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**Original Research Article**

# Physicochemical Parameters and Antibacterial Activity of Biosynthesized Silver Nanoparticles from *Carica papaya* Leaf Extract

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## Abstract

In this research work, Silver nanoparticles were synthesized from Carica papaya leaf extract via green route. The physicochemical parameters including boiling point, color, odor, density, pH as well as the solubility of papaya leaf extract were first determined before proceeding with the synthesis of silver nanoparticles. The formation of Silver Nanoparticles first, was identified by color change from light brown to dark brown after the nucleation of the metal ions indicating that phytoconstituents of Carica papaya resulted in the reduction of $Ag^+$ to $Ag^0$, a phenomenon that could be attributed to the surface Plasmon absorption. The bio fabricated silver nanoparticles were characterized using UV – Visible and SEM to be certain of its formation before being deployed in the antibacterial studies. The UV-Vis spectral analysis showed maximum absorbance of 1.05 at a corresponding wavelength (λ max) of 400nm reflecting the surface Plasmon resonance of silver NPs from papaya leaves which is characteristic of Silver Nanoparticles. SEM image revealed that, the synthesized silver nanoparticles have a spinel like structure and an average size of about 50nm. The antibacterial studies of Silver nanoparticles were conducted against B. subtilis, K. pneunoniae, P. aeruginosa, E. coli, and S. typhi. 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; 24.44mm, 17.64mm, 17.52mm, 16.88mm, and 16.00mm for B. subtilis, P. aeruginosa, E. coli, K. pneumoniae and S. typhi respectively. The zone of inhibition for Augmentin was found to be higher compared to silver nanoparticles for each pathogen, except for P. aeruginosa where it is almost the same, an indication of high activity of silver nanoparticles against P. aeruginosa due to the comparability with Augmentin. For each concentration investigated, B. subtilis demonstrated higher zone of inhibition as compared to other pathogens studied in this work, hence, Ag NPs may be a potential antibiotic.

**Keywords:** Physicochemical Parameters, Antibacterial Activity, Biosynthesized, Silver Nanoparticles, *Carica papaya*, Leaf Extract.



## 1. INTRODUCTION

Owing to the wide application of silver nanoparticles (Ag NPs) in some disciplines, such as pharmacy, medicine, catalysis, solar energy, and water disinfection, the literature has focused attention on the synthesis of Silver nanoparticles with minimum hazards and maximum advantage. Recently, several studies have shown that plant extracts act as potential originators for the synthesis of the nanomaterials in some ecofriendly ways as they are used successfully in the synthesis of several greener nanoparticles (Fatemeh B, *et al.*, 2017; Mela Y, *et al.*, 2022). This therefore, means Increasing awareness towards green chemistry and other biological processes led to countless interests in fabricating an environmentally friendly approach for the synthesis of nanoparticles(Sharmila B.A, *et al.*,2018; Bashpa P, *et al.*, 2017; Mahesh and Shivayogeeshwar, 2018; Elisha K, *et al.*, 2020).Quite a number of transition elements (metals) including Silver, Cobalt, Iron, Copper, Gold, Platinum, Nickel, Platinum among others, have over the years, been deployed in the synthesis of plants mediated nanoparticles due to their numerous applications, hence, the reason for their popularity((Flora P.J, *et al.*, 2018; Suriyavathana M, *et al.*, 2018; Mani P, *et al.*, 2016; Ravindra D.K, *et al.*, 2018). In the same vein, these metallic nanoparticles show unique characteristic properties that made them stand out as reported in many literatures (Shailesh C.K, *et al.*,2018; Darshana R, *et al.*, 2020). Bio fabrication of Silver nanoparticles, in particular, has attracted wide audience since the demand to synthesize nanoparticles in an environmentally benign way has increased significantly consequence upon their inherent characteristic of acting as an antimicrobial agent (Mela Y, *et al.*, 2022). Some researchers described Nanoparticles as submicron moieties with diameters ranging from 1-100 nm made of inorganic or organic materials having novel properties as compared to the bulk materials (Kuchekar S. R, *et al.*, 2018; Mandeep and Dimple, 2018). Furthermore, some researchers including Wilson L.D, *et al.*, 2020; Mela Y, *et al.*, 2022; Zaccheus S, *et al.*, 2020 and Mela Y, *et al.*,2022 in separate studies all have demonstrated that coupling two metals in a fascinating way, would lead to the formation of bimetallic nanoparticles having improved properties than their counter monometallic nanoparticles. This study therefore explores the physicochemical properties of *Carica papaya* leaf extract, green synthesis and characterization of Ag NPs from the papaya leaf extract as well as deployment of the same in antimicrobial study on some selected pathogens.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The materials used during this work include but not limited to $AgNO_3$ solution, *Papaya leaf*, deionized water, hot plate, Whatman no. 1 filter paper, crucible, beaker. All the reagents used during this work were of analytical grade.

### 2.2 METHODS

#### 2.2.1 Collection of Plant Samples

Fresh leaves of papaya were collected from within the Garden located at Banganje, Billiri Local Government Area of Gombe State, Nigeria. The leaf sample was transported via road to Federal University of Kashere, Gombe State, Nigeria. The same was identified and authenticated at the Taxonomy Section.

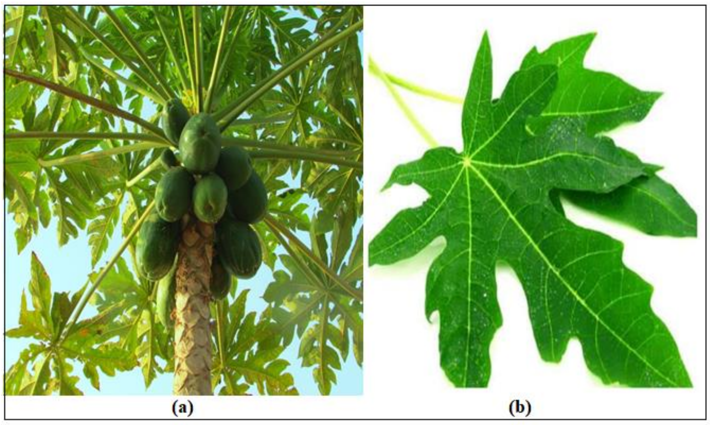


**Figure 1: (a) *Carica papaya plant,* (b) *Carica papaya* Leaf**

#### 2.2.2 Preparation of Aqueous Leaf Extract

The collected papaya leaves were thoroughly washed under running tap water and rinsed severally with distilled water followed by sun-drying to remove residual moisture. The dried materials were cut into tiny sizes and ground with the aid of a crucible. About 60g of it was weighed and dispersed in 200 ml of sterile distilled water in a 500 ml glass beaker and boiled at $70^0$C for 30 min and were allowed to cool. After that, the solution was filtered through Whatman No.1 filter paper (Springfield Mill. Maid stone. Kent, England) and the filtrate was used immediately for the synthesis of Silver nanoparticles.

#### 2.2.3 Physicochemical Properties of Papaya Leaf Extract

**Boiling Point:** About two 2mL of *papaya* leaf extract was placed in a test tube. The test tube with the extract was heated in bath oil, and when it starts to boil the temperature was recorded. This process was repeated three times to have an accurate result.

**Color:** The color of *papaya leaf extract* was observed by the researcher and five respondents using their sense of sight. Having five respondents is the way of getting an accurate result. A majority is a rule to be noticed in this test.

**Density:** The *papaya leaf extract* was poured into a graduated cylinder, then its volume was recorded and weighed in analytical balance. The density of the *papaya leaf extract* was calculated by the mass divided by its volume.

**Odor:** The odor of *papaya* leaf extract was observed by the researcher and five respondents using their sense of smell.

**pH:** The pH of *papaya* leaf extract was determined by using pH indicator strips. About 10 mL of the sample was placed in a beaker and three pH indicator strips were dipped into the extract. After the three pH indicator strips dried, the color pattern was then analyzed and the pH of the extract was obtained.

**Solubility:** Three solvents were used namely; hexane, water, and ethanol. Nine test tubes were used, each containing 2 mL of the plant sample extract. These nine test tubes were divided into three groups, each group having two milliliters of solvent to be poured in. The nine test tubes were then observed for one minute to determine the solubility of the plant sample extract in three different solvents, and the results were recorded. If the solute is miscible in both ethanol and water the solute is said to be polar, on the other hand, if the solute is miscible to hexane, it is non-polar.

#### 2.2.4 Preparation of Silver Nitrate Solution

An accurately weighed 0.017 grams of silver nitrate was dissolved in 90mL of de-ionized water and stored in a bottle for further use. The bottle with the solution was covered with aluminum foil to avoid degradation of the silver nitrate.

#### 2.2.5 Biosynthesis of Silver Nanoparticles

Here, the method reported by Mela Y *et al.*, 2022 was adopted with slight modifications as follows: A solution containing 200 ml of 0.01mol/dm$^3$ AgNO3 was gradually mixed with one hundred milliliters of the prepared aqueous leaf extract of *Carica papaya* on a hot plate at 70$^o$C with constant stirring for 30 minutes in a 1000 ml beaker. A noticeable change in color of the reaction mixture was recorded. 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 1nm (from 200 to 800 nm)

#### 2.3.2 SEM Analysis

The surface morphology of the nanomaterial (Ag NPs) was characterized by scanning electron microscope (SEM).

### *2.4. Antimicrobial analysis*

The antimicrobial efficacy was tested by using the agar-well diffusion method as described by Shehu *et al.*2020 as follows. The bacterial isolates; gram-positive bacteria (*Bacillus subtilis*, *Klebsiella pneunoniae*) and gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*) were first grown in a nutrient broth for 12–18 h before use and standardized to 0.5 McFarland standards (106 cfu ml$^{-1}$). One hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Hi Media) and the agar medium was punched with a 6 mm diameter wells and filled with different concentration (100, 200, 300, 400 and 500 µg/L) of AgNPs solutions in equal amounts. The plates were observed for zone of inhibition after 24 h incubation at 37$^o$C.

## 3. RESULTS AND DISCUSSION

### *3.1 Physical Properties*

The test tube containing papaya leaf extract when placed in bath oil started boiling at an average boiling point of 80.4°C. Similarly, Color and odor of the extract were determined by a panel of evaluators. The majority of evaluators said that the aqueous leaf extract of the sample has light brown color and pleasant odor. In the same vein, the density of the extract was determined by weighing (5 mL) of the extract and then dividing the weight in grams by the volume in mL. After the computation, it was found out that the density of the aqueous leaf extract was 0.93g/mL. The pH was determined using pH indicator strips by pouring 10 mL of leaf extract and dipping the pH indicator strips into the extract and analyzing the change of its color pattern. After analyzing the change in color pattern, the researcher found out that the aqueous leaf extract is slightly basic with a pH level of 8. Again, the solubility of the sample leaf extract was evaluated using water, hexane, and ethanol as solvents. The selection of solvents was based on polarity. Water and ethanol are polar while hexane is non-polar. For easy accessibility, the physicochemical properties findings are here documented in table 1 below.

**Table 1: Summary of Physicochemical Properties Finding**

| Physical Properties | Observation | Interpretation |
|---|---|---|
| Boiling Point | 80.4°C | Lower Boiling Point than Water |
| Color | Light brown | Light brown |
| Odor | Pleasant | Pleasant |
| Density | 0.93g/mL | Less dense than water |
| pH | 8 | Slightly basic |
| **Solubility in:** | | |
| Ethanol | Miscible | Polar |
| Hexane | Immiscible | Non Polar |
| Water | Miscible | Polar |

### 3.2 Silver Nanoparticles' Formation

The formation of Silver Nanoparticles initially, was identified by color change from light brown to orange immediately at the spot and later changed to dark brown (Figure 2) after the nucleation of the metal ions indicating that phytoconstituents of *Carica papaya* caused the reduction of $Ag^{+}$ to $Ag^{0}$, a phenomenon that could be attributed to the surface Plasmon absorption.

### 3.3 UV- Visible Spectrophotometric Analysis

UV-visible spectroscopy measures the extinction of light passing through a sample. It is a valuable tool for identifying, characterizing, and studying nanoparticles. From the UV-Vis spectral analysis, it can be seen that highest absorption peaks appeared at 400nm (Figure 2) reflecting the surface Plasmon resonance of silver NPs from papaya leaves which is characteristic of Silver Nanoparticles. This finding is in consonance with the previous result reported by separate researchers (Varaprasad T, *et al.*, 2017). Furthermore, the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation resulted to the surface Plasmon absorption in the metal nanoparticles.

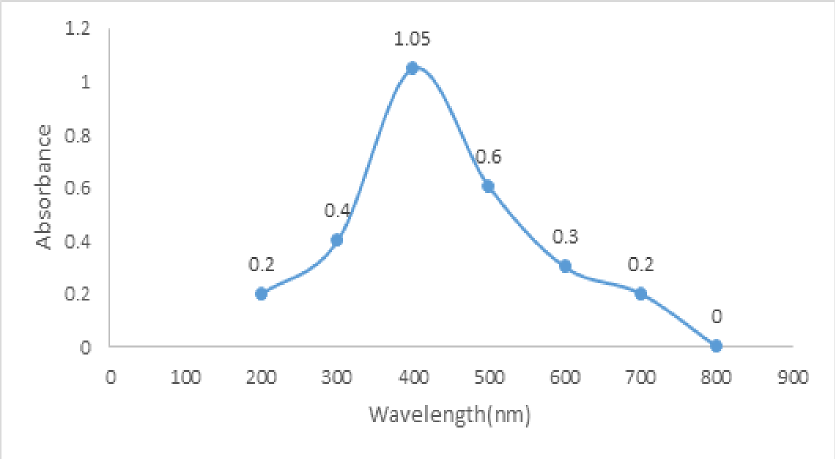


**Figure 2: UV Spectrum of Ag NPs from Papaya Leaf Extract**

### 3.3 SEM Results

The surface morphology of the AgNPs were investigated using Scanning electron microscope (SEM) and findings are presented in Figure 3. It is clear from the SEM image that the whole matrix is filled with small AgNPs indicating uniform distribution of nanoparticles. It is notable from the SEM image that, the synthesized silver nanoparticles have a spinel like structure and an average size of about 50nm, a result that is similar to the one reported by some researchers (Igwe and Ekebo, 2018; Igwe and Mgbemene, 2014; Mela Y *et al.*, 2022) and from nanomaterials point of view, this is an ideal size range.

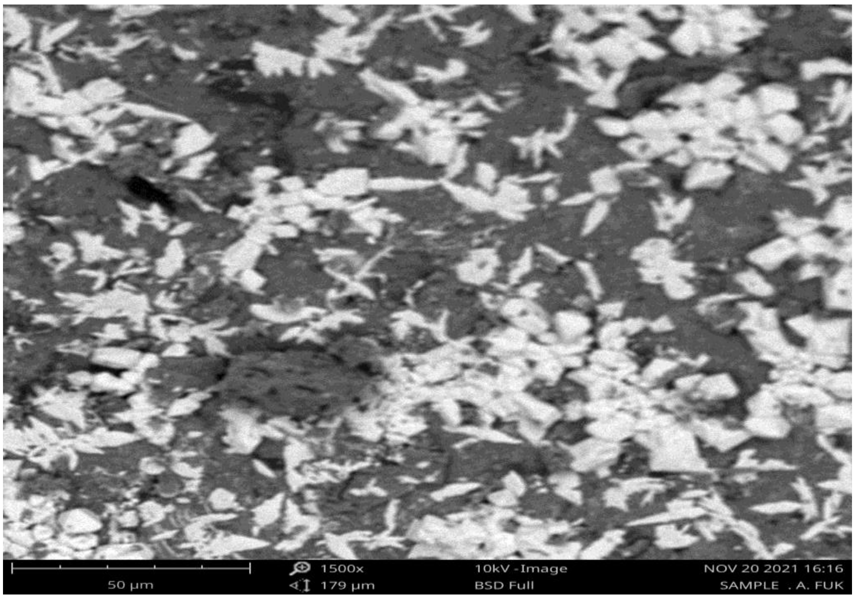


**Figure 3: SEM Spectrum of Ag NPs from Papaya leaf extract**

### 3.4 Antibacterial Results

Throughout this study, Augmentin was used as control at concentration of 300µg/L. Presented in Table 2 below are the results of antibacterial studies of Silver nanoparticles against *Bacillus subtilis*, *Klebsiella pneunoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*. Varying concentrations of 100, 200, 300, 400 and 500µg/L of Silver nanoparticles were tested against each pathogenic bacterium. The inhibition zone increases generally with increase in concentrations of silver nanoparticles of all the bacteria. Similar observations were reported in the literature (Nathaniel A. D, *et al.*, 2022). At higher concentration of 500µg/L, the zones of inhibition were in the following order; 24.44mm, 17.64mm, 17.52mm, 16.88mm, and 16.00mm for *B. subtilis*, *P. aeruginosa*, *E. coli*, *K. pneumonia*e and S. *typhi* respectively. The zone of inhibition for control (Augmentin) was found to be higher compared to silver nanoparticles for each pathogen except for *P. aeruginosa* where it is almost the same, an indication of high activity of silver nanoparticles against *P. aeruginosa* due to the comparability with Augmentin (control). For each concentration investigated, *B. subtilis* demonstrated higher zone of inhibition as compared to other pathogens (Table 2). This phenomenon indicates that, Ag NPs are more potent against *B. subtilis* than any other pathogen investigated in this study.

**Table 2: Antibacterial Activity of AgNPs**

| AgNPs | TEST Organism | Concentration (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **100**µg/L | **200**µg/L | **300**µg/L | **400**µg/L | **500**µg/L | **Control (Augmentin) 300**µg/L |
| | *E. coli.* | 9.00mm | 11.00mm | 13.00mm | 14.10mm | 17.52mm | 20.00mm |
| | *B. subtilis* | 10.33mm | 13.22mm | 16.55mm | 21.00mm | 24.44mm | 22.22mm |
| | *P. aeruginosa* | 8.10mm | 12.55mm | 13.50mm | 16.60mm | 17.64mm | 23.00mm |
| | *K.pneunoniae* | 7.42mm | 10.00mm | 14.60mm | 16.55mm | 16.88mm | 18.44mm |
| | *S. typhi* | 9.10mm | 9.22mm | 12.77mm | 14.11mm | 16.00mm | 25.00mm |

## 4. CONCLUSION

The Physicochemical parameters of the *Carica papaya* leaf extract were determined before proceeding with the synthesis of the Silver nanoparticles using green method. The formation of Silver Nanoparticles was identified by color change from light brown to dark brown. The UV-Vis spectral analysis revealed highest absorption peak (λ max) at 400nm with a corresponding absorbance of 1.05. From the SEM analysis, the synthesized silver nanoparticles have a spinel like image and an average size of about 50nm. The antibacterial studies revealed that the inhibition zone increases generally with increase in concentrations of silver nanoparticles of all the bacteria. The zone of inhibition for control (Augmentin) was found to be higher compared to silver nanoparticles for each pathogen except for *P. aeruginosa* where it is almost identical, an indication of high activity of silver nanoparticles against *P. aeruginosa.* It could therefore be concluded that, Ag NPs can act as an antibacterial agent.

**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, wrote the first draft of the manuscript and managed the analyses of the study. Authors JDS, PDB and JWKJ managed literature searches. All authors read and approved the final manuscript.

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**Conflict of Interest:** The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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