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Original Research Article

Green Synthesis of Silver Nanoparticles from *Parinari curatellifolia* Methanol Stem Bark Extract and Evaluation of Antioxidant and Antimicrobial Activities

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ABSTRACT

Parinari curatellifolia Planch ex. Benth (Chrysobalanaceae) has been used traditionally for the treatment of cancer, pneumonia, fever, dressing of wounds and fractures. The green synthesized nanoparticles from phytochemicals have been utilized as effective antimicrobial and anticancer agents. The research was designed to synthesize nanoparticles from methanol stem bark extract of *P. curatellifolia* and to investigate its antioxidant and antibacterial activities. The stem bark was powdered and then extracted by maceration. Silver nanoparticles were synthesised using 5 mM $AgNO_3$ solution. DPPH free radical and hydrogen peroxide scavenging assays were employed to determine the antioxidant activity. The antibacterial activity was determined using agar disk diffusion method on *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Escherichia coli*. The phytochemical screening of the extract revealed the presence of saponins, flavonoids and tannins. XRD analysis of the synthesized silver nanoparticles revealed a crystalline structure with face cantered cubic orientation. The SEM revealed particle size of 1 nm and consisted of highly agglomerated particles which formed large aggregates. The IC_{50} of DPPH free scavenging activity of the extract, silver nanoparticles and ascorbic acid (standard) were 2.804 mg/mL, 2.367 mg/mL and 2.065 mg/mL respectively. Also, that of H_2O_2 against the extract, silver nanoparticles and ascorbic acid (standard) were 4.986 mg/mL, 36.56 mg/mL and 6.414 mg/mL respectively. The nanoparticles inhibited the growth of the organisms with zone of inhibition between 8-12 mm whereas that of standard antibiotic (gentamicin) ranged from18-26 mm. This therefore implies that PC extract can be used in the synthesis of nanoparticles for its use against bacteria strains and as antioxidants.

***Keywords*:** Antioxidant, Antibacterial, Green synthesis, *Parinari curatellifolia*, silver nanoparticles .

Introduction

Green synthesis of herbal nanoparticles from medicinal plants is rapidly growing in the field of nanotechnology as it substitutes using toxic chemicals, and lesser time is used for the process.[1] Nanotechnology deals with the synthesis of nanoparticles with controlled size, shape and dispersity of material at the nanometer scale length.[2,3] Gold, silver and platinum have numerous applications in information technology, electronics, biomedicals used in targeted drug delivery and antibacterial applications.[4] Several studies have given attention to silver amongst other nanoparticles, this is due to its physical and chemical properties as well as its application in biomedicine as antiangiogenic, anti-cancer and antibacterial agent.[5,6,7]
Nanoparticles inhibits bacterial and fungal growth as they can penetrate the cell wall and cause damage to deoxyribonucleic acid (DNA) and proteins thereby liberating silver from the silver nanoparticles which makes it effective against bacteria and fungi.[8]

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Plants chemicals have been used for synthesis of nanoparticles due to their reductive properties. [9] Flavonoids, tannins, saponins are classes of phytochemicals that have been reported to act as reducing agents.[10]
Nanoparticles have been utilized by researchers to improve health as antioxidants, and antimicrobial agents.[11] Due to the high prevalence of bacterial resistance to antibiotics and also the increasing rate of cancer that arises from free radicals, there is the need for detailed scientific investigations on the silver nanoparticles synthesized from *Parinari curatellifolia* as a potential therapeutic agent. Researches on medicinal plant extracts and their synthesized nanoparticles have shown that, herbal nanoparticles are effective in the treatment of bacterial infections.[1]
Parinari curatellifolia (PC) belongs to the family Chrysobalanaceae. It is eaten as food for its nutritional benefits and its usage in treatment of diseases in traditional medicine.[12] The leaves, seeds, stem bark and roots of the plant have powerful antimicrobial properties and it is used in treatment of pneumonia, abdominal complaints, cancer and toothache.[13,14] The fruit extract possesses cardiotonic properties and it is use to improve the function of the heart and in the treatment of hypertension.[15] The activities of the plant extract have been attributed to phytochemicals such triterpenoids, saponins, alkaloids, flavonoids, tannins and phenols.[16,17]
The search for effective antibiotics against resistant bacterial strains; effective free radical scavengers and the toxicity associated with administration of a high dose of drug is a continuous. Therefore, this research was aimed at synthesis of silver nanoparticles using methanol extract of PC as well as to investigate the antibacterial and antioxidant activities of the nanoparticles and the methanol extract.

Materials and Methods

Collection of plant material
The plant was collected in Zaria-Nigeria (Latitude: 11.1247° N; Longitude: 7.7254°E) in July, 2021 by the Herbarium curator (Abdullahi Shehu) of the Department of Plant Science, Ahmadu Bello University, Zaria. The plant has been identified previously via taxonomic means and voucher specimen with number 903 was deposited at the herbarium of the department for reference. The stem bark of the plant was air dried and then ground to powder using pestle and mortar. It was stored in an air tight container until required for use.

Extraction of plant
The plant powder (30 g) was macerated in 300 mL of methanol and allowed to stir for 6 h with magnetic stirrer. It was then allowed to macerate for 24 h. The mixture was filtered using Whatman filter paper in a conical flask and the filtration was repeated three times to ensure no solid particle escaped into the liquid extract. The marc was then washed further with 300 mL of methanol and was filtered as described earlier. The extracts obtained were combined and then kept for further studies.

Preliminary phytochemical screening
The screening for the presence of alkaloids, tannins, saponins and flavonoids was done according to the methods described by Halilu et al[12]

Preparation of silver nitrate solution and synthesis of silver nanoparticles
The 5 mM silver nitrate ($AgNO_3$) solution was prepared by dissolving 0.424 g of silver nitrate in sufficient distilled water to make up to 500 mL in a volumetric flask.[18]

Preparation of silver nanoparticles from the methanol extract
The silver nanoparticles were synthesized at room temperature using the methanol extract. The silver nitrate solution was reacted with the methanol extract in the of 9:1 ratio respectively. In this method, 90 mL of the silver nitrate solution was mixed with 10 mL of the methanol extract in a conical flask and was allowed to stir for 24 h using magnetic stirrer at 300 rpm. The mixture was kept in the dark for 72 h. The solution was then centrifuged at a speed of 4500 rpm for 20 min to separate the particles from solution. The supernatant layer was decanted and the sedimented particles at the bottom of the centrifuge tube was transferred to a watch glass and then dried in the oven for 24 h at 50˚C.[18]

Characterization of silver nanoparticles
Ultra-violet (UV) spectroscopy analysis of plant extract and silver nanoparticles
The methanol extract and the silver nanoparticles solution were analysed separately using the UV spectrophotometer. The cuvette was rinsed and filled with methanol to obtain the baseline for the methanol extract and the silver nanoparticles solution. The samples were scanned between 200 to 800 nm to obtain the absorbance of extract and silver nanoparticles solution.

FTIR analysis
The liquid methanol extract, silver nanoparticle solution and the synthesized powder silver nanoparticles were analysed using FTIR. The samples were scanned between 4000-400 cm^{-1}. The spectra obtained were compared with reference chart to identify the functional groups present in each sample.

Scanning Electron Microscopy (SEM)
The silver nanoparticles were subjected to SEM analysis at magnifications of ×1000, ×1500 and ×2000. The images were captured and then recorded.

X-Ray Diffraction (XRD) Analysis
The powdered silver nanoparticles (AgNPs) was placed in a square grating glass slide and arranged until it was levelled out and evenly squared, then placed in the XRD machine for analysis.

Determination of antioxidant activity
DPPH and H_2O_2 radical scavenging assay
The method previously described by[19] was adopted. The absorbance was measured and percentage inhibitions were calculated. From the percentage inhibitions the IC_{50} were determined using GraphPad version 7.

Antibacterial studies
Test microorganisms
Clinical isolates of two Gram negative (Escherichia coli and Salmonella typhi) and two Gram positive (Bacillus subtilis and Staphylococcus aureus) bacteria were obtained from the Microbiology Laboratory, Cyprus International University.

Preparation of media and growth of microbes
The nutrient agar was prepared according to the manufacturer's specifications. In order to prepare 20 plates of nutrient agar, 8 g of the nutrient agar was dissolved in 400 mL of distilled water. Furthermore, in order to prepare 20 plates of Mueller Hinton agar, 15.2 g of the agar was dissolved in 400 mL of distilled water. For 25 plates of lysogeny broth (LB), 12.5 g of the broth was dissolved in 500 mL of distilled water. A strain of each microbe from glycerol stocks were picked up using a sterilized loop and streaked on the nutrient agar plates and kept in an inverted position. This was allowed to incubate for 24 h to observe growth of the various bacteria.

Preparation of plant extract and silver nanoparticles
The plant extract 20 mg/mL was first prepared by dissolution in distilled water. Another extract of 20 mg/mL was prepared in 15% DMSO. The silver nanoparticles (10 mg/mL) was prepared as above. The extracts were carefully filtered using a 45/25 nm syringe filter under aseptic techniques. Silver nitrate (5 mM) solution, distilled water and DMSO were used as negative controls. Gentamycin was used as positive control.

Antimicrobial activity
Agar disk diffusion method
A drop of LB broth was spotted at the centre of the nutrient agar plate. Using a sterile loop, a colony of bacteria was picked from the plate having the cultures and dissolved in the drop of LB broth then streaked on the plate. A sterilized spreader was then used to spread the bacteria throughout the plate. A sterile Whatman filter paper of 6 mm in diameter was placed on each labelled site and using a micropipette, 2µL each of the various extracts and controls were injected into the paper disk. This was kept to incubate for 24 h at 37˚C. This was done for the different concentrations; the antibiotic paper disk was placed last. All this was done under aseptic conditions. Bacteria inhibition on the different extracts for all the different plates were checked after incubation. The formation of zone of inhibition (restricted bacteria growth) around the cavity indicates anti-bacterial activity. This was compared to the controls (distilled water, silver nitrate and gentamicin). The analyses were done in triplicates and zones of inhibition of growth were measured in millimetres using a ruler.

Statistical Analysis
The GraphPad prism 7 was used to determine the IC_{50} of the test samples.

Results and Discussion

Preliminary phytochemical screening
The percentage yield of the extract in methanol was 4.5%. The phytochemical screening (Table 1) revealed the presence of flavonoids, tannins and saponins which are responsible for the bio-reduction Ag^+ to silver nanoparticles. The result agrees with previous studies reported by Halilu *et al*[12]

Characterization of the plant extract and synthesized nanoparticles
Visual observation of the synthesized Nanoparticles
The visual observation of the synthesis of the silver nanoparticles showed transient colour change from reddish brown to a dark yellow

colour due the excitation of surface plasmon resonance phenomenon.[20] The change in colour was a demonstration that there was a reaction between the plant extract and the silver ions in solution which led to the reduction of the Ag^+ to Ag.[21] A clear demonstration of the colour changes is shown in Plates 1 (brown), 2 (yellowish brown), 3 (Brown) and 4 (Dark brown).

Synthesis of Nanoparticles

The plant extract acts as both reducing and capping agents, hence mediating the synthesis as well as stabilizing the silver nanoparticles.[9] The phytochemical screening reveals the presence of tannins, saponins and flavonoids which have been shown to have the ability to reduce silver ions (Ag^+) to silver nanoparticles due to the presence of the hydroxyl group they contain. Literatures have shown that the phenolic hydroxyl group in phenolic compounds such as flavonoids and tannins are effective in reduction of silver ions to silver nanoparticles.[22] This study indicated that, higher amounts of silver nanoparticles could be produced when the reaction mixture is allowed to incubate in a dark place for more days. Also, the concentration and incubation time are important factors in nanoparticles formation. The higher the concentration of $AgNO_3$, the higher the nanoparticles formed.

UV Spectroscopy in monitoring stability of plant extract and silver nanoparticles

The absorbance of the plant extract and the silver nanoparticles solution were scanned between wavelength of 200- 800 nm. The peak of the maximum absorption of the extract was observed at a wavelength of 480 nm and that of the silver nanoparticles showed three absorption peaks at 380, 460 and 480 nm. The formation and stability of the silver nanoparticles in aqueous solution was established using UV-VIS spectroscopy which generally could be used for characterization of colloidal particles of extracts and nanoparticles in aqueous solution.[23] The Surface Plasmon Resonance (SPR) bands are known to be influenced by the size, shape, morphology, composition and dielectric environment of prepared silver nanoparticles as reported by Nafe *et al*.[23]

FTIR analysis

The FTIR spectrometry was used to analyse the plant extract, the silver nanoparticles solution and the solid silver nanoparticles. The IR peaks of the plant extract were observed at 3343 cm^{-1}, 3035 cm^{-1}, 2847cm^{-1} and 1425cm^{-1} (Table 2; Figure 1). The IR peaks of the silver nanoparticles solution were observed at 3353 cm^{-1}, 2968 cm^{-1}, 2148 cm^{-1}, 2009 cm^{-1}, 1615 cm^{-1}, 912 cm^{-1}, 603 cm^{-1} (Table 3; Figure 2). The solid silver nanoparticles showed peaks of 3346 cm^{-1}, 2932 cm^{-1}, 1602 cm^{-1}, 1288cm^{-1} and 1008cm^{-1} (Table 4; Figure 3). IR analysis was used to study the nature of capping ligands that stabilizes the silver nanoparticles, formed by the bio reduction process.[21] The N-H band observed in the plant extract indicated the stretching of an amino group. Furthermore, in the solid silver nanoparticles, the absorption band at 1602 cm^{-1} indicated the presence of carboxylates. Also, the absorption bands at 2148 cm^{-1} and 1615 cm^{-1} observed in the aqueous solution indicated the presence of an amide functional group.[23] The OH stretching at 3353cm^{-1} shows the presence of phenolic groups[24] and this agrees with the result of the qualitative phytochemical screening.

Table 1: Phytochemical screening

Phytochemical/Test	Inference
Saponins	Saponins present
Phenolic OH group	Phenolic compounds present
Tannins	Tannin present
Flavonoids	Flavonoid present

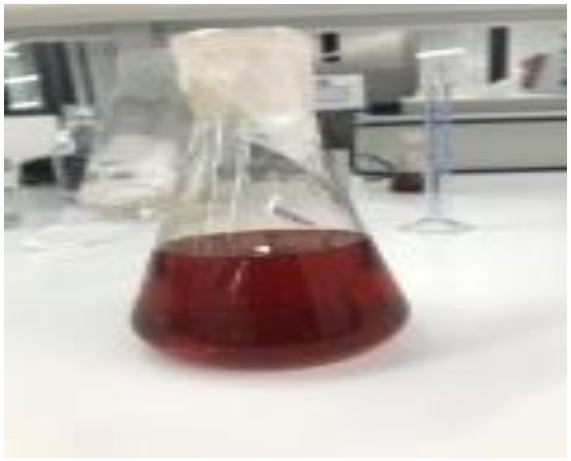

Plate 1: Methanol extract

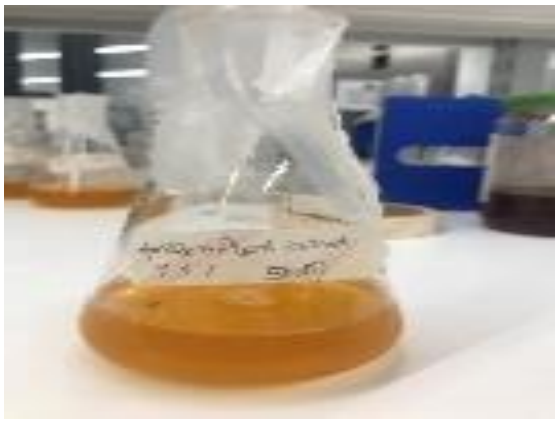

Plate 2: Reaction of methanol extract and $AgNO_3$ after 3 minutes

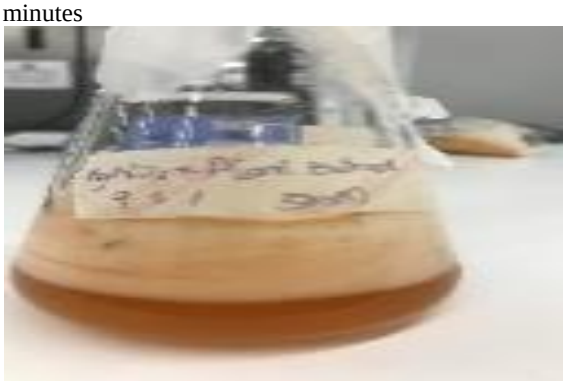

Plate 3: Reaction of methanol extract and $AgNO_3$ after 24 hours

Plate 4: Reaction of methanol extract and $AgNO_3$ after 5 days

SEM Analysis

The SEM was used to produce detailed images of surface structures. The SEM images are shown in Plates 4 A, B and C. The Detailed structure of the solid silver nanoparticles was examined under the scanning electron microscopy and it revealed particle a size of 1 µm. It was clearly observed that the sample consisted of a highly agglomerated nanoparticles which formed large aggregates. This indicates stable grain of size according to literatures.[25,26]

The result of the XRD showed the atomic arrangement, crystalline structure, crystallite size silver NPs from PC and imperfections of silver NPs. The XRD peaks (Figure 4), confirmed the presence of four significant peak between 30-70º. The XRD peaks obtained from this study conforms with the patterns of silver nanoparticles. The peaks of diffractions were observed at 32.5º, 38.2º, 46.5º and 65.5º relates to the facets 111, 200, 220 and 311 respectively of the face-centred cubic (FCC) which shows that the silver nanoparticles were crystalline in nature.[27]

Antioxidant studies

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging activity

The results of the antioxidant activity are shown in Table 5 and Figure 5. The free radical scavenging capacity of extract and silver nanoparticles have been expressed in terms of percentage inhibitions and IC_{50} values. The activity of the extract and silver nanoparticles have been compared with ascorbic acid (standard). From Table 5, the percentage inhibition increases as the concentration decreases. On the basis of the IC_{50}, the silver nanoparticle had better activity than the extract. The lower the IC_{50}, the higher the activity.[28] The ascorbic acid being a standard drug had higher activity than extract and nanoparticles. The activity demonstrated by the extract may be due to the phenolic compounds.[28,29]

Hydrogen peroxide (H_2O_2) scavenging activity

The result of the hydrogen peroxide scavenging activity is presented in Table 6 and Figure 6. The H_2O_2 is highly important because of its ability to penetrate biological membranes. The scavenging activity of H_2O_2 by extracts maybe attributed to the phenolic compounds they contain.[28] The OH group donates electrons to H_2O_2, thus neutralizing it to water.[30] On the basis of the IC_{50} values of the extract, silver nanoparticle and the ascorbic acid, the extract had better activity than the silver nanoparticle and this may be due to the inability of the silver nanoparticle to dissolve in water. The ascorbic acid had better activity.

Antibacterial studies

The antibacterial activities (Table 7) showed that the silver nanoparticles inhibited the growth of the microorganisms with mean zone of inhibition of growth ranging between 9-12 mm. The extract in water showed mean zone of inhibition between 2.3-3.0 mm. The extract in DMSO showed mean zone of inhibition between 5.7-8.0 mm. Gentamycin showed mean zone of inhibition ranging between 20-26.7 mm. while the $AgNO_3$ showed 9.7-13.0 mm. Comparatively, the antibacterial activity of the nanoparticle was higher than the methanol extract and this may be due to surface area of the nanoparticle.[11] The result obtained from this research is in agreement with.[31] Furthermore, the mechanism of action of silver nanoparticles is based on premise that silver nanoparticles can penetrate the cell walls of bacteria and then cause a structural change in the cell membrane which could cause an increase in cell permeability.

This may lead to an uncontrolled transport of the nanoparticles through the cytoplasmic membrane and then result in cell death.[31] On the other hand, gentamycin had better activity than the nanoparticle and the extract. Also, it was observed that on comparing the activity of the plant extract dissolved in distilled water and the other dissolved in DMSO; The one dissolved in DMSO had higher activity and this may be attributed to the high degree of solubility of the extract in DMSO. The responses of each bacterial strain against the samples are presented in Figures 7, 8, 9 and 10.

Table 2: IR frequency of plant extract

Frequency (cm^{-1})	Functional group	Peak appearance
3343	-N-H stretch	Strong
3035	=C-H stretch	Weak
2847	-C-H aldehydic	Variable
1425	-CH_3 Bend	Medium

Table 3: FTIR frequency of silver nanoparticle solution

Frequency (cm^{-1})	Functional group	Peak appearance
3353	OH Carboxylic acid stretch	Strong
2968	C-H Stretch	Weak
2148	C=C Stretch	Variable
2009	C=C Stretch	Variable
1615	C=C Alkene	Weak

Table 4: Frequency of silver nanoparticles

Frequency (cm^{-1})	Functional group	Peak appearance
3348	N-H stretch	strong
2932	-C-H stretch	variable
1602	C=C Aromatic	weak
1288	C-O-C stretch	strong

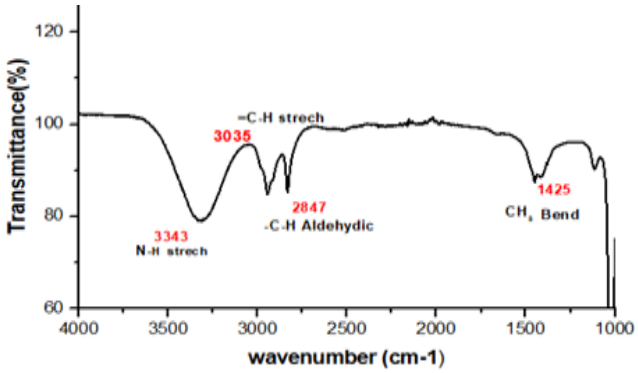

Figure 1: FTIR spectrum of plant extract

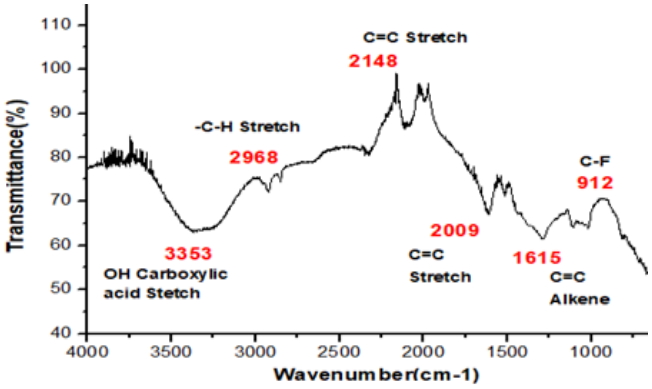

Figure 2: FTIR spectrum of silver nanoparticle solution

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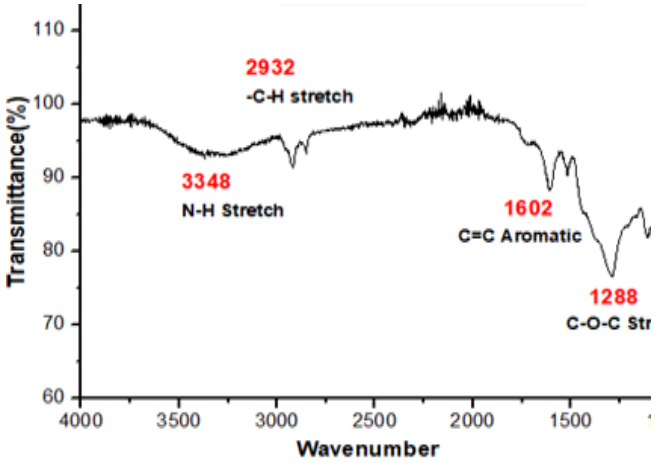

Figure 3: FTIR spectrum of solid silver nanoparticles

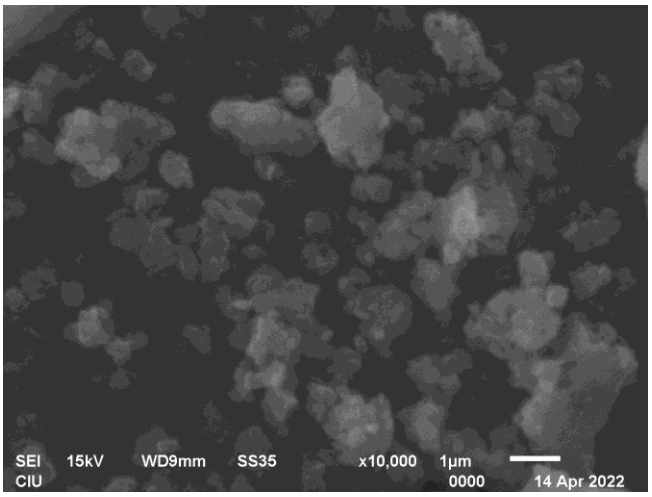

Plate 2A: SEM images of silver nanoparticles at ×10,000 Magnification

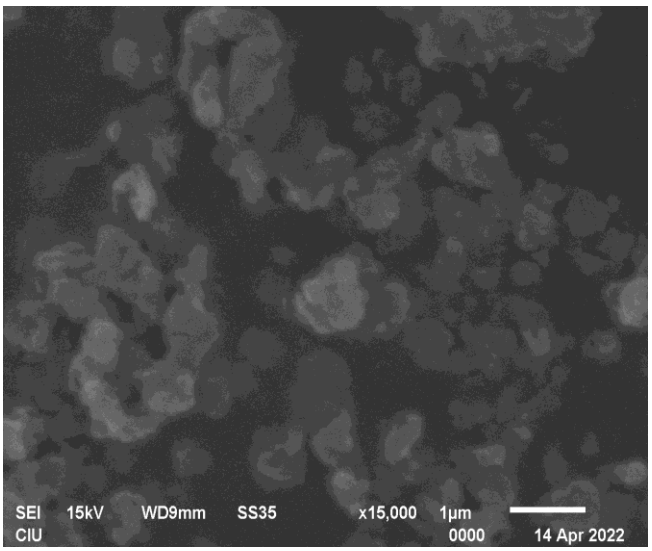

Plate 2B: SEM images of silver nanoparticles at × 15,000 Magnification

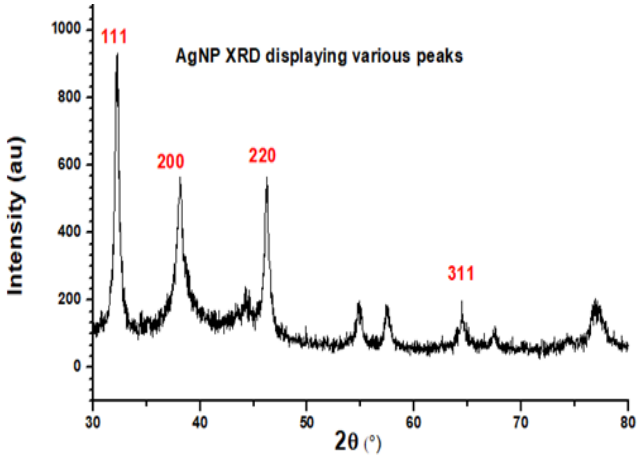

Figure 4: XRD Peaks of silver nanoparticles

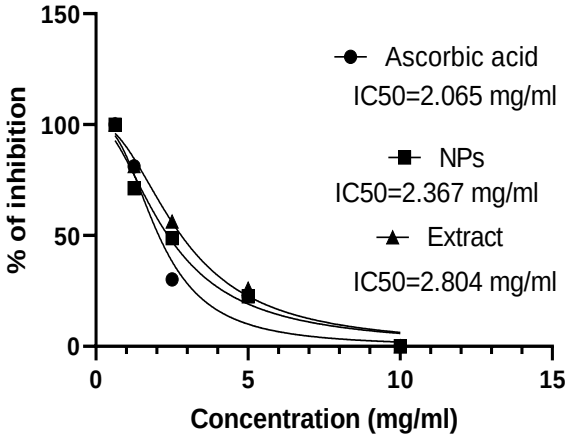

Figure 5: DPPH IC_{50} value of methanol extract, nanoparticles (NPs) and ascorbic acid

Conclusion

The bio-reduction of the Ag^{+} ions to silver nanoparticles have been attributed to flavonoids, tannins and saponins. The methanol extract and the silver nanoparticles had free radical scavenging activity with IC_{50} 2.804 mg/mL and 2.367 mg/mL respectively against DPPH. The IC_{50} of the methanol extract and silver nanoparticles against H_2O_2 were 4.986 mg/mL and 36.56 mg/mL respectively. PC methanol extract and the nanoparticle inhibited the growth of *E. coli*, *S. typhi*, *B. subtilis* and *S. aureus* with zone of inhibition ranging between 8-12 mm.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 5: Scavenging activity against DPPH free radical

Conc. (mg/mL	% SCA methanol extract	%SCA nanoparticles	%SCA of Ascorbic
10.00	80.56	57.22	99.22
5.00	85.04	62.54	99.34
2.50	90.22	68.72	99.38
1.25	94.52	74.02	99.65
0.625	97.72	80.76	99.75

Table 6: Hydrogen peroxide scavenging activity

Conc. (mg/mL	% SA methanol extract	%SA nanoparticles	%SA of ascorbic acid
10.00	70.54	63.65	81.39
5.00	80.21	78.79	87.53
2.50	82.98	82.88	90.02
1.25	92.13	89.14	94.04
0.625	94.67	92.67	96.62

SA = Scavenging Activity

Table 7: Mean zone of inhibition of growth in (mm)

Organisms	Nanoparticle	Extract in water	Extract in DMSO	Gentamycin	$AgNO_3$	H_2O	DMSO
S. aureus	9.0	2.3	8.0	20.0	9.7	0	0
E.coli	8.3	2.4	6.0	26.7	13.0	0	0
B. subtilis	9.0	2.2	5.7	18.7	13.0	0	0
S. typhi	12.0	3.0	7.7	22.3	9.0	0	0

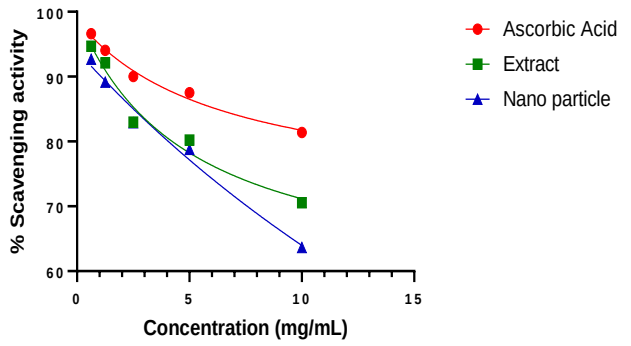

Figure 6: H_2O_2 IC_{50} value of methanol extract, nanoparticles (NPs) and ascorbic acid

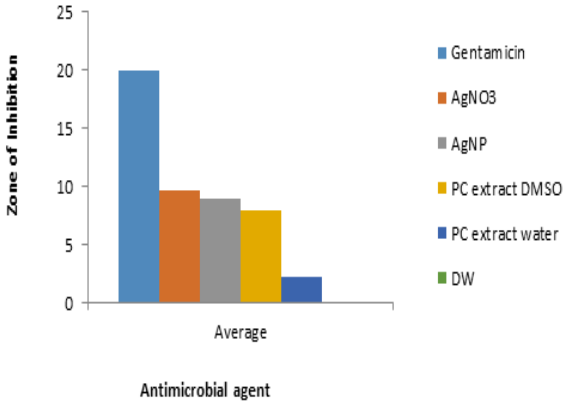

Figure 8: Zone of inhibition of samples against *S. aureus*

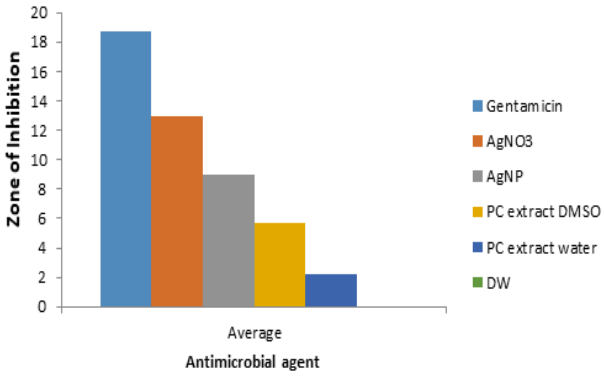

Figure 7: Zone of inhibition of test samples against *B. subtilis*

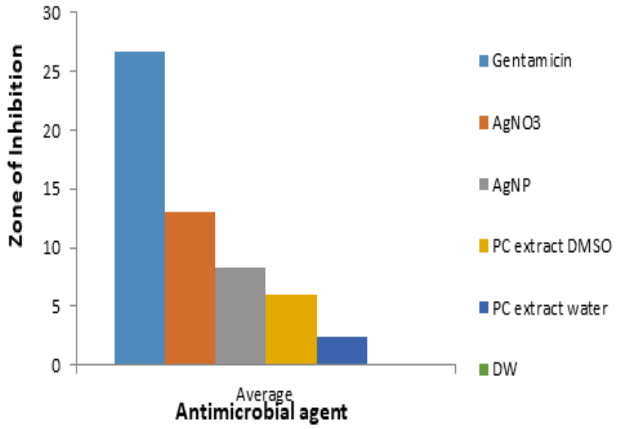

Figure 9: Zone of inhibition of samples against *E. coli*

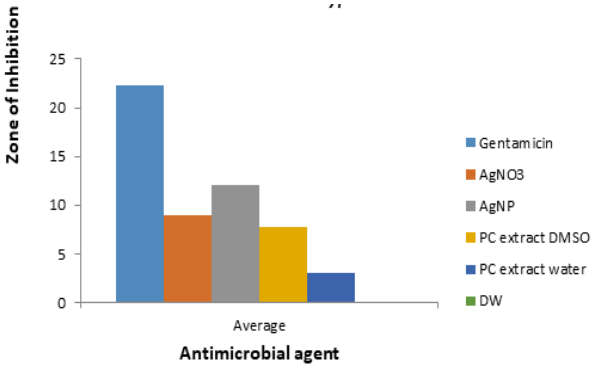

Figure 10: Zone of inhibition of samples against *S. typhi*

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