International Journal of Academic Information Systems Research (IJAISR)
ISSN: 2643-9026
Vol. 8 Issue 10 October - 2024, Pages: 35-45

Evaluation Of Antimicrobial Activity Of Fabricated Silver Nitrate Particles Obtained From Soursop Seeds Against Isolated Indoor Bacteria

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Abstract: Background*: The antimicrobial potential of silver nitrate particles derived from soursop seeds against indoor bacteria was investigated in the Microbiology Departmental Laboratory, Uli, Anambra State, Nigeria. The study aimed to prepare and analyze the phytoparticle constituents of soursop seed extracts and evaluate the antibacterial efficacy of fabricated silver nanoparticles (Ag-NPs) against isolated indoor bacteria.* ***Methodology****: An extraction method was employed to obtain silver nanoparticles from soursop seeds, followed by antimicrobial assays using different concentrations of Ag-NPs and ciprofloxacin The setup was allowed to stand for 6-12 hr for diffusion to take place after which the plates were inverted and incubated at room temperature for 24 hr. After 24 hr, zones of inhibition were measured from two different angles and recorded.* ***Result****: The finding revealed that Isolate E (Pseudomonas, vibrio) was the most susceptible (27.5 ± 7.5) to the silver nitrate particles from Annona muricata seeds at 30mg/ml, while Isolate B (Aeromonas) had the lowest susceptibility (16.5 ± 0.5) to the extract at concentration of 30mg/ml. The results indicated significant susceptibility of indoor bacteria to Ag-NPs, with Pseudomonas spp. being the most susceptible. Moreover, Ag-NPs exhibited higher antimicrobial activity compared to ciprofloxacin* ***Conclusion****: This study has elaborately demonstrated the antimicrobial activity of silver nanoparticles extracted from Annona muricata seeds against isolated indoor bacteria. Therefore it is necessary to utilize the deduction from this research in developing novel therapies to combat multi-drug resistant organisms.*

INTRODUCTION

In recent years, the rise of antimicrobial resistance has become a significant global health concern, prompting the search for novel antimicrobial agents to combat infectious diseases. Natural products have emerged as promising sources of antimicrobial compounds, with silver nanoparticles (AgNPs) gaining attention for their potent antimicrobial properties. This research focuses on evaluating the antimicrobial activity of fabricated silver nitrate particles derived from soursop seeds against isolated indoor bacteria.

Indoor environments harbor a diverse array of bacteria, some of which can pose health risks to occupants. Common indoor bacteria include *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella* spp., which can lead to infections and contribute to the spread of disease [1]. Moldoveanu [2] identified two groups of bacteria, that is, *Legionella* and *Actinomycetes*, as important bacteria in indoor air. These indoor bacteria release structures which include but are not limited to; bacterial spores, peptidoglycans, microbial volatile organic compounds, exotoxins, etc. Some of these structures pose a serious threat to humans who occupy these indoor spaces while conferring resistance to the implicated bacteria.

Annona muricata commonly known as Soursop is a species of the genus Annona, of the Annonaceae family, order Magnoliales, and division Magnoliophyta. The soursop tree is about 5–10 m tall and 15–83 cm in diameter with low branches, producing fruits containing 55–170 black seeds when fresh which turn light brown when dry [3]. Raw soursop is 81% water, 17% carbohydrates, 1% protein, and has negligible fat. The leaves, bark, fruit, and seed of A. *muricata* have been the subject of countless medicinal uses. The fruit is not only appreciated as food, but the juice is used as galactagogue to treat diarrhea, heart and liver diseases [4]. The medicinal uses of A. *muricata* leaves include treatments for hypertension [5,4,6], diabetes [7,8,5], and cancer [9,10]. Unripe fruit, seeds, leaves, and roots are also used as biopesticides, bio insecticides, and topical insect repellents [3].

Nanoparticles, best described as micro molecules synthesized through either physical, chemical, physicochemical, or biological means, are useful in consumer goods, pharmaceutical, chemical, environmental, energy, agricultural, and communication industries [11]. Silver nanoparticles have attracted interest as antimicrobial agents due to their broad-spectrum activity against bacteria, viruses, and fungi. The antimicrobial mechanism of AgNPs involves the release of silver ions, which disrupt cellular processes and inhibit microbial growth. Additionally, AgNPs exhibit low toxicity to human cells, making them promising candidates for antimicrobial applications. Recent studies have shown that the production of metallic NPs via plant parts including the seed, leaf, and stem, is the most reproducible approach, cost-cost-effective, and simplest [12]. Silver nanoparticles which are the most important metal nanoparticles have been shown to have both antibacterial and antifungal properties. Various literatures have revealed increased antimicrobial activities of plant bio-synthesized silver nanoparticles [13,14,15,16].

1.2 Objectives

i. Isolation and identification of indoor bacteria present in Chukwuemeka Odumegwu Ojukwu University.

ii. Preparation and determination of phytoparticle/chemical constituents of soursop seed extracts.

iii. Synthesis and characterization of the silver nitrate nanoparticle.

iv. Evaluation of the antibacterial activity of fabricated silver nanoparticles obtained from soursop seeds against the isolated indoor bacteria.

MATERIALS AND METHOD

2.1 Sample Collection

The samples were collected from ripe soursop sold at Umuru/Amadunu Nnobi in Idemili south local government area of Anambra state. Samples were transported to the Microbiology laboratory in Chukwuemeka Odumegwu Ojukwu University Uli, in a sterile polyethylene bag.

2.2 Sterilization of Material

All materials that were used in the course of this project such as glass wares were properly washed with detergent and water to remove dirt and contaminations and dried properly. The washed glass wares were sterilized in a portable laboratory hot air oven.

2.3 Isolation of Indoor Bacteria

The settle plate method using 16 open 8.5cm diameter Petri dishes containing different cultures was used as described by[11,17]. This method allows bacteria or fungi-carrying particles to settle on culture media. Bacteria were collected on Nutrient agar (NA) to which an antifungal agent (Nystatin) has been incorporated to inhibit the growth of fungi. The media plates were placed on a table with the sampling height 1 m above the floor which approximated the human breathing zone, while the sampling area was at the corner and middle of the selected lecture room and the tops of the office doors. A set of 8 plates were exposed with their lids open. The plates were allowed to stay for 30 min. Duplicate samples for each culture medium were collected to ensure sampling accuracy. Afterward, the plates were covered, kept in tightly sealed cases, and transported to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University and incubated at 37 °C for 24 hr. To avoid self-contamination of agar plates during air sampling, sterile gloves, mouth masks, and protective gowns were worn, and before use the agar plates were also checked visually for any microbial growth. After 24 hr, Bacterial colonies were counted with the aid of the colony counter and a magnifying lens.

2.4 Characterization of isolates

Individual bacterial isolates were identified using standard methods including colonial morphology (The colonies were differentiated based on size, color, and pigmentation), microscopy, and biochemical tests as described by [17]

2.5 Morphology

The colony of the pure culture of each bacterium isolates were observed for morphological features using Bergey’ s manual of determination bacteriology. The pigmentation, colony form, colony margin, and elevation of the bacterial isolates were observed. The Cells' shape was determined under the x100 objective of an Oil immersion microscope after the gram staining procedure [15].

2.5.2. Gram Staining

A thin smear of the culture was prepared on a clean grease-free glass slide, air-dried and heat-fixed. The smear was flooded with crystal violet solution for 60 seconds and rinsed with water. Upon drying, the primary stain was fixed using Lugol’ s iodine. Alcohol

(95%) was used to decolorize the slide content for 10 seconds. The smear was then counter-stained with safranin solution for 60 seconds, rinsed, and air-dried. The slide was then viewed under the light microscope using an x100 oil immersion objective lens. This was carried out using a modified method of [17].

2.5.3 Biochemical identification

2.5.3.1 Catalase test

A small quantity of 24 hr-old culture was transferred into a drop of 3% Hydrogen peroxide solution on a clean slide with the aid of a sterile inoculating loop. Gas seen as white froth indicates the presence of catalase enzyme (Cheesbrough, 2006).

2.5.3.2 Citrate test

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. About 2.4 g of citrate agar was dissolved in 100 ml of distilled water. About ten milliliters (10 ml) of citrate medium was dispensed into each tube and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate.

2.5.3.3 Oxidase test

A piece of filter paper was soaked with a few drops of oxidase reagent. A sterile inoculating loop was used to pick a colony of the test organism and smear it on the filter paper. If the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple color [17].

2.5.3.4 Indole test

Tryptone broth (5 ml) was placed into different test tubes after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes uninoculated to serve as control. The test tubes were then incubated at 37℃ for 48 hr. After incubation, 0.5 ml of Kovac's reagent was added and shaken gently; it was allowed to stand for 20 min to permit the reagent to rise. A red or red-violet color at the top surface of the tube indicates a positive result while yellow coloration indicates a negative result [17]).

2.5.3.5 Starch hydrolysis

This is used to assay the ability of microorganisms that produce enzymes that degrade substrate with carbon compounds. Nutrient agar was prepared with 1% soluble starch and was sterilized. The medium was poured into sterile plates and was inoculated by streaking the organisms once across the plates after solidifying. The plates were incubated at 37℃ for 24 hr after which they were flooded with Gram's iodine. Unhydrolyzed starch forms a blue color with the iodine. Hydrolyzed starch appears as a clear zone due to alpha-amylase activity while reddish-brown zones around the colony, indicate partial hydrolysis of starch (to dextrans).

2.5.3.6 Motility test

A sterile needle was used to pick a loop of a 24 hr-old culture and was stabbed onto nutrient agar in glass vials. The vials were incubated at 37℃ for 24-48 hr. Non-motile bacteria had growth confined to the stab line with definite margins without spreading to the surrounding area while motile bacteria gave diffused growth extending from the surface (Olutiola et al., 2000).

2.5.3.7 Triple Sugar Iron Test

With a straight inoculation needle, a well-isolated colony was picked. The TSI was then inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. The tube was sealed loosely and incubated at 35-37℃ in ambient air for 18-24 hr. The tube was examined afterward and the result was recorded.

2.5.3.8 Urease test

This test was carried out to identify bacterial isolates capable of hydrolyzing urea using the enzyme urease. A loopful of a well-isolated colony was taken with an inoculating loop and inoculated on the agar slants. The inoculation was done on just the slant, and the bottom of the tube was not stabbed. The tubes were then incubated with loosened caps at 35-37℃. The tubes were observed for the development of pink color for as long as 7 days.

2.6 Extraction of the Plant Constituent

The soursop seeds were washed and dried after which the coating of the seed was separated from the main seed. The main seeds were ground into powder using a grinder. 21.5 g of the grounded soursop seed was weighed out and added into a clean conical flask containing 100 ml of deionized water. The mouth of the flask was sealed using non-absorbent cotton wool and aluminum foil. Using a magnet stirrer heating mantle, at 70℃, the flask and its content were heated till a change in coloration was observed (Fig 2). Upon color change, the sample extract was filtered into a clean beaker using a cheesecloth filter and was allowed to cool.

2.7 Preparation of Silver Nitrate (AgNO3) Solution

0.01 g of Silver nitrate reagent was added into a clean conical flask containing 50 ml of deionized water. The flask was kept in a dark place to reduce photochemical reaction.

2.8 Synthesis of the Nanoparticle

The prepared Silver nitrate solution in a conical flask was placed on the stirrer (Fig 3). A retort stand with a burette clamped to it was set after which the burette was fixed into the conical flask. The solution contained in the flack was heated to a hot temperature. 27 ml of the sample extract was poured into the burette and was used to titrate (in drops) the Silver nitrate solution. Titration continued until there was a change in color of the Silver nitrate solution from pink to gold with a corresponding formation of silver nanoparticles (Fig 4). The nanoparticles were centrifuged and the sediments were dried in a dry air oven for 1 hr.

Figure 1: Heated grounded soursop seeds

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Figure 2: Silver nitrate solution

Figure 3: Synthesis of silver nanoparticles

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Figure 4: Wet silver nanoparticles

2.9 Antibacterial assay

The antimicrobial assay was done by weighing 0.18 g of the dry Ag-NPs into 10 ml of deionized water and a 2-fold serial dilution was done in four test tubes to get four concentrations: 30 mg/ml, 15 mg/ml, 7.5 mg/ml and 3.25 mg/ml. 0.3 ml of DMSO (Di-methylsulfoxide) was weighed into 10 ml of distilled water. A 2-fold dilution of ciprofloxacin was prepared to obtain four antibiotic concentrations. One hundred milliliters of Mueller-Hinton agar was prepared, sterilized, and poured into 5 sterile glass petri dishes and was allowed to gel. The 5 organisms were centrifuged, and washed thrice and 1 ml of each organism was spread on an appropriately labeled agar plate. Using a 1 cm cork-borer, 9 holes were bored on each petri dish; 4 for the Ag-NP, 4 for the various concentrations of ciprofloxacin, and 1 for DMSO. Using a micropipette, 20 µL of Ag-NP for each concentration was added into the holes. A similar process was carried out for the various concentrations of ciprofloxacin whereas 1 ml was taken for DMSO and placed in the respective holes. The setup was allowed to stand for 6-12 hr for diffusion to take place after which the plates were inverted and incubated at room temperature for 24 hr. After 24 hr, zones of inhibition were measured from two different angles and recorded.

2.10 Procedure for FTIR

Buck Scientific M530 USA FTIR was used for the analysis. This instrument was equipped with a detector of deuterated triglycine sulphate and a beam splitter of potassium bromide. The software of Gram A1 was used to obtain the spectra and to manipulate them. Approximately 1.0 g of samples and 0.5 ml of nujol were added, they were mixed properly and placed on the salt pellet. During measurement, FTIR spectra were obtained at frequency regions of 4,000 – 600 cm-1 and co-added at 32 scans and at 4 cm-1 resolution. FTIR spectra were displayed as transmitter values (Van der Weerd et al., 2004).

2.11 UV-VIS Spectroscopic Analysis

UV-visible spectrophotometric analysis was conducted on the sample using a UV-visible spectrophotometer (Apel 3000 UV) with a slit width of 2 nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200-1100 nm.

RESULTS AND DISCUSSION OF FINDINGS

Table 1: Morphological characteristics of bacterial isolates

Isolate	Pigmentation	Colony form	Colony margin	Elevation
A	White cloudy	Irregular	Undulate	Raised
B	Lightly yellow	Irregular	Undulate	Raised
C	Brownish white	Irregular	Undulate	Raised
D	White	Irregular	Undulate	Raised
E	White cloudy	Irregular	Undulate	Raised

Table 2: Gram staining characteristics of the bacterial isolates

Isolate	Shape	Arrangement	Gram reaction	Suspected organisms
A	Straight rod	Single, pairs	Negative	*Aeromonas, Pseudomonas*
B	Rod	Single	Negative	*Aeromonas, Pseudomonas*
C	Cocci	Single, pairs	Positive	*Staphylococcus*
D	Rod, Cocci	Single. Pairs	Positive	*Staphylococcus*
E	Rod	Clustered, single	Negative	*Aeromonas, Pseudomonas, Vibrio*

Table 3: Biochemical identification of the isolates

Test	Catalase	Oxidase	Urea	Indole	Motility	Citrate	TSI			
							H_2S	Fru	Glu	Suc
Isolate A	+	+	+	+	+	+	+	–	–	–

Isolate B	+	+	+	+	+	+	+	+	–	+
Isolate C	+	–	+	+	+	+	+	+	–	+
Isolate D	+	–	+	+	+	+	+	+	–	+
Isolate E	+	+	–	+	+	+	+	+	–	+

Hint: + = Positive reaction; – = Negative reaction

3.4 FTIR result

The result of FTIR analysis of A. muricata seeds silver-nanoparticles is shown in Fig. 5. The vibration frequencies at about 886.6515, 1050.146 are attributed to carbonyl group (C–Cl) stretching. Vibration frequencies at about 1297.237, and 1387.877 attributed to Cyano functional group (C–N) stretching. Ranging from 1631.851, 1861.839, 2003.006, 2281.031, 2457.452 vibration frequencies attributed to Carbonyl/hydroxyl functional groups (C=O) stretching. Vibration frequencies at about 2520.073, 2624.198, 2874.754, 2982.065, 3160.414, 3262.022, are attributed to hydrocarbon functional group stretching. Then, vibration frequencies at about 3531.646, and 3668.566 attributed to amine and hydroxyl functional groups stretching.

3.5 UV-VIS Spectroscopic analysis

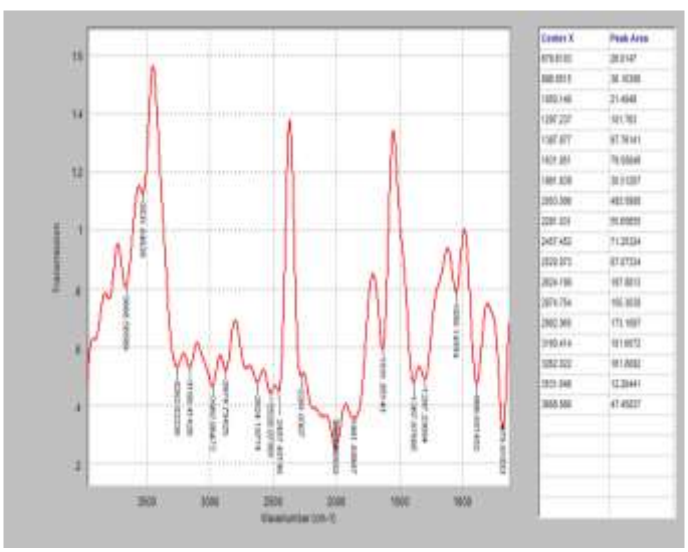

Figure 5: FTIR chart of biosynthesized silver nanoparticles

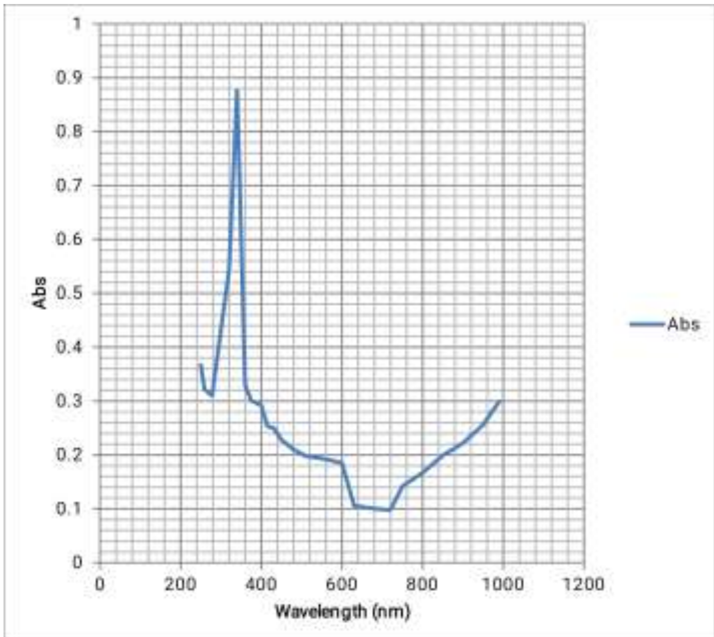

Figure 6: shows the absorbance spectra of the synthesized silver nanoparticles.

3.6 Antibacterial assay

The antibacterial effects of the Ag-NPs extracted from *Annona muricata* seeds are summarized in Table 4.

Table 4: Antibacterial effects of the Ag-NPs extracted from Annona muricata seeds

Isolates	***Annona muricata* seeds (Ag-NP**				**Ciprofloxacin**				**DMSO (Negative control)**
	30	**15**	**7.5**	**3.25**	**30**	**15**	**7.5**	**3.25**	
A	21.5 ± 1.5	14.5 ± 0.5	15.5 ± 0.5	19.5 ± 0.5	17.5 ± 0.5	15 ± 0.00	14 ± 1	10.5 ± 0.5	0 ± 0.0
B	16.5 ± 0.5	16 ± 1	16.5 ± 1.5	16 ± 2	16 ± 1	15 ± 0	13 ± 1	10.5 ± 0.5	0 ± 0.0
C	18 ± 1	15 ± 0	14 ± 0	11 ± 0	15 ± 0	16.5 ± 0.5	14.5 ± 0.5	10.5 ± 0.5	0 ± 0.0
D	19 ± 1	20 ± 0	17 ± 1	22.5 ± 2.5	20.5 ± 0.5	19.5 ± 0.5	18 ± 0	16.5 ± 0.5	0 ± 0.0
E	27.5 ± 7.5	18 ± 0	16.5 ± 0.5	7.5 ± 0.5	20 ± 0	18.5 ± 0.5	17 ± 1	15 ± 0	0 ± 0.0

Discussion of Finding

The cultural and morphological characteristics revealed a similar colony form, colony margin, and elevation, while distinctly distinguishing the various pigmentations such as white cloudy, lightly yellow, and brownish white on the nutrient agar as shown in Table 1.

The biochemical characteristics of the isolates revealed that the isolates were positive for catalase, income, citrate, hydrogen sulfide, and motility test, as shown in Table 3. The isolates differ in their variation in the utilization of sugar (Fructose and sucrose), but they all cannot utilize glucose in their metabolism. The isolates also differ in their reaction to oxidase and urea test. Table 2 revealed the gram-staining characteristics of the bacterial isolates such as the shape, cell arrangement, gram reaction, and the suspected organisms.

Isolate E (*Pseudomonas* spp) was the most susceptible (27.5 ± 7.5) to the silver nitrate particles from Annona muricata seeds at 30mg/ml, while Isolate B (Aeromonas and Pseudomonas spp had the lowest susceptibility (16.5 ± 0.5) to the extract at concentration o 30mg/ml. At the lowest concentration of the extract (3.25), isolate A (*Aeromonas* and *Pseudomonas* spp) had the highest susceptibility of (19.5±0.5), while isolate E (*Pseudomona* spp) had the least susceptibility of (7.5±0.5). This is by the work of Ojemaye et al. (14), where they discovered that the silver nanoparticles mediated by the leaves and fruit extracts of Crataegus ambigua exhibited strong antibacterial activities against seven test bacterial strains. The bacterial strains included L. ivanovii ATCC19119, S. aureus NCIB50080, M. smegmatis ATCC700084, V. *paraheamolyticus*, E. *cloacae* ATCC13047, E. coli 180 ATCC700728, and S. uberis ATCC700407. The silver nanoparticles (AgNPs) synthesized from the plant's leaf extract displayed significant higher zones of inhibitions ranging from 11–25, 9.5–15, and 7.5–10 mm at 60, 30.00, and 15 mg mL−1 respectively against L. ivanovii, S. aureus, and M. smegmatis when compared to fruit AgNPs at same concentrations.

The study identified and characterized indoor bacteria, revealing similarities and differences in their morphological and biochemical characteristics. Notably, the isolates exhibited varying susceptibility to the synthesized Ag-NPs, with Pseudomonas spp. being the most susceptible. These findings align with previous research indicating the antimicrobial efficacy of silver nanoparticles synthesized from plant extracts. The observed higher lethality of Ag-NPs compared to ciprofloxacin suggests their potential in combating multi-drug resistant organisms. Overall, the study emphasizes the importance of exploring natural antimicrobial agents for indoor environments to mitigate the spread of infectious diseases.

CONCLUSION

This study underscores the antimicrobial activity of silver nitrate particles derived from soursop seeds against isolated indoor bacteria. By synthesizing and characterizing these nanoparticles and conducting antimicrobial assays, the research contributes to the development of natural antimicrobial agents for indoor environments. These findings hold promise for addressing the challenge of antimicrobial resistance and enhancing infection control measures in indoor settings.

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