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Plant Based Green Synthesis, Characterization and Antimicrobial Investigation of Silver Nanoparticles from Khaya senegalensis Aqueous Leaf Extract

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ABSTRACT

In this research article, Silver nanoparticles were synthesized from Khaya senegalensis leaf extracts via green route. Spectroscopic study revealed color change of the solution from yellow to light brown within 25 min of addition of the AgNO3 against leaf aqueous extract with constant stirring. Beyond this time, no further change in color after the nucleation of the metal ions indicating that phytoconstituents of Khaya senegalensis resulted in the reduction of Ag+ to Ag0, a phenomenon that could be attributed to the surface Plasmon excitement of AgNPs. The bio fabricated silver nanoparticles were characterized using UV – Visible, FTIR and RXD so as to be certain of its formation before being deployed in the antimicrobial studies. The UV-Vis spectral analysis of the AgNPs from the leaf extract showed maximum absorbance of 2.01AU at a corresponding wavelength (λ max) of 500nm. The X-Ray Diffraction patterns of green synthesized AgNPs showed that the structure of the nanoparticles under research have face centered cubic (fcc) and spinel like structures with biosynthesized AgNPs having the average particle size of 65.5nm. The antimicrobial studies of Silver nanoparticles were conducted against B. subtilis, K. pneumonia (gram +ve bacteria) and two fungi, A. niger and C. albicans. Different concentrations of 100, 200, 300, 400 and 500μg/L of Silver nanoparticles were tested against each pathogen. The inhibition zone increases generally with increase in concentrations of silver nanoparticles. At higher concentration of 500μg/L, the zones of inhibition were in the following order; 18.5mm, 26.3mm, 23.5mm, and 24.4mm for Bacillus subtilis, Klebsiella pneumonia, Aspergillus niger and Candida albicans respectively. For each concentration investigated, C. albicans, demonstrated higher zone of inhibition as opposed to all other pathogens under investigation. The results of this research therefore indicated that Silver Nanoparticles synthesized from Khaya senegalensis plant extracts demonstrated potent antimicrobial activity on the selected pathogenic microbes, hence be used as antimicrobial agent against the organisms in question.

Keyword: Antimicrobial, Characterization, Green synthesis, Khaya senegalensis, Silver Nanoparticles

I. INTRODUCTION

The field of nanotechnology is one of the most explored areas of research in modern materials science, as nanoparticles exhibit novel properties depending upon their morphology, size and shape, thereby potently enabling them to interact with animals, plants and microbes. Silver nanoparticles (Ag NPs), in particular, are among the most promising materials that have attracted much attentions and have been extensively studied in different fields including materials science and engineering, biomedical, antimicrobial and catalytic applications simply because they have unique properties due to their surface area and particle size [1-2]. Their unique physical and optical properties such as high surface to volume ratio, Surface Plasmon Resonance (SPR), as well as Surface Enhance Raman scattering (SERS) have resulted in the metallic nanoparticles recent development [3]. These distinct features result in increased applications of metal nanoparticles in the field of sensing & bio imaging, medicine, cosmetics, agriculture, purification, treatment of water and textile waste treatment [4-11]. Research investigation based on advanced nanomaterials of noble metals like silver has conquered a lot of interest among scientists during the past years for its physiochemical properties such as size, distribution and morphology, they have been studied for magnetic properties, catalytic activity, optical properties, electronic properties and antimicrobial properties [12-16].The plant based synthesis of nanomaterials is now a leading branch of nanotechnology as it has many merits over chemical and physical methods of nanoparticle synthesis. The merits of this approach are but not exhaustible to its; cost-effectiveness, eco-friendliness, simplicity, easily scaled up for mass-scale synthesis, relatively reproducible, more so, there is no need of: high pressure, energy, toxic chemicals, high temperature, often results in more stable materials and most importantly, it is environmentally friendly. Furthermore, the integration of the principles of green chemistry with nanotechnology has become a key area in nanoscience and has received great attention in recent years [17-18]. Biological methods are being utilized in the synthesis of metal and metal oxide nanoparticles nowadays, since the particles obtained are of desirable size and morphology and the properties of the particles are enhanced in a greener way. Due to the rich biodiversity of plants and their potential secondary metabolites, plants and plant parts have been well utilized in recent years in the bio fabrication of a variety of nanoparticles. More so, the use of chemical as reductants and stabilizers have been avoided since plant extracts can act as both reducing and stabilizing agents for the formation of nanoparticles. These plants and their parts help to produce metal nanoparticles that are much stable as compared to the other organisms and can reduce the metal ions faster than that of bacteria and fungi [19].

They equally reduce the cost of isolation and culturing bacteria and fungi, hence, increasing their cost-competitive feasibility of nanoparticle production [20] and improved antimicrobial properties compare to their monometallic entities [21–23] In this research study, silver nanoparticles have been successfully synthesized by using *Khaya senegalensis aqueous* leaf extract. The synthesized Ag NPs were characterized using UV-Visible Spectroscopy FTIR, XRD. These Ag NPs were further subjected to antimicrobial studies.

II. MATERIALS AND METHODS

2.1 Materials

The materials used during this research work include, *Khaya senegalensis* leaves, distilled water, Silver nitrate ($AgNO_3$), Nutrient agar, culture bottle, incubator, among others.

2.2. Methods

2.2.1 Sample Collection as Well as Preparation of Plant Extract

Healthy plant samples were collected from the vicinity of Kashere and were identified by the botanist. The collected samples were thoroughly washed under running tap water and rinsed severally with distilled water followed by shade-drying to remove residual moisture. The dried materials were ground using mortar and pestle into fine powder. About 10g of the samples were weighed and dispersed in 100 ml of sterile distilled water in a 250 ml glass beaker and boiled at 60˚C for 25 min on a hot plate. This was allowed to cool. After that, the solution was filtered through Whatman No. 1 filter paper and the filtrate collected was used immediately for the synthesis of silver nanoparticles.

Figure 1: *Khaya senegalensis* Leaves

2.2.3 Synthesis of Silver Nanoparticles

The synthesis of Silver Nanoparticles was carried out according to a method reported by the literature [24] with slight modifications as follows:

In a typical reaction procedure, 10 ml of *Khaya senegalensis* leaf extract was added to 100ml of 0.01 M aqueous $AgNO_3$ solution at room temperature. The mixture was heated on the hot plate at a temperature of 60˚C with constant stirring. The color of the resulting solution indicating the formation of AgNPs was noted.

2.2.4 Antimicrobial Analysis

Here, the green synthesized Silver nanoparticles using *Khaya senegalensis* leaves extract were investigated for its antibacterial and antifungal activity by Agar well diffusion method against some selected gram positive bacteria and fungi

2. 3. Characterization of the Sample Synthesized

2.3.1. UV-Visible Spectral Analysis

The silver nanoparticles were confirmed by measuring the wavelength of reaction mixture in the UV-vis spectrum at a resolution of 1 nm (from 200 to 800 nm)

2.3.2. FT-IR Analysis:

The characterization of the active functional groups on the surface of silver nanoparticles (AgNPs) synthesized from *Khaya senegalensis* leaves extract was investigated by FTIR analysis and the spectra was scanned in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

2.3.3. X-ray Diffraction (XRD) Analysis

The particle size of the green synthesized silver nanoparticles was determined using X-ray diffractometer operating at a voltage of 45 kV and current of 40 mA with Cu K (α).

3. Results and Discussion

3.1. Color change identification during Formation of AgNPs from leaf extract

Here, the formation of Silver Nanoparticles first, was identified by color change as the addition of crude extract resulted in a quick shift in color from yellow to light brown after 25 min, demonstrating the rapid reduction of Ag^+ to Ag^0 in $AgNO_3$ solution.

This phenomenon could be attributed to the surface Plasmonic excitement of AgNPs, a report that corresponds to that of the literatures [25]

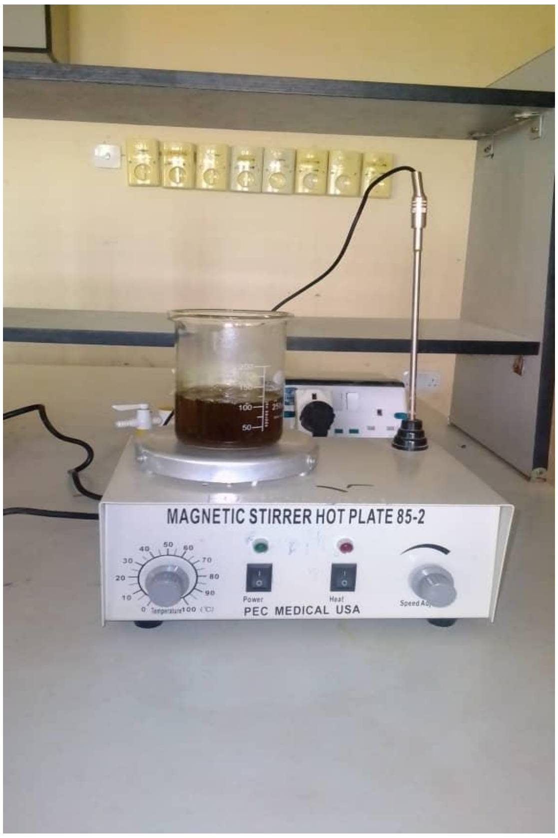

Figure 2:(a) Set up for Synthesis of AgNPs

(b) Leaf extract, $AgNO_3$ & AgNPs from the Leaf extract

3.2 UV- Visible Spectrophotometric Study

The reduction of Ag^+ to Ag^0 was measured periodically at 200-800nm, using distilled water as the blank. A spectrum of Ag NPs was plotted with wavelength on x-axis and absorbance on y-axis. The maximum absorption peak was observed at absorbance of 2.01AU with corresponding wavelengths (λ max) at 500nm indicating the formation of Ag NPs due to the excitation of the surface Plasmon vibration in the Ag NPs. The UV result is in agreement with the ones reported from the literatures [26-27]. The UV–Vis absorption spectrum of the synthesized Ag NPs is shown in Figure 3.

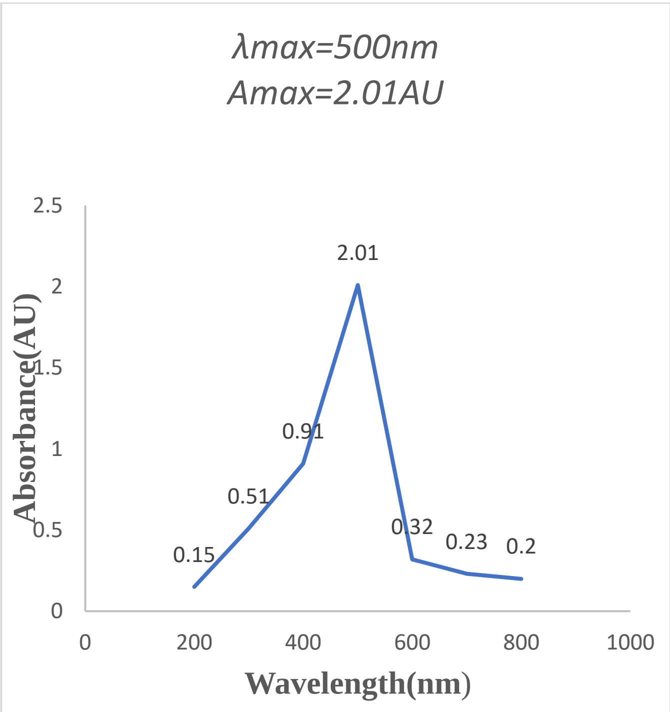

Figure 3: UV-Vis of AgNPs Synthesized from *Khaya senegalensis leaf extract*

NB: The reduction of Ag was measured periodically at 200-800nm, using distilled water as the blank. A spectrum of NPs was plotted with wavelength on x-axis and absorbance on y-axis

3.3. FT-IR Interpretation of AgNps from Khaya senegalensis leaves

FT-IR identifies the functional groups present in the synthesized silver nanoparticles and understanding their changes from inorganic silver nitrate ($AgNO_3$) to elemental silver using different phytochemicals which would function as reducing, stabilizing and capping agent. From the FT-IR spectrum of the sample under investigation, the bands 1643.99cm^{-1}, 1633.69cm^{-1}, 1384.53cm^{-1}, 1094.14cm^{-1}, and 602.19cm^{-1}, were noted in which the absorption peak at 1643.99cm^{-1}, corresponds to the stretching due to N-H, while the peak at 1633.69cm^{-1}, is probably associated with C-H stretch of alkane and O-H stretching, 1384.53cm^{-1}, peak possibly depicts C=C stretching, 1094.14cm^{-1}reveals the existence of C=O bond and 602.19cm^{-1}depicted C-O stretching. The disparity in the FT-IR spectrum signifies the presence of bioactive molecules in plant extracts that participated in the reduction of silver nitrate ($AgNO_3$) and the formation of silver nanoparticles. Interestingly, this finding corresponds to most of the existing research works [26-27]

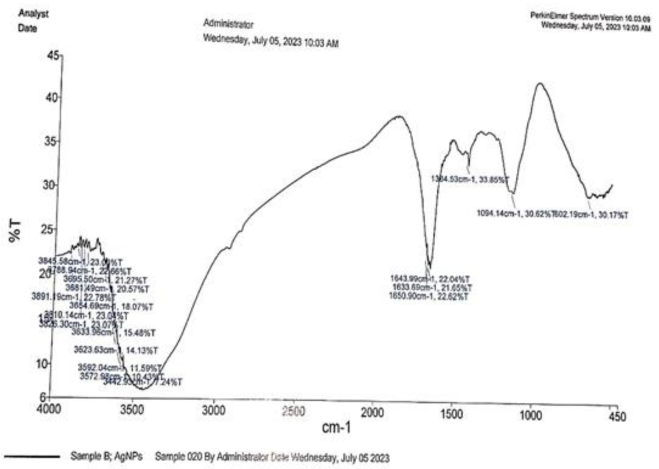

Figure 4: Spectra of AgNps from *Khaya senegalensis* leaves.

Table 1: results summary of FTIR of AgNps from *Khaya senegalensis leaves extract*

peak (cm^{-1})	Functional group /Bonds	V. mode	Intensity
1643.99	N-H	stretch	Strong
1633.69	C-H	stretch	Weak
1384.53	C=C	Bend	Strong
1094.14	C-O	stretch	Weak
602.19	C-O	bend	Medium

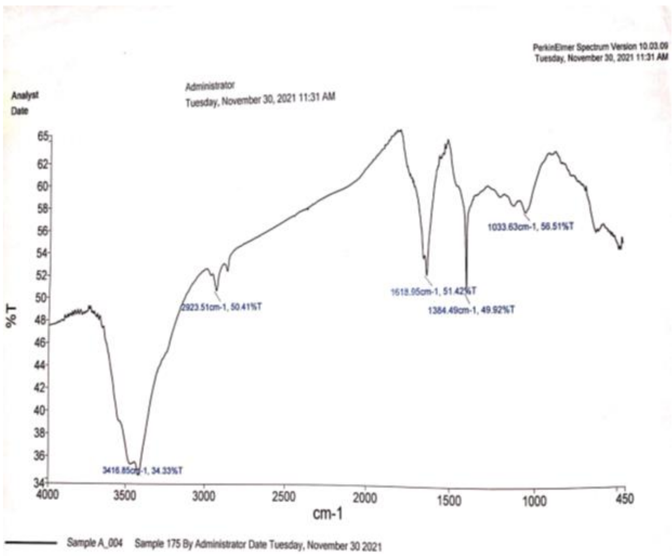

Figure 5. FT-IR Spectrum of Green Synthesized Silver Nanoparticles from *Khaya senegalensis leaf Extract*

3.4. XRD Analysis

The XRD pattern indicates AgNPs synthesized has face centered cubic and a spinel like structures with the average particle size of 65.5nm using the Scherer's formula: D=Kλ/βcosθ

Where:

K is a constant equal 1,

λ is the X-ray 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

The result of this finding aside conforming to the true definition of what nanoparticles are (tiny materials with size ranging from 1-100nm), corresponds to the earlier literatures reported by some researchers [28]. More to that, there were also three intense peaks in the spectrum ranging between 10^0 and 80^0 The Bragg reflections were prominent with 2θ values of 32.49, 33.86, 38.31, 44.48 and 46.42.

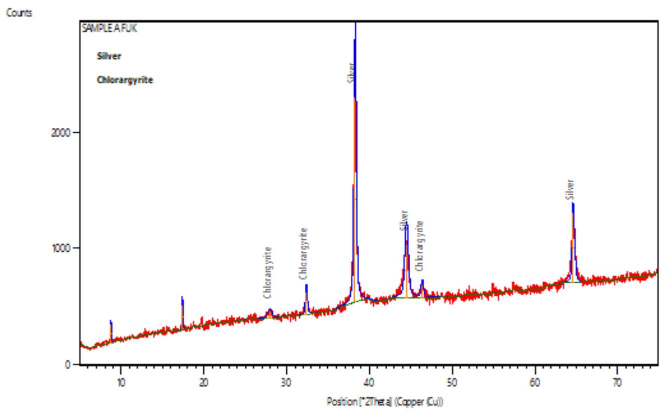

Figure 6. XRD Diffractogram of Green Synthesized AgNPs from *Khaya senegalensis Leaf Extract*

3.5. Antimicrobial Activity

Owing to the rapid increase of antibiotic resistance in this period, Silver nanoparticles (AgNPs) have demonstrated significant application in the reduction of pathogenic microbes and also in the treatment of microbial infections. This has attracted the attention of the researchers investigating the therapeutic abilities of AgNPs systems as potential antimicrobial agents. Presented below (Table 2) is the result of antimicrobial activity of Silver Nano-particles against *Bacillus subtilis*, *Klebsiella pneumonia* (gram +ve bacteria) and two fungi, *Aspergillus niger* and *Candida albicans.* Throughout the study, Augmentin was used as control at concentration of 300µg/L. Different concentrations of 100, 200, 300, 400 and 500µg/L of Silver Nanoparticles was tested against each pathogen. As the concentrations of Silver Nanoparticles of all the pathogens increase, there generally appeared to be increase in inhibition zone. At higher concentration of 500µg/L, the zones of inhibition were in the following order; 18.5mm, 26.3mm, 23.5mm, and 24.4mm for *Bacillus subtilis*, *Klebsiella pneumonia*, *Aspergillus niger* and *Candida albicans respectively.* For each concentration investigated, *C. albicans*, demonstrated higher zone of inhibition as opposed to all other pathogens under investigation. The results of this research therefore indicated that Silver Nanoparticles synthesized from *Khaya senegalensis* leave extract demonstrated potent antifungal activity on the selected pathogenic microbes. The finding corresponding to the reports given by the earlier researcher [28]

Table 2 : Antimicrobial activity of AgNps synthesized *from Khaya senegalensis* leaf

TEST of organism	**Concentration (mm)**					**Control (Augmentin)**
	100µg/L	**200µg/L**	**300µg/L**	**400µg/L**	**500µg/L**	**300µg/L**
B. subtilis	9mm	10.5mm	11.5mm	13.5mm	18.5mm	25.5mm
K.neumonia	12.5mm	13.7mm	16.5mm	19.00mm	26.3mm	29.5mm
Asp. Flavus	11.5mm	13.5mm	15.5mm	20mm	23.5mm	30.5mm
C. albicans	16mm	18mm	20.5mm	22.5mm	24.4mm	24.9mm

III. CONCLUSION

Silver Nanoparticles were biofabricated from *Khaya senegalensis* extract. The method is considered to be environmentally friendly because the synthesis is carried out at ambient temperature, using *Khaya senegalensis* extract and without the addition of any chemical reductant, it does not therefore, generate any environmental pollution. Various Characterization techniques including UV-Visible, FT-IR, and XRD were all employed to determine the absorption peaks, functional group, and crystalline size of the nanoparticles in question. Characterization results obtained from UV-Spectroscopic, FT-IR, SEM and XRD analysis showed that the particles synthesized are in nanoscale range and crystalline in nature. The small size and stability of the particles can be attributed to heat applied during preparation of the extract and the concentration of $AgNO_3$. The antimicrobial activity of the AgNPs is dependent on the size and capping agents used. Since the particles are in nanoscale range as proven by characterization studies, their potency as an antimicrobial agent is further established by the antimicrobial assay performed against four different pathogens namely, *Bacillus subtilis*, *Klebsiella pneumonia* (gram +ve bacteria) and two fungi, *Aspergillus niger* and *Candida albicans* and the investigation showed that the Silver nanoparticles synthesized were effective against the selected microbes

Authors' Contributions: This work was carried out in collaboration among all authors. Author MY conceived and designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors BAD, RB, JJ, JWKJ and TAS managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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