ANTIMICROBIAL ACTIVITIES OF SILVER NANOPARTICLES AGAINST SKIN DISEASE-ASSOCIATED OPPORTUNISTIC PATHOGENS

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Abstract: Silver nanoparticles (AgNPs) is known to possess effective antimicrobial properties against a wide range of skin diseases. The use of green synthesis from plant extracts has also attracted attention because it is inexpensive, simple and environmentally friendly. This study focuses on evaluating the activity of silver nanoparticles on *Staphylococcus epidermidis* and *Candida albicans* which have been implicated in various cases of opportunistic infections of the skin. Potato capped silver nanoparticles were synthesized through a green route and characterized using UV, XRD, and TEM. Their antimicrobial effect was compared with conventional tetracycline and nystatin using an agar well diffusion assay. The zone of inhibition (ZOI), minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were assessed. The ZOI for AgNPs was 34 mm and 42 mm at 90 mg/mL for *S. epidermidus* and *C. albicans* respectively while the ZOI of tetracycline and nystatin were 41mm and 18mm respectively at 90 mg/mL.The MIC and MBC for AgNPs was 5.63 mg/mL for S. epidermidis and <2.82 mg/mL for C. albicans. Meanwhile, the MIC and MBC for tetracycline was <3.13mg/mL for *S. epidermidis* while MIC and MFC was >100mg/mL for nystatin. AgNPs therefore had a better performance on *C. albicans* than the conventional nystatin. The Ultra-violet visible Spectrophotometer showed absorbance peak at 520 nm, the Transmission Electron Microscopy (TEM) showed pentagonal AgNPs with an average particle size of 76 nm while the XRD shows the crystalinity of the NPs and also confirms the nanoparticles possessing the face centred cubic structure (fcc). The results of this study gives credence to the scientific basis of AgNPs for skin care formulations.

Keywords: Antimicrobial activity, Green synthesis, Pathogens, Skin infection, Silver nanoparticles.

Introduction

Metal nanoparticles are a unique group of nanoparticles that possess novel electronic, optical and chemical properties. They are particles of metal atoms with diameters between 1nm and 100nm (Gong et al 2007). There are various types of metal nanoparticles namely Magnesium, gold (Gu et al, 2003), Copper, Zinc, titanium (Retchkiman Schabes et al, 2006) and silver. Silver nanoparticles have been found to be the most effective as an antimicrobial agent in water treatment, medical devices, texiles (Khaydarov et al, 2009) , treatment of infections in open wounds, chronic ulcers and brucellosis (Alizadeh et al ,2013, Rawaani et al, 2013, paint coatings (kumar et al, 2008).

Synthesis of silver nanoparticles have been extensively studied with the use of physical and chemical methods. These methods require the use of large amount of energy a lot of time for thermal stability (Krus et al, 2000, Magnusson et al 1999) and harmful organic solvents (Krutyakor et al, 2008). The synthesis of silver nanoparticles through green routes in which plant extracts serve as both capping agent and reducing agent is on the rise. The silver ions Ag^+ are reduced to Ag^o, the plant extract helps to prevent agglomeration of the nanoparticles. Potato is a root vegetable, a starchy tuber of the plant *Solanum tuberosum*. The word "Potato" may refer to the plant itself or to the edible tuber. Potato serves as a plant extract, where it acts as both a reducing agent as well as a stabilizer for the nanoparticles.

Skin infections occur when parasite, fungi or germs such as bacteria penetrate the skin and spread. When this happens, it can cause pain, swelling, other types of discomfort and skin colour changes. A skin infection may be mild or serious. In recent times, silver nanoparticles have found significance in its antimicrobial activity. We investigate the antimicrobial activity of Silver nanoparticles synthesised through green routes using Potato leaf extract as a capping agent against skin disease inducing pathogens.

Materials and Method

All reagents and solvents were analytical grade and used without further purification. Silver nitrate (>99.9% pure), methanol, acetone were purchased from Sigma–Aldrich.

Preparation of Potato Leaf Extract

20 g of potato leaves were thoroughly washed in distilled water for 5minutes. The leaves were dried at room temperature and cut into fine pieces, then boiled in 250 mL Erlenmeyer flask with 100 mL of distilled water for 15 min. It was then filtered thrice to remove particulate matter and to get a clear solution which was refrigerated at 4^{o}C for further use. **Preparation of *Silver Nanoparticles***

10 mL of plant extract was added to the aqueous solution of 1 mM Silver nitrate. The sample was incubated in the dark for 24 hours to minimize photo activation of silver nitrate at room temperature. Reduction of Ag^+ to Ag^o was confirmed by the colour change of solution from colourless to brown.

Microbial strains, growth media and culture conditions

The bacterial and fungal strains used in this study were mainly reference strains obtained from the stock culture collection of the department of Microbiology, Faculty of

Science, University of Lagos, Nigeria. They include a bacterium [*Staphylococcus epidermidis* (ATCC 12228) and a fungus [*Candida albicans* (ATCC 10231)]. Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), Sabouraud dextrose agar (SDA) and Sabouraud Dextrose broth (SDB) (SRL, India) were used for cultivation and sensitivity tests. Media were prepared according to manufacturer's instructions and autoclaved for 15 min at 121°C. All agar and broth cultures were incubated at 28 ±2°C for 3-5 days for the fungal strain and at 37°C for 24 h for the bacterial strain. The population of viable cells in suspensions were confirmed by viable counts.

Preparation of Standardized Inoculum

The test organisms were subcultured onto fresh plates of Mueller Hinton agar (MHA) (SRL, India) for 24 hr at 37 °C and Sabouraud dextrose agar (SRL, India) for 3 - 7 days at 28±2 °C for bacteria and fungi respectively. The inoculum was prepared by colony suspension method. Colonies were picked with a sterile loop, followed by a direct colony suspension of each test isolate in Mueller Hinton broth (MHB) and Sabouraud Dextrose broth (SDB). The turbidity was subsequently adjusted to a 0.5 McFarland standard (1.5 x 10^8 cfu/mL).

Well Diffusion Assay

The antimicrobial screening assays carried out included zones of inhibition (ZOI), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). The antimicrobial activity of silver nanoparticles and conventional tetracycline (antibacterial agent) and nystatin (antifungal agent) were evaluated for the test organisms by agar well diffusion method as described by (Sherlock et al., 2010).

Agar well diffusion assays were performed using Mueller Hinton Agar (SRL, India). Each agar-containing medium was poured into sterile 90 mm diameter sterile Petri-dishes to depths of 5 mm, swirled gently to allow distribution of agar media and allowed to solidify. The agar plates were seeded with 0.1 mL inoculum of the test suspension with sterile cotton swabs. Cylindrical wells (4 mm deep x 6 mm in diameter) were subsequently cut into the centre of the agar with a 7-mm diameter sterile cork borer. Silver nanoparticles solution of concentrations of 90 mg/ml, 80 mg/ml, 70 mg/ml and 60 mg/ml were utilized for the assay. A sterile pipette was used to introduce 0.1 mL of the silver nanoparticles into each well. Tetracycline and nystatin served as controls. The plates were allowed to stand for one hour for proper diffusion of antimicrobial agents before they were incubated at 37 °C for 24 hr for bacteria and at room temperature (28±2 °C) for 48 hr for fungi. The antimicrobial activity was determined based on the inhibitory effect of the AgNPs and conventional antimicrobial agents on the bacterial and fungal growth on agar plates. After incubation, appearance of clear zones around the well was indicative of inhibition. The diameters of the growth inhibition zones were measured in millimetres to the nearest 0.1mm using a meter rule and compared.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the silver nanoparticle and conventional antimicrobial agents that showed potent antimicrobial activity during the sensitivity test were determined by serial dilution in Mueller Hinton broth with concentrations of conventional antimicrobial agents ranging from 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.13mg/mL. Concentrations of AgNPs utilized were 90 mg/ml, 45 mg/ml, 22.5 mg/ml, 11.25 mg/ml, 5.63 mg/ml and 2.82 mg/ml. The various test tubes were inoculated with inoculum prepared from fresh overnight cultures and then incubated at 37 ^{0}C for 24 hr for bacteria and at room temperature for 48 hr for fungi. The lowest concentration of the silver nanoparticles and conventional antimicrobial compounds that inhibited the growth of the test organism were noted and recorded as the minimum inhibitory concentration (MIC).

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined using content from the broth dilution of MIC test. The surface of sterile Mueller Hinton Agar (MHA) medium (20 mL) in petri dishes were aseptically inoculated using sterile inoculating loop with the contents of the test tubes from MIC test. The plates were incubated at 37 ^{0}C for 24 hr. The minimum bactericidal concentration was recorded as lowest concentration of the antimicrobial compound that showed no growth on the agar plate (Igwo-Ezikpe *et al*., 2013).

Minimum Fungicidal Concentration (MFC)

The Minimum Fungicidal Concentration (MBC) was determined using content from the broth dilution of MIC test. Sterile Sabouraud Dextrose Agar (SDA) plates (20 mL) were aseptically inoculated using sterile inoculating loop with the contents of the test tubes from MIC test. Subsequently, the plates were incubated at room temperature (28±2 °C) for 3-5 days. The minimum fungicidal concentration was recorded as lowest concentration of the antimicrobial compound that showed no growth on the incubated plates (Igwo-Ezikpe *et al*., 2013).

Results and Discussion

The silver nanoparticles diffused through the agar and resulted in larger clear zones in *Candida albicans* when compared with the less effective and conventional nystatin (Tables 1-3). No clear zones that were indicative of antimicrobial activity were identified in the nystatin which served as the control. The antimicrobial activity was also evidenced by the MIC which was 5.63mg/mL of the AgNP against *S. epidermidis* which was relatively close to that of conventional tetracycline which had an MIC of <3.13mg/mL

The TEM reveals size and morphology of the nanoparticles as shown in Fig 1. The micrograph showed the pentagonal shape of the nanoparticles with an average particle size of 52.94 nm revealed from the histogram in Fig 2. The Ultraviolet Spectra (Fig 3) of the prepared AgNPs showed absorption at 512 nm. Fig4 shows the XRD analysis of the synthesised silver nanoparticle from potato leave extract

with diffraction peaks at 2Θ= 38.3º, 44.5º, 64.8º, 77.5º respectively which corresponds to the standard powder diffraction card of JCPDS, No 04-0783 with values (111), (200), (220) and (311) planes of silver.. The XRD study confirms that the particles have a face centered cubic crystal structure.

The Pentagonal Silver nanoparticles with an average particle size of 52-94 nm were tested against bacteria and fungi. Due to the large surface area of the nanoparticle as a result of the size, there was an interaction with both the bacteria and fungus. The size of nanoparticles have been found to influence the antimicrobial activity Studies show that the shape of the nanoparticles plays a major role in the antimicrobial efficacy of the nanoparticle (Sukdeb et al , 2007).

Table 1 shows the maximum ZOI of 42 mm at 100 mg/ml on *Candida albicans* and minimum ZOI of 22 mm at 60 mg/ml on *Staphylococcus epidermidis*. This showed that silver nanoparticles have antimicrobial activity. Table 2 shows that silver nanoparticles indicated MIC of 5.63 mg/ml against *Staphylococcus epidermidis* and 2.82 mg/ml against *Candida albicans*. Table 3 indicate that silver nanoparticles showed a Minimum Bactericidal Concentration against *Staphylococcus epidermidis* at 5.63 mg/ml and a Minimum Fungicidal Concentration on *Candida albicans* at 2.82 mg/ml. This showed that silver nanoparticles have bactericidal and fungicidal effects on test organisms in this study.

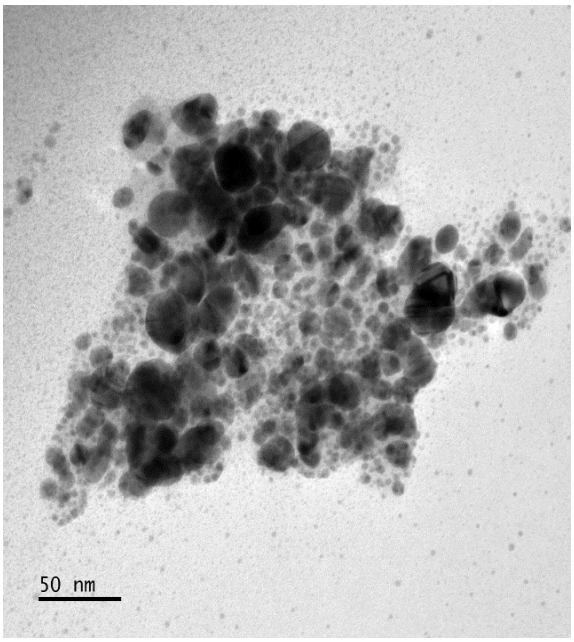

Fig 1: TEM image of potatoe capped silver nanoparticles.

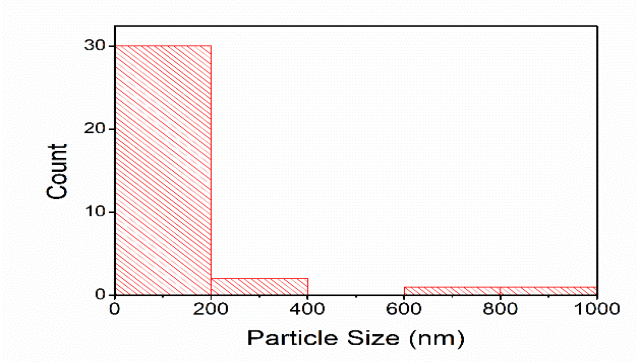

Fig2: Histogram showing the particle Size

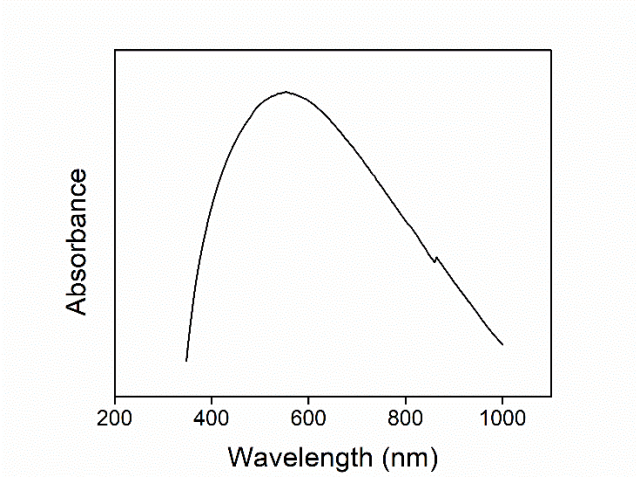

Fig3: UV spectra of potato capped silver nanoparticles

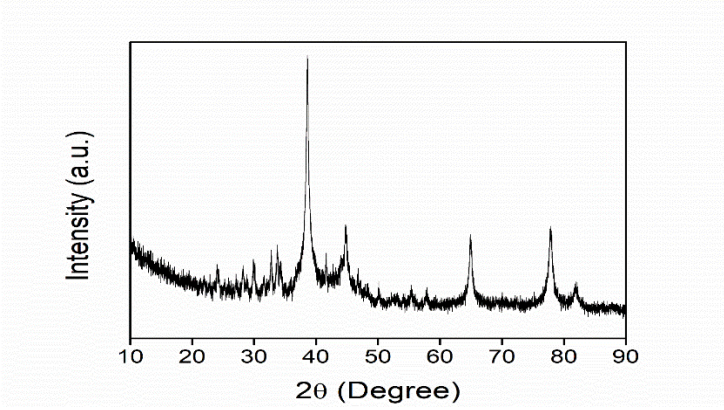

Fig 4: XRD of Potato Capped Silver nanoparticles

Table 1: Zone of inhibition (mm) values of Silver nanoparticles against test organisms

Test Organisms	**Concentrations (mg/ml)**								
	Silver nanoparticles				Tetracycline/Nystatin				
	90mg/ ml	80mg/ ml	70mg/ ml	60mg/ ml	100mg/ ml	90mg/ ml	80mg/ ml	70mg/ ml	60mg/ ml
Staphylococcus epidermidis	34	28	24	22	42	41	41	39	38
Candida albicans	42	39	36	34	20	18	17	17	15

Table 2: Minimum inhibitory concentration of Silver nanoparticles against test organisms

Test Organisms	**Concentrations (mg/ml)**											
	Silver nanoparticles						Tetracycline/Nystatin					
	90mg/ml	45mg/ml	22.5mg/ml	11.25mg/ml	5.63mg/ml	2.82mg/ml	100	50	25	12.5	6.25	3.13
Staphylococcus epidermidis	-	-	-	-	-	+	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-	+	+	+	+	+	+

- = no visible growth
+ = visible growth

Table 3: Minimum Bactericidal/Fungicidal concentration (MBC/MFC) of Silver nanoparticles against test organisms

Test Organisms	**Concentrations (mg/ml)**											
	Silver nanoparticles						Tetracycline/Nystatin					
	90mg/ml	45mg/ml	22.5mg/ml	11.25mg/ml	5.63mg/ml	2.82mg/ml	100	50	25	12.5	6.25	3.13
Staphylococcus epidermidis	-	-	-	-	-	+	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-	+	+	+	+	+	+

- = no visible growth
+ = visible growth

Conclusion

In this work, we have reported the evidence of antimicrobial effects of silver nanoparticles nanoparticles (AgNPs) on two opportunistic microorganisms implicated in skin-diseases. Silver nanoparticles were synthesised through a green route from potato leaf extract. This method is cost effective, without the use of toxic chemicals and lower reaction time. This antimicrobial activity may be due to the mechanical interactions on cell walls and/or membranes of microorganisms.

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