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Evaluation of Antibacterial Efficacy of Green Synthesized Silver Nanoparticles from *Moringa Oliefera* Aqueous Root Extract

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Abstract
In this Research study, Silver Nanoparticles were green synthesized from Moringa oliefera plant aqueous root extract and different Characterization techniques including UV-Visible, FT-IR, SEM and XRD were all employed to ascertain the absorption peaks, functional group, surface morphology and crystalline size of the nanoparticles respectively. These nanoparticles green synthesized were applied against four different pathogens namely, S. typhi, S. aureus, Bacillus, Strep A. and the investigation showed that the silver nanoparticles synthesized were effective against the selected pathogens. From the UV-Vis spectral analysis, it was observed that highest absorption peaks appeared at 300nm reflecting the surface plasmon resonance of silver Nanoparticles from Moringa oliefera plant aqueous root extract which is characteristic of Silver Nanoparticles. From the FT-IR spectrum of the sample investigated, the peaks 3436.44 cm-1, 1638.75cm-1, 1384.50cm-1, 1090.80cm-1 and 798.08cm-1 were observed where the absorption band at 3436.44 cm-1, corresponds to the stretching due to N-H, while the band at 1638.75cm-1 is associated with C-H stretch of alkane and O-H stretching. The peak at 1384.50cm-1 shows C=C stretching, 1090.80cm-1 reveals the existence of C-H bending and 798.08cm-1 depicted C-O stretching. The SEM analysis revealed the shape of the nanoparticles as being crystalline in nature while XRD result admits that the average size of the green synthesized Ag NPs was 47.54nm using the Scherer's formula. Augmentin was used as control at concentration of 300µg/L throughout antimicrobial studies. Different concentrations of 100, 200, 300, 400 and 500µg/L of Silver Nanoparticles were tested against each bacterium. It was discovered that with increase in concentrations of Silver Nanoparticles of all the pathogens, there generally appeared to be increase in inhibition zone. At higher concentration of 500µg/L, the zones of inhibition were in the following order; 26.5mm, 19.0mm, 17.5mm, and 17.0mm for S. typhi, Strep. A, S. aureus and Bacillus respectively. Although the green synthesized silver nanoparticles exhibited a good antibacterial activity against gram negative and gram-positive bacteria, for each concentration investigated, S. typhi, demonstrated higher zone of inhibition as opposed to all other bacteria investigated in this research.

Keywords: Nanoparticles, Moringa Oliefera, Aqueous Root Extract, Antibacterial Activity

1. Introduction

Nanoparticle research is presently an area of strong scientific interest due to a wide variety of Potential applications in biomedical, optical and electronic fields. Many plant parts have been used in green synthesis of silver nanoparticles including Mulberry Leaves Extract [1-2], *Orthosiphon thymiflorus, Rosa rugosa, Chenopodium album, Jatropa Gossypifolia, Polyalthia longifolia, Coriandrum Sativum and moringa oliefera* leaf extract [3-11]. Biosynthesis of NPs is a reliable, eco-friendly and important aspect of green chemistry approach that intersects biotechnology and nanotechnology [12]. Research has shown that Microbes and Plants are supportive in the biosynthesis of NPs with good surface and size characteristics [13]. Development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. To meet the increasing demands for commercial nanoparticles, new eco-friendly "green" methods of synthesis are being discovered [14-20]. Silver has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms [21]. Antibacterial activity of the silver containing materials used in medicine to reduce infections in burn treatment [22] and arthroplasty [23], as well as to prevent bacteria colonization on prostheses [24], catheters [25], vascular grafts, dental materials [26], stainless steel materials [27], and human skin [28]. Silver nanoparticles also exhibit a potent cytoprotective activity towards HIV-infected cells [29]. Because of such wide range of applications, numerous synthetic methods have been developed [30]. Biological route of nanoparticles synthesis using microorganism [31-33], enzyme [34] and plant or plant extract [35-41] have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [42]. It can also be suitably scaled up for large-scale synthesis of nanoparticles. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles.

2. Materials and methods

2.1 Apparatus / Instruments

All the glass wares and apparatus used in this study are Pyrex products. For this research the following apparatus were used; beakers, conical flasks, test tubes, measuring cylinder, whatman filter paper No.1, watch glass, oven, weighing balance, funnels, hot plate, autoclave, petri dish, incubator, refrigerator, laminar flow cabinet, cotton wool, aluminum foil paper, candle, matches, universal container, spatula, glass rod stirrer, syringes, micropipette, wire loop, Ultraviolet –Visible spectrophotometer (modelJENWAY 630), SEM, and FTIR (PerkinElmee Spectrum Version 10.0309) Incubator (DRB0092), Refrigerator (Thermo cool HTF 219 H), Laminal flow carbine (Isocide TM 5237), Hotplates (CB 160)

2.2 Reagents

The chemicals/reagents used were of analytical grade and include: Silver nitrate ($AgNO_3$), Potato dextrose agar, Nutrient agar for bacterial culture, Mueller–Hinton broth, agar for antimicrobial activity and Double distilled water.

2.3 Preparation of aqueous plant extract

The preparation of *Moringa oliefera* aqueous root extract was carried out according to a method reported from the literature [20] as follows: The collected samples were thoroughly washed 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 pieces 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. After that, the solution was filtered through Whatman No. 1 filter paper and the filtrate was used immediately for the synthesis of silver nanoparticles.

2.4 Synthesis of Silver Nanoparticles

The method reported in the literature [2] was employed in the synthesis of Silver Nanoparticles viz: In a typical reaction procedure, 5 ml of *moringa oliefera* root extract was added to 50 mL of 0.01 M aqueous $AgNO_3$ solution at room temperature, the changed in color of the resulting solution indicated the formation of AgNPs. The concentrations of $AgNO_3$ solution and *Moringa oliefera* root extract was varied at 1–5 mM and 5–10% by volume, respectively. The formed solution was tested using UV-Vis Spectroscopy.The silver nanoparticles obtained by *Moringa oliefera* root extract was centrifuged at 35,000 rpm for 10 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

3. Results and discussion

The primary test for ascertaining the formation of AgNps is by color change. As the solution was heated, a change in color was observed from yellow to black on heating for 30 minutes and the color change indicates the formation of silver nanoparticles by plant materials, this is the primary test for the checking of formation of AgNps as shown in the fig 1.

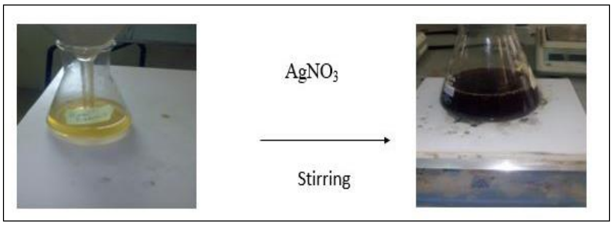

Fig 1: (a) Root extract of moringa oliefera (b)Nanoparticles of moringa oliefera root

3.1 Characterization

3.1.1 UV spectroscopy

UV spectroscopy is one of the most important techniques to determine and evaluate the formation and stability of metal nanoparticles in aqueous solution. The reduction of Ag^+ to Ag^0 was measured periodically at 200-800nm, using distilled water as the blank. A spectrum of AgNPs was plotted with wavelength on x-axis and absorbance on y-axis. The absorbance was observed around 300nm, 400nm and 500nm indicating the formation of Nps due to the excitation of the surface plasmon vibration in the AgNps. This result agrees with the one reported from the literature [15] which talked about the possibility of finding the plasmon band of AgNps in range from 400-420nm.

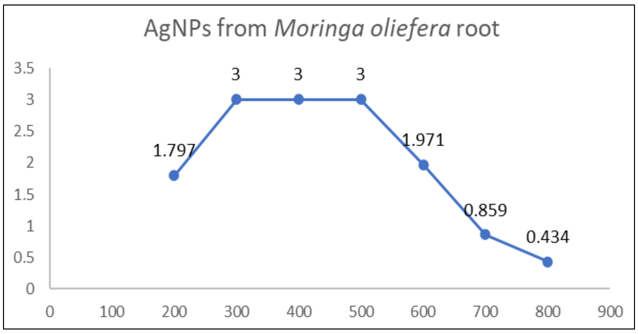

Fig 2: UV visible absorption spectra of AgNps from Moringa oliefera root

3.1.2 FTIR Analysis

Fig 3 shows the FTIR spectrum of silver nanoparticles from Moringa Oliefera root. The FTIR analysis was carried out to determine the number of phytoconstituents in root of Moringa oliefera plant acting as a stabilizing and capping agent in the presence of green synthesis. From the FT-IR spectrum of the sample investigated, the peaks 3436.44 cm-1, 1638.75cm-1, 1384.50cm-1, 1090.80cm-1 and 798.08cm-1 were observed where the absorption band at 3436.44 cm-1, corresponds to the stretching due to N-H, while the band at 1638.75cm-1 is associated with C-H stretch of alkane and O-H stretching. The peak at 1384.50cm-1 shows C=C stretching, 1090.80cm-1 reveals the existence of C-H bending and 798.08cm-1 depicted C-O stretching. The FTIR study indicates the presence of ketones (C=O), hydroxyl

(OH), alkyne (C≡C) and alkene (C=C) groups in Moringa oliefera root primarily involved in reduction of Ag^+ ions to Ag^O. This result is in concord with the ones reported from the literatures [9, 14].

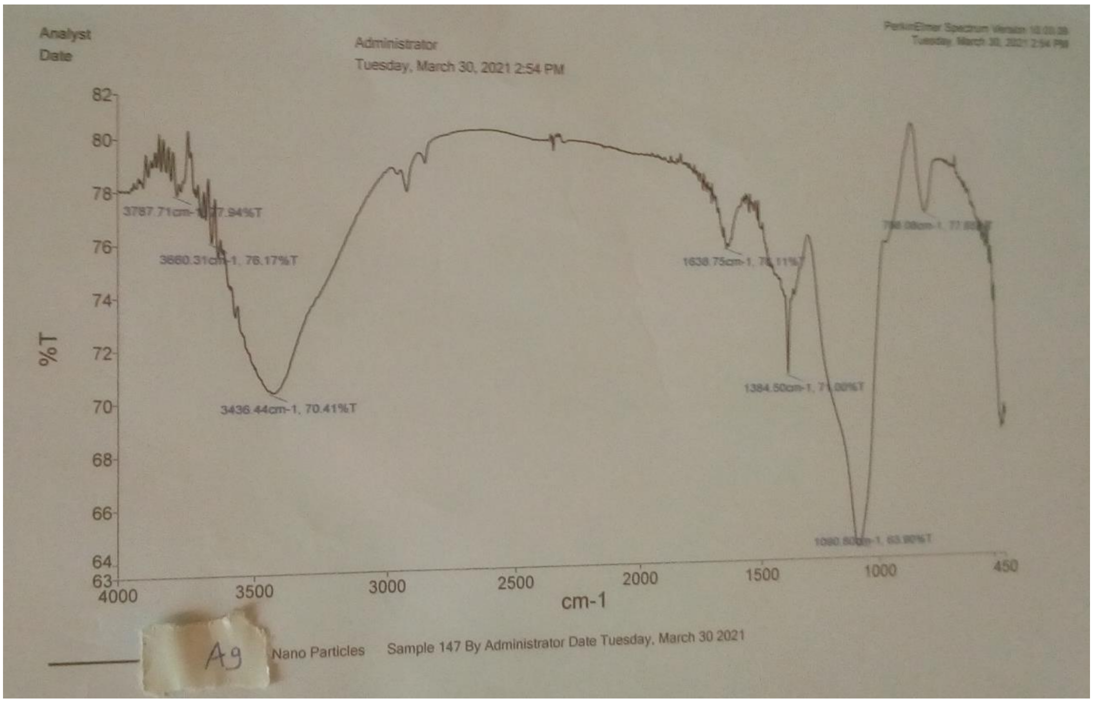

Fig 3: FT-IR Spectra of AgNps from Moringa oliefera root

3.1.3 SEM analysis

It is clearly seen from the SEM image (fig 4) that the particles are merely crystalline in nature. The Scherrer rings characteristics of silver clearly observed shows that the structure seen in the SEM image is nano crystalline in nature. It is observed that the silver nanoparticles are scattered over the surface and no aggregates are noticed under SEM, the difference in size is possibly due to the fact that nanoparticles were formed at different times. Similar work done on *Papaver sonmiferum* [36] showed the morphology of the nanoparticles to be crystalline in nature.

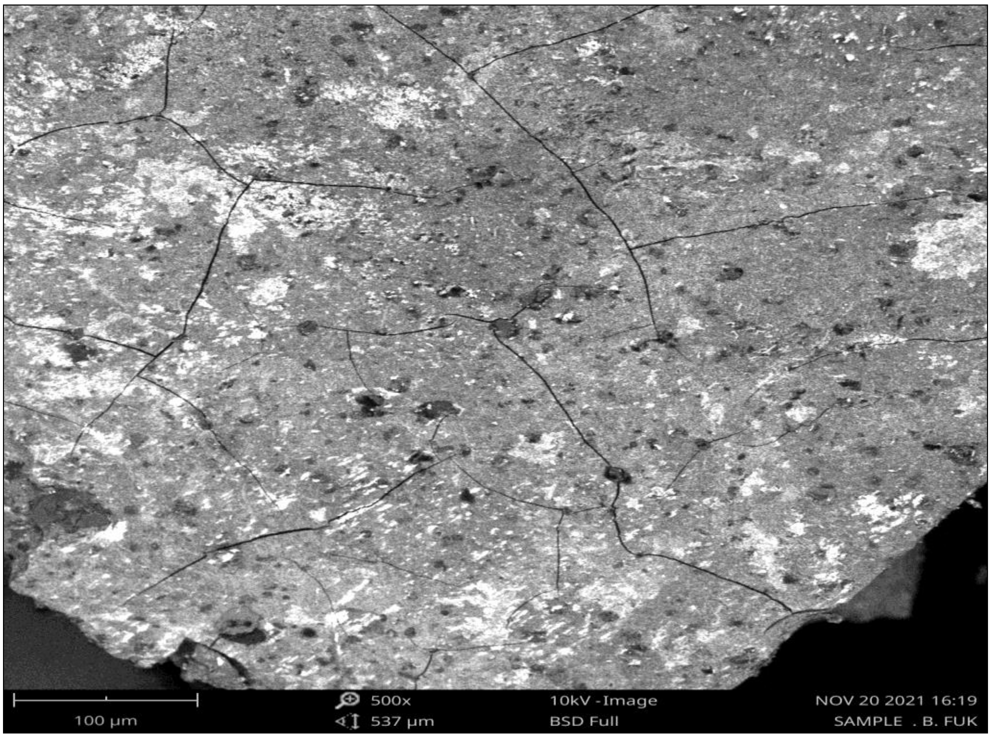

Fig 4: SEM image of silver nanoparticles for moringa oliefera roots

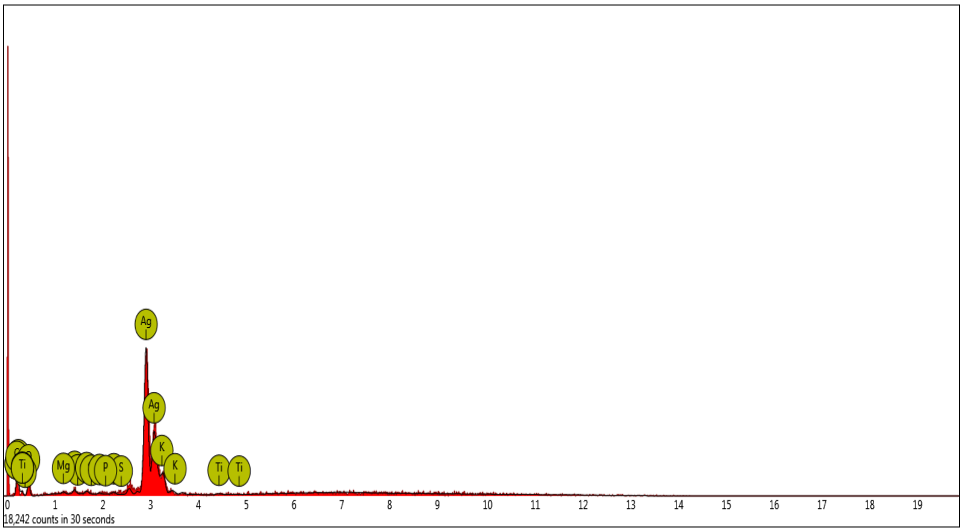

Fig 5: Spectrum of silver nanoparticles from moringa oliefera root extract

3.1.4 EDX analysis of silver nanoparticles from moringa oliefera root extract

Energy dispersive x-ray analysis (Elemental analysis) is one more evidence to strongly confirm the presence of elemental silver from the reduction of silver ions. EDX peak which strongly suggested the peak of silver element which is higher as shown in fig 5 above, oxygen, carbon, potassium and Nitrogen also showed high peak. In addition to that, other small trace of element of Phosphorus, sulfur, magnesium, silicon, titanium and aluminum were also predicted during the entire scan rate procedure.

Table 1: Elemental composition of the synthesized silver NPs

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
47	Ag	Silver	46.42	84.87
8	O	Oxygen	25.79	6.63
6	C	Carbon	15.12	3.72
19	K	Potassium	3.26	1.60
7	N	Nitrogen	5.73	1.20
13	Al	Aluminium	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

3.1.5 XRD result

The X-ray diffraction patterns of green synthesized silver nanoparticles is shown be-low, and a meticulous investigation of the spectrum revealed that the structure of AgNPs under in-vestigation is a cubic crystal system.

For the synthesized silver nanoparticles of *Moringa oliefera root* extract, the average size of the green synthesized Ag NPs was obtained to be 47.54 nm using the Scherrer equation:

$$D=K\lambda/\beta\cos\theta,$$

Where,

K is a constant equal 1,

λ is the Xray source wavelength

β is the full width half maximum,

θ is the corresponding diffraction angle to the lattice plane and finally,

D denotes the diameter of silver nanoparticles

This particle size is indeed within the acceptable range of the nanoparticles.

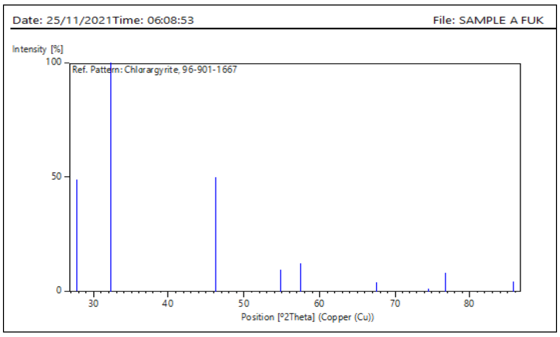

Fig 6: XRD Spectrum of *Moringa oliefera* root extract

3.2 Antibacterial analysis for silver nanoparticles from *Moringa oliefera* root extract

This nano particle was given most significant antibacterial activity against gram negative and gram-positive bacteria. Nano particles are more efficient in the drugs delivery processes, the results obtained from antibacterial study show that the effect of the nanoparticle is not dose dependent as the doses used in some organisms gave a higher effect while also giving lower effects in the other organisms this can be as a result of the biochemical mechanisms of adaptation by these organisms in the given conditions. This is similar to the literature report on antimicrobial activity of *Polyalthia longifolia* leaf extract along with D-sorbitol [8].

Table 2: Antibacterial activity of AgNps synthesized *from Moringa oliefera* root

AgNPs	Test of organism	Concentration (mm)					
		100 μg/L	**200** μg/L	**300** μg/L	**400** μg/L	**500** μg/L	**Control (Augmentin) 300** μg/L
	S. typhi	11.5mm	18.5mm	22.0mm	25.0mm	26.5mm	26.5mm
	S. aureaus	11.5mm	12.0mm	17.0mm	19.0mm	17.5mm	20.0mm
	Bacillus	10.5mm	14.5mm	16.5mm	15.5mm	17.0mm	26.5mm
	Strep. A	11.5mm	15.5mm	17.5mm	17.0mm	19.0mm	22.0mm

4. Conclusion

In this study simple approach was attempted to obtain a green eco-friendly, non-toxic way for synthesis of silver nanoparticle from *Moringa oliefera* root extract. The primary confirmation for silver nanoparticle was due to colour changes and UV/Vis absorbance was observed around 300nm, 400nm and 500nm indicating the formation of AgNps due to the excitation of the surface plasmon vibration in the AgNps. The SEM analysis identified the particles to be merely crystalline in nature. Edax study revealed the elemental compositions of each element present in the nanoparticles. The nano particle gave most significant antibacterial activity against gram negative and gram-positive bacteria

5. References

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