



Susceptibility of *Escherichia coli* and Selected Microorganisms to Antibiotic and Silver Nanoparticles (AgNps)

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ABSTRACT

Therapeutic effect of antibiotics is threatened due to increasing resistance exhibited by pathogenic microorganisms, hence a need for other antimicrobial substitute. In this study, Silver nanoparticles (AgNps) was synthesized using aqueous solution of *Azadirachta indica* leaf extract and silver nitrate. The AgNps were characterized by UV-Vis (Ultra Violet Visible) and FTIR (Fourier Transform Infrared) spectrophotometer. The antibacterial susceptibility was investigated against *Escherichia coli* (from wastewater and faecally contaminated soil), *Staphylococcus aureus* 29918, *Pseudomonas aeruginosa* 27853 and *Proteus mirabilis* using AgNps in different concentrations of 100-400 mg/mL by agar diffusion method, DMSO (Dimethyl sulfoxide) without the AgNps was used as negative control. Zones of inhibition were measured using CLSI standard. Standard antibiotics used were compared with the AgNp, all the organisms were susceptible to the antibiotics except *P. mirabilis*. At 100-400 mg/mL AgNps was bacteriostatic to the isolates. AgNps synthesized was a very effective antibacterial agent compared with the standard antibiotics. Leaf extract of other medicinal plants should also be explored for AgNps and antibacterial effectiveness.

Keywords: Silver nanoparticles, *Escherichia coli*, antibacterial susceptibility, zone of inhibition.

INTRODUCTION

Infectious illnesses caused by microorganisms are widely spread, persistent and almost difficult to treat due to the rapid development in antibiotic resistance mechanisms in most microorganisms (Li and Thomas, 2018) which are often a step ahead of the conventional antibiotics. The continuous requirement for effective therapeutic agents at low cost have also been a motivating factor as many researchers have attempted to develop new antimicrobial agents that are cost-effective and potent in combating these resistances.

Silver have been known to possess excellent medicinal properties since the nineteenth century (Klasen, 2000) and silver ion and silver-based compounds have been widely utilized in several antimicrobial applications including dental work (Herrera *et al.*, 2000) antimicrobial properties (Mody *et al.*, 2010).

Nanoparticle is referred to a particle with size range of 1nm-100nm, at least in one of the three possible dimensions. Research on the synthesis of nanosized material has been of great interest because of their unique physical, chemical and biological properties which are usually better in qualities than the corresponding bulk material of the same element (Atul *et al.*, 2010). Nanoparticles can be categorized broadly as inorganic and organic NPs. Inorganic NPs incorporate semi-conductor NPs like (ZnO, ZnSCdS), metallic NPs (Au, Ag, Cu, Al) and magnetic NPs (Co, Fe, Ni) while organic NPs coating of medical devices and in managing wound (Atiyeh *et al.*, 2007). It has also been used in some part of the world over the years for food storage and in designing food utensils as a result of its distinctive incorporate carbon NPs (fullerenes, quantum dots, carbon nanotubes).

Silver nanoparticles are mostly smaller than 100 nm and consists of about 20-15,000 silver atoms. They exhibit outstanding physicochemical and biological activities in its nanoscale form, due to this, they have been essential for antimicrobial and anti-inflammatory activities (Vinod *et al.*, 2014). Oluwaniyi *et al.* (2015) added that silver nanoparticles have honorable qualities and are the most widely synthesized metal nanoparticles as a result of their non-toxicity to man. However, the use of silver ions (Ag^+) have been limited as they are easily inactivated by complexation and precipitation, as a result of these, Silver nanoparticles (AgNps) can be a valuable alternative to ionic silver as they have no charge, are of a small size and they have high surface area to volume ratio (Sintubin *et al.*, 2012), all of which improves their biocompatibility. Many studies have reported that AgNps have powerful antimicrobial activity, mediated by the inhibition of bacterial cell division and finally leading to cell death through destroying bacterial membranous structure (Lee *et al.*, 2014). The AgNps are also capable of precipitating bacterial cellular proteins, blocking the microbial respiratory chain system and could sustain the release of Ag^+ once inside the bacterial cells which may create free radicals and induce oxidative stress, hence, enhancing their bactericidal activity (Song *et al.*, 2006). It should be clear that AgNps by themselves have no antibacterial or antifungal properties, but it is the release of silver ions due to the destabilization of AgNps which confers such properties (Vance *et al.*, 2015).

The use of plant extracts to synthesize nanoparticles has been embraced in recent times due to the growing demand for an eco-friendly process, apart from these, Plant extracts may also act as both reducing and stabilizing agents in the synthesizing of nanoparticles (Ahmed *et al.*, 2016). It is also simple, cost effective, and can be easily

scaled up for large scale synthesis compared to the use of chemicals and microorganisms which impose high toxicity from chemicals and requires high maintenance of hygienic conditions respectively. In addition, microbial mediated nanoparticles are complex, time consuming and not yet commercially viable. As a result of these, enzymes and plant or plant extract have been suggested as possible eco- friendly alternatives to other physicochemical methods (Mishra *et al.*, 2014).

Plant mediated synthesis is purely a green synthesis route and are considered better candidates among the different biological entities as they use naturally occurring precursors such as vitamins, sugars, plant extracts, and biodegradable polymers as reductants and capping agents (Jitendra *et al.*, 2014). The biological approach is close to principles of 'Nature' and involves natural phenomenon that take place in biological systems. The accumulation of nanoparticles (NPs) varies with reduction potential of ions and the reducing capacity of plant depends on the presence of various polyphenols and other heterocyclic compound (Vedpriya, 2010). More so, plants are known to harbor a big range of metabolites that are most likely to be responsible in the green synthesis of metal nanoparticle. It has been hypothesized that several factors together determine the synthesis of NPs, such as plant source, organic compounds in the crude plant extract and even the pigments, alkaloids, polyphenols and proteins, etc., in the plant extract. Number of biomolecules present in the plant extract reduces the monovalent silver ion to uncharged atoms and these atoms aggregate to reach nano-size, other biomolecules of the plant extract then envelope or cap them to prevent their further aggregation (Ganaie *et al.*, 2014). The rich source of metabolites with negatively charged functional groups may be possibly responsible for reduction of metal ions and

efficient stabilization of synthesized NPs at natural conditions (Banerjee and Narendhirakannan, 2011).

Azadirachta indica (neem) belongs to the Meliaceae family, it is a very active medicinal plant which contains rich phytochemicals such as the flavanones and terpenoids which act as good stabilizers and reducing agents for silver nanoparticles. Neem tree is found in abundance in tropical and semitropical regions like Nigeria, India, Bangladesh, Pakistan and Nepal. It is a fast-growing tree with 20-30 m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are compound imparipinnate with

MATERIAL AND METHODS

Test Isolates

Escherichia coli was isolated from wastewater and faecally contaminated soil. Three test isolates which are *Staphylococcus aureus* 29918, *Pseudomonas aeruginosa* 27853 and *Proteus mirabilis* were obtained from the culture collection unit of the Department of Microbiology, University of Ilorin. They were maintained on appropriate agar slants and kept in the fridge, and also sub-cultured periodically to maintain their purity.

Determination of phytochemicals (Qualitative) in the neem leaf extract

Testing for Alkaloids

One cm³ of HCl was added to 3 cm³ of the extracts in a test tube. The mixture was heated for 20 minutes, cooled and filtered. Two drops of Wagner's reagent were added to 1 cm³ of the extracts. A reddish brown precipitate indicates the presence of alkaloids (Victor and Chidi, 2009).

Testing for Phenolics

Two drops of 5 % FeCl₃ was added to 1 cm³ of the extracts in a test tube. A greenish

each comprising 5-15 leaflets. Its fruits are green drupes which turn golden yellow on ripening in the month of June-August (Brahmachari, 2004).

The present work aims to evaluate the antibacterial activity of Silver nanoparticles (AgNps) synthesized using leaf extract of *Azadirachta indica* (neem) on *Escherichia coli*, isolated from wastewater and faecally contaminated soil and *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 29918, and *Proteus mirabilis* with their corresponding antibiotic susceptibility profiles.

precipitate indicates the presence of phenolics (Victor and Chidi, 2009).

Testing for Glycosides Ten cm³ of 50 % H₂SO₄ was added to 1 cm³ of the extracts, the mixture was heated in boiling water for 15 minutes. 10 cm³ of Fehling's solution was added and the mixture was boiled. A brick red precipitate indicates the presence of glycosides (Victor and Chidi, 2009).

Testing for Saponins

Frothing test: two cm³ of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins (Victor and Chidi, 2009).

Testing for Flavonoids

One cm³ of 10 % NaOH was added to 3 cm³ of the extracts. A yellow colouration indicates the presence of flavonoids (Victor and Chidi, 2009).

Preparation of leaf extracts from *Azadirachta indica* leaves

Collection of plant

Azadirachta indica leaves were collected from the tree early in the morning, located 8.4813985, and 4.6762772 at the frontage of Chemistry Department, University of Ilorin, Ilorin, Nigeria.

Identification and Authentication: it was carried out at the Department of Plant Biology Herbarium unit, University of Ilorin

with specimen Voucher number:
UILH/001/1280.



Plate 1: *Azadirachta indica* leaves x 30
Source: EzGro Garden, 2017.

Preparation of extract: Fresh leaves of *Azadirachta indica* were thoroughly washed with deionized water, and then cut into small pieces. Approximately 20 g of leaves were then boiled with 200 ml of deionized water for 10 minutes. The aqueous solution was filtered with Whatman No. 1 filter paper (Elemike *et al.*, 2014). This solution was used for green synthesis of Silver nanoparticles (AgNps) or reducing the silver ions.

Synthesis of silver nanoparticles: Silver nitrate was used to prepare 100 ml of 1 mM solution of silver nitrate, which was prepared with a deionized water. In a typical reaction procedure, 5 ml of *Azadirachta indica* aqueous leaf extract was added to 20 ml of 1 mM silver nitrate solution. The solution was heated and stirred at 80°C, the resulting colour change of the resulting mixture signified the formation of silver nanoparticle. The nanoparticles precipitated out of the reaction medium after 24 hours of incubation in a dark cupboard. This was filtered with the aid of a vacuum pump and the residue was

scrapped and kept for further analysis (Oluwaniyi *et al.*, 2015).

Characterization of silver Nanoparticles

Ultra Violet Visible Spectroscopy (UV-vis): The optical property of AgNps was determined by UV-Vis spectrophotometer (Beckman). Colloidal solution of AgNps was introduced into the curvet of the spectrophotometer and the spectra was taken between the wavelength range of 390-450 nm, and the resulting spectra was observed and recorded (Oluwaniyi *et al.*, 2015).

Fourier Transform Infrared Spectrometer (FTIR) analysis: The functional groups responsible for the synthesis of silver nanoparticles were identified by (Nicolet iS5) FTIR spectrophotometer using KBr pellet. The infrared Spectrum of the nanoparticles were recorded on the FTIR spectrometer (Oluwaniyi *et al.*, 2015).

Antimicrobial Sensitivity test with Silver nanoparticles

This was carried out using the agar well diffusion technique as described by Thombre *et al.* (2012) with slight modifications. The bacteria isolates were grown on nutrient agar for 18 hours before use and each isolate was inoculated into peptone water which was standardized using 0.5 of McFarland standard and allowed to stand for 15 minutes. Mueller Hilton agar plates were prepared and the isolates was inoculated from the peptone water to the Mueller Hilton agar plates using a sterile swab stick, and was allowed to stand for 10 minutes.

With the aid of sterile cork borer wells were made on the surface of the solidified agar, and the agar plugs were carefully removed using sterile loops. One mL of the various concentrations: 100-400 mg of silver nanoparticles dissolves in DMSO was introduced into the wells. Control experiment was carried out using DMSO (Dimethyl sulfoxide) only without silver nanoparticles. The plates were allowed to stand for 30 minutes to allow for proper diffusion of the silver nanoparticles into the medium before they were incubated at 37°C for 24 hours.

After incubation, the plates were observed for zones of inhibition and measured using meter rule at right angles from the edge of the well to the end of the zone of inhibition. The assay was done in triplicates and the zones expressed as mean. The presence of these zones of clearance around the well was taken as evidence of the antibacterial activity of the extract.

Antibiotics susceptibility test

Antibiotics susceptibility test was carried out on the test organisms using the Kirby-Bauer disk diffusion method, and results obtained were used to classify isolates using the standard reference value of the Clinical and Laboratory Standard Institute ((CLSI, 2016).

Determination of minimum inhibitory concentration

Macro broth dilution method was used, the least concentration that showed zone of inhibition was further tested to determine the Minimum Inhibitory Concentration (MIC). The stock of 100 mg/mL of the extract was re-suspended in DMSO to produce two-fold dilution, this was done to reduce the concentration of the solution by half by adding 1 ml of the stock to 1 ml of DMSO. This makes the concentration 50 mg/ml; and this was also re-suspended in DMSO to give 25 mg/ml and this was done up to 1.56 mg/ml. each dilution (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.126 mg/ml, and 1.56 mg/ml) were dispensed inside a sterilized test tube and labelled appropriately. About 4.5 ml of the nutrient broth was poured inside a test tube with the aid of syringe and sterilized in an autoclave, the sterilized nutrient broth inside the seven test tube was allowed to cooled, and it was seeded with 0.5 ml of each dilution and 0.1 ml of the standardized test organism was inoculated into each test tube. The control used was 4.5 ml of nutrient broth + 0.5 ml of diluted silver nanoparticles and 4.9 ml of nutrient broth + 0.1 ml of each standardized organism. These were then incubated for 24 hours at 37°C. After incubation, the growth of the bacterial isolates in the test tubes was observed as turbidity using visibility and comparing them with that of the two controls. The lowest concentration of Silver nanoparticles that shows no growth of the microorganisms is the MIC (NCCLS, 2000).

Minimum Bactericidal/Lethal Concentration

The test tubes from the Minimum Inhibitory Concentration (MIC) assay that showed no growth were used. One ml of each test tube containing different organisms at different concentrations were streaked onto solidified sterile nutrient agar plates and then incubated

at 37°C for 24 hrs, after which the plates were observed for growth. The lowest concentration that shows no growth on the

plates was the minimum bactericidal/ lethal concentration (NCCLS, 2000).

Results

Table 1: Qualitative Phytochemical Screening of *Azadirachta indica* leaf extract

PHYTOCHEMICALS	RESULT
Saponin	+
Phenolics	+
Flavonoids	+
Coumarins	+
Glycosides	+
Alkaloids	+

Key: + Present

Ultra Violet- Visible Spectroscopy

The extent of absorbance of light through the Silver nanoparticles was determined and the

peak value was obtained at a wavelength of 420 nm. The spectrum of the absorbance is as shown in Figure 1.

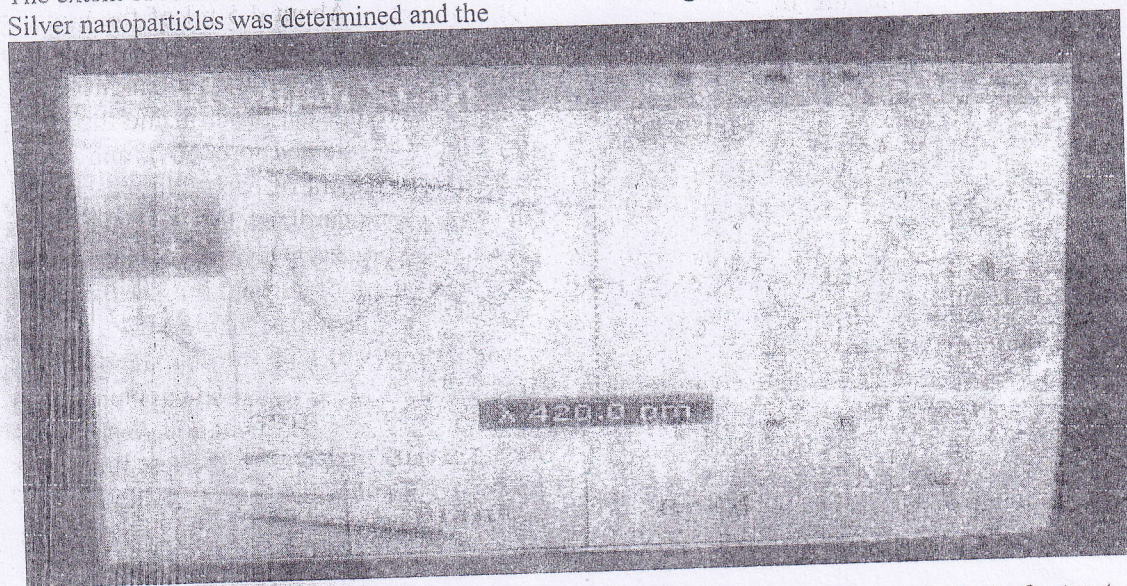


Figure 1: UV-Vis of silver nanoparticles of AgNps synthesis from *Azadirachta indica* leaf extract

Fourier Transmission Infrared Ray (FTIR)

The result of the FTIR revealed the presence of four obvious peak at 3600 cm^{-1} , 2650 cm^{-1} , 1700 cm^{-1} and 1400 cm^{-1} as shown in Figure 2.

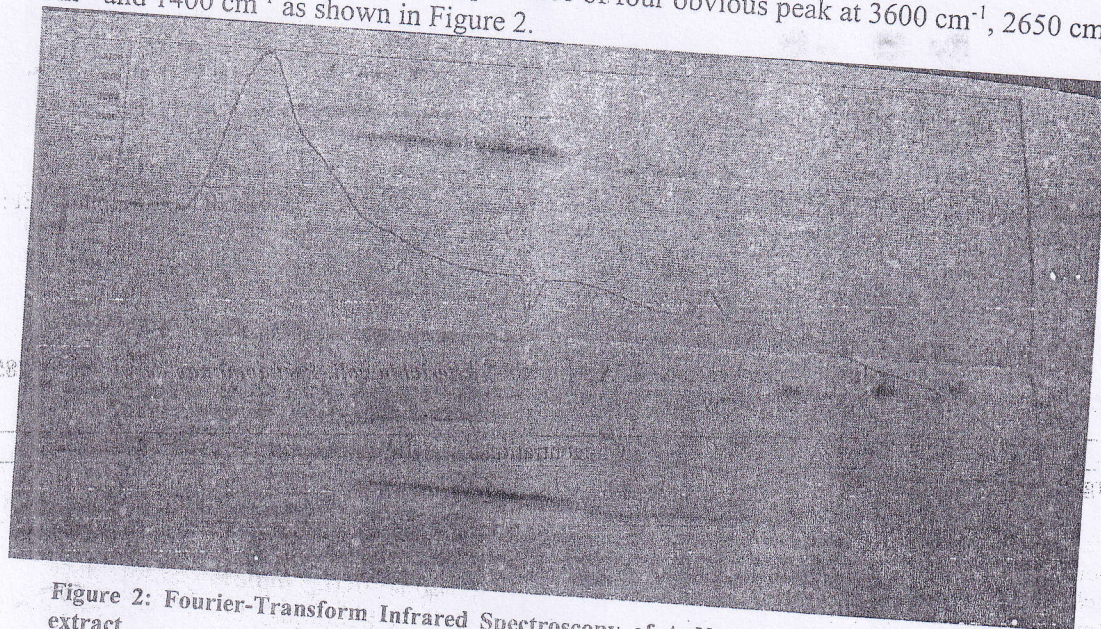


Figure 2: Fourier-Transform Infrared Spectroscopy of AgNps synthesis from *Azadirachta indica* leaf extract.

Table 1: Antibacterial activity of silver nanoparticles (100-400mg/ml) on the Gram negative isolates in comparison with standard antibiotics

Isolates	AgNps (100mg/ml) (mm)	AgNps (200mg/ml) (mm)	AgNps (300mg/ml) (mm)	AgNps (400mg/ml) (mm)	Ofl 5µg (mm)	Amo 30µg	Nit 300µg (mm)	Amp 10µg	Cef 30µg	Gen 10µg (mm)	Crx 30µg	Cip 5µg (mm)
<i>E. coli</i> (Soil)	10.33	10.67	13.67	16	24.66	R	19	R	R	14.6	R	29
<i>E. coli</i> (water)	10.67	11.33	11.67	12.33	25	R	22.6	R	R	18	R	26.33
<i>P. aeruginosa</i> 27853	12.33	15	R	R	14.6	R	R	R	R	R	R	R
<i>Proteus mirabilis</i>	R	R	10.67	11.33	R	R	R	R	R	R	R	R

Key: OFL- Ofloxacin; AMO- Amoxicillin/Clavulanate; NIT- Nitrofurantoin; AMP- Ampicillin CEF- Cefazidine;
CRX- Cefuroxime; GEN- Gentamicin; CIP- Ciprofloxacin. R: Resistance

Table 2: Antibacterial activity of silver nanoparticles (100-400mg/ml) on the Gram positive isolates (*Staphylococcus aureus* 29918) in comparison with standard antibiotics

Isolate	AgNPs (100mg/ml) (mm)	AgNPs (200mg/ml) (mm)	AgNPs (300mg/ml) (mm)	AgNPs (400mg/ml) (mm)	Pef 10µg (mm)	Amp 30µg	Zin 20µg	Amo 30µg	Roc 25µg (mm)	Str 30µg (mm)	Sep 30µg	Ery 10µg
<i>S. aureus</i> 29918	12.33	12.67	13.33	15.33	22.6	R	R	R	20.33	19	R	R

Key: R: Resistance; PEF: Pefloxacin; AMP: Ampiclox; Zin: Zinnacef; AMO: Amoxacillin; ROC: Rocephin; SEP: Seprtin; STR: Streptomycin; ERY: Erythromycin.

Table 3: Minimum inhibitory concentrations of AgNps on *Escherichia coli*, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 29918 and *Proteus mirabilis*

Organisms	Concentration (mg/ml)							MIC (mg/ml)
	100	50	25	12.5	6.25	3.12	1.56	
<i>Escherichia coli</i> (Soil)	-	-	-	+	+	+	+	25
<i>Escherichia coli</i> (water)	-	-	+	+	+	+	+	50
<i>Staphylococcus aureus</i> 29918	-	-	-	+	+	+	+	25
<i>Pseudomonas aeruginosa</i> 27853	-	-	-	+	+	+	+	25
<i>Proteus mirabilis</i>	-	-	+	+	+	+	+	50

Key: - No visible growth; + Visible growth

Table 4: The Minimum Bactericidal Concentration (MBC) of silver nanoparticles on *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus* 29918 and *Pseudomonas aeruginosa* 27853

Concentration (mg/ml)	Organisms				
	<i>Escherichia coli</i> (Soil)	<i>Escherichia coli</i> (water)	<i>Staphylococcus aureus</i> 29918	<i>Pseudomonas aeruginosa</i> 27853	<i>Proteus mirabilis</i>
100	++	++	++	++	++
50	++	++	++	++	++
25	++	++	++	++	++

Key: ++ Growth

DISCUSSION

Color changes of the solution of *Azadirachta indica* leaf extract from yellow to brown were observed when the silver nitrate was added to the aqueous leaf extract. The bioreduced (Ag^0) gave reddish brown coloured solution

which turned to deep brown after incubating in the dark cupboard for 24 hours, same color changes were observed by Elemike *et al.* (2014) using different plant extract. This indicates the green route to the reduction of silver ions and stabilization of silver

nanoparticles produced. The reduction of silver ions to silver atom in the solution was observed by UV-Visible Spectrophotometer. The peak intensity of the surface plasmon absorption for the *Azadirachta indica* synthesis silver nanoparticles occurred in the optical region of the UV-Visible spectrum at 420 nm. The 420 nm signify the formation of silver nanoparticles. This result obtained is in consonance with Oluwaniyi *et al.* (2015), which has a wavelength of 460 nm. As shown in Fig. 2, the FTIR was spectra (from 4000-500 cm^{-1}) taken to detect the major functional groups in plant extract and their possible role in reduction process during the synthesis of AgNPs and stabilization of Silver nanoparticles, the band 3600 cm^{-1} corresponds to O-H (Hydroxyl groups) stretching H-bonded alcohols and phenols. The peak found around 2650 showed a stretch for C-H bond (saturated aliphatic), peak around 1700 cm^{-1} showed the bond stretch for N-H and 1400 cm^{-1} show glycosides or other (C-O-C) groups (Kumar *et al.* (2017) whereas the stretch for Ag-NPs were found around 500-650 cm^{-1} . Therefore, the synthesized nanoparticles were surrounded by proteins and metabolites such as phenolics, alkaloids, saponin, flavonoids, coumarins, and glycosides having the functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form the metal nanoparticles (i.e. capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Antimicrobial assay indicated that the AgNPs synthesized from the extract leaf of *A. indica* showed antibacterial activity against all test isolates, *E. coli* isolated from wastewater and

faecally contaminated soil displayed zone of inhibition of 10.67 mm-12.33 mm and 10.33 mm-16 mm respectively between the concentrations of 100-400 mg/ml. *P. aeruginosa* displayed the highest susceptibility to the silver nanoparticles with the zone of inhibition ranging from 12.33 mm-17.33 mm between the concentrations of 100-400 mg/ml. *S. aureus* had zone of inhibition between 12.33 mm-15.33 mm between the concentrations of 100-400 mg/ml. *P. mirabilis* had no zone of inhibition at 100 and 200 mg/ml concentrations but had zones of inhibition ranging from 10.67 mm-11.33 mm at 300 and 400 mg/mL concentrations respectively, all of which did not conform with findings of Ahmed *et al.* (2016) and Oluwaniyi *et al.* (2015). However, this could be as a result of variation in strain of the test organisms, site of collection, environmental condition during the assay and the activity of the silver nanoparticles produced. Antibiotics susceptibility showed that all the gram negative isolates (*P. aeruginosa*, *P. mirabilis*, and *E. coli* from wastewater and faecally contaminated soil showed complete resistance to Amoxicillin, Ampicillin, Cefazidime and Cefuroxime while both *E. coli* isolates from wastewater and faecally contaminated soil were highly susceptible to Ciprofloxacin (29 mm and 26.33 mm respectively), *P. aeruginosa* was susceptible to Nitrofurantoin (14.6 mm) while *P. mirabilis* showed no susceptibility to the tested antibiotics. Gram positive isolate (*S. aureus*) was completely resistant to Ampiclox, Zinnacef, Amoxicillin, Septrin and Erythromycin, but highly susceptible to Pefloxacin and Rocephin (22.6 mm and 20.33 mm respectively) which disagrees with the reports of Bhatt *et al.* (2014) in which susceptibility to Erythromycin was observed. The Minimum Inhibitory Concentration for *Escherichia coli* isolated from wastewater and faecally contaminated soil was at 50

mg/ml and 25 mg/ml respectively, and *P. aeruginosa*, *S. aureus* and *P. mirabilis* was at 25 mg/ml, 25 mg/ml and 50 mg/ml respectively. The Silver nanoparticle at the entire concentration (100 mg/ml, 50 mg/ml and 25 mg/ml) did not show bactericidal/lethal effects on all test isolates. Therefore, the result shows that at the above mention concentration the Silver nanoparticles has no bactericidal effect but rather, bacteriostatic effect on all the test isolates.

Both *E. coli* isolated from wastewater and faecally-contaminated soil showed susceptibility to the silver nanoparticles, but *E. coli* isolated from faecally-contaminated soil showed more susceptibility to the silver nanoparticles and the standard antibiotics, than that from wastewater. *P. aeruginosa* showed wide array of susceptibility than other which may be due to the Gram negative nature of the cells and for this reason, silver ions which are positively charge therefore easily react with the cell wall (Gogoi *et al.*, 2006). *S. aureus* were also very susceptible, but not as susceptible as *P. aeruginosa*. *P. mirabilis* was the least susceptible of all the test isolates to the Silver nanoparticles.

Comparing the susceptibility pattern of the silver nanoparticles to the standard antibiotics used, *P. aeruginosa* and *P. mirabilis* were more susceptible to the silver nanoparticles than the standard antibiotics. However, *E. coli* from both wastewater and faecally contaminated soil was more susceptible to the antibiotics than silver nanoparticles as well as *S. aureus*, these could be due to the concentration employed, as increased activity on these organisms could be achieved at higher concentrations of the AgNps. Other reasons could be the strain of the organisms, where they were isolated and the environmental conditions.

It is therefore pertinent to note that high antibacterial effect of the Silver nanoparticles

was observed compared to standard antibiotics. Also, the phytochemical screening of the Silver nanoparticles of the *A. indica* leaf extract used in this work revealed the presence of 5 secondary metabolites, which help in the stabilization and reduction of Silver ion to Silver nanoparticles. It is the presence of these phytochemicals that determine the effectiveness of the Silver nanoparticles.

CONCLUSION

This study therefore shows that Silver nanoparticles synthesized from leaf extract of *Azadirachata indica* poses good antibacterial activity and can be used in combating and managing infections emanating from these microorganisms. This will help overcome the global menace of multi-drug resistance. However, *E. coli* and *S. aureus* were more susceptible to the standard antibiotics than the Silver nanoparticles synthesized.

RECOMMENDATIONS

Findings here suggests the use of Silver nanoparticles synthesized with *A. indica* leaf extract for eliminating *P. aeruginosa* and *P. mirabilis* which are frequently associated in malignant infections as they were more susceptible to the synthesized silver nanoparticles. Also, large scale production of silver nanoparticles should be employed, and further studies should focus on synthesizing silver nanoparticles with other desirable plant extracts capable optimizing the antimicrobial properties of AgNps.

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