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Antifungal Activity of Nano Silver against *Candida Albicans* and *Aspergillus Niger*

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Abstract: Owing to development of resistance by microorganism to the existing drugs, it has become very imperative to develop alternatives. This led to the delivery of nanotechnology, and recently, researchers have paid attention to this field to solve the problem of resistance. The green synthesis of Ag NPs from *Moringa Oliefera* roots and their efficacy on two human fungi pathogens is reported in this work. The reduction of Silver ion to elemental silver as well as characterization was evident from the visual colour change to dark brown and the information deduced from UV-Visible, FT-IR, SEM, EDX and XRD spectrophotometers. The promising silver NPs possess good antifungal effect against *Candida Albicans* and *Aspergillus Niger* from the measured zone of inhibition values. These NPs are capable of inhibiting the growth of the two pathogens.

Keywords: Antifungal Activity; *Aspergillus Niger; Candida Albicans*; Green Synthesis; *Moringa Oliefera*; Silver Nanoparticles

1. Introduction

Diseases of microbial infections and increased resistance to antibiotics are urgent problems that require immediate solutions.[1] For this study, two human fungal pathogens were selected. These are *Candida Albicans* and *Aspergillus Niger*. The former is a microorganism frequently isolated from human infections.[2] Usually it is harmless but can turn into an opportunistic organism in immune-compromised or immunologically deficient individuals.[3] The use of antifungal in an indiscriminate form has increased the resistance of the existing drugs.[4] While *Aspergillus Niger* is one of the most important microorganisms used in biotechnology. It is associated with fewer problems, but some few medical reports such as lungs ailment in severely immune-compromised patients.[5] It is economically important fermentation agent as a source of citric acid.[6]

Nanotechnology nowadays is a promising field that provides wide variety of applications involving the synthesis and potential uses of nanoparticles usually of sizes in the range of 1-100 nm.[7] Nanoparticles are synthesized as alternative agents against the drug-resistant microorganisms to deal with the bacterial strain resistance.[8, 9] Example of those NPs is the green synthesized Silver NPs, an important alternative agent that is biocompatible, less toxic and environmentally friendly. The synergy of microbial inhibition of Silver NPs improves antimicrobial effect.[10, 11]

Moreover, Ag NPs comparatively is usually preferred over other monometallic NPs in almost all fields. Its application has increased significantly especially because they have unique photoelectric, physicochemical properties, high reactivity which makes them useful in molecular diagnostics, drug delivery, electronics, and catalysis. They are used as antibacterial agents and are chemically stable due to their large surface area-to-volume ratio.[12-14]

Many physical and chemical methods have been used for the synthesis of nanoparticles, but usually employ the use of toxic and expensive reducing agents which in turn pose great environmental hazards.[15] However, green chemistry approach minimizes the use of chemicals by using biological materials such as medicinal plants, animals and fungi. It is thus more popular because it provides clean, eco-friendly, cost effective, safe, conveniently utilizable and beneficial way to the synthesis of metal NPs for the large-scale production.[16,17] In this present study, Ag NPs were synthesized using the rich reducing potential of *Moringa oliefera roots*. The synthesis was followed by spectroscopic investigation using UV-visible for optical measurement, FT-IR for functional group investigation, and SEM-EDX for morphology and elemental analysis as well as XRD for phase's variety and size determination.

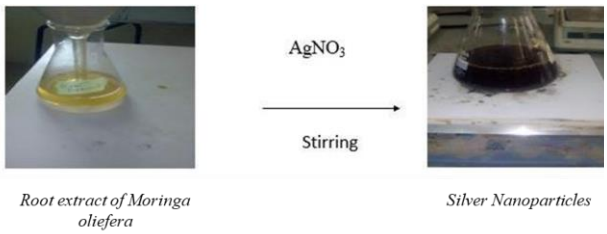

Fig. 1. Formation of Ag NPs.

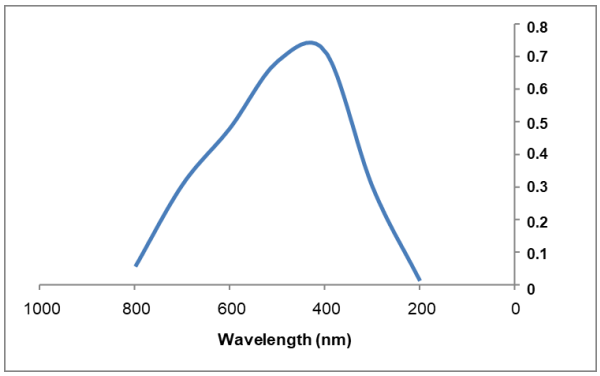

Fig. 2. UV-Visible spectra for Ag NPs.

2. Materials and Methods

2.1. Sample Collection

Fresh roots of *Moringa oliefera* were collected in an open space from Gombe metropolis, Gombe State, Nigeria. The samples were identified and authenticated by a botanist in the department of Botany Gombe State University. The microbial cultures were procured from the general Laboratory at the Federal Teaching Hospital, Gombe.

2.2. Sample Preparation

The aqueous root extract was prepared by washing the collected sample thoroughly under running tap water and rinsed severally with distilled water followed by shed- drying to remove residual moisture. The dried materials were ground using mortar and pestle into fine particles. A 30 g portion of it was weighed and dispersed in 200 ml of sterile distilled water in a 500 ml glass beaker and boiled at 100˚C for 30 min and was allowed to cool. Thereafter, the solution was filtered through Whatman No. 1 filter paper and the filtrate was used immediately for the synthesis of silver nanoparticles.

2.3. Synthesis of Ag NPs

Two hundred millilitres of the filtrate obtained after boiling the plant extract was measured in a measuring cylinder and transferred into a 2000 ml beaker. A 500 ml of 0.001M $AgNO_3$ solution was added gradually to the boiling root extract with occasional stirring using a glass rod for 20 minutes. There was a visual color change indicating the formation of NPs, nanoparticles were allowed to settle was centrifuged at 35000 rpm for 10 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

2.4. UV-Vis Spectrophotometric Analysis

Carey Series UV-Vis spectrophotometer Agilent Technology was employed for optical measurement and investigation of the bio-reduction of silver ions operated between the ranges of 250-800 nm wavelengths by sampling 1 ml aliquot liquid obtained after the synthesis against distilled water as the reference solvent. A spectrum was plotted with absorbance against wavelength (nm).

2.5. FT-IR Spectroscopy Measurement

For removing the biochemical compounds or uncapping ligands of the nanoparticles, the 500 mL residual solution of reaction mixture was centrifuged at 10000 rpm for 30 min and the precipitate re-suspended in 10 mL organic free water. The centrifugation and re-suspension processes were repeated 5 times. The purified suspension was dried in an oven at 60°C to obtain the stable powder and analysed by Fourier transform infrared spectrum (FTIR), Perkin Elmer-RX1 spectrophotometer.

2.6. Scanning Election Microscopy (SEM) and (Energy Dispersing X-ray Analysis (EDX)

Scanning Electron Microscope (SEM) analysis was carried out using scanning electron microscope machine compatible with EDX machine. This was used to investigate the morphology and structure of Ag NPs.

2.7. X-ray Diffraction Spectroscopy (XRD)

XRD was applied to investigate the phase variety of the solid Ag NPs. The result it produced is very sensitive because it is directly from the molecular arrangements of the crystalline material. The synthesized silver nanoparticles were studies with CUK α radiation at voltage of 45 kV and current of 20 MA with scan rate of 0.030/s.

2.8. Fungal Culture

The effect of antifungal activity of Ag NPs was assessed by agar well diffusion method. The bio-synthesized Ag NPs were evaluated against two human fungal pathogens, *Aspergillus Niger*, and *Candida Albicans*. About 38 g of potato dextrose agar was dissolved in 1000 ml of de-ionized water, the mixture was heated on a hot plate and then autoclaved for 15 minutes at the temperature of 121˚C. The mixture was allowed to cool and then poured on petri dishes after the nutrient agar has solidified inside the petri dish, pure isolate of the fungi *Aspergillus Niger* and *Candida Albicans*. Test fungal samples each were grown on different agar plate at 27˚C for 24 hours in an incubator. Four wells were made in PDA plates of 6 mm diameter using sterile cork borer.

3. Results and Discussions

3.1. Ultra Violet Spectroscopy Measurement

The formation of nanoparticles was visually observed from the color change from yellow to dark brown after addition of the metal precursor ($AgNO_3$) to the root extract, and heating for 30 minutes with occasional stirring. An illustration of the color change is depicted

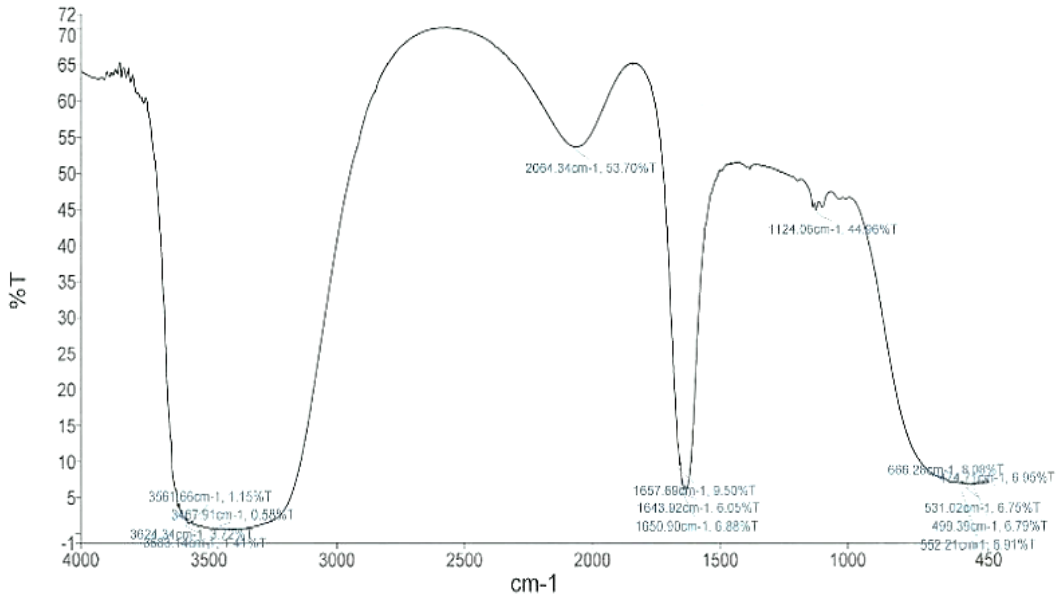

Fig. 3. FTIR Spectra of *Moringa oliefera* root extract.

Table 1. EDX analysis of Ag NPs

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
47	Ag	Silver	42.42	82.87
8	O	Oxygen	29.79	8.63
6	C	Carbon	17.12	3.72
19	K	Potassium	2.26	1.60
7	N	Nitrogen	4.73	1.20
13	Al	Aluminums	1.28	0.63
14	Si	Silicon	0.65	0.33
22	Ti	Titanium	0.35	0.30
12	Mg	Magnesium	0.67	0.30
16	S	Sulfur	0.48	0.28
15	P	Phosphorus	0.25	0.14

Table 2. Result for Antifungal Studies of Silver Nanoparticles against *Candida Albicans* and *Aspergillus Niger*

	Zone of Inhibition of Ag NPs (mm)					
Organisms	**100µg/L**	**200µg/L**	**300µg/L**	**400µg/L**	**500µg/L**	**Control (Fulcin)**
Candida a.	8.5	11.5	12.5	17	17	15.5
Asp. n.	7	9	12.5	12.5	13.5	16

in Fig. 1. After cooling the solution, the supernatant liquid was decanted and Ag NPs settled at the button, thereafter, evaporated and dried. The supernatant liquid was subjected to UV spectroscopy. The maximum absorption wavelength was 400 nm. Mathur *et al*.[18] had similar finding with max at 400 nm. Mini *et al*., [19] obtained his maximum wavelength at 428 nm. Plot of absorbance against wavelength of the synthesized Ag NPs from root extract is shown in Fig. 2.

3.2. FTIR Analysis

The analysis was carried out to determine the phyto- chemicals in the root extract of *Moringa Oliefera* (Fig. 3) involved in the formation of Ag NPs. The noticed functional groups in the raw extract were peaks at 3561.66 cm^{-1}, 3624.34 cm^{-1} likely due to hydroxyl group present in water, 2064 cm^{-1} due to sp carbon of alkyne, 1657.69 cm^{-1} corresponding to sp^2 carbon of alkene. Also observed were sharp peak at 1500 cm^{-1}, 1124 cm^{-1}. These were the possible functional group as they were replaced in the spectra of silver nanoparticle. The new absorption bands that were displayed in the spectra of the Ag NPs (Fig. 4) were at 3420 cm^{-1}, 2390 cm^{-1}, 1762 cm^{-1}, 1620 cm^{-1}, 1383 cm^{-1} Danbature et al.,[20] and Flora et al.,[21]

3.3. SEM Analysis

The SEM image obtained for silver nanoparticles from *Moringa oliefera* root is shown in Fig. 5. The Scherrer rings characteristics of silver clearly seen in the SEM image implied the particles are crystalline in nature, they are scattered over the surface and no aggregates are noticed. The difference in size is possibly due to the fact that nanoparticles are been formed at different times. Nanoparticles synthesized from *Papaver sonmiferum* by Wali et al.[22] in a similar research were reported to be crystalline in nature.

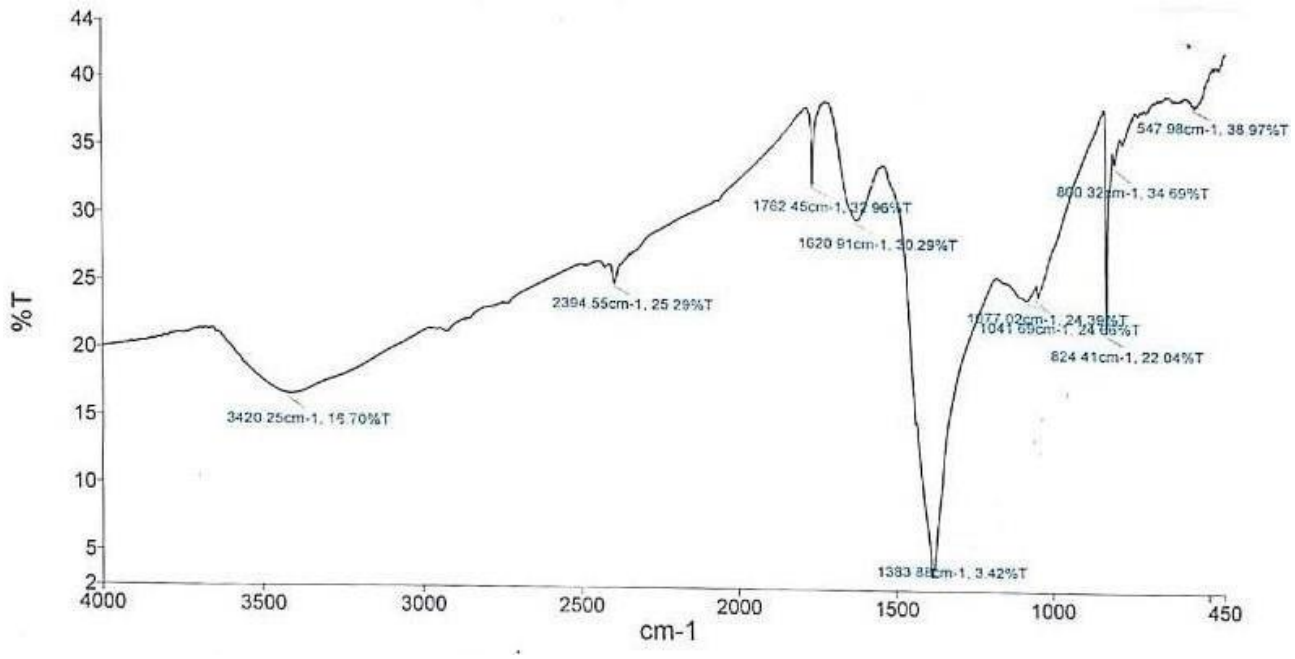

Fig. 4. Spectra of AgNps from *Moringa oliefera* root.

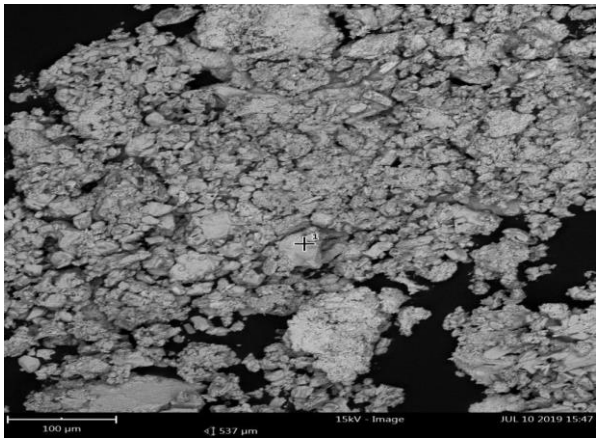

Fig. 5. SEM analysis of silver nanoparticles for *moringa oliefera* roots.

3.4. Energy Dispersing X-Ray Analysis (EDX)

Energy dispersive x-ray analysis (Elemental analysis) was employed to confirm the presence of reduced metallic silver as well as other elements possibly present. EDX peak strongly suggests the peak of silver element which is shown in Fig. 6 below. It is also clear from the quantitative elemental analysis presented in Table 1 that the atomic concentration of silver was about 42% followed by oxygen, carbon, nitrogen and potassium and some other elements in trace amounts. Similarly, the weight concentration of silver was very high, about 83% and residues of other elements in the composition completing the percentage. Work done by Siavash[23] also showed that *Alfalfa* plants contain similar elements having similar atomic concentrations.

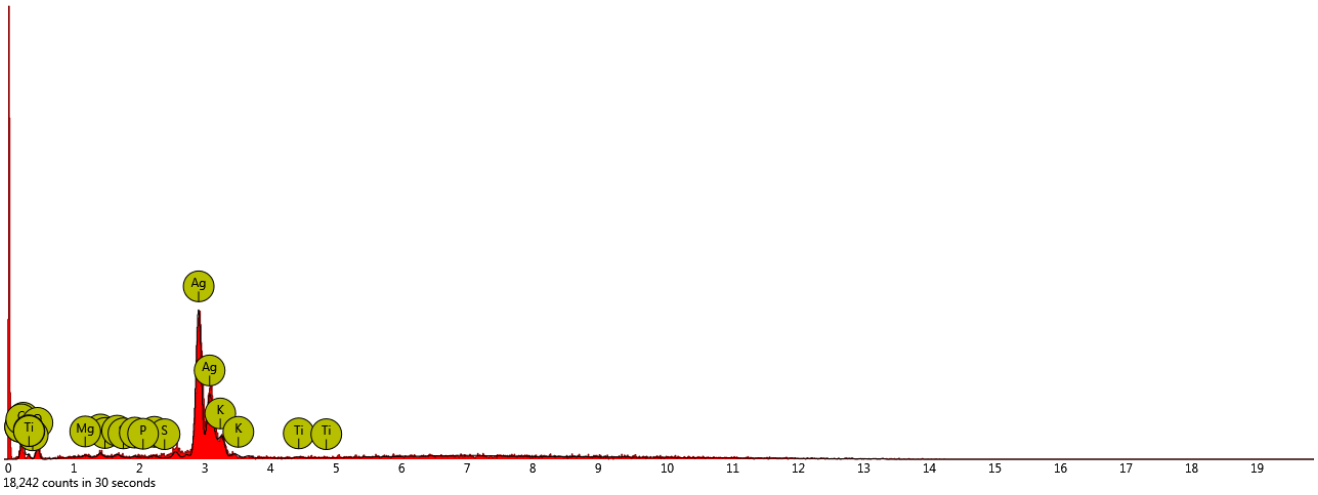

Fig. 6. EDX (energy dispersing X-ray analysis) for root nanoparticles.

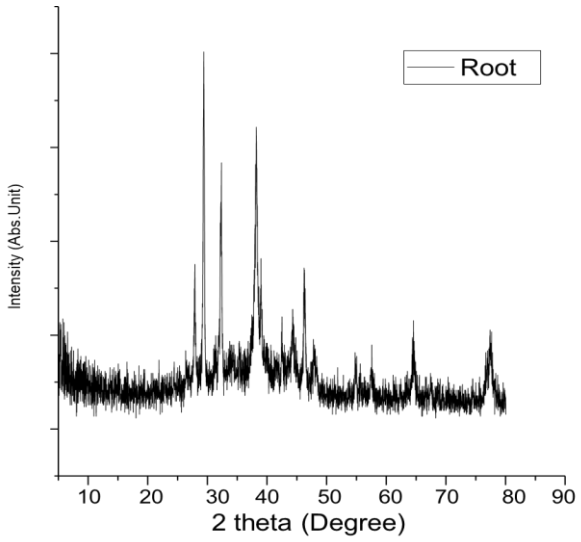

Fig. 7. XRD Spectrum for Silver Nanoparticles

3.5. XRD Analysis for Root Nanoparticles

Outstanding sharp and high intense peak reflections were conveyed in the root- mediated green synthesis. The XRD image in Fig. 7 below displayed crystalline Ag NPs. Characteristics reflections which appeared at 2ɵ values of approximately 30, 33, 40, 48, 66 and 80 assigned to be Ag planes of the face-centred cubic structure.

3.6. Antifungal Activity Result

The antifungal activity of green synthesized silver nanoparticle from *Moringa oliefera* root was tested against two human pathogens, *Aspergillus Niger* and *Candida Albicans*, presented in Table 2 above. In both organisms, the zones of inhibition values were more significant at higher concentrations as also reported by Basavaraja *et al.*,[24] Comparatively, the NPs were more sensitive against *Candida Albicans* than *Aspergillus Niger* with zones of inhibition values of 8.5 mm and 7 mm at 100µg/L, 17mm and 13.5mm at 500 µg/L respectively. The activity of the Ag NPs at 400 µg/L and 500 µg/L for *Candida Albicans* was better than the positive control, Fulcin. The results can be compared to that reported by Magesh et al.,[25] for the same microorganisms at the same concentrations. The root extract and $AgNO_3$ were not evaluated for this antifungal property because literature has shown that NPs possess better results than the two categories. For example Balashanmugam et al.,[24] reported that $AgNO_3$ had very weak activity and the plant extract had zero activity.

4. Conclusions

Bio-molecular and promising Ag NPs were successfully synthesized using the reducing property of *Moringa oliefera* roots. The formation was monitored and confirmed using some characterization techniques. Antifungal activity test carried out confirmed the efficacy of the NPs against two human pathogens viz. *Candida Albicans* and *Aspergillus Niger*. It is expected that this research will be useful in developing effective antifungal agents that would counteract the increasing resistance by microorganisms.

Conflicts of Interest

The authors declare no conflict of interest.

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