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Impact of plant-based nanoparticles synthesized from *Carica papaya* and *Bryophyllum pinnatum* against selected microorganisms

Francis Aibuedefe Igiebor[1]✉, Favour Chinaza Michael[1], Ochoyama Haruna[2], and Beckley Ikhajiagbe[3]

[1]Department of Biological Sciences, College of Science and Computing, Wellspring University, Benin City, Nigeria; [2]Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria; [3]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria;
✉***Corresponding author, E-mail: francis.igiebor@lifesci.uniben.edu.***

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Abstract. Plant-based nanoparticles offer sustainable, eco-friendly alternatives to conventional methods, promising antibacterial properties in the face of antibiotic resistance and addressing global health concerns. Five urine and stool samples were collected from the Benin Medical Centre in Benin City, Edo State, and sent to the Wellspring University Research Laboratory for microbiological analysis. *Carica papaya* and *Bryophyllum pinnatum* were used for fresh utilization by washing, weighing, and crushing their leaves, then mixing them with distilled water and heating at 85 °C and 60 °C for 60 minutes. Silver and copper nanoparticles (AgNPs and CuNPs) were synthesized using standard procedures. The NPs were preliminary validated by visual detection of color changes and characterized using a UV-visible spectrophotometer at 300 nm and Fourier transform infrared. The *in vitro* antimicrobial activity of plant-mediated NPs was investigated using five isolates: *S. aureus*, *B. alvei*, *H. pylori*, *P. aeruginosa*, and *E. coli*. The *in vitro* antimicrobial activity of plant-mediated NPs was investigated using five clinical strains displaying multiple resistance to antibiotics: *S. aureus*, *B. alvei*, *H. pylori*, *P. aeruginosa*, and *E. coli*. The agar-well diffusion method showed inhibition of the isolates by plant-mediated NPs but no inhibition by the plant extract alone. The study indicates that plant-mediated NPs

exhibit promising antimicrobial activity, promoting sustainability and eco-friendliness, but further research is needed to assess their safety and efficacy in clinical settings.

Keywords: nanoparticles, resistant, antimicrobial, plant-mediated, MAR index.

Introduction

Plant-based nanoparticles are the green synthesis of nanoparticles (NPs) mediated by plant leaves, stems, soot, fruits, and roots (Ikhajiagbe *et al*., 2021; Igiebor *et al*., 2023). These NPs offer a sustainable and eco-friendly alternative to conventional methods of synthesis (Igiebor *et al*., 2023). By harnessing the natural compounds present in plants, such as flavonoids, phenols, and terpenoids, researchers can create NPs with unique properties and applications (Marslin *et al*., 2018). The versatility of these NPs is remarkable, as they can be tailored for various purposes, including drug delivery systems, water purification, catalysis, and even solar cells (Gupta and Xie, 2018; Joseph *et al*., 2023; Yusuf *et al*., 2023). Moreover, the abundance and diversity of plant sources make this approach highly scalable and cost-effective. With ongoing advancements in this field, plant-based nanoparticles hold great promise for revolutionizing numerous industries while minimizing environmental impact.

NPs are classified based on their physical and chemical properties. Different types of nanoparticles are carbon-based NPs, metal NPs, semi-conductor NPs, lipid-based NPs, ceramic NPs, and polymeric NPs (Troncarelli *et al*., 2013; Igiebor *et al*., 2023). Under the category of metal NPs, silver (Ag), copper (Cu), zinc (Zn), iron (Fe), and gold (Au) are currently used NPs with great antibacterial properties (Sánchez-López *et al*., 2020; Skłodowski *et al*., 2023).

According to Kuppusamy *et al*. (2016), plant-based NPs have excellent antibacterial activity against organisms that are resistant to antibiotics. The great stability and quick rate of plant-based NPs generated interest in understanding and describing the mechanisms of metal ion absorption and bioreduction by plants as a result of the biosynthesis of metal NPs. In this way, numerous investigations have supported this. As a result, a number of plant-based NPs have been successfully synthesized (Ikhajiagbe *et al*., 2021; Igiebor *et al*., 2023). Plants such as *Brassica juncea* (mustard greens, Brassicaceae), *Medicago sativa* (alfalfa, Fabaceae), and *Helianthus annuus* (sunflower, Asteraceae) can accumulate a significant amount of silver when it is present in the substrate (Aswini *et al*., 2021).

The most researched plant-based NPs are silver nanoparticles (AgNPs), which have mainly been linked to the production of powerful antibacterial and antifungal capabilities. In order to synthesize silver nanoparticles (AgNPs), Malabadi *et al*. (2012) employed cell cultures from the leaves, callus, and roots of *Catharanthus roseus* (Apocynaceae). The maximum antimicrobial activity of stabilized AgNPs against all pathogens tested has been demonstrated, demonstrating the efficacy, affordability, and environmental friendliness of nanoparticles with the desired features. It has also been reported that AgNPs can be synthesized quickly, in just 5 hours, by reducing aqueous Ag^{+} ions with *Dioscorea bulbifera* tuber extract (Dioscoreaceae). The resulting AgNPs have potent antibacterial properties against both Gram-negative and Gram-positive bacteria. Due to its distinctive phytochemistry, this plant species also has significant therapeutic potential (Ghosh *et al*., 2012).

Moreover, copper nanoparticles (CuNPs) have also shown promising antimicrobial properties. These NPs have been synthesized using various methods, including chemical reduction and green synthesis approaches. In other studies (Ali *et al*., 2021; Ahmed *et al*., 2023), CuNPs were successfully prepared using the leaf extract of *Azadirachta indica* (Neem). The synthesized CuNPs exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria, making them a potential candidate for combating microbial infections. Also, CuNPs have been found to possess antifungal properties, as demonstrated in a study by Singh *et al*. (2020). They reported that CuNPs synthesized using the fruit extract of *Syzygium cumini* showed significant inhibition of fungal growth, highlighting their potential as an alternative treatment for fungal infections. Therefore, the development of silver and copper nanoparticles offers a promising avenue for the production of effective antimicrobial agents with broad-spectrum activity against bacteria.

Materials and methods

Sample collection

A total of five (5) samples were obtained from the Benin Medical Centre (BMC), Benin City, Edo State. The samples were immediately transported to the laboratory for microbiological analysis. Fresh leaves of *Carica papaya* and *Bryophyllum pinnatum* were obtained from the agricultural farm at Wellspring University, Benin City, Edo State.

Preparation of leaves extract

Carica papaya leaves were washed with distilled water to remove dust and dirt on the surface of the leaves and to leave no impurities. For fresh use, 20g of the leaves was weighed using the weighing balance, and thereafter transferred into mortar and pestle, the leaves were carefully crushed and transferred into a beaker containing 100 ml of distilled water. The mixture was heated in a water bath at 85 ℃ for 60 minutes, and then kept to cool at room temperature. The mixture was filtered using whatman filter paper.

The surface *Bryophyllum pinnatum* leaves were washed with distilled water and crushed into tiny pieces on a foil paper and was placed into the oven at 60 ℃ until fully dried, the dried plant leaves was grinded to fine powder using a dry electrical blender, 5 g of the plant powder was dissolved into 50 ml of distilled water and was placed in the water bath at 60 ℃ for 60 minutes, when it cooled to room temperature, the plant extract was filtered using whatman filter paper.

Synthesis of silver nanoparticles (AgNPs) using plant extracts

For the synthesis of silver nanoparticles, 1.7 g of $AgNO_3$ salt was dissolved in 1000 ml of distilled water to prepare 10 mM of $AgNO_3$ stock solution. Thereafter, 45 ml of precursor $AgNO_3$ solution was measured in a measuring cylinder and poured into a beaker covered in foil paper and labelled for each plant extract, 5 ml of the aqueous extract of *Carica papaya* filtrate is dropped (using dropping pipette) into the beaker containing silver nitrate ($AgNO_3$), while stirring using magnetic stirrer for an hour, during this synthesis the lights in the laboratory was switched off. There was an observation for change in colour. The same procedure was done for *Bryophyllum pinnatum* filtrate, 5 ml of the plant extract filtrate of *Bryophyllum pinnatum* was dropped into the beaker (the beaker was also wrapped in foil paper) containing 45 ml of silver nitrate, while stirring on the magnetic stirrer, during this synthesis the lights in the laboratory was also switched off, there was an observation for colour change. The formation of AgNPs was characterized with the development of colour, which was produced as a result of the reduction of silver ion by biomolecules present in the plant extract. The colour of the solute changed (Melkamu and Bitew, 2021; Asif *et al*., 2022).

Synthesis of copper nanoparticles (CuNPs) using plant extract

The synthesis was done according to the method of Rajesh *et al*. (2018). 24.9 g of copper sulphate ($CuSO_4$) was dissolved in 1000 ml distilled water to prepare 10 Mm of $CuSO_4$ stock solution. CuNPs were produced by dropping

1 ml (using a dropping pipette) of *Bryophyllum pinnatum* extract into a beaker (the beaker was wrapped in foil paper and labelled), containing 20 ml of the precursor $CuSO_4$, while stirring using the magnetic stirrer for an hour, there was an observation for change in colour. The same procedure was done for *Carica papaya* leave extract filtrate, 1ml of the leave extract filtrate was poured into a beaker (the beaker was labelled and wrapped in foil paper) containing 20 ml of precursor $CuSO_4$, while stirring on the magnetic stirrer, an observation of colour change was expressed.

Antibacterial activity of AgNPs and CuNPs on the selected pathogens

The *in vitro* antimicrobial activity of AgNPs and CuNPs was investigated using the method of Balouiri *et al*. (2016). Filter paper disc diffusion and Agar wells methods were used to test the antibacterial capabilities of CuNPs and AgNPs, to test if silver nitrate ($AgNO_3$) and copper sulphate ($CuSO_4$) has inhibitory effects, as well as compare the nanoparticles synthesised from each plant (*Bryophyllum pinnatum* and *Carica papaya*) for their inhibition activities.

Filter paper disc diffusion test

This was carried out using the methods of Zia *et al*. (2018). A 3.8 g of Mueller Hinton agar was dissolved in 100 ml of distilled water and autoclaved at 121 °C for 25 minutes. After sterilization the medium was kept to cool, then poured into sterile Petri plates/dish (one dish for each isolate) to solidify. Sterile swab sticks were used to collect an inoculum and spread evenly on the surface of Mueller Hinton agar plates, filter papers were carefully cut to small circular pieces and soaked with CuNPs, AgNPs, plant extract, $CuSO_4$, and $AgNO_3$ separately. The filter paper discs loaded with CuNPs, AgNPs, plant extract, $CuSO_4$, and $AgNO_3$ was placed on the surface of the inoculated plates (each solute was placed according the label at the back of the plates). Before incubation, the plates were kept at room temperature for few minutes to allow the diffusion of the solutions, and then afterwards placed at 37 °C for 24 hours. The zones of inhibition was measured (diameter in mm). This procedure was done for each extract solution; this implies the nanoparticles synthesized from each plant extract followed this filter disc diffusion step.

Agar wells diffusion method

Zia *et al*. (2018) protocol was adopted with few modifications. A 9.5 g of Mueller Hinton agar to 250 ml of distilled water was prepared and sterilized using the autoclave at 121 °C. The medium was transferred to Petri plates and

allowed to solidify. A sterile swab stick was used to collect an inoculum and spread evenly on the surface of the agar plates. After some time, five wells were punched into the inoculated Mueller Hinton agar plates that was well separated using 6 mm cork borer, thereafter 10 μL (using a micropipette) of CuNPs, AgNPs, $CuSO_4$, $AgNO_3$ and plant extract solution are poured into each well, the samples were allowed to diffuse into the agar by keeping them under room temperature for few minutes, before proceeding to keep in the incubator, after 24 hours of incubation, the diameter of the zone of inhibition was evaluated and measured in mm. the inhibition zone of the different nanoparticles (CuNPs and AgNPs) that was gotten from the two plants (*Bryophyllum pinnatum* and *Carica papya*) used was compared and recorded.

Nanoparticles characterization

UV-visible characterization

UV-Visible spectroscopy analysis was carried out on a UV Visible absorption spectrophotometer. Equal amounts of the suspension were taken and analysed at room temperature. The progress of the reaction between metal ions and the leaf extract was monitored at different wavelength, between spectra ranges of 300 to 800 nm.

Fourier Transform Infrared (FT-IR) characterization

This technique is a powerful tool used to identify the chemical bonds in a molecule by producing an IR spectrum that is similar to a molecular fingerprint. FT-IR characterization was also done to analyse the functional groups of samples. About 1-2 mg of powdered leaves samples were mixed with potassium bromide, pressed into a pellet (KBr pellet) and placed in the machine. The FT-IR instrument sent infrared radiation of about 10,000 to100 cm^{-1} through the sample, with some radiation absorbed and some passed through. The absorbed radiation was converted into rotational and vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm^{-1} to 400 cm^{-1}, representing a molecular fingerprint of the sample. Each molecule or chemical structure thus produces a unique spectral finger print (Wang and Weller, 2006).

Identification of the isolates collected

Isolates were identified following the procedure of Cheesbrough (2006). The colony morphology of the isolates were observed and recorded. Gram staining of the isolates was carried out. The biochemical tests used to confirm

the collected isolates were oxidase, citrate, catalase, motility, sugar fermentation, indole, urease, coagulase (Maduka *et al*., 2022).

Antimicrobial Susceptibility Test (AST)

Antimicrobial susceptibility test was carried out on each isolate using the disc diffusion method, to evaluate the sensitivity of test organisms to various antibiotics. Mueller hinton agar was used for this medium, 3.8 g of Mueller Hinton agar was dissolved in 100 ml of distilled water, an sterilized in the autoclave at 121 ℃ for 25 minutes, the medium was poured into petri plates/dish to solidify, using a sterile swab the test organism was carefully spread on the agar plate, waited for few seconds before placing the discs, the discs was placed on the surface of the inoculated Mueller Hinton agar plate (using a sterile forceps) then placed inside the incubator 37 ℃ for 24 hrs. After incubation, diameters (measured in mm) of zones of inhibition were measured using a ruler, the results for sensitivity and resistance was evaluated using Clinical Laboratory Standard Institute (CLSI, 2020).

Multiple antibiotic resistance (MAR) index

The MAR was determined for each isolate by dividing the number of antibiotics the isolate was resistant to by the total number of antibiotics tested (Ehiaghe *et al*., 2022).

$$MAR\ index = \frac{a}{b}$$

Key: a = the number of antibiotics which the test isolate showed resistance; b = the total number of antibiotics used in subjecting the isolates to susceptibility test.

Results

Table 1 shows the qualitative phytochemical screening of *Bryophyllum pinnatum* and *Carica papaya* leaves. The content of cardiac glycosides was very high in *Bryophyllum pinnatum* but high in *Carica papaya*. Flavonoids, tannins, saponin, steroids, phenols, alkaloids and terpenoids contents were present in both plants, whereas phlabotannins, coumarin and anthraquinone contents were absent.

Table 1. Qualitative phytochemical screening of *Bryophyllum pinnatum* and *Carica papaya* leaves

Active ingredients	*Bryophyllum pinnatum*	*Carica papaya*
Flavonoids	++	++
Tannins	++	++
Cardiac glycosides	+++	++
Saponin	-	+
Steroids	++	+
Phenols	++	++
Phlabotannins	-	-
Coumarin	-	-
Alkaloids	+	++
Anthraquinone	-	-
Terpenoids	+	++

Key: - Negative (Absent); + Positive (Present) But low; ++ (High); +++ (Very high)

Table 2 shows the results of the antibiotics susceptibility test for Gram-positive bacteria. Two clinical isolates were tested against twelve antibiotics, *Staphylococcus aureus* was resistant to ten antibiotics and sensitive to only two antibiotucs. However, *Bacillus alvei* was resistant to all the twelve antibiotics.

Table 3 shows the results of the antibiotics susceptibility test for Gram-negative bacteria. Three clinical isolates were tested against twelve antibiotics. *Pseudomonas aeruginosa* and *Helicobacter pylori* were resistant to all the twelve antibiotics. However, *Escherichia coli* was only sentivie to only one antibiotic (ofloxacin), but resistant to eleven antibiotics.

Table 4 shows the multiple antibiotic resistance (MAR) index of the isolates. It was observed that the isolates MAR index was greater than 0.2 signified that the organisms have originated from high- risk sources of contamination, where antibiotics are often used.

Table 2. Antibiotics susceptibility test for Gram-positive bacteria

Antibiotic disks			**Inhibition zone diameter (mean±standard deviation, mm)**		****CLSI standard (mm)**	
Test/ Report group	**Antimicrobial agent**	**Disk content**	****Staphylococcus aureus***	****Bacillus alvei***	**S ≥**	**R ≤**
Aminoglycosides	Gentamicin	10µg	R	R	15	12
Macrolides	Azithromycin	15µg	R	11.67±2.08	18	13
	Erythromycin	15µg	R	R	23	13
Quinolones & Fluoroquinolones	Ciprofloxacin	5µg	14.67±1.33	13.67±1.45	21	15
	Levofloxacin	5µg	R	R	19	15
	Ofloxacin	5µg	R	R	18	14
Carbapenems	Imipenem	5µg	20.67±1.76	11.00±0.58	19	15
β-lactam combinations	Amoxicillin-clavulanate	30µg	19.00±1.53	15.33±1.76	18	13
Cephems (parenteral)	Cefotaxime	25µg	R	R	26	22
	Ceftriaxone	45µg	11.00±0.58	R	23	19
	Cefuroxime	30µg	R	14.00±2.00	18	14
Cephems (oral)	Cefixime	5µg	R	R	19	15

*CLSI standard for *Bacillus alvei* could not be determined;

**CLSI standard for *Staphylococcus aureus*

Key: S = Sensitive; I = Intermediate; R = Resistant.

Figure 1 shows the absorbance spectrum of plant-mediated nanoparticles. It was observed that *Carica papaya*-mediated AgNPs and *Bryophyllum pinnatum*-mediated CuNPs had the highest absorbance, of 1.9 at 300 nm. *Bryophyllum pinnatum*-mediated AgNPs had absorbance of 1.6 and *Carica papaya*-mediated CuNPs had absorbance of 1.3 at 300 nm.

Table 3. Antibiotics susceptibility test for Gram-negative bacteria

Antibiotic disks			**Inhibition zone diameter (mean±standard deviation, mm)**			****CLSI standard (mm)**	
Test/Report group	**Antimicrobial agent**	**Disk content**	*****Pseudomonas aeruginosa***	****Helicobacter pylori***	*****Escherichia coli***	**S ≥**	**R ≤**
Aminoglycosides	Gentamicin	10µg	13.33±1.76	12.00±1.15	12.67±1.76	15	12
Quinolones & Fluoroquinolones	Levofloxacin	5µg	14.00±2.31	14.00±2.31	16.00±2.31	22	14
	Ofloxacin	5µg	R	R	23.33±1.76	16	12
	Nalidixic acid	30µg	R	R	13.33±1.76	19	13
Carbapenems	Imipenem	10µg	R	R	R	19	15
β-lactam combinations	Amoxicillin-clavulanate	30µg	R	R	R	18	13
Cephems (parenteral)	Cefotaxime	25µg	R	R	13.33±2.40	26	22
	Ceftriaxone	45µg	11.33±1.33	14.00±1.15	R	23	19
	Cefuroxime	30µg	R	10.67±0.67	R	18	14
Cephems (oral)	Cefixime	5µg	R	12.00±1.15	14.67±2.91	18	14
Nitrofurans	Nitrofurantoin	300µg	14.00±1.15	10.00±0.00	R	17	14
Penicillins	Ampiclox	10µg	R	R	13.33±1.76	17	13

*CLSI standard for *Helicobacter pylori* could not be determined;

**CLSI standard for *Pseudomonas aeruginosa* and *Escherichia coli*

Key: S = Sensitive; I = Intermediate; R = Resistant.

Table 4. Multiple antibiotic resistance (MAR) index of the isolates

Isolates	**Sample**	**MAR index**	**Standard (≤0.2)**
Staphylococcus aureus	Urine	0.83	
Escherichia coli	Urine	0.92	
Pseudomonas aeruginosa	Urine	1.00	**High risk**
Helicobacter pylori	Stool	1.00	
Bacillus alvei	Stool	1.00	

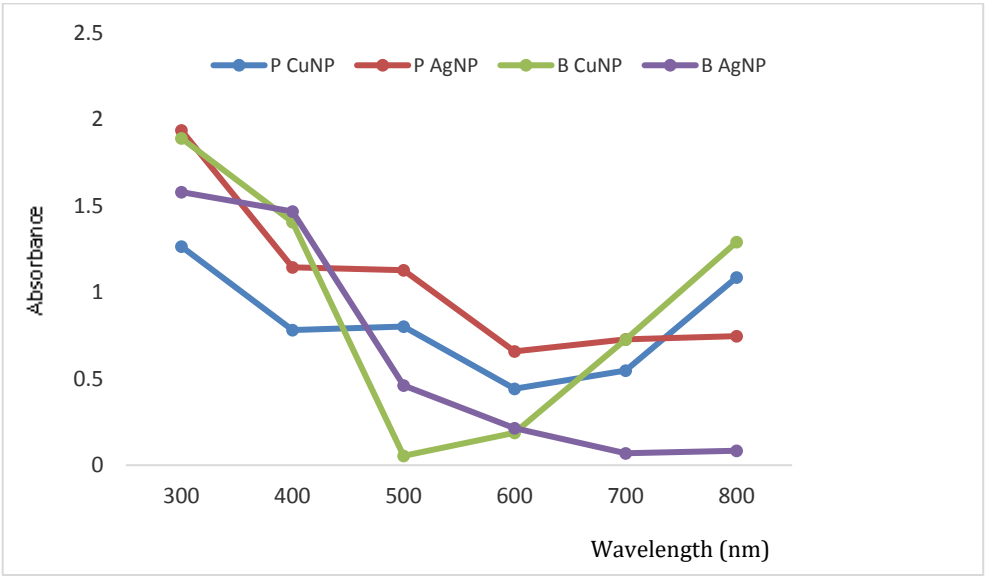

Figure 1. UV-VIS absorbance spectrum of P CuNPs (*Carica papaya* copper nanoparticles), P AgNPs (*Carica papaya* silver nanoparticles), B CuNPs (*Bryophyllum pinnatum*), B AgNPs (*Bryophyllum pinnatum* silver nanoparticles), at room temperature.

It is evident that the major peak positions (Figure 2) for *B. pinnatum*-mediated AgNPs were observed at 3,464.18 and 1,640.57 cm^{-1}, while for *B. pinnatum*-mediated CuNPs, the peaks were found at 3,437.41 and 1,643.49 cm^{-1}. Similarly, *C. papaya*-mediated AgNPs exhibited peaks at 3,439.28 and 1,643.73 cm^{-1}, whereas *C. papaya*-mediated CuNPs displayed peaks at 3,438.82 and 1,643.78 cm^{-1}.

These results demonstrate that there are slight variations in peak positions among the different plant extracts used, indicating the presence of residual plant extract as a capping agent for both AgNPs and CuNPs. The similarities observed in the spectra further support this conclusion, as they suggest a common mechanism of nanoparticle synthesis involving the plant extracts as stabilizing agents.

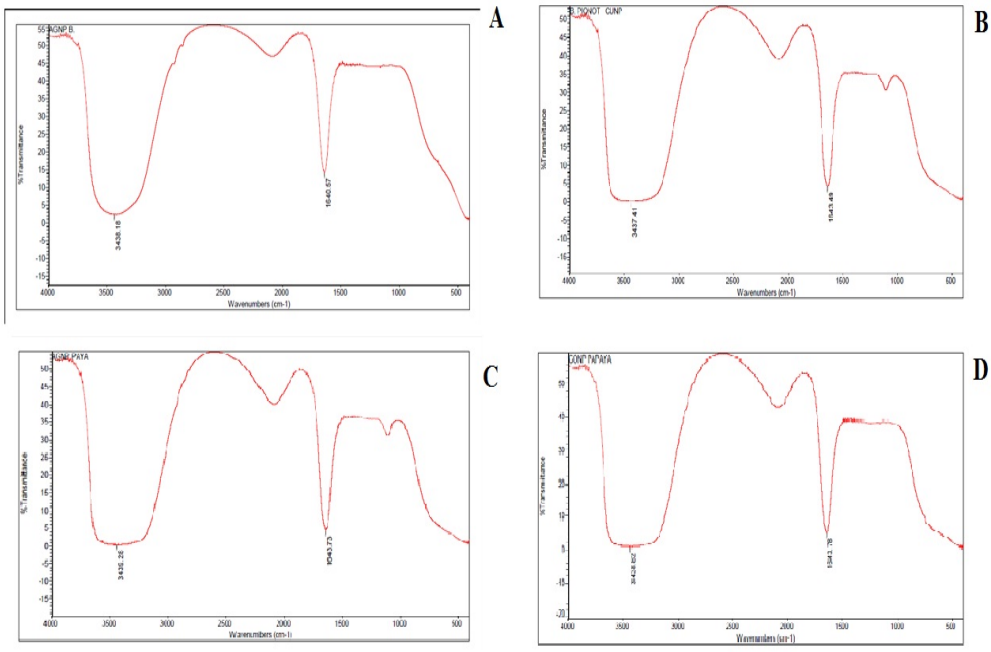

Figure 2. FT-IR spectrum of synthesized nanoparticles
(a) *B. pinnatum*-mediated AgNPs (b) *B. pinnatum*-mediated CuNPs
(c) *C. papaya*-mediated AgNPs (d) *C. papaya*-mediated CuNPs

Table 5 shows the antimicrobial activities of copper sulphate ($CuSO_4$) and silver nitrate ($AgNO_3$). The results revealed that there were high inhibitory activity of *Escherichia coli*, *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Bacillus alvei* in $CuSO_4$ and $AgNO_3$ ranging from 7 – 24 mm using the filter and Agar-well diffusion methods. However, there was no activity of inhibition of *Staphylococcus aureus* by $AgNO_3$. Generally, $CuSO_4$ had a better inhibition in both filter discs and agar wells methods.

Table 6 shows the antimicrobial test using filter paper discs diffusion method. The result showed that *Carica papaya* and *Bryophyllum pinnatum*-mediated CuNPs and AgNPs inhibited the activity of the *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Bacillus alvei* However, *Carica papaya*-mediated AgNPs could not inhibit the activity of *Pseudomonas aeruginosa* and *Helicobacter pylori*. Similarly, the plant extracts alone could not inhibit the activity of the isolates. Interestingly, *Carica papaya*-mediated CuNPs and *Bryophyllum pinnatum*-mediated AgNPs had better inhibition levels.

Table 5. Antimicrobial activities of $CuSO_4$ and $AgNO_3$

Isolates	Filter disc Inhibition zone diameter (mean±standard deviation, mm)		Agar wells Inhibition zone diameter (mean±standard deviation, mm)	
	$AgNO_3$	**$CuSO_4$**	**$AgNO_3$**	**$CuSO_4$**
Staphylococcus aureus	NA	20.33±1.53	6.67±1.53	21.67±1.53
Escherichia coli	19.33±1.53	22.00±1.00	9.33±2.08	20.33±2.08
Pseudomonas aeruginosa	23.33±1.53	16.67±1.53	6.33±2.08	13.00±2.64
Helicobacter pylori	24.67±2.08	17.00±2.64	7.00±2.64	18.00±2.64
Bacillus alvei	23.00±2.64	25.33±1.53	6.33±2.08	17.67±1.53

Mean±Standard error values in triplicate

Key: NA = No activity

Table 6. Antimicrobial activity using filter paper method

Isolates	*Carica papaya* Inhibition zone diameter (mean±standard deviation, mm)			*Bryophyllum pinnatum* Inhibition zone diameter (mean±standard deviation, mm)		
	CuNPs	**AgNPs**	**CA extract**	**CuNPs**	**AgNPs**	**BP extract**
S. aureus	20.67±1.53	5.67±1.53	NA	17.00±2.00	12.67±2.52	NA
E. coli	31.00±2.00	20.33±1.53	NA	13.00±2.00	19.67±3.06	NA
P. aeruginosa	26.67±1.53	NA	NA	13.67±1.53	19.67±1.53	NA
H. pylori	17.00±2.00	NA	NA	17.00±2.00	30.00±3.00	NA
B. alvei	30.00±2.00	20.00±2.00	NA	21.67±2.52	19.67±2.52	NA

Mean±Standard error values in triplicate

Key: NA = No activity; CuNPs = copper nanoparticles; AgNPs = silver nanoparticles; CA extract = *Carica papaya*; BP extract = *Bryophyllum pinnatum*.

Table 7 shows the antimicrobial test using agar wells diffusion method. The result reveals 100 % inhibition of the isolates by *Carica papaya* and *Bryophyllum pinnatum*-mediated copper and silver nanoparticles. However, there no inhibition of isolates by the plant extract alone.

Table 7. Antimicrobial activity using agar wells diffusion method

Isolates	*Carica papaya* Inhibition zone diameter (mean±standard deviation, mm)			*Bryophyllum pinnatum* Inhibition zone diameter (mean±standard deviation, mm)		
	CuNPs	**AgNPs**	**CA extract**	**CuNPs**	**AgNPs**	**BP extract**
S. aureus	21.33±1.04	6.00±2.00	NA	24.00±2.00	9.83±2.25	NA
E. coli	18.33±2.52	6.00±1.00	NA	12.67±1.53	4.33±0.58	NA
P. aeruginosa	34.83±1.52	6.50±1.32	NA	11.33±1.15	18.33±0.58	NA
H. pylori	18.17±0.76	15.83±0.76	NA	14.83±1.26	15.17±0.76	NA
B. alvei	20.33±1.52	6.83±1.26	NA	17.83±1.26	19.17±0.76	NA
Mean±Standard error values in triplicate						

Key: NA = No activity; CuNPs = copper nanoparticles; AgNPs = silver nanoparticles; CA extract = *Carica papaya*; BP extract = *Bryophyllum pinnatum.*

Discussion

The impact of plant-based nanoparticles synthesized from *Carica papaya* and *Bryophyllum pinnatum* against selected microorganisms has been investigated. The time of addition of the plant extracts to the metal ion solution was considered the start of the reaction. It is well known that silver nanoparticles exhibit a yellowish-brown colour in aqueous solutions due to the excitation of surface plasmon vibrations in silver nanoparticles (Sulochana *et al*., 2012). The colour change of the solution to green after the reaction of plant extract with copper ions can be used to illustrate the synthesis of copper nanoparticles (Gebremedhn *et al*., 2019).

The phytochemical constituents of *Bryophyllum pinnatum* and Carica papaya are known for their diverse biological activities and potential health benefits. However, it is worth noting that phlabotannins, coumarin, and anthraquinone were not detected in either *Bryophyllum pinnatum* or *Carica papaya*. This information sheds light on the unique chemical profiles of these two plants and underscores their potential applications in various fields, such as medicine and nutrition. According to Ahmad *et al*. (2010), flavonoids, which were one of the phytochemicals detected in this study, contain various functional groups that

have an enhanced ability to reduce metal ions. The reactive hydrogen atom is released due to tautomeric trans-formations in flavonoids, through which the enol-form is converted into the keto-form. This process is realised by the reduction of metal ions into metal nanoparticles.

The absorbance spectrum in this study reveals that *Carica papaya*-mediated AgNPs and *Bryophyllum pinnatum*-mediated CuNPs exhibit the highest absorption at 300 nm, reaching an impressive value of 1.9. On the other hand, *Bryophyllum pinnatum*-mediated AgNPs display a slightly lower absorbance of 1.6, while *Carica papaya*-mediated CuNPs exhibit a comparatively lower absorbance of 1.3 at the same wavelength. These findings indicate that both *Carica papaya* and *Bryophyllum pinnatum* have the ability to synthesize nanoparticles with significant absorbance properties, thus making them promising candidates for various applications in fields such as medicine, catalysis, and environmental remediation.

Five clinical isolates were tested against 12 antibiotics, *B. alvei*, *P. aeruginosa* and *H. pylori* showed resistance to 12 antibiotics, whereas *E. coli* and *S. aureus* showed resistance to 11 and 10 antibiotics respectively, indicating a significant challenge in managing infections caused by these pathogens as well. The consistent presence of multidrug-resistant *P. aeruginosa* and *S. aureus* isolates reported by Ehiaghe *et al*. (2022) and *H. pylori* reported by Wang *et al.* (2019)reinforces the notion that this bacterium poses a persistent threat to patient health in Medicare facilities. The collective evidence from these studies highlights the urgent need for comprehensive infection control measures, including prudent antibiotics.

In this study, the multiple antibiotic resistances (MAR) index of all the isolates was greater than 0.2, indicating they likely originated from high-risk sources of contamination. This is similar to the study by Ayandele *et al*. (2020), Serwecińska (2020) and Ehiaghe *et al*. (2022), who also found a high MAR index in isolates from hospitals. The presence of multidrug resistance in these isolates highlights the urgent need for effective surveillance and control measures to prevent the spread of resistant bacteria. Furthermore, it emphasises the importance of prudent antibiotic use in both human and veterinary medicine to minimise the selection pressure for resistance.

This study revealed that copper sulphate ($CuSO_4$) and silver nitrate ($AgNO_3$) showed high antimicrobial activity against *E. coli*, *P. aeruginosa*, *H. pylori*, and *B. alvei* using filter and agar-well diffusion methods. However, there was no inhibitory activity against *S. aureus* by the plant extracts; this is similar to the report of Ahmed *et al*. (2016) who reported that the plant extract showed no antimicrobial activity.

Interestingly, *Carica papaya* and *Bryophyllum pinnatum*-mediated CuNPs and AgNPs effectively inhibited the activity of *S. aureus*, *E. coli*, *P. aeruginosa*, *H. pylori*, and *B. alvei*, demonstrating their broad-spectrum antimicrobial potential. These findings suggest that the combination of metal salts with plant-mediated

nanoparticles could be a promising approach for combating bacterial infections. The result of this study is in agreement with the report by Anandalakshmire *et al*. (2016) and Bhat *et al*. (2021), who reported that plant-mediated nanoparticles showed significant antimicrobial activity against a range of bacterial strains. Also, Ahmed *et al*. (2016) reported that the produced NPs displayed equal efficacy against *E. coli* and *S. aureus*, while the plant extract showed no antimicrobial activity.

The mechanisms by which AgNPs and CuNPs exert their antimicrobial effects are multifaceted and involve various mechanisms. AgNPs and CuNPs have been shown to disrupt bacterial cell membranes, leading to leakage of intracellular components and subsequent cell death (Bruna *et al*., 2021; Lai *et al*., 2022). They can interact with bacterial DNA, inhibiting replication and causing genetic damage. Furthermore, these nanoparticles can generate reactive oxygen species (ROS), which induce oxidative stress in bacteria and impair their vital cellular processes (Manke *et al*., 2013; Mammari *et al*., 2022). CuNPs have the ability to interfere with bacterial enzyme systems, disrupting crucial metabolic pathways necessary for bacterial survival (Kaur *et al*., 2023). Moreover, copper ions released from CuNPs can generate ROS within bacterial cells, causing oxidative damage and inhibiting microbial growth (Orta-Rivera *et al*., 2023). Moreover, the use of Carica papaya and Bryophyllumpinnatum as green synthesis agents offers a sustainable and eco-friendly alternative to conventional chemical methods (Ikhajiagbe *et al*., 2022).

Conclusions

The use of *Carica papaya* and *Bryophyllum pinnatum*-mediated copper and silver nanoparticles has demonstrated promising results in terms of their antimicrobial activity against various bacterial strains. This approach not only provides an effective alternative to conventional chemical methods but also promotes sustainability and eco-friendliness. It is crucial to evaluate their safety and efficacy in clinical settings to ensure their suitability for practical applications. By continuing to explore these aspects, this will help to unlock the full potential of these green synthesis agents and pave the way for a safer and more sustainable future in antimicrobial research..

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