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Original Article

Characterization and Antimicrobial Investigation of Synthesized Silver Nanoparticles from *Annona muricata* Leaf Extracts

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Abstract

Green synthesis is an ecological system for the production of eco-friendly and well characterized metallic nanoparticles using plants. In this research work, the synthesis of silver nanoparticles was from the extract of *Annona muricata* leaves. The characterization of silver nanoparticles was done with Fourier Transform Infrared spectroscopy (FTIR), UV-vis spectrometry and transmission electron microscope to determine functional groups, shape, size and morphology of synthesized nanoparticle. The anti-antimicrobial potency of the synthesize nanoparticle was investigated via well diffusion method. The UV spectrum of synthesized nanoparticle revealed absorbance at 435.00nm which confirmed the formation of silver nanoparticle. The FTIR analysis shows bands corresponding to -OH, C=O and -NH_2 nanoparticle are spherical shape of the functional group. The micrograph obtained from TEM analysis confirmed that the synthesized silver is spherical in shape. The antimicrobial investigation of the silver nanoparticles shows good antibacterial activity due to high zones of inhibition against test bacteria. This work revealed that the synthesized silver nanoparticles possess great antimicrobial potency.

Keywords: *Annona muricata*; Antimicrobial activity; Characterization; Silver nanoparticles

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Introduction

The usage of plants extracts for nanoparticles synthesis has various applications in life [1]. The reduction and elimination of the elegant process of upholding cell cultures associated with biological processes is an advantage of nanoparticle synthesis from plants. The use of plants in production of nanoparticles in large quantity can be aptly scaled up [2]. The multi-drug resistance of commercially available antibacterial drug against these pathogens calls for search of new bioactive compounds to eradicate this major health challenge [3].

Different nanomaterials like copper, nickel, gold, zinc and titanium has been reported [4,5]. Among all silver nanoparticles have demonstrated most effectiveness against bacteria there by showing good antimicrobial efficacy. Diverse approach such as the biological and chemical method for the synthesis of nanoparticles has been reported. Whoever, chemical methods have been reported to be very expensive and produce some toxic byproducts which are dangerous [6,7]. In quest to synthesis stable metal nanoparticles that are not expensive, safe, reliable and less toxic. Green synthesis is the application of chemistry in the scheming, developing and implementing of chemical yields to facilitate the eradication of hazardous substances that are dangerous to man and his environment [8]. Lately, the development of interest in the use of green chemistry approach in metal nanoparticles synthesis has greatly increased [9-12]. An innovative methods referred to as green/biosynthesis have been developed recently where extract of plants such as *Coriandrum sativum, Petroselinum crispum* [13,14]. *Murraya koenigii* and *Ocimum sanctum* were used as metal nanoparticles synthesis [10,15].

Among numerous plants *Annona muricata* leaves extract, was chosen for this study because of many pharmacological effects including anti-cancer, anti-inflammatory, anti-diabetic, antioxidant and hypotensive activities it possess [16,17]. The results obtained from the phytochemical screening of *Annona muricata* confirmed the presence of geranyl acetate, camphor, geraniol, coumarins and quercetin 3-glucoronide [18]. Also a lot of organic compounds such as xylopine, isolaureline, chlorogenic acid, chatechin, vomifoliol , roseoside, liliolide and epicatechin isolated from *Annona muricata* leaf has been reported to exhibit good inhibitory concentration against some bacterial has also been [18]. This study was aimed on the investigation of synthesized silver nanoparticles that is environmental friendly from the leaves of *Annona muricata* and evaluates their antimicrobial activity against some selected human pathogens.

Materials

Silver nitrate ($AgNO_3$; 99.9%, Sigma Aldrich, Germany). Leaves of *Annona muricata* were obtained from the premises of Obafemi Awolowo University, Ile-Ife, Nigeria. All chemicals used were of analytical grade.

Extraction procedure

Annona muricata leaf extract was prepared using green process technique; via standard procedure [19]. The leaves of *Annona muricata* collected was properly washed with distilled water so as to

remove foreign particles and dust available on the leaves surfaces. About Twenty (20) grams of leaves of *Annona muricata* were pulverized and boiled in 100 mL distilled water with 250 mL flask for 30 minutes. The extract was filtered. The removal of heavy molecular weight constituents in the extract was removed by centrifuging at 7500 rpm. The pH of the *Annona muricata* extract was 7.5 and kept at 4°C for supplementary experimental analysis. Extract of about 5 mL was added to 45 ml of 0.01M $AgNO_3$ solution and was left to react at room temperature. The colour of the solution changes from transparent yellow to dark brown and the intensity of the colour also increase with time which signifies the formation of silver nanoparticle.

Characterization of synthesized nanoparticles

Shimadzu UV-1800 model of UV-vis spectrometer operated on a resolution of 1 nm with absorbance in the region 250 and 900 nm wavelength was used for the examination of the reduction mechanism of silver ion. The (FTIR) spectrum was used to determine the functional groups in the *Annona muricata* extract and the nanoparticle produced. FTIR spectra were measured by Shimadzu spectrometer in the wavelength of 500-4000 cm^{-1}. The particle size and surface morphology of silver nanoparticle synthesized was measured with transmission electron microscopy.

Antimicrobial activity of synthesized nanoparticles by well diffusion method

The antimicrobial activity of the synthesized nanoparticles was tested against some bacterial and fungal clinical pathogens by agar disc diffusion method. The cultures were swab in pre-sterilized Muller Hinton agar plates under aseptic condition was used. The Plates were permitted to stay for 10 minutes to absorb the cultures. Then three 8mm wells were punched into the Muller Hinton agar plates to examine the antimicrobial activity of the synthesized nanoparticles. 20µl of each test sample at different concentrations (50,75,100 µg/ml) was transferred into each of the punched wells and closed using 0.8% molten agar. Tetracycline and Chloramphenicol were used as control for bacteria and fungi test pathogen respectively. The incubation of the plates was done at 37°C for 22 hours. The inhibition zones of test pathogens were measured after incubation period.

Results and Discussion

UV-Vis spectroscopy

The noticeable peaks at 435nm showed on the UV-Vis spectra illustrated in figure 1 correspond to silver nanoparticles surface plasmon resonance. This suggested that the reduction Ag^+ ions have been ascertained. Absorbance at 0.556 around 431.6nm wavelength has been reported for silver [20]. The maximum absorbance detected at 435.00nm on the spectrum of synthesized silver nanoparticle was in the range earlier reported for biosynthesized silver nanoparticles, 350-550nm [21-24].

FTIR analysis

The FTIR spectrum of the extracts shows number of absorption peaks, as showed in figures 2a and 2b. Strong absorption bands at 3431 to 3433cm^{-1} are a signal of bonded -OH of alcohol or possibly -NH of amine. The absorption peaks at 2924cm^{-1} could be allotted to alkane stretching vibrations and that at 1664cm^{-1} can be allotted to tertiary amides C=O stretching [25]. Reported that bands in the range of 1400-1600, 1640-1690, 2850-3000 and 1000-1300cm^{-1} are groups assisting reduction of silver nanoparticle [26]. The FTIR analysis confirmed (-OH) and amine (-N-H) as functional groups accountable for the reduction of Ag^+ ions to Ag^0.

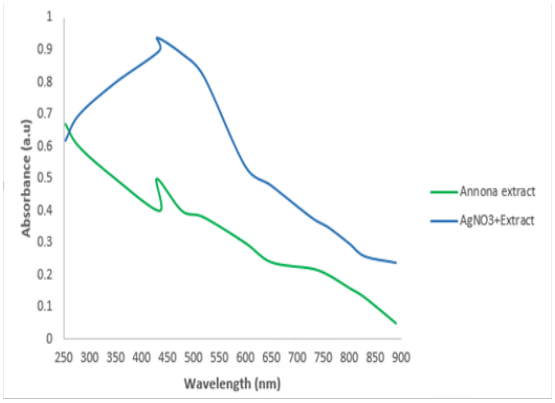

Figure 1: UV-visible absorption spectrum of silver nanoparticle produced.

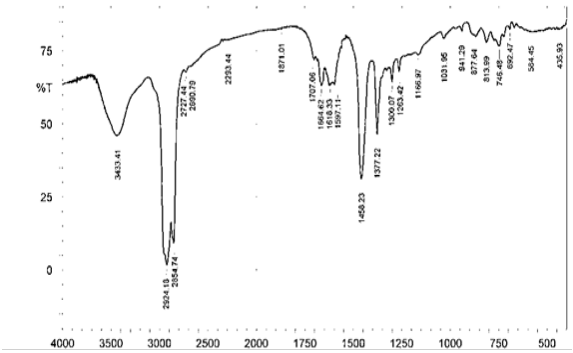

Figure 2a: FTIR spectrum of the *Annona muricata* extract cm^{-1}.

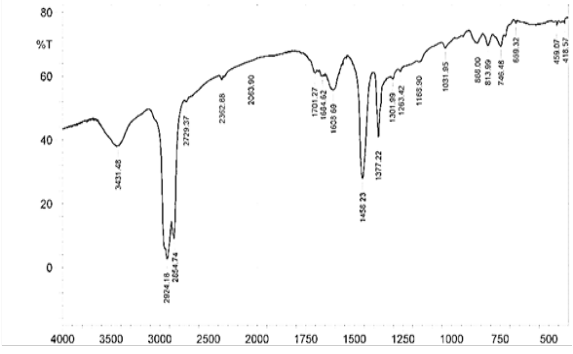

Figure 2b: FTIR spectrum of synthesized silver nanoparticle.

Transmission Electron Microscopic (TEM) analysis

The morphology and particle size of nanoparticles synthesized from Annona muricata extract was scanned with transmission electron

microscopic. The TEM micrograph showed in figure 3 indicated mono-dispersed silver nanoparticles with spherical shape. The result obtained from the TEM analysis is consistent with previous findings of [27]. The TEM micrograph of synthesized nanoparticles at 100nm magnification indicates an average size ranging from 22-28nm and was spherical in shape.

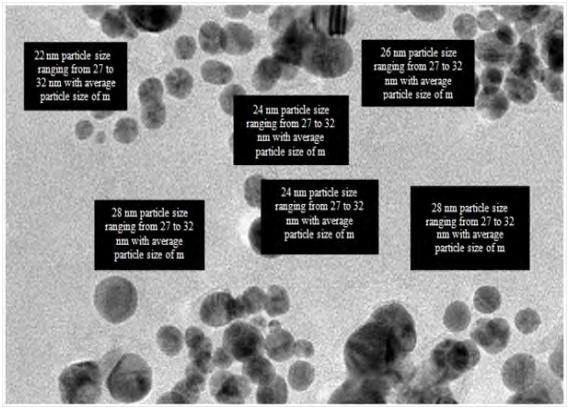

Figure 3: TEM micrograph of synthesized nanoparticles.

Antimicrobial analysis of synthesized silver nanoparticle

The zones of inhibition of the synthesized nanoparticles against the selected fungi (*Aspergillus flaws*, *Penicillium camemeri* and *Candida albican*) at concentrations of 50, 75 and 100µg are showed in table 1, figures 4 and 5: The highest zone of inhibition against the test fungi was observed at the 100µg of silver nanoparticles against *Candida albican* while the least zone of inhibition was detected at concentration of 50µg of silver nanoparticles against *Penicillium camemeri*. The higher the concentration of silver nanoparticle against the test fungi the higher the zones of inhibition observed. The silver nanoparticles especially at high concentration compete moderately with the control (Chloramphenicol) when compared.

Bacterial test pathogens	Inhibition zone of silver nanoparticle(mm)			
	Concentration (µg)			Tetracycline
	50	75	100	
Pseudomonas aeruginosa	12	17	22	28
Klebsiella pneumonia	10	15	20	23
Bacillus subtilis	13	16	18	10
Fungi test pathogens	Concentration (µg)			Chloramphenicol
	50	75	100	
Aspergillus flaws	12	13	15	24
Penicillium camemeri	5	15	17	20
Candida albican	13	17	19	23

Table 1: Zones of inhibition of silver nanoparticles against test bacteria and fungi.

The synthesized silver nanoparticles showed high inhibition zones against selected bacterial namely; *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Bacillus subtilis* especially at 100µg where the Inhibition zones are 22, 20 and 18mm as showed in table 1. Lowest inhibition zone was observed when 50µg of silver nanoparticle was used against *Klebsiella pneumonia* while highest inhibition zone was observed when 100µg of silver nanoparticle was used against *Pseudomonas aeruginosa.* The potency of the synthesized nanoparticle increases as concentrations of synthesized nanoparticles used against the test bacterial increase due to the observed zones of inhibition. The antimicrobial assay used in this study shows a better inhibition zones when compared with inhibition zones obtained from the antimicrobial assay used by on *Juglan regia* extract and that used by on some novel antibiotics [29,30]. The inhibition zones in this study were higher than that obtained from the study of at concentration of 50, 75 and 100µg [28]. The synthesized silver nanoparticles compete better than the control (tetracycline) at all concentration against *Bacillus subtilis.* This suggests that silver nanoparticles synthesized using *Annona muricata* can inhibit both gram positive and gram negative bacteria. This is evidence that the synthesized silver nanoparticle exhibit good antibacterial activity.

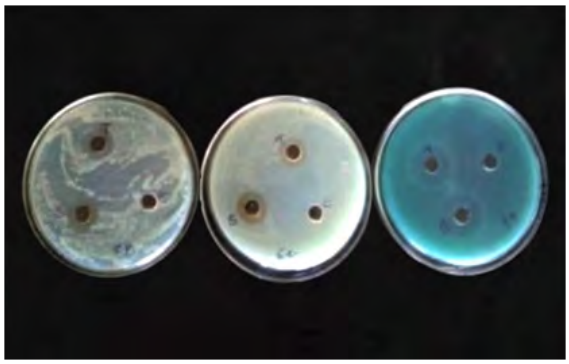

Figure 4: Antibacterial activity of synthesized silver nanoparticles from *Annona muricata* leaf.

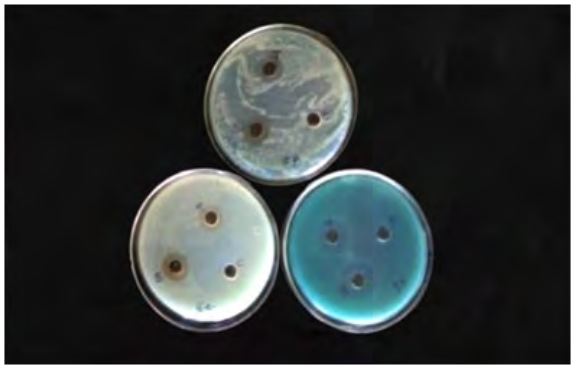

Figure 5: Antifungal activity of synthesized silver nanoparticles from *Annona muricata* leaf.

Conclusion

The synthesized silver nanoparticle from *Annona muricata* leaf was fairly uniform in size, shape and spherical in shape. The FTIR spectrum shows absorption bands corresponding to -OH, -NH and C=C functional groups. The synthesized silver nanoparticles from *Annona muricata* leaf in this study was eco-friendly, cost effective and also possess good antibacterial and antifungal activities.

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