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# Green Synthesis of Silver Nanoparticles Using Stem Bark Extract of Prosopis africana and Their Antimicrobial Activity

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**ABSTRACT**
Silver nanoparticles (AgNPs) were synthesized using aqueous stem bark extract of Prosopis africana. The synthesis was done using 1mM silver nitrate solution and aqueous extract derived from the stem bark of Prosopis africana at 60°C.The AgNPs were characterized using UV-Visible spectroscopy, FT-IR, SEM, XRF and XRD analyses. The combination of pale yellow to dark brown coloration and the characteristics peak at 445nm from the UV-Visible spectroscopic analysis confirmed the formation of the silver nanoparticles.The XRD analysis confirmed the crystalline nature of the synthesized AgNPs and the crystallite size was 10nm approximately. The FT-IR spectrum revealed the possible biomolecules that served as reducing and as well as stabilizing agents. The SEM images showed multiple shapes with spiky and spherical shapes dominant. The XRF analysis confirmed the existence of silver in the AgNPs and its quantification. The antimicrobial sensitivity of the AgNPs was also tested against Staphyloccus aureus, Bacislus subtilis, Escherichia coli and Proteus vulgarcus. The AgNPs was effective against Staphyloccus aureus and Bacislus subtilis and non-effective against Escherichia coli and Proteus vulgarcus. The minimum inhibitory concentrations (MIC) were 0.5µg/ml, 0.25µg/ml for Staphyloccus aureus and Bacislus subtilis respectively.

**Keywords**: Green synthesis, silver nanoparticles, Prosopis africana, antimicrobial activity

## I. INTRODUCTION

Nanotechnology is one of the most active fields of research in material sciences. The term "nanoparticles" is used to describe a particle with size in the range of 1nm-100nm. The synthesis of metal nanoparticles is a huge area of research due to its potential applications which was implemented in the development of novel technologies. The nanoparticle exhibits completely new or improved properties, such as morphology, size, and reactivity etc. Novel applications of nanoparticles are emerging rapidly on various fields [1]. In general, nanotechnology involves the synthesis, development and application of different types of nanoparticles [2]. Noble metals nanoparticles like silver, gold, platinum e.t.c. are prepared using different physical and chemical methods [3,4] and these methods are not environmentally friendly [5-7]. Examples of these methods include laser ablation, gamma irradiation, electron irradiation, chemical reduction, photochemical and microwave processing [8]. There is a need to develop a safer and eco-friendly method that does not involves the use of toxic chemicals. Currently, green method approach is commonly used for the biosynthesis of nanoparticles using microorganisms and plant extracts [9-11]. Plant-mediated synthesis has been receiving attention globally because it is very rapid, non-pathogenic, and economical and involves a single step procedure [12]. In the recent years, silver nanoparticles have been synthesized from various plant sources such as green tea, Azadirachta indica, leguminous shrub, leaf broth, natural rubber, starch, etc.

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Silver is a nontoxic inorganic antibacterial agent since ancient times [12].Silver nitrate was once described in Pharmacopoeia in 69BC, possibly to prevent and treat wound infections [13, 14]. Silvernitrate and silver sulfadiazine are used in burnt treatment since the late 20$^{th}$ century [15].

Prosopis africana, African mesquites or iron tree refers to same plant as it is the only Prosopis species native to tropical Africa. P. africana is a flowering plant from the family of Leguminosae-Mimosoideae and genus Prosopis. It has different names among Nigerian ethnic groups which include Kiriya (Hausa), Ayan (Yoruba), Ibwa (Igbo), Okpehe (Idoma) and Kpaaye (Tiv). In some parts of Nigeria, the seed of P. africana when fermented serve as food condiment which contains large amount of both proteins and fatty acids [16]. P. africana has an average height of 4-20m with an open crown and slightly rounded buttresses. The bark is very dark, scaly, slash orange to reddish brown with white streaks. It occurs from Senegal to Ethiopia in the zone between the Sahel savanna forests. Due to its extreme exploitation, it has disappeared from many parts of the Southern Sahel and the adjacent Sudan savanna [17].

## II. MATERIALS AND METHODS

### 2.1 Materials

Silver nitrate $AgNO_3$, nutrient agar, Muller Hilton broth and Whatman No. 1 filter paper were acquired and used for the experimental analysis. The stem bark of Prosopis africana was obtained from the forest located at Dogon Kawo, Doguwa Local Government, Kano State. Four bacteria Staphyloccus aureus, Bacislus subtilis, Escherichia coli and Proteus vulgarcus were obtained from University of Ilorin Teaching Hospital Kwara State, Nigeria.

### 2.2 Sample Collection and Identification

Stem bark of Prosopis africana (Iron tree), was collected from a forest located at Dogon Kawo, Doguwa Local Government, Kano State, Nigeria. It was put in a polyethene bag and transported to identification unit. The sample was identified in the herbarium unit of plant science department, Bayero University Kano, Nigeria. A certificate with identifiable number BUKHAN0193 was issued.

The sample was transported to the Federal Polytechnic, Offa. It was washed under running tap water to remove dust impurities and dried at room temperature. The pulverization is done by the aid of mortar and pestle and then sieved using 0.2mm sieve. It was then stored in a sample bottle, inside the laboratory cupboard for later use.

### 2.3 Chemicals and Extract Preparation

1mM solution of silver nitrate was prepared by weighing 0.2g of $AgNO_3$ and transferred to a1L volumetric flask containing about 400ml of distilled water. The solution was stirred for complete dissolution and topped to the mark using distilled water.

About 12.0g of the powdered sampled was weighed using a digital balance and transferred to a big beaker. 120cm$^3$ of distilled water was added and heated at 80°C for about 15 minutes. It was then filtered using Whatman no. 1 filter paper.

### 2.4 Green Synthesis of the Silver Nanoparticles

15cm$^3$ of the filtrate was measured out using a measuring cylinder. This was followed by addingit in drops into 120cm$^3$ freshly prepared 0.001M silver nitrate solution. This was carried out at 60 °C.

### 2.5 Formation of Silver Nanoparticles

The synthesis of the AgNPs started immediately after mixing the two solutions. A gradual color change was observed from light brown to yellowish brown and finally to dark brown color. The yellowish color remained for about 20 minutes before the finally dark brown coloration. Silver ions ($Ag^+$) were reduced to neutral silver ($Ag^0$) and lastly the AgNPs were produced.

### 2.6 Characterization of Silver Nanoparticles

The growth formation of the nanoparticles was monitored using IMPLEN Uv-Visible spectrophotometer (C40, NPOS 4.2 build, version 14900, with serial number S40727) from the wavelength range of 350-800nm, Fourier Transform Infrared Spectroscopy (FT-IR) (Agilent Cary 630 FTIR, USA), Scanning Electron Microscopy (SEM), the X-ray fluorescence analysis (XRF) was carried out using NITON XL722S portable x-ray fluorescence instrument, the X-ray diffraction analysis (XRD) was performed using PHILIP W 1800 X-ray diffraction at 40mA and 45kV with Cu Kα radiation in θ-2θ configuration. For the antimicrobial sensitivity, dilution of silver nanoparticles was made in distilled water at concentrations of 0.5µg/ml, 0.25µg/ml and 0.13µg/ml. Sterile filter paper disks of approximately 5 mm in diameter with 1 µL of each concentration was dispensed into a sterile Petri dish. Sterile swabs was then used to inoculate Mueller Hinton Agar plates, immersing the tip of the latter in the culture of Staphyloccus aureus, Bacislus subtilis, Escherichia coli and Proteus vulgarcus and passing the wet swab over the entire surface of the plates. Using sterile forceps, the dry

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disc was taken, placing it on the inoculated plate and pressed gently. Three discs of each were placed per plate and incubated at 35 ℃ for 24 h [18].

## III. RESULTS AND DISCUSSION

### 3.1 Ultraviolet-visible Spectroscopy Analysis

The synthesis of the AgNPs was confirmed through UV-Visible spectroscopic analysis. The Surface Plasmon Resonance (SPR) observed at 445nm which is a physical phenomenon arises due to the vibration motions of the plane-polarized light on the metal surface during its reflection. Figure 5 shows the UV-Visible absorption spectrum of the AgNPs with a broad-band absorption between 425-445nm which was characteristics of polydispersed AgNPs in solution as reported by [19]. Other broad absorption peaks of AgNPs were observed in previous researches [20] and [2].

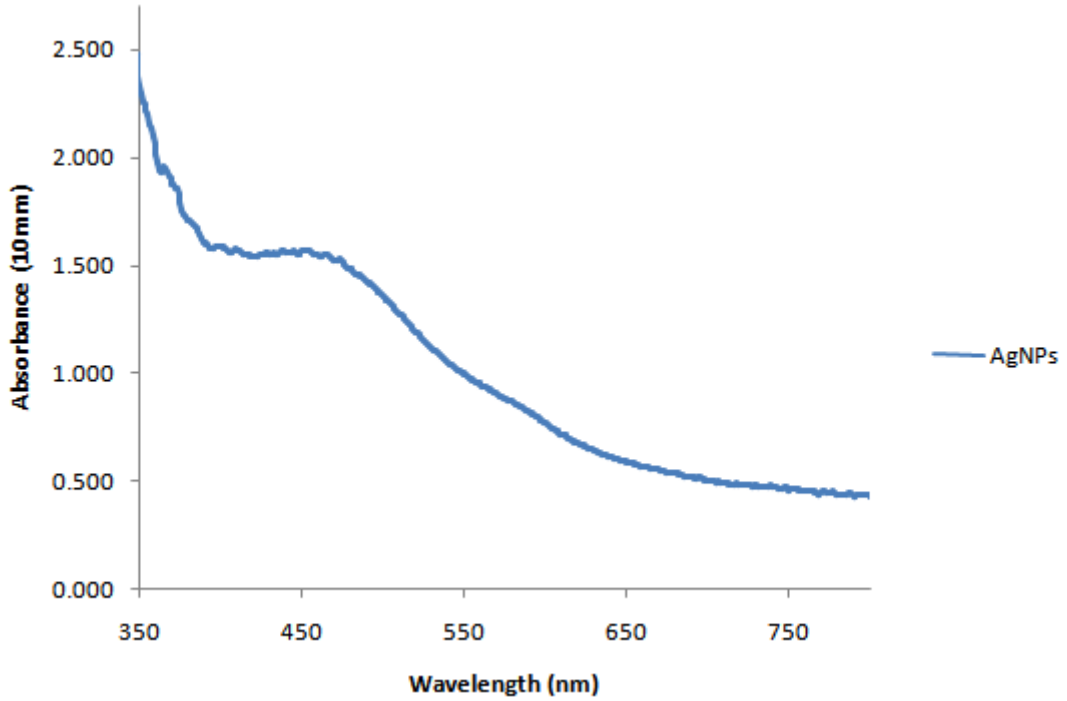


**Figure 1:** Uv-visible absorption spectrum of AgNPs synthesized from Prosopis africana at 60°C.

### 3.2Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

FTIR analysis was done to know the probable functional groups responsible for the reduction of silver ion ($Ag^{+}$) to ($Ag^{0}$) and served as capping as well as stabilizing agents in the stem bark extract of Prosopis africana. Figure 2 shows the FTIR spectrum of the AgNPs with different peaks for the synthesis done at 60 ℃. A broad absorption band at 3313.60$cm^{-1}$ is attributed to the existence of O-H stretch which could be from phenols and hydrogen bonded alcohols as reported by [19] and is followed by other absorption bands at 1636 and 1017$cm^{-1}$ which also indicated C=O stretching as reported by [21] and C-O, C-N stretching vibrationsof aliphatic amine respectively [22]. The specific peak at 2762.0$cm^{-1}$ was assigned to the C-H stretching of an aldehyde functional group [23]. The bands at 2195.4 $cm^{-1}$, 2128 $cm^{-1}$ and 2064.9$cm^{-1}$ indicated C≡C [24] which was also followed by absorption at 626.3$cm^{-1}$ - 540.5$cm^{-1}$ assigned to S-S and C-S stretching [24]. Thus, it can be deduced that the extract consists of hydroxyl, carbonyl, aldehyde, thio-group and alkyne functional groups.

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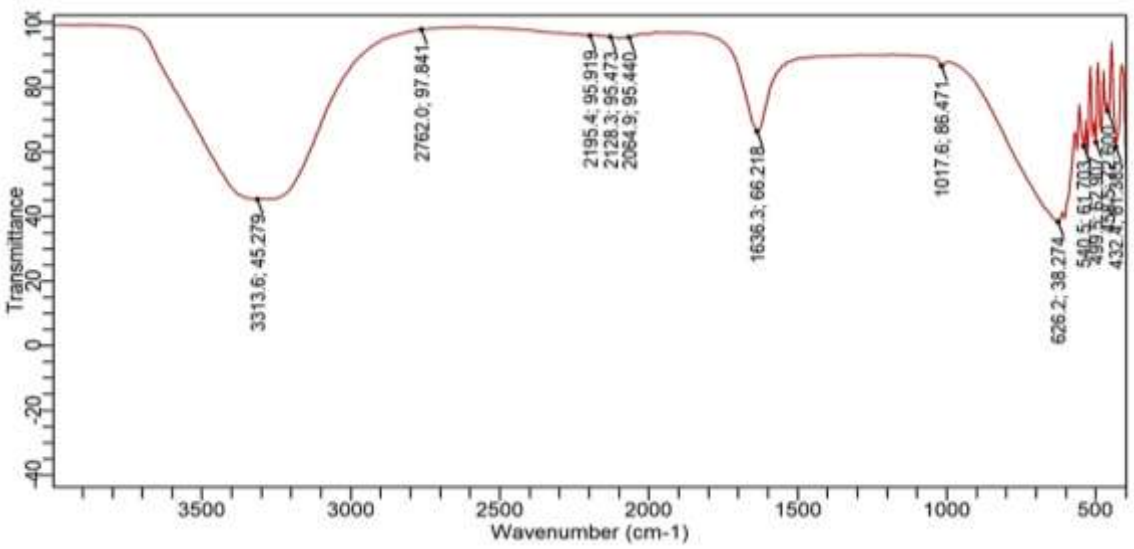


**Figure 2**FTIR spectrum of the AgNPs with different peaks for the synthesis done at 60 °C.

### 3.3 X-ray Fluorescence Analysis (XRF)

X-ray fluorescence analysis was carried out to confirm the existence of silver in the AgNPs. The analysis also ascertained silver element as the major constituent with 67.67±2.9% as shown in Figure 3. The peaks at 22.16KeV and 24.94KeV were characteristics of Kα1 and Kβ1 emission energies of silver element; this is in line with X-ray data booklet [25]. Other prominent trace elements were Fe, Rh, and Pd.

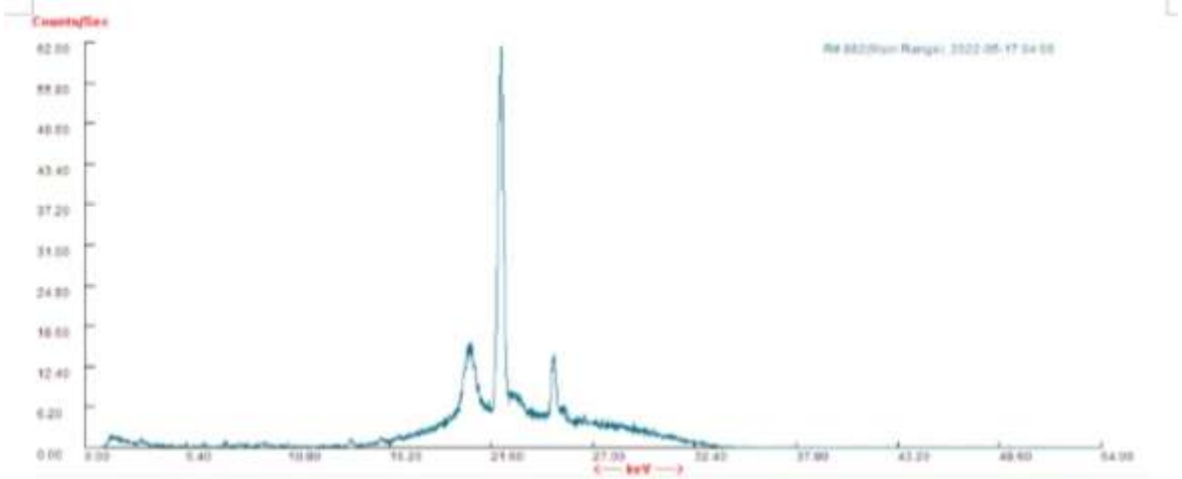

**Figure 3 XRF image of the AgNPs**

### 3.4 Scanning Electron Microscopy Images (SEM)

To check the morphology of the AgNPs, the SEM was done. The SEM image in Figure 4 showed multiple shapes resembling spiky, rods and spherical nanoparticles. Thus, it was polydispersed. It also showed that the particles agglomerated to form crystallites.

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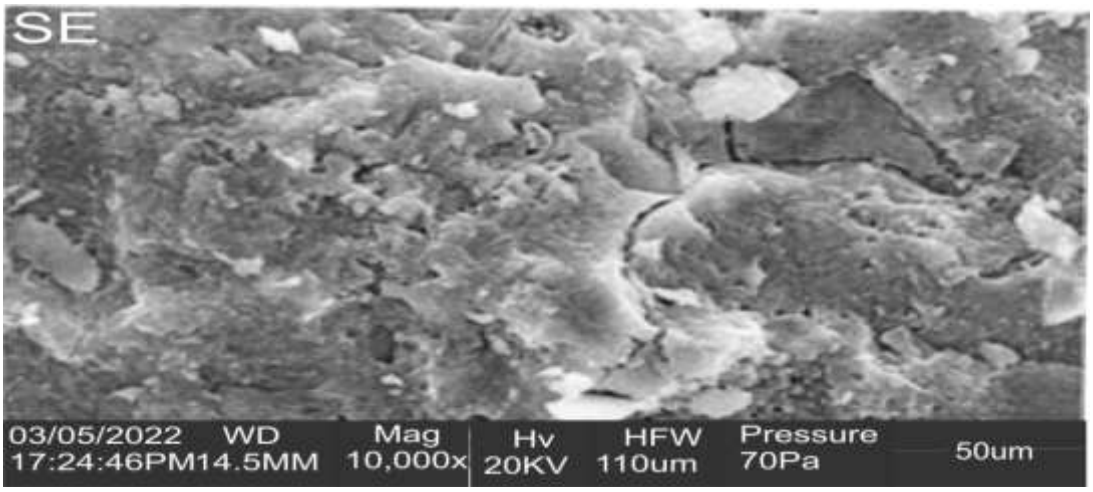


**Figure 4 The SEM image of the AgNPs**

### 3.2 X-ray Diffraction (XRD) Analysis

The crystalinity and the crystallite size of the biosynthesized nanoparticles were investigated using XRD analysis. The XRD spectrum in Figure 5 showed three sharp and distinct diffraction peaks at 2θ values of 38.06, 44.23 and 67.43 respectively. These values correspond to **(111)**, **(200)** and **(220)** planes of the Bragg reflection based on the face centered cubic structure of AgNPs. These 2θ values were in excellent agreement with the ones reported by [2] and in good agreement with [26]. The crystallite size was calculated using Debye-Scherrer equation as shown in equation (1) below:

$$d = {}^{k\lambda}/_{\beta(\cos\theta)} \quad (1)$$

Where d is the particle size of the crystal, k is Sherrer constant (0.9), λ is the wavelength of X-ray source used in XRD (0.15406 nm), β is the breadth of the observed diffraction peak at its half maximum and θ is the Bragg angle and was found to be 10nm approximately.

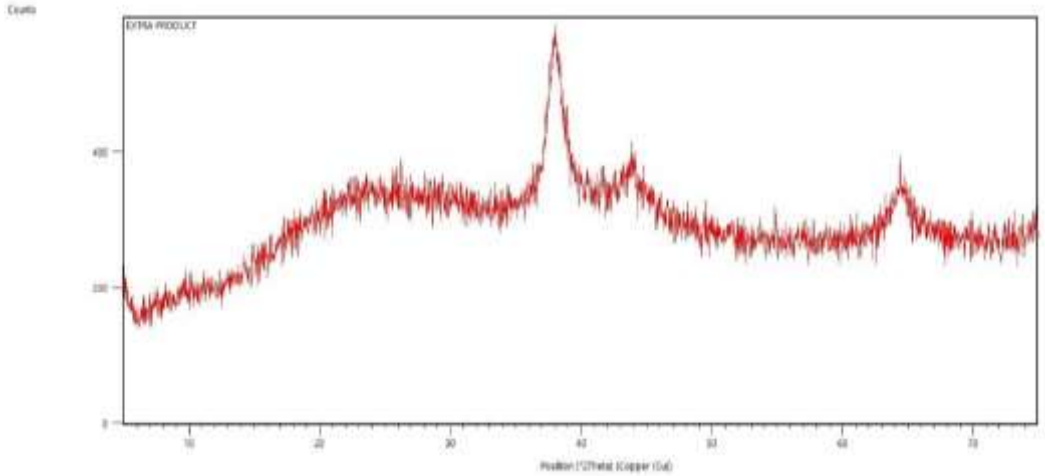

**Figure 5 the XRD pattern of the AgNPs**

### 3.6 Antimicrobial Sensitivity

The antimicrobial sensitivity of the synthesized nanoparticles was tested against some isolated clinical strain bacteria (Staphyloccus aureus, Bacislus subtilis, Escherichia coli and Proteus vulgarcus) obtained from the University of Ilorin Teaching hospital. Various concentrations of 0.5µg/ml, 0.25µg/ml and 0.13µg/ml of the nanoparticles were used. From Table 1 below, concentration of 0.5µg/ml has the highest inhibition efficiency on Bacillus subtilis and Staphyloccus aureuswhich are gram positive bacteria. On the other hand, there is no cleared zone at all concentrations on Escherichia coli and Proteus vulgarcus which are gram negative. However, there

was 4mm of inhibition on Bacillus subtilis at concentration of 0.25µg/ml.

**Table 1** Zones of inhibition of AgNPs by P. africana stem bark extract

| AgNPs Concentration (µg/ml) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | Staphyloccus aureus | Bacilus subtilis | Escherichia coli | Proteus vulgarcus |
| 0.5 | 10 | 12 | - | - |
| 0.25 | - | 4 | - | - |
| 0.13 | - | - | - | - |

## IV. CONCLUSION

A stem bark extract of Prosopis africana was successfully used for the biosynthesis of silver nanoparticles. Characterization of the silver nanoparticles was done using Ultraviolet-visible spectroscopy, FT-IR, XRF, XRD and SEM analyses. The crystallite size of the AgNPs was calculated from the XRD data approximately 10nm. The biosynthesized nanoparticles were tested for the growth inhibition against Staphyloccus aureus, Bacilus subtilis, Escherichia coli and Proteus vulgarcus which were clinically isolated from patients. However, it only inhibited the growth of Staphyloccus aureus and Bacilus subtilis at concentrations of which are gram-positive bacteria but has no effect against Escherichia coli and Proteus vulgarcus which are gram-negative bacteria.

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