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Momordica charantia stem extract mediated biogenic synthesis of silver nanoparticles: optical and antimicrobial efficacy

Anuoluwa Abimbola Akinsiku[1,*], Kolawole Oluseyi Ajanaku[1], Abimbola Augustine Adebisi[1], Abiola Edobor-Osoh[1], Olanrewaju Aladesuyi[1], Taiwo Olugbenga Samson[2], Enock Olugbenga Dare[3,]**

[1] Department of Chemistry, Covenant University, Canaan Land, Nigeria
[2] Department of Biological Sciences, Covenant University, Canaan Land, Nigeria
[3] Faculty of Chemistry and Pharmacy, Institute for Organic Chemistry, Regensburg University, Germany
Corresponding author: * anu.akinsiku@covenantuniversity.edu.ng;
** enock-olugbenga.dare@chemie.uni-regensburg.de

Abstract. In this study, potential of aqueous stem extract of *Momordica charantia* for phytosynthesis of silver nanoparticles (Ag NPs) was evaluated using reduction method. The locally sourced biodiversity was extracted in water and methanol by cold extraction, after which it was screened for the presence of phytochemicals. Optical measurement was carried out with Uv-visible spectrophotometer, evidence of capping and reduction of Ag^{+} to Ag° by the biomolecules in the plant extract was achieved using Fourier transformed infra-red (FT-IR). Antimicrobial activity of the green synthesized nanoparticles was tested on clinical isolates. Visual evidence of Ag NPs formation was detected by change in reaction solution colour from light brown to deep brown, which resulted in excitation of surface plasmon resonance (SPR) between 400- 450 nm. Growths of microbes used were significantly inhibited ($P < 0.05$) with increasing concentrations of Ag NPS. Moderate activity was displayed by Ag NPs with the same value of MIC and MBC (12.5 mg/mL) on all the organisms considered except *P. aeruginosa* in which low activity was observed. The enhanced SPR displayed by Ag NPs qualifies them as potential materials for therapeutic and diagnostic uses.
Keywords: *Momordica charantia*, optical, antimicrobial, phytochemicals, silver nanoparticles

1. Introduction

Manipulation of particles structure and size on atomic scale (approximately 1-100 nm) is highly relevant in many fields, as the properties by atoms/molecules on nanoscale differ physically and chemically from their equivalent bulk. These new properties by nanostructured materials are of great advantages in various sectors like health, biomedical, drug delivery, chemical industries, optics, mechanics, energy science, photo-electrochemical, non-linear optical devices, food packaging industries, corrosion amongst others [1-5]. Also, bio-science uses vibrational spectroscopic for analysis or early diagnosis of diseases [6-9].

Optically active nanomaterials due to presence of surface plasmon resonance either by electromagnetic mechanism (vibrational spectroscopy techniques) or chemical mechanism (charge transfer that takes place between adsorbed molecules and nanostructured surfaces) can be applied to improve vibrational spectroscopic signals and amplimers as a result of their pure enhancing surface. Silver, copper and gold nanoparticles are often used in electromagnetic mechanism due to the possession

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of aforementioned properties [10]. *Momordica charantia* Linn. belongs to Cucurbitaceae family. It is a climber with tendrils, glabrous and lobed palmate leaves. Yoruba, Ibo and Hausa tribes in Nigeria named it *Ejinrin were, Alo-ose* and *Kakayi* respectively. It is used in traditional medicine for the treatment of diabetes, night blindness, convulsion, dysmenorrhoea, piles, sore, jaundice and aphrodisiac. The plant also possesses anthelmintic and antimicrobials properties. In China, *M. Charantia* is used for averting fever polydipsia [11-13].

"Green" synthesized nanoparticles are considered to be eco-friendly among various synthetic routes because of their biocompatibility. Ag NPs among metal nanoparticles obtained by plant-mediated green synthesis are mostly used for medical applications. Moreover, some plants have been exploited for the synthesis of Ag NPs [14-24]. Possibility of bio-nanoparticles formation is as a result of phytochemicals present in the plants considered for the syntheses. It is worth mentioning that phytochemical constituents vary among plants.

Issue of drug resistance (antibiotics) by disease causing microbes has been a lingering menace in public health. Report has shown that many new antibiotics that were developed in last decades exhibited less activity against these multi drug resistance bacteria [25]. Hence, in view of eco-friendly, bio-reducing agent and cost effectiveness, we report optical property and antimicrobial efficacy of green synthesized silver nanoparticles using stem extract of locally sourced *Momordica charantia* (*M. charantia*). Characterization of the newly synthesized Ag NPs was carried out with Fourier transform infra-red (FT-IR), photoluminescence and uv-visible spectrophotometer.

2. Experimental details

2.1. Materials and chemicals

M. charantia plant was obtained from open space in Nigeria (Atan-Iju, Ogun State) (Fig. 1), it was identified and authenticated at Forest Research Institute of Nigeria (FRIN). Voucher (FHI No. 109926) was also deposited at the herbarium headquarters in Nigeria. Pure silver nitrate ($AgNO_3$) of analytical grade purchased from Sigma-Aldrich company, UK was used without further purification, distilled de-ionized water (d-d water) and Whatman number 1 filter paper.

2.2. Preparation of aqueous leaf extract

Stem parts of *M. charantia* (20 g) were thoroughly washed with distilled water and homogenized using mortar and pestle. Slurry formed was then extracted with d-d water (200 mL), filtered through Whatman number 1 filter paper and then kept at 4ºC for phytochemical screening [26] and synthesis of silver nanoparticles (Ag NPs).

2.3. Phytochemical screening

Using standard procedure, *M. charantia* aqueous extract was screened to identify possible biologically active compounds present in it [27].

2.4. Phyto-synthesis and isolation of Ag NPs at room temperature

Nano silver was synthesized by phyto-reduction of Ag^+ to Ag°. Stem extract of *M. charantia* (40 mL) was mixed with 200 mL $AgNO_3$ solution of varied concentrations (0.5 – 3.0 mM) in 250 mL conical flasks. Resulting mixtures were stirred strongly and left at room temperature. Aliquot samples were taken at different time intervals and check for appearance of surface plasmon resonance (SPR). Ag NPs formation was informed by change in colour of the reaction mixture, which was confirmed by Uv-vis spectrophotometry. Isolation and purification of Ag NPs were carried out by repeated centrifugation at 15,000 rates per minute for 20 minutes. Colloidal nanosilver formed was re-dispersed in distilled deionized water and then centrifuged again for 20 minutes. The obtained suspension was oven dried at 70ºC and used for further analyses.

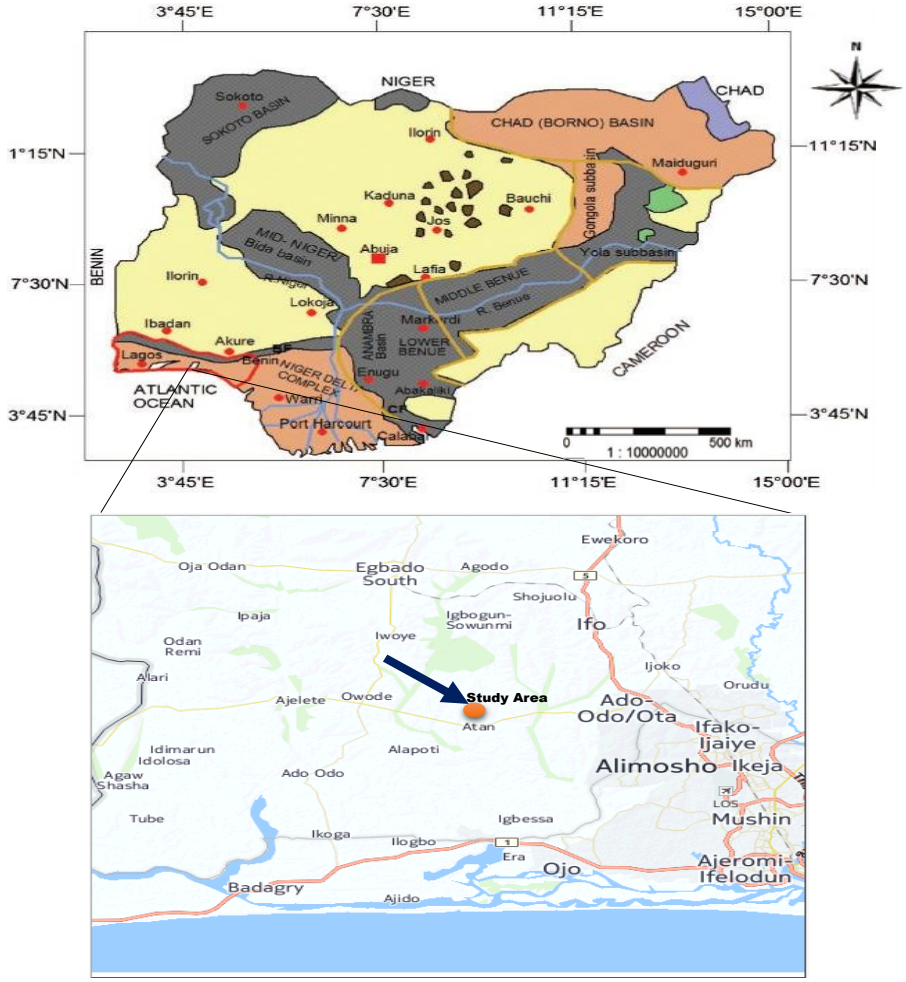


Figure 1. Map of Nigeria indicating plant collection site.

3. Characterization

3.1. Optical characterization

Uv-visible spectrophotometer (Double Beam Thermo Scientific GENESYS 10S) at a resolution of 1 nm was used to monitor the optical properties of Ag NPs. The spectra were measured at different time intervals between 200 and 800 nm wavelength. Double distilled water was the blank used. Photoluminescence (PL) emission of Ag NPs was measured by Perkin-Elmer 55 spectrophotometer at room temperature, each sample was placed in 1 x 1 cm quartz cell.

3.2. FT-IR analysis

For Fourier transform infra-red (FT-IR) analysis, spectrum indicating functional groups present in the green Ag NPs was recorded with Perkin-Elmer 100 series FT-IR by the KBR pellet over the range of 4000-500 cm^{-1} wavenumber, functioned at 4 ms^{-1} scan rates.

3.3. Antimicrobial study

3.3.1. Test microorganisms. Gram–negative bacteria - *Escherichia coli* and *Pseudomonas aeuruginosa*, Gram–positive bacteria - *Staphylococcus aureus* and *Streptococcus pyogenes*, fungi –*Candida albicans* and *Trichophyton rubrum* were the test organisms used. The freshly cultured clinical isolates were

obtained from Sacred Heart Hospital, Department of Medical Microbiology and Parasitology, Lantoro, Abeokuta in Nigeria.

3.3.2. Sensitivity of test organisms. Sensitivity testing was carried out using standard methods endorsed by National Committee for Clinical Laboratory Standards [28]. Antimicrobial activity was carried out using standard methods of agar well diffusion and Minimum Inhibitory Concentration (MIC), Minimum Fungicidal concentration (MFC) and Minimum bactericidal concentration (MBC).

3.3.3. Agar well diffusion method. The *M. charantia* stem extract-mediated Ag NPs were evaluated for their antimicrobial activities on clinical isolates using well plate agar diffusion method [14]. Inoculation was done on 9 cm diameter Mueller Hinton agar plate; 0.5 McFarland turbidity standard was used by adjusting the microbial cultures in it. The plate was flooded with 1 mL of each standardized test organism and then swirled. Excess inoculum was carefully decanted. A sterile cork borer was used to make wells (6 mm in diameter) on the agar plates. Aliquots of the Ag NPs dilutions (0.1 mL) was reconstituted in 50% DMSO at concentration of 100 mg/mL and applied on each of the well in the culture plates previously inoculated with the test organisms. However, each extract was tested in duplicate with 0.1 mL of 5 µg/mL ciprofloxacin as positive control for bacteria and fluconazole for fungi. These were then left on the bench for 1 hour for proper diffusion of the Ag NPs, [29]. Thereafter, the plates were incubated at 37ºC for 24 hours for bacteria and yeast; 28ºC, 72 hours for *T. rubrum*. Antimicrobial activity was determined by measuring the zone of inhibition around each well (excluding the diameter of the well) for each nanoparticle obtained from the plant extract. Duplicate tests were conducted against each organism.

3.3.4. Minimum inhibitory concentration (MIC) by tube dilution method. Sterilized test tubes (12) were arranged in a rack, afterwards 1 mL of sterile nutrient broth was added to tube labelled 2 to 10. Known nutrient broth concentration (1 mL) was added to tubes 1 and 2. Serial doubling dilution from tube 2 to tube 10 was made, while the remaining 1 mL was discarded. 1 mL of ciprofloxacin was added to tube 11 (positive control); and water to tube 12 (negative control). 1 mL of 0.5 McFarland was added overnight and broth culture to all the tubes and then covered. The experiment was incubated overnight at 37ºC and observed for the highest dilution showing no turbidity. The zone of inhibition was then verified and interpreted according to CLSI guidelines [28] and the MIC was determined.

3.3.5. Minimum bactericidal concentration (MBC). MBC was determined by using modified method by [30] Doughari *et al*. (2007) where 0.5 mL of the sample was removed from those tubes from MIC which did not show any visible sign of growth, it was then inoculated on sterile Mueller Hinton agar by streaking. The plates were then incubated at 37ºC for 24 hours. The concentration at which no visible growth was seen was recorded as the minimum bactericidal concentration [30].

3.3.6. Minimum fungicidal concentration (MFC). The method described by Doughari *et al.* [30] was used to determine MFC. 0.5 mL of the sample which showed no visible sign of growth during MIC screening was taken from the test tubes, and then inoculated on sterile potato dextrose agar by streaking. The plates were then incubated at 37ºC for 24 hours. The concentration at which no visible growth was seen was recorded as the minimum fungicidal concentration (MFC) [28].

4. Results and Discussion

4.1. Phytochemical analysis

Phytochemical screening carried out on the stem extract of *M. charantia* indicated the presence of saponin and alkaloids in water extraction which is less compared with alcohol medium solvent extraction (Table 1). The identified biomolecules when alcohol solvent was used are similar to previous work [27].

4.2. Optical properties of the Ag NPs

Results of uv-visible spectrophotometry analysis of samples taken at different intervals are shown in Fig.2. Colour dispersion formed with the appearance of SPR due to collective oscillation of the

conducting electrons in the Ag NPs was an evidence of Ag NPs formation. Observed surface plasmon band (400-450 nm) corresponds to silver nanoparticles [10]. It is obvious that the alkaloids and saponins present in water cold extraction of *M. charantia* stem were able to reduce Ag^{+} to Ag° at room temperature. However, the rate of reaction was very slow when compared with the same biosynthesis carried out at 70ºC in our previous study [31]. The nucleation and onset growth commenced at 120th hour (0.5 mM precursor solution), 90th hour (1.0 mM precursor solution) and 24th hour in 2.0 and 3.0 mM precursor solutions. The sluggish reaction was an indication that the reacting solutions were not supersaturated to generate extremely small particle size. Likewise, the biomolecules which acted as the reducing and capping agents in the stem were considered of less concentration compared with the quality in the leaf parts. The observed broad peaks proposed large particle sizes with higher level of aggregation, which were inevitable due to the longer reaction time. Biosynthesized Ag NPs also exhibited fluorophores with strong intensity of emission at 440 nm when excited at 329 nm in earlier study at elevated temperature of 70ºC (Fig. 4) [31]. The optical property displayed by the as-prepared nanoparticles is an indication for application as potential optical material.

Table 1. Qualitative analysis of *Momordica charantia* aqueous stem extract

Phytochemicals	*Momordica charantia* (stem)	
	Water extract	Methanolic extract
Tannins	-	+
Phenols	-	+
Flavonoids	-	+
Saponins	+	++
Glycosides	-	-
Anthraquinones	-	-
Alkaloids	+	+
Carbohydrates	-	-

(+) = Weak presence of phytochemical; (++) = Strong presence of phytochemical; (-) = Absence of phytochemical

4.3. FT-IR analysis

FT-IR spectrum indicating possible functional groups which were responsible for capping and stabilizing the as-prepared Ag NPs using stem extract of *M. charantia* is shown in Fig. 5. Bands at 3237, 2913, 1620, 1513 and 1021 cm^{-1} assigned to (−OH) stretching in saponins, (−CH) stretching in alkaloids, N-H bending, C=C stretching and C-N group respectively. Schemes 1-2 are the proposed mechanisms for the reaction, as the phytochemicals were adsorbed on the surface of nanoparticles. There is coordination via lone pair of electrons existing in the nitrogen attached to the methyl group via the C-N present in the alkaloid, resulting in formation of double bond, thereby reducing Ag^{+} to Ag°. The nanocluster was then capped by the alkaloid present in the phytochemical to provide stability.

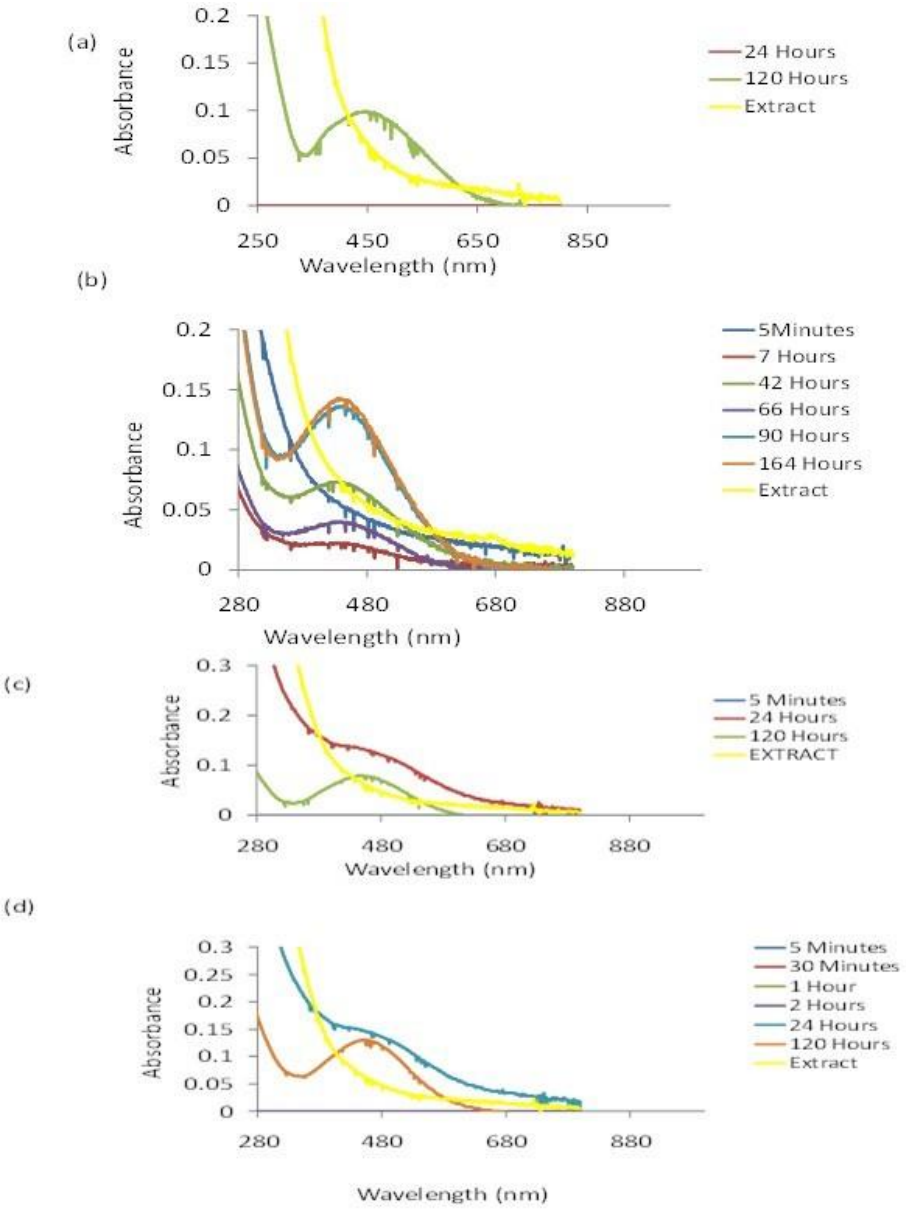


Figure. 2. Room temperature time-resolved uv-visible spectra of Ag NPs prepared from the reduced (a) 0.5 mM (b) 1.0 mM (c) 2.0 mM (d) 3.0 mM $AgNO_3$ solutions using the stem extract of *M. Charantia.*

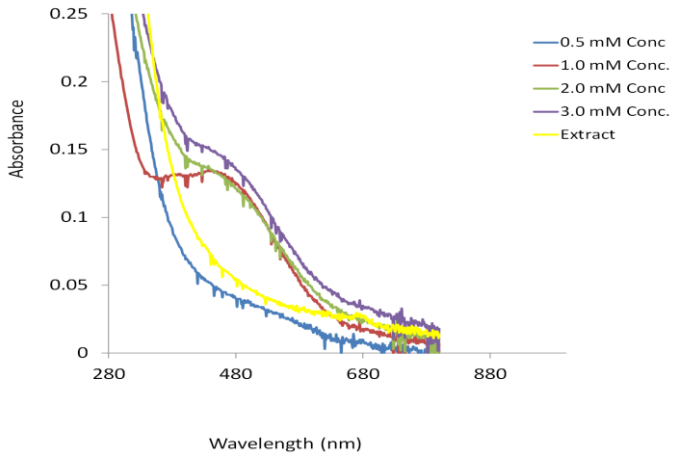


Figure. 3. Comparison of onset growth in the Ag NPs prepared from varied precursor concentrations using *M. charantia* stem extract at room temperature, 24 hours.

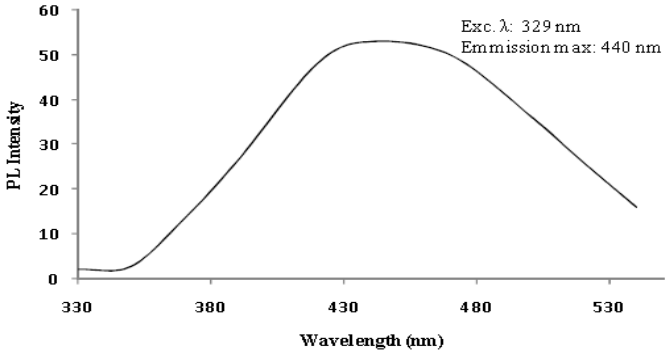


Figure. 4. The PL emission spectrum of Ag NPs synthesized using *M. charantia* stem extract at 70°C [31].

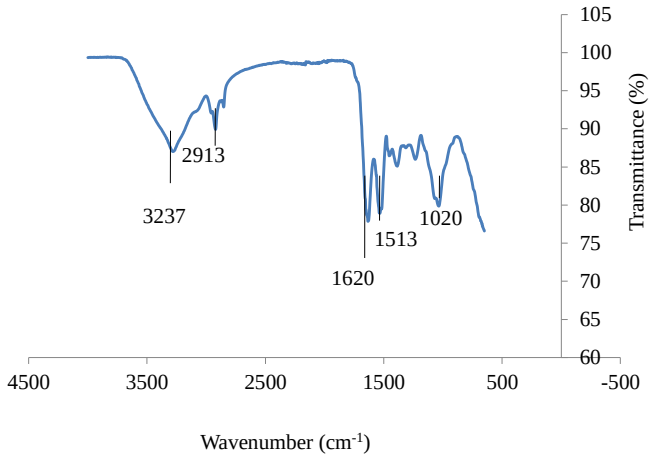
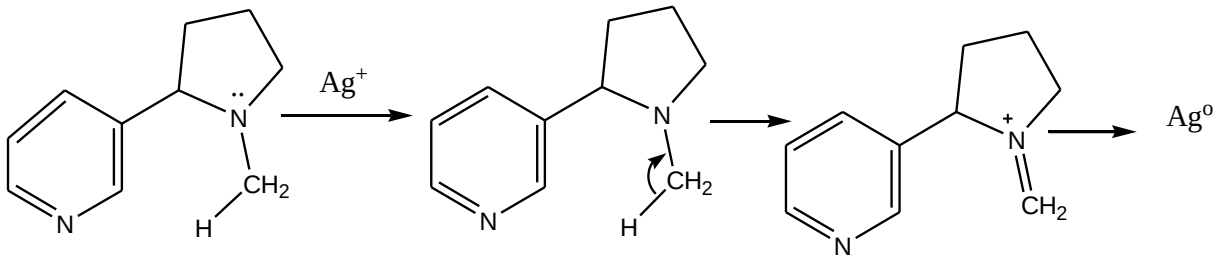
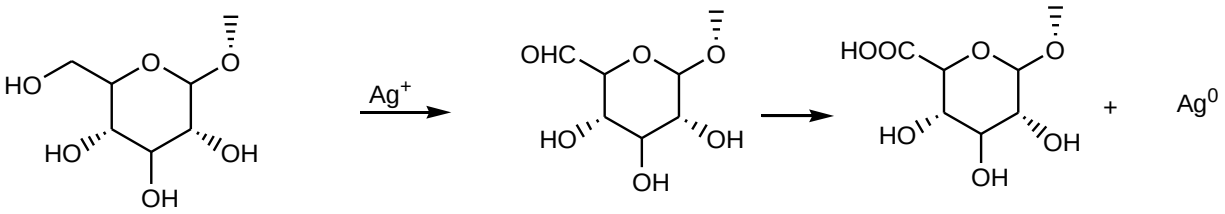


Figure. 5. FT-IR spectrum of Ag NPs.



Alkaloid

Scheme. 1. Reduction of silver ion to silver nanoparticles by alkaloid.



Saponin

Scheme. 2. Reduction of silver ion to silver nanoparticles by saponins.

4.4. Antimicrobial study

Sensitivity testing revealed activity of the phyto-synthesized Ag NPs based on size of zones of inhibition in millimetre (mm) (Fig. 6). Result from agar diffusion test showed that Ag NPs possessed antifungal and antibacterial properties as displayed in their zones of inhibition, which was pronounced at higher nanoparticles concentration on all the organisms except *P. aeruginosa* where very low activity was observed. There was no significant difference on the growth inhibition against the microbes at all concentrations of the Ag NPs as exposed in the ANOVA, SPSS statistical tool ($P > 0.05$). This indicated similar activity at all concentrations of the nanoparticles compared with the standard - ciprofloxacin (Bacteria) and fluconazole (Fungi).

Results of MIC, MBC and MFC showed activity of Ag NPs on *S. aureus, S. pyogenes, E. coli, P. aeruginosa, C. albicans* and *T. rubrum* with MIC and MBC value of 12.5 mg/mL. Least activity was detected against *P. aeruginosa* (50 mg/mL MIC and 100 mg/mL MBC) (Tables 2-3). This was due to penetration of the Ag NPs through the layer with the outer membrane composed by phospholipids and lipopolysaccharides (LPS) of the *E. coli* (complex gram-negative bacterium). Ag NPs was also able to pass through the thicker peptidoglycan cell wall layer which is accountable for rigidity and low activity in gram-positive bacteria, as detected in the MIC test carried out on *S. aureus* and *S. pyogenes* [32]. According to Marini *et al.* [33], the observed growth inhibition in bacteria could be related to reaction of thiol groups present in bacteria protein with the release of Ag^+ which slowed down or changed the replication of DNA.

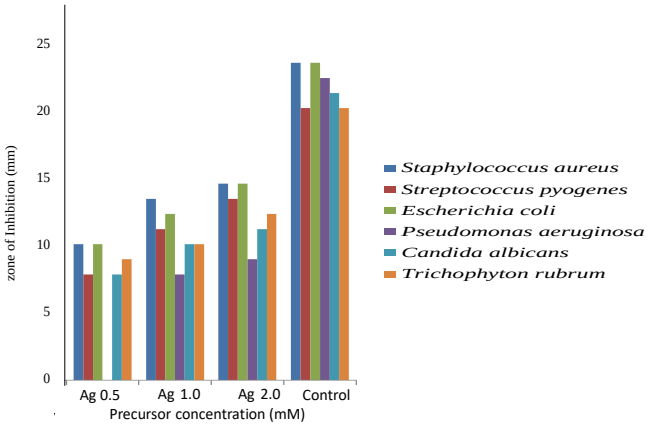


Figure. 6. Zones of inhibition by Ag NPs prepared from stem extract of *Momordica charantia.*

Table. 2. Sensitivity testing of organisms with standard deviation in zones of inhibition (Agar Diffusion Test)

Nanoparticles	Organisms/Mean zone diameter (mm) ± SD					
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Trichophyton rubrum*
Ag 0.5	9 ± 0.5	7 ± 0.2	9 ± 0.3	Nil	7 ± 0.2	8 ± 0.3
Ag1.0	12 ± 0.4	10 ± 0.2	11 ± 0.4	7 ± 0.1	9 ± 0.1	9 ± 0.1
Ag 2.0	13± 0.5	12 ± 0.4	13 ± 0.2	8 ± 0.2	10 ± 0.2	11 ± 0.3
STAT	***P > 0.05***	***P > 0.05***	***P > 0.05***	***P > 0.05***	***P > 0.05***	***P > 0.05***
Control	21 ± 0.8	18 ± 0.3	21 ± 0.2	20 ± 0.4	19 ± 0.6	18 ± 0.3
STAT	***CoControl vs Aa, Ba, Ca, Da, Ea, A, B, C, E, F-value 21.45, P < 0.05***					

Control -Ciprofloxacin (Bacteria) and Fluconazole (Fungi), mean zone inhibition (mm) ± standard deviation of three replicates. Ag 0.5 = Silver nanoparticles formed by reducing 0.5 mM $AgNO_3$ concentration solution, Ag 1.0 = Silver nanoparticles formed by reducing 1.0 mM $AgNO_3$ concentration solution, Ag 2.0 = Silver nanoparticles formed by reducing 2.0 mM $AgNO_3$ concentration solution, Ag 3.0 = Silver nanoparticles formed by reducing 3.0 mM $AgNO_3$ concentration solution.

Table. 3. Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC).

Organisms / Nano particles	Organisms/MIC, MBC & MFC (mg/mL)					
	Staphylococcus aureus MIC, MBC	*Streptococcus pyogenes* MIC, MBC	*Escherichia coli* MIC, MBC	*Pseudomonas aeruginosa* MIC, MBC	*Candida albicans* MIC, MFC	*Trichophyton rubrum* MIC, MFC
Ag 0.5	100, 100	100, 100	50, 100	100, 100	100, 100	100,100
Ag1.0	12.5, 25	25, 50	25, 50	100, 100	50, 100	50, 100
Ag 2.0	12.5, 12.5	12.5, 25	12.5, 25	50, 100	50, 50	50, 50
STATISTICS	***P < 0.05***	***P < 0.05***	***P < 0.05***	***P < 0.05***	***P < 0.05***	***P < 0.05***
Control	3.13	6.25	6.25	6.25	6.25	6.25
STATISTICS	***CoControl vs Aa, Ba, Ca, Da, Ea, A, B, C, E, F-value 34.06, P < 0.05***					

Control- Ciprofloxacin (Bacteria) and Fluconazole (Fungi). Ag 0.5 = Silver nanoparticles formed by reducing 0.5 mM $AgNO_3$ concentration solution, Ag 1.0 = Silver nanoparticles formed by reducing 1.0 mM $AgNO_3$ concentration solution, Ag 2.0 = Silver nanoparticles formed by reducing 2.0 mM $AgNO_3$ concentration solution, Ag 3.0 = Silver nanoparticles formed by reducing 3.0 mM $AgNO_3$ concentration solution.

5. Conclusion

In this study, simple and environmentally friendly protocol was adopted for the synthesis of Ag NPs using aqueous stem extract *M. charantia* as a reducing/stabilizing agent. The phyto-reduced Ag NPs were characterized by uv-visible spectrophotometer and FT-IR. In vitro antimicrobial study revealed that the Ag NPs possessed moderate antimicrobial activities. Moreover, the nanoparticles displayed optical characteristics which qualify them as potential materials for therapeutic and early diagnostic of diseases by providing message during optical imaging.

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