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Biosynthesis of Silver Nanoparticles using Almond Plantleaf extract and their Antibacterial Activity

Ayandiran D.Aina[1], OluwafayokeOwolo[2], OkaroGinikachukwu[3], Folasade O.Aina[4], Olusola N. Majolagbe[5], Olumide D.Olukanni[6], Mary C. Stephen[7], Aderiike, G.Adewumi[8]
Department of Microbiology, Babcock University, PMB 21244, Ikeja, Lagos, Nigeria[1, 2, 3]
Department of Maternal and Child Health, Babcock University, PMB 21244, Ikeja, Lagos, Nigeria[4]
Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria[5]
Department of Chemical Sciences, Redeemer's University, Gbongan-Osogbo RoadPMB 230, Ede.[6]
Department of Pharmaceutical Microbiology, University of Ibadan, PMB 5116, Ibadan, Oyo State, Nigeria[2, 7]
Department of Basic and Applied Sciences, Babcock University, PMB 21244, Ikeja, Lagos, Nigeria[8]

Abstract
This work reports the synthesis of silver nanoparticles (AgNPs) using almond leaf extract and the evaluation of its antibacterial activity. Antibacterial activity of the biosynthesized AgNPs was evaluated against some selected pathogenic microorganisms. The biosynthesised AgNPs were characterised using UV-Visible, Fourier Transmission Infrared Spectroscope (FTIR), Energy Dispersive X-ray Spectroscope (EDX) and Field Emission Scanning Electron Microscope (FESEM). The absorption spectrum of the synthesized AgNPs showed a maximum spectrum of 430 nm while FTIR analysis showed different functional groups present on the surface of the AgNPs with broad peak between 3000 and 3800 cm^{-1}. The FESEM showed a large number of spherically shaped nanoparticles with sizes ranging from 8.34 to 78.96 nm. The almond leaf extract possess biomolecules which aided the bioreduction, formation and stabilization of the AgNPs, which in turn inhibited distinctly the growth of the selected microorganisms with zones ranging from 9-10 mm. This study showed that almond leaf can be used for the synthesis of AgNPs possessing antibacterial properties.

Key words*:* Almond leaves, antibacterial, silver nanoparticles, green synthesis

INTRODUCTION

Nanotechnology is fundamentally concerned about the synthesis of nanoparticles and their application in different fields of medicine, science, physical science, materials science, and designing. Metal nanoparticles, for example, gold (Au) and silver (Ag) have perceived significance in science, material science, and science due to their exceptional optical, electrical, and photothermal properties [1].

As of late, nanotechnology has demonstrated a quick and effective development, since it enables known materials to be created with unique properties [2]. Diminishing the size of any material to nanoscale may change its natural properties. Along these lines, the properties of a nanostructured material can be very divergent from those of the bulk material, making it fit for various applications. Specifically, metallic nanoparticles (NPs) for example, silver and copper NPs (AgNPs and CuNPs) have been connected in a wide assortment of fields, including prescription, farming, bioengineering, among others [3], in light of the fact that they have been demonstrated and have been known to be antibacterial and biocidal agents [4].

Nanoparticles have been reported as having various medicinal applications. Studies such as Adelere*et al*. [5] have reported the antimicrobial activity of a green synthesized nanoparticle. Nasrollahi*et al*. [6] alsodemonstrated the antifungal activities of silver nanoparticle.In addition, nanoparticles have been reported to have anti-inflammatory [7], antiangiogenesis [8] andantiviral activity [9]. Plant extracts have recently been used for nanoparticles green synthesis since they are rich in bioactive compounds [5, 10-12] and hence this study was also aimed at the synthesis and application of nanoparticles obtained from plant extract.

Almond (*Prunusdulcis* L.), a specie of Prunus, belongs to the subfamily Prunoideae of the family Rosaceae. Nutritiously and therapeutically, almond has been accounted for to be a valuable food item [13-14].The leaves of this plant contains a wide variety of biomolecules which can serve as both capping and reducing agents in the synthesis of nanoparticles; it is hencefascinating to evaluate the viability of the aqueous extract from almond leaves (frequently disposed of as an agrowaste) towards the green synthesis of silver nanoparticle.

As a result of the very little utilization of this plant in the synthesis of nanoparticles and in addition to lacking information from works on the effect of *P. dulcis* on antimicrobial activity, the principal aim of this investigation is to use the aqueous extract of the plant leaves for synthesis of silver NPs and to assess the in vitro antimicrobial activity of the biosynthesized silver nanoparticles.

MATERIAL AND METHODS

Sample collection

Fresh *Prunusdulcis* leaves were collected from the local area of the University campus. It was washed thoroughly with distilled water several times to remove dust and dried under shade (30°C ± 2°C) for 5 days. The dried leaves were cut into small pieces, and blended into powder. The powdered samples were kept in air tight containers at room temperature for further use.

Preparation of extract

One gram of the milled leaves of *Prunusdulcis* was weighed and suspended in 100ml of distilled water. The extract was

obtained by heating in water bath at 60°c for 1 hour. The extract was filtered using Whatman No. 1 filter paper and then centrifuged at 4000RPM for 20minutes. The supernatants were collected and used without further purification [15].

Synthesis of Silver nanoparticles

1mM aqueous solution of silver nitrate was prepared for synthesis of silver nanoparticles. Approximately 1 ml of the extract was added to a reaction vessel containing 40ml of a 1mM silver nitrate ($AgNO_3$) solution to reduce the amount of silver ions. The reaction was carried out under static condition at room temperature (30 ± 2°C) for 2hr. The formation of AgNPs was observed as a change in the solution colour [15].

Characterization of Synthesized Silver nanoparticles

The formation of the synthesized nanoparticles was confirmed by measuring its absorbance spectrum using UV–Visible spectrophotometer (Cecil, USA) operated at 190–1100 nm. The identity of the biomolecules that took part in the green synthesis was determined by FTIR spectroscopy. The measurements were performed between 4000-400 cm^{-1} to see the attachment of biomolecules on the surface of the AgNPs using Shimadzu FTIR spectrometer, model 8400S (Shimadzu, Japan). To achieve this, purified silver nanoparticles were dried and blended with KBr in the ratio 5: 95 to form a pellet which was used for the measurement.The size, morphology, and elemental composition of the synthesized nanoparticles were unravelled by Field Emission Scanning Electron Microscopy (FESEM) and EDX analyses. The Field Emission Scanning Electron Microscopy (FESEM) micrograph was obtained as follows. A drop of nanoparticles in suspension was placed on a 200 mesh hexagonal copper grid (3.05 mm) (Agar Scientific, Essex, UK) coated with 0.3 % formvar dissolved in chloroform. The particles were allowed to settle for 3–5 min on the grid, the excess liquid flicked off with a wick of filter paper and the grids were then air dried before FESEM viewing. Micrograph was obtained using a JEM-1400 (JEOL, USA) operating at 200 kV.

Antimicrobial Properties of the synthesized Nanoparticle

The antibacterial activity of the synthesized AgNPs was evaluated against bacterial pathogens including *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* using agar well diffusion method as described by Perez *et al.* [16]. The organisms were grown overnight in peptone water and incubated at 37°C for 18 h. The 18h old cultures were used to seed the plates of Muller Hinton agar with the aid of sterile cotton swab stick. The seeded plates were allowed to stand for 3-5h. The plates were then bored using 6mm cork borer at 6 different points, one for each concentration. The wells were then irrigated with 100µl of graded concentrations of the silver nanoparticle (10, 20, 40, 60, 80 and 100). Control experiments were set up with almond leaves extract, silver nitrate (positive control) and distilled water (negative control). The plates were incubated for 18 h at 37 °C after which the zone of inhibition was computed. The agar well diffusion test was performed in triplicate

RESULTS AND DISCUSSION

Synthesis and Characterization of the Silver nanoparticle

The phytosynthesis of the silver nanoparticles was catalyzed by the aqueous extract of the almond leaves. Within the first 10 mins, a colour change was observed. The initial solution which was colorless was transformed to light brown and then stabilized at a dark brown color as indicated in figure 1.It is known that when the surface plasmon vibrations in silver nanoparticles are excited, the silver nanoparticles exhibit some yellowish brown color in the aqueous solution [17].

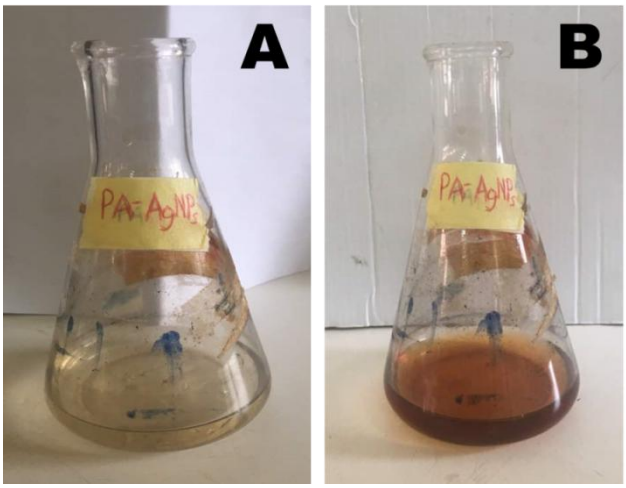

Figure 1: Synthesis of the Almond leaf extract mediated silver nanoparticles (a) immediately after the addition of the almond leaf extract to the silver nitrate; (b) Formation of deep brown colouration after 30 min.

The biosynthesized silver nanoparticle showed a maximum absorbance wavelength at 430nm which is indicated in figure 2, a value within the range previously reported for AgNPs [17].

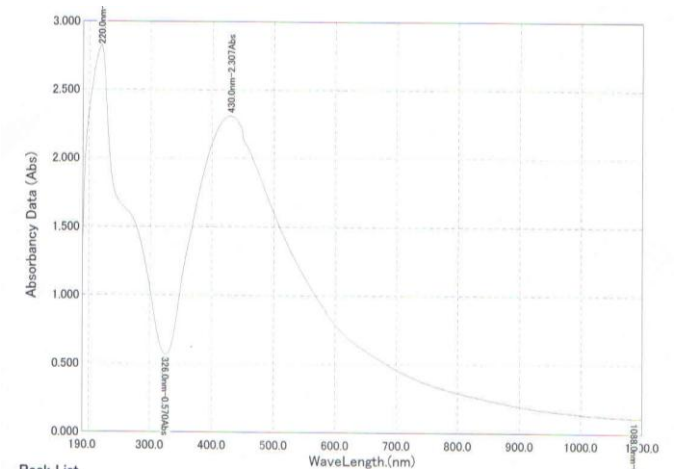

Figure 2: UV-Vis spectra of silver nanoparticles synthesized from almond leaf extract

The NP shows a broad peak between 3000 and 3800 cm^{-1}, which are identified as those of O-H vibrations and/or N-H stretching associated with N-substituted amide [18] 2359 cm^{-1} peak is that of CO_2 from air, the 2000 cm^{-1} peak is probably from C=N and/or C=O bond, the distinct peak at 1635 cm^{-1} is the –N–H bend of amino acids/proteins[19-20] and the one at 1384 cm^{-1} is due to in plane bending of alkenes and aromatics [21]. The Ag-O stretching modes are observed at 669 cm^{-1} and 420 cm^{-1} [22]. In essence, the FTIR analyses suggested the presence of phytochemicals on the surface of the NPs as capping agents, whichare then released systematically as drugs in the study.

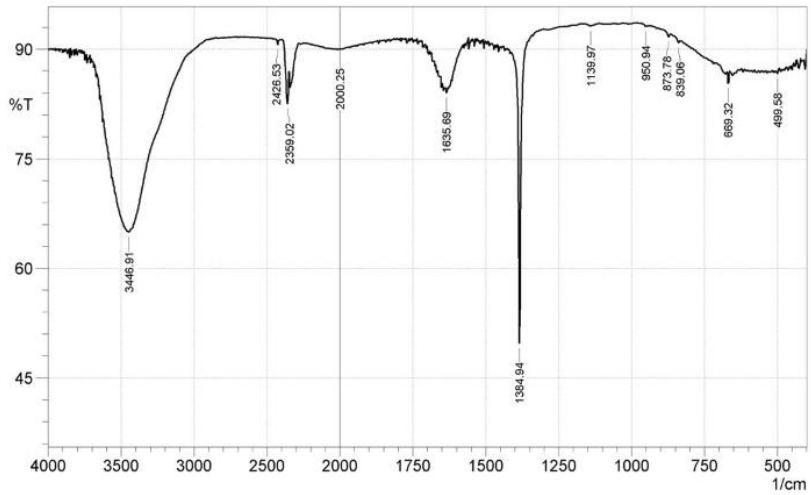

Figure 3: The FTIR spectra of the silver nanoparticles synthesized from almond leaf extract

The chemical analysis of the almond leaf extract - mediated silver nanoparticle was shown (Figure 4) by the EDX spectroscopy. Elemental signals were observed around the Ag atom within the ranges of 2.5 to 3.2keV. Other elements present include copper, oxygen, and carbon). Similar observations have been reported by other researchers [23-24] with silver signals at the range of 1.5 to 5.0 keV

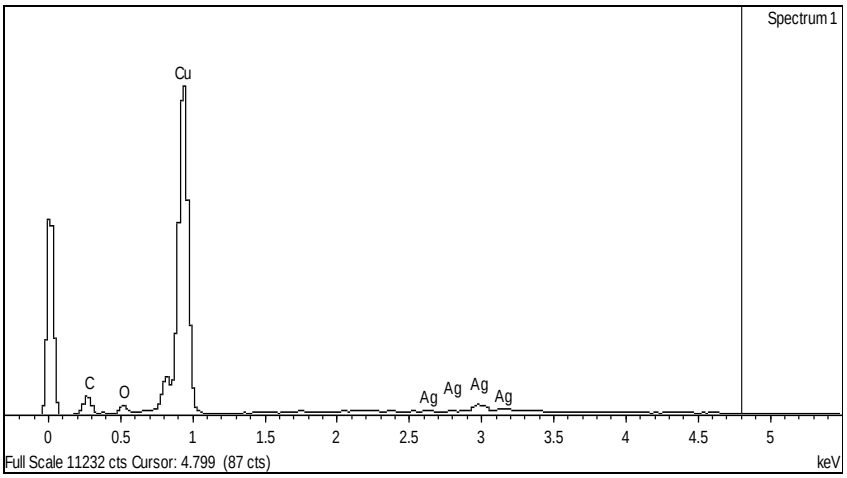

Figure 4: Energy dispersive spectra of the synthesized AgNPs

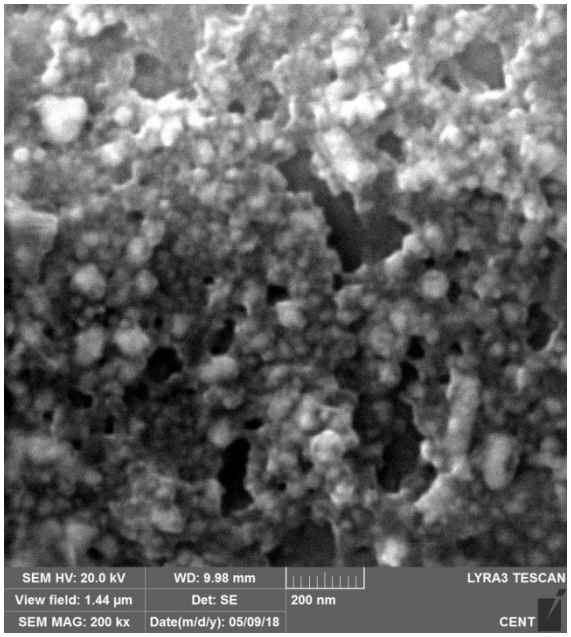

Figure 5: Field Emission Scanning Electron Micrograph of the Synthesized AgNPs

The size and morphology of the biosynthesized nanoparticle were captured by (Figure 5) FESEM. The particles which ranged from 8.34 to 78.96 nm were spherical in shape. This size range falls within the ranges reported when other plants were used in the biosynthesis of silver nanoparticle[15, 25].

Antimicrobial activity of synthesized nanoparticle

Of all the four selected pathogens tested, the synthesized silver nanoparticles inhibited just the growth of *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, both of which were inhibited at only the 100µg/ml concentration with zones ranging from 9mm-10mm. Biosynthesized silver nanoparticles have been reported to have successfully inhibited various pathogenic organisms [26-30]. *Staphylococcus aureus* and *E.coli* were totally resistant to all the concentrations of the synthesized silver nanoparticles. Panacek*et al.* [31] who reported similar resistance pattern to silver nanoparticle attributed the resistance to the production of Flagellin, an adhesive protein of the bacterial flagellum which causes aggregation of silver nanoparticles and thereby eliminates their antibacterial effect. Overall, the results of this study indicated that the nano-sized silver produced by *Prunusdulcis* leaves showed antibacterial property

Table 1: Zone of inhibition of the biosynthesized AgNPs against some selected pathogens

Mean Zone of Inhibition (mm) ± standard deviation (SD)									
	AgNPs						$AgNO_3$	CDE	Distilled H_2O
	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml			
Isolate									
Staphylococcus aureus	NZ	NZ	NZ	NZ	NZ	NZ	6.1±0.2	NZ	NZ
Klebsiella pneumoniae	NZ	NZ	NZ	NZ	NZ	9.4±0.1	6.4±0.1	NZ	NZ
Pseudomonas aeruginosa	NZ	NZ	NZ	NZ	NZ	10.2±0.2	6.3±0.1	NZ	NZ
Escherichia coli	NZ	NZ	NZ	NZ	NZ	NZ	6.1±0.1	NZ	NZ

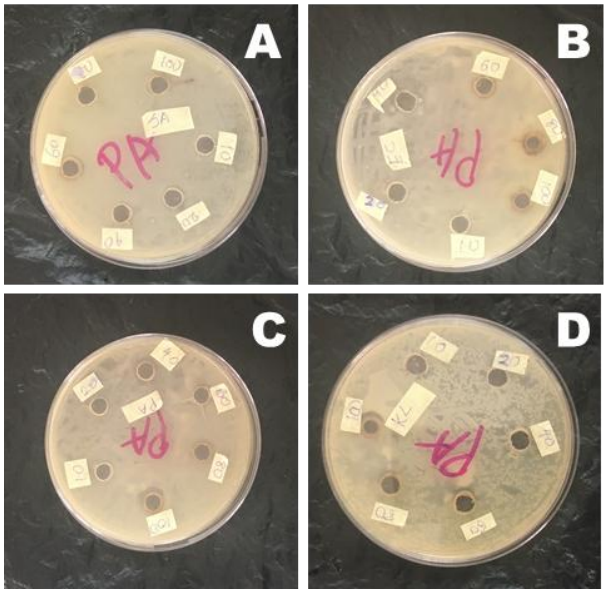

Figure 6: The antibacterial activity of the synthesized AgNPs against some clinical bacterial isolates (a) against *Staphylococcus aureus* (b) against *Escherichia coli* (c) against *Pseudomonas aeruginosa* (d) against*Klebsiella pneumoniae*

CONCLUSION

In this work, almond leaf extract was used to synthesize nanoparticles. The synthesised AgNPs was analysed using UV Spectrophotometer, FTIR, FESEM and EDX. The biosynthesised silver nanoparticle had antibacterial activities against some selected bacterial isolates. Thus almond leaf extract mediated-AgNPs could have various pharmaceutical applications.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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