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Antimicrobial activities of biosynthesized silver nano particles from leaves extract of some medicinal plants

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Abstract

The antimicrobial activity of biosynthesized silver nanoparticles (AgNPs) from the leaves of four medicinal plants, *C*arica *papaya (CP), Moringa oleifera (MO), Mangifera indica (MI) and Garcinia kola (GK)* were assessed against selected gram positive and gram-negative bacteria. Method of synthesis of nanoparticles utilized was the eco-friendly Bio-based method using plant leaves extract as reducing and stabilizing agents. Two different ratios for each plant extract and silver nitrate (1:1 and 1:2) respectively were used. Particle characterization was carried out using visual inspection and UV-Vis spectrophotometry. Antimicrobial activity was assessed using agar diffusion method. Visual inspection revealed gradual color change from golden yellow to dark brown, confirming nanoparticles formation. The surface plasmon resonance peak was between 416 and 438 nm for the silver nanoparticles. The minimum inhibitory concentration ranged from 3.125 - 12.5µg/ml. In conclusion, all four biogenic silver nanoparticles have reasonable antimicrobial activity with ratio 1:2 being more potent.

Keywords: Silver nanoparticles; Antimicrobial; Characterization; Leaves; Chemical reduction

1. Introduction

Scientifically, a nanometer is one-billionth of a meter in the metric scale of linear measurements [1]. Nanotechnology is an emerging field of research that involves design, synthesis, and manipulation of particle structures ranging from approximately 1-100 nm [2, 3]. Nanoparticles are small-sized nanospheres designed at the atomic or molecular level [4]. Application of nanotechnology is found in several areas such as targeted drug delivery [5], sensing [6], artificial implants [7] and tissue engineering [8]. Some modern drugs are formulated as nanoparticles used for the treatment of cancer [9], and for combatting human pathogens like bacteria [1, 10, 11]. Nanomedicine is an emerging field of medicine that utilizes the knowledge and techniques of nanoscience in medical biology and disease prevention and remediation [12]. In Nanomedicine, the use of nanostructures such as delivery agents involve encapsulating drugs or attaching therapeutic agents and precisely delivering them to target tissues with a controlled release [13,14].

The use of Silver nanoparticles have particularly received attention as a result of their physical, chemical, and biological properties that enhances their catalytic activity and bactericidal effects, making them of interest in nanobiotechnological research [15,16]. Therapeutically, they are used as anticancer agents [17], antimicrobial agents

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in wound dressings [18-20], and as topical creams to prevent wound infections [21]. Several chemical and physical methods have been developed to synthesize silver nanoparticles, the most popular of which is the chemical reduction method [2]. It involves the use of expensive and hazardous chemicals such as reducing agent, stabilizers, and organic solvent. It may also involve special techniques such as high energy radiation and microwave irradiation [22]. Hence, there is an increasing necessity for development of environmentally and economically friendly methods which do not utilize toxic chemicals in the biosynthesis of nanoparticles [2]. The eco-friendly, Bio-based method have become valuable alternatives to chemical reduction method [2, 22]. The potential organisms used in the bio-based method of nanoparticle synthesis ranges from simple prokaryotic bacterial cells to eukaryotic fungi and plants [23]. About 25% of the major pharmaceutical compounds and their derivatives in use today are derived from natural resources [24, 25]. Synthesis of silver nanoparticles have been carried out using plant materials such as citrus fruits extracts [22], *Camellia sinensis* (green tea) extract [26], leaf extract of *Datura metel* [27], *Capsicum annuum* [28], leaf extract of *Euphorbia hirta* [29] and lots more. The present study aims at carrying out the biosynthesis of silver nanoparticles from leaves extract of *Carica papaya, Garcinia Kola, Moringa oleifera* and *Mangifera indica,* as well as determining the antimicrobial activities of the synthesized nanoparticles against Gram-positive and Gram-negative organisms.

2. Material and methods

2.1. Materials

Plant leaves extract of *Carica papaya, Garcinia Kola, Moringa oleifera* and *Mangifera indica*. Silver nitrate (Sigma Aldrich, USA); Mueller-Hinton broth culture media. Other chemicals and reagent were of analytical grade. Organisms used include: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus pyogenes* and *Salmonella paraptyphi*.

2.2. Plant material collection and extraction

Fresh leaves of *Carica papaya* (Linn) (CP), *Garcinia Kola* (Heckel) (GK), *Moringa oleifera* (Lam) (MO) and *Mangifera indica* (MI) were harvested from selected farm sites in Ibiono Ibom Local Government Area of Akwa Ibom State, Nigeria. Plants identification and authentication were carried out by Prof. M. E. Bassey, a plant Taxonomist in the Department of Botany and Ecological Studies, University of Uyo. Voucher specimen numbers assigned were UUPH18A, UUPHA501, UUPH21A and UUPH3C for *C. papaya, M. oleifera, G. kola and M. indica* respectively. These specimens were deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. The leaves were separately washed with tap water to remove dust particles and other unwanted materials. The dust free leaves were air dried under room temperature for 3 weeks then powdered using an electric blender.

2.2.1. Extraction procedure

50g powder each of each plant material was separately put in 500 mL conical flask and 250 mL of distilled water added. The conical flask was covered with aluminum foil and kept in a reciprocating shaker for 24 h for continuous agitation at 150 rpm for thorough mixing. The extract was filtered using muslin cloth followed by Whatman no 1 filter paper and the resultant solution kept for the nanoparticle synthesis.

2.3. Biosynthesis of Silver Nanoparticles

About10 mL of 1% silver nitrate (AgNO3) was prepared by dissolving 0.1 g of silver nitrate (AgNO3) in 10ml of water and stirred for a few minutes using a magnetic stirrer. This was followed by drop-wise addition of the extract using 10ml syringe, with continuous stirring to obtain a $[Ag]^+$ dispersion evident by brown coloration. About 10 ml of each plant extract was mixed with 10ml of 0.1 % silver nitrate (1:1), while another 10ml of extract was mixed with 20ml of 0.1% silver nitrate (1:2). The mixtures were further diluted by adding 50ml of distilled water and maintained at 40 ^oC for 24 hours. Aliquots of each extract was taken and kept in sample bottles in a refrigerator at -4 ^oC for characterization.

2.4. Characterization of Biogenic Silver Nanoparticles

2.4.1. UV-VIS Spectroscopic analysis to determine surface plasmon resonance for Silver Nanoparticles

The optical property of synthesized Silver nanoparticles was determined using a double-beam spectrophotometer (Hitachi, U-3010). The photoluminescence emission spectra from the samples which were dispersed in distilled water were recorded by a spectro-fluorometer (LS 55, Perkin Elmer) at room temperature using a four sided polished quartz cuvette with a path length of 10 mm. The spectra were taken at different time intervals (up to 24 hours) between 350nm to 700nm and after 24 hours.

2.5. Antimicrobial activity of silver nanoparticles biosynthesized from *Carica papaya* (Linn) (CP), *Garcinia Kola* (Heckel) (GK), *Moringa oleifera* (Lam) (MO) and *Mangifera indica* (MI) leaves.

2.5.1. Collection of microbial isolates

Clinical isolates used for this research work were obtained from microbial stock cultures at the Department of Microbiology, University of Uyo. Gram-positive species used were *Staphylococcus aureus* and *Streptococcus pyogenes* while *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella paraptyphi* were the gram-negative species. Their identities were confirmed by their morphological characteristics on specific media, followed by biochemical tests. The biochemical tests include; Coagulase test, Mannitol salt agar test, Indole test, Oxidase test, Citrate test and Staining test. These isolates were inoculated with their differential medium and incubated for 24 h at 37 ^{o}C. These were further sub-cultured into nutrient agar slant and broth.

2.5.2. Preparation of Inoculum

Inocula were prepared by direct colony suspension as recommended by Clinical and Laboratory Standards Institute [30]. The pure cultures of these test organisms were inoculated into sterile peptone water and incubated for 24 hours at 37°C. Gram positive isolates i.e. *Staphylococcus aureus and Streptococcus pyogenes* were serially diluted to factor 3 using tenfold serial dilution and Gram negative isolates i.e. *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella paraptyphi* were diluted up to factor 5 using tenfold serial dilution. These were carried out to standardize the inoculum size.

2.5.3. Determination of Antimicrobial Activity using Well Agar Diffusion technique

The silver nanoparticles biosynthesized from the leaves extract were screened for antimicrobial activity using the agar well diffusion method described by Okeke et al. [31]. About 0.1 ml of each of the test organisms was aseptically spread on the surface of the Muller Hinton agar plates using sterile bench hockey stick. These were allowed for 30 minute s to pre-diffuse into the medium. A sterile cork-borer of 5mm in diameter was used to bore holes on the agar plates. 0.5 ml of each silver nanoparticle was used to fill the agar wells made on the Muller-Hinton agar plates. The plates were allowed to stand for 1h to allow the compounds to diffuse into the medium. Chloramphenicol (30µg/disc) was used as standard for the test for antibacterial activity. Agar plates were incubated at 37 ^{o}C for 24-48 hours. Antimicrobial activities of each silver nanoparticle was determined by measuring the zones of inhibition in millimeters. Tests were carried out in triplicates.

2.5.4. Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of test microorganisms and reference antibiotics was determined by the serial broth micro-dilution method. Different concentrations of each silver nanocomplex were prepared to obtain 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. These different concentrations were mixed with agar plates to obtain plates of different concentrations of the silver nanocomplexes. The test organisms were streaked on the surface of each plate of different concentration. These were carried out such that all the silver nanocomplexes that gave antimicrobial activity on test organisms were inoculated with the microorganisms. Plates were incubated at 37 ^{o}C for 24 hours. The least of the silver nanocomplexes that inhibit growth of test organisms were taken as the MIC of the nanocomplex to that microorganism. Testing was carried out in triplicates.

3. Results

3.1. Particle Characterization

3.1.1. U.V Analysis

Figures 1 and 2 show the U.V spectra of biogenic silver nanoparticles using two ratios 1:1 and 1:2 (extract to silver nitrate concentration).

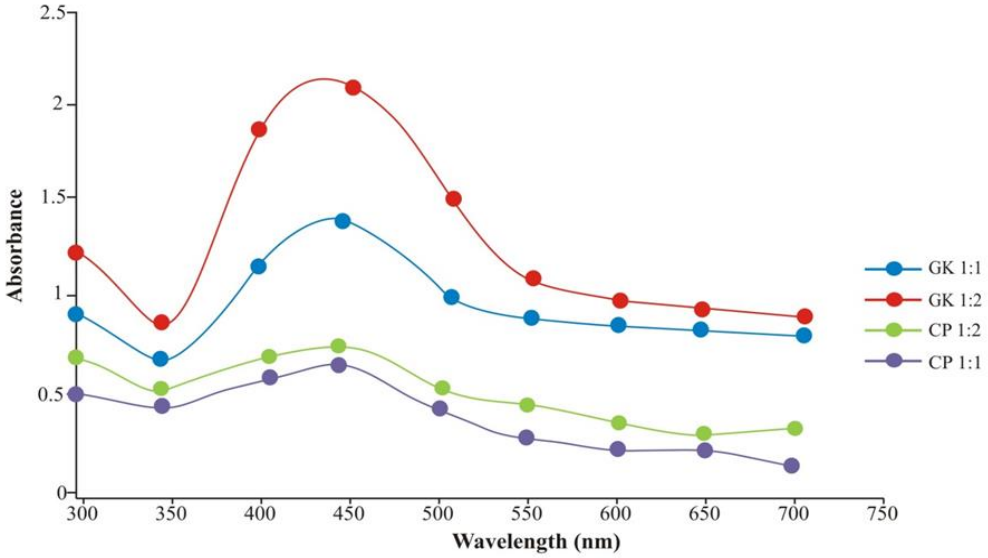

Figure 1 UV spectra showing the rapid synthesis of GK and CP nanoparticles.

GK: *Garcinia kola*
CP: *Carica papaya*

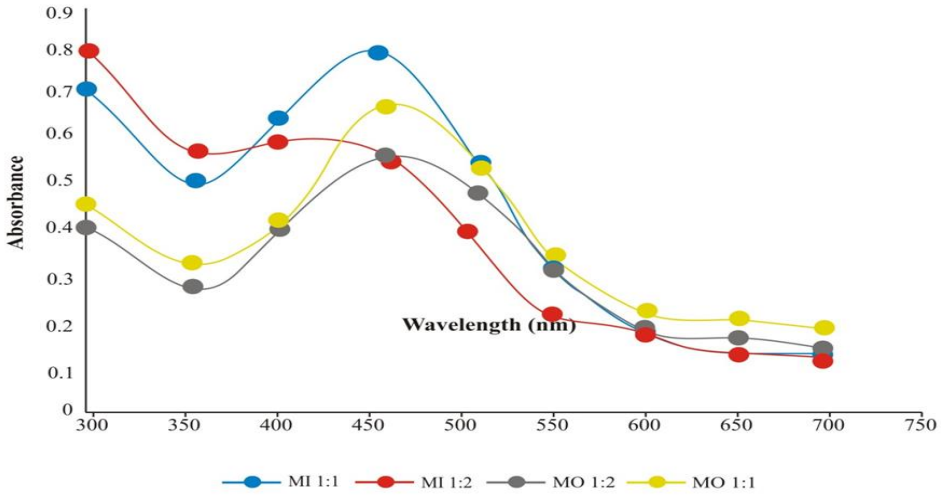

Figure 2 UV spectra showing the rapid synthesis of MI and MO nanoparticles.

MI: *Mangifera indica*
MO: *Moringa oleifera*

3.1.2. Visual Inspection

Biosynthesized silver nanoparticles were inspected visually using the naked eye. It was observed that upon the addition of 0.1% silver nitrate solution, plant extracts changed color from light yellow to dark brown.

3.2. Antimicrobial activity of biogenic silver nanoparticles

Tables 1 and 2 show the antimicrobial activity of plant-synthesized nanoparticles: at two different ratios for each silver nanoparticle subtypes on selected gram positive and gram negative bacteria. Table 1 represents the summary for the calculation of the minimum inhibitory concentration (MIC) for each nanoparticle type and their distinct ratios respectively. The degree of activity is represented by the Inhibition zone Diameter (IZD) in millimeters (mm) [Table 2]

Table 1 Minimum Inhibitory Concentration (MIC)

MIC (µg/ml) **Organism**					
Ag+ nanoComplex	E. coli	S. aureus	S. pyogenes	S. typhi	P. aeroginosa
GK-AgNP (1:1)	-	-	-	12.5	12.5
GK-AgNP (1:2)	12.5	6.25	6.25	12.5	12.5
MO-AgNP (1:1)	-	12.5	12.5	12.5	12.5
MO-AgNP (1:2)	12.5	6.25	6.25	3.125	3.125
CP-AgNP (1:1)	-	-	-	-	-
CP-AgNP (1:2)	12.5	12.5	12.5	6.25	12.5
MI-AgNP (1:1)	-	-	-	-	-
MI-AgNP (1:2)	12.5	6.25	6.25	12.5	12.5
Chloramphenicol	1.0	0.5	1.0	0.5	1.0

Key: GK-AgNPs = *Garcinia kola silver nanoparticles;* MO-AgNPs = *Moringa oleifera silver nanoparticles*
CP-AgNPs = *Carica papaya silver nanoparticles;* MI-AgNPs = *Mangifera indica silver nanoparticles*

Table 2 Inhibition Zone Diameter.

Inhibition zone diameter (mm) **Organism**					
Ag+ NanoComplex	*E. coli*	*S. aureus*	*S. pyogenes*	*S. typhi*	*P. aeroginosa*
GK-AgNP (1:1)	-	-	-	20±0.01	12±0.00
GK-AgNP (1:2)	8±0.02	20±0.00	25±0.01	34±0.01	22±0.00
MO-AgNP (1:1)	-	4±0.00	7±0.00	4±0.01	9±0.02
MO-AgNP (1:2)	11±0.01	11±0.01	9±0.00	15±0.00	12±0.01
CP-AgNP (1:1)	-	-	-	-	-
CP-AgNP (1:2)	4±0.00	2±0.01	12±0.00	22±0.00	11±0.00
MI-AgNP (1:1)	-	-	-	-	-
MI-AgNP (1:2)	-	12±0.00	22±0.00	15±0.01	12±0.00
Chloramphenicol	25±0.00	30±0.00	34±0.00	35±0.00	30±0.01

Key: - means no activity was recorded; ( ) indicates the different ratios of plant extract to silver nitrate salt.
GK-AgNPs = *Garcinia kola silver nanoparticles;* MO-AgNPs = *Moringa oleifera silver nanoparticles*
CP-AgNPs = *Carica papaya silver nanoparticles;* MI-AgNPs = *Mangifera indica silver nanoparticles*

4. Discussion

The change in color from light yellow to dark brown when silver nitrate was added to the plant extracts signifies the reduction of silver ions to silver nanoparticles. This change in color is due to the Surface Plasmon Resonance (SPR) phenomenon. It is a collective vibration of free electrons of the metal nanoparticles in resonance with the frequency of the light wave interactions causing the SPR band to appear in the visible and infrared region [32]. The spectra for the silver nanoparticles for all four medicinal plants range from 416 to 438 nm. Surface Plasmon resonance within this wavelength range usually indicates silver nanoparticle formation [33, 34]. Silver nanoparticles biosynthesized from the leaves extract of *Carica papaya, Garcinia kola, Moringa oleifera* and *Mangifera indica* showed broad spectrum antibacterial activities against both Gram negative and Gram-positive bacteria-*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella paraptyphi.* Antimicrobial activity of plant-synthesized silver nanoparticles in this study were seen to increase with increased concentration of silver ions per plant extract ratio. GK-AgNP (1:2) was seen to exhibit the highest antimicrobial activity among the eight nanoparticle variants based on the zones of inhibition observed across the test isolates, though not as effective as the prototype drug, Chloramphenicol. At the highest ratio (1:2) of Ag^+ equivalent, all four biogenic silver nanoparticles displayed reasonable antimicrobial activity. The minimum inhibitory concentration ranged from 3.125 - 12.5µg/ml and varied with type of

bacteria, with *Pseudomonas aeruginosa* and *S. typhi* being the most sensitive to MO-AgNP (1:2) at MICs of 3.125µg/ml for both bacteria.

5. Conclusion

In conclusion, the rapid biosynthesis of nanoparticles using the leaves extract of *Carica papaya, Garcinia Kola, Moringa oleifera* and *Mangifera indica* has the potential to provide efficient, non-toxic, cost-effective and environmentally friendly alternatives. These nanoparticles have shown broad spectrum inhibitions against both Gram negative and Gram positive bacteria and may be effective as drug delivery systems for loading resistance-prone antibiotics so as to enhance their antimicrobial activities.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

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