Original Research Article

Synthesis of silver nanoparticles from *Adansonia digitata* leaf extract and its antimicrobial properties

Abstract

Silver nanoparticles (AgNPs) were green synthesized using *Adansonia digitata* leaf extract. The synthesized silver nanoparticles were characterized in terms of synthesis, size, shape, morphology and capping functionalities by UV-Visible Spectroscopy, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). Antimicrobial activity of the synthesized silver nanoparticles was investigated by well diffusion method. The antibacterial activity of the nanoparticle was studied against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis, Pseudomonas aeurigunosa*, *Salmonella typhi* and *Klebsiella pneumonae* while the antifungal activity was studied against *Candida albicans, Aspergillus niger*, *Penicillum notatum* and *Rhizopus stolomifer*. The synthesized AgNPs was active against all the studied microorganisms. *Staphylococcus aureus* was the most susceptible bacterium (inhibition zones ranging from 12.00 to 28.00mm, MIC : 30µl, MBC : 50µl) while *Aspergillus niger* was the most susceptible fungi (inhibition zones ranging from 10.00 to 18.00mm, MIC : 90µl, MFC : 120µl. In conclusion the synthesized silver nanoparticles was found to have antimicrobial activity against the pathogenic bacteria and fungi tested and hence has a great potential in biomedical application for the treatment of microbial infections.

Keywords : Silver nanoparticles; *Adansonia digitata;* antimicrobial activity; nanoparticles; methanolic extract.

INTRODUCTION

In recent years, transmission of infectious pathogens to the community has caused outbreak of diseases such as influenza, diarrhea, cholera etc. throughout the world (Rasko *et al.,* 2011). These infectious diseases have not only occurred in developing countries with low levels of hygiene and sanitation but have also been recognized in developed countries. Food and water borne pathogens are the main factors for the outbreak of these diseases, and the outbreak of re-emerging and emerging infectious diseases are a significant burden on global and public health (Jones *et al.,* 2008). Their emergence is thought to be driven largely by socio-economic, environmental and ecological factors. The comprehensive treatments of environments containing infectious pathogens using advanced disinfectant nanomaterials have been proposed for prevention of the outbreak (Erick and Thomas, 2011; XiZhu *et al*., 2015) Among these nanomaterials silver nanoparticles (AgNPs) with unique properties of high antimicrobial activity have attracted much interest from scientists and technologies to develop nanosilver based disinfectant products (Saxena *et al.,* 2010; Elumalai *et al.,* 2010). AgNPs have been synthesized by physio-chemical techniques such as chemical reduction (Khan, *et al.,* 2011), gamma ray radiation (Chen *et al.,* 2007), micro emulsion (Zhang *et al.,* 2006), electrochemical method (Reicha *et al.,* 2012), laser ablation (Abid *et al.,* 2002), autoclave (Yan and Pan, 2012), microwave (Khan *et al.,* 2011) and photochemical reduction (Alarcin *et al.,* 2012). These methods have effective yield but they are associated with some limitations like use of toxic chemicals and high operational cost and energy needs (Esumi *et al*., 2000 ; Bakshi *et al*., 2008) Considering the drawbacks of physiochemical methods, cost effective and energy efficient new alternative for AgNPs synthesis using microorganisms (Sharma *et al.,* 2009), plant extracts (Elumalai *et al.,* 2010) and natural polymers (Huang and Yang, 2004) as reducing and capping agents are emerging very fast. Several authors have used plant extracts, bacteria, fungi and algae

for the synthesis of metal nanoparticles (Lateef *et al*., 2015; Rajeshkumar *et al.,* 2015) . *Adansonia digitata* L is commonly known as Baobab tree native to Africa. Baobab is a multipurpose tree which offers protection and provides food clothing as well as raw materials for many useful items. The fruit pulp, seeds leaves, flowers, roots and bark of baobab are edible and they have been studied by scientists for their useful properties (Simon, 2015). The fruit pulp have very high vitamin C, calcium, phosphorus, carbohydrates, fibers, potassium, proteins and lipid content which can be used in seasoning as an appetizer and also make juices (Food Standard Agency, 2002 ; Sidibe and Williams, 2002 ; Chadare *et al*., 2009) . Seeds contain appreciable quantities of phosphorus, magnesium, zinc, sodium,iron and high levels of lysine and thiamine (Chadare *et al*., 2009). Baobab has numerous biological properties including antioxidant, antimicrobial, antimalarial, diarrhea, ,anaemia ,asthma and anti-inflammatory activities amongst others (Hussain and Denni, 1999 ; Vertuani *et al.*,2002 ; Vimalanathan and Hudson, 2009 ; Kabore *et al*., 2011; Deooyem *et al*., 2014). Currently there are no reports on the use of *Adansonia digitata* for the biosynthesis of silver nanoparticles. In this study, green synthesis of silver nanoparticles using the leaf extract of *Adansonia digitata* and evaluation of its antimicrobial activity against various human pathogenic bacteria and fungi was reported.

Materials and Methods

Reagents

All reagents were of analytical grade and obtained from Sigma-Aldrich Chemical, Germany.

Plant Materials

Fresh leaves of *Adansonia digitata* were collected from Ladoke Akintola University of Technology, Ogbomoso school farm. The plant was authenticated at the botanical unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Healthy leaves with no sign of damage were air dried under shade at room temperature for four weeks and pulverized into powdered form.\

Fig 1 : *Adansonia digitata* plant

Microorganisms

The microorganisms used in this study were clinical isolates obtained from the Department of Pharmaceutical Microbiology, University of Ibadan. The organisms were maintained on agar slant at 4^{o}C and subcultured on a fresh appropriate agar slant, 24 hours prior to the antimicrobial test. Two Gram-positive bacteria : *Staphylococcus aureus*, *Bacillus subtilis;* four Gram-negative bacteria : *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonae*

and four fungi : *Candida albicans Aspergillus niger*, *Penicillum notatum* and *Rhizopus stolomifer* were used for the bioassay.

Preparation of Plant Extract

Two kilogrammes of the pulverized material was soaked in 6.0 litres of methanol for two weeks. After soaking period, the liquid portion was separated from the shaft with the aid of Whatman (No1) filter paper and the filtrate was concentrated using a rotary evaporator and dried on a water bath to obtain a dry residue which is the crude methanolic extract (yield weight 110g) which was stored in a dessicator until used.

Phytochemical Screening

The methanolic extract was screened for the presence or absence of alkaloids, saponins, tannins, flavonoids, steroids, and phenols using standard methods (Banso and Ngbede, 2006; Ngbede *et al.,* 2008).

Synthesis of Silver Nanoparticle

The extract was centrifuged at 4,000 rpm for 20 minutes. The supernatant obtained was used to synthesize silver nanoparticles as described by Lateef *et al*., (2016). About ImL of the supernatant was added to the reaction vessel containing 40ml of 1mM silver nitrate (AgNO3) solution for the reduction of silver ion. The reaction was carried out in static condition at room temperature (30 ± 2^{o}C) for 2hrs . The formation of silver nanoparticles was monitored through visual observation of the change of colour and measurement of the absorbance spectrum of the reaction mixture using UV-Visible spectrophotometer.

Characterization of Silver Nanoparticle

UV-Visible Spectroscopy Analysis

The formation of silver nanoparticles by the reduction of the aqueous silver ion during exposure of *Adansonia digitata* methanolic leaf extract was monitored by UV-VIS spectroscopy. The reduction of silver ions was monitored from 200 – 900nm using UV-VIS spectrophotometer (UV – 2450 Shimadzu). The spectrum data recorded was then plotted.

FTIR Analysis

Fourier transform infrared spectroscopy (FTIR) was employed to detect organic compound present in the leaf extract that was responsible for the reduction of silver ions to form silver nanoparticles and for stabilization of the nanoparticle. The emission spectrum was recorded using an LF-45 fuorescence spectrophotometer Shimadzu IR Prestige FTIR instrument in the wavelength range 4000 – 500cm^{-1}.

SEM Analysis

Scanning electron microscopy micrograph was obtained using a Hitachi scanning electron microscope (model S-2600N, Tokyo, Japan) operating in the high vacuum anode with an acceleration voltage of 20KV by a filter paper soaked in the silver nano particle solution.

Antimicrobial Assay

The assessment of antimicrobial activity of the synthesized silver nanoparticles was carried out using the well diffusion method (Lino and Deogracious, 2006). Two gram-positive bacteria, four

gram-negative bacteria and six fungi were used for the bioassay. The bacteria cultures were inoculated in nutrient broth and incubated for 24 hours at 37^{o}C while the fungal cultures were inoculated on potato dextrose agar and incubated for 48 hours at 28^{o}C. From freshly cultured bacteria and fungal colonies, 100µl of the inoculum were taken and spread on Mueller Hinton agar plates. A sterile cork borer was then used to create wells (6mm diameter) for different concentration of the synthesized silver nanoparticle. Using a micropipette, 30µl, 60µl, 90µl, 120µl, 150µl and 180µl of the nanoparticle solution samples were poured into the wells on all plates. Wells containing standard antimicrobials: Gentamycins (10µg/ml) and Tiaconazole (70%w/v) were included to serve as positive control. A 0.5ml portion of sterile methanol was introduced into another well to serve as negative control. The bacteria plates were incubated at 37^{o}C for 24 hours while the fungal plates were incubated at 28^{o}C for 48 hours. After incubation, the plates were examined for the presence of zone of inhibition, indicated by clear zone around the wells and the diameters of zones were measured.

Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

MIC, MBC and MFC were determined using the agar dilution method (Oyeleke *et al.*, 2005). For the bacterial and fungal isolates, 30µl, 60µl, 90µl, 120µl, 150µl and 180µl of the silver nanoparticles were separately added to 18mL of agar in test tubes. Then 1mL of 18hours old of each bacterial and fungal cultures earlier adjusted at 10^{-7}CFU/mL was added to each test tube. For bacteria cultures, the tubes were incubated at 37^{o}C for 24 hours while for fungal cultures, the tubes were incubated at 28^{o}C for 48 hours and observed for growth in form of turbidity. The lowest concentration of nanoparticles that produced no visible bacterial and fungal growth (no turbidity) by visual inspection was considered the MIC. The MBC and MFC were determined by

removing 100µl of bacterial and fungal suspension from MIC tube that did not show any growth and subcultured onto Mueller Hinton agar plates and incubated at 37^{o}C for 24 hours for bacterial cultures and 28^{o}C for 48 hours for fungal cultures. After incubation, the concentration at which no visible growth was seen was recorded as the MBC or MFC.

RESULTS AND DISCUSSION

Phytochemical screening

Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins and steroids.

Visual Inspection

Preliminary identification of nanoparticles formation was carried out by observing the colour change of the reaction solution. The formation of silver nanoparticles by leaf methanolic extract of *Adansonia digitata* was facilitated within a period of 10 minutes, with the colour stabilizing within 20 minutes.. The colour of the solution changed from light yellow to dark brown after 20 minutes of addition of the extract to silver solution (Fig 2) suggesting the formation of silver nanoparticles. Synthesised silver nanoparticles exhibit dark-yellowish-brown colour due to the surface plasmon resonance phenomenon

(a) **(b)**

Fig 2 : Colour change in the reaction mixture (silver nitrate + *Andansonia digitata* extract)

(a) Colour of the mixture after mixing the extract with silver nitrate solution (b) Colour change of the mixture after 20 minutes

UV-Visible Spectroscopy

The reduction of Ag^{+} into Ag particles was further confirmed using UV-VIS spectroscopy. (Fig 3) depicts the UV-VIS spectrum of the synthesized silver nanoparticles from *Adansonia digitata* extract. Absorption spectrum of the silver nanoparticle formed in the reaction medium has absorbance peak at 418nm which is a characteristics band for silver. No other peak was observed in the spectrum which confirms that the synthesized products were silver nanoparticles.

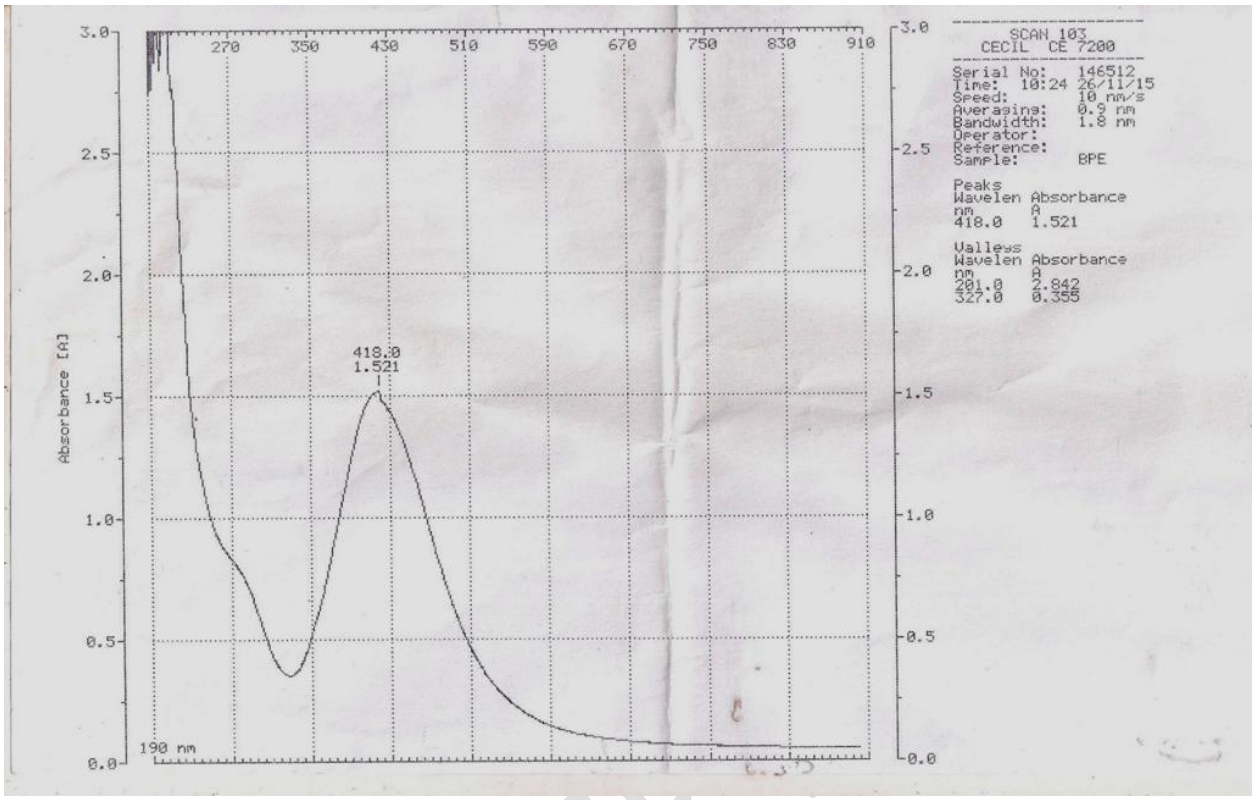
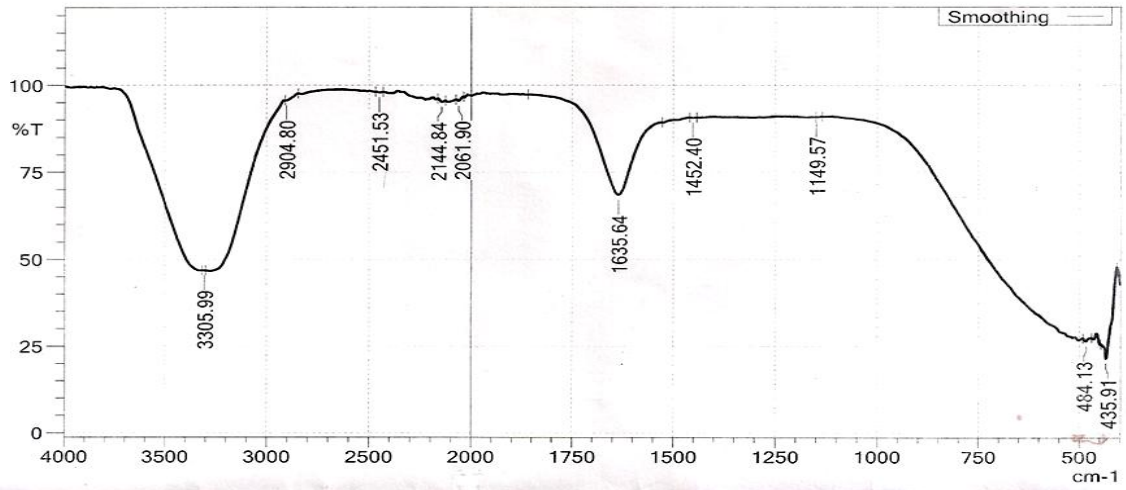


Fig. 3 : UV-VIS spectrum of silver nanoparticles synthesised from methanolic leaf extract of *Adansonia digitata*

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR measurement was carried out in order to identify the presence of various functional groups in the biomolecules responsible for the bioreduction of Ag^{+} and capping/stabilization of silver nanoparticles (Fig. 4). The observed intense bands were compared with standard values to identify the functional groups. FTIR spectrum showed absorption bands at 3305.39, 2904.80, 2451.53, 2144.84, 2061.90, 1635.64, 1452.40, 1149.57, 484.13 and 435.91cm^{-1} indicating the presence of capping agent with the nanoparticle. The bands at 3305.39cm^{-1} correspond to N-H stretching from the secondary amine. Bands at 2904.80, 2451.53, 2144.84, and 2061.90cm^{-1} arising from C – H stretching of aromatic compounds. The band at 1635.64cm^{-1} in the spectrum

corresponds to C – N and C – C stretching indicating the presence of proteins (Prakash *et al.*, 2013). The band at 1452.40cm^{-1} was assigned for N – H stretch vibration present in the amide linkage of protein. The band at 1149.54cm^{-1} exemplifies C – O – C stretching typical of propionates and higher esters. Band at 484.13 and 435.91cm^{-1} might be attributed to C – H stretching of the aromatic compounds. From the FTIR study, it may be concluded that protein could play the role of reducing and capping agents resulting in the formation of silver nanoparticles in the medium.



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	435.91	21.55	10.71	447.49	408.91	2666.945	224.151	
2	484.13	26.49	0.91	491.85	470.63	1547.259	7.182	
3	1149.57	90.95	0.01	1151.50	1136.07	139.166	0.193	
4	1452.40	90.70	0.05	1460.11	1442.75	161.049	0.472	
5	1635.64	68.58	23.39	1859.38	1527.62	4223.896	2025.286	
6	2061.90	95.47	0.71	2075.41	2036.83	153.368	10.760	
7	2144.84	95.18	0.66	2164.13	2127.48	163.354	11.686	
8	2451.53	97.91	0.22	2468.88	2432.24	72.596	3.891	
9	2904.80	95.64	0.21	2910.58	2846.93	223.812	11.163	
10	3305.99	46.79	0.07	3317.56	3300.20	923.114	0.656	

Fig. 4 : FTIR spectrum of silver nanoparticles synthesized from methanolic leaf extract of *Adansonia digitata*

Scanning Electron Microscopy (SEM)

SEM revealed information about the size, shape and morphology of the synthesized silver nanoparticles. The SEM image showed relatively spherical shaped nanoparticles with diameter range of 40 – 50nm (Fig. 5).

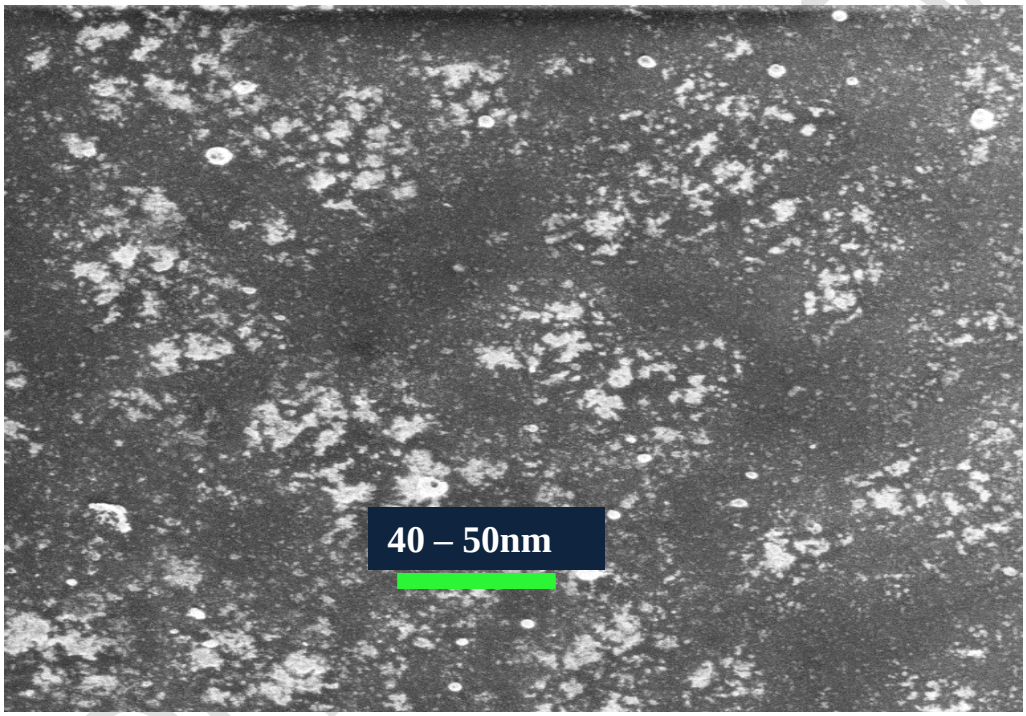


Fig 5: SEM image of silver nanoparticles synthesized from methanolic leaf extract of *Adansonia digitatata*

Antimicrobial Study

Table 1 depicts the result of the antimicrobial screening of the silver nanoparticle synthesized from *Adansonia digitata.* In this study, the synthesized silver nanoparticle was evaluated against ten microorganisms: six bacteria and four fungi. It was observed that the synthesized silver

nanoparticle was active against all the studied microorganisms with bacteria being more susceptible than the fungi. *Staphylococcus aureus* was the most susceptible bacteria of all the tested bacteria with inhibition zones ranging from 12.00 to 28.00mm while *Candida albicans* and *Aspergillus niger* were the most susceptible fungi with inhibition zones ranging from 10.00 to 18.00mm. Result of the minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the synthesized silver nanoparticles from *Adansonia digitata* leaf extract are presented in Table 2. The synthesized silver nanoparticles demonstrated good antimicrobial activity with the MIC value against the bacteria ranging from 30µl to 120µl, MIC for the fungal was 100µl. The synthesized silver nanoparticle showed the lowest MIC against *Staphylococcus aureus* (30µl). The broadest activity of the silver nanoparticle against most of the tested bacteria was 90µl as MIC while broadest activity of 150µl was recorded against most fungi tested. The silver nanoparticle synthesized from methanolic extract of *Adansonia digitata* also showed bactericidal and fungicidal activities on the bacteria and fungi isolates with *Staphylococcus aureus* being the susceptible microorganism. At a small concentration of 90µl, the synthesized silver nanoparticle was able to kill the organism. These findings are in agreement with previous studies that examine the antimicrobial activity of silver nanoparticles against many human pathogens (Kaushik *et al*.,2014 ; Wael Mahmoud *et al*., 2017). Introduction of silver into bacteria cells induces a high degree of structural and morphological changes which can lead to cell death. As the silver nanoparticles come in contact with the bacteria, they adhere to the cell wall and cell membrane (Klasen, 2000). Once bound, some of the silver passes through to the inside and interacts with phosphate containing compounds like DNA and RNA while another portion adheres to the sulphur-containing proteins on the membrane. The silver-sulphur interactions at the membrane cause the

cell wall to undergo structural changes like the formation of pits and pores (Feng *et al.,* 2000). Through these pores, cellular compounds are released into the extracellular fluid simply due to the osmotic difference. Within the cell, the integration of silver creates a low molecular weight region where DNA then condenses (Feng *et al.,* 2000). Having DNA in a condensed state inhibits the cells replication proteins contact with the DNA. Thus the introduction of silver nanoparticles inhibits replication and is sufficient to cause death of the cell (Chen *et al*., 2014) . This has been correlated to the suppression of enzymes and inhibited expression of proteins that relate to the cell's ability to produce ATP. Although, it varies from every type of the cell proposed, as their membrane composition varies greatly. It has been seen that in general, silver nanoparticles with an average size of 10μm or less show electronic effect that greatly increase their bactericidal activity (Pal *et al.,* 2007). This could be partly due to the surface area to the volume ratio. The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cell dies (Fig. 6). Some studies suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death (Daniel *et al.,* 2006; Kim *et al.,* 2007). It has also been proposed that there can be release of silver ions by the nanoparticles (Feng *et al.,* 2008) and these ions can interact with the thiol groups of many vital enzymes and inactivate them (Matsumara, 2003). The bacteria cells in contact with silver take in silver ions which inhibit several functions in the cell and damage the cells. Then there is generation of reactive oxygen species which are produced possibly through the inhibition of respiratory enzyme by the silver ions and attack the cell. Silver is a soft acid and there is a natural tendency of an acid to react with a soft base (Morone *et al.,* 2005). The cells are majorly made up of sulfur and phosphorus which are soft bases. The action

of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to a death. Another fact is that the DNA has sulphur and phosphorus as its major components, the nanoparticles can act on the soft bases and destroy the DNA which would definitely lead to cell death. The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes. It has also been found that the nanoparticles can modulate the signal transduction in bacteria. It is a well established fact that phosphorylation of protein substrate in bacteria influences bacteria signal transduction. Dephosphrelyation is noted only in tyrosine residue of Gram-negative bacteria. The phosphotyrosine profile of bacteria peptides is altered by the nanoparticle. It was found that the nanoparticles dephosphorylated the peptide substrate on tyrosine residues which lead to signal transduction inhibition and thus the stoppage of growth. It has been noted that silver nanoparticles establish synergistic activity with common antibiotics already in use today such as penicillin G, ampicillin, erythromycin, chindamycin and vanomycin against *E. coli* and *Staphylococcus aureus* (Shahverdi *et al.,* 2007). Silver nanoparticles can prevent bacteria from growing on or adhering to the surface. This can be useful in surgical setting where all surfaces in contact with the patient must be sterile. Interestingly, silver nanoparticles can be incorporated in materials used for coating many type of surfaces including metals, plastics and glass (Kee *et al.,* 2014). In medical equipments, it has been shown that silver nanoparticles lower the bacterial count on devices used compared to the old techniques. Silver nanoparticles are more commonly used in skin graft for burn victims as the silver nanoparticle embedded with the graft provide better antimicrobial activity and result in significantly less scarring of the victim. Now silver nanoparticles are used in bandages and patches to help heal certain burns and wounds.

Table 1 : Antimicrobial activity of silver nanoparticles synthesized from methanolic leaf extract of *Adansonia digitata*

	Diameter of zone of inhibition (mm)									
Conc. (µl)	***S. aureus***	***E. coli***	***B. subtilis***	***P. aeuriginosa***	***S. typhi***	***K. pneumonae***	***C. albicans***	***A. niger***	***R. stolomifer***	***P. notatum***
180	28	20	24	26	24	20	18	18	16	18
150	26	18	20	22	20	18	16	16	14	16
120	24	16	18	18	16	16	14	14	12	12
90	16	14	14	14	14	14	12	12	10	10
60	14	12	12	12	12	12	10	10	-	-
30	12	10	10	10	10	10	-	-	-	-
Gentamycin (10µg/ml)	38	38	40	40	40	40	-	-	-	-
Tiaconazole (70% w/v)	-	-	-	-	-	-	28	28	26	28
Methanol	-	-	-	-	-	-	-	-	-	-

- means no zone of inhibition

Table 2 : Minimum inhibitory concentration (MIC), Mimimum bactericidal concentration (MBC) and Minimum fungicidal concentration of the silver nanoparticles synthesised from methanolic leaf extract of *Adansonia digitata*

TEST ORGANISMS	MIC (µl)	MBC/MFC (µl)
Staphylococcus aureus	30	90
Escherichia coli	90	120
Bacillus subtilis	90	120
Pseudomonas aeruginosa	90	120
Salmonella typhi	90	120
Klbesiella pneumonae	90	150
Candida albicans	120	150
Aspergillus niger	90	120
Penicillium notatum	120	150
Rhizopus stolomifer	120	150

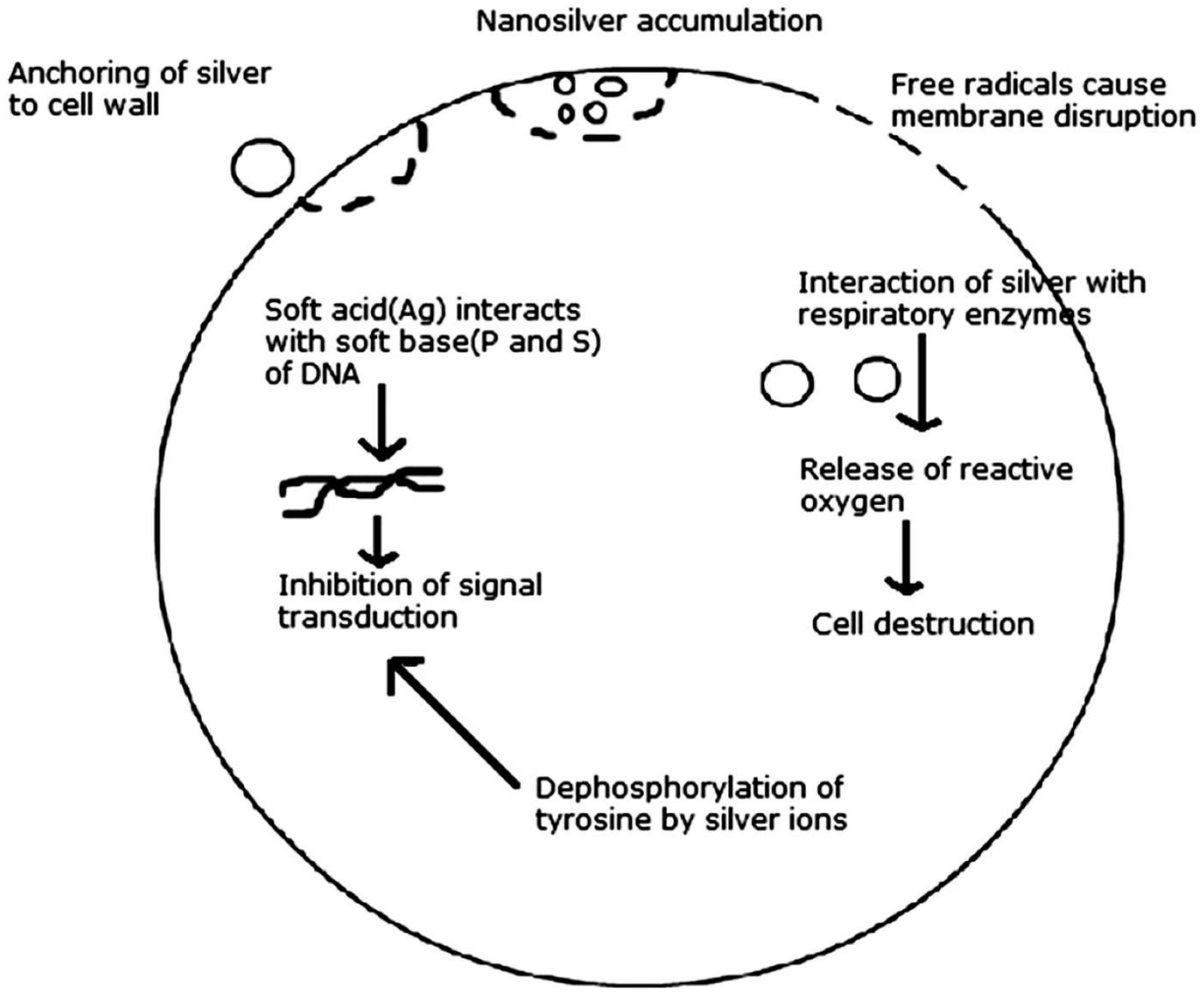


Fig 6 : How silver nanoparticles kill bacteria

CONCLUSION

This study reported that silver nanoparticles were green synthesized by simple efficient and ecofriendly method using the leaf extract of *Adansonia digitata*. The leaf extract contains reducing agents (mainly protein) that reduces silver ions and formed silver nanoparticles and this was confirmed by UV-VIS and FTIR studies. The biologically synthesized silver nanoparticles inhibited the growth of all the tested microorganism and hence could be of immense use in medical field as a potential antimicrobial agent.

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