The antibacterial efficacy of AgNPs was presumed to be linked to their sizes; the smaller the size, the larger the surface area-to-volume ratio [46]. This characteristic enables the interaction with bacterial cells. Findings had also shown that the antibacterial activity of AgNPs depends on shape [47]. It has also been documented that the interaction of metal nanoparticles with cysteine residues results in ROS generation by inhibiting electrons at terminal oxidase, thereby prompting bacterial cell death [48]. AgNPs attack bacterial cells through the release of silver ions in the cells which prompts antibacterial effects such as denaturation of cell membrane and interference with DNA replication and respiratory chain finally leading to death [49].

4. Conclusion

In summary, AgNPs were successfully synthesized through eco-friendly method using *Garcinia kola* bark extract. The characterization of the synthesized AgNPs confirmed *Garcinia kola* bark as a good source for the synthesis of silver nanoparticles. The antibacterial investigation of synthesized silver nanoparticle revealed its promising antibacterial potential against some human pathogens. AgNPs from *Garcinia kola* bark is hence recommended for usage in the field of nanobiotechnology and nanomedicine as product of value.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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