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Eco-Friendly Synthesis of Silver Nano Particles Using *Carica papaya* Leaf Extract

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Abstract

Silver nanoparticles were synthesized using eco-friendly method with extract of *Carica papaya* as reducing and stabilizing agent. The silver precursor used was silver nitrate solution. A visible colour change from colourless to reddish brown confirmed the formation of the nanoparticles and the UV-Vis spectroscopy showed surface plasmon resonance of 435 nm for the silver nanoparticle. The mean particle size was 250 nm while the polydispersity index was 0.22. The antimicrobial activity of the synthesized nanoparticles was studied against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis and Staphylococcus aureus*. The silver nanoparticles biosynthesized showed satisfactory antimicrobial activity against the test isolates. Antimicrobial property of the nanoparticles was similar ($P > 0.05$). Generally, MIC values of the samples against the microorganisms tested ranged from 25 - 100 mg/ml. *Pseudomonas aeruginosa* was most sensitive while *Staphylococcus aureus* and *Bacillus subtilis* were least sensitive to the silver nanoparticles.

Keywords

Silver Nanoparticles, Eco-Friendly Method, Characterisation, Antimicrobial

1. Introduction

Bio-synthesis of nanoparticles is a fast growing research in the field of nanotechnology. Extensive research has been carried out on silver nanoparticles as an important group of nanomaterials due to their peculiar biological, optical and physio-chemical properties [1].

Nanoparticles can be synthesized easily by using various physical and chemical methods. Chemical reduction of metal salts using various reducing agents in the presence of stabilizer is currently of interest in the preparation of silver na-

noparticles. Reducing agents such as sodium borohydride ($NaBH_4$), hydrazine (N_2H_4), formaldehyde, etc. can be used to reduce a silver containing salt to produce silver nanoparticles [2].

But most of the chemical methods used for the synthesis of nanoparticles involve the use of toxic hazardous chemicals that create biological risk and sometimes these chemical processes are not eco-friendly. Therefore, there is a growing need to develop cost-effective, non-toxic and eco-friendly methods for the synthesis of silver nanoparticles using simple techniques and readily available equipment. The use of plants and microorganisms in the synthesis of nanoparticles has emerged as an eco-friendly and exciting approach [2] [3].

In recent times, plant extract has been used as reducing and capping agent for the synthesis of nanoparticles. The use of plant extract is more beneficial as it does not involve sophisticated processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell culture [4]. Plants extracts from *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica* and *Citrus sinensis* have been used as reducing agents in the synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution [5]. Synthesis of silver nanoparticles has also been done using *Citrullus lanatus* [6], *Murraya koenigii* [7] and *Eriobotrya japonica* leaf extract [1].

Nanoparticles are now considered viable alternatives to antibiotics as they seem to possess a high potential to address the problem of the emergence of bacterial multidrug resistance [8]. Silver nanoparticles have attracted much attention in the science [9]. Silver has always been used against various infections and functions as both antiseptic and antimicrobial agent against gram-positive and gram-negative bacteria [10]. Silver nanoparticles were considered, in recent years, particularly attractive for the production of a new class of antimicrobials [8] [11] [12], opening up a completely new way to combat a wide range of pathogenic bacteria.

This study aims to synthesise silver nanoparticles using *Carica papaya* leaf extract and also to determine the antimicrobial of the nanoparticles synthesised.

2. Materials and Method

Silver nitrate (Sigma U.S.) and plant extract (*Carica papaya*) were the materials used. Other chemicals and reagent were of laboratory grade.

2.1. Microorganisms Used

Pseudomonas aeruginosa, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* collected from Microbiology Postgraduate laboratory, University of Uyo purified by sub-culturing several times to obtain pure cultures.

2.2. Plant Materials Collection and Processing

Fresh leaves of *Carica papaya* (pawpaw), was collected separately from a local farm in Uyo, Akwa Ibom State. Plant species were identified and authenticated

in the Department of Pharmacognosy and Natural Medicine, University of Uyo. The plant leaves were thoroughly washed with tap water to remove dust particles and other unwanted materials accumulated on the leaves. The dust free leaves were pulverized and kept to dry under shade in the Pharmaceutics laboratory for 24 h. The dried leaves were then powdered by using an electric blender.

2.3. Extraction Procedure

50 g of the powdered plant material was put in 500 mL conical flask and 250 mL of distilled water was 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. Then, the extract was filtered by using muslin cloth followed by Whatman no 1 filter paper. The resultant solution was kept for the nanoparticle synthesis.

2.4. Synthesis of Silver Nanoparticles Using Aqueous Extracts of *Carica papaya* (Pawpaw Leaves) with Model Drug

10 mL of 1% silver nitrate ($AgNO_3$) was prepared by dissolving 0.1 g of silver nitrate ($AgNO_3$) in 10ml of water followed by incorporation of 5 ml of the extract in drops under constant stirring using a magnetic stirrer assembly for 5 min, to obtain $[Ag]^+$dispersion. 25 mL aliquot of a freshly prepared aqueous extract of *Carica papaya* leaves (reducing agent) was added to the resultant mixture and maintained at 40˚C temperature for 24 h. The resultant suspension of Silver nanoparticle was lyophilized (using Virtis 2KBTXL-75 Benchtop SLC Freeze Dryer) and subjected to further analysis.

2.5. Characterisation of Silver Nano-Composites: UV-VIS Spectroscopy to Determine Surface Plasmon Resonance for Silver Nanoparticles

UV-Vis spectral analysis was done using a double - beam spectrophotometer (Hitachi, U-3010) with the samples dispersed in distilled water and kept in a quartz cuvette with a path length of 10 mm. The photoluminescence emission spectra from the samples (dispersed in distilled water) were recorded by a spectrofluorometer (LS 55, Perkin Elmer) at room temperature using a four sided polished quartz cuvette with a path length of 10 mm.

2.6. Antimicrobial Studies of *Carica* Silver-Nanocomposites

The silver nanoparticles biosynthesised from the *Carica papaya* leaf extract was screened for antimicrobial activity using the agar well diffusion method described by Okeke *et al.*, 2001 [13].

0.1 ml of each of the organisms was aseptically spread on the surface of the Muller-Hinton agar plate using sterile bench Hockey stick. These plates were left on the bench for thirty minutes to pre-diffuse into the medium. A sterile cock borer of 5 mm was used to bore holes on the agar plates. The silver nanoparticles concentrations were graded as 500 mg/ml, 400 mg/ml, 200 mg/ml, 100 mg/ml.

About 0.5 ml volume of each diluted silver nanoparticle was used to fill the agar wells made in the Muller-Hinton agar plates. The plates were allowed to stand for one hour to allow the extract to diffuse into the medium.

1% Silver nitrate was used as control. All plates were incubated at 37˚C for 24 - 48 hours.

Antimicrobial activities of the silver nanoparticles and the control against microbial isolates were determined by measuring the inhibition zone diameter in cm.

The Minimum inhibitory concentrations were determined by preparing different concentrations 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and mixed with the medium and then the organisms were streaked on the plates and incubated for 24 hours at 37˚C. The minimum inhibitory concentration was determined by checking the plate for the line of streaking of the minimum concentration of the silver nanoparticle without growth.

3. Results and Discussion

The results for characterization of synthesized nanoparticles are shown in Table 1. The results for the determination of antimicrobial activities of synthesized silver nanoparticles are shown in Table 2 and Table 3.

3.1. Antimicrobial Studies

The antimicrobial activity of silver nanoparticles was carried out against both Gram positive and Gram negative bacteria. The synthesized silver nanoparticles exhibited good antibacterial activity against both Gram positive and Gram negative bacteria.

Silver has been known to impart antimicrobial activity to bacteria. Dilute solutions of silver nitrate were used as far back as the 19th century for the treatments of infections [14]. Hence, silver nitrate solution was used as a control system in this research work.

Table 1. SPR bands of silver nanoparticles characterization.

Distilled water (ml)	$AgNO_3$ (g)	Reducing agent (*Carica papaya* leaf extract)	Colour change	SPR peak (nm)
10	0.1	5	Reddish brown	435

Table 2. Antimicrobial activities of Silver nanoparticles synthesized.

Microorganism	Zones of Inhibition (cm)	
	Control (Silver nitrate solution)	*Carica papaya* silver nano-composite
Pseudomonas aeruginosa	1.0	1.5
Escherichia coli	1.5	1.5
Bacillus subtilis	1.5	1.5
Staphylococcus aureus	1.2	1.3

Table 3. Minimum inhibitory concentrations of *Carica papaya* silver nano-composites.

Microorganism	Minimum Inhibitory Concentrations (mg/ml)	
	Control (Silver nitrate solution)	*Carica papaya* silver nano-composite
Pseudomonas aeruginosa	100 mg/ml	25 mg/ml
Escherichia coli	100 mg/ml	50 mg/ml
Bacillus subtilis	100 mg/ml	100 mg/ml
Staphylococcus aureus	100 mg/ml	100 mg/ml

The antibacterial activity of the extract was indicated by the production of inhibition zones for *Pseudomonas aeruginosa* (1.5 cm), *Escherichia coli* (1.5 cm), *Bacillus subtilis* (1.5 cm) *and Staphylococcus aureus* (1.3 cm) on the agar plate. The minimum inhibitory concentration ranged from 25 - 100 mg/ml with *Pseudomonas aeruginosa* being the most sensitive with an MIC of 25 mg/ml while *Staphylococcus aureus* and *Bacillus subtilis* were the least sensitive to silver nanoparticles synthesized.

From the work done and results obtained, silver nanoparticles synthesized using *Carica papaya* had antibacterial activity against the four strains of pathogens used *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis and Staphylococcus aureus*.

3.2. Colour Change

Reduction of silver ions into silver nanoparticle by the plant extract was confirmed by a colour change from colourless to reddish brown. The colour change was due to the surface plasmon resonance (SPR) phenomenon. The metal nanoparticles have free electrons, giving the SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with light.

4. Conclusions

The rapid biosynthesis of silver nanoparticles using the leaf extract of *Carica papaya* provides an efficient, cost-effective and eco-friendly route for the synthesis. The colour change from colourless to reddish brown observed is the characteristics of silver nanoparticles due to SPR phenomenon. UV-Vis spectroscopy confirmed the formation of silver nanoparticles with absorption peak at 435 nm for the entire nanoparticle.

The antimicrobial activity of synthesized nanoparticle was studied against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The nanoparticles synthesized have satisfactory inhibitions against the four mentioned microorganisms with *Pseudomonas aeruginosa* being the most sensitive.

Conflict of Interests

The authors have not declared any conflict of interests.

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