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Original Research Article

Green-synthesized silver nanoparticles by using fresh *Justicia Secunda*, *Telfairia Occidentalis,* and *Jatropha Tanjorensis* aqueous leaf extracts against clinical and environmental bacterial isolates

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ABSTRACT

Synthesis of silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are picking up significantly worldwide as a result of their importance in biomedical applications. This study aimed to investigate the antibacterial activities of green synthesized silver nanoparticles by using three Nigerian fresh leaf extracts (*Justicia secunda*, *Telfairia occidentalis,* and *Jatropha tanjorensis*) on clinical and environmental bacterial isolates. The green synthesized AgNPs were characterized by using UV-Vis spectrophotometry, Fourier transform infrared (FT-IR), and Scanning electron microscopy (SEM), and screened for their antibacterial activity by using Agar diffusion method. Our result showed that the green synthesized AgNPs from the three Nigerian vegetables exhibited the moderate antibacterial potentials against selected clinical and environmental bacterial isolates. In conclusion, the green synthesized AgNPs by using fresh *Justicia secunda*, *Telfairia occidentalis,* and *Jatropha tanjorensis* leaf extracts have antibacterial properties with therapeutic potential in treatment, management, and/or prevention of bacterial infections, and may be promising source for the development of chemotherapeutic (antibiotic) agents in the future.

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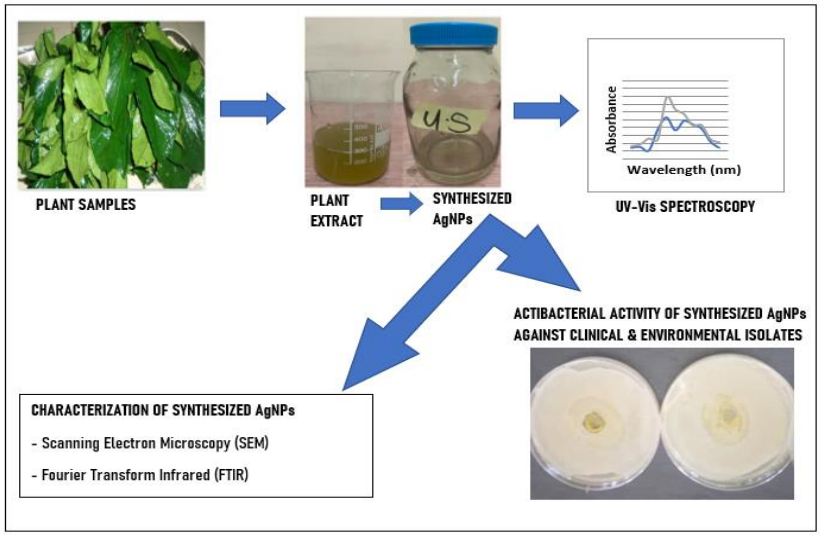
Graphical Abstract



Introduction

The prolonged use, misuse, and overuse of antibiotics in humans and animals have become a major global threat to the public health. Currently, several hundred thousand deaths yearly can be attributed to the infections with antibiotic-resistant bacteria [1] and the uncontrolled use of antibiotics is considered the major driver of the development of antibiotic resistance in humans and animals. Hence, the need for new antibacterial agents that are able to kill or inhibit the growth of bacteria. Recently, scientists around the world are focusing on nano-bioscience due to its potential in several applications, one of which is the ability to combat bacterial resistance [2]. The nanotechnology application in the development of the effective antibacterial agents is a growing research area and a new promising alternative.

The antibacterial action of nanoparticles (NPs) has been associated with variety of mechanisms compared with antibiotics. Moreso, the surface region of NPs is a vital part in their action; however, it fluctuates based on the NP type. The antibacterial action of silver is as of now deeply grounded [3], yet investigation into nano-size silver (<100 nm) has expanded because of its multidisciplinary application and the remarkable protection against a wide range of bacteria, including Gram-negative and Gram-positive forms [4]. The main physicochemical factors influencing the antibacterial capability of silver nanoparticles (AgNPs) includes size, shape, surface charge, concentration, and colloidal state. Method of activity might be related to the different mechanisms including concerning grip to bacterial cells, penetration inside the cells, ROS (reactive oxygen species), and generation of free radicals, or regulation of microbial sign transduction pathways [5]. AgNPs are now utilized in textile and cottage industries and in wastewater treatment. However, they are utilized most regularly in biomedical applications [6, 7], for example, bio-detecting, imaging and medication conveyance, as antimicrobial [8], anticancer, and larvicidal agents [9]. More recently, interest was centered around the antiviral action of AgNPs considering the COVID-19 pandemic [10].

Since the beginning of time, the medicinal plants have been utilized to treat illnesses and relieve symptoms. It has been essential to health care systems where a sizable portion of the global population relies on the use of herbs as medication [11]. Medicinal plants are essential to both human and animal health care systems [12]. Several plants have been explored for their antimicrobial activities but, the use of green synthesis method [13, 14] has been reported to be more effective against the microbial activities [15]. Here in, three Nigerian vegetables (*Justicia secunda*, *Telfairia occidentalis,* and *Jatropha tajorensis*) were used as reducing agents in the AgNPs synthesis.

Justicia secunda belonging to the *Acanthaceae* family, is commonly called ‘‘blood root” or ‘‘ewe eje” in the Yoruba language of South-western, Nigeria [16]. The red aqueous extracts of the leaves have been reportedly used to treat a number of ailments including anemia [17], wounds, menstrual and abdominal aches [11], abortions [18], dilation, and curettage in cases of miscarriage [19]. These biological activities have been attributed to its phytochemical makeup including polyphenols such as flavonoids, tannins, leuco-anthocyanins, and anthocyanins [20]. *Telfairia occidentalis*, a fluted pumpkin and a member of *Cucurbitaceae* family is indigenous to West Africa and is primarily farmed in Sierra Leone, Ghana, and Nigeria [21]. It is a tropical, dioecious vine and herbaceous nutritious vegetable creeping over the ground with a lobed leaves and twisted tendrils. The present phytoconstituents includes alkaloids, saponins, vitamins, minerals, and essential and non-essential amino acids [22]. The plant extracts have been documented to have antioxidant, anti-diabetic, hematological, anticancer, anti-inflammatory, analgesic, male fertility, hepatoprotective, antimicrobial, and antimalarial activities [23, 24]. *Jatropha tanjorensis*, on the other hand, is a prevalent weed of field crops in the rainforest regions of West Africa, particularly Nigeria belonging to *Euphorbiaceae* family. It is commonly known as “hospital too far” and “ewe Iyana ipaja” in Yoruba tribe of South-western Nigeria [25]. It has been reported to possess the antioxidant and hemagglutination in addition to its hypoglycemic and antiparasitic activities [26], hypolipidemic [27], antimicrobial [28], anti-plasmodial [29], anti-anaemic [30], antioxidant [31], renal, and hepatic protective activities [32]. The plant's juice and the crushed leaves are applied to wounds, ulcers, scabies, eczema, and ringworm [33]. Hence, this present study aimed to investigate the antibacterial activity of green synthesized silver nanoparticles using aqueous extracts of *Justicia secunda, Telfairia occidentals*, and *Jatropha tanjorensis* on the selected clinical and environmental pathogens.

Experimental

Plant collection

A fresh matured *Justicia secunda, Telfairia occidentalis,* and *Jatropha tanjorensis* leaves were harvested in January 2022 from Erunwen Area in Ikorodu, Lagos State, South-western Nigeria. It was identified and authenticated with a voucher number LSH001047, LSH001048, and LSH001049, respectively. Silver nitrate ($AgNO_3$) and other chemicals were of analytical-grades.

Preparation of extracts

The leaves of *Justicia secunda, Telfairia occidentalis*, and *Jatropha tanjorensis* were individually cleaned under running water before being rinsed with de-ionized water. To obtain the fresh aqueous extracts, the cleaned fresh leaves were individually ground (10% w/v aqueous solution) and filtered with

Whatman filter 1 paper. The filtrates were then concentrated by using the rotary evaporator and kept at 2-4 °C in the refrigerator for further use.

Synthesis of sliver nanoparticles

The synthesis of silver nanoparticles was carried out (1 mM $AgNO_3$ in 90 mL of de-ionized water) at room temperature in an Erlenmeyer flask for each plant extracts. In summary, 90 milliliters of silver nitrate solution were added to 10 milliliters of each extract and the mixture was agitated at room temperature. The reduction of silver nitrate into AgNPs was demonstrated by the solution's change in color. Then aliquots were obtained and absorbance (between 200 and 700 nm) measured by using a UV visible spectrophotometer after one hour and two weeks. The solution was centrifuged at 5000 rpm for 15 minutes after the reaction was finished, and the supernatant was stored in the refrigerator for future study.

Characterization of sliver nanoparticles

FT-IR (Fourier Transform Infra-Red spectroscopy) and scanning electron microscopy (SEM) were used to evaluate the synthesized AgNPs after the UV visible spectroscopy. The synthesized AgNPs size, shape, and capping agents were examined by using SEM, and their surface coatings were examined by using FT-IR.

Phytochemical screening

The methods provided by [34] were used to identify the phytochemical components of the synthesized AgNPs by using the aqueous leaf extracts of *Justicia secunda, Telfairia occidentals*, and *Jatropha tanjorensis* together with their corresponding quantities.

Bacterial culture collection

Organisms of clinical importance, namely *P. aeruginosa, K. pneumoniae, E. coli, S. aureus, S. Typhi*, and *Proteus* spp. were obtained from the culture repository of the Microbiology Department, Lagos State University (LASU). Bacterial cultures of environmental importance, namely *E. amnigenus, B. lacterosporus, A. mallei*, and *A. hydrophila* were also collected from the same center. All the organisms were obtained in the pure form and sub-cultured on nutrient agar slants kept at room temperature until they were ready for use.

Antibacterial activity by using agar diffusion assay

This was done by using the method previously described by [35]. Sterile inoculating loop was used to pick two-loopful of pure colonies on the cultured plates and emulsified in 10 mL of normal saline. The cultures were swabbed on the surface of sterile Mueller Hinton agar plates by using a sterile cotton swabs. The sterile swab sticks were used to spread by streaking the organism across the agar surface and allowed to dry for 5 minutes. Sterile cork borers and inoculating needles were used to form wells of 9 mm at the center of the inoculated agar plates. By using a micropipette, 100 µL of the extracts were added to the wells in the plates and allowed to settle for about an hour for thorough diffusion. The plates were then incubated in an upright position at 37 ˚C for 24 hours. The diameter of the resulting inhibition zones were measured in millimeter (mm), and then they were recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by the broth dilution method described by [36]. The extracts were

diluted to the different concentrations ranging from 50 to 3.125 mg/mL in sterile distilled water. The different concentrations were added to 2 mL of sterile nutrient broth in test tubes. Then, 1 mL of the standardized test organism was added to the content of the test tubes and the tubes were incubated at 37 °C for 24 hours. The MIC was taken as the lowest concentration of extracts that did not allow any visible growth for each of the test bacteria.

Results and Discussion

Green synthesis is an easy, popular, and valuable biological way of synthesizing nanoparticles. The use of plant to synthesis nanoparticles have been documented to be non-toxic, safe, eco-friendly, and cost-effective. Silver and its compounds are well-known for their wide range of application in biomedical sciences and is considered as one of the most universal antibacterial substances against a wide range of microorganisms from different sources.

Table 1 represents the qualitative phytochemical analysis of synthesized AgNPs by using aqueous leaf extracts of *Justicia secunda*, *Telfairia occidentalis,* and *Jatropha tanjorensis*. Phenols, tannins, alkaloids, and saponins were presented in the three extracts. Flavonoids, reducing sugar and cardiac glycosides were common to all the extracts except *J. tanjorensis* extract, while phlobatannin was absent in the three extracts. Steroid and terpenoid were also absent in *T. occidentalis* and *J. tanjorensis*, but they were present in *J. secunda*

Table 1. Preliminary qualitative phytochemical screening of the synthesized AgNPs by using *Justicia secunda*, *Telfairia occidentalis*, and *Jatropha tanjorensis* aqueous leaf extracts

Phytochemicals	Js-ALE	To-ALE	Jt-ALE
Phenol	+	+	+
Flavonoid	+	+	-
Tannin	+	+	+
Alkaloid	+	+	+
Saponin	+	+	+
Reducing sugar	+	+	-
Steroid	+	-	-
Terpenoid	+	-	-
Cardiac glycoside	+	+	-
Phlobatannin	-	-	-

Key: **+** denotes present, and **–** denotes absent. Js= *Justicia secunda*, To= *Telfairia occidentalis*, Jt= *Jatropha tanjorensis*, ALE= Aqueous leaf extract

The UV-Visible spectrophotometry

The UV-Visible spectrophotometer was used to determine the absorbance for wavelengths ranging between 200 to 700 nm. The addition of silver nitrate solutions to the different aqueous leaf extracts gave a visible color change from red to dark brown for *Justicia secunda* and yellow to almost colorless for *Telfairia occidentalis* and *Jatropha tanjorensis* (Figure 1). The observed color change confirmed the AgNPs synthesis which is in agreement with the findings of [37] who also reported a color change that is expected in the nanoparticles synthesis. The UV-visible spectra of the synthesized AgNPs, as depicted in Figure 2**a-c**, revealed the absorbance peak of 419 nm, 408 nm, and 403 nm for *Justicia secunda, Telfairia occidentalis*, and *Jatropha tanjorensis*, respectively, which is within the absorbance peak range (400-450 nm) of AgNPs as reported in the findings of [38].

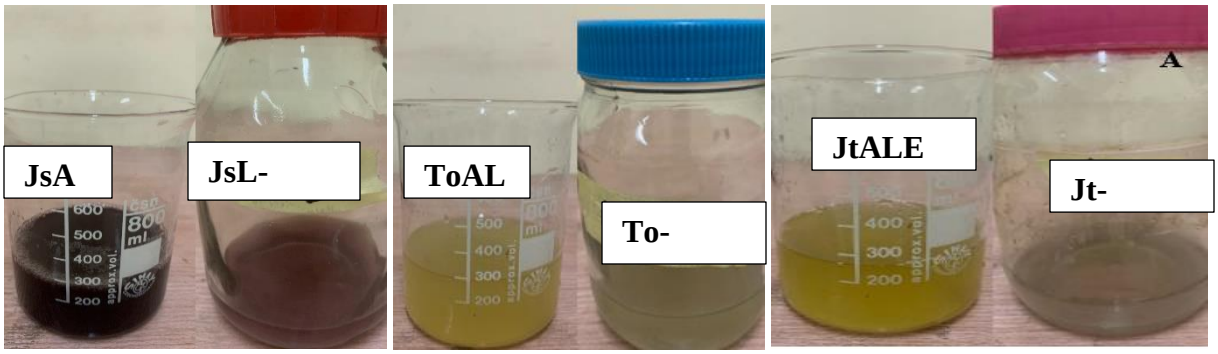
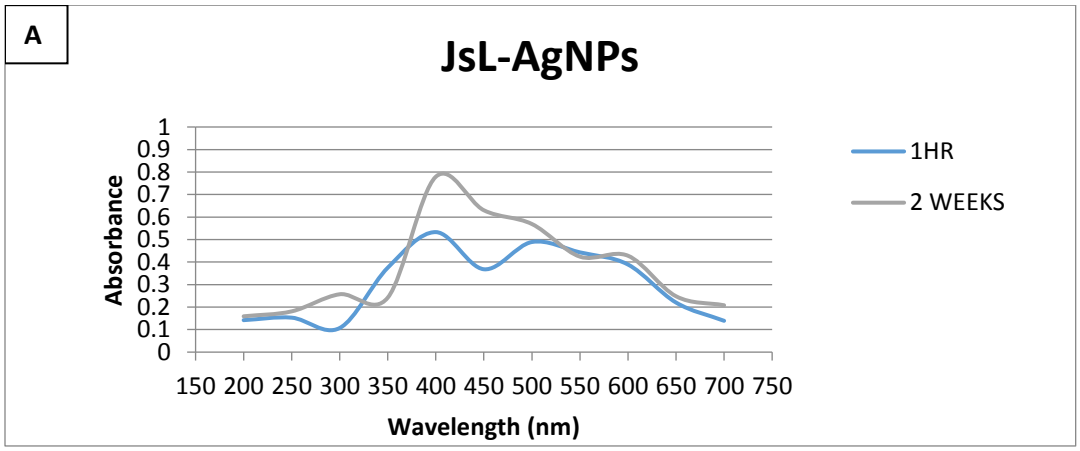
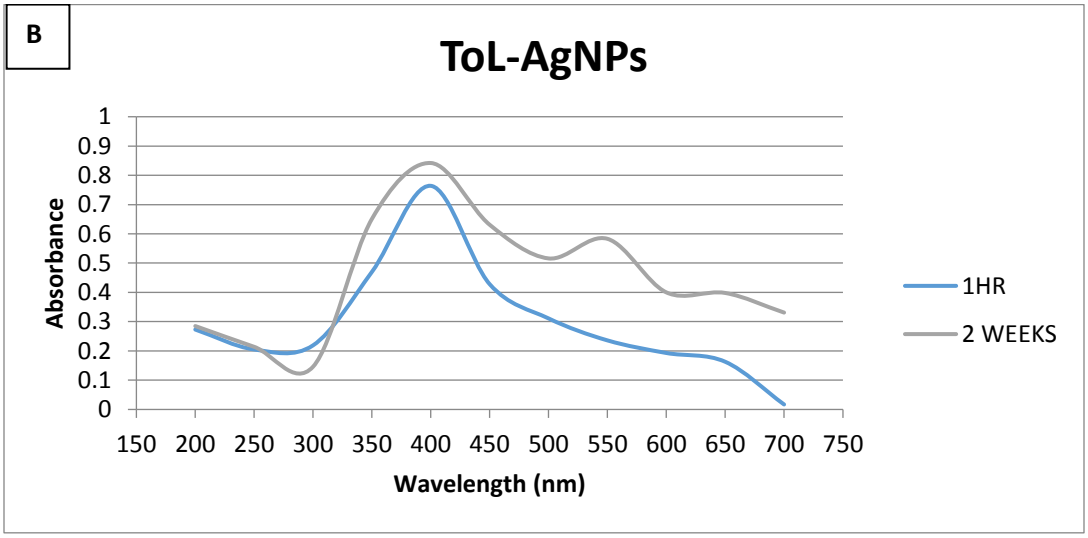


Figure 1. Visual analysis of color change from when the plant extract was added to silver nitrate solution. Js= *Justicia secunda*, To= *Telfairia occidentalis*, Jt= *Jatropha tanjorensis*, ALE= Aqueous leaf extract, and AgNPs= silver nanoparticles





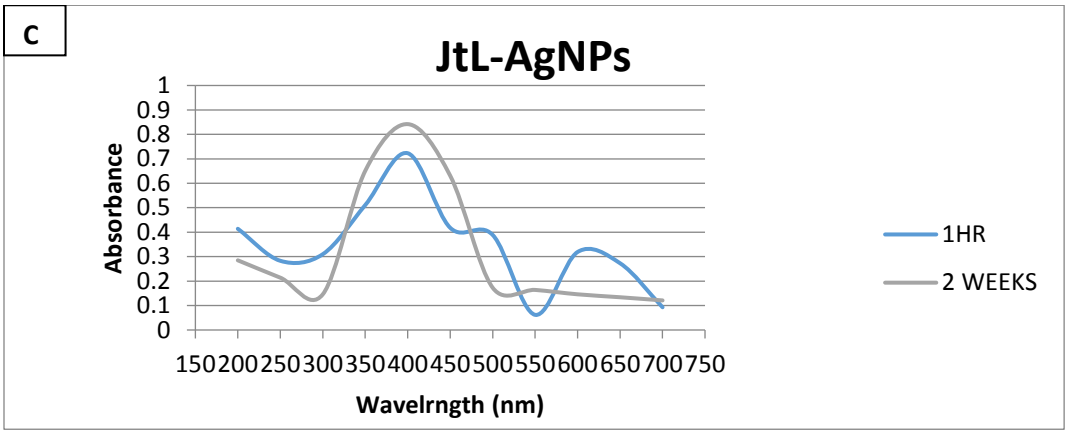


Figure 2. The UV-visible readings (200-700 nm) of the synthesized silver nanoparticles by using (a) *Justicia secunda,* (b) *Telfairia occidentalis, and* (c) *Jatropha tanjorensis*

This is a confirmation of the rapid bio-reduction of silver nitrate into silver nanoparticles by using the extracts as a reducing agent [39].

Scanning electron microscope (SEM) analysis

The morphology of the nanoparticles was depicted by using the scanning electron microscopy (SEM) analysis (Figure 3). In the present study, the SEM results revealed the AgNPs to be pseudo-spherical in shape with sizes of 50.47 nm, 43.66 nm, and 42.66 nm for *J. secunda*, *T. occidentalis*, and *J. tanjorensis*, respectively. These sizes and shape may be as a result of the inter-link between the compounds presented in the extracts and nanoparticles. The silver nanoparticles exhibited a surface plasmon resonance band due to the free electron excitation, which is similar to results obtained in a report by [40].

Fourier Transform Infra-red Spectroscopy (FT-IR)

Figure 4 below displays the presence of possible functional groups involved in the reduction of Ag^{+} to Ag^{0} which may be attributed to the embedded phytochemical constituents in the synthesized AgNPs by using *J. secunda*, *T. occidentalis*, and *J. tanjorensis*. The absorbance peak observed at 3265.1, 3268.9, and 3268.9 cm^{-1} for Js-AgNPs, To-AgNPs, and Jt-AgNPs, respectively by using FT-IR techniques predicted alkynes (C-H stretch), phenol (O-H stretch), carboxylic acids, and alcohols (O-H stretch), while 1636.3 cm^{-1} predicted alkenes (C=C stretch) and amines (N-H bend). It may be inferred that these organic compounds are responsible for capping and the efficient stabilization of the synthesized nanoparticles coating the extracts [41].

Antibacterial Activity and Minimum Inhibitory Concentration (MIC)

Herein, the synthesized AgNPs by using the three plant leaf extracts were tested against two (2) Gram-positive, eight (8) Gram-negative clinical, and the environmental bacterial isolates by using the agar well-diffusion method. Figure 5**a-c** demonstrates the antibacterial activity of the synthesized AgNPs after 24 hours of incubation. The obtained results revealed that *Escherichia coli* and *Acinetobacter mallei* were resistant to the synthesized AgNPs by using the leaf extracts of *Justicia secunda*, *Telfairia occidentalis*, and *Jatropha tanjorensis,* meanwhile the clear inhibition zones were observed for other bacterial isolates.

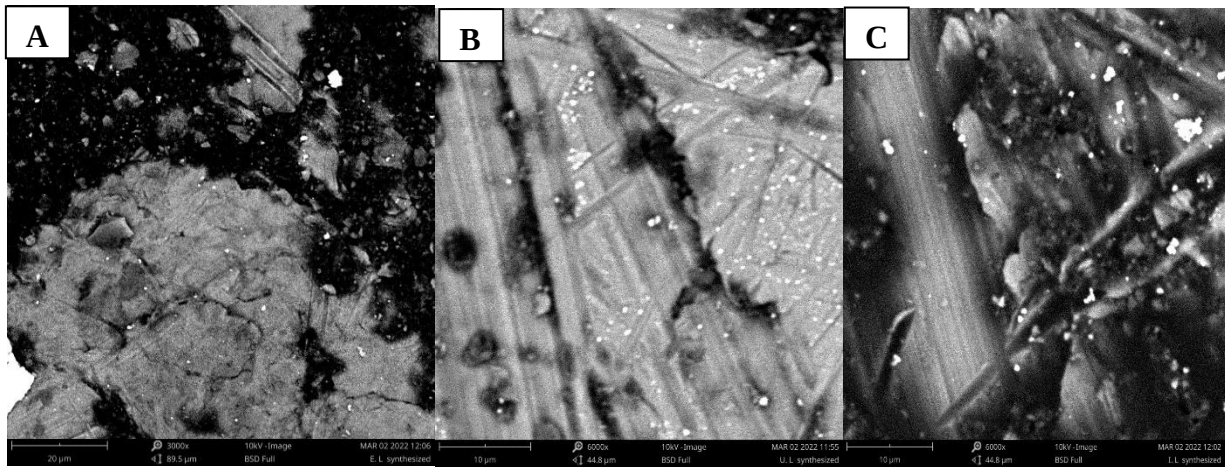


Figure 3. Scanning Electron Microscopy (SEM) image of synthesized silver nanoparticles by using (a) *Justicia secunda*, (b) *Telfairia occidentalis, and* (c) *Jatropha tanjorensis*

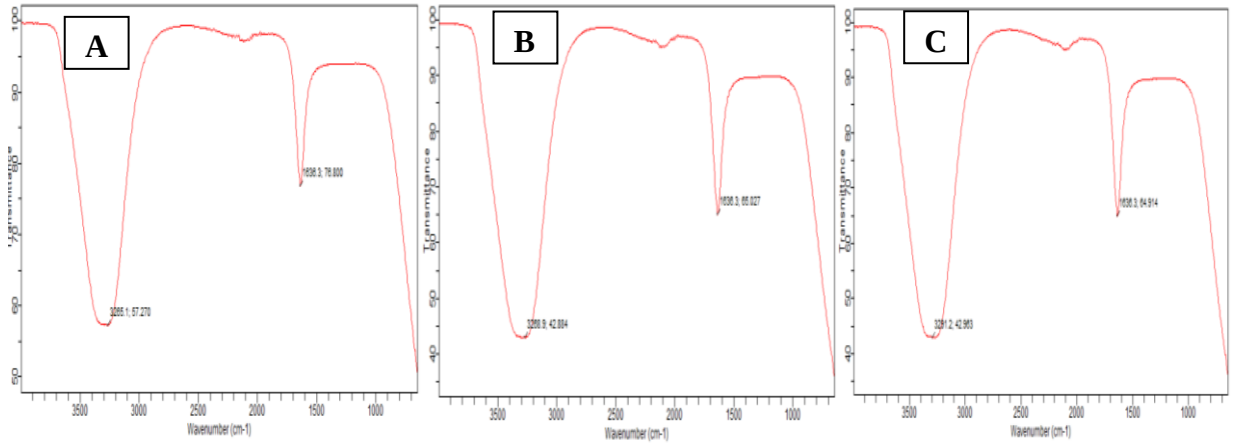


Figure 4. Fourier Transform Infra-Red spectroscopy (FT-IR) analysis of both extract only and synthesized silver nanoparticles by using (a) *Justicia secunda,* (b) *Telfairia occidentalis, and* (c) *Jatropha tanjorensis*

The increased antibacterial activity of the synthesized AgNPs may be attributed to their large surface area which provides more surface contact with microorganisms [42]. Another important reason for the enhanced antibacterial activity of AgNPs, as documented in a recent study, may be as a result of a synergistic effect between nanoparticles and natural compounds reported that the synergy between phenazine-1-carboxamide and AgNPs increased the antibacterial effect by 32-fold against MRSA strains, causing morphological alterations to the cell wall of bacteria [43, 44]. The mechanism of action of the antibacterial activity of AgNPs may result from the attack of respiratory chain and cell division which ultimately leads to the cell death. The silver nanoparticles have also been reported to release silver ions inside the bacterial cells, further enhancing their bactericidal activity [45]. However, the resistance observed in *E. coli* and *A. mallei* has not been fully explored but, [46] suggested that Gram-negative bacteria may develop the resistance to silver nanoparticles after the repeated exposures.

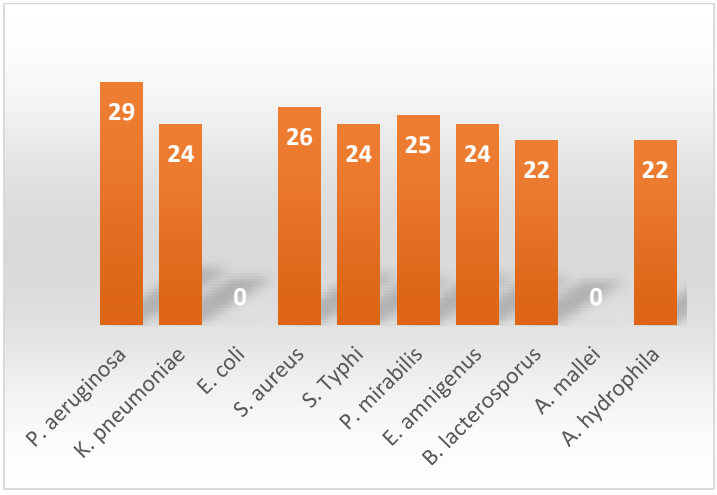


Figure 5A. Antibacterial activity of the synthesized AgNPs by using *Justicia secunda* aqueous leaf extracts

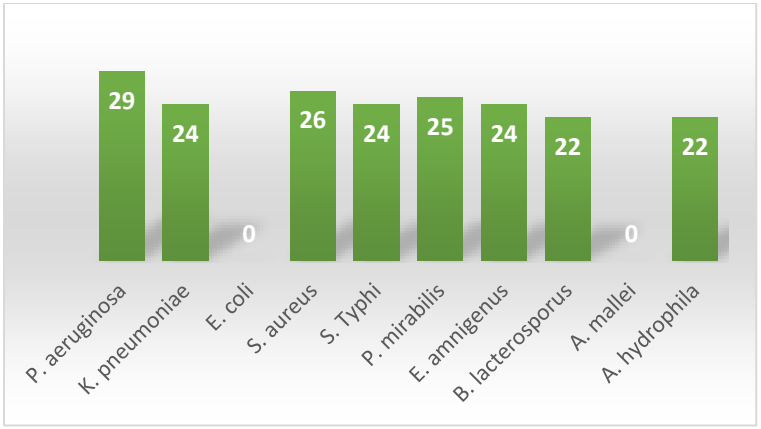


Figure 5B. Antibacterial activity of the synthesized AgNPs by using *Telfairia occidentalis* aqueous leaf extracts

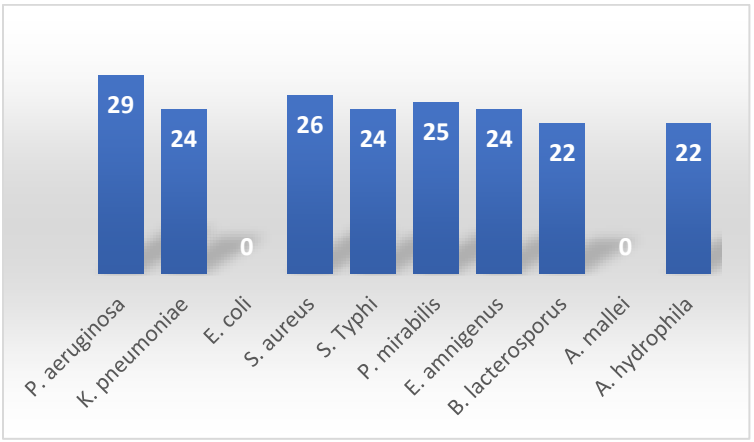


Figure 5C. Antibacterial activity of the synthesized AgNPs by using *Jatropha tanjorensis* aqueous leaf extracts

Table 2 summarizes the least concentration at which the synthesized AgNPs by using the three plant extracts is capable of inhibiting the growth of test bacteria. The differently synthesized AgNPs were found to be an effective antibacterial agent against the test bacteria and the recorded MIC values at which no visible growth of test bacterial strains was found are presented in Table 2. The minimum inhibitory concentration for Js-AgNPs against *K. pneumonia*, *S. aureus*, *S. typhi*, *P. mirabilis*, *E. amnigenus*, and *B. lacterosporus* was found to be 3.125 mg/mL, 6.25 mg/mL, 3.125 mg/mL, 6.25 mg/mL, 3.125 mg/mL, and 6.25 mg/mL, respectively. The MIC for To-AgNPs against *P. aeruginosa* and *S. aureus* were found to be 3.125 mg/mL, while *S. typhi*, *P. mirabilis*, *E. amnigenus*, and *B. lacterosporus* was found to be 6.25 mg/mL.

Table 2. The minimum inhibitory concentration of the synthesized AgNPs against the bacterial isolates

Bacteria	Concentration of Extracts (mg/µl)				
	3.125	6.250	12.500	25.000	50.000
			P. aeruginosa		
JsL-AgNPs	+	+	+	+	+
ToL-AgNPs	-	+	+	+	+
JtL-AgNPs	+	+	+	+	+
			K. pneumonia		
JsL-AgNPs	-	+	+	+	+
ToL-AgNPs	+	+	+	+	+
JtL-AgNPs	+	+	+	+	+
			E. coli		
JSL-AgNPs	+	+	+	+	+
ToL-AgNPs	+	+	+	+	+
JtL-AgNPs	+	+	+	+	+
			S. aureus		
JsL-AgNPs	-	-	+	+	+
ToL-AgNPs	-	+	+	+	+
JtL-AgNPs	-	-	-	+	+
			S. Typhi		
JsL-AgNPs	-	+	+	+	+
ToL-AgNPs	-	-	-	+	+
JtL-AgNPs	+	+	+	+	+
			P. mirabilis		
JsL-AgNPs	-	-	+	+	+
ToL-AgNPs	-	-	+	+	+
JtL-AgNPs	+	+	+	+	+
			E. amnigenus		
JsL-AgNPs	-	+	+	+	+
ToL-AgNPs	-	-	+	+	+
JtL-AgNPs	+	+	+	+	+
			B. lacterosporus		
JsL-AgNPs	-	-	+	+	+
ToL-AgNPs	-	-	+	+	+
JtL-AgNPs	-	+	+	+	+
			A. mallei		

JsL-AgNPs	+	+	+	+	+
ToL-AgNPs	+	+	+	+	+
JtL- AgNPs	+	+	+	+	+
			A. hydrophila		
JsL-AgNPs	+	+	+	+	+
ToL-AgNPs	+	+	+	+	+
JtL-AgNPs	-	+	+	+	+

Key: **+** denotes the growth of the test bacteria, and **–** denotes the inhibition of the test bacteria

Conclusion

The pseudo-spherical shaped synthesized AgNPs by using the three Nigerian vegetables (*Justicia secunda*, *Telfairia occidentalis*, and *Jatropha tanjorensis*) showed the antibacterial activities on both Gram-positive and Gram-negative bacteria which may possibly be due to their embedded polyphenol phytoconstituents revealed by FTIR analysis. Therefore, this may serve as a therapeutic potential in the treatment, management, and/or prevention of bacterial infections, and can be a promising source for the development of chemotherapeutic (antibiotic) agents in the future.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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