

Original Research Article

ANTIMICROBIAL EVALUATION AND CHARACTERIZATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING *Annona muricata* L. leaf Extract

ABSTRACT

AIM: This study was aimed at assessing the antimicrobial activity of Green synthesized Nanoparticles of *annona muricata* L leaf extract.

STUDY DESIGN: Plant preparations-Green synthesis of Silver Nanoparticles-Characterization (Ultra Violet-Visible, Fourier Transform-Infrared, Particle size Analysis)- Antimicrobial Evaluation using the clinical isolates.

PLACE and DURATION of STUDY: Department of Pharmaceutical Chemistry, Madonna University, Elele, Department of Microbiology and Biotechnology University of Nigeria, Nsukka between June 2021 to November 2021

METHODOLOGY: Fresh plant leaves were collected, dried and grinded. 20g of the powder was used to generate the extract. 0.1698g of silver was used to produce silver nitrate (AgNO_3). 50mls of the extract was mixed with 250mls of AgNO_3 and incubated at 27°C in the dark. The synthesized silver nitrate was centrifuged for 30minutes at 5000rpm. Green synthesis of silver Nanoparticles (AgNPs) was done and the AgNPs produced were scrapped off the plate and placed in sample tubes. Characterization was done using UV-Vis, FT-IR and Particle size analysis. Five clinical isolates were used for Antimicrobial evaluation of the green synthesized nanoparticles.

RESULTS: The silvery brown color was observed at 1 hour, indicating the formation of AgNPs. Characterization was done by UV-Vis, FT-IR and Particle Size Analysis. Antimicrobial Evaluation was carried out with several clinical isolates. The UV- VIS showed a peak between 370-450nm. The FT-IR results confirmed phytochemicals in *Annona muricata* L and its roles as a bio-reductant and stabilizer on the surface of silver nanoparticles. The Particle size analysis result indicates an average particle size of 570nm. AgNPs were screened against both Gram-positive and Gram negative Bacterial and the minimum inhibition concentrations (MIC) on the microbes were obtained. The results signify that the silver nanoparticles synthesized using the aqueous leaf extract of *A. muricata* L. were found to have promising antimicrobial activity.

CONCLUSION: The synthesized silver nanoparticles of *Annona muricata* leaf extract exhibited an effective antimicrobial activity.

KEYWORDS: Green Synthesis, *Annona muricata* Anti-Microbial, Silver-Nanoparticles, UV-VIS, FT-IR.

1. INTRODUCTION

It is a piece of common knowledge that almost everyday use of antimicrobial agents in hospitals and by the community paves way for antimicrobial-resistant pathogens^[1]. The multi-drug resistance of most commercially available antibacterial against these pathogens calls for a search for new bioactive compounds and methods to eradicate this major health challenges.^[2] The most extensive studies on Silver Nanoparticles is because of its large surface area which allows them to be in better contact with microorganisms, thereby impacting good antimicrobial activity^[3]. Nanoparticles refer to small particles that measure around 1 nm to 100 nm in size^[4]. Nanoparticles great role in the field of high-sensitivity bio-molecular detection, catalysis, biosensors and medicine; it is been recognized to have strong inhibitory and bactericidal effects alongside anti-fungal, anti-inflammatory and anti-angiogenesis activities^[5]. Antimicrobial resistance to virtually every available medicine is a proven fact and the need for a continuous search for alternative, especially from plant sources has been acknowledged^[6]. *Annona muricata l* is of the family of Annonaceae. It is native to Mexico, Cuba, Central America and parts of Asia like India and Malaysia. It is a flowering evergreen tree that belongs to the subclass *Magnoliidae*. *Annona muricata l* is commonly called Soursop or Graviola or Guyabano and in Latin America, Guanabana^[7] and it is gaining global popularity for being a miracle tree in the field of cancer research and can pave way for research in many fields^[8]. Hence, this study was mapped out to provide information on the antimicrobial effect of the aqueous extract of *Annona muricata l* leaf.

2. MATERIALS AND METHODS

2.1 Materials Used

Mueller Hinton agar, Sabourad dextrose agar, Nutrient broth, Sterile swab sticks, Filter paper, Beakers (pyrex), Volumetric flask (pyrex), Plastic test tubes (micro point diagnostics), Filter paper (whatman #1 and #3), Measuring cylinders, Thermostat water bath (SANFA DK420), UV/VIS spectrophotometer (JENWAY, 6705), Fourier Transformed Infrared Spectrophotometry (FTIR- Shimadzu Model-8400 spec), Electronic weighing balance (Shimadzu, Japan), Porcelain plates, Sample bottles, Autoclave health team instrument (model YX-280A), Laboratory incubator (model: DNP-9022A), Oven: spring field instrument England (model 9022A), Centrifuge (model 908), Cotton wool (agary), Funnel, Spatula (Stainless steel), Petri dishes, Refrigerator (Thermocool), Masking tape, Syringes, Stirring rod, Conical flask , Glass funnel, Silver nitrate (AgNO₃), Distilled water

2.1.1 Clinical Isolates Used Includes: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*

2.2 Methodology

2.2.1 Preparation of plant extract

The fresh leaves of *Annona muricata* were collected from a farm in Abakaliki, Ebonyi State, Nigeria in August 2020 and dried for 7 days. The leaves were then milled using a mechanical grinder and stored in an air-tight container. 20g quantity of the powdered leaves was added into 400ml of distilled water and heated at 100°C for 30 minutes, it was left to cool and then filtered using a whatman filter paper no 1 and 3

2.2.2 Green Synthesis of Silver Nanoparticles

A 0.1698g of silver nitrate was dissolved in 10mls of distilled water in a 500ml beaker which was then transfer to a 1L volumetric flask where the volume of the solution was made up to the 1L mark of the volumetric flask to produce 1mM of AgNO₃. 50ml of the plant extract was mixed with 250ml of silver nitrate solution 1.00mM. The reaction was incubated at a temperature of 27⁰ in the dark to avoid photochemical activation of silver nitrate. Observation of the reaction was taken at 0 minutes, 30 minutes and 1hour. Silvery brown color was observed at the end of 1hour, indicating the formation of AgNPs. The synthesized silver nitrate was centrifuged for 30minutes at 5000rpm. The pellet containing silver nanoparticle was rinsed using a small volume of distilled water and was poured into a Porcelain plate and placed in the oven at 115⁰ to evaporate the solvent for 20minutes. Using a spatula, the AgNPs were scrapped off the plate and placed in sample tubes^[9,10]

2.2.3 Characterization of Silver Nanoparticles

Ultraviolet-Visible Spectrophotometry Analysis (UV-VIS)

The extracted AgNPs were scanned in the wavelength ranging from 300-800nm using the UV/VIS spectrophotometer (JENWAY, 6705), and the characteristic peaks were detected at different times (0.5, 1, 1.5, 2, 2.5, 3 hours).

2.2.4 Fourier Transform Infrared Spectroscopy Analysis (FT-IR)

The FT-IR analysis was done to determine the presence of functional groups. The FTIR analysis was carried out using Shimadzu FTIR Spectrophotometer (FTIR-8400S) with wave numbers ranging from 4000-500 cm⁻¹

2.2.5 Particle Size Analysis

This particle size analysis was done to determine the size of the AgNPs. The analysis was done using dynamic light scattering. The Malvern machine (Version 2.2) was used between the range of 0 – 10000 nm.

2.2.6 Antimicrobial Evaluation

Agars were prepared and solidified. The solidified agars were dried in the hot air oven at 45°C for 30 minutes. A suspension of the tested organisms was uniformly swabbed each on agar plates. The filter paper was cut 5mm by 5mm and autoclaved at 121°C for 15 minutes and allowed to cool. The plates containing different microorganisms were divided into 4 quadrants and properly labeled (quadrant 1=100µg, quadrant 2=50µg, quadrant 3=AgNO₃ solution, quadrant 4=standard antibiotics). The reconstituted silver nanoparticle (AgNp) extract was impregnated and placed on the inoculated agar plates. The plates were incubated for 24 hours and the minimum zone of inhibition was calculated. Minimum Inhibitory Concentration was done by Serial dilutions of the reconstituted silver nanoparticle in different concentrations (1µg/ml - 10µg/ml). A 4 ml of the prepared nutrient broth + microorganism was added into the dilutions of the silver nanoparticles in the test tubes. Then the setup was incubated for 24 hours. This was done for the different microorganisms (*Escherichia coli*, *Aspergillus niger*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*). Then the serial dilution was observed for microbial growth usually indicated by the presence of turbidity. The last test tube without turbidity is the minimum inhibitory concentrate

3. Results and Discussion



At 0 min

At 30 min

At 1 hour

Fig 1: Color change at different intervals during the process of green synthesis



Fig 2: Agar plates

3.3 Ultra Violet Visible Spectroscopy

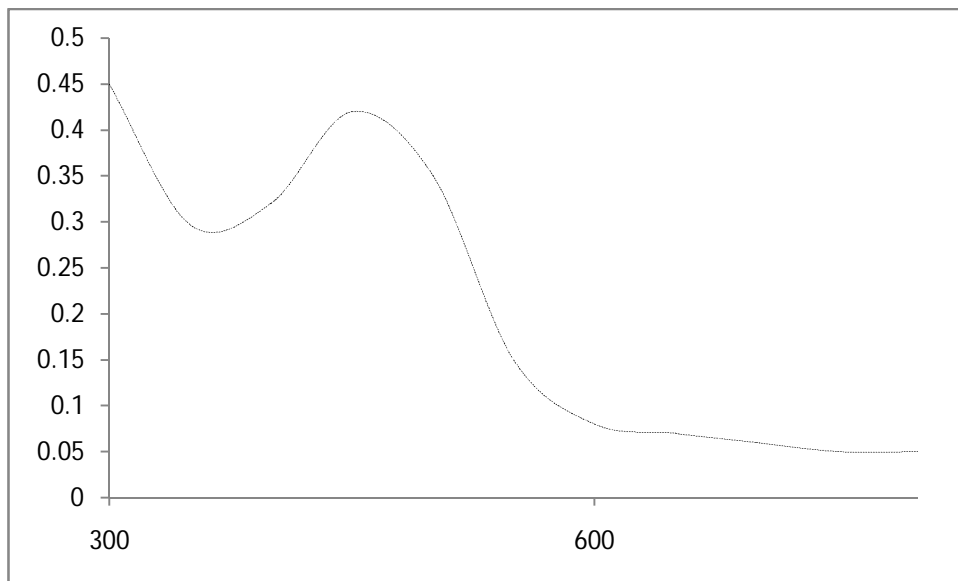


Figure 3 Uv – Vis spectrum result at 0.5 hour after mixing the AgNO_3 with the leaf extract.

Observation showed that the maximum wavelength was 370nm.

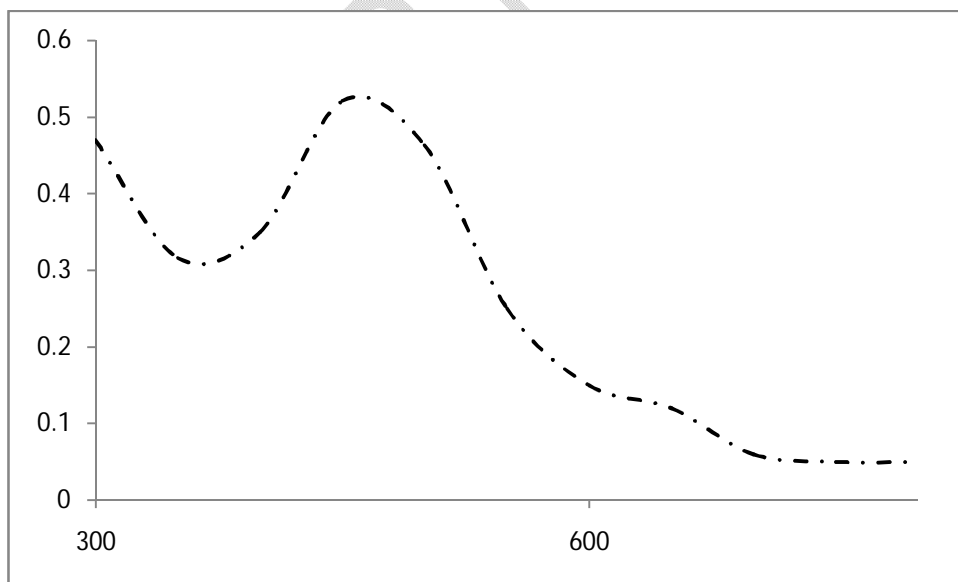


Figure 4 Uv – Vis spectrum result at 1 hour

The maximum wavelength was noted to be 400nm with a visible peak

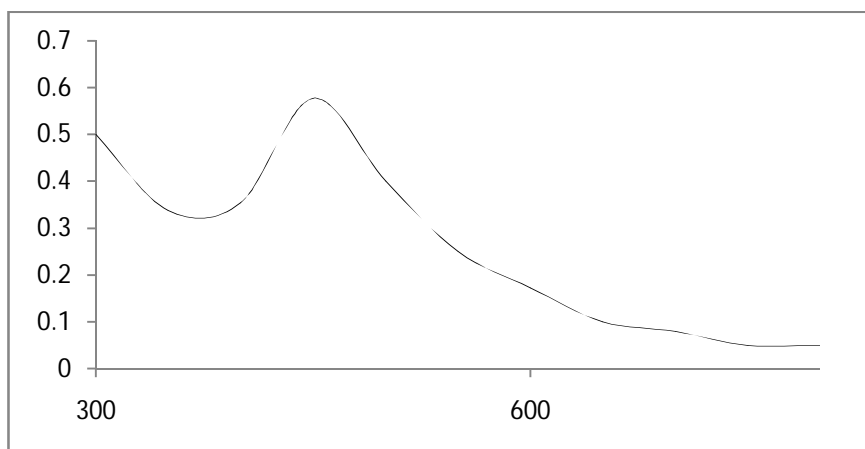


Figure 5 Uv – Vis spectrum result at 1.5 hour

The maximum wavelength was noted to be 450nm and there was a visible peak

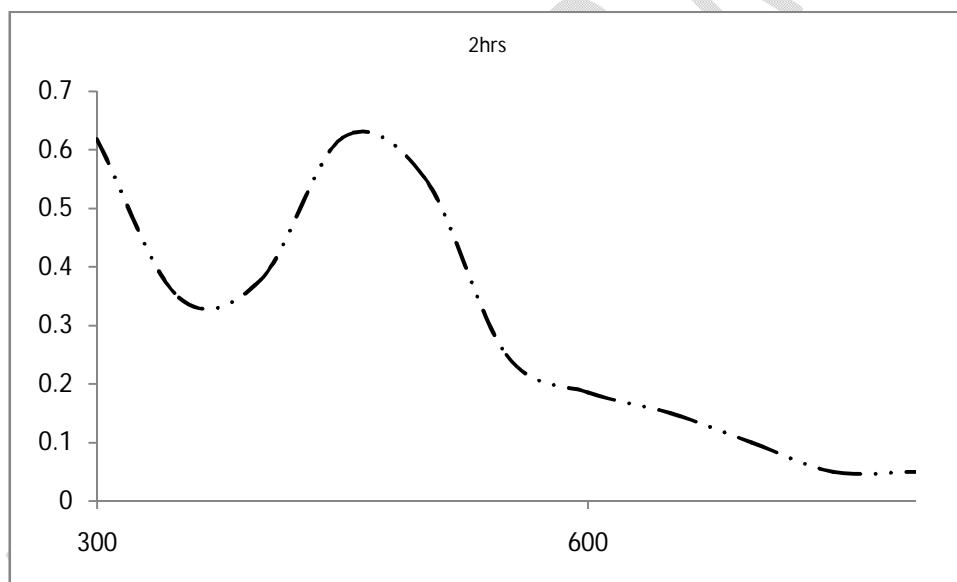


Figure 6 Uv – Vis spectrum result at 2 hours

The maximum wavelength was noted at 430nm. A visible peak was observed

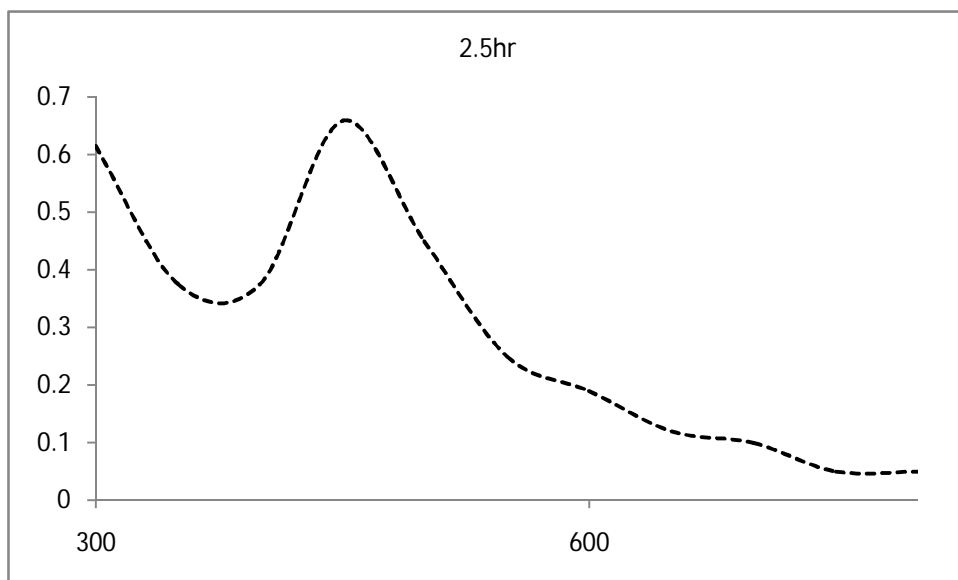


Figure 7 Uv – Vis spectrum result at 2.5 hours

The maximum wavelength was noted at 450nm. A visible peak was observed

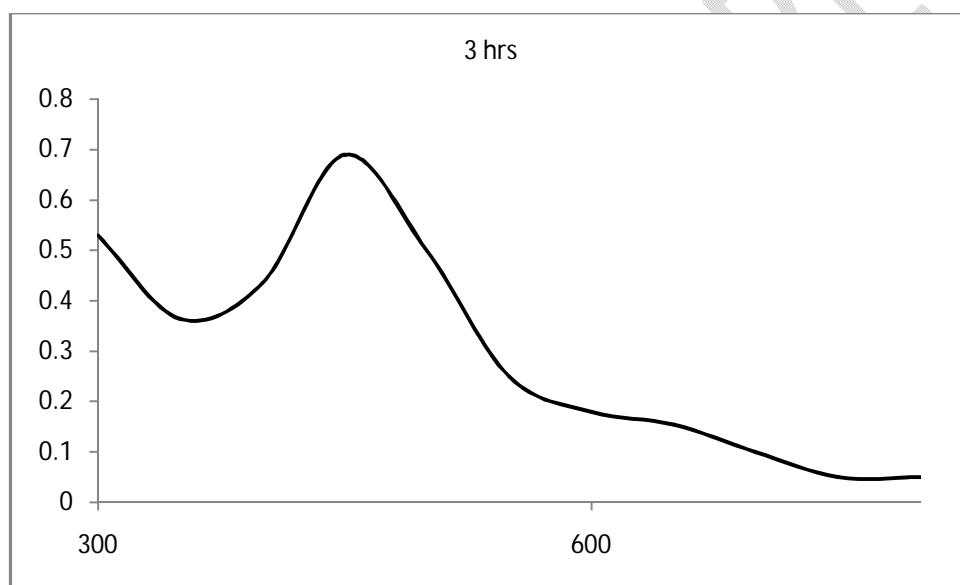


Figure 8 Uv – Vis spectrum result at 3 hours

The maximum wavelength was noted at 450nm with a visible peak

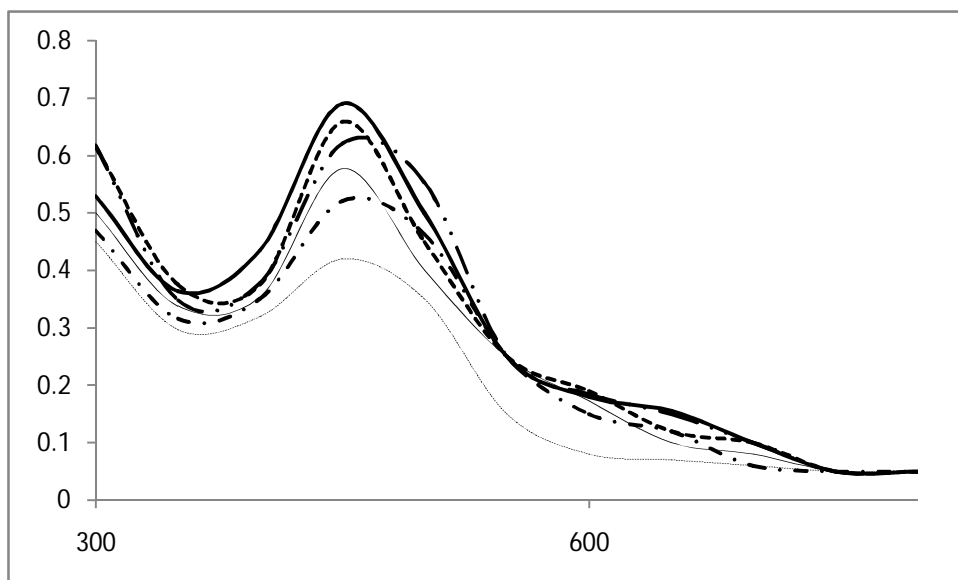


Figure 9 Uv – Vis Spectra result at different times

The maximum wavelength was noted at 450nm and as wavelength of absorption increased, time increased also.

3.10 Fourier transform infrared spectroscopy analysis

Table 1 Results of FTIR analysis

S/N	Absorption frequency (cm ⁻¹)	Possible bonds	Possible functional group
1	3873.19	O-H	Alcohol
2	3456.55	N-H	Primary amine
3	1604.83	C-H	Aromatic compound
4	1527.67	N-O	Nitro compound
5	1450.52	C-H	Alkane
6	1388.79	C-H	Aldehyde
7	1327.07	S=O	Sulfone
8	1180.47	C-O	Aliphatic ether
9	1049.31	S=O	Sulfoxide
10	910.43	-	-
11	848.71	C-Cl	Halo compounds
12	748.41	C=C	Alkene
13	594.1	C-Br	Alkyl halides
14	540.09	C-I	Halo compounds

3.11 PARTICLE SIZE ANALYSIS

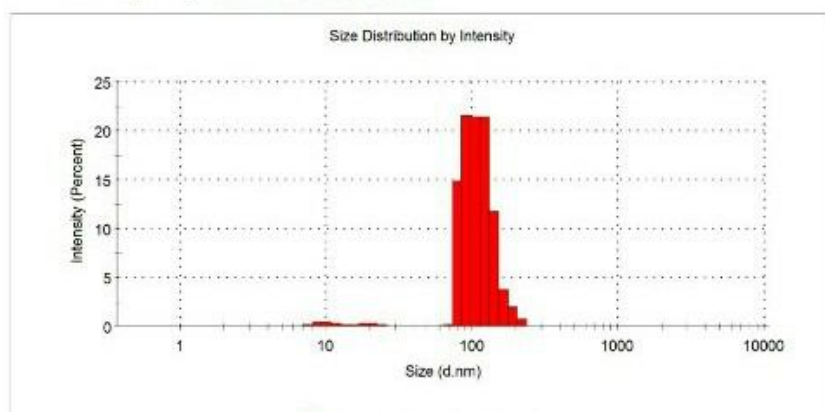


Figure 10 this shows the graph of size distribution by intensity of the synthesized AgNPs using *Annona muricata*.

Table 2 Results of particle size analysis

Peak No	Size (d.nm)	Intensity (%)
Peak 1	111.1	97.6
Peak 2	10.09	1.3
Peak 3	19.49	1.0
Z – AVERAGE (d.nm): 570 nm		
POLY DISPERSITY INDEX (PDI)		
SIZE RANGE: 0 – 10000 nm		

3.12 Antimicrobial screening results

Table 3 Results of antimicrobial sensitivity testing

S/N	CONCENTRATION	<i>Escherichia Coli</i>	<i>Bacillus Subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus Aureus</i>
1	AgNPs (100 µg)	NZ	NZ	NZ	NZ
2	AgNPs (50 µg)	NZ	NZ	NZ	NZ
3	Silver nitrate	7 mm	NZ	NZ	NZ
4	Gentamycin	12 mm	12 mm	8 mm	8 mm
5	Ofloxacin	20 mm	20 mm	21 mm	NZ

Keyword: NZ - No zone

Table 4 Results MIC of AgNPs against various organisms

S/N	MICROORGANISM	MINIMUM INHIBITORY CONCENTRATION (µg)
1	<i>Salmonella typhi</i>	10
2	<i>Escherichia coli</i>	8
3	<i>Bacillus subtilis</i>	5
4	<i>Staphylococcus aureus</i>	5
5	<i>Candida albicans</i>	10
6	<i>Aspergillus niger</i>	10

4.1 DISCUSSION

The Silver Nanoparticles formed were shown by a color change from yellowish to silvery brown. This was because of the excitation of surface plasmon vibration^[11]. Fig 3-9 illustrates the graphs of UV-VIS analysis at different time intervals showing their peaks at different times. The spectrum shown in Fig 5 had a maximum absorption peak at a wavelength of about 450nm and absorbance at 0.695, which is in the range of the surface plasmon resonance for AgNPs reported to have an absorption maximum between 400 nm to 450 nm. According to^[12], the maximum absorption peak was at a wavelength of 435.00nm and absorbance at 0.556, which is still within the range of the surface Plasmon resonance for synthesized silver nanoparticles. Fig 9 illustrates a combination graph of the various reaction time at different times. It was noticed that as time increases the peak increases as well.

The binary character of the plant extract as a reducing and capping agent and the presence of some functional groups was confirmed by FT-IR analysis of AgNPs in fig 10. The FT-IR spectrum of the extracts shows several absorption peaks as seen in fig 10. Strong absorption bands at 3873.19 to 3456.55cm⁻¹ are a signal of bonded -OH of alcohol or possibly -NH of amine. The absorption peaks at 1450.52cm⁻¹ could be allotted to alkane stretching vibrations and that at 1180.47cm⁻¹ can be allotted to aliphatic ether C=O stretching. This aligns with previous studies by^[13,14] having similar results. The FTIR analysis confirmed (-OH) and amine (-N-H) as functional groups accountable for the reduction of Ag⁺ ions to Ag⁰. Fig 10 shows the functional group analysis of the FTIR spectrum of the synthesized AgNPs from plant extracts of leaves of *Annona muricata*. The functional groups behind the formation of the AgNPs included; alcohol, primary amine, aromatic compound, nitro compound, alkane, aldehyde, sulfone, aliphatic ether, sulfoxide, halo compound, alkene and alkyl halides.

Particle size analysis which is also known as Dynamic light scattering(DLS) is a method that depends on the interaction of light with particles and the method can be used for computing narrow particle size distributions, especially in the range of 1–100 nm .The Z average is 570.8nm as indicated in fig 10. The DLS gives a hydrodynamic size (wet) which could be above 100nm, unlike the Transmission Electron Microscopy size (dry) which have particle sizes around 30-40nm^[15]. Particle size obtained does not depend on the method used. An example is the TEM analysis of green synthesized silver nanoparticles using *Azadirachta indica* showed particle size around 34nm^[16]. Furthermore, a study conducted by^[17], shows the size of TEM analysis of silver

nanoparticles to be around 50 nm while that of DLS size distribution ranged from 10-150nm. Another possible reason the particle size exceeded 100 nm could be due to the long duration of storage in the course of transporting samples for analysis. Polydispersity Index (PDI) estimates the cohesive nature of nanoparticles. The binary value of PDI ranges from 0.0 (for a perfectly uniform sample for the particle size) to 1.0 (for a highly polydisperse sample with multiple particle size populations). Values of 0.2 and below are most commonly considered acceptable in practice for polymer-based Nano-materials while nanoparticles with PDI greater than 0.3 is considered acceptable for Drug Delivery. The synthesized AgNPs had an average PDI of 0.537, which is a great indication that they are highly homogenous and would be effectively used in various applications.

The zones of inhibition of the synthesized nanoparticles against the selected microorganism (*E.coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus*) at concentrations of 100µg, 50µg, silver nitrate solution and control which includes Gentamycin and Ofloxacin were showed in fig 10. The highest zone of inhibition against the test was observed at the control (Gentamycin) even though there was no zone of inhibition on *Staphylococcus*. There was a zone of inhibition on all microorganisms used in control (Ofloxacin) with *Pseudomonas aeruginosa* having the highest sensitivity. In silver nitrate solution, there was a zone of inhibition only in *E.coli*. Even though there was no zone of inhibition in concentrations 100µg and 50µg as seen in fig 4, this is similar to the previous study by^[18], which also proved that *Annona muricata* has antimicrobial properties.

The different microorganisms used for the MIC, *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger* as seen in fig 10 showed they had minimum inhibition at different concentrations. The synthesized AgNPs yielded growth in all microorganisms which makes a good antimicrobial agent, 50% diluted dimethylsulphuroxide (DMSO) yielded growth in all microorganisms, Cypro 10µg/ml yielded growth only in *Candida albicans* and *Aspergillus niger*, Fluconazole 10ug/ml yielded growth in all except *Candida albicans* and *Aspergillus niger*. The results show that green synthesized silver nanoparticles of *Annona muricata* exhibit better anti-bacterial activity than anti-fungal activity.

4.2 CONCLUSION

The synthesized silver nanoparticles of *Annona muricata* leaf extract exhibited an effective antimicrobial activity. It was eco-friendly and also cost-effective. The FT-IR analysis showed the presence of the functional groups from *Annona muricata* / leaf extract which played a double role as a reducing and stabilizing agent. The particle size analysis indicated that the silver nanoparticles were within the nano range and that it was fairly homogenous which made it a suitable pharmaceutical product

4.4 RECOMMENDATION

In this study, *Annona Muricata* l. Can serve as a potential anti-microbial agent against various micro-organisms. *Annona Muricata* l. Silver nanoparticles can be used for the development of anti-microbial agents. Also, further work can be carried out on green synthesized-silver nanoparticles using *Annona muricata* leaf extract to determine its mechanism of action, side effects, and toxicity.

REFERENCES

1. Nagel, J. L., Kaye, K. S., LaPlante, K. L., & Pogue, J. M. (2016). Antimicrobial stewardship for the infection control practitioner. *Infectious Disease Clinics*, 30(3), 771-784. <http://dx.doi.org/10.1016/j.idc.2016.04.012>. PMID:27515147.
2. Bhatt P, Negi PS (2012) Antioxidant and antibacterial activities in the leaf extracts of Indian Borage (*Plectranthus amboinicus*). *Food and Nutrition Sciences* 3: 146-152
3. Muzammil S, Hayat S, Fakhar EM, Aslam B, Siddique MH, Nisar MA. Nano-antibiotics: future nanotechnologies to combat antibiotic resistance. *Front Biosci*. 2018; (Elite Ed) 352-374
4. He, X., Deng, H. Hwang, H. 2019. The current application of nanotechnology in food and agriculture. *J. Food Drug Anal.* 27, 1–21
5. Shakeel Ahmed, Saifullah, Mudasir Ahmad, Babu Lal Swami & Saiqa Ikram (2016) Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract, *Journal of Radiation Research and Applied Sciences*, 9:1, 1-7, DOI: 10.1016/j.jrras.2015.06.006
6. Carlotta, L, Anna, R.F, Mauro, C., and Alberto Vergara (2022). Prevalence and antimicrobial profile in *salmonella* spp. isolates from swine food chain. *Italian journal of Food Safety* 11:9980
7. Emmanuel A, Kubmarawa D, Yahaya SG (2019). Phytochemical screening and microcidal activity of the ethanolic and aqueous extracts of *Annona muricata* against some pathogenic bacteria. *South Asian Research Journal of Natural Products*, 2(2):1-6.
8. Mithun PBH, Gururagavendra R, Ramya S, Ashwini R (2016). Anti-microbial efficacy of soursop leaf extract (*Annona muricata*) on oral pathogens: An in-vitro study. *Journal of Clinical and Diagnostic Research*, 10(11):1-4.
9. Valsalam, S., Agastian, P., Arasu, M.V., Al-Dhabi, N.A., Ghilan, A.K.M., Kaviyarasu, K., Ravindran, B., Chang, S.W., Arokiyaraj, S., 2019. Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of *Tropaeolum majus* L. and

its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. *J. Photochem. Photobiol. B: Biol.* 191, 65–74.

10. Anand, K., Kaviyarasu, K., Muniyasamy, S., Roopan, S.M., Gengan, R.M., Chuturgoon, A.A., 2017. *Bio-synthesis of silver nanoparticles using agroforestry residue and their catalytic degradation for sustainable waste management. J. Clust. Sci.* 28(4), 2279–2291
11. Kumari J, Mamta B, Ajeet S (2016) Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. Leaves and their synergistic effect with antibiotics. *J Radiat Res Appl Sci* 9(3):217–227.
12. Akintelu SA, Folorunso AS (2019) Characterization and Antimicrobial Investigation of Synthesized Silver Nanoparticles from *Annona muricata* Leaf Extracts. *J Nanotechnol Nanomed Nanobiotechnol* 6: 022
13. Banjeer P, Satapathy M, Mukhopahayay A, Das P. Leaf extract mediated green synthesis of silver nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular spectroscopy.* 2014; 135:373-378.
14. Elangovan KD, Elumalai S, Anupriya R, Shenbhagaraman PK, Kaleena KM. Phyto mediated biogenic synthesis of silver nanoparticles using leaf extract of *Andrographis echinoides* and its bio-efficacy on anticancer and antibacterial activities. *J Photochem Photobiol B: Biol.* 2015; 151:118-124
15. Sugunan, A. (2016). Re: The hydrodynamic size of nanoparticles is mostly greater than 100nm, then how can we say that these are nanoparticles? Retrieved from: https://www.researchgate.net/post/The_hydrodynamic_size_of_nanoparticles_is_mostly_greater_than_100nm_then_how_can_we_say_that_these_are_nanoparticles_Is_there_any_justification
16. Ahmed, S., Ahmad, M., Swami, B.L., Ikram, S., 2016. A Review on Plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J. Adv. Res.* 7, 17–28. Balouiri, M., Sadiki, M., and Ibensouda, S. K. (2016). Methods for in vitro evaluating Antimicrobial Activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.
17. Anandalakshmi, K.; Venugobal, J.; Ramasamy, V. *Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. Appl. Nanosci.* 2016, 6, 399–408.
18. Ezealisiji KX, Noundou S, Ukwueze SE. Green synthesis and characterization of mono dispersed silver nanoparticles using root bark aqueous extract of *Annona muricata* Linn and their antimicrobial activity. *Appl Nanosci.* 2017; 7:905-911