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GREEN SYNTHESIS, ANTIBACTERIAL SCREENING AND ANTIOXIDANT ACTIVITY OF SILVER NANOPARTICLES (AGNPS) CAPPED WITH METABOLITES FROM ANDROGRAPHIS PANICULATA (BURM.F.) WALL. EX NEES LEAVES

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ABSTRACT

The study presents a novel method for synthesizing silver nanoparticles (AgNPs) using A. paniculata leaves extract as a bioreducing agent for Ag+ ions derived from AgNO3. The biomolecules within the extract are credited with the reduction process. Characterization techniques including UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD) analysis, and Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX) analysis were employed to analyze the properties of the synthesized nanoparticles. UV-Vis spectroscopy revealed a prominent Surface Plasmon Resonance (SPR) peak at 550 nm, indicative of the presence of AgNPs with efficient light absorption and scattering properties. SEM analysis provided insights into the morphology and size distribution of the nanoparticles. XRD analysis confirmed the crystalline nature of the nanoparticles, while EDX analysis corroborated the presence of elemental silver in the nanoparticle composition. The antimicrobial activity of the synthesized AgNPs against a spectrum of human pathogens, particularly noteworthy inhibition against E. coli and S. aureus, highlights their potential as antimicrobial agents. Furthermore, the antioxidant activity assessed through the DPPH scavenging assay underscores the potential health benefits of these nanoparticles. A notable observation was the variation in activity between A. paniculata extract and A. paniculata-AgNPs, with the latter exhibiting reduced inhibitory effects attributed to fewer functional groups on the nanoparticle surface. This finding contributes to a deeper understanding of structure-function relationships in nanoparticle-based applications.

Keywords: Green Synthesis, Nanotechnology, Silver Nanoparticles, Metabolites

1. INTRODUCTION

The field of nanotechnology is the most active area of research in modern material sciences. Though there are many physical and chemical methods,

environment-friendly synthesis is the most emerging method of synthesis. One of the environment-friendly methods is green synthesis that has many possible applications in environmental and biomedical fields. Green synthesis is considered as a key tool in reducing the destructive effects associated with the traditional methods of synthesis for nanoparticles commonly utilized in laboratory and industry Singh et al. (2018). Plant-based extracts have drawn consideration over conventional due to its numerous advantages such as non-hazardous, economical, and feasible methods with variety of applications in biomedicine, and nanotechnology, etc.

Nanoparticles have unique physicochemical properties such as high surface area, high reactivity, tunable pore size, and particle morphology. Nanoparticles can serve as "magic bullets", containing herbicides, nano-pesticide fertilizers, or genes, which target specific cellular organelles in plant to release their content. So, nanoparticle synthesis from plant extracts tentatively offers a route for large scale production of commercially attractive nanoparticles. Silver nanoparticles (AgNPs), among other nanoparticles, acquired more attention because of their unique properties Alharbi et al. (2022). Silver particles are used in different fields including medical, food, healthcare, consumer and industrial, as antibacterial agents, medical device coatings, optical sensors, anticancer agents, etc. Its applications in the life sciences and medicine have focused on antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, and antiangiogenic properties. Thus, AgNPs are reported to have potential anticancer, antimicrobial, antioxidant, antifungal, anti-inflammatory, antiviral, antiangiogenetic, and antiplatelet activities Luhata et al. (2022). Different studies of plant extracts used for the synthesis of AgNPs were in the literature namely *Memecylon edule, Callicarpa maingayi, Terminalia chebula, Trachyspermum ammi and Papaver somniferum, Bauhinia variegate L., Hevea brasiliensis*, Aloe vera and tea leaf Keshari et al. (2020). *Andrographis paniculata (Burm.f.) Wall. ex Nees in Wall* belong to the family *Acanthaceae*. This plant used as traditional medicine to treat infectious diseases and fevers. *Andrographis paniculata* grows in moist and shady places. This plant is erect with 30 -110 cm in height. It has slender stem, dark - green lance - shaped leaves. The blades measure from 8 cm long by 2.5 cm wide. The small flowers produce a capsule from 2cm long and few millimeters wide. Yellow brown seeds ae within these capsules Kumar et al. (2021). This paper will use extracts from *Andrographis paniculata* for the AgNPs that may be a possible source of antimicrobial or antioxidant, anti – inflammatory or antifungal properties.

The use of *Andrographis paniculata* as an effective medicine lasted for centuries especially in Asia. Diseases related to blood abnormalities like skin eruptions, boils, scabies and chronic undetermined fever are treated because of its "blood purifying" effect. The presence of chemical like lactones, diterpenoids, diterpene, glycosides, flavonoids and flavonoid glycosides made this plant a common treatment for upper respiratory tract infection.

The utilization of *Andrographis paniculata* as a medicinal plant for treating a number of diseases in most Asian countries poses critical evaluation since these cases are considered self- limiting Okhuarobo et al. (2014). In the study conducted by Jayakumar et al. (2013), the leaves contained the highest amount of andrographolide, the bitter constituent of the leaves. In addition, this chemical is present in great amount in leaves Jayakumar et al. (2013). The range is from12.44 to 33.52 mg/g in dried leaves found at 90 - 120 days. It is on this premise that the leaves are used for the characterization and antioxidant activity of silver

nanoparticles (AgNPs) capped with metabolites from *Andrographis paniculata leaves.*

2. MATERIALS AND METHODS

2.1. MATERIALS AND TOOLS

Plant samples was collected from Purok 3, Ibung, Villaverde, Nueva Vizcaya. The plant specimen was identified and authenticated at the Institute of Biology, Jose Vera Santos Memorial Herbarium (PUH), College of Science, University of the Philippines, Diliman, Quezon City. Aqueous solution of silver nitrate (AgNO3, 1mM) was freshly prepared at SMU CNS Laboratory from Merck.

The *A. paniculata*-AgNPs was characterized using BK UV 1000 Spectrophotometer, Nicolet 6700 FT-IR Spectrometer, PHYWE X-ray diffraction equipment and JEOL 5310 scanning electron microscope (SEM) - AMETEK EDAX ELEMENT Energy Dispersive Spectroscopy (EDS) System.

2.2. METHOD OF QUALITATIVE AND QUANTITATIVE ANALYSIS

The study utilized the quantitative research using the experimental method used to gather data and to guarantee the accuracy and reliability of the data vital to attain substantial and thorough interpretations of the results.

The research focused on the development of a method for the synthesis of AgNPs that do not include the use of toxic and flammable chemicals. This study made use of *Andrographis paniculata*, also known as Serpentina. The experiment was conducted at Saint Mary's University Center for Natural Sciences Laboratory in December 2022.

Procedures

Extract Preparation

The plant samples were washed with distilled water and oven dried. After drying, the leaves then crushed and soaked in distilled water for 24 hr. at room temperature. The extract was filtered using Whatman filter paper to separate the solid powder affording a clear light-yellow solution.

Biosynthesis of Silver Nanoparticles

Biosynthesis of AgNPs was performed following the procedure used by Song & Kim (2008). A freshly prepared aqueous solution of silver nitrate (AgNO3, 1mM) was used for AgNPs synthesis. Plant extracts (5mL) will be mixed with a 45 mL AgNO3 solution. To observe the effect of temperature on the synthesis of AgNPs, biosynthesis will be carried out at room temperature and 60C.

Characterization of AgNPs

UV-Vis Spectroscopic Analysis

UV-Vis spectroscopic analysis of biosynthesized AgNPs has been performed by continuous scanning from 250 to 750 nm and 1 mM AgNO3 solution was used for baseline correction.

X-Ray Diffraction (XRD) analysis

X-ray diffraction (XRD) analysis of purified AgNPs was performed to describe the diffraction pattern pf the biosynthesized AgNPs.

Fourier Transform Infrared (FTIR) analysis

The fine powder of the biosynthesized AgNPs was analyzed using FTIR to study the biomolecules presence as capping agents on AgNP's surface.

Scanning Electron Microscopy (SEM) and Electron Diffraction Spectroscopy (EDX)

SEM-EDX analysis of purified AgNPs was performed to describe the morphology of the biosynthesized AgNPs.

Free Radical Scavenging Activity

Samples (1 mL) contains different concentrations (50, 100, 150, 200, 250, 300, 350, and 400 uL/mL) of AgNPs, were mixed with 1 mL of freshly prepared DPPH (0.004% w/v in absolute methanol) solution. The reaction solution was incubated for 30 minutes in the dark at room temperature. Absorbance has been recorded at 517 nm using UV-Vis spectrophotometer. Methanol was used as blank, and DPPH was used as control. Free radical scavenging activity was expressed as the percentage of inhibition.

Antibacterial screening

E. coli, and S. aureus was used for the antibacterial screening of AgNPs.

3. RESULTS AND DISCUSSIONS

3.1. RESULT OF QUALITATIVE AND QUANTITATIVE ANALYSIS

1) UV-Vis Spectroscopic analysis

UV-Vis Spectrophotometer was used to detect the visual characteristic and spectrum absorption of the biosynthesized silver nanoparticles. Table 1 shows the absorbance values of the silver nanoparticles.

Table 1

Table 1 The Average Absorbance of the Raw Extract and Synthesized A. Paniculata-AgNPs

Wavelength, nm	Mean absorbance, $\bar{x}$ Raw extract	*A. paniculata*-AgNPs at room temp	*A. paniculata*-AgNPs at 60°C
250	3.000	3.000	3.000
350	3.000	3.000	3.000
450	3.000	3.000	3.000
550	3.000	3.696	2.060
650	3.000	3.000	2.398
750	3.000	2.081	1.377

After addition of *A. paniculata* extract to silver nitrate solution, a UV-Vis scan was taken from 250 to 750 nm. The fixed ration of extract to metal ion solution led the change of color due to the formation of silver nanoparticles. The change in color is primarily attributed to the Surface Plasmon Resonance (SPR) phenomenon exhibited by the silver nanoparticles. SPR is a collective oscillation of free electrons on the surface of metal nanoparticles when they interact with electromagnetic radiation, such as visible light. This interaction leads to the absorption of specific wavelengths of light and the generation of a characteristic absorption spectrum.

Figure 1 shows the SPR peak intensity determined spectrophotometrically using BK UV 1000 Spectrophotometer at 550 nm. AgNPs absorb and scatter light with extraordinary efficiency centered at 550 nm. The strong interaction with light

at 550 nm occurs because the conduction electrons on the silver nanomaterial surface undergo a collective oscillation when they are excited by light at this wavelength. Observation of this strong broad plasmon peak has been well documented for various metal NPs, with sizes ranging all the way from 2 to 100 nm Henglein (1993). A different set of conditions was performed at 60°C and the SPR was measured at room temperature.

Figure 1

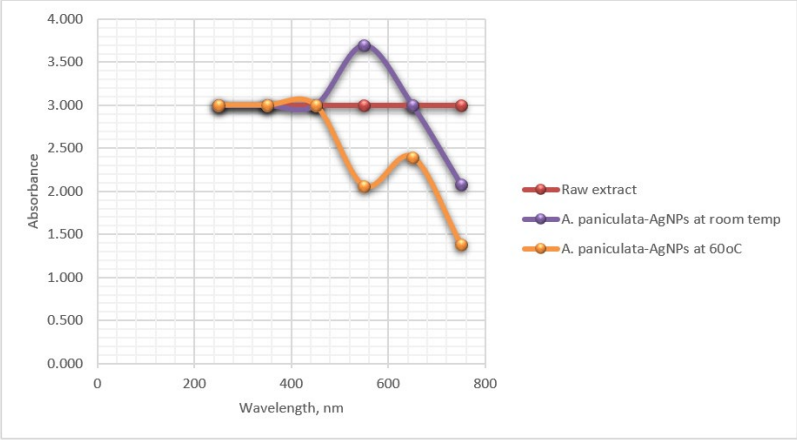


Figure 1 Optimal Synthesis Condition of A. Paniculata-AgNPs

2) FTIR Spectroscopic analysis

The infrared (IR) spectrum of *A. paniculata* exhibits strong peaks at 1064.41 cm^{-1} and 1136.32 cm^{-1}, indicating O-H bending and C-O stretching vibrations, respectively, indicative of hydroxyl (OH) and carbonyl (C=O) or ether (C-O-C) functional groups in the extract. A peak at 3487.15 cm^{-1} corresponds to O-H stretching and N-H extension vibrations, suggestive of hydroxyl and amine (NH_2) or amide (NH-C=O) groups, with mention of inter hydrogen bonds implying molecular hydrogen bonding networks. These spectral features provide insights into the chemical composition and structural characteristics of *A. paniculata*, aiding in the identification of potential bioactive constituents and understanding its pharmacological properties.

Figure 2

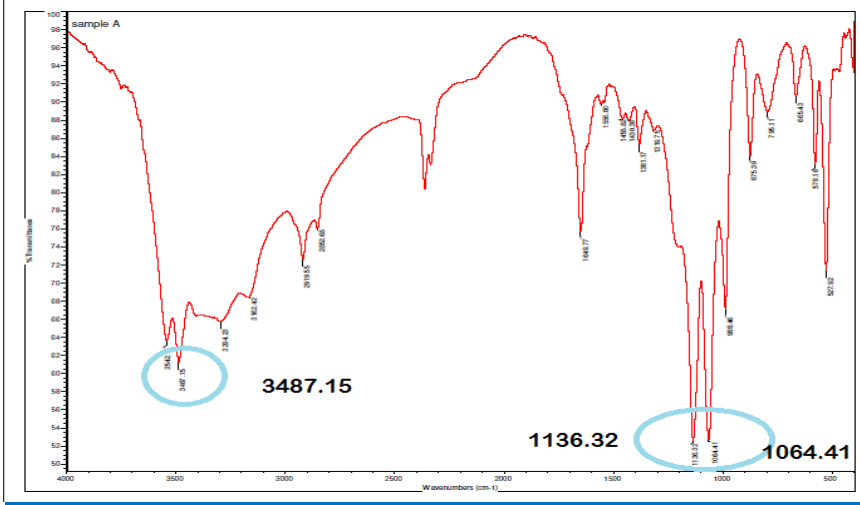


Figure 2 Infrared Spectrum of *A. Paniculata* Extract.

In the infrared (IR) spectrum of *A. paniculata* with Ag at 0H, the multiple bond region from 1500 cm^{-1} to 2500 cm^{-1} reveals numerous absorptions, particularly between 1500 cm^{-1} to 2000 cm^{-1}, indicative of C-C aromatic bonds within the aromatic rings present in the plant's organic compounds. Aromatic C-H stretching vibrations typically appear at higher wavenumbers, specifically around 3100-3000 cm^{-1}, further confirming the presence of aromatic hydrocarbons in the extract. These spectral features are characteristic of compounds containing benzene rings or other aromatic structures, providing valuable information about the aromatic constituents in *A. paniculata* and contributing to the understanding of its chemical composition and potential pharmacological activities.

Figure 3

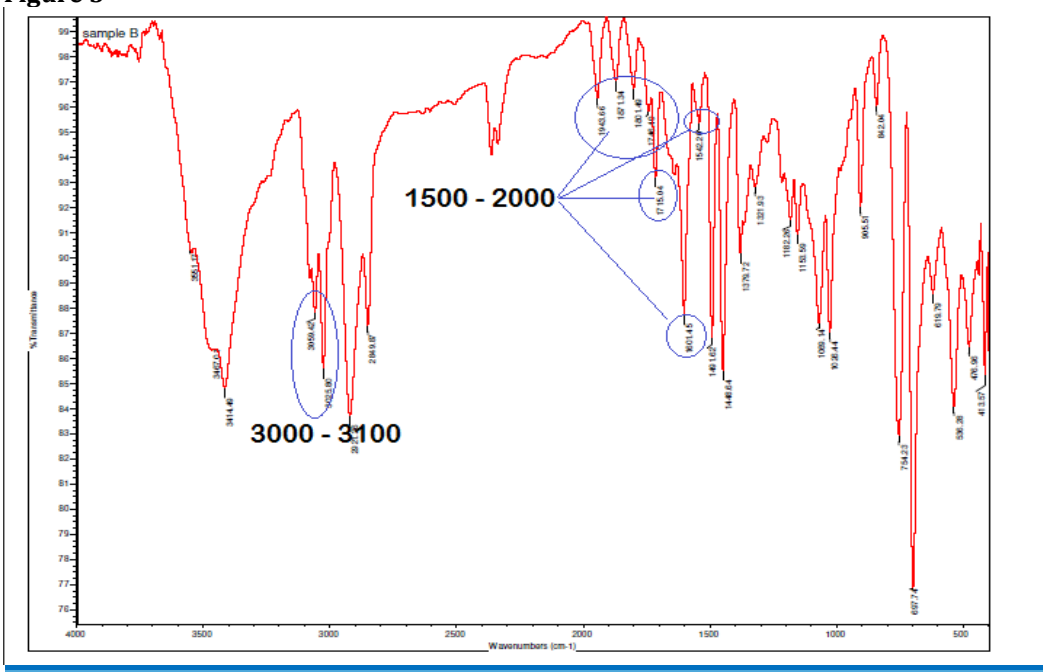


Figure 3 Infrared Spectrum of Interaction of *A. Paniculata* with Ag at 0H.

The presence of characteristic bands at 3000-3100 cm^{-1}, corresponding to C-H stretching vibrations in aromatic compounds, along with bands specific to phenyl groups at 1561.23 cm^{-1}, 1338.68 cm^{-1}, and 926.75 cm^{-1} in the IR spectrum, serves as strong evidence confirming the grafting of silver (Ag) onto the backbone of *A. paniculata*. The C-H stretching bands in the 3000-3100 cm^{-1} range are indicative of aromatic hydrocarbons, aligning with the phenyl groups' vibrations at 1561.23 cm^{-1} (C=C stretching), 1338.68 cm^{-1} (C-H bending), and 926.75 cm^{-1} (C-H out-of-plane bending). These specific bands corroborate the integration of silver nanoparticles onto the aromatic structure of *A. paniculata*, providing insight into the interaction and bonding between Ag and the plant extract, which is crucial for applications such as nanoparticle synthesis and functionalization for various biomedical or environmental uses.

Figure 4

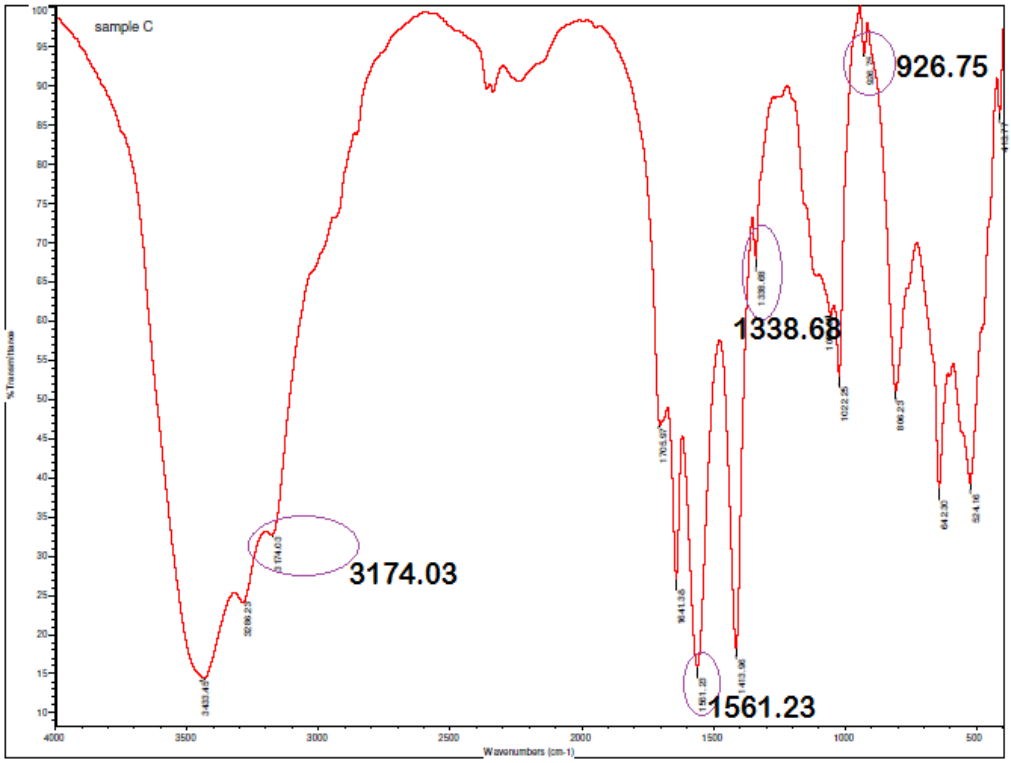
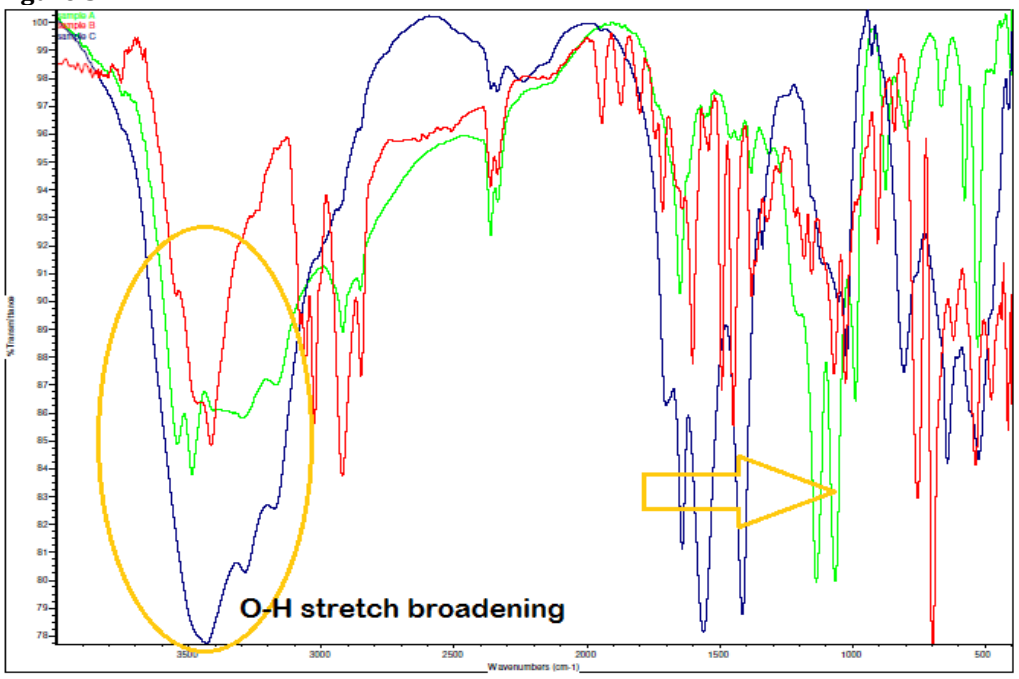


Figure 4 Infrared Spectrum of *A. Paniculata* AgNPs at 1H

Figure 5



*green – A. paniculata extract, red – *A. paniculata* extract with Ag, blue – *A. paniculata* AgNPs

Figure 5 Comparison of IR Spectrum (Overlaid IR Spectra)

The observed shift to slightly higher wavenumbers (rightward shift) in the bands around 1500 cm^{-1} in the IR spectrum, particularly in the *A. paniculata*-AgNPs sample, can be attributed to the phenomenon where heavier atoms tend to absorb at lower frequencies. This shift indicates changes in molecular environments or interactions involving heavier elements, possibly related to the presence of silver nanoparticles (AgNPs) in the *A. paniculata* extract. Additionally, the broadening of the O-H stretch compared to the spectra of *A. paniculata* extract and *A. paniculata* extract with Ag alone is attributed to the complex hydrogen bonding network within the *A. paniculata*-AgNPs sample. Since a significant quantity of compounds is present in the sample, each molecule may engage in hydrogen bonding to varying extents, leading to a range of bond strengths and resulting in adsorptions at varying frequencies during IR spectroscopy. This variation in bonding strengths and frequencies contributes to the broadened appearance of the O-H stretch peak in the IR spectrum, reflecting the average of these slightly different absorptions across the sample.

3) XRD Analysis

Figure 6

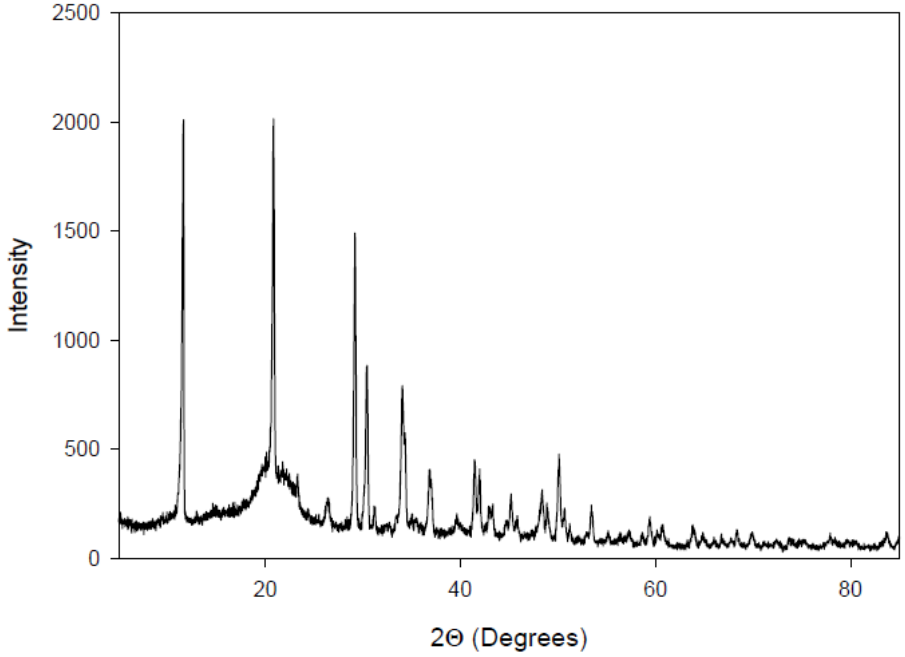


Figure 6 X-Ray Diffractogram of *A. Paniculata* Extract.

The X-ray diffraction (XRD) profile of *A. paniculata* extract reveals characteristic peaks at specific angles, represented as 2Θ (2 times the angle of diffraction). In this case, the XRD pattern exhibits peaks at 2Θ = 11º and 21º, indicating the crystallographic form of the extract. These peaks correspond to the diffraction of X-rays by the crystalline components present in the extract, providing information about their arrangement and structure within the sample. The positions and intensities of these peaks in the XRD pattern are unique to the crystalline phases present in the extract, allowing for identification and characterization of the extract's crystalline form.

Figure 7

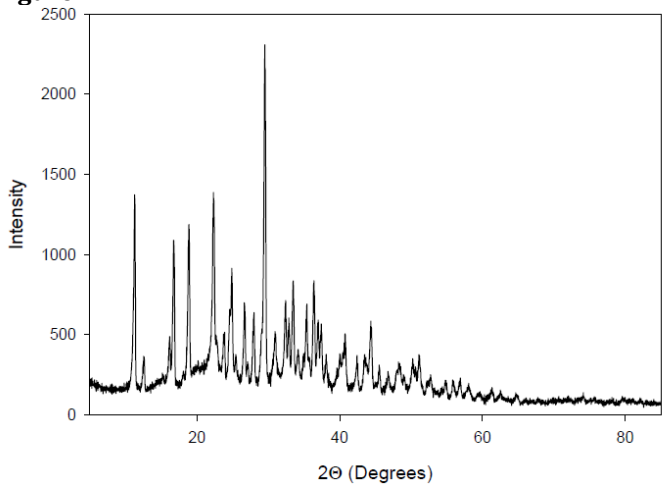


Figure 7 X-Ray Diffractogram of the AgNPs.

The X-ray diffraction (XRD) spectrum of the silver nanoparticles (AgNPs) derived from *A. paniculata* extract exhibits multiple crystalline areas between 11º-28º and 32º-45º in the XRD pattern. This indicates that the AgNPs possess a diverse range of crystal structures within these angular regions. The complexity of crystal structures in the *A. paniculata* extract is reflected in the XRD pattern, where each crystalline form exhibits its distinctive peaks. These peaks are characteristic of the specific arrangement of atoms in the crystal lattice, providing insights into the crystallographic properties and structural diversity of the AgNPs synthesized from *A. paniculata* extract.

Figure 8

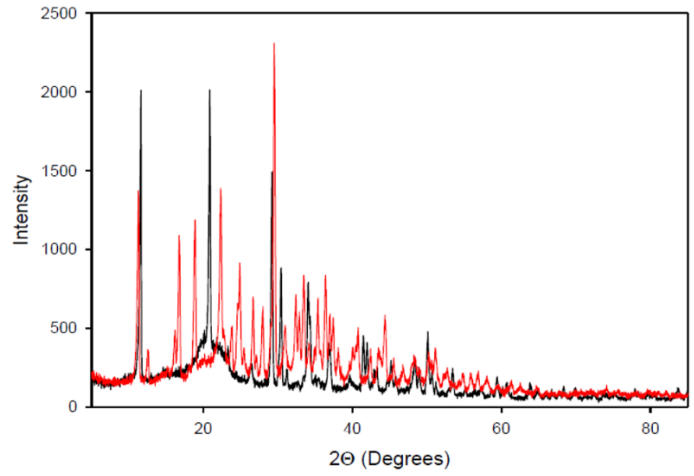


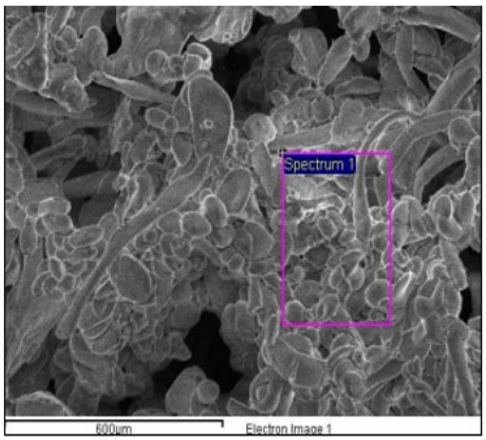
Figure 8 Overlaid X-Ray Diffraction Patterns

The overlaid peak intensities of *A. paniculata* extract (black) and *A. paniculata*-AgNPs (red) in Figure 8 reveal an increase in the number of peak intensities in the resulting diffractogram. This observation suggests that the interaction of silver (Ag) with A*. paniculata* extract leads to the formation of bonds contributing to several diffractions observed. The Bragg reflections in the diffractogram indicate the presence of sets of lattice planes, which can be indexed as a face-centered cubic (FCC) structure typical of silver. This indexing is based on the arrangement of atoms

in the crystal lattice, confirming the crystalline nature of the silver nanoparticles (AgNPs). The XRD differences between *A. paniculata*-AgNPs and the *A. paniculata* extract demonstrate that the crystallographic structure has changed post-reduction, further supporting the formation of AgNPs. Overall, the X-ray diffraction pattern clearly indicates that the AgNPs derived from *A. paniculata* extract are crystalline in nature Shameli et al. (2011).

4) SEM-EDX Analysis

Figure 9



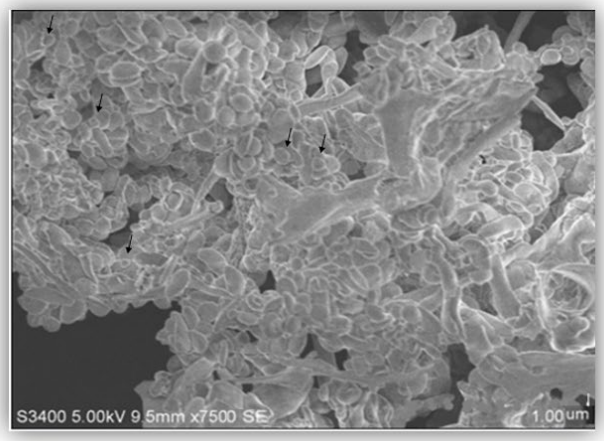
Processing option : All elements analysed (Normalised)

Spectrum	In stats.	N	Ag	Total
Spectrum 1	Yes	96.39	3.61	100.00
Mean		96.39	3.61	100.00
Std. deviation		0.00	0.00	
Max.		96.39	3.61	
Min.		96.39	3.61	

Figure 9 EDX Image of AgNPs: Confirmatory Test for Ag

The Energy Dispersive X-ray Spectroscopy (EDX) spectra of the sample revealed that silver (Ag) and nitrogen (N) were the most abundant elements present. This finding aligns with the synthesis process involving *A. paniculata* extract and silver nitrate solution, where AgNPs are formed with the assistance of nitrogen-containing compounds from the plant extract. The EDX analysis provides quantitative information about the elemental composition of the sample, highlighting the presence of Ag and N as the predominant elements, which is consistent with the expected composition of *A. paniculata*-AgNPs based on the synthesis method used.

Figure 10



Particle	Size (nm)
1	15.2
2	13.09
3	12.08
4	10.50
5	10.1

Figure 10 SEM Micrograph of the AgNPs

The Scanning Electron Microscopy (SEM) analysis of the sample provided insights into the surface morphology and actual size of the silver nanoparticles (AgNPs) synthesized using *A. paniculata* extract. The SEM images showed that the particles were polydispersed, indicating a range of sizes, and exhibited circular shapes. The high density of AgNPs observed in the SEM images, attributed to the *A. paniculata* extract, further confirmed the successful development of Ag nanostructures. This morphology and distribution of AgNPs are crucial for understanding their potential applications, as polydispersed nanoparticles with uniform shapes can offer enhanced properties in various fields such as catalysis, sensing, and biomedical applications.

5) Antibacterial Assay

Table 2

Table 2 Antibacterial Assay of *A. Paniculata*-AgNPs

	Mean zone of inhibition	
	Test organism	
	Escherichia coli	***Staphylococcus aureus***
A. paniculata raw extract	7.18 mm	7.29 mm
A. paniculata-AgNPs at room temperature	12.15 mm	14.38 mm
A. paniculata-AgNPs at 60°C	11.06 mm	14.18 mm
Meropenem (positive control)	28.56 mm	23.19 mm
Negative control	6 mm	6 mm

Legend: <10 mm=inactive 10-13mm= partially active 14-19mm=active >19mm= very active

The results showed that the *A. paniculata* extract was inactive against gram-negative bacteria *Escherichia coli* and gram- positive bacteria *Staphyloccocus aureus.* Meropenem was used as positive control. Meropenem is known for exhibiting strong antibacterial properties. According to the legend above, results that are less than 10 mm are inactive, 10-13 mm is partially active, 14-19 mm is active, and greater than 19 mm is very active. Three petri dishes were used for each bacterium. Both *A. paniculata*-AgNPs produced at room temperature and at 60°C were partially active against *E. coli* having a $\overline{x}$ zone of inhibition of 12.15 mm and 11.06 mm, respectively. Meanwhile, the synthesized AgNPs were active against S. aureus with 14.38 mm $\overline{x}$ mean zone of inhibition for *A. paniculata*-AgNPs at room temperature and 14.18 mm for *A. paniculata*-AgNPs at 60°C.

Synthesized silver nanoparticles have shown superior antimicrobial activity against all tested human pathogens among which exhibited potent inhibitory activity against *E. coli* and *S. aureus*.

6) DPPH Radical Scavenging Assay

The antioxidant activity of the *A. paniculata* extract and *A. paniculata*-AgNPs was assesses using DPPH scavenging assay. Table 3 shows the dose dependent increase in the inhibition percentage of the synthesized *A. paniculata*-AgNPs (60°C) at 50, 100, 150, 200, 250, 300,350, 400 µL/mL concentration. As the concentration of *A. paniculata*-AgNPs increases, the percentage inhibition was found to be increase. Decreasing activity was observed with *A. paniculata* extract and *A. paniculata*-AgNPs (room temp). In comparison to the extract, AgNPs have shown

less percentage of inhibition this may be due to the presence of less amount of functional groups adhered to the nanoparticles.

Table 3

Table 3 DPPH Radical Scavenging Assay Results			
	% Radical Scavenging Activity		
Concentration, µL/mL	*A. paniculata* extract	*A. paniculata*-AgNPs at room temp.	*A. paniculate*-AgNPs at 60°C
50	50.41	25.06	17.31
100	49.35	23.43	18.77
150	42.99	23.20	21.55
200	42.87	21.44	23.71
250	42.63	20.26	28.15
300	42.40	19.43	19.84
350	38.87	18.37	34.94
400	28.74	18.37	31.25

4. CONCLUSIONS AND RECOMMENDATIONS

Synthesis of AgNPs capped with metabolites from *A. paniculata* leaves extract was confirmed by the color change from clear to yellowish brown which indicates formation of AgNPs. AgNPs are crystalline in nature as described by the diffraction pattern from XRD analysis. The EDX spectra of the sample showed that the silver and nitrogen were the most abundant element present in the samples. The SEM analysis revealed the surface morphology of and actual size of the AgNPs. It is concluded that synthesized AgNPs have antioxidant activity due to the capped metabolites. Also, the synthesized AgNPs have shown potential bacterial activity against human pathogenic bacteria. Synthesized AgNPs exhibited slightly equivalent antibacterial activity as compared to meropenem. These results suggest that in the future, AgNPs can be selected as potential antibacterial agent.

CONFLICT OF INTERESTS

None.

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