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 from *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 Pharmaceutical and Medicinal chemistry,University of Nigeria Nsukka and 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 27^0C 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 characterization 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. 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. The results signify that the silver nanoparticles synthesized using the aqueous leaf extract of *A. muricata l.* were found to have promising antimicrobial activity against both Grampositive and Gram negative bacteria with the minimum inhibitory concentrations ranging from 5µg to 10µg.

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 have great roles in the field of high-sensitivity bio-molecular detection, catalysis, biosensors and medicine. It has 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].Annona muricata with ethanol and aqeous extracts has shown reasonable activity against various bacteria strains.[9]. Hence, this study was mapped out to evaluate the antimicrobial activity of silver nanoparticles synthesized from *Annona muricata* 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 Transform Infrared Spectrophotometry (FTIR-ShimadzuModel-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 ($AgNO_3$),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 100^0C 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 100ml 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 $AgNO_3$. 50ml of the plant extract was mixed with 250ml of silver nitrate solution 1.00mM. The reaction was incubated at a temperature of 27^0 in the dark to avoid photochemical activation of sliver nitrate. Observation of the reaction was taken at 0 mintues, 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.[10,11]

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 dnm.

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℃ 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=$AgNO_3$ 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 was the minimum inhibitory concentration.

3. Results and Discussion

At 0 min At 30 min At 1hour

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

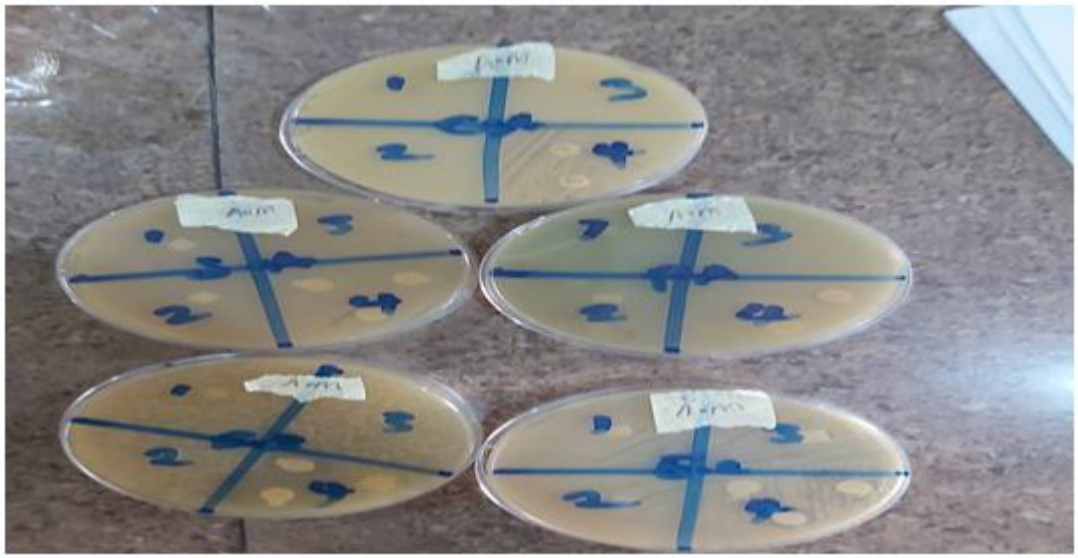

Fig 2: Agar plates

3.3 Ultra Violet Visible Spectroscopy

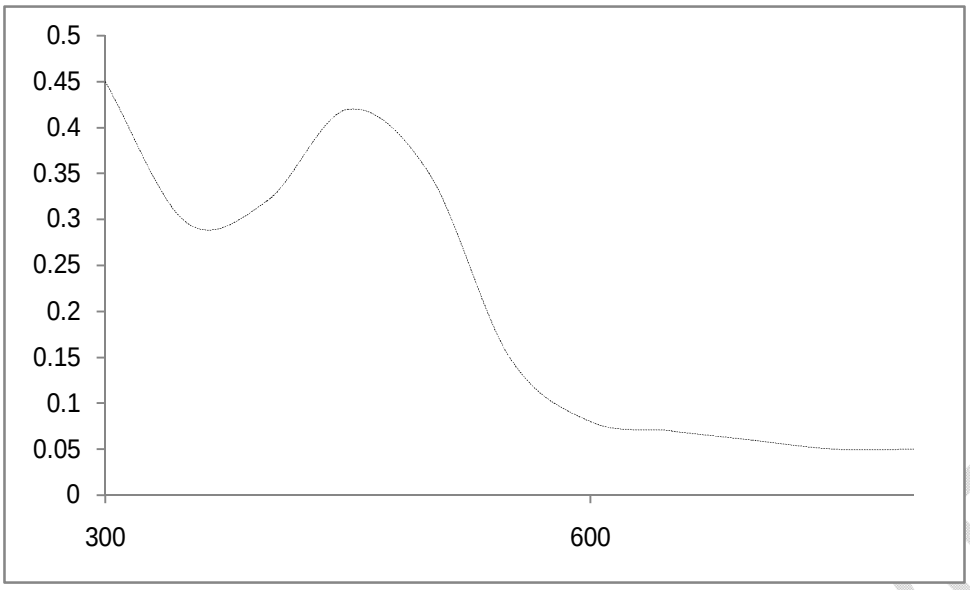

Figure 3. At 0.5 hour after mixing the silver nitrate and leaf extract. Observation showed that thewavelength of maximum absorption was 370nm.

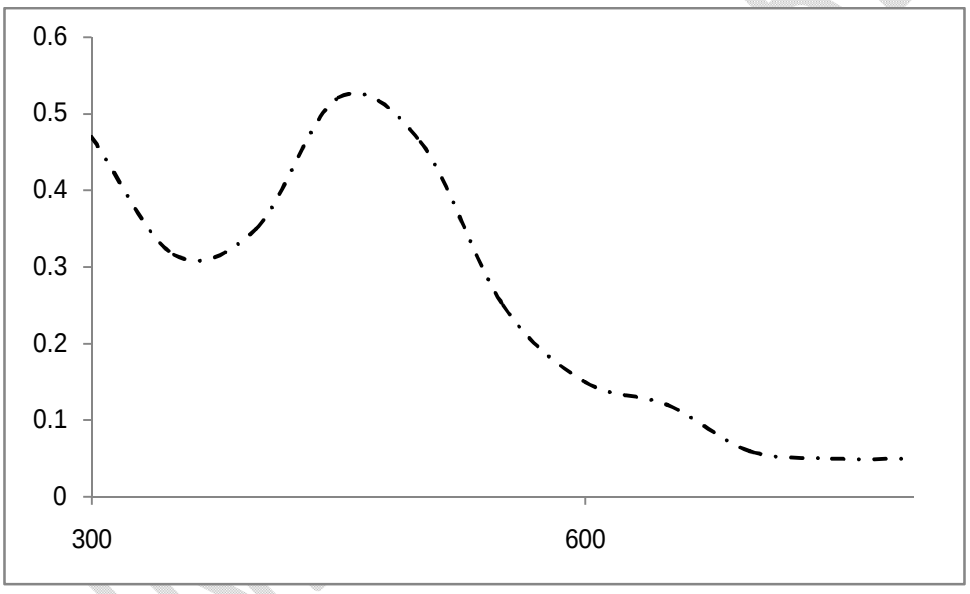

Figure 4 : At 1 hour the wavelength of maximum absorption was noted to be 400nm.

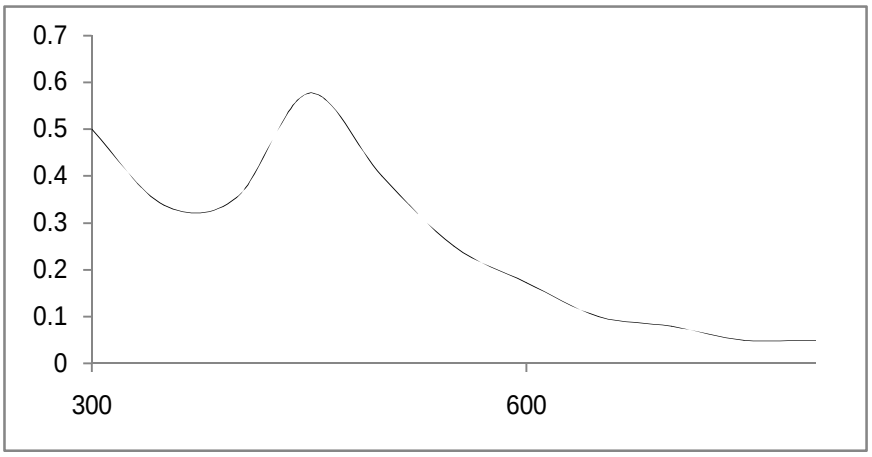

Figure 5 Uv – Vis spectrum result at 1.5 hour

The wavelength of maximum absorption after 1.5 h was 450nm.

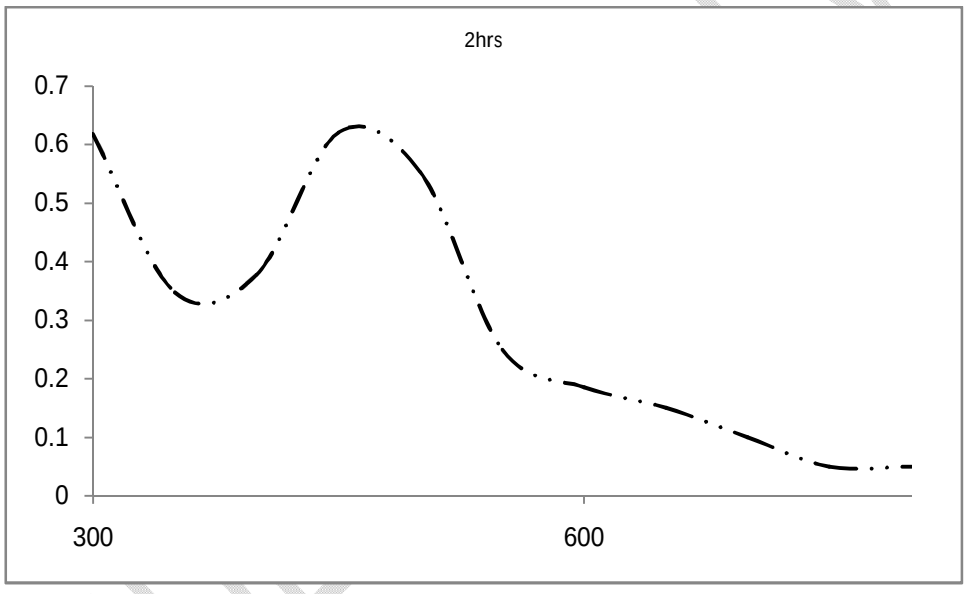

Figure 6 Uv – Vis spectrum result at 2 hours

At 2 h, the maximum wavelength was noted at 430nm.

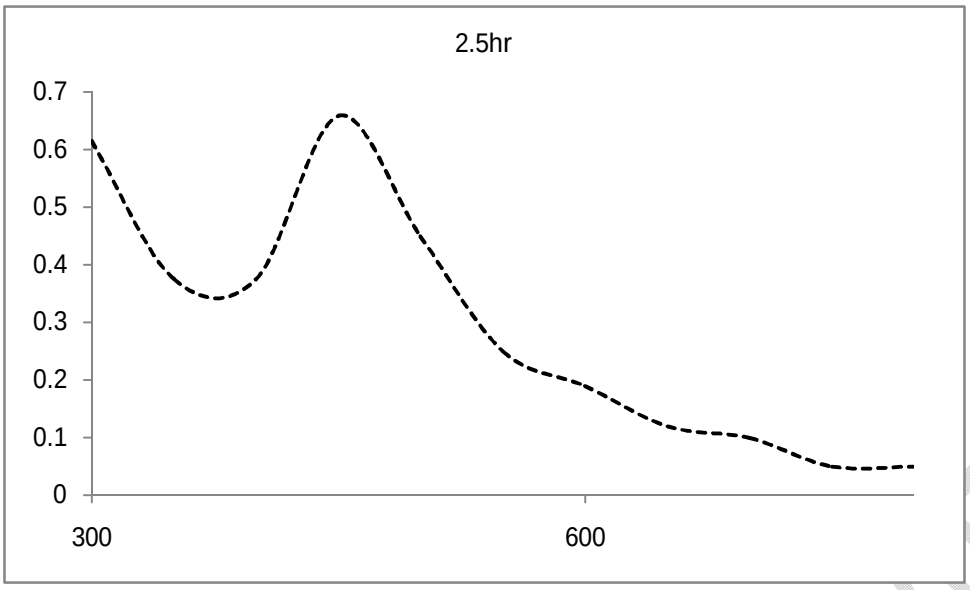

Figure 7 Uv – Vis spectrum result at 2.5 hours

At 2.5 h the maximum wavelength was noted at 450nm.

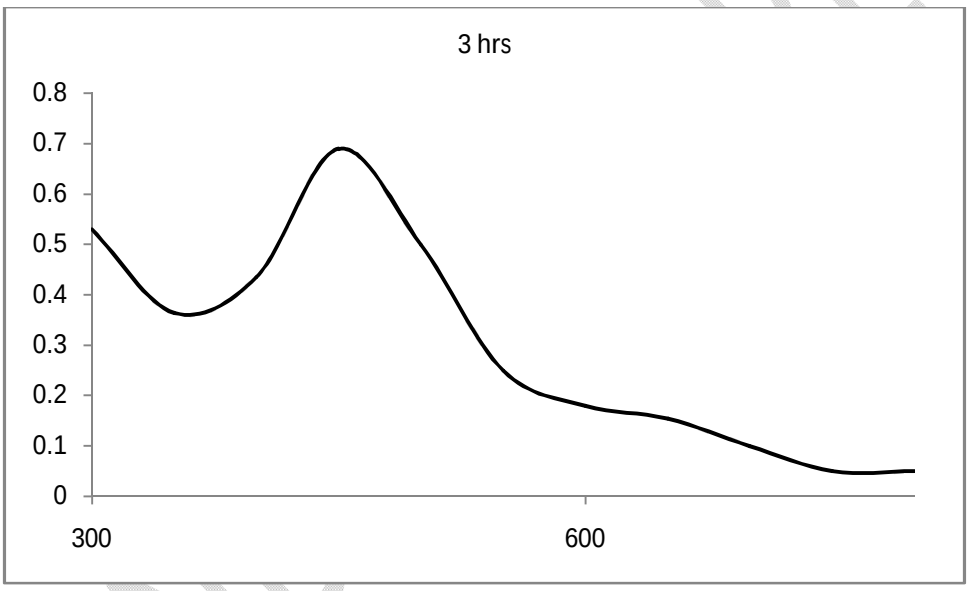

Figure 8 Uv – Vis spectrum result at 3 hours

The maximum wavelength after 3 h was noted at 450nm.

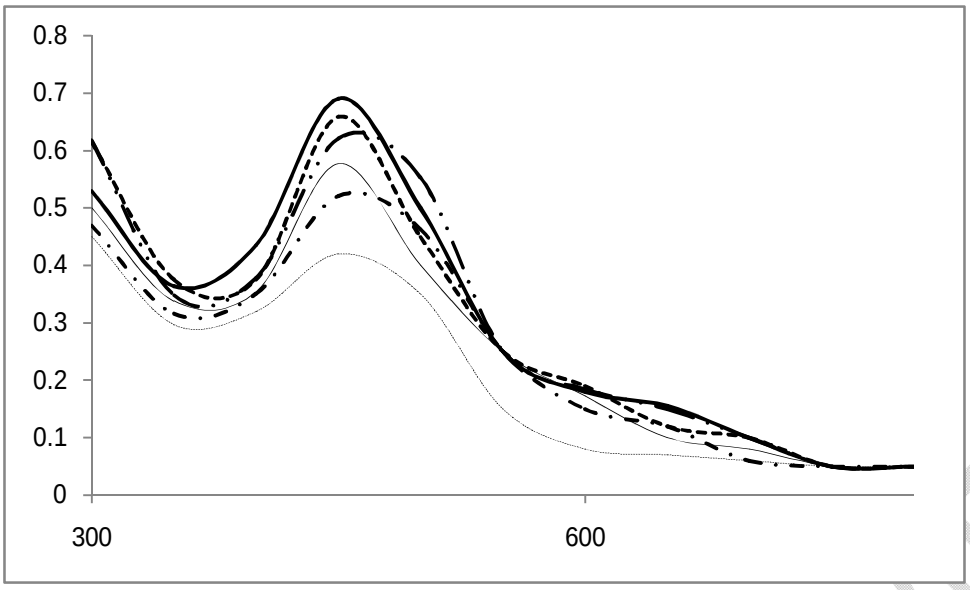

Figure 9 Uv – Vis Spectra result at different times

Figure 9 shows the combined graph of the absorbance against wavelength for various times. It was observed that as time increased, wavelength of maximum absorption increased

The wavelength of maximum absorption 450nm.

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

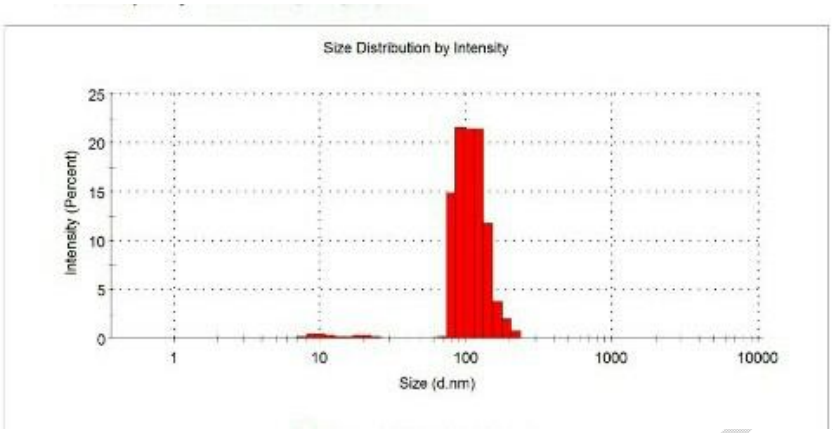

Figure 10 this shows the graph of size distribution by intensity of the synthesized AgNPs using *Annona muricata.*

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): 1.2** **SIZE RANGE: 0 – 10000 nm**		

Table 3. Results MIC of AgNPs against various organisms

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

4.1 DISCUSSION

The silver nanoparticles formed were shown by a color change from yellowish to silvery brown. This could be because of the excitation of surface plasmon vibration[12]. 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. 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[13]**.** Fig 9 illustrates a combination graph of the various reaction time at different times. It was noticed that as time increased the peak increased 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[14,15] 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 by 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 was 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[16]. Particle size obtained does not depend on the method used. An example is the TEM analysis of green synthesized silver nanoparticles using *Azadirachta indica* which showed particle size around 34nm[17]. Furthermore, a study conducted by[18],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 different microorganisms used for the MIC, *Salmonella typhi, E. coli, Bacillus subtilis, Staphylococcus aureus, Candida albicans, Aspergilus 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 Aspergilus niger, Fluconazole 10ug/ml yielded growth in all except Candida albicans and Aspergilus niger. The results show that green synthesized silver nanoparticles of *Annona muricata l* exhibit better anti-bacterial activity than anti-fungal activity. Fungal invasion in food,feed, food products and other monuments by *Aspergillus niger* has been a serious threat. Therefore inhibition of this toxin is studied all over the world. In this regard, nanotechnology has given a promising approach for fungi inhibition with the application of nanoparticles[19]. With the development of nanotechnology,various nanoparticles and nano-quantum dots have been used as labels to enhance the sensitivity of the electrochemical immunoassay technique used in detecting Typhoid fever caused by *salmonella typhi*[20]. "Bacterial pneumonia is one of the leading causes of death worldwide and it is caused by Bacillus subtilis. However, antibiotic therapy and traditional antibiotic delivery are associated with important challenges, including drug resistance, low bioavailability, and adverse side effects; the existence of physiological barriers further hampers treatment. Fortunately, these limitations may be overcome by the application of nanotechnology, which can facilitate drug delivery while improving drug stability and bioavailability"[21].As earlier stated that there has been some challenges in the conventional treatment methods for some diseases like Gastroenteritis caused by E.coli, nanotechnology and in particular nanoparticles have been of great importance in serving as a good drug delivering system. This is because of parameters like its shape and geometry[22].

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 l* 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

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.

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