Antibacterial potency of silver nanoparticles synthetized using *Boerhaavia diffusa* leaf extract as reductive and stabilizing agent

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Abstract

This study was aimed on the use of simple and eco-friendly method in synthesis of silver nanoparticles (AgNPs) using *Boerhaavia diffusa* leaf extract and to investigate the antimicrobial activity of the synthesized silver nanoparticles. The characterization of the synthesized silver nanoparticles was investigated with UV-Visible spectroscopy, Fourier Transmission Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM). The reduction of the Ag^{+} to Ag^{0} by *Boerhaavia diffusa* leaf extract was followed by color change of the solution from colorless, yellow to dark brown within 24 hours. The surface Plasmon resonance peaks of the maximum absorbance of synthesized silver nanoparticles was observed at 425 nm, signifying the formation of AgNPs. The participation of –OH, C=O, C=C and alkane functional group in the synthesized AgNPs was detected from the FTIR spectra. The TEM micrograph reveals that the silver nanoparticle are spherical in shape with particle size ranging from 30- 40 nm. The high percentage zone of inhibition (26 – 51%) against selected microbes shows that the silver nanoparticle exhibit good antimicrobial potency. From this study we can conclude that *Boerhaavia diffusa* leaf extract could be considered as good antimicrobial agent, a good source for simple, low cost and eco-friendly synthesis of stable AgNPs.

Keywords: *Boerhaavia diffusa* leaf extract, silver nanoparticles, antimicrobial and Eco-friendly.

1.0 Introduction

The occurrence of antibacterial multi-drug resistant infections, which affects the public health sector worldwide, has been increasing to worrisome levels and has stimulated investigations on plant species for the treatment of bacterial infections [1]. Reports from previous study have shown that inorganic nanoparticles possess the ability of interacting with microorganisms and consequently exhibit antibacterial activity [2-4]. Biosynthesis of nanoparticles has become a green research area in recent years in the view of replacing the hazardous and non-renewable chemicals effect of the chemical method of synthesis with utilization of plant extract as a reductant in the synthesis of metal nanoparticles [5,6]. The proficiency of flavonoids and alkaloids in medicinal plants to reduce metal ion precursor is the principle behind the biosynthesis of metal nanoparticles from plant extracts [7,8]. The physical, biological, and pharmaceutical application of silver nanoparticles has been recognized in the past decades [9,10]. Silver nanoparticle synthesis from plant extract is widely preferred to various known synthesis methods due to its cost-effectiveness, less toxicity and safety level when use for healing purpose. Recent studies have reported the use of silver nanoparticles as an anti-bacterial agents. The role of silver nanoparticles as an anti-bacterial agent might offer new application in pharmaceutical and medical sciences [11]. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as contain natural capping agents [12]. Among other plants, *Boerhaavia diffusa* leaves was chosen for this study due to its reported pharmacological effects such as anti-asthmatic, antibacterial, anti-diabetic, anti-inflammatory, antioxidant, anti-nociceptive, diuretic and immunomodulator The phytochemical screening of *Boerhaavia diffusa* confirmed the presence of phenolic glycoside, methyl flavone and rotenoids such as Boeravinone-A, Boeravinone-B, Boeravinone-M, Coccineone-B, 6-O-Demethylboeravinone and 2'-O-

methylabronisoflavone [13]. The aim of this study was to investigate the antimicrobial activity of green synthesis of silver nanoparticles using *Boerhaavia diffusa* leaf extracts as both reducing and stabilizing agent against Gram positive and Gram negative bacteria.

2.0 Materials and Methods

2.1 Preparation of Boerhaavia diffusa Leaves Extract and Silver Nitrate Solution

Boerhaavia diffusa were collected in the month of January from tropical regions from Lumeko farmland in Irele Local government of Ondo State Nigeria. The obtained *Boerhaavia diffusa* leaves were at their prime (neither too young nor too old). The *Boerhaavia diffusa* leaves were rinsed with fresh water and air dried at room temperature by laying them evenly. 10 g of air dried leaves were milled and transferred into a 200 mL conical flask. The content in the conical flask was boiled with distilled water for 5 minutes and cooled. The cooled mixture was filtered with Whatman filter paper to obtain a light yellow color *Boerhaavia diffusa* leaves extract. The extracted solution was stored in a fridge for further use. 40 ml of 1mM silver nitrate ($AgNO_3$) solution obtained from Sigma Aldrich was mixed with 8 mL of the extract. These mixtures were placed on a shaker and stirred for 30 min, 150 rpm at room temperature. After 30 min, the change in color from light brown to dark red was observed and monitor till 48 hours of the reaction, the change in the color intensity after the reduction of silver ion to silver nanoparticles by *Boerhaavia diffusa* extracts with increasing time of reaction was recorded. This confirm the formation of silver nanoparticle.

3.0 Characterization of the silver nanoparticles

3.1 UV-visible Spectroscopy

The reduction of pure silver ions to silver was examined by measuring the UV-Vis spectrum of the reaction. The UV-vis absorption spectrum of the synthesized silver nanoparticles from *Boerhaavia diffusa* extract was done with spectrophotometer (Schimadzu 1601 spectrophotometer) in the wavelength range from 250 to 900 nm to determine its maximum absorption [14]. The aqueous silver ions was reduced by addition of *Boerhaavia diffusa extract.*

3.2 Fourier Transformed Infrared (FTIR) Analysis

Fourier Transformed Infrared analysis of *Boerhaavia diffusa* extract and synthesized AgNPs was conducted in order to know the main functional groups in them and function groups responsible for the reduction of silver ion. The *Boerhaavia diffusa* extract and the colloidal solution of synthesized AgNPs was run as inert on the Fourier Transformed Infrared (FTIR) Spectrophotometer (FTIR-8400s, SHIMADZU) with wavelength 4,000-400 cm^{-1}.

3.3 Transmission Electron Microscopy (TEM) Analysis

The surface morphology and particle size distribution of synthesized silver nanoparticle was determined with transmission electron microscopy JEOL JEM model 1010 (JEOL, Japan), at an accelerating voltage of 80 kilo voltage). Few drop of the synthesized silver nanoparticle solution was dropped on Lacey carbon grids, 300 mesh and the grid was allowed to dry before taking the measurement

Table 1.0 Pathogenic bacteria species used

Bacteria	
Gram positive	Gram negative
Bacillus subtilis	*Pseudomonas aeruginosa,*
Staphylococcus aureus	*Klebsiella pneumonia,*
Clostridium ramosum	

3.4 Antimicrobial activity of biosynthesized Silver nanoparticles

The antimicrobial activity of the synthesized silver nanoparticles was tested against some clinical isolates gram-positive and gram-negative bacteria species showed in table 1. The diffusion method was used for this study. The pure cultures of the organisms were sub cultured on Muller–Hinton broth at 35 ^{0}C on rotary shaker at 200 rpm. Sterile cotton swab was used to homogenously spread each strain on the individual plates. 8 mm size wells were made on the Muller–Hinton agar plates by gel puncture. Fifty microliters of 50 µl, and 100 µl of silver nanoparticles solution were

poured into wells on all plates with a micropipette and streptomycin was used as a control. After incubation at 35 ^{0}C for 18 hours, the zones of inhibition were measured with ruler and their percentage zone of inhibition were calculated with the given formula.

$$PGI = \frac{(BDC-BDT)}{(BDC)} \times 100$$

PGI = Percent growth inhibition,

BDC = Bacteria colony diameter in control

BDT = Bacteria colony diameter in treatment

4.0 Result and discussion

4.1 UV–visible absorbance analysis:

The formation of AgNPs was monitored with UV-Vis spectrum and observed colour change. The reduction of silver ions into AgNPs commenced after 30 minutes of reaction and was monitored for 24 hours. A single sharp surface plasmon resonance band at 425 nm was observed on the absorption spectra of AgNPs solution (Figure 1). The absorption peak observed at wavelength 425 nm is a sign of reduction of silver. This finding is in line with the result obtained from [15,16].

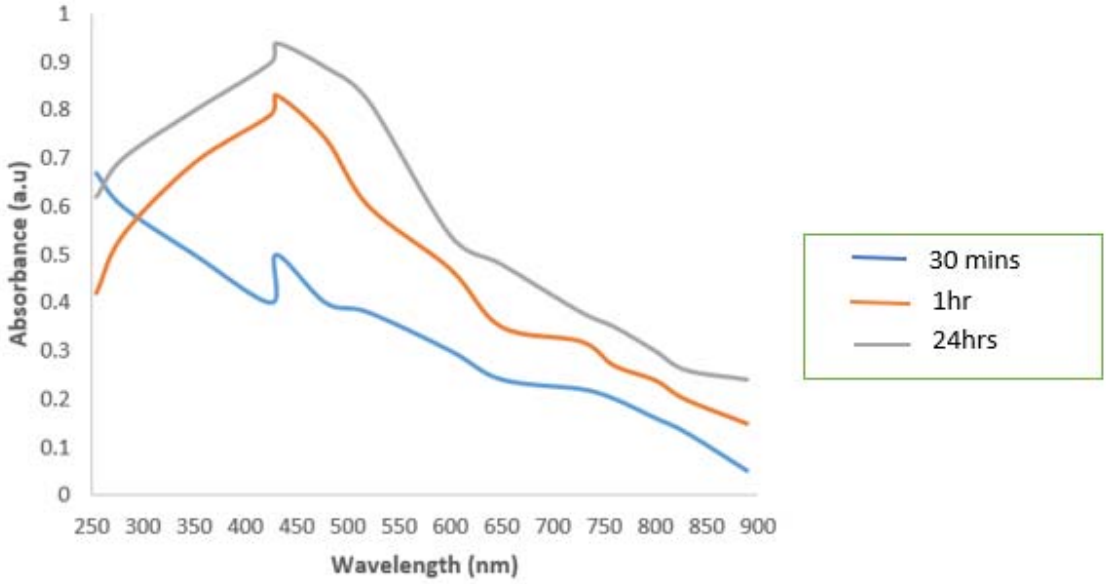

Figure 1: UV–visible spectra of synthesized AgNPs.

4.2 FTIR Analysis

The major absorption peaks on FTIR spectra of *Boerhaavia diffusa* extract and synthesized AgNPs are recorded in the range of 4000-400cm^{-1} as represented in Figures .The comparison of positions and intensities of various peaks appearing on the FTIR spectra of *Boerhaavia diffusa* extract (Figure 2A) and synthesized AgNPs (Figure 2B) showed that there were appearances of additional new peaks indicating the existence of interaction between the *Boerhaavia diffusa* extract and synthesized AgNPs thereby confirming the formation of silver nanoparticles.

The peaks in the region of 1456.30 to 1458.23 cm^{-1}, 1608.69 to 1618.33 cm^{-1}, 1701.27 cm^{-1}, 2854.74 cm^{-1} , 2924.18 to 2924.16 cm^{-1} and 3439.19 cm^{-1} correspond to asymmetric C-CH_3 bend, C=C stretching of poly-aromatic compounds, C=O stretching of carbonyl, C-H out of plane stretch in CH_2, C-H asymmetric stretch in CH_3 and correspond to OH stretching of alcohol respectively on both spectrum. The disappearance of absorption peaks at 3988.92 cm^{-1}, 1911.52 cm^{-1}, 1219.05 cm^{-1}, 1168.90 cm^{-1}, and 974.06 cm^{-1} on the FTIR spectrum of synthesized AgNPs

might be due to the physical interaction of the AgNPs with *Boerhaavia diffusa* extract. This indicate the reduction of silver ion by *Boerhaavia diffusa* extract. This finding is in line with the result obtained by previous study [17,18].

4.3 Transmission Electron Microscopy Spectroscopy (TEM)

The TEM analysis of silver nanoparticles in optimal conditions is showed in (Figure 3). The TEM micrograph of synthesized silver nanoparticles shows that the particles are nano-scale and uniform based on the particle size and shape. The produced nanoparticles using the transmission electron microscopy (TEM) was spherical in shape with a size distribution ranging from 30 to 40 nm. These findings also agrees with result obtained by previous study [19,20].

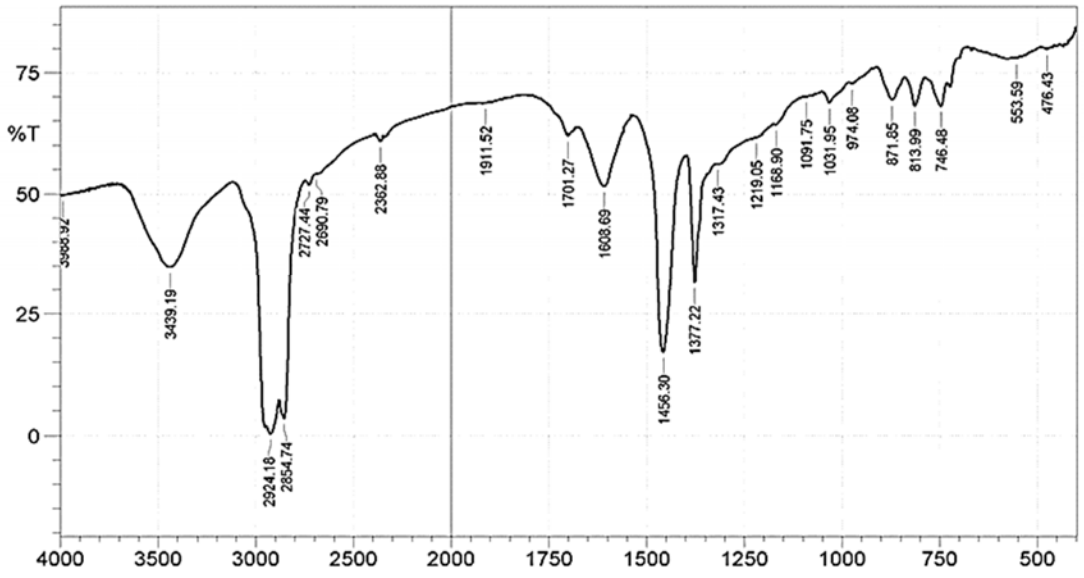

Figure 2A: FTIR spectrum of sample

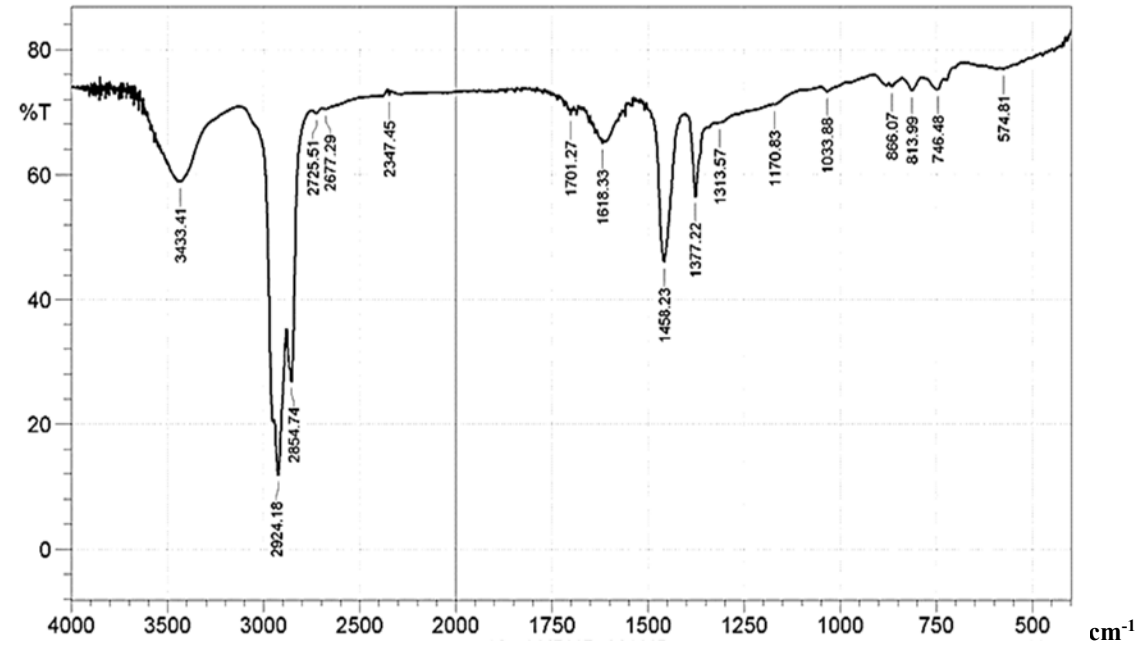

Figure 2B: FTIR spectrum of synthesized AgNPs

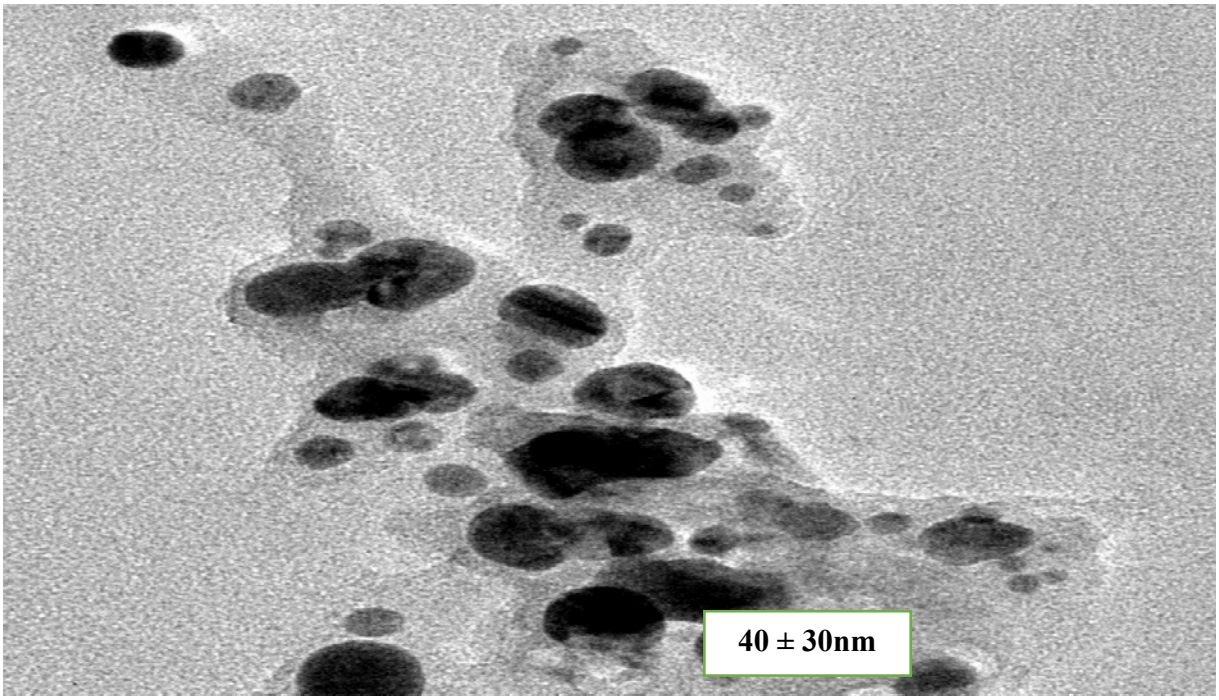

Figure 3: TEM micrograph of the synthesized silver nanoparticles

4.4 Antimicrobial Analysis

The results obtained from the investigation of the antimicrobial property of AgNPs against selected bacteria was showed in Figure 4. The percentage inhibition zones of the AgNPs at 50µl and 100µl are compared with streptomycin as control. The percentage zones of inhibition of Gram-positive bacteria in the presence of 50µl and 100µl AgNPs are in the range of 28 to 35%, 26 -30% and 26- 32% for *Bacillus subtilis, Staphylococcus aureus* and *Clostridium ramosum* are in the range of respectively. The percentage zones of inhibition of Gram-negative bacteria in the presence of 50µl and 100µl AgNPs ranges from 45 to 51% and 42 to 45% for *Pseudomonas aeruginosa* and *Klebsiella pneumonia* respectively. The control (streptomycin) has percentage zones of inhibition of 75 and 82% at 50µl and 100µl respectively. The percentage zones of inhibition increases with increase in volume of AgNPs. This investigation reveals that Gram-negative bacteria are more sensitive to AgNPs compare to the Gram-positive bacteria species. This finding is supported by the fact that Gram-negative bacteria have very thin cell wall thickness (8-10nm) and a single thin peptidoglycan layer compare to thick cell wall (20-80nm) and multiple layers of peptidoglycan found in Gram-positive bacteria. This suggest that the synthesized nanoparticles has good antimicrobial properties. The mechanism of bactericidal activity of AgNPs in this study is possibly as a result of binding of the silver nanoparticles to the cell wall and the generation of free radicals. This study agrees with the result obtained from previous study [21,22].

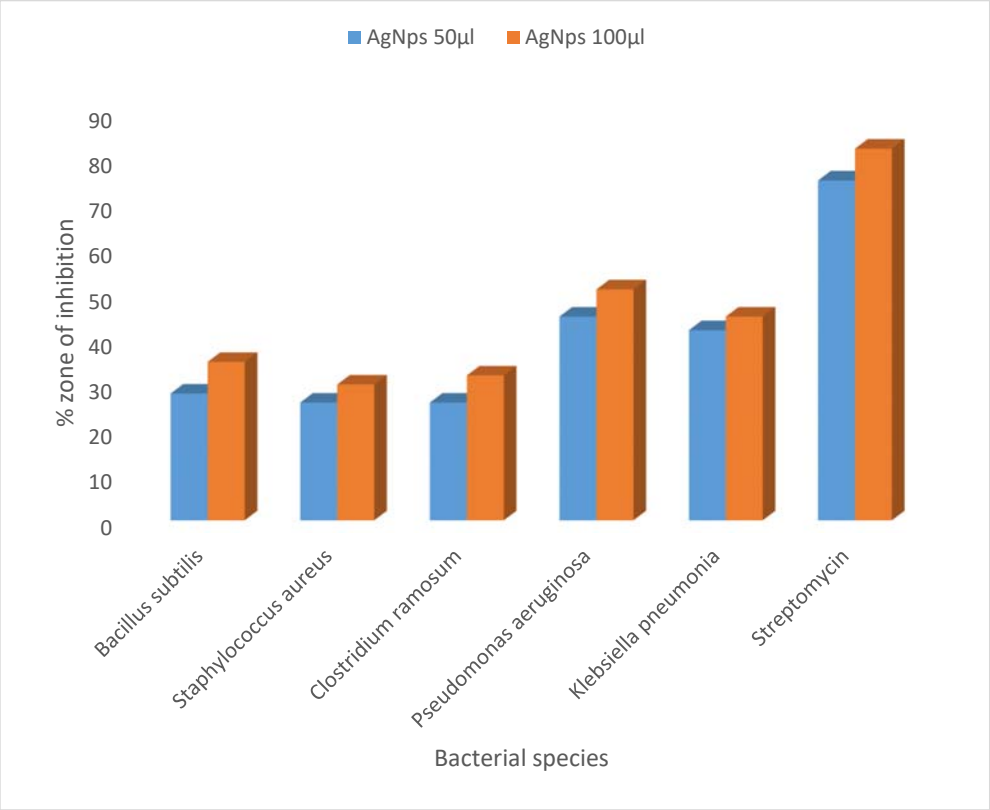

Figure 4: Percentage Inhibition zone of antibacterial activity test of the synthesized AgNPs.

5.0 Conclusion

Silver nanoparticles (AgNPs) were successfully synthesized using *Boerhaavia diffusa* leaf extract as a bioreductor and stabilizer. UV–visible spectrophotometry, FTIR spectrophotometry and TEM analyses denote the formation of AgNPs and complete reduction of Ag^{+} from $AgNO_3$ precursor at the nano-size range. Furthermore, the high zones of inhibition of synthesized AgNPs against test bacterial strain confirms it as a good antibacterial agent capable of combating some worrisome challenges of infections caused by bacteria.

Acknowledgements

The authors would like to appreciate the staff of the Microbiology unit of Ladoke Akintola University of Technology Teaching Hospital Ogbomoso, who provided part of the instruments and materials used for this study.

Disclosure statement

No prospective conflict of interest was reported by the authors.

Author's Contribution statement

Aderonke Similoluwa **FOLORUNSO** and Sunday Adewale **AKINTELU** designed the experiments, interpreted, and discussed the results. Abel Kolawole, **OYEBAMIJI** did the antibacterial analysis of the study and drafted part of the manuscript. Ehimen Annastasia **Erazua** conducted the plagiarism check of the manuscript. The manuscript was read by the author's and approved it for submission.

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