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Research Article

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Green ultrasonic synthesis, Characterization and Antibacterial activity of Silver and Gold Nanoparticles mediated by *Ganoderma lucidum* extract

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Abstract

Metal nanoparticles possess an extensive scientific and technological significance due to their unique physiochemical properties and their potential applications in different fields like medicine. Silver and gold nanoparticles have shown to have antibacterial and cytotoxic activities. Conventional methods used in the synthesis of the metal nanoparticles involve use of toxic chemicals making them unsuitable for use in medical field. In our continued effort to explore for simple and eco-friendly methods to synthesize the metal nanoparticles, we here describe synthesis and characterization of gold and silver nanoparticles using Gonaderma lucidum, wild non-edible medicinal mushroom. G. lucidum mushroom contain bioactive compounds which can be involved in the reduction, capping and stabilization of the nanoparticles. Antibacterial activity analysis was done on E. coli and S. aureus. The synthesis was done on ultrasonic bath. Characterization of the metal nanoparticles was done by UV-VIS., High Resolution Transmission Electron Microscope (HRTEM) and FTIR. HRTEM analysis showed that both silver and gold nanoparticles were spherical in shape with an average size of 15.82±3.69 nm for silver and 24.73±5.124nm for gold nanoparticles (AuNPs). FTIR analysis showed OH and -C=C- stretching vibrations, an indication of presence of functional groups of biomolecules capping both gold and silver nanoparticles. ***AgNPs showed inhibition zones of 15.5±0.09mm and 13.3±0.14mm while AuNPs had*** *inhibition zones of 14.510±0.35 and 13.3±0.50mm on E. coli and S. aureus respectively. The findings indicate the potential use of AgNPs and AuNPs in development of drugs in management of pathogenic bacteria.*

Introduction

Nanotechnology is a field of great demand in modern research due to its application in multiple biomedical fields. Nanotechnology deals with synthesis, strategy and manipulation of particle of size 1 – 100 nm [1]. The use of nanomaterials is particularly intriguing due to their high surface area to volume ratio and their unique physical and chemical properties. The application and properties of nanoparticles depend on their size, shape, morphology and composition [2]. The metal nanoparticles have potential applications in the fields of catalysis, photonics, medical and bioengineering [3]. The conventional methods used for their synthesis are not environmentally friendly as they involve use of toxic chemicals [4].

Green synthesis of metal nanoparticles offers an alternative method. It is simple, rapid and environmentally friendly. Green synthesis involves use of fungi, plant extracts, enzymes and bacteria [5-7]. Mushrooms contain bioactive components like proteins, flavonoids and phenols which can be used in reducing, capping and stabilizing nanoparticles [8].

The rise in emerging infectious diseases and their impact in increased incidences of drug resistance are well documented [9]. Thus, there is a pressing demand to discover novel strategies and identify new antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections [10]. Metal nanoparticles of different sizes tend to have different antimicrobial activities. The greatest challenge in biosynthesis is that, different plants produce metal nanoparticles of different sizes and shapes [11].

AgNPs have been reported to exhibit strong antiseptic, antibacterial, antifungal and antiviral properties thus making them to be of great interest in the medical field [12]. Gold nanoparticles (AuNPs) have also shown antibacterial, antifungal and anticancer activity [13]. AuNPs have also been used in the treatment of cancer tumors. Cancer cells along with bacteria and viruses can be damaged by nanophotothermolys with laser and gold nanoparticles [14].

In this study, *G. lucidum* was used to synthesize AgNPs and AuNPs. *G. lucidum* is known for its bioactive compounds for anti-oxidant and anti-inflammatory activities [15]. It contains phytochemicals like flavonoids, polyphenols, terpenes and steroids which can be used as reducing, and capping agents in the assembly of AuNPs and AgNPs [16]. An ultrasonic bath was used as an agitator in the synthesis.

Keywords: Gold, Silver Nanoparticles, *Ganoderma Lucidum*, Antibacterial Activity, Sonication

Materials and Methods

Materials

G. lucidum (Figure 1) was collected from Trans Nzoia County in Western Kenya. It was transported to Kenyatta University, identified by taxonomist from Museums of Kenya, Nairobi and voucher specimen deposited at their herbarium.

Figure 1: *Gonaderma lucidum*

Sample Preparation

The *G. lucidum* was washed thoroughly with tap water to remove dust particles, debris and any other impurities. It was then washed with distilled water and shade dried. The dry *G. lucidum* was cut into small pieces then pulverized using Retsch 200 grinder. 10 g of the *G. lucidum* was mixed with 100ml of water in a conical flask then immersed in an ultrasonic bath for 3 hours at 60 ºC. The extract was filtered using what-man filter paper No. 1. The filtrate was centrifuged for 20 minutes at 3500 rpm to remove any fine plant materials. The supernatant liquid was stored at-4ºC until further use.

Synthesis of Gold and Silver Nanoparticles

5 ml of 5g/ml *G. lucidum* extract and 45 ml of 0.1M gold (III) chloride solution was mixed in a conical flask. The mixture was then immersed in ultrasonic bath and formation of the AuNPs monitored via colour change and by the use of UV-Vis spectrometer. Similarly the synthesis of AgNPs was done by mixing 5 ml of 5g/ml *G. lucidum* extract and 45 ml of 0.001M $AgNO_3$ solution in conical flask then immersed in sonicator.

UV-Vis Spectroscopy

UV-Vis spectrometer (Specord 200 Analytik jena) was used to monitor the formation of AuNPs and AgNPs. Scanning was done at regular intervals to check the intensity of the optical density of the absorption band in the range from 300 nm to 800 nm. Surface plasmon resonance(SPR) band for AgNPs absorb in the range 400nm to 450nm while those of AuNPs appear in the range of 500nm to 600nm. Water was used as blank [17, 18].

Fourier Transform Infra-Red (FTIR) Spectroscopy

Measurements were done using FTIR (Shidmanzu IRt racer-200) to determine the functional groups of biomolecules capping and stabilizing the nanoparticles. The sample was centrifuged at 5,000 rpm for 20 minutes. The liquid was decanted from the centrifuge tube to obtain solid nanoparticles. The solid was mixed with KBr, ground then minipressed to a pellet for FTIR analysis.

High Resolution Transmission Electron Microscope (HRTEM)

The size, shape and morphology of the nanoparticles were determined by HRTEM (FEI Tecnai F20). The samples for HRTEM analysis were prepared by drop coating the AgNPs/AuNPs solution on to carbon-coated copper HRTEM grids [19].

Results and Discussion

Visual Observation

Formation of the nanoparticles was monitored by observing the color change of the solution. The mixture of *G. lucidum* extract and $AgNO_3$ solution changed from light yellow to orange after 3 hours. Thereafter, there was no observable change (Figure 2). The colour change was an indication of formation of AgNPs [20].

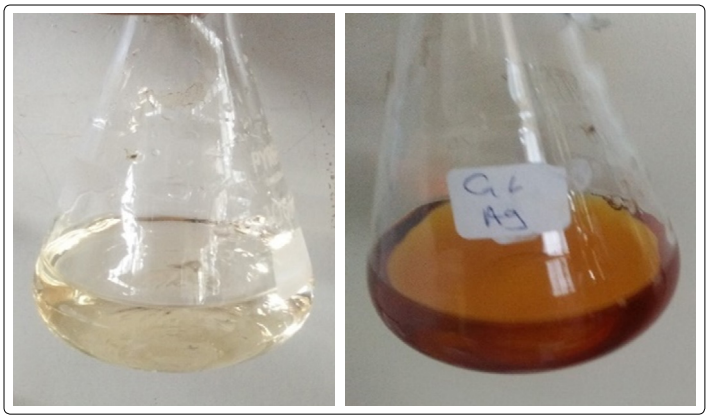

Figure 2: Colour change of *G. lucidum* extract and $AgNO_3$ mixture at (i) 0 minutes (ii) 180 minutes on sonication.

The mixture of the *G. lucidum* extract and gold (III) chloride solution changed from light yellow to dark purple in one hour (Figure 3). The same colour change was observed by Priya Tharishini *et al.*, when they synthesized gold nanoparticles using *Cassia auriculata* [21].

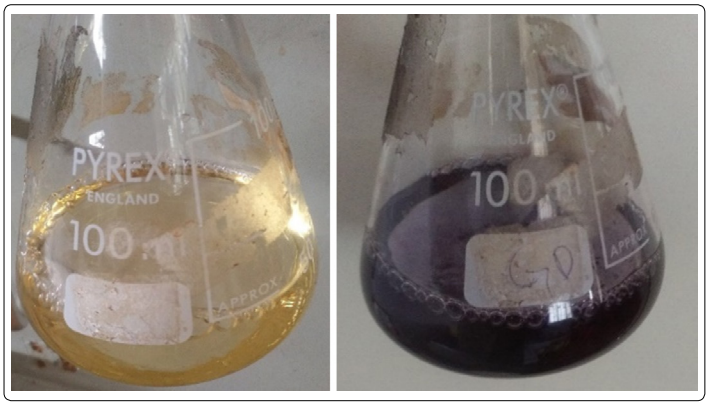

Figure 3: Colour change of *G. lucidum* extract and Au_2Cl_6 mixture at (i) 0 minutes (ii) 60 minutes on sonication.

UV-Vis Spectra Analysis

The formation of metal nanoparticles was further characterized using UV-Vis spectroscopy. The surface plasmon resonace (SPR) band for AgNPs appeared at λ_{max} 417 nm (Fig. 4). The band was due to collective oscillation of electrons in the conduction band of AgNPs in resonance with a specific wavelength of incident light [22]. The optical density increased steadily up to 3 hours. Increase in optical density was due to increase in the concentration of the AgNPs as observed earlier by Maillard *et al.*, on his study on silver growth by surface plasmon enhanced photoreduction of adsorbed silver ions (Ag^{+}) [23]. There was no shift of absorption band with reaction time. This was an indication that the AgNPs formed were stable, uniform in size and well dispersed in the solution.

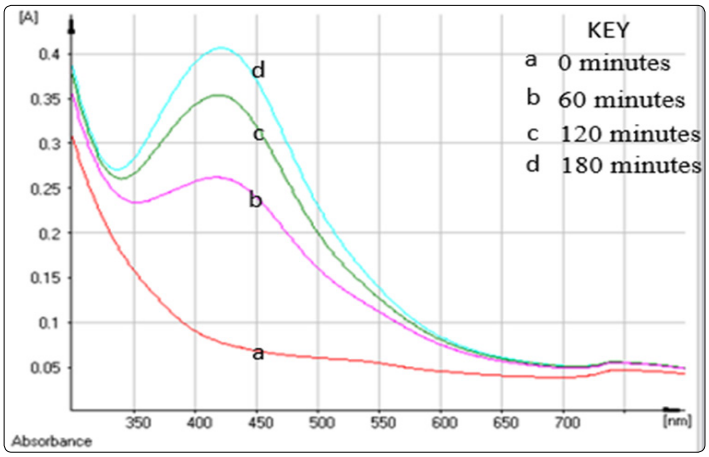

Figure 4: UV-VIS spectra for *G. lucidum* AgNPs

The UV-Vis absorption spectrum of the synthesized AuNPs appeared at λ_{max} 550 nm (Fig. 5), characteristic of AuNPs [18]. The shape, broadness and absorbance intensity of the spectrum remained constant. This observation indicated that the synthesized AuNPs were stable and well dispersed.

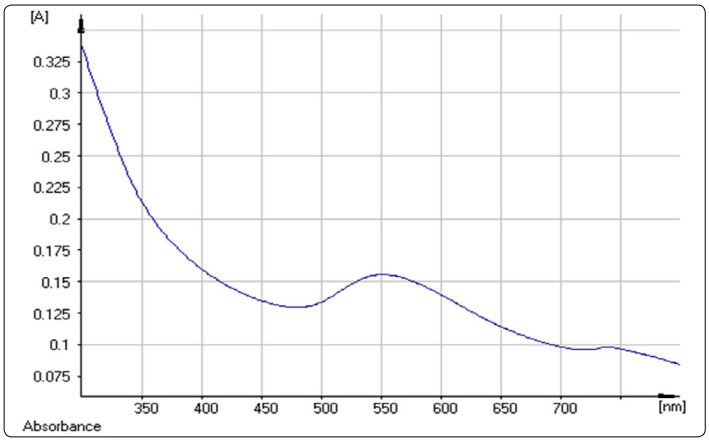

Figure 5: UV-Vis spectrum of *G. lucidum* AuNPs

High Resolution Transmission Electron Microscopy (HRTEM)

The TEM analysis confirmed that the synthesized AgNPs were spherical, with smooth surface and mono dispersed with no agglomeration as depicted in Figure 6. The AgNPs had an average size of 15.82±3.69nm.

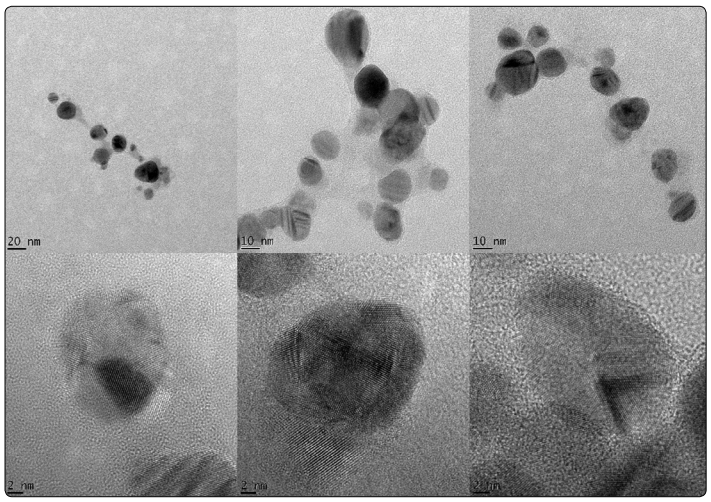

Figure 6: HRTEM micrographs of AgNPs at different magnification

The results of Energy Dispersive X-ray (EDX) analysis (Figure 7) affirmed that the nanoparticles were pure silver with peaks at 3.0KeV, characteristics of pure silver [24].

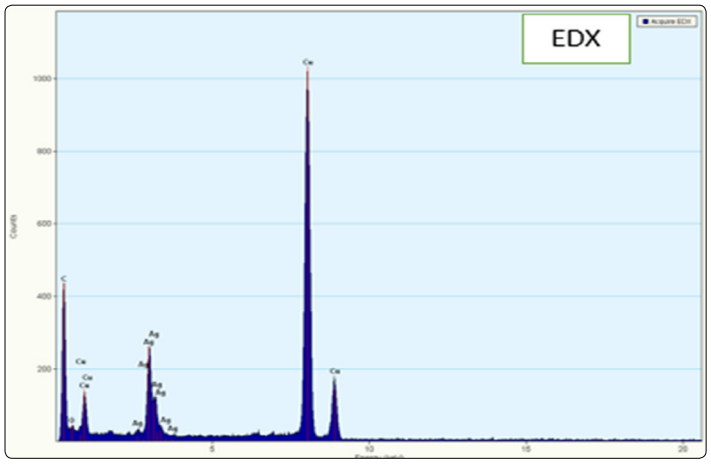

Figure 7: EDX spectrum of the AgNPs

Selected Area Electron Diffraction (SAED) analysis showed the synthesized AgNPs and AuNPs were crystalline in nature (Fig.8). The circular shinny sports were characteristic of crystalline nanoparticles [25].

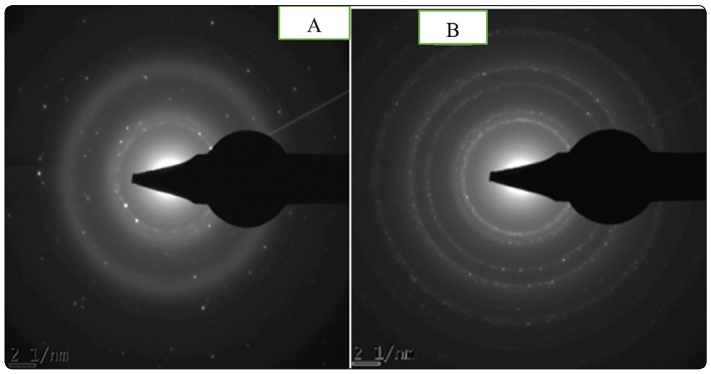

Figure 8: SAED micrographs, A-for AgNPs and B-for AuNPs

The TEM images (Fig. 9) of AuNPs showed that the nanoparticles had a smooth surface, monodispersed with no agglomeration. The average size of the nanoparticles was 24.73±5.124 nm (Figure 9), bigger in size than AgNPs.

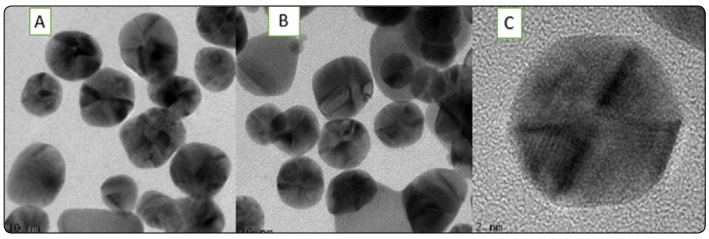

Figure 9: A, B and C-HRTEM images of AuNPs at different magnification

AuNPs EDX spectrum (Figure 10) had peaks at 2.0KeV characteristics of pure gold [26].

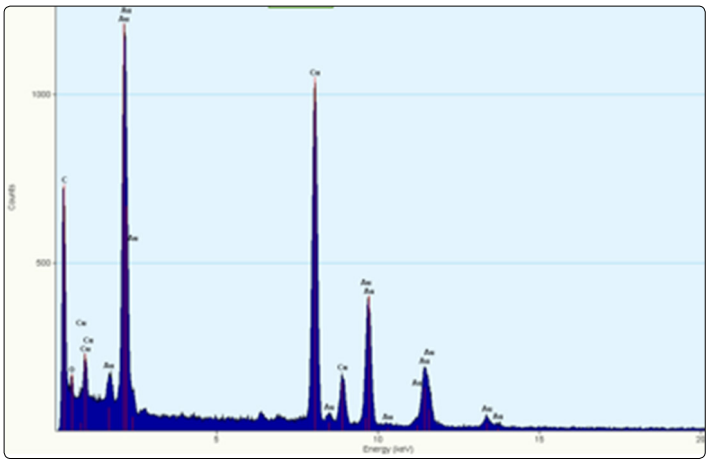

Figure 10: EDX spectrum of the AuNPs

FTIR Analysis

The possible functional groups of potential biomolecules responsible for the synthesis of the nanoparticles were identified using FTIR analysis. FTIR spectrum analysis of both AgNPs and AuNPs showed absorption peaks at 3297.37 cm^{-1} and 1637.59 cm^{-1} (Figure 11) for O-H and C=C stretching. This showed that phenolic compounds could be involved in capping and stabilizing the nanoparticles.

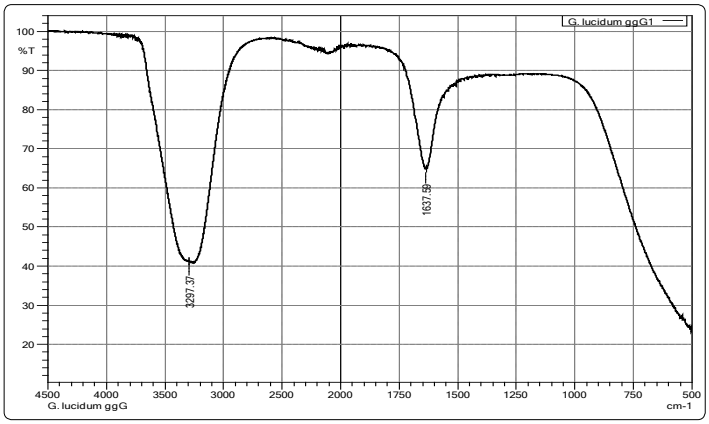

Figure 11: FTIR spectrum of *G. lucidum* mediated silver and gold nanoparticles.

Antibacterial Activity of Synthesized Gold and Silver Nanoparticles

The AgNPs antibacterial activity was tested on Gram-negative and Gram-positive bacteria by disk diffusion method. The zones of inhibition were recorded after 24 hours of inoculation (Table 1). The AgNPs had inhibition zone of 15.5±0.09mm towards *E. coli* compared to the ciprofloxacin (positive control) which had an inhibition of 33.4±0.542. AgNPs on *S. aureus* showed an inhibition zone of 13.3±0.14 mm against vancomycin (positive control) with inhibition of 22.1±0.12 mm. *G. lucidum* extract (negative control) had inhibition zone of 7.0±0.00 mm on *E. coli* and 6.75±0.15 mm on *S. aureus*. Water had no effect on the bacteria under the study. The bacteria were susceptible to AgNPs.

Table 1: Inhibition zones of AgNPs and AuNPs on *E. coli* and *S. aureus*

Sample	zones of inhibition (mm)	
	E. coli	***S. aureus***
AgNPs	15.5±0.09	13.3±0.14
AuNPs	14.510±0.35	13.3±0.50
G. lucidum extract	7.0±0.00	6.75±0.15
Vancomycin	N/A	22.1±0.12
Ciprofloxacin	33.4±0.542	N/A
Distilled water	6	6

NB: N/A- not applicable

AuNPs showed inhibitory effects on both *E. coli and S. aureus* (Table 1). The nanoparticles. exhibited inhibition zones of 14.510±0.35mm and. 13.3±0.50mm against *E. coli and S. aureus respectively.* 7.0±0.00mm. The positive controls, ciprofloxacin and Vancomycin had inhibition zones of 33.4±0.443mm and 22.1±0.12 mm respectively.

AuNPs and AgNPs showed potential to inhibit growth of *E. coli* and *S. aureus.* The nanoparticles can penetrate the bacteria cell membrane by biophysical interactions through biosorption and cellular intake causing depletion of the membrane and toxicity to bacteria [27, 28].

Conclusion

The study showed that, AgNPs and AuNPs were successfully prepared cheaply, rapidly and in a ecofriendly method using *G. lucidum* extract.

Both AuNPs and AgNPs had smooth surface, pure, crystalline and were quasi-spherical. Both *E. coli* and *S. aureus* were susceptible *to* AuNPs and AgNPs.

Potential Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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