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Antibacterial Action of Silver Nanoparticles of Extract of Leaf of *Thevetia nerifolia*

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ABSTRACT

Silver nanoparticles (AgNPs) were synthesized using a combination of aqueous extract of *Thevetia nerifolia* and 1mM of silver nitrate ($AgNO_3$) solution to obtain concentrations of 100mg/ml–400mg/ml. Characterization of the particles was done by UV–Vis spectroscopy Fourier transform infrared (FTIR). Antibacterial activity was investigated against *Staphylococcus aureus, Escherichia coli and Psedomonas aeruginosa* using standard agar well diffusion method. Gentamicin and tetracycline were used as reference antibiotics. The Minimum Inhibitory Concentration (MIC) was achieved by microbroth dilution technique. Minimum Bactericidal Concentration (MBC) was done by plate assay. The mode of action of the particles was evaluated by direct exposure of the cells of selected isolates to the nanoparticles. The presence of phytochemical constituents was examined using standard methods. The total yield of AgNPs of the plant extract was 0.6g. Characterization by UV-visible spectrometry revealed peak absorbance of 0.465 at 452.0nm, while FTIR showed the presence of two (2) functional groups. According to the antibacterial sensitivity assay, the four concentrations exhibited considerable effects against all test isolates. At 400mg/ml, the highest inhibitory activities were observed with

S. aureus and *E. coli* with zones of inhibition measuring 22mm and 20mm respectively. The activity of synthesized particle compared favourably with reference antibiotics. The MIC was obtained at 40mg/ml while MBC was at a higher concentrations. The mode of action showed disruption of cellular components as a result of increased permeability of cell membrane. Following the results of the phytochemical analysis, a total of five (5) bioactive constituents were obtained. It is evident from this study that AgNPs synthesized could be a good candidate in the treatment of conditions caused by the test isolates.

Keywords : *Thevetia nerifolia,* Nanoparticles, Phytochemicals, Agar well diffusion, FTIR

INTRODUCTION

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles ranging from 1 to 100 nm for applications ranging from catalysts and sensing to optics, antibacterial activity as well as data storage (Choi *et al.,* 2007; Hutter and Fendler, 2004;Sudrik *et al.,* 2006; Sun *et al.,* 2000; Vilchis-Nestor *et al.,* 2008;Yoosaf *et al.,* 2007). These particles can be compared to biological molecules which find their way to required sites within the body (Ackerman *et al.,* 2002). Catalytic activities of nanoparticles differ from the chemical properties of bulk plant materials (Kholoud *et al.,* 2010).

Colloidal silver is of particular interest because of it's distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities (Frattini *et al.,* 2005). Such nanoparticles can be synthesized using physical, chemical and biological reduction methods (Sun *et al.,* 2002; Taleb *et al.,* 1997; Esumi *et al.,* 1990; Henglein *et al.,* 2001; Zhu *et al.,* 2000; Pastoriza-Santos *et al.,* 2002; Naik *et al.,* 2002).

The development of the biological synthesis of nanoparticles using microorganisms or

plant extracts plays an important role in the field of nanotechnology, as it is environmentally friendly with minimal toxicity (Yuet *et al.,* 2012). This green synthesis method has emerged as simple and alternative means to chemical synthesis, as it is cost effective and easily scaled up for large scale synthesis (El-Shishtawy *et al.,* 2011).

Thevetia nerifolia is a small ornamental tree, which grows to about 1.5 - 2.3m in height. It is a native plant of central and southern Mexico and Central America. It is a relative of *Nerium oleander*, giving it a common name Yellow Oleander, and also lucky nut in the West Indies. It has been discovered to contain bioactive compounds, such as alkaloids, anthroquinones, flavonoids, phenolic compounds, saponins, steroids and tannins (Buvaneswari *et al.,* 2011).

It is reported to possess curative effects against skin infections, in addition to its healing potential against conditions such as edema, insomnia, hemorrhoids, malaria, snake bites, etc (Nellis, 1997). All parts of the plant, especially the seeds are useful in treating scorpion stings, snake bites, leprosy, ringworm and other skin diseases (Udayan *et al.,* 2009). Also, its antimicrobial activities against several pathogenic microorganisms have been well documented.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves of *Thevetia nerifolia* were obtained at the early hours of the morning from the University of Ilorin, Nigeria in the month of February, 2015. Identification and authentication was carried out at the Department of Plant Biology Herbarium unit, University of Ilorin with specimen voucher number: UIH 001/018.

Collection and maintenance of microorganisms

Three (3) test isolates namely *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* were obtained from the culture collection unit of the Department of Microbiology, University of Ilorin. They were maintained on appropriate agar slants and sub cultured periodically for the purpose of purity.

Plant preparation and extraction

The method of John *et al.* (2012) was adopted with slight modifications. The plant leaves were thoroughly rinsed with sterile distilled water, dried with water absorbent paper and cut into small pieces. Ten grams (10g) of cut leaves were dispensed into 100ml of sterile distilled water and boiled for an hour at 80ºC. The resulting mixture was filtered and collected in a conical flask by standard filtration method.

Preparation of Silver nanoparticles from *Thevetia nerifolia* leaves extract

Silver nanoparticles were synthesized by addition of the filtrate of leaves to 1mM silver nitrate solution in the ratio 1:10. The reaction mixture was stirred using a magnetic stirrer for 4hours. Color changes were observed in the reacting solution. A dark brown solution was aged for 24 - 48hours at room temperature for full formation of silver nanoparticles crystals. After 48hours, the solution was decanted and filtered with a membrane filter using a vacuum pump (Richardson *et al.*, 2006).

Characterization of silver nanoparticles of extract of leaves of *Thevetia nerifolia*

Ultra violet visible spectroscopy (UV-vis):

The optical property of AgNPs was determined by UV-Vis spectrophotometer (Beckam couler DU®730). Distilled water was added to the AgNPs of extracts of leaves of *T. nerifolia* to get a solution after which the solution was introduced into the

cuvet of the spectrophotometer and spectra was taken between the wavelengths of 200nm – 800nm. The resulting spectra were observed and recorded (Tamasa, 2013).

Fourier Transform Infrared Spectroscopy (FTIR) analysis:

The chemical composition of the particles was studied by FTIR spectrophotometer **(**Perkin-Elmer LS-55- Luminescence spectrophotometer**)** (Pradhu *et al.,* 2010).

Antibacterial sensitivity assay (Agar diffusion technique)

The antibacterial screening of the silver nanoparticles of extract of leaves of *T. nerifolia* was done using agar well diffusion method against test isolates as described by Thombre *et al.* (2012) with slight modifications. Sterile nutrient agar was poured into sterile Petri dishes and allowed to solidify. Inoculum of 24hours old of each test isolates was separately swabbed on the surface of the solidified agar plates and allowed to diffuse. Thereafter, a sterile cork borer was used to bore hole, 1ml of each concentration of the silver particles was separately introduced into the well and incubated at 37^{o}C for 24hours. The plates were observed for the presence of clear zones around the agar wells. Diameter of zones of inhibition were measured to the nearest millimeter (mm) and recorded.

Determination of antibacterial susceptibility of standard antibiotics

Gentamicin and Tetracycline (standard antibiotics) were used as reference antibiotics to compare with the particles. This was carried out using Kirby-Bauer (1966) method, with slight modification. Sterile nutrient agar was introduced into sterile Petri dishes and allowed to solidify. Inoculum of 24hours old of each test isolate was separately swabbed on the surface of the agar plates and left for few minutes. Thereafter, the standard antibiotic discs were placed at the centre of the inoculated nutrient agar plates and incubated at 37^{o}C

for 24hours. The plates were observed for the presence of clear zones around antibiotic discs. Diameter of zones of inhibition were measured to the nearest millimeter (mm) and recorded.

Determination of minimum inhibitory concentration

Broth dilution method was employed in the determination of minimum inhibitory concentration. Broth (9.5ml) was dispensed into nine (9) test tubes containing 0.3ml of various concentrations (100, 90, 80, 70, 60, 55, 50, 45, 30 mg/ml) of the particles. To the test tubes, 0.2 ml of each of the test isolates was separately added. The turbidity of the contents of tubes was taken with the aid of a spectrophotometer after which they were incubated at 37^{o}C and turbidity reading recorded again after 18 – 24 hours (NCCLS, 2000).

Determination of minimum bacterial concentration

The test tubes from the minimum inhibitory concentration (MIC) assay that showed no growth were streaked onto freshly prepared sterile nutrient agar plates and incubated at 37^{o}C for 24hrs after which they were observed for growth. The lowest concentration that showed no growth on the recovery plate was regarded as minimum bactericidal concentration or minimum lethal concentration (NCCLS, 2000).

Determination of the mode of action of AgNPs of *Thevetia nerifolia* against test isolates

Formalin (0.2 ml) was dispensed into four (4) test tubes, to each of the tubes, 1ml of the synthesized AgNPs and 1ml of test isolate were added and left for 5 min. This was done separately for each test isolates. The content was centrifuged at 12,168x10^{3} g (MSE Minor 35 Centrifuge) for 15 min. The

cells in tubes were resuspended in 0.1 ml demineralised water. Smears of this were made on glass slides, dried and stained with dilute carbol fuschin for 30 sec, it was rinsed in water, air-dried and examined under the microscope. Photomicrographs were taken at a magnification of X400 (Alli *et al*., 2011). A control experiment was carried out on test isolates without the addition of extracts and formalin. This was done by carrying out Gram stain reaction on the test isolates (Fawole and Oso,2007).

Phytochemical analysis of silver nanoparticles of leaf extract of *Thevetia nerifolia*

Phytochemical screening was carried out on methanolic extract of the test plant as well as the silver nanoparticles to compare between the plant secondary metabolites present in both. This was done following standard tests described by Sofowora (1993); Edeoga *et al.* (2005) and Sahuvinod *et al.* (2010).

RESULTS

Recovery of silver nanoparticles from chemical reduction of silver nitrate

A total yield of 0.6g was recovered as crystals from the synthesis. The color of the crystal gave a black dry appearance.

Evaluation of the physical and chemical characteristics of silver nanoparticles of *T. nerifolia*

The extent of absorbance of light through the particles was determined and a peak value was obtained to be 0.465 at a wavelength of 452.0nm. The spectrum of the absorbance is as shown in Figure 1. The result of the FTIR revealed the presence of two obvious peaks at 3439.08cm^{-1} and 1639.49cm^{-1} as shown in Figure 2.

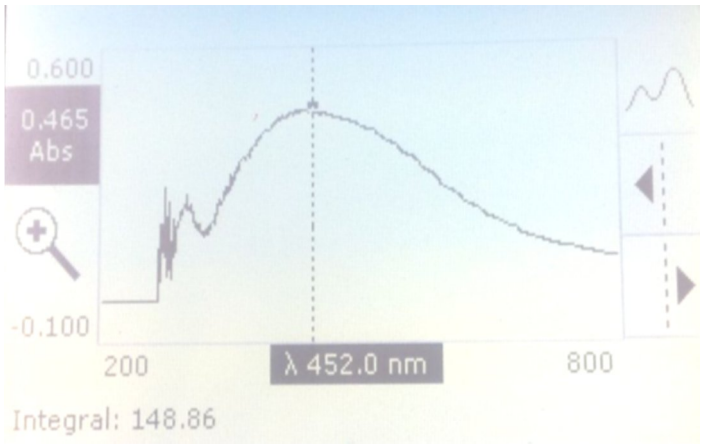

Figure 1: UV-Vis of silver nanoparticles of *T. nerifolia* leaf extract

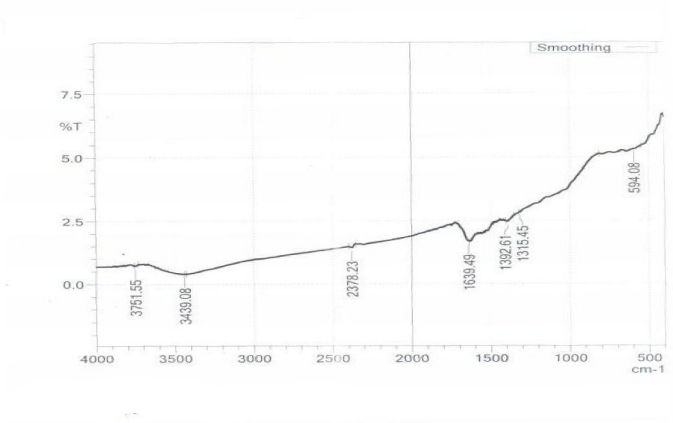

Figure 2: Fourier-Transform Infrared Spectroscopy of $AgNO_3$ synthesized from *Thevetia nerifolia* leaf extract

Effect of different concentrations of silver nanoparticles of *Thevetia nerifolia* on test isolates

It was observed that three of the test isolates showed varying susceptibility to the silver nanoparticles, with increase in the diameter of zone of inhibition as concentration increased. The largest zone of inhibition was recorded when the particles was assayed against *S. aureus* (22mm) at 400mg/ml, while the lowest diameter of zone of inhibition was observed with *P. aeruginosa* (8mm) at the lowest concentration of 100mg/ml. This result is shown in Table 1.

Effects of Standard antibiotic on test isolates

It was observed that all test isolates showed varying susceptibility to the antibiotics. The largest zone of inhibition was recorded when gentamicin was assayed against *E. coli* (21mm). This result is shown in Table 2.

Evaluation of Minimum Inhibitory Concentration (MIC)

The minuimum inhibitory concentration

against test isolates was obtained at 40mg/ml as shown in Table 3.

Evaluation of Minimum Bactericidal Concentration (MBC)

The particles had a minimum bactericidal effect at a concentration of 100mg/ml for all tested isolates, implying a higher dose compared with the MIC.

Effect of particles on cell wall of isolates

Mode of action of the silver nanoparticles against the test isolates as revealed by photomicrograph, altered the normal cellular architecture of test isolates as observed in Plates 1-6.

Table 1: Antibacterial activity of AgNPs of *T. nerifolia* leaf extract

	Concentrations			
Test isolates	**100mg/ml**	**200mg/ml**	**300mg/ml**	**400mg/ml**
S. aureus	12.00 ± 1.15^{b}	15.00 ± 1.15^{a}	18.00 ± 1.15^{a}	22.00 ± 1.15^{a}
E. coli	11.00 ± 1.15^{b}	13.50 ± 1.15^{ab}	16.00 ± 1.15^{ab}	20.00 ± 1.15^{b}
P. aeruginosa	8.00 ± 1.115^{b}	10.50 ± 1.15^{b}	13.00 ± 1.15^{b}	15.00 ± 1.15^{b}
Total mean	10.33 ± 0.83	13.00 ± 0.87	15.66 ± 0.92	19.00 ± 1.19
$P \leq 0.005$	0.11	0.08	0.5	0.1

Values in the same group and column carrying the same letter/s are not significantly different at ($P \leq 0.005$).

Table 2: Antibacterial activity of standard antibiotics

Test isolates	Diameter of zone of inhibition(mm)		
	400mg/ml concentration of AgNPs of *T. nerifolia*	Gentamicin	Tetracycline
S. aureus	22	19	20
E. coli	20	18	17
P. aeruginosa	15	14	13

Table 3: Determination of Minimum Inhibitory Concentration (MIC)

CONCENTRATION (mg/ml)	*S. aureus*		*E. coli*		*P. aeruginosa*		MIC
	(0-hrs)	(24-hrs)	(0-hrs)	(24-hrs)	(0-hrs)	(24-hrs)	
100	1.906	1.790	1.434	0.992	1.596	1.324	+
90	1.422	1.103	1.203	0.805	1.452	1.195	+
80	1.757	1.485	0.833	0.548	1.183	0.992	+
70	0.592	0.437	0.651	0.400	0.902	0.743	+
60	0.481	0.394	0.609	0.376	0.820	0.611	+
50	0.491	0.314	0.558	0.349	0.778	0.570	+
40	0.478	0.227	0.531	0.313	0.516	0.364	+
CONTROL	0.423	0.897	0.492	0.955	0.476	0.872	++

KEY: + = Inhibition present

++ = Inhibition absent

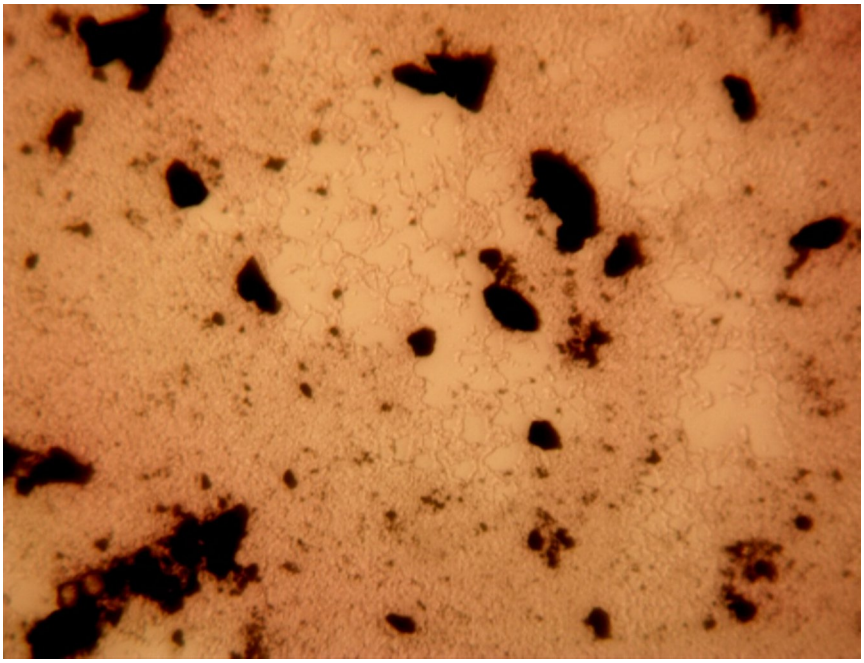

PLATE 1: Photomicrograph of cells of *Staphylococcus aureus* treated with silver nanoparticles of *T. nerifolia* leaf extract (X400)

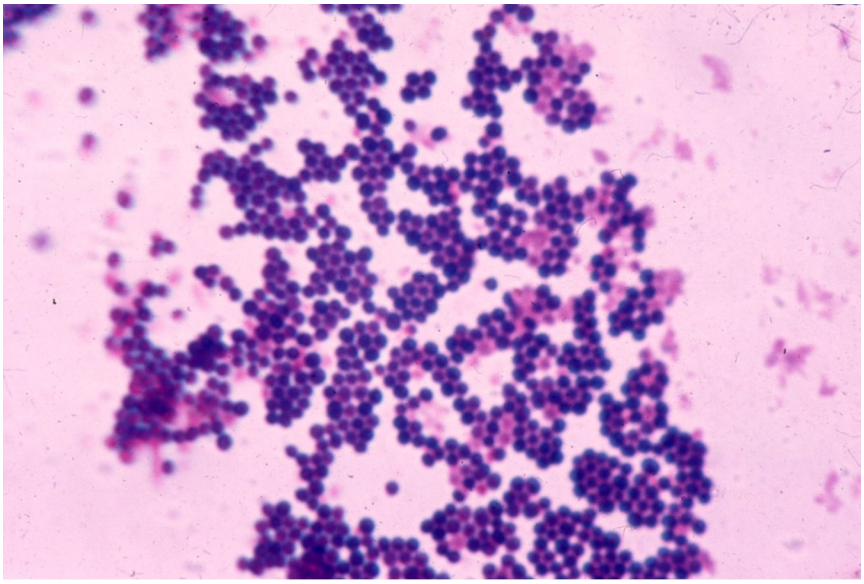

PLATE 2: Photomicrograph of normal cells of *S. aureus* without treatment (Control) X400

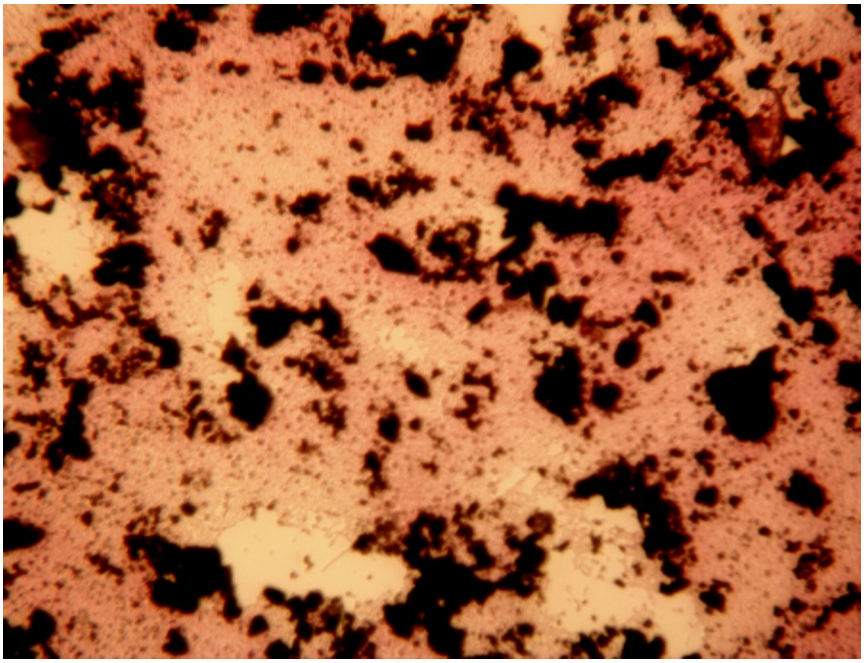

PLATE 3: Photomicrograph of cells of *Escherichia coli* treated with silver nanoparticles of *T. nerifolia* X400

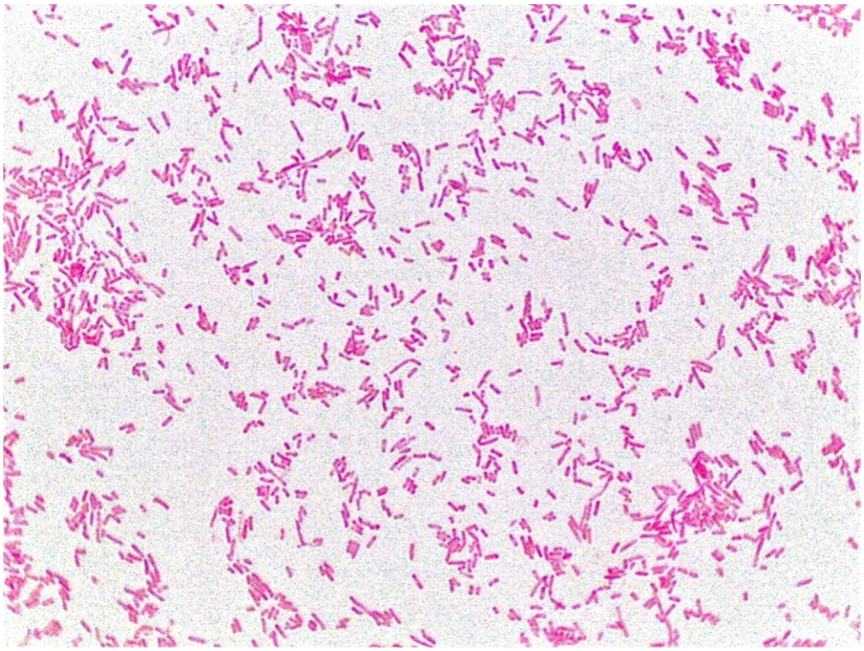

PLATE 4: Photomicrograph of normal cells of *E. coli* without treatment (Control) X400

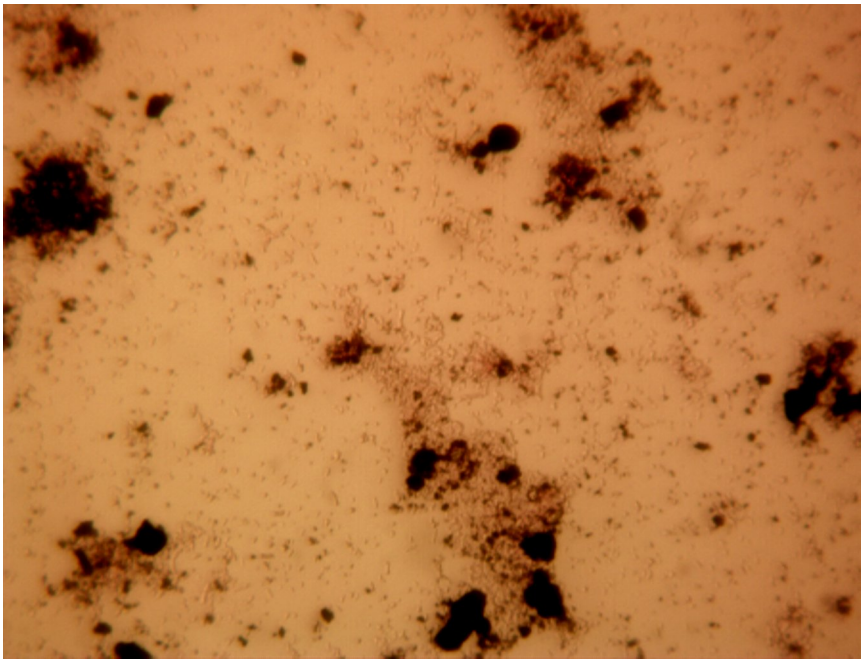

PLATE 5: Photomicrograph of cells of *Pseudomonas aeruginosa* treated with silver nanoparticles of *T. nerifolia* X400

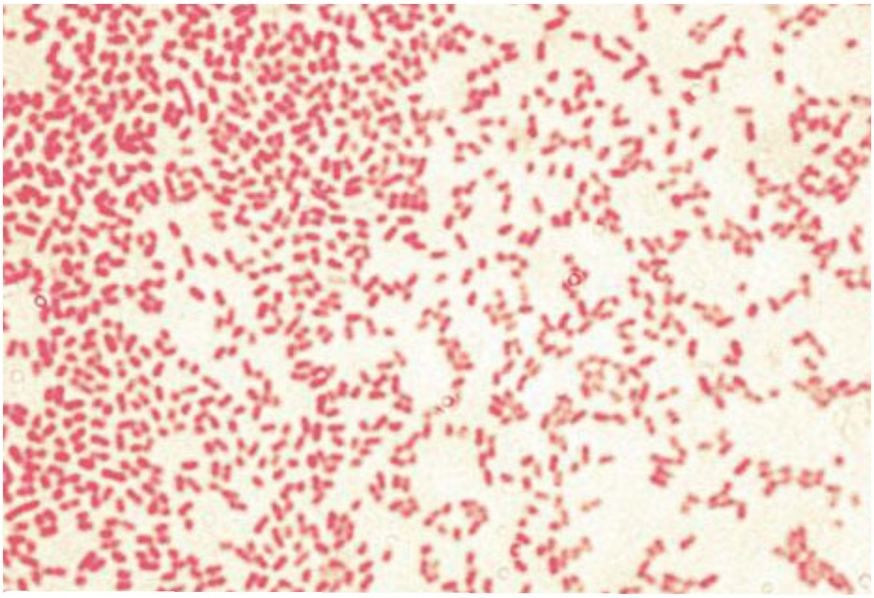

PLATE 6: Photomicrograph of normal cells of *P. aeruginosa* without treatment (Control) X400

Table 4: Comparism of the phytochemical analysis of silver nanoparticles of Thevetia nerifolia with Methanolic leaf extract

Phytochemicals	Silverparticles	Methanolic extract
Saponins	+	-
Tannins	+	+
Flavonoids	+	+
Terpenes	+	+
Anthraquinones	+	+
Steroids	-	-

KEY: + = Present

\- = Absent

DISCUSSION

The analysis of UV-Visible spectroscopy showed an appearance of surface plasmon resonance (SPR) peak at 452nm wavelength range, which corresponds to silver particles production. Silver particles absorbs radiation intensely at a wavelength of 400nm due to the transition of electrons. No other peak was observed in the spectrum which confirms that the synthesized products are of silver only. This result is in consonance with Thombre *et al.* (2012) reporting that a unique property of silver nanoparticles is that the SPR peak wavelength lies in the range of 400nm to 570nm.

FTIR was carried out to identify the possible biomolecules responsible for capping and reducing agent for the silver nanoparticles synthesized by leaf extract of *T. nerifolia*. The band at 3439.08cm^{-1} is due to stretch of -N-H while 1639.49cm^{-1} corresponds to C=C of amide. According to Naheed *et al.* (2011), the peak at around 3439.08cm^{-1} was characteristic of -N-H stretching of amide I band. The bonds or functional groups such as -C-O-C-, -C-O- and -C=C- derived from heterocyclic compounds and the amide I bond derived from the proteins, which are present in the extract are the capping ligands of the nanoparticles (Kiruba *et al.,* 2011; Harekrishna *et al.,* 2009; Manish *et al.,* 2012; Naheed *et al.* 2011).

In the assay of the antibacterial activity, all test isolates showed zones of inhibition at all concentrations.The silver particles demonstrated higher efficacy as compared to the reference antibiotics, this might be attributed to the crude nature of the bioactive components of the plant, together in an active matrix with the metallic silver ions, thereby producing synergistic effects better than the single active compounds in each of the reference antibiotics.

The minimum inhibitory concentration was observed to be at 40mg/ml for *E. coli, S. aureus and P. aeruginosa.* It was observed that the MBC for all tested isolates were obtained at a much higher dose than MIC, in the total extermination of the microbial cells (Table 2). This result agrees with Nazneen *et al.* (2014) indicating that MBC values of extract of *T. nerifolia* were achieved at much higher concentrations than MIC.

The exact mode of action of the silver nanoparticles, causing the antibacterial effect is not clearly known and is a debated topic (Prabhu and Poulose, 2012). There are various theories on the action of silver nanoparticles on microbes to cause microbicidal effect. There is formation of 'pits' as well as accumulation of nanoparticles on the cell surface (Sondi and Salopek-Sondi, 2004). However, in this study the silver nanoparticles are thought to have caused increased permeability of the cell membrane and subsequently intrusion into the cytosol of the cells thereby resulting in altered cellular architecture of test isolates. This is in line with the investigation carried out by Prabhu and Poulose, (2012), reporting that silver nanoparticles have the ability to anchor to bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane.

Another mechanism may be due to the high surface area to volume ratio of the silver particles, which allows their easy penetration into bacterial cells. Due to their unique size and greater surface area, silver nanoparticles can easily reach the nuclear content of bacteria (Chen *et al.,* 2010; Chudasama *et al.,* 2009). 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 of the microbial cells and inactivate them

(Matsumura *et al.,* 2003). Another fact is that the DNA of the microbial cell possesses sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA, which would definitely lead to cell death (Hatchett and Henry, 1996). Furthermore, it was reported by Dibrov *et al.* (2002) and Hamouda *et al.* (2000) that the positive charge on the silver ion is the reason for antimicrobial activity as it can attract the negatively charged cell membrane of microorganisms through the electrostatic interaction.

The phytochemical screening of the silver nanoparticles of *T. nerifolia* leaf extract used in this work, revealed the presence of five (5) secondary metabolites (Table 3). Ayoola *et al.* (2008) investigated the phytochemicals present in *T. nerifolia* leaf extract and discovered the presence of all of the bioactive metabolites found in this study. Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antiviral, and vasodilatory activities (Miller, 1996). Tannins have been known to precipitate proteins in microbial cells thereby hindering the process of protein synthesis (Okwu and Josiah 2006).

The available conventional antimicrobial agents are increasingly becoming resisted by a wide array of microorganisms. Therefore an alternative way to overcome this menace is to comb the earth for other sources of medicinal agents from natures' own pharmacy, which obviously is the green synthesis of antimicrobials due to bioactive components as well as silver ions.

REFERENCES

Akerman, M.E., Chan, W.C., Laakkonen, P., Bhatia, S.N., Ruoslahti, E., Chan, Laakkonen, Bhatia, and Ruoslahti.

(2002). "Nanocrystal targeting in vivo". *Proceedings of the National Academy of Sciences of the United States of America* **99** (20):12617–21.

Ayoola, G.A., Folawewo, A.D., Adesegun, S.A., Abioro, O.O., Adepoju-Bello, A.A. and Coker, H.A.B. (2008). Phytochemical and antioxidant screening of some plants of apocynaceae from South West Nigeria. *African Journal of Plant Science* Vol. 2 (9), pp. 124-128.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1966). Antibioticsusceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*; 45:493-496.

Buvaneswari, K., Ramamoorthy, D. and Velanganni, J. (2011). Preliminary Phytochemical and Antimicrobial Activity Studies on the Leaves of the Indian Plant *Thevetia neriifolia* Juss. *World Journal of Agricultural Sciences* 7 (6):659-666, 2011.

Chen, S.F., Li, J.P., Quin, K. and Xu, W.P. (2010). Large scale photochemical synthesis of M@TiO2 nanocomposites (M = Ag, Pd, Au, Pt) and their optical properties, Co oxidation performance and antibacterial effect. *Nano. Res.* 3:244–255.

Choi, Y., Ho, N. and Tung, C. (2007). *Angew. Chem. Int. Ed.* 707, 46.

Chudasama, B., Vala, A.V., Andharya, N., Upadhyay, R.V. and Mehta, R.V. (2009). Enhanced antibacterial activity of bifunctional Fe_3O_4 core-shell nanostructures. *Nano. Res.* 2:955–965.

Dibrov, P., Dzoiba, J., Gosink, K.K. and Häse, C.C. (2002). Chemiosmotic mechanism of antimicrobial activity of Ag(+) in vibrio cholera.

Antimicrob Agents Chemother 46:2668–2770.

Edeoga, H.O., Okwu, D.E. and Mbaeble, B.O. (2005). Phytochemical Constituents of some Nigerian Medicinal Plants. *Afr. J. of Biotech,* 4: 685-688.

El-Shishtawy, R.M., Asiri, A.M. and Al-Otaibi, M.M. (2011). Synthesis and spectroscopic studies of stable aqueous dispersion of silver nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*.79 (5):1505–1510.

Esumi K, Tano T, Torigoe K, Meguro K. (1990). *Chem. Mater.* 2:564-567.

Fawole, M.O. and Oso, B.A. (2007). Laboratory manual of Microbiology. Spectrum books limited. Pg. 16. ISBN: 978-029-256-X.

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N. and Kim, J.O. (2008). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater. Res.* 52, 662–668.

Frattini, A., Pellegri, N., Nicastro, D. and De Sanctis, O. (2005). Effect of amine groups in the synthesis of Ag nanoparticles using aminosilanes. *Mat. Chem. Phys.* 94, 148–152.

Hamouda, T., Myc, A., Donovan, B., Shih, A., Reuter, J.D. and Baker, R.J. (2000). A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial against bacteria, enveloped viruses and fungi. *Microbial Res*. 156:1.

Harekrishna, B., Dipak, K.B., Gobinda, P.S., Priyanka, S., Sankar, P. D. and Ajay, M. (2009). Green synthesis of silver nanoparticles using latex of *Jatropha curcas Colloids and Surfaces A*: *Physicochem. Eng. Aspects* **339** 134-139.

Hatchett, D.W. and Henry, S. (1996). Electrochemistry of sulfur adlayers on low-index faces of silver. *J. Phys. Chem.* 100, 9854–9859.

Henglein, A. (2001). Langmuir 2001, 17:2329-2333.

Hutter, E. and Fendler, J.H. (2004). *Adv. Mater*. 1685,16.

John, D.A. and Steena, R.S. (2012). Biosynthesis of silver nanoparticles and its antibacterial activity against human pathogens. *Int J Pharm Pharm Sci, Vol 5, Issue 1, 257-259*.

Kholoud, M.M.A., Ala'a, E., Abdulrhman, A. and Reda, A.A.A. (2010). Synthesis and applications of silver nanoparticles. *Arabian Journal of Chemistry.* 3, 135–140.

Kiruba, D.S.C.G., Kumar, R., Sathish, V., Sivakumar, M., Sunitha, S. and Anitha, S. T. (2011). Green Synthesis (*Ocimum tenuiflorum*) of Silver Nanoparticles and Toxicity Studies in Zebra Fish (*Danio rerio*) Model *International Journal of NanoScience and Nanotechnology* **2** (2)103-117.

Manish, H., Shreeram, J. and Mayur, D.K.K. (2012). Latex-mediated synthesis of ZnS nanoparticles: green synthesis approach *J Nanopart Res* **14** 865.

Matsumura, Y., Yoshikata, K., Kunisaki, S. and Tsuchido, T. (2003). Mode of bacterial action of silver zeolite and its comparison with that of silver nitrate. *Appl. Environ. Microbiol.* 69, 4278–4281.

Miller, A.L. (1996). Antioxidant Flavonoids: Structure, Function and Clinical Usage. *Alt. Med. Rev*. 1(2):103-111.

Naheed, A., Seema, S., Singh, V. N., Shamsi, S. F., Anjum, F. and Mehta, B. R. (2011). Biosynthesis of Silver Nanoparticles from Desmodium triflorum: A Novel Approach Towards Weed.

Utilization *Biotechnology Research International* 8 pages.

Naik, R.R., Stringer, S., Agarwal G. *Nat. Mater*. 1:169-172.

Nazneen, R., Riaz, M., Haseebur, R. and Mir, H. (2014). Spectrophotometric screening of potent bactericidal property of *Thevetia peruviana* schum. leaf and fruit rind extracts on clinical and plant pathogens. *Int J Appl Sci Biotechnol*, Vol. 2(4):451-459.

NCCLS. (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 5th ed. NCCLS document M7-A5. NCCLS, Wayne, Pa.

Nellis, D.W. (1997). Poisonous Plants and Animals of Florida and the Caribbean, Pineapple press, Florida.

Okwu, D.E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology* vol. 5 (4):357-361.

Pastoriza-Santos, T. and Liz-Marzan, L.M. (2002). Langmuir. 18:903-905.

Prabhu, N., Raj, D.T., Yamuna, G.K., Siddiqua, S.A and Innocent, J.P. (2010). Synthesis of silver photo nano particles and their antibacterial efficacy. *Dig J Nanomater Bios* 5(1): 185-189.

Prabhu, S. and Poulose, E.K. (2012). Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *International Nano Letters*. 2:32.

Richardson, A., Chan, B.C., Crouch, R.D., Janiec, A., Chan, B.C. and Crouch R.D. (2006). Synthesis of silver nanoparticles: An undergraduate laboratory using green approach. *Chem. Educ.* 11:331–333.

Sahuvinod, K., Irehhaiya, R., Shashi, A. and Gujar, H. (2010). Phytochemical investigation and chromatographic evaluation of the ethanolic extractof whole plant extract of *Dendrophthoe falcata* (L.F) Ettingsh. *International Journal of Pharmaceutical sciences and Research* 1(1):39-45.

Sofowora, E.A. (1993). *Medicinal Plants and Traditional Medicine in Africa.* Spectrum Books Ltd, Ibadan, Nigeria. 289 pp.

Sudrik, S., Chaki, N., Chavan, V. and Chavan, S. (2006). Chem. Eur. J. 859, 12.

Sun, S., Murray, C., Weller, D., Folks, L. and Moser, A. (2000). Science 1989, 287.

Sun Y, Yin Y, Mayers B.T, et al. Chem. Mater. (2002). 14:4736-4745.

Taleb, C., Petit, M. and Pileni, P. (1997). *Chem. Mater*. 9:950-959.

Tamasa P. (2013). Synthesis and characterization of silver nanoparticles using leaf extract of *Azadirachta indica.* Pg 35.

Thombre, R., Parekh, F., Lekshminarayanan, P. and Francis, G. (2012). Studies on antibacterial and antifungal activity of silver nanoparticles synthesized using *Artocarpus heterophyllus* leaf extract. *Biotechnol. Bioinf. Bioeng*. 2(1):632-637.

Udayan, P.S. and Balachandran, I. (2009). *Medicinal plants of Arya Vaidya Sala.* Department of Publications, AryaVaidyaSala. Kottakkal, Kerala.

Vilchis-Nestor, A., Sa´ nchez-Mendieta, V., Camacho-Lo´ pez, M., Go´ mez-Espinosa, R., Camacho-Lo´ pez, M. and Arenas-Alatorre, J.(2008). Mater. Lett. 62, 3103.

Yoosaf, K., Ipe, B., Suresh, C.H. and Thomas, K.G. (2007). *J. Phys. Chem.* C 1287, 111.

Yuet, Y.L., Buong, W.C., Mitsuaki, N. and Son, R. (2012). Synthesis of silver nanoparticles by using tea leaf extract from *Camellia Sinensis. International Journal of Nanomedicine*. 7 4263–4267.

Zhu, J., Liu, S.W., Palchik, O. (2000) Langmuir. 16:6396-6399