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Characterization, Antimicrobial and Toxicity Studies of Silver Nanoparticles Using *Opuntia ficus indica* Leaves Extracts from Effurun, Delta State

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Abstract

The silver nanoparticles were synthesized by reacting silver nitrate, a metal precursor with the extract obtained from the leaves of *Opuntia ficus indica*, which acts as a reducing and stabilizing agent. Formation of silver nanoparticles was indicated by colour change from light yellow to dark brown which was confirmed by ultraviolet-visible (UV-Vis) spectroscopy showing surface plasmon band at 369 nm resulting from relatively small amount of reductive biomolecules for the silver ion reduction. Scanning electron microscope **(**SEM) analysis gave the morphology of the nanoparticles and showed that the silver particles were of spherical shape. Fourier transform infrared (FTIR) spectrometer presented the functional groups and it showed that the reduction of silver was as a result of the presence of the absorption band of -OH stretching of alcohols in biomolecules such as glycosides, terpenoids, phenols, and alkaloids which made capping and stability of the particles. The antimicrobial activities of silver nanoparticles were determined using agar well diffusion method with some clinical pathogenic microbes such as methicillin resistant *Staphylococcus aureus,* Vanocomycin resistant *Enterococci, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Candida krusei, Candida stellatoidea* and *Candida tropicalis*. High anti-microbial activities were seen in *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus* having zone of inhibition of 30 mm and 29 mm with minimum inhibitory concentration (MIC) value of 1.25 mg/L and minimum bactericidal concentration (MBC) value of 2.5 mg/L, respectively. Toxicity study revealed that the silver nanoparticles were not toxic at 100 mg/L indicating that it is eco-friendly.

Keywords: Characterization, Antimicrobial, Toxicity, Silver nanoparticles, *Opuntia ficus indica*.

Introduction

Nanoparticles are particles with size dimensions ranging from 1 to 100 nm with a surrounding interfacial layer which consists of ions, inorganic, and organic molecules. They show characteristics based on structures between bulk materials and atoms, making the reactivity of bulk materials of the same composition different due to their large surface areas which give them the ability to be used in wide ranges of applications (Moritz and Geszke-Moritz 2013, Nagajyothi et al. 2012).

The synthesis of metallic and semiconductor nanoparticles through physical and chemical routes was extensively reported in a comparative study which showed that the use of aqueous leaf extract of *Costus afer* in

the synthesis of silver nanoparticles (CA-AgNPs) and the electrochemical characterization of CA-AgNPs/multiwalled carbon nanotubes (MWCNT)-modified electrode confirmed charge transfer properties of the nanocomposites. The CA-AgNPs/MWCNT-modified electrode demonstrated faster charge transport behavior (Elemike et al. 2018). Silver nanoparticles are very common and have numerous applications in the field of oxidation catalysis (Derikvand et al. 2010), sensors (Wang et al. 2011), fuel cells (Petrov et al. 2008), photovoltaic cells (Sanli et al. 2008), optical switching devices (Ida et al. 2008), optical data storage systems (Li et al. 2003) and in diagnostic biological probes (Wang and Gu 2009).

Nanoparticles over the years have been synthesized using various methods, such as physical, chemical and biological methods have been employed for this purpose. The biological methods have an edge or more compensation over the chemical methods, because the biological methods follow highly controlled assembly, availability of biological entities and eco-friendly procedures (Bar et al. 2009a, 2009b). The electrochemical analysis by cyclic voltammetry (CV) and the differential pulse voltammetry (DPV) confirmed the effective reduction capacity of *Mimosa albida* leaves extract to reduce Ag ions to AgNPs. A study confirmed that aqueous extract of *Mimosa albida* contains reducing agents capable of synthesizing silver nanoparticles which can be used in the phytochemical industry (Pilaquinga et al. 2020). Silver nanoparticles in particular, have great properties but one of the most interesting and important property been studied by various researchers all over the world is the anti-microbial activity. Aritonang et al. (2019) reported that the aqueous leaf extracts of *I. balsamina* and *L. camara* were separately tested for antimicrobial activities against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria. The results showed that bacterial growths inhibited by the extracts containing Ag nanoparticles were comparable to ciprofloxacin in inhibiting bacterial growth. The results recorded from UV–Vis spectrum, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive spectroscopy (EDS) support the biosynthesis and characterization of silver nanoparticles from *Acalypha indica* leaf extracts and the antibacterial activities of synthesized silver nanoparticles from the leaf of *A. indica* showed effective inhibitory activity against water borne pathogens, i.e., *Escherichia coli* and *Vibrio cholera* with silver nanoparticles having 10 µg/ml recorded as the minimum inhibitory concentration (MIC) against *E. coli* and *V. cholera* (Krishnaraj et al. 2010).

The plant *Opuntia ficus indica* has been reported in literature as a source of bioactive compounds for nutrition, health and disease control (El-Mostafa et al. (2014). In traditional medicine, *Opuntia ficus indica* has been used for the treatment of burns, wounds, edema, hyperlipidemia, obesity and catarrhal gastritis and also the alcoholic extracts are indicated for anti-inflammatory, hypoglycemic, and antiviral purposes (Kaur et al. 2012). This study was intended to validate, amongst others, the ability of the extract from the leaves of *Opuntia ficus indica* found in Effurun to reduce silver metal based on its known bimolecular contents to form silver nanoparticles and in turn check its ability to inhibit the growth of microbes and other possible applications in the world of medicinal chemistry and other chemical sciences.

Materials and Methods

Plant collection: Fresh mature leaves of *Opuntia ficus indica* were purchased from a well-kept garden in a very good state which is located in Effurun, Delta State, Nigeria. The plant was identified by Dr. Gloria Omoregie, a botanist in the department of Environmental Management and Toxicology,

Federal University of Petroleum Resources, Effurun.

Plant preparation: The leaves of *Opuntia ficus indica* were carefully stripped off the sharp spines protruding from the pad using a knife and a pair of hand gloves. The flat pads were washed thoroughly repeatedly for about four times using distilled water to remove dust particles and other impurities. They were air dried at room temperature (27 °C) and cut into smaller pieces. A subsample (25 g) of the shredded leaves of *Opuntia ficus indica* was weighed using an analytical balance and transferred into a 250 mL beaker, then 100 mL of distilled water measured using a standard measuring cylinder and was added into the beaker which contained the *Opuntia ficus indica* extract. It was subjected to stirring for 10 min and was boiled for about 30 min at 60 °C, after which an extract was obtained. The extract was cooled at room temperature (27 °C) after which it was filtered to obtain a filtrate. The filtrate was stored in a conical flask at room temperature for the synthesis of silver nanoparticles.

Preparation of 0.01 M silver nitrate: In the preparation of 0.01 silver nitrate solution, 1.67 g of analytical grade pure silver nitrate salt was weighed using an analytic balance and was used to prepare 0.01 M of silver nitrate solution.

Synthesis of silver nanoparticles: In the green synthesis of silver nanoparticles, 100 mL of the *Opuntia ficus indica* extract was added to 500 mL of 0.01 M $AgNO_3$ solution and a spontaneous reaction was allowed. The solution was then subjected to heating and stirring at 90 °C. As the reaction proceeded, it was observed that the colour change was from a clear visible solution to a dark brown solution which indicated the formation of silver nanoparticles (Khan et al. 2018). The mechanism of reduction was determined using UV-Visible spectroscopy and the silver nanoparticles were collected after centrifugation at 2500 rpm and drying in an oven at 50 °C.

Characterization of silver nanoparticles

Fourier transform infrared spectrometer: The FT-IR spectra of the silver nanoparticle metal complexes were recorded in between the spectra range of 4000-650 cm^{-1}. FTIR analysis of the dried Ag NPs was carried out through the potassium bromide (KBr) pellets (FTIR grade) method in 1:100 ratio and spectrum was recorded using FT/IR-6303 Fourier transform infrared spectrometer equipped with JASCO IRT-7200 Intron infrared microscope using transmittance mode operating at a resolution of 4 cm^{-1}.

Scanning electron microscope (SEM): A scanning electron microscope is a description of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. A scanning electron microscope (SEM) Model No. JEC-1611 was used. The colloidal solutions containing Ag NPs were centrifuged at 4,000 rpm for 15 min, and the pellets were discarded and the supernatants were again centrifuged at 25,900 rpm for 30 min. This time, the supernatants were discarded and the final pellets were dissolved in 0.1 mL of deionized water. The pellets were mixed properly and carefully placed on a glass cover slip followed by air-drying. The cover slip itself was used during scanning electron microscopy (SEM) analysis.

Ultraviolet-visible spectroscopy: The electronic spectrum of the silver nanoparticles was recorded in the UV-visible region. Samples (1 mL) of the suspension were collected periodically to monitor the completion of bioreduction of Ag^+ in aqueous solution, followed by dilution of the samples with 2 mL of deionized water and subsequent scan in UV-visible (vis) spectra, between wave lengths of 200 to 700 nm in a spectrophotometer (Model No. DU-48, Fullerton, CA, USA), having a resolution of 1 nm. UV-vis spectra were recorded at intervals of 0 min, 0 min, 5 min, 10 min, 15 min and 20 min.

Anti-microbial analysis

The antimicrobial activities of silver nanoparticles were determined using some clinical pathogenic microbes such as methicillin resistant *Staphylococcus aureus, Vanocomycin Resist enterococci, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Candida krusei, Candida stellatoidea,* and *Candida tropicalis*. The microbes were obtained from the department of Medical Microbiology, ABU Teaching Hospital, Zaria.

Agar well diffusion method: Antibacterial activities of synthesized nanoparticles were evaluated by the agar well diffusion method as described by Aida et al. (2001). 0.5 g of the silver nanoparticles was weighed and dissolved in 10 mL of dimethyl suphur oxide (DMSO) to obtain a concentration of 50 mg/mL. 0.1 g of silver nanoparticles was weighed and dissolved in 1 mL of DMSO to obtain a concentration of 10 g/mL.

Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturer's instructions sterilized at 121 °C for 15 min and poured into sterile petri dishes and were allowed to cool and solidify. The medium was seeded with 0.1 mL of the standard inoculums of the test microbes such as methicillin resistant *Staphylococcus aureus,* vanocomycin resistant *Enterococci, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Candida krusei, Candida stellatoidea,* and *Candida tropicalis* using a sterile swab. By the use of a standard sterile cork borer of 6 mm in diameter, a well was cut at the center of each inoculated medium. 0.1 mL of the extract concentration of 50 mg/mL was then introduced into the well on the inoculated media. Incubation was made at 37 °C for 24 hrs, after which the plates were observed for zone of inhibition of growth. The zone of inhibition was measured with a transparent ruler and the results recorded in millimeters.

Determination of minimum inhibition concentration (MIC)

The minimum inhibition concentration (MIC) of the silver nanoparticles (AgNPs) was determined using the broth dilution method. 10 mL of the broth was dispensed into test tubes and was sterilized at 121 °C for 15 minutes; then, the broth was allowed to cool. McFarland's standard scale number 0.5 was prepared to give turbid solution.

Normal saline was prepared, 10 mL was dispensed into sterile test tube and the test microbes were inoculated and incubated at 37 °C for 6 hrs. Dilution of the test microbes was done in the normal saline until the turbidity matched that of McFarland's scale by visual comparison of which at this point the test microbes had a concentration of 1.5×10^8 cfu/mL.

Two-fold serial dilutions of the extract were made to obtain the concentrations of 50 mg/mL, 25 mg/mL, 6.25 mg/mL, 3.13 mg/mL, 1.57 mg/mL and 1.25 mg/mL. Two-fold serial dilutions of the silver nanoparticles (AgNPs) were made to obtain the concentrations 10 mg/mL, 5 mg/mL, 2.5

mg/mL, 1.25 mg/mL, 0.63 mg/mL and 0.31 mg/mL. Having obtained the different concentrations of the silver nanoparticles in the sterile broth, 0.1 mL of the test microbes in the normal saline was then inoculated into the different concentrations. Incubation was made at 37 °C for 24 hrs, after which the test tubes of the broth were observed for turbidity (growth). The lowest concentration of the extract in the sterile broth which showed no turbidity was recorded as the minimum inhibition concentration.

Determination of minimum bactericidal/fungicidal concentration

Minimum bactericidal/fungicidal concentration (MBC/MFC) was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared and sterilized at 37 °C for 15 mins, and thereafter poured into sterile petri dishes and was allowed to cool and solidify. The contents of the MIC in the serial dilutions were then sub-cultured onto the prepared medium. Incubation was made at 37 °C for 24 hrs, after which the plates were observed for colony growth, MBC/MFC were the plates with the lowest concentrations of the silver nanoparticles without colony growth.

Toxicity analysis

Study area

The snails used during the course of this study were obtained from Iteregbi Community, which is made up of fresh water environment. It is located at Ugheli North Local Government Area of Delta State, Nigeria. The coordinates for sampling was latitude 5°3'59.6" N and longitude 5°49'59.8" E. The samples were collected by hand sorting.

Test chemicals: For the purpose of this study, the biosynthesized silver nanoparticles were used as the test chemicals for toxicity analysis.

Snail toxicity bioassay: The snails that were used were the brown black African giant snails with a mean weight of 1.81 g and length of 2.42 cm. The procedure of the International Organization for Standardization (ISO 2006) was adopted for the snail bioassays.

Acclimatization of the test organism: The snails were acclimatized in clean soils for seven days before the commencement of the experiment. For each of the test concentrations, 1000 g (1 kg) of natural soil of the organisms' habitats were placed into the test tank and sprinkled with 100 mL of different concentration of the silver nanoparticles solution. Cellulose was also added to the soil as food for the organisms to ensure they were not starved thereafter; ten healthy active organisms were cleaned, weighed and carefully transferred into the test containers (ISO 2006). The experiment began with a range finding test to establish a working range in order to determine the concentration to be used in the definitive test. From the prepared stocked solution, a range finding test using concentrations of 1 mg/L, 10 mg/L, and 100 mg/L was carried out using the silver nanoparticles solution, which was later used for the definitive test. Three replicates per treatment were prepared for the five concentrations for the silver nanoparticles. The control setup which contained distilled water was prepared alongside with the silver nanoparticles solution. The organisms were kept in the laboratory and each of the test concentrations was labelled. The set up was covered with net and held with rubber band to prevent the organisms from dying as shown in the experimental set up in Figure 1.

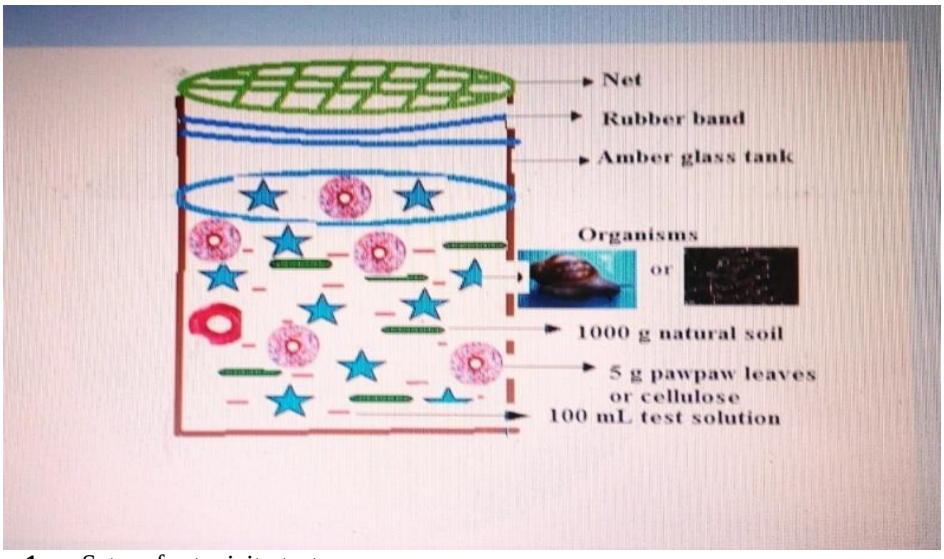

Figure 1: Set up for toxicity test.

Assessment of response (mortality): Mortality and the lethal test were evaluated on the 7th day, and for the sub lethal experiments in all the replicates. Physical changes (morphology) and behavioural responses were also noted. The organisms were considered dead if there was no movement when the snails were prod with a metal rod or if there was no activity after 5 min of placing the snails on a white paper.

Evaluation of growth rate and toxicological risk assessment: The weight of the organisms was measured again after exposure for the sub lethal assessment to evaluate the growth rate and to estimate any inhibition resulting from exposure to the silver nanoparticles solution. The specific growth rate, which is the average percentage increase in body mass per day over a given time interval was done using the Equations 1 and 2. The percentage growth rate inhibition efficiency was also obtained from Equations 3 and 4.

$$\text{Specific growth rate (SGR)} = \frac{\text{Log } W_2 - \text{Log } W_1}{T_2 - T_1} \quad (1)$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Log } L_2 - \text{Log } L_1}{T_2 - T_1} \quad (2)$$

Where W_1 or L_1 mean initial weight or length of organism at T_1 (on day one) W_2 or L_2 means final weight or length of organism. T_2-T_1 means time interval in days.

The percentage growth rate relative to the control and growth rate inhibition efficiency was calculated using the following formula

$$\%\text{ growth rate relative to control} = \frac{\text{Sample growth rate}}{\text{Control growth rate}} \times 100 \quad (3)$$

$$\%\text{ growth rate inhibition efficiency (\%)} = \frac{\text{Control growth rate} - \text{Sample growth rate}}{\text{Control growth rate}} \times 100 \quad (4)$$

Statistical analysis: The susceptibility of the snails to the silver nanoparticles solution test was determined using the probity method of analysis for median lethal concentration LC_{50} at day 7 and all the data collected were subjected to descriptive statistics and analysis of variance (ANOVA) using SPSS (Statistical Product and Service Solutions) software version 21.0. These were used to determine the mean statistical differences between the

controls and treatment groups at significance toxicity assessment endpoints.

Soil samples: The soil samples were obtained from Iteregbi Community and at a depth of 0–15 cm. The soil samples were air dried, crushed and sieved through a 2 mm mesh size sieve and used for fertility analyses.

Results and Discussion

The results of characterization of silver nanoparticles obtained from the green synthesis of the leaves extract of *Opuntia ficus indica* revealed the results presented below.

UV spectroscopy showed surface plasmon band at 369 nm. The reduction of silver ions into silver nanoparticles was observed as a result of the colour change from light yellow to dark brown at absorbance of 369 nm when silver nitrate was reacted with the extract of *Opuntia ficus indica*. The colour change is due to the surface plasmon resonance (SPR) phenomenon (Krishnaraj et al. 2010). The metal nanoparticles are composed of free mobile electrons, which give the SPR absorption band, due to the combined vibrations of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 369 nm in case of *Opuntia ficus indica*.

Looking at studies on silver nanoparticles synthesis, it was found that the silver nanoparticles showed SPR peak at around 385-450 nm (Huang et al. 2007). From our studies, we found that the SPR peak for *Opuntia ficus indica* was at 369 nm (Figure 2). The band at 369 nm is as a result of relatively small amounts of reductive biomolecules for the silver ion reduction, which resulted in small number of particles formed as stated by Silva et al. (2012).

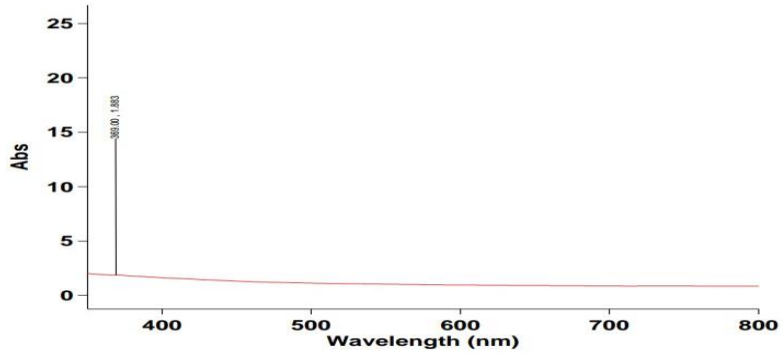

Figure 2: Ultra-violet visible spectrum of silver nanoparticles bound.

FTIR showed absorption bands of functional groups. The possible biomolecules identified in the capped silver nanoparticles showed characteristic peaks at 3619.2, 3570.8, 2918.5, 2851.4, 2374.3, 2113.4, 1990.4, 1893.5, 1543.1, 1319.5, 1032.5 and 823.7 cm^{-1} (Figure 3). These bands denote stretching vibrational bands responsible for compounds like glycosides, terpenoids, alkaloids, alkane, alkyne, Siddiqui et al (2000). The absorption bands were assigned to OH stretching (alcohols) in glycosides, terpenoids, alkaloids at 3619.2–3570.8 cm^{-1}, C–H stretching vibrations for alkane was assigned to 2918.5–2374.3 cm^{-1}, 2113.4 cm^{-1} for alkyne, strong N–O stretching indicating nitro compound at 1543.1 cm^{-1}, O-H indicating phenol at 1319.5 cm^{-1}, C–N for aliphatic amine at 1032.5 cm^{-1}. The peaks observed from the analysis showed that there was capping of the nanoparticles by alkaloids, terpenoids and glycosides in the

extract of *Opuntia ficus indica*. According to various literatures, most plant bio synthesized nanoparticles were characterized by the presence of numerous peaks as a result of the organic compositions in the plant as the chemicals synthesized are characterized by few, strong peaks (Elemike et al. 2017). This gave insight to the fact that capping and stability of the silver nanoparticles were by the action of bio reducing agent, *Opuntia ficus indica* extract.

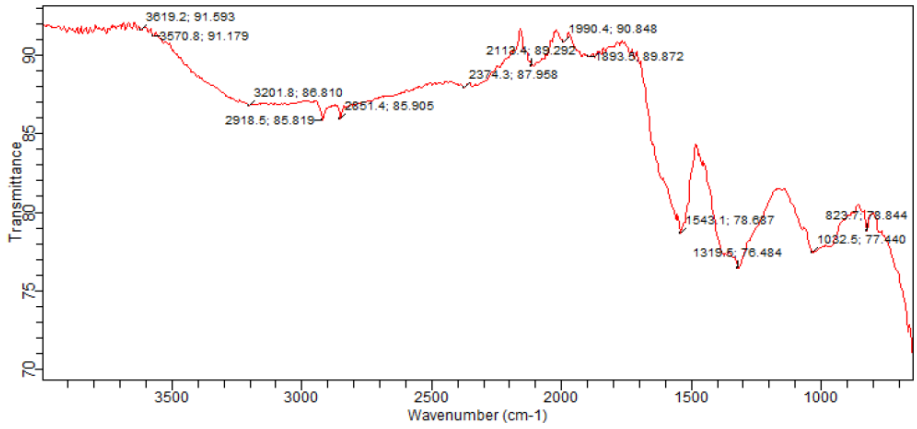

Figure 3: Infrared spectrum of silver nanoparticles bound.

SEM analysis showed that the silver particles were of spherical shapes (Figure 4). The SEM analysis carried out provided information on the surface morphology. The results showed that the particles were of spherical shapes. Literature on other studies showed that the shapes of silver nanoparticles vary due to increase in the concentration ratio of silver nitrate to plant extract (Krishnaraj et al. 2010).

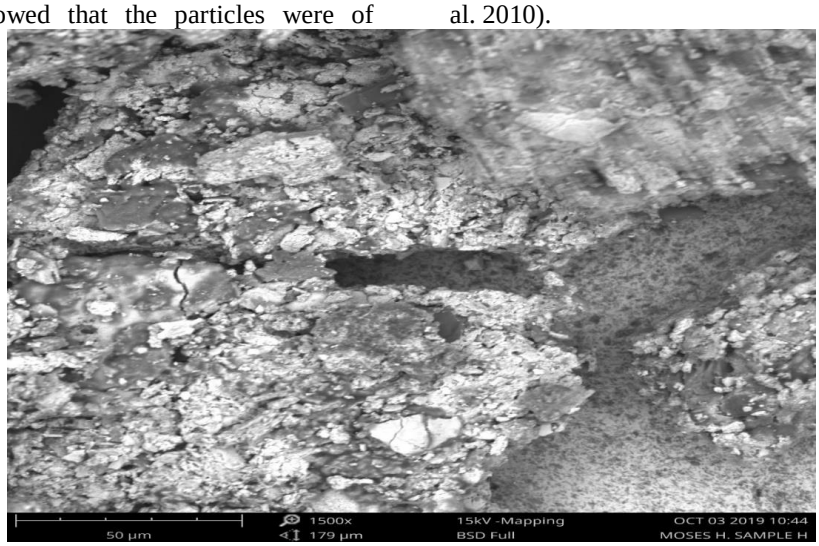

Figure 4: SEM imaging on silver nanoparticles bound.

The anti-microbial tests showed that the activities of the bio-synthesized silver nanoparticles were based on the zone of inhibition which was in millimetre (mm). The agar diffusion test carried out on the silver nanoparticles showed that they possessed antibacterial and antifungal properties. It was observed that the biosynthesized silver nanoparticles showed higher activities in methicillin resistant *Staphylococcus aureus, Pseudomonas aeruginosa* and relatively less for *Escherichia coli* and vancomycin resistant *Enterococci* but could not inhibit the growth *Staphylococcus aureus* and some others.

The minimum inhibition concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) tests were also determined. The results showed activities for only methicillin resistant *Staphylococcus aureus,* vancomycin resistant *Enterococci, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Candida krusei*, but could not inhibit the growth of others as shown in Table 1. The inhibitory effects on the biosynthesized silver nanoparticles in the in-vitro antimicrobial assays are concentration dependent (Andrighetti-Fröhner et al. 2009). It was observed that silver nanoparticles inhibited the growth of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* at the same MIC value of 1.25 mg/L, while inhibited the growth of *Escherichia coli* and *Streptococcus pyogenes* at the same MBC value of 5 mg/L. *Escherichia coli and Streptococcus pyogenes* showed the highest antimicrobial activities with MIC values of 2.5 mg/L, 1.25 mg/L and an MBC value of 5 mg/L, respectively (Table 2 and Table 3). No activity was recorded for *Staphylococcus aureus* as there was no sensitivity and zone of inhibition. Biosynthesized silver nanoparticles are able to advance through the thicker peptidoglycan cell wall layer which may be the reason for rigidity and low or no activity of the Gram positive bacteria as detected in the MIC tests for *Staphylococcus aureus* and *Streptococcus pyogenes* (Nazzaro et al. 2013).

Anti-microbial analysis results

The preliminary assays (toxicity range finding) revealed a relative delay in the snails' mortality (Figure 5, Table 4). After 7 days of exposure, there was mortality only at the concentration of 100 mg/L. The data obtained from the toxicity tests revealed that the highest concentration (100 mg/L) of silver nanoparticles resulted in no significant deaths of the organisms. This indicated that the silver nanoparticles had no significant effects on snails and can be explained by the slow time release of silver ions from the silver nanoparticles (Kittler et al. 2010). In nature, soil constitutes the support in which snails take around 40% of their nutrients. Studies have shown that the longer the exposure of snails to silver nanoparticles, the higher the effects in the digestive gland, foot and mantle of the snails (Oliveira-Filho et al. 2005). The interaction between silver nanoparticles and the soil may be of practical significance because of possible inhibition in microbial activities contributing to soil fertility due to its anti-microbial properties. According to some literatures, studies revealed that speciation has strong roles in silver nanoparticles toxicity (Wood et al. 1996), alongside shape and size (Fabrega et al. 2011, Lapresta-Fernández et al. 2012, Ivask et al. 2014).

Table 1: Zone of inhibition of silver nanoparticles bound isolate against test microorganisms in millimetres

Test organism	Zone of inhibition (mm)
Methicillin resistant *Staphylococcus aureus*	29
Vanocomycin resistant *enterococci*	25
Staphylococcus aureus	0
Streptococcus pyogenes	27
Escherichia coli	25
Klebsiella pneumonia	0
Proteus mirabilis	0
Pseudomonas aeruginosa	30
Salmonella typhi	28
Candida albicans	0
Candida krusei	26
Candida stellatoidea	0
Candida tropicalis	0

Table 2: Minimum inhibition concentration of synthesized silver nanoparticle against the test organisms

Test organism	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.63 mg/mL	0.31 mg/mL
M RSA	–	–	–	0*	+	++
VRE	–	–	0*	+	++	+++
S. pyogenes	–	–	-	0*	+	++
E. coli	–	–	0*	+	++	+++
P. mirabilis						
P. aerginosa	–	–	–	0*	+	++
S. typhi	–	–	–	0*	+	++
C. albicans						
C. krusei	–	–	–	0*	+	++

Key: – no colony growth, 0* MIC, + scanty colonies growth, ++ moderate colonies growth, +++ heavy colonies growth.

Table 3: Minimum bacterial/fungal concentration of synthesized silver nanoparticle against the test organisms

Test organism	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.63 mg/mL	0.31 mg/mL
M-RSA	–	–	0*	+	++	+++
VRE	–	0*	+	++	+++	++++
S. pyogenes	–	0*	+	++	+++	++++
E. coli	–	0*	+	++	+++	++++
P. aerginosa	–	–	0*	+	++	+++
S. typhi	–	–	0*	+	++	+++
C. krusei	–	0*	+	++	+++	++++

KEY: – no colony growth, 0* MBC, + scanty colonies growth, ++ moderate colonies growth, +++ heavy colonies growth.

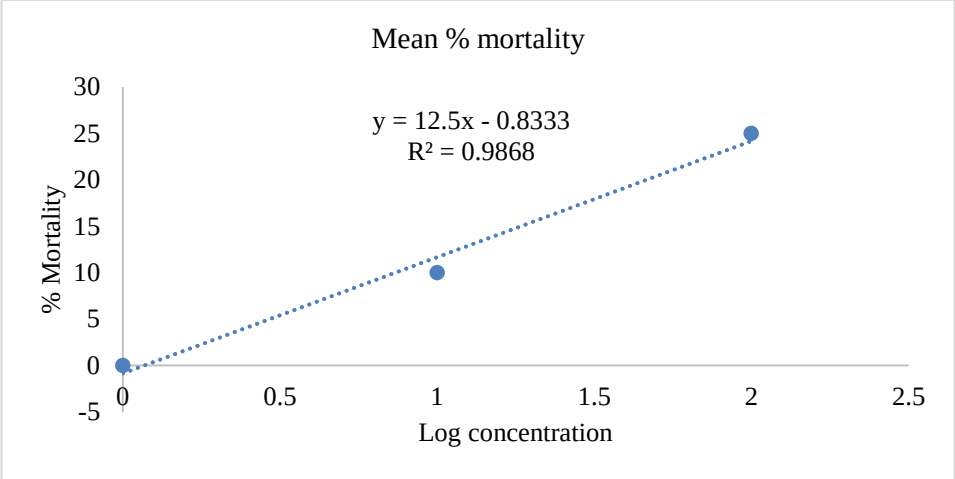

Figure 5: Mean percentage mortality of snails.

Table 4: Results of percentage mortality of snails when expose to silver nanoparticles

	Number dead					% Mortality				
Conc., mg/kg	Tank 1	Tank 2	Mean	SD	SE	Tank 1	Tank 2	Mean	SD	SE
0	0	0	0.0	0.00	0.00	0	0	0	0	0
1	0	0	0.0	0.00	0.00	0	0	0	0	0
10	1	1	1.0	0.00	0.00	10	10	10	0	0
100	2	3	2.5	0.71	0.41	20	30	25	7	4

Key: SD, SE, mg/kg

Conclusion

The synthesis of silver nanoparticles was achieved using the leaves of *Opuntia ficus indica* extract as a reducing agent. The formation of the silver nanoparticles was achieved by colour change from light brown following the standard procedures on addition of the leaves extract of *Opuntia ficus indica* to silver nitrate solution which was confirmed by the UV-Vis spectroscopy at 369 nm. FTIR showed that reduction was as a result of the presence of phytochemicals such as glycosides, terpenoids, phenols, alkaloids in the extract of *Opuntia ficus indica*. SEM analysis gave insights on the morphology of the biosynthesized silver nanoparticles; the nanoparticles were of spherical shapes. The biosynthesized silver nanoparticles inhibited growth of some clinical pathogens such as Gram positive bacteria *S. pyogenes*, Gram negative bacteria *E. coli* and fungi *Candida krusei* which makes them excellent anti-bactericidal and fungicidal agents. This property makes them suitable for applications in medicine, which is our main focus. The results obtained from the toxicity study showed that the silver nanoparticles were not toxic but at higher concentrations other than the one used in this study they may have adverse effects on the survival, and at non-lethal concentrations, they may affect the reproduction rates of the snails.

Conflict of Interest

We declare that there are no conflicts of interest in this research work.

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