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Characterization and Pathogenic Study of Plant Mediated Silver Nanoparticles Utilizing *Psidium guajava* Stem Bark Extract

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ABSTRACT

The formation of Silver Nanoparticles first, was recorded by color change as the addition of crude extract resulted in a quick shift in color from light brown to orange instantly. After 5 h later, dark brown color was obtained. The UV–Visible optical findings validated the production of AgNPs in the mixture, with a surface plasmon resonance spectra at 400nm. From the FT-IR spectrum of the sample investigated, the absorption peak at 3416.85 cm^{-1} corresponds to the stretching due to N-H, while the peak at 2923.51 cm^{-1} is probably associated with C-H stretch of alkane and O-H stretching, 1618.95 cm^{-1} peak possibly depicts C=C stretching, 1384.49 cm^{-1} reveals the existence of C-H bending and 1033.63 cm^{-1} depicted C-O stretching. The SEM result confirmed the shape of the sample in question as having mixed cubic and hexagonal structures. The XRD result revealed that the size of the particles was 44.5nm using the Scherer's formula. Different concentrations of 100, 200, 300, 400 and 500µg/L of Silver Nanoparticles was tested against four different pathogens, namely; Staphylococcus aureus, Escherichia coli, Candida albicans, and Aspergillus niger. 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; 23.44mm, 18.52mm, 17.66mm, and 15.99mm for E. Coli, S. aureus, C. albicans and Aspergillus niger respectively. For each concentration investigated, E. coli, demonstrated higher zone of inhibition as opposed to all other pathogens under investigation.

Keywords : Characterization, Pathogenic Study, Plant Mediated, Silver Nanoparticles Psidium guajava, Extract

I. INTRODUCTION

Due to a wide variety of potential application in biomedical, optical and electronic fields, Nanoparticles research is presently an area of strong scientific interest. Nanoparticles are simply considered to be tiny materials with size ranging from 1-100nm. Nanoparticles possess unique physical and chemical properties due to their high surface area and nanoscale size [1]. Nanoparticles have found various applications in the fields of medicine, biology, catalysis among others, as a result of the uniqueness in their physical and chemical properties [2] These particles, though can be synthesized by physical and chemical methods, the green approach is now widely acceptable due to its numerous advantages such as eco friendliness, cost effectiveness, less production time, to mention a few [3].Silver nanoparticles gain utmost position when compare to other nanoparticles because of their wide mode of activity and their application in almost all the fields[4-5]. Currently, Biosynthesis of nanoparticles by plant extracts is under exploitation. Utilization of plants or their extracts for synthesis of nanoparticles could be advantageous over other environmentally friendly biological processes as this eliminates the elaborate process of maintaining cell culture [6]. Research had indicated that bio molecules like protein, phenols, & flavonoids not only play a role in reducing the ions to the nano size scale, but also play a vital function in the nanoparticles' shaping [7-8]. Most plant parts including leaves, roots and stems are deployed in the synthesis of nanoparticles [9-10]. Furthermore, owing to the rich biodiversity of plants and their potential secondary metabolites, plants and plant parts have been well exploited in modern times in the synthesis of a variety of nanoparticles. In the synthesis of nanoparticles, plant extracts can act as both reducing and stabilizing agents, hence the use of chemical reductants and stabilizers can be avoided [11-12]. Although various nanoparticles of different transition metals such as Co, Pt, Ni, and Fe have proven to be effective antimicrobial agents, research based on advanced nanomaterials of silver has conquered a lot of interest among scientists during the past decades for its physiochemical properties such as size, distribution and morphology, they have been studied for catalytic activity, optical properties, electronic properties, magnetic properties, antibacterial and antifungal properties [13-16]. More so, Literatures have reported that Extracts from different plant parts such as Lepidium draba root, Saudi's Dates, *Aegle marmelo* aqueous leaf, Orthosiphon thymiflorus leaf were used to fabricate silver nanoparticles using green method all to avoid toxic, complicated and time consuming conventional approach [17-20]. Interestingly, nanotechnology being the art and science of manipulating matter at the nanoscale to create new and unique materials and products with enormous potential to change society, has succeeded in green synthesizing Zinc Oxide(ZnO), Copper- Cobalt, and Ag-Fe Bimetallic Nanoparticles as these hybrids were found to have improved antimicrobial properties compare to their monometallic entities [21–23]

II. METHODS AND MATERIAL

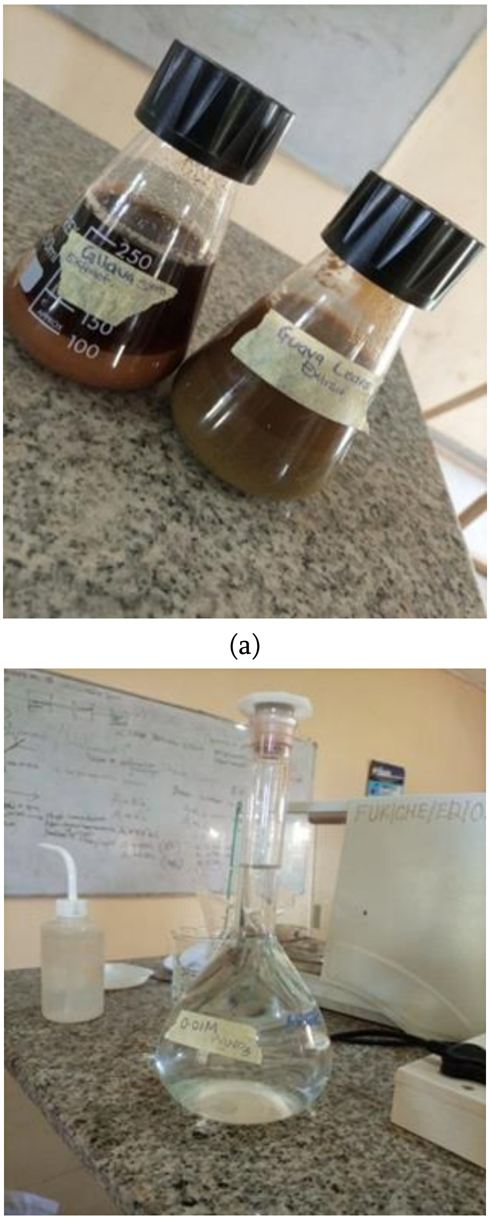
2.1 Materials

The materials used during this research work include, Psidium guajava stem bark, distilled water, Silver nitrate ($AgNO3$), 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 Banganje and were washed properly under running tap water. The samples were shade dried and homogenized to fine powder using a mortar and pestle. 10g of powdered *Psidium guajava* Stem bark was dissolved in 100ml of distilled water and heated for about 10 minutes at 60^{o}C. The extract was filtered using a whatman No. 1 filter paper and kept for further use.



(a)

(b)

Figure 1: (a) guava stem bark extracts (b) silver nitrate solution

2.2.2 Biosynthesis of Silver Nanoparticles using *Psidium guajava* Stem Bark Extract:

A solution containing 250 ml of 0.01mol/dm3 AgNO3 was gradually mixed with one hundred milliliters of the prepared aqueous stem bark extract of *Psidium guajava* (1:5 v/v) on a hot plate at 80^{o}C while stirring for 30 minutes in a 500 ml beaker. A noticeable change in color of the reaction mixture from light brown to reddish brown was observed. The mixture was then stored for about 24 hours after which the nanoparticles settled down. This was evaporated and centrifuged in an oven at 105^{o}C.

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 *Psidium guajava* leaf 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}. The sample was prepared by dispersing the silver nanoparticles uniformly in distilled water as a matrix.

2.3.3. SEM Analysis:

The morphological surface of the nanoparticles (AgNPs) was determined by Scanning Electron Microscope (SEM).

2.3.4. X-ray Diffraction (XRD) Analysis

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

2.4. Antimicrobial analysis

Here, the green synthesized Silver nanoparticles using *Psidium guajava* stem bark extract were investigated for its antibacterial and antifungal activity by Agar well diffusion method against some selected gram positive and gram negative bacteria and fungi

III. RESULTS AND DISCUSSION

3.1. Formation of Silver Nanoparticles and UV-Visible Spectrophotometric Analysis

Here, the formation of Silver Nanoparticles first, was identified by colour change as the addition of crude extract resulted in a quick shift in colour from light brown to orange immediately at the spot and later changed to reddish brown (Figure 2) after 5 h, 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. Similarly, UV–Visible optical findings validated the production of AgNPs in the mixture, with a surface plasmon resonance (SRP) spectra at 400nm of maximum absorption (figure 3). The results obtained conform to those published in the literature, in which a *Dovyalis caffra* as well as *Datura stramonium* extracts were employed to generate silver nanoparticles, and UV–Visible absorbance spectra suggested that the SPR band for Ag particles was in the 400–450 nm range [24-25].

(a)

(b)

(c)

Figure 2 : Colour change of Psidium guajava stem bark extract before (a), immediately(b), and after addition of silver nitrate (c).

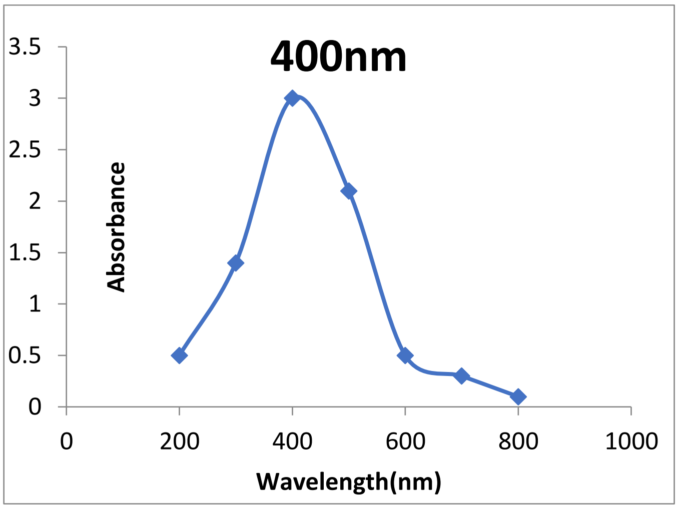


Figure 3: UV-Vis silver nanoparticles synthesized from *Psidium guajava* stem bark

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

3.2. FT-IR Interpretation

FT-IR seeks knowledge about the functional groups present in the synthesized silver nanoparticles for understanding their changes from inorganic silver nitrate (AgNO3) 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 3416.85cm^{-1}, 2923.51cm^{-1}, 1618. 95cm^{-1}, 1384. 49cm^{-1}, and 1033. 63cm^{-1}, were noted in which the absorption peak at 3416.85 cm-1 corresponds to the stretching due to N-H, while the peak at 2923.51 cm-1 is probably associated with C-H stretch of alkane and O-H stretching, 1618.95 cm-1peak possibly depicts C=C stretching, 1384.49 cm-1 reveals the existence of C-H bending and 1033.63 cm-1 depicted C-O stretching. The dichotomy in the FT-IR spectrum signifies the presence of bioactive molecules in plant extracts that participated in the reduction of silver nitrate (AgNO3) and the formation of silver nanoparticles. Interestingly, this finding corresponds to most of the published research works [26-27]

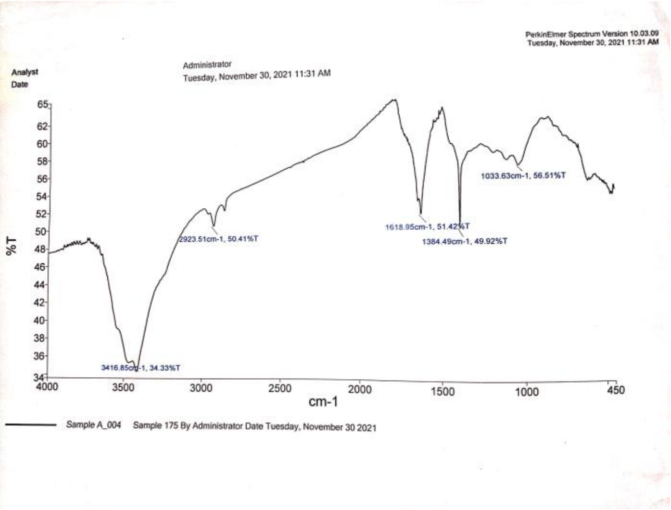


Figure 4. FT-IR Spectrum of Green Synthesized Silver Nanoparticles from *Psidium guajava* Stem Bark Extract

3.3. Scanning Electron Microscopy (SEM)

The surface morphology and crystalline structures of green synthesized AgNPs were studied using SEM (Figure 5). The result of the analysis confirms the shape of the sample in question as having mixed cubic and hexagonal structures. The evidently recognized mixed cubic and hexagonal biosynthesized AgNPs were dispersed within the size range of 20–80 nm. It was however, interesting that with increasing in the amount of the stem bark extract, the SEM images of AgNPs vary. It is therefore, crucial to infer that the shapes and sizes of AgNPs depend significantly, on the plant extract concentration, which consequently alters the optical and electronic characteristics of nanoparticles. Furthermore, it is also to note that, the reduction of Ag^+ to Ag^0 may be mainly due to the presence of secondary metabolites from the plant extract and other bioactive molecules [26-27].

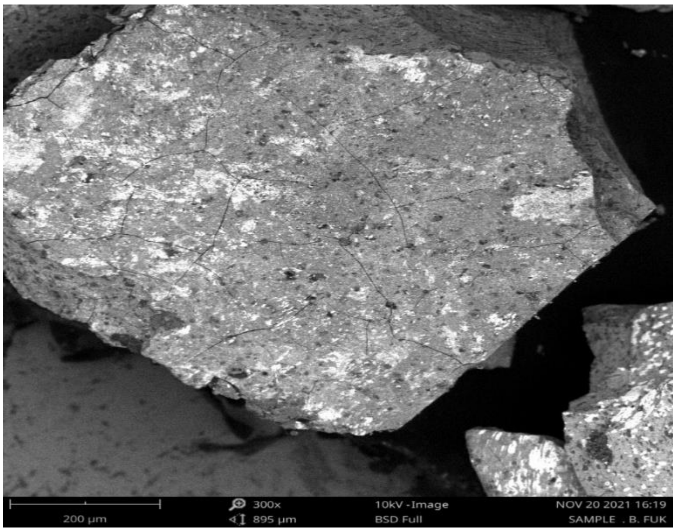


Figure 5. SEM Spectrum of Silver Nanoparticles from *Psidium guajava* Stem Bark Extract.

3.4. XRD Analysis

As depicted in figure 6, the XRD pattern indicates AgNPs' mixed cubic and hexagonal structures with particles ranging between 20 and 70 nm. 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 38.5^0, 44.5^0, and 64.5^0. Using the Scherer's formula: $D=K\lambda/\beta\cos\theta$

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 average size of the particles was determined to be 44.5nm

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 [28-31]

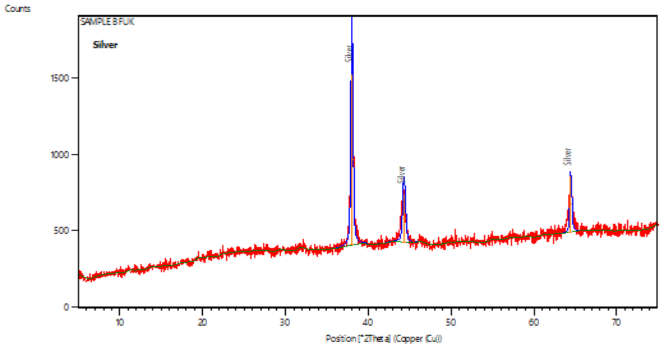


Figure 6. XRD Diffractogram of Green Synthesized AgNPs from *Psidium guajava* Stem Bark Extract.

3.5. Antimicrobial Activity

Silver nanoparticles (AgNPs) have demonstrated significant application in the reduction of pathogenic microbes and also in the treatment of microbial infections. Owing to the rapid increase of antibiotic resistance in this period, this has revived the attention of the researchers investigating the therapeutic abilities of AgNPs systems as potential antimicrobial agents. Presented below (Table 1) is the result of antimicrobial analysis of Silver Nano-particles against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger.* Throughout the investigation, 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; 23.44mm, 18.52mm, 17.66mm, and 15.99mm for *E. Coli, S. aureus, C. albicans* and *Aspergillus niger* respectively. For each concentration investigated, *E.* coli, 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 *Psidium guajava* stem bark extract demonstrated effective antimicrobial activity on the selected Pathogenic microbes. Interestingly, this finding validates the reports by the earlier researcher [32-34].

Table 1. Antimicrobial activity of Silver Nanoparticles

AgNPs	TEST Organism	Concentration (mm)					
		100µg/L	**200µg/L**	**300µg/L**	**400µg/L**	**500µg/L**	**Control (Augmentin) 300µg/L**
	S. aureus.	12.50mm	12.55mm	13.50mm	15.00mm	18.52mm	28.00mm
	E. Coli	13.33mm	14.22mm	16.55mm	19.00mm	23.44mm	25.22mm
	C. albican	12.11mm	12.55mm	13.55mm	15.66mm	17.66mm	27.55mm
	Aspergillus Niger	11.44mm	12.00mm	13.66mm	14.00mm	15.99mm	23.00mm

IV.Conclusion

Silver Nanoparticles were green synthesized from *Psidium guajava* stem bark extract. The method is considered to be green because the synthesis is carried out at ambient temperature, using *Psidium guajava* stem bark and without the addition of any chemical reductant, it does not therefore, generate any environmental pollution. Different Characterization techniques such as 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 in question. Characterization results obtained from UV-Spectroscopic, FT-IR, SEM and XRD analysis proved 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 effectiveness as an antimicrobial agent is further established by the antimicrobial assay performed against four different pathogens namely, *S. aureus, E. coli, C. albican and Aspergillus niger* and the investigation showed that the Silver nanoparticles synthesized were potent against the selected pathogens.

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 AG and JWKJ 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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