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In vitro Antimicrobial Evaluation of Biologically Synthesised Silver Nanoparticles from *Terminalia avicennioides* Extracts on Antibiotic Resistant *Pseudomonas aeruginosa Isolates*

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Abstract: Most available antimicrobials are now ineffective and the whole world healthcare system is currently under threat of antimicrobial resistant infections. Consequently, study of plant bioactive compounds with potentials infectious diseases therapeutic values is of significant risen interest with biologically synthesized plant extracts derived silver nanoparticles on the greater focus. This research was aimed at determining the *in vitro* antimicrobial activity of silver nanoparticles synthesized from extracts of *Terminalia avicennioides* on antibiotic resistant *Pseudomonas aeruginosa* isolates from wounds. Standard phenotypic and genotypic techniques were used for the Isolation and identification of *Pseudomonas aeruginosa* isolates. Selected antibiotics, *Terminalia avicennioides* extracts and the extracts derived silver nanoparticles antimicrobial activities on the antibiotic resistant *Pseudomonas aeruginosa* were determined using standard tests methods. Findings showed the isolates to be resistant to 18.18% - 100% of the antibiotics used, but 100% sensitive to imipenem. Analysis of the plant extracts for bioactive compounds showed the presence of tannins, alkaloids, flavonoids, cardiac glycosides, phenols, saponins and terpenoids. Antimicrobial profile of *Terminalia avicennioides* extracts on the antibiotic resistant *Pseudomonas aeruginosa* isolates showed zones of growth inhibition ranged from 10.04±9.39 – 18.08±10.62 mm with no significant difference ($P > 0.05$), minimum inhibitory concentration ranged from 60.000+65.8281 -40.000 + 21.0821 mg/ml with no significant difference ($p < 0.05$), and minimum bactericidal concentration ranged from 100.00 ± 89.4427 – 63.6364 ± 50.4525 mg/ml with no significant difference ($p > 0.05$). The antimicrobial activity of the biologically synthesized silver nanoparticles on the antibiotic resistant *Pseudomonas aeruginosa* showed zone of growth inhibition ranged from 28.00 ± 13.51 – 53.00 ± 76.97 mm with no significant difference ($p > 0.05$). *Terminalia avicennioides* extracts and silver nanoparticles antimicrobial activity showed significant difference ($p > 0.05$). In comparison, the silver nanoparticles zones of growth inhibition was larger (28.39 ± 2.98 mm) than that of the extracts (16.83 ± 12.70 mm). This inferred that the synthesized silver nanoparticles possess potential of being used as a good chemotherapeutic agent for wound infections.

Keywords: *Pseudomonas aeruginosa*, Antibiotic Resistant, Nanoparticles, Wound, Antimicrobial, *Terminalia avicennioides*

1. Introduction

Pseudomonas aeruginosa carries multi-resistance plasmids and developed mutational resistance to cephalosporins class of antibiotics; and most *Pseudomonas aeruginosa* isolates are resistant to all reliable antibiotics. The organism is described as a unique problematic opportunistic pathogen due to combination of the specie inherent resistance to many antimicrobial classes [1]. Scientific researches focusing on dicovery and development of new and effective antimicrobial

agents aganist drug resistant bacteria, including development of new types of antibiotics that do not possess characteristics of cross - and co - resistance to the available classes of antibiotics has been recammended by WHO [2].

Interestingly, because of the potential therapeutic sources of new antimicrobial molecules in medicinal plants, they have received greater attention in traditional herbal medicines worldwide for treatment of various infectious diseases many years ago [3, 4]. Moreso, because of the global threat of antimicrobial resistant infections to health care system, most scientific researches are now shifting focus to exploring novel compounds including Silver nanoparticles (AgNPs) to halt multdrug-resistant microorganisms. This is due to the silver nanoparticles unique high antibacterial activity characteristics against a wider range of bacteria without any toxicity to animal cell [5].

The combining uses of traditional healing agents with modern scientific products or practiced like the nanofibres consisting of silver nanoparticles have been the current approach in ensuring innovative wound therapy [6]. In this regards, *Terminalia cuneata* and many other medicinal plants have receives much application for biological synthesis of plant derived silver nanoparticles [7]. This study aimed at determination of the *in vitro* antimicrobial activity of biologically synthesized silver nanoparticles from *Terminalia avicennioides* extracts against antibiotic resistant *Pseudomonas aeruginosa* isolated from wounds.

2. Materials and Methods

2.1. Ethical Permission

Ethical permission Reference number; HREC - 20-0004 was obtained from the research ethical committee of Barau Dikko Teaching Hospital, Kaduna, Nigeria, before proceeding to collect patient's wound swabs for isolation of *Pseudomonas aeruginosa.* Patient from different clinics with wounds infections were given inform consent forms for their consent prior to obtaining helpful data along with wound swabs. In the case of children with wound infections, their parents or guardians were asked to give assent for them.

2.2. Collection of Wound Swabs and Isolation of Pseudomonas aeruginosa Isolates

Sixty wound swabs samples were collected base on convinience from in - and out - patients with wound in various clinics at Barau Dikko Teaching Hospital Kaduna, Nigeria. With the assistance of the clinics nurses, sterile swab cotton tips were used to aseptically obtained exudate or pus or purulent discharge from the patients' wounds. The swabbed tip were broken into a sterile brain heart infusion broth (BHI), and transported in ice packed thermo flasks to postgraduate Medical Microbiology Laboratory, Department of Microbiology, Kaduna State University, for isolation of *Pseudomonas aeruginosa* isolates.

Brain Heart infusion (BHI) broth, Cetrimide agar, and MacConkey agar used in this research work were prepared based on their manufacturers instruction. *Pseudomonas aeruginosa* isolates were isolated from the swab wound samples in the laboratory following the procedures described by Vallis *et al*. and Cheesbrough [8, 9]. The Swab samples were incubated at aerobic condition in a Brain Heart Infusion (BHI) broth at the temperature of 37°C for 24 hours. This was subcultured on cetrimide agar plates for selective isolation of *Pseudomonas aeruginosa*. Presumptive *Pseudomonas aeruginosa* colonies from the cetrimide agar plates were transferred onto MacConkey agar plates using streak plate method and then incubated earobically at 37°C for 24 hours. Pure single colonies from this medium were subcultured on nutrient agar slant and preserved at refrigerated temperature for further study.

2.3. Phenotypic amd Molecular Identification of Pseudomonas aeruginosa Isolates

2.3.1. Morphological and Biochemical Characterisation

Morphological and biochemical characterisation of the pure isolates was done according to the procedures described by Aneja, Ochai and Kolhatkar and Cheesbrough [9-11]. The biochemical test carried out for the identification of *Pseudomonas aeruginosa* include; motility, oxidase, indole, triple sugar iron (TSI), citrate utilization, urease, methyl red and Voges-Proskauer.

2.3.2. Molecular Identification Using Chromosomal DNA Extraction and Polymerase Chain Reaction (PCR)

The DNA extraction was done usimg bioneer bacterial extraction (Genomic DNA extraction) kits. This extraction was carried out following the protocols as described by Bobai *et al*. [12]. Using the forward primer - GGACTACAGGGTATCTAAT 16S (RIBOSE-1) and reverse primer - AGAGTTTGATCCTGG 16S (RIBOSE-2), the PCR of the extracted genomic DNA carried out following the protocol described by Bobai *et al.*(12). The electrophoresis of the PCR product was carried out using 1.5% agarose gel at 125 volt for 35 minutes and gel DNA bands were visualised using UV Biorad gel imaging system. Online BLASTn was employed to search for the 16SrRNA genes nucleotides sequences.

2.4. Susceptibility Profile of Some Antimicrobial Agents Conventionally Used for Wound Therapy

The susceptibility profile of *Pseudomonas aeruginosa isolates* was performed using Kirby-Buaer disc diffusion method described by Arora [13] and Bobai et al. [12]. single disc Gram-negative antibiotics (Oxoid); Ceftriazone (30 µg), chloramphenicol (30 µg), Gentamycin (10 µg), Imipenem (10 µg), Ampicillin (10 µg), Ciprofloxacin (5 µg), Kanamycin (30 µg), Meropenem (10 µg), Nalidixic acid (30 µg), Amoxicillin-Clavulanic acid (30 µg), and Tetracycline (30 µg) were used. Diameters of zones of growth inhibition were taken and interpreted as either sensitive, intermediate, or resistant following the Clinical and Laboratory Standard Institute (CLSI) guidelines [14].

2.5. Authentication of Terminalia avicennioides Plant Materials

Fresh *Terminalia avicennioides* plant's parts was collected and transported for identification at the Herbarium Unit of Department of Biological Science, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria; where the voucher number (900239) of the plant was obtained. Fresh *Terminalia avicennioides* plant's parts was collected after the authentication of the plant in large quantity and cut into small pieces and dried under shade at 30°C in a clean laboratory cabinet. The dried plant materials wasl first pounded in a mortar, followed by dry-milling with an electric blender and then sieved to obtained fine powder using 20 µm mesh size sieve.

2.6. Preparation of Plant Extracts

Acetonic, ethanolic and aqueous extracts from the plant powder of root bark, stem bark and leaf were prepared following the method described by Bobai *et al.* [12].

2.7. Qualitative and Quantitative Phytochemical Screening

Standard procedures described by Trease and Evans, Harborne, and Sofowara [15-17] were used to qualitatively determine the presence of bioactive constituents such as; saponins, tannins, phenolic compounds, anthraquinones, cardiac glycosides, alkaloids, and flavonoids. While the standard procedures described by; Harborne, AOAC, Chang *et al*., Edeoga *et al.* and Oloyed [16, 18-21] were used for the quantitative determination of some of these phytochemicals such as; Phenol, Flavonoids, Alkaloids, Saponins, Tannins, and terpenoids.

2.8. Biosynthesis of Silver Nanoparticles from Terminalia avicennioides Extracts

The Silver nanoparticles was biologically synthesized following standard procedures described by Balashanmugam and Kalaichelvan, Henry *et al.*, and Suresh *et al*. [22-24]. In the synthesis, 0.0425 g was dissolved in forty-five millilitres of sterile distilled water to obtained 5mMl Silver nitrate. Using a magnetic stirrer, the mixture was stirred for 10 minutes, followed by addition of five millilitres (5 ml) of the extract drop-by-drop until an initial colour changed observed. The mixture of silver nitrate solution and plant extract was held at 60°C for 60 minutes to control colour rapid changes. The mixture was incubated at room temperature for 24 hours away from light rays along with the plant extracts as negative control. A final appearance of colour changed different from the negative control was noted as formation of the plant extract derived Silver nanoparticles (AgNPs).

2.9. Evaluation of Antimicrobial Activity of the Terminalia avicennioides Extracts and AgNPs on Antibiotic Resistant Pseudomonas aeruginosa

2.9.1. In Vitro Determination of Antimicrobial Potency

Spread-plate and agar-well diffusion method described by Ochai and Kolhatkar, Cheesbrough [9, 11] and Bobai *et al*. [12] were used to determine the plants extracts as well as the AgNPs antimicrobial activity against all the antibiotic resistant *Pseudo on as aeruginosa.*

2.9.2. In Vitro Determination of the Minimum Inhibitory Conentration (MIC)

Considering the antimicrobial activity of *Terminalia avicennioides* extracts and that of the AgNPs, the concentrations of the plant extracts that showed cleared antimicrobial activity were selected for the determination of the MIC following the method described by Bobai *et al.* [12].

2.9.3. In Vitro Determination of the Minimum Bactericidal Conentration (MBC)

Considering the result from the minimum inhibitory concentrtion test of *Terminalia avicennioides* extracts, a loopful of the broth cultures were transfered from each of the MIC test tubes that lack visible growth, and inoculated by streaking on a fresh sterile antimicrobial agent free nutrient agar plates, and incubated for 24 hours at 37°C. The concentrations at which no growth was observed were noted and recorded as the minimum bactericidal concentration (MBC) [9].

2.10. Data Analysis

Research data were analysed using; Analysis of Variance (one way-ANOVA), Duncan multiple test, and Independent T-test using SPSS version 23, and presented in bar chart and tabular form.

3. Results and Discussion

3.1. Results

3.1.1. Morphological and Biochemical Characteristics of Presumptive Pseudomonas aeruginosa

Table 1 showed the colonial morphology of presumptive *Pseudomonas aeruginosa* isolates on cetrimide agar and MacConkey agar after 24 hours incubation at 37°C. On centrimide agar, the presumptive isolates of *Pseudomonas aeruginosa* colonies appeared as blue-green and yellow-green, circular, with smooth edge, flat and moderate shape. On MacConkey agar, the colonies appeared colourless, flat and circular with smooth edges - a typical characteristic of *Pseudomonas aeruginosa.* Morphology of the isolates were shot rod arranged mostly in single form under microscopic examination. Gram stain cells under the microscopic evaluation appeared pinkish in colour indicating that they are gram negative rod. The biochemical results showed that these isolates are motile, oxidase positive, catalase positive, indole negative, methyl red (MR) negative, Voges-Proskauer (VP) negative, citrate utilization negative, hydrogen sulphide production negative, gas production negative, with alkaline butt and acid slant.

3.1.2. Molecular Characteristics of Pseudomonas aeruginosa Isolates

Figure 1 showed the amplified 16SrRNA genes bands of

Pseudomonas aeruginosa at 789 bp. The BLAST results (table 2) of the amplified 16SrRNA genes of the presumptive *Pseudomonas aeruginosa* isolates revealed 92.71%, 97.67%, and 91.81% similarity with Genbank database for P1, P2 and P3 respectively.

Table 1. *Morphological and Biochemical Characteristics of Presumptive Pseudomomas aerugenosa Isolates.*

Isolate Identification Code	Morphological Characteristics		
	Colonial morphology on cetrimade and MacConkey agar	**Cellular/microscopic morphology**	***Gram reaction***
DR1	Blue-green, smooth circular, flats & moderate colonies on cetrimide agar. Colourless, flat, circular and smooth edge colonies on MacConkey agar	Shot rod appeared mostly single formed	*Gram negative*
DR12, DR14, DR17 DR19, DR23, DR24 DR30, MSW1 FSW3, FSW4	Yellow-green, circular and smooth edges, flat and moderate colonies on centrimide agar	Shot rod appeared mostly in single formed	*Gram negative*

Table 1. *Continued.*

Isolate Identification Code	*Biochemical characteristics*								*TSI*				Probable organism
	Matility	***oxidase***	***Catalase***	***Indole***	***Methyl red***	***Voge-proskaver***	***Citrale-utilization***	***Ureas utilization***	***Butt***	***slat***	***H_2S***	***GAS***	
DR1	+	+	+	–	–	–	–	–	*AL*	*A*	–	–	*Pseudomonas aeruginosa*
DR12, DR14, DR17 DR19, DR23, DR24 DR30, MSW1 FSW3, FSW4	+	+	+	–	–	–	–	–	*AL*	*A*	–	–	*Pseudomonas aeruginosa*

Key: + = positive, - = negative, TST = triple sugar iron, H_2S = hydrogen sulphide, A = Acid and Al = Alkaline
A = acid, AL = alkali, D = dressing room unit, FS = female surgical ward, and MS= male surgical ward.

Table 2. *BLAST Characteristics of Pseudomonas aeruginosa Isolates.*

S/N	Isolate Code	Isolate identity	Sequence Searched Gene	Scores	Percentge Similarity (%)	E-Value	Query cover (%)	Sequence Searched Accession No
4.	P1	*Pseudomonas aeruginosa*	16SrRNA	756	92.71	0.0	100	JX232010.1
5.	P2	*Pseudomonas aeruginosa*	16SrRNA	73.1	97.67	2e-10	5	MT572506.1
6.	P3	*Pseudomonas aeruginosa*	16SrRNA	815	91.81	0.0	93	JX292018.1

Key: P1 = D_1, P2 = FS_4, P3 = MS_1 (D = dressing room unit, FS = female surgical ward, and MS= male surgical ward).

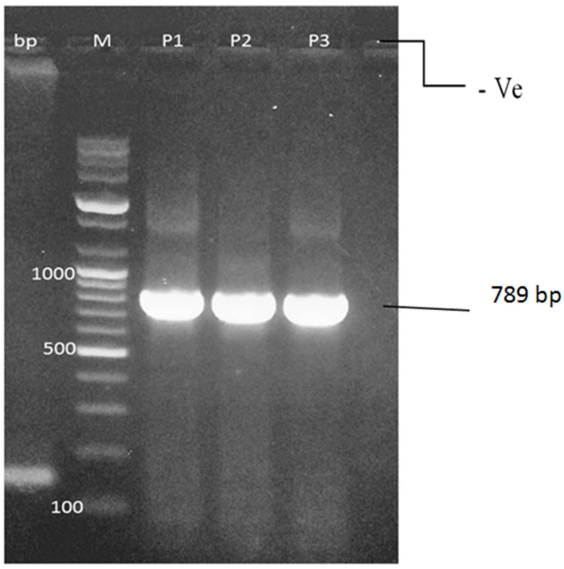

Figure 1. *Amplified 16SrRNA genes bands of Pseudomonas aeruginosa isolates.*

Key: M = 100 bp Marker, P = *Pseudomonas aeruginosa*, bp = base pair, - Ve = Negative Control

3.1.3. Antimicrobial Profile of Some Antimicrobial Agents Against Pseudomonas aeruginosa Isolates

Figures 2 and 3 revealed all the *Pseudomonas aeruginosa* to be multi-drug resistant isolates. From the total of eleven *Pseudomonas aeruginosa* isolates, 11 (100%) were resistant to chloramphenicol, ampicillin, meropenem, and nalidixic acid, 8 (72.73%) resistant to kanamycin and tetracycline, 7 (63.64%) resistant to ceftriazone and amoxicillin-clavulanic acid, 4 (36.36%) resistant to Gentamycin, 2 (8.18%) resistant to Ciprofloxacin; however, the 11 (100%) were susceptible to imipenem (Figure 2). Figure 3 showed that three isolates; D_{14}, D_{17}, and D_{19} were resistant each to 6 (54.55%) Antimicrobial agents; D_{12} and D_{24} were resistant each to 7 (63.64%) antimicrobial agents; D_1, D_{23}, and D_{30} were resistant each to 8 (72.73%) antimicrobial agents; FS_3 and FS_4 were resistant each to 9 (81.18%) antimicrobial agents; and only MS_1 was resistant to 10 (81.82%) antimicrobial agents. According to the results; imipenem, ciprofloxacin and gentamycin were the very effective antimicrobial agents against the *Pseudomonas aeruginosa* isolates.

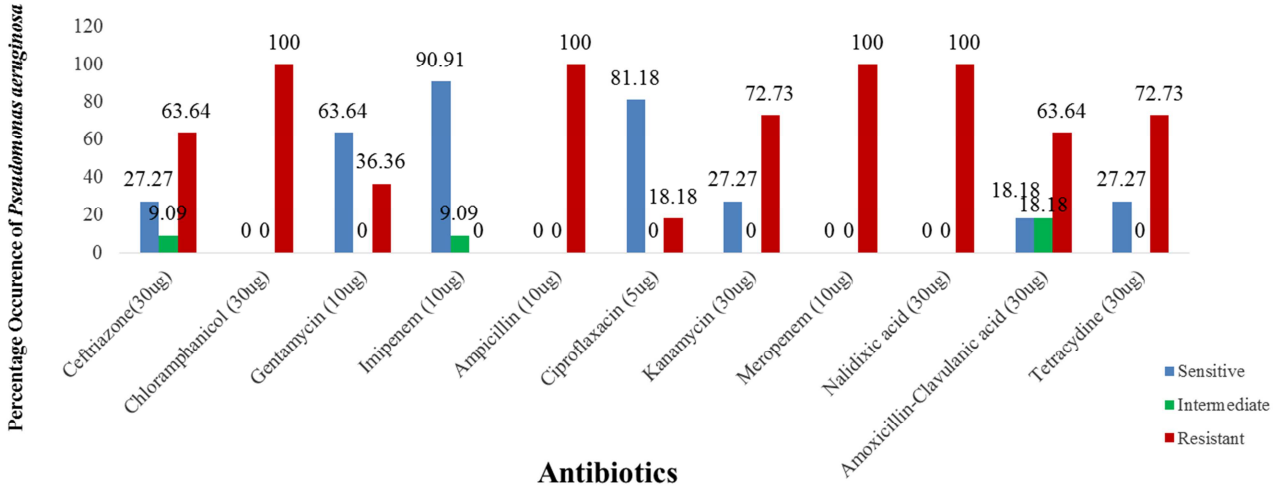

Figure 2. *Susceptibilty Profile of Pseudomonas aeruginosa isolates against some antimicrobial agents.*

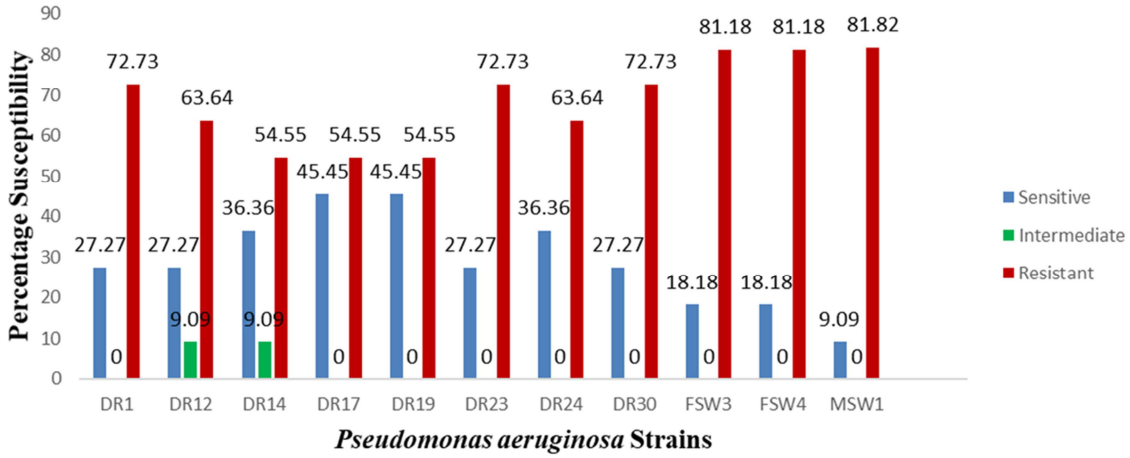

Figure 3. *Susceptibility Pattern of Some Antimicrobial Agents Tested against Pseudomonas aeruginosa Isolates.*

3.1.4. Qualitative and Quantitative Phytochemical Characteristics of Terminalia avicennioides Extracts

Table 3 revealed that the root bark, stem bark, and leave extract of all the three types of solvents contain; flavonoids, tanins, saponins and phenol. Similarly, ethanolic extracts of root bark, stem bark as well as acetone and aqueous stem bark extracts were found to have alkaloids. All stem bark, ethanolic and aquous root bark extracts as well as ethanolic leaves extracts were found to contains cardiac glycoside. All leave extracts, acetone stem bark as well as ethanol root bark extracts contains terpenoids. However, anithroquinone was not found in all category of the extracts analysed. The quantitative analysis (Table 4) showed that the extracts generally had higher phenol content (2331-34 mg/100 g), followed by terpenoids (887-35 mg/100 g), and then Saponins (47.27-22.72 µg/g) as the lowest.

Table 3. *Qualitative Phytochemical Characteristics of Terminelia avecenoides Extracts.*

S/No	*Terminelia avecenoides* Plant Part	Type of Solvent Extract	Phytochemical Characteristics							
			Alkaloids	Flavonoids	Tannins	Saponins	Cardiac glycosides	Phenols	Anthraquinones	Terpenoids
1.	Root Barks	E	+	+	+	+	+	+	-	+
		A	-	+	+	+	-	+	-	-
		Aq	-	+	+	+	+	+	-	-
2.	Stem Barks	E	+	+	+	+	+	+	-	-
		A	+	+	+	+	+	+	-	+
		Aq	+	+	+	+	+	+	-	-
3.	Leaves	E	-	+	+	+	-	+	-	+
		A	-	+	+	+	-	+	-	+
		Aq	-	+	+	+	+	+	-	+

Key: + = Positive; - = Negative, E = Ethanol, A = Acetone, Aq = Aqueous.

Table 4. *Quantitative Phytochemical Analysis of Extracts of Terminelia avecenoides.*

	T. Phenols (mg/100 g)	Flavonoids (mg/100 g)	Tannins (mg/100 g)	Terpenoids (mg/100 g)	Saponins (µg/g)	Alkaloids (mg/100g)
E L	176.00 ± 10.50	84.00 ± 3.30	120.00± 2.60	887.00 ± 4.20	24.31 ± 0.76	129.52 ± 1.96
ERB	273.00 ± 10.70	84.40 ± 1.30	102.00 ± 1.50	68.00 ± 1.20	47.27 ± 1.72	298.33 ± 1.12
ESB	123.00 ± 20.80	88.00 ± 4.20	89.00 ± 11.00	Not Detected	45.93 ± 2.20	122.48 ± 4.96
AQL	362.00 ± 20.10	77.00 ± 8.10	104.00± 1.60	35.00 ± 1.70	19.90 ± 1.02	312.43 ± 0.96
AQRB	34.00 ± 10.12	100.00 ± 13.00	83.00 ± 3.70	Not Detected	37.35 ± 3.14	236.40 ± 0.48
AQSB	540.00± 20.10	111.00 ± 10.00	112.00 ± 10.00	Not Detected	22.72± 1.31	275.28 ±1.48
A L	2331.00 ± 23.00	106.00 ± 4.30	114.00 ± 3.50	388.00 ± 3.00	37.76 ± 3.20	131.73 ± 1.21
ARB	96.00 ± 10.10	104.00± 13.00	91.00 ± 3.60	Not Detected	37.79± 2.30	127.60 ± 0.72
ASB	1660.00 ± 12.00	104.00 ± 17.00	108.00± 3.50	56.00 ± 3.00	45.22 ± 4.21	323.82 ± 3.12

Key: EL: Ethanol Leave Extract, ERB: Ethanol Root Bark Extract; ESB: Ethanol Stem Bark Extract, AQL: Aquoues Leave Extract, AQRB: Aquoues Root bark Extract; AQSB: Aquoues Stem Bark Extract, AL: Acetone Leave Extract; ARB: Acetone Root Bark Extract; ASB: Acetone Stem bark Extract.

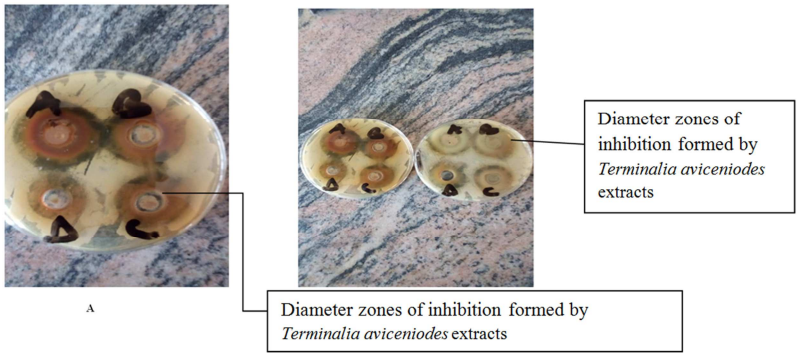

Figure 4. *A and B: growth inhibition zones formed by the activity of Terminalia aviceniodes extracts.*

3.1.5. Antimicrobial Activity of Terminalia avicennioides Extracts Against Antibiotic Resistant Pseudomonas aeruginosa Isolates

Figure 4 is the growth inhibitions zone formed by the activity of the extracts of the *Terminalia avicennioides.* The antimicrobial activity of *Terminalia avicennioides extacts* against antibiotic resistant *pseudomonas aeruginosa* result in tables 5 and 6 showed in vitro activity of the ethanol, acetone, and aqueous root bark, stem bark, and leave extracts as diameter zones of growth inhibition in millimeter for four varying concentrations. The growth inhibition zones formed ranged from 10.04±9.40 – 18.08±10.62 mm with no significant difference (P> 0.05). Comparatively, acetone root bark, leave and root bark extracts showed larger zone of growth inhibition than the ethanol stem bark which showed the smaller zone.

Table 5. *Antimicrobial Activity of Terminalia avicennioides Extracts Against Antibiotic Resistant Pseudomonas aeruginosa Isolates.*

Isolate	Extract	Mean±SD Diameter of Inhibition Zone (mm)	P-value at α = 0.05	Interpretation
	Leave, Stem and Root Bark Extracts Activity			
	AL	17.75 ± 9.58[a]		
	EL	16.88 ± 10.73[ab]		
	AQL	16.99 ± 10.44[ab]		There is significant difference between
	ASB	17.62 ± 9.40[a]	0.005	the inhibition zones formed, with
Pseudomonas	ESB	10.04 ± 9.40[c]	(P< 0.05)	Acetone extracts having larger zones
aeruginosa	AQSB	11.98 ± 10.01[ac]		compared to other extracts.
Strains	ARB	18.08 ± 10.62[a]		
(D_1, D_{12},	ERB	16.68 ± 10.34[ab]		
D_{14}, D_{17},	AQRB	14.04 ± 10.79[abc]		
D_{19}, D_{23},	Plant Parts Extracts Activity			
D_{24}, D_{30},	Leave	16.95 ± 10.19[a]	0.018	There is significant difference between
FS_3, FS_4, and	Steam bark	13.21 ± 10.05[b]	(p < 0.05)	the extracts inhibition zones, with leave
MS_1)	Root bark	16.26 ± 10.62[a]		and root extracts forming larger zone
	Extracts Strength (mg/ml)			than the stem bark extracts.
	200	24.32 ± 8.06[a]		
	100	20.16 ± 8.33[a]	0.0001	Inhibition zone based on extracts
	50	15.51 ± 8.23[a]	(P < 0.05)	strengths revealed significant difference,
	25	7.78 ± 8.27[a]		with 200mg/ml and 100mg/ml producing
				larger zone than the others.

Key: D = dressing room unit, FS = female surgical ward, MS = male medical ward, EL: Ethanol Leave Extract, ERB: Ethanol Root Bark Extract; ESB: Ethanol Stem Bark Extract, AQL: Aquoues Leave Extract, AQRB: Aquoues Root bark Extract; AQSB: Aquoues Stem Bark Extract, AL: Acetone Leave Extract; ARB: Acetone Root Bark Extract; ASB: Acetone Stem bark Extract.

Table 6. *Antimicrobial Activity of Terminalia avicennioides Extracts Against Antibiotic Resistant Pseudomonas aeruginosa Isolates.*

Isolate	Extract	Mean±SD Diameter of inhibition Zone (mm)	P-value at α = 0.05	Interpretation
Pseudomonas aeruginosa Strains (D_1, D_{12}, D_{14}, D_{17}, D_{19}, D_{23}, D_{24}, D_{30}, FS_3, FS_4, and MS_1)	Leave Extracts Activity			
	AL	17.75 ± 9.58[a]	0.8130 (P > 0.05)	There is generally no significant difference. However, concenttations zone of inhibitions showed significant difference with higher concentration having larger zone compared to the lower concentration.
	EL	16.88 ± 10.73[a]		
	AQL	16.19 ± 10.45[a]		
	ASB	19.93 ± 10.30[a]	0.0001 (P < 0.005)	
	ESB	14.94 ± 9.76[b]		
	AQSB	11.85 ± 7.81[a]		
	Stem Bark Extracts Activity	6.15± 7.07[c]	0.0033 (P < 0.05)	Zone of growth inhibition between the acetone, ethanol and root bark extracts showed significant difference. Acetone stem bark extract showed larger zone compared to ethanol and aqueous stem bark extracts.
	ASB	17.63 ± 9.41[a]		
	ESB	10.04 ± 9.40[b]		
	AQSB	11.99 ± 10.08[b]		
	Extracts Strength (mg/ml)			
	200	23.11 ± 9.94[a]	0.0001 (p < 0.005)	Concenttations zone of inhibitions showed significant difference with higher concentration having larger zone compared to the lower concentration.
	100	19.03 ± 8.81[a]		
	50	13.77 ± 9.08[b]		
	25	9.15± 9.47[b]		
	Root Bark Extracts Activity			
	ARB	18.08 ± 10.62[a]	0.2641 (P > 0.05)	The antimicrobial activity showed no significant different. However, acetone and ethanol root bark extracts showed larger zones compared to aqueous root bark extract zone of growth inhibition.
	ERB	16.68 ± 10.34[a]		
	AQRB	14.04 ± 10.80[b]		
	Extracts Strength (mg/ml)			
	200	22.12± 8.40[a]	0.0001 (p < 0.005)	Concenttations zone of inhibitions showed significant difference with higher concentration having larger zone compared to the lower concentration.
	100	19.31± 7.81[a]		
	50	14.45± 8.40[b]		
	25	13.98±6.91[b]		

Key: D = dressing room unit, FS = female surgical ward, and MS= male surgical ward, EL: Ethanol Leave Extract, ERB: Ethanol Root Bark Extract; ESB: Ethanol Stem Bark Extract, AQL: Aquoues Leave Extract, AQRB: Aquoues Root bark Extract; AQSB: Aquoues Stem Bark Extract, AL: Acetone Leave Extract; ARB: Acetone Root Bark Extract; ASB: Acetone Stem bark Extract.

Table 7. *Minimum Inhibitory Concentration (MIC) of Terminalia avicennioides Extracts against Antibiotic Resistant Pseudomonas aeruginosa Isolates.*

Isolate	Extract	Mean±SD MIC (mg/ml)	P-value at α = 0.05	Interpretation
Pseudomonas aeruginosa Strains (D_1, D_{12}, D_{14}, D_{17}, D_{19}, D_{23}, D_{24}, D_{30}, FS_3, FS_4, MS_1)	Leave, Stem and Root Bark Extracts Activity			
	AL	57.50 ± 40.91[a]	0.9941 (P > 0.05)	MIC showed no significant difference.
	L	52.50 ± 44.79[a]		
	AQL	60.00 ± 61.46[a]		
	ASB	50.00 ± 40.83[a]		
	ESB	55.00± 64.33[a]		
	AQSB	60.00 ± 65.83[a]		
	ARB	55.00 ± 28.38[a]		
	ERB	40.00 ± 21.08[a]		
	AQRB	50.00 ± 40.82[a]		
	Plant Parts Extracts Activity		0.7625 (P > 0.05)	MIC do not showed significant difference
	Leave	56.67 ± 46.22[a]		
	Stem bark	55.00 ±56.25[a]	0.9431 (p > 0.05)	MIC do not revealed significant difference. However, ethanol extracts showed higher
	Root bark	48.33 ±30.75[a]		
	Leave Extracts Activity			
	AL	57.50 ± 40.91[a]	0.9289 (p > 0.05)	MIC compared to acetone and aqueous leave extracts.
	EL	52.50 ± 44.79[b]		
	AQL	60.00 ± 61.46[a]		
	Stem Bark Extracts Activity			
	ASB	50.00 ± 40.83[a]	0.5560 (p > 0.05)	MIC do not revealed significant difference.
	ESB	55.00 ± 64.33[a]		
	AQSB	60.00 ± 65.83[a]		
	ARB	55.00 ± 28.38[a]		
	ERB	40.00 ± 21.09[a]		
	AQRB	50.00 ± 40.82[a]		

D = dressing room unit, FS = female surgical ward, and MS= male surgical ward, EL: Ethanol Leave Extract, ERB: Ethanol Root Bark Extract; ESB: Ethanol Stem Bark Extract, AQL: Aquoues Leave Extract, AQRB: Aquoues Root bark Extract; AQSB: Aquoues Stem Bark Extract, AL: Acetone Leave Extract; ARB: Acetone Root Bark Extract; ASB: Acetone Stem bark Extract.

Table 8. *Minimum Bactericidal Concentration (MBC) of Terminalia avicennioides Extracts Against Antibiotic Resistant Pseudomonas aeruginosa.*

Isolate	Extract	Mean±SD MBC (mg/ml)	P-value at α = 0.05	I interpretation
Pseudomonas aeruginosa (D_1, D_{12}, D_{14}, D_{17}, D_9, D_{23}, D_{24}, D_{30}, FS_3, FS_4, MS_1)	Leaves, Stem and Root Bark Extracts Activity			
	AL	95.45 ±78.91[a]		
	EL	86.36 ±83.94[a]		
	AQL	81.82 ±75.08[a]		
	ASB	63.64 ±50.45[a]	0.9766	Results generally showed no
	ESB	86.36±95.10[a]	(P > 0.05)	significant difference
	AQSB	81.81 ±75.08[a]		
	ARB	81.81 ±60.30[a]		
	ERB	100.00 ±77.46[a]		
	AQRB	100/00 ±89.44[a]		
	Plant Parts Extracts Activity			
	Leaves	87.87±77.08[a]	0.5696	Results generally showed no
	Stem bark	74.24 ±79.18[a]	(P > 0.05)	significant difference
	Root bark	93.94±74.74[a]		
	Leave Extracts Activity			
	AL	95.46±78.91[a]	0.9195	Results generally showed no
	EL	86.36±83.94[a]	(P > 0.05)	significant difference
	AL	81.82±75.08[a]		
	Stem Bark Extract Activity			
	ASB	63.64±50.45[a]	0.8051	Results generally showed no
	ESB	86.36 ± 95.11[a]	(P > 0.05)	significant difference
	AQSB	72.73±90.45[a]		
	Root Bark Extracts Activity			
	ARB	81.82 ±60.30[a]	0.8148	Results generally showed no
	ERB	100.00 ±77.46[a]	(P > 0.05)	significant difference
	AQRB	100.00 ± 89.44[a]		

Key: D = dressing room unit, FS = female surgical ward, and MS= male surgical ward, EL: Ethanol Leave Extract, ERB: Ethanol Root Bark Extract; ESB: Ethanol Stem Bark Extract, AQL: Aquoues Leave Extract, AQRB: Aquoues Root bark Extract; AQSB: Aquoues Stem Bark Extract, AL: Acetone Leave Extract; ARB: Acetone Root Bark Extract; ASB: Acetone Stem bark Extract.

3.1.6. Minimum Inhibitory Concentration (MIC) of Terminalia avicenode Extracts Against Antibiotic Resistant Pseudomonas aeruginosa

As presented in table 7, the MIC of leave, stem and root bark extracts for all types of solvent extracts tested against antibiotic resistant *Pseudomonas aeruginosa* isolates ranged from 60.00±61.46 - 40.00±21.08 mg/ml, but no significant difference (P> 0.05). However, ethanol root bark extracts showed higher MIC value of 40.00±21.08 mg/ml, and aqueous leave and stem bark extracts showed the lower MIC values of 60.00±61.46 mg/ml and 60.00±65.83 mg/ml respectively.

3.1.7. Minimum Bactericidal Concentration (MBC) of Terminalia avicennioides Extracts Against Antibiotic Resistant Pseudomonas aeruginosa Isolates

As presented on table 8, MBC for leave, stem and root bark extracts for all types of solvent extracts tested against antibiotic resistant *Pseudomonas aeruginosa* strains ranged from 100.00±89.44 – 63.64±50.45 mg/ml, but no significant difference (P> 0.05). However, acetone stem bark extracts showed higher MBC (63.64±50.45 mg/ml), and aqueous and ethanol root bark extracts showed the lower MBC values of 100.00±89.44 mg/ml and 100.00±77.46 mg/ml respectively.

3.1.8. Visual Characteristics of Biologically Syntheisesd Terminalia avicennioides Extracts Derived Silver Nanoparticles (AgNPs)

Table 9 showed the biologically synthesised Silver nonaparticles from the *Terminalia avicennioides* extracts visual characteristics. The colours of these AgNPs generally appeared brown. Hence, NPs1, NPs2 and NPs3 appeared light-brown, greenish-brown and light brown respectively. NPs4, NPs5 and NPs6 appeared redish- brown, coffee brown and redish-brown respectively, while NPs7, NPs8 and NPs9 appeared dark brown, coffee-brown and dark-brown respectively.

3.1.9. Antimicrobial Activity of Biologically Synthesized Silver Nanoparticles (AgNPs) from Terminalia avicenode Extracts on Antibiotic Resistant Pseudomonas aeruginosa Isolates

Table 10 revealed generally, the growth inhibition zones formed by the biologically sythesised silver nanoparticles tested against *Pseudomonas aeruginosa* isolates. The growth inhibition zone values ranged from 26.50±3.04 to 53.00±76.97mm. Between the zone of growth inhibition for the various AgNPs tested against the *Pseudomonas aeruginosa* isolates, there was no no significant diferrence (P> 0.05). However, in comparison, the antimicrobial zone of growth inhibition produces by the acetone root bark extracts derived AgNPs (NPs7) revealed larger zone of growth inhibition (53.00±76.97mm) than the other AgNPs and the standard antibitiotic. The growth inhibition zones between different AgNPs and the standard antibiotic showed significant difference (P < 0.05),

3.1.10. Comparative Antimicrobial Activity of Terminalia avicennioides Extracts and Their Biologically Synthesised Silver Nanoparticles (AgNPs) Tested Against Pseudomonas aeruginosa Isolates

The growth inhibitions zones formed by the biological

synthesised silver nanoparticles and the standard antibiotic (Ciprofloxacin) activities have been displayed on Figure 5. The growth inhibition zones formed by *Terminalia avicennioides* extracts and their respective biologically sythesised silver nanoparticles tested against *Pseudomonas aeruginosa* isolates have been presented in table 11. The comparative results analysis revealed significant difference ($P < 0.05$), with the *Terminalia avicennioides* extracts antimicrobial activity having lower inhibition zone value (16.83 ± 12.70 mm) compared to that of the respective *Terminalia avicennioides* extracts derived AgNPs (28.40 ± 2.98 mm). Similarly, between the *Terminalia avicennioides* extracts derived AgNPs activity and that of the standard antibiotic (ciprofloxacin), there was a significant difference ($P < 0.05$), with the standard antibiotic (ciprofloxacin) activity having larger diameter zones (40.50 ± 0.59 mm) than that produced by the synthesised AgNPs. Commendably, the antimicrobial activity of the biologically synthesised extracts derived AgNPs exhibit higher inhibitory effect with the root bark extracts derived AgNPs forming the largest zone of growth inhibition (28.99 ± 2.40 mm) against the *Pseudomonas aeruginosa* isolates.

Table 9. *Visual Characteristics of Biologically Synthesised Terminalia avicannodes Extracts Derived Silver Nanoparticles.*

S/N	Extract	nanoparticle code	Mixture of Extracts with Silver Nitrate reacted at 60°C for 1hour	Plant Extracts Derived Silver Nanoparticle after 24 hours Incubation
1	AL	NPs_1	G - brown	D – brown
2	EL	NPs_2	G - brown	D – brown
3	AQL	NPs_3	G - brown	L – Brown
4	ASB	NPs_4	R - brown	R - brown
5	ESB	NPs_5	L - brown	C – brown
6	AQSB	NPs_6	R - brown	R - brown
7	ARB	NPs_7	L - brown	D – brown
8	ERB	NPs_8	L - brown	C – brown
9	AQRB	NPs_9	L - brown	D – brown

Key: NPs1 to NPs9 = Synthesised Nanoparticles 1 to 9, G = green, R = red, L=light, C= coffee, D = dark, EL: Ethanol Leave Extract, ERB: Ethanol Root Bark Extract; ESB: Ethanol Stem Bark Extract, AQL: Aquoues Leave Extract, AQRB: Aquoues Root bark Extract; AQSB: Aquoues Stem Bark Extract, AL: Acetone Leave Extract; ARB: Acetone Root Bark Extract; ASB: Acetone Stem bark Extract.

Table 10. *Antimicrobial Activity of Biologically Synthesised Terminalia avicennioides Extracts Derived Silver Nanoparticles Against antibiotic Resistant Pseudomonas aeruginosa Isolates.*

Isolate	Extract	Mean±SD inhibition Zone (mm)	P-value at α = 0.05	Interpretation
	Leave, Stem and Root Bark Silver Nanoparticles Activity			
	NPs_1	28.00 ± 3.51^b		
	NPs_2	28.68 ± 2.98^b		
	NPs_3	28.95 ± 2.98^b		
	NPs_4	29.04 ± 3.04^b	0.1823	Antimicrobial activity of leave and stem bark extracts do not revraled significant difference. However, ethanol and aqueous root bark activity revealed significant differentce with ethanol extracts and standard antibiotic (Ciprofloxacin) having higher zone of growth inhibition compared to other extracts activity.
	NPs_5	26.50 ± 3.04^b	($p > 0.05$)	
	NPs_6	27.81 ± 2.6007^b		
	NPs_7	53.00 ± 76.97^a		
	NPs_8	28.59 ± 3.21^b		
	NPs_9	40.50 ± 0.59^a		
	Silver Nanoparticles and Ciprofloxacin Activity			
Pseudomonas aeruginosa Strains (D_1, D_{12}, D_{14}, D_{17}, D_{19}, D_{23}, D_{24}, D_{30}, FS_3, MS_4, MS_1)	Leave = NPs_1, NPs_2& NPs_3	28.54 ± 2.76^a	0.2534 ($P > 0.05$)	The antimicrobial activity showed no significant difference.
	Stem Bark = NPs_4, NPs_5& NPs_6	27.79 ± 3.00^a		
	4Root Bark= NPs_7, NPs_8& NPs_9	36.73 ± 44.66^a		
	Standard (Ciprofloxacin)	40.50 ± 0.59^a		
	Leave Silver Nanoparticles Activity			
	NPs_1	28.00 ± 3.51^a	0.7177	The antimicrobial activity do not revealed significant difference.
	NPs_2	28.68 ± 2.98^a	($P > 0.05$)	
	NPs_3	28.96 ± 1.62^a		
	Stem Bark Silver Nanoparticles Activity			
	NPs_4	29.05 ± 3.04^a	0.1383	The antimicrobial activity do not revealed significant difference.
	NPs_5	26.50 ± 3.05^a	($P > 0.05$)	
	NPs_6	27.82 ± 2.60^a		
	Root Bark Silver Nanoparticles Activity			
	NPs_7	53.00 ± 3.21^a	0.1414	The antimicrobial activity do not revealed significant difference.
	NPs_8	28.59 ± 3.21^a	($P > 0.05$)	
	NPs_9	29.59 ± 3.20^a		

Key: NPs1 to NPs9 = biologically Synthesised Nanoparticles 1 to 9, D = dressing room unit, FS = female surgical ward, and MS= male surgical ward.

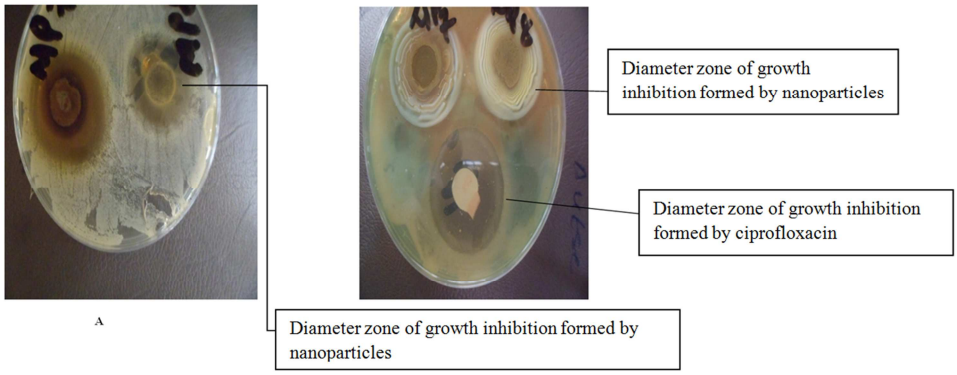

Figure 5. *A and B: inhibition zones of Biologically synthesized Terminalia avicennioides extract silver nanoparticles and Standard antibiotic.*

Table 11. *Comparative Antimicrobial Activity of Terminalia avicennioides Extracts and their Biologically Synthesised Silver Nanoparticles (AgNPs) Against pseudomonas aeruginosa Isolates.*

Isolate	Extract		Mean ±SD inhibition Zone (mm)	Df	t_{cal}	P-value at α = 0.05	Interpretation
Pseudomonas aeruginosa Strains (D_1, D_{12}, D_{14}, D_{17}, D_{19}, D_{23}, D_{24}, D_{30}, FS_3, MS_4, MS_1)	Extracts Nanoparticles		16.83 ±12.70 28.40 ±2.98	196	-8.83	0.0001 (P<0.005) 0.0001 (P<0.005)	There is a significant difference between the extract activity and their derived AgNPs, with AgNPs having larger zone of growth inhibition compared to that of extracts.
	Leave	Extracts Nanoparticles	17.12 ±12.73 28.55 ±2.76	64	-5.04	0.0001 (P<0.005)	
	Stem bark	Extracts Nanoparticles	14.11 ±12.64 27.67 ±3.19	64	-5.98	0.0001 (P<0.005)	
	Root bark	Extracts Nanoparticles	19.26 ±12.59 28.99 ±2.90	64	-4.33	0.0001 (p<0.005)	
		Ciprofloxacin	40.50 ±0.59		-13.41	0.0001 (p<0.005)	There is significant difference between the activity of the standard antibiotic (ciprofloxacin) and extracts derived AgNPs, with Ciprofloxacin having larger zone of growth inhibition compared to AgNPs.
		Nanoparticles	28.40±2.98		34.76	0.0001 (p<0.005)	

Key: D = dressing room unit, FS = female surgical ward, and MS= male surgical ward.

3.2. Discussion

Cultural morphology of *Pseudomonas aeruginosa* on centrimide agar revealed a characteristic blue-green colour for some colonies and yellow-green for other colonies. These colonies all appeared circular with smooth edges, flat and moderate shape. Prasanna *et al.* reported similar findings about the cultural characteristics of this organism on cetrimide agar [25]. Moreso, Ochai and Kolhakar stated that the production of distinctive blue-green and yellow-green colour by *Pseudomonas aeruginosa* strains on Centrimide agar is due to its water-soluble pigments, pyocyanin and pyoverdine which diffuses through the medium; and that *Pseudomonas aeruginosa* is the only Gram - negative bacteria species well known with production of pyocyanin [11]. The combination of this characteristic colour and grape-like smell of growth colonies on Centrimide agar due to production of 2-amino acepophenone distniquishes *Pseudomonas aeruginosa* from other Pseudomonas species. In addition to the identification application, they do chelate ions to support the metabolic physiology and the expression of *Pseudomonas aeruginosa* virulence factors such as exotoxin A, enoprotease and pyoverdine [26, 27]. In this study, *Pseudomonas aeruginosa* revealed colourless, flat and smooth, and non-lactose fermenters colonies on MacConkey agar. Generally, the colonies showed regular margins and alligator skin-like appearanced from top viewed. The colourless nature of the colonies is attributed to the fact that this organism does not ferment lactose. This agreed with Prasanna *et al.* report about the cultural characteristics of this organism on MacConkey agar [25].

Cellular morphology and biochemical characteristics revealed *Pseudomonas aeruginosa* to be oxidase positive - an important biochemical characteristic which indicates that Psedomonas species produces indophenol oxidase, an enzyme. This is a unique characteristic that distinguishes it from other Gram-negative bacteria [11]. It was revealed by biochemical test in this study that this organism is motile, indole negative, methyl red negative, hydrogen sulphide production negative, Voges-Proskauer negative, citrate utilization positive, urea

utilization negative, andnon-lactose, glucose, mannitol and sucrose fermenter. The Gram stain and microscopy revealed the organism to be Gram negative rod. This agreed with the findings reported by Walthiq and Mohammed [28]. Typical *Pseudomonas aeruginosa* identitfication characteristics was established in this study using the classical identification approach. However, it became necessary to identified the *Pseudomonas aeruginosa* isolates by molecular identification methods in this study for the purpose of conducting ethnobotanical on pathogen-specific wound in relation to the reported historical used of the plant *Terminalia avicennioides.* This is for reproducibility of studies according to Vanvuuren [29]. The genotypical identification approach was used was to ascertained the genomic closeness of *Pseudomonas aeruginosa* isolated from the patients' wound in this study with *Pseudomonas aeruginosa* strains available in the Genbank database according to Prescott *et al.* [30]. This study gel electrophoresis of amplified 16SrRNA genes bands of *Pseudomonas aeruginosa* isolates formed at 789 bp of the DNA marker. The searched online BLASTn of the 16SrRNA genes sequences of the study *Pseudomonas aeruginosa* isolates; Ps1, Ps2 and Ps3 showed 92.71%, 97.67% and 91.81% respectively as the percentage identity and similarity of these isolates to those from Genbank database. This confirmed the isolates identity to be *Pseudomonas aeruginosa* strains. The *Pseudomonas aeruginosa* similarity percentage ranged from 76.87%, to 99.67% and according Prescott *et al.* [30]. Prokaryotes with genomes homology of at least 70% are from the same species [30]. This confirmed the identity of these research study isolates as *Pseudomonas aeruginosa.*

The current study outcome showed that all the *Pseudomonas aeruginosa* strains as multidrug resistant to the selected conventional antibiotics - chloramphenicol, ampicillin, amoxicillin-clavulanic acid, kanamycin, tetracycline, ceftriazone, gentamycin, ciprofloxacin, meropenem and nalidixic acid; while all the isolates were sensitive to imipenem (figures 1 and 2). According to the findings in this study, imipenem, Ciprofloxacin and Gentamycin were found to be the very efficacious antimicrobial agents on all the *Pseudomonas aeruginosa* isolates. The multidrug resistance exhibited by *Pseudomonas aeruginosa* might be attributed to some factors which include; possession of unique outer membrance that prevents some antimicrobial agents from entering the bacterial cell [31], inherent resistance and capacity to acquire multidrug resistance to antimicrobial agents [32, 33], ability of resistant strain in clinical settings to be distributed in an environment with the presence of large antimicrobial agents [34], and rapid spreading of high risk “clone” of *Pseudomonas aeruginosa* ST235 carrying high antimicrobial identity resulting to infectious diseases that are difficult to treat [35].

Also, it has been reported that *Pseudomonas aeruginosa* characteristics multidrug resistance to be due to; numerous multidrug resistant efflux pump mechanism, obtaining drug resistance gene readily from bacteria by transformation, conjugation and transduction [35], biofilm formation-preventing host defenses and the antibiotic from reaching the bacteria, use of broad-spectrum beta-lactamases and metallo-B-lactamases (MBL) through alteration of penicillins binding proteins (PBP), Porin mutabion/porin impermeability (OPrD), plasmid enzymatic modification, and DNA gyrase mutations [32, 33]. The major cause of *Pseudomonas aeruginosa* resistance to beta-lactam antibiotics used in this study could due to beta-lactamases production. Both chromosomally-mediated and plasmid-mediated beta-lactmases characteristically produce AmPC-type which mediated resistance to the third generation cephoalosporin and the monobacterm [36] and also the metalo-beta-lactamase (MBL) production is considered important as a mechanism of resistance to antibiotics [32].

Imipenem was the effective drug against the *Pseudomonas aeruginosa* isolates according to the susceptibility test outcome in this study (figures 1 and 2), and this was followed by Gentamycin. This calls for the need for careful prescription of these antibiotics by health professionals for the purpose of avoiding development of resistance. In addition, susceptibility results should always be requested for used as the basis for the prescription of the antibiotics to patients. In respect to this, educating health professionals on the findings and the public health importance as contained in this study will be helpful.

Terminalia avicennioides were obtained and the bioactive compounds qualitatively and quantitatively detected from these extracts were flavonoids, alkaloids, tannins, cardiac glycosides, phenolic compounds, terpenoids and saponnins. The results showed the absence of anthroquinones from all the extracts tested. Interestingly, higher phenol content (2331-34 mg/100 g) was quantitatively detected, followed by terpenoids (887-35 mg/100 g), and the Saponins (47.27-22.72 µg/g) from the extract phytochemicals analysis. This results agreed with findings reported by Odebumin *et al*. and Alaje *et al*. [37, 38]. Irshad *et al*. reported that many medicanal plant possess these phytochemicals constituents. Including; carotenoids, steroids and ketones [39]. The most important of these according to Radhika *et al*. [40] are the alkaloids, tannins, sapponins, flavonoids and phenolic compounds. According to Cragg and Newman, the key defence and therapeutic principles of madicnal plants are derives from these plant key metabolites or phytoconstituents [41]. The important bioactive compounds such as; steroids, tannins and saponins are derivable from the medicinal plant selected for this study - *Terminilia avecennoides* parts [42]. Interestingly, these bioactive constituents have been known to provide antimicrobial activity on bacteria pathogens [43]. In this regard, flavonoids has been reported to be singly responsible for antimicrobial activity associated with most potential medicinal plant [44]. Worth noting is that, confering protection against some microbial infections has been well established by the activity of plant extracts rich in tannins and phenolics compounds [45]. The *Terminilia avecennoides* plant can be described among otjer medicinal plant that possess potent antiseptic, bactericidal and other medicinal properties due to the presence of the important phytochemicals compounds in its extracts as detected in this

study. Each of the compounds identified may have one or more therapeutic application and may be acting singly or in consortium to bring about cidal or static effect on the organism [46]. Thus, the presence of the phytochemical compound recorded in this study could be responsible for the active antimicrobial activity of this medicinal plant extracts.

Various *Terminilia avecennoides* extracts were study and their susceptibility test against antibiotic resistant *Pseudomonas aeruginosa* showed diameter zone of growth inhibition of the various concentrations, extracting solvents and parts of the plant. Antmicrobial activity of the plant extracts to the tested bacteria was revealed by formation of clear diameter zone of growth inhibition. Factors such as; the presence of important phytochemicals compounds, strength of the extracts used, and their ability to migrade throughout the medium influences the level of the antibacterial activity on the antibiotic *Pseudomonas aeruginosa* in this study. It was clearly observed that the zone of inhibition of the extracts increases as the extracts concentration increases. Thus, the linear relationship between the concentrations of the extract zone of inhibition could be that the extracts used were able to diffused into the inoculated nutrient agar. Moreso, this might be responsible for the presence of variation in size of diameter zones of growth inhibition, given larger and smoller, and a total absence of zone of growth inhibition in this study. The presence of flavonoids and a mixture of phenolic compounds and tannins is said to be responsible for the interesting antimicrobial and therapeutic activities of *Terminilia avecennoides* as revealed in this study [47]. The phenolic compounds always act as protoplasmic poison by destroying bacterial cell wall in addition to precipitation of cell proteins. Alkaloids and tannins are other metabolites known to always inhibit enzymes and protein synthesis, while glycosides are antidiarrhea [48]. The antibacterial activity of the *Terminilia avecennoides* extracts in this study formed larger diameter zone of growth inhibition at concentration of 200 mg/ml and 100 mg/ml, and this is similar to findings reported by Shedidi [49]. The present study showed *Terminalia avicennioides* extracts having potent antibacterial activity. The ability of the extracts of *Terminilia avecennoides to* inhibit the growth of the antibiotic resistant *Pseudomonas aeruginosa* explains why it is been effectively used in folk medicine for treatment of wound infection. The *Terminalia avicennioides* is the most widely used plants for traditional medicinal purposes worldwide including wound healing [50, 51]. The pulverized leaves are used in Northern Nigeria on burns and bruises. The root bark is made into a decoction along with other medicinal plants by the Baule of Ivory coast for severe jaundice and non-healing old sores. In Casamance of Senegal, the root bark is considered cleasing and healing on refractory sores. The powdered root bark is applied topically to sores and ulcers and is rubbed on the gums of toothache in Ivory Coast. The root bark is being used for treatment of skin infection and separate examination of antimicrobial activity against *Sarcina lutea, Staphylococcus aureus, Mycobacterium phlei,* and some Gram-positive organisms [52, 53]. It can therefore, be deduced from the result obtained in this study that *Terminilia avecennoides* is a source of bioactive compounds with potential therapeutic benefit, because it portrays a good inhibitory effect against the multi drug resistant *Pseudomonas aeruginosa.*

The *Terminilia avecennoides* extracts showed MIC values at different concentration depending on extracting solvent and parts of the plants. The MIC of the plant extracts tested against the antibiotic resistant *Pseudomonas aeruginosa* isolates ranged from 60.00±65.83 - 40.00±21.08 mg/ml and showed no significant difference (P> 0.05) with ethanol root bark extracts having higher MIC value of 40.00±21.08 mg/ml, and aqueous leave and stem bark extracts showed the lower MIC values of 60.00±61.46 mg/ml and 60.00±65.83 mg/ml respectively. Similarly, the MBC of the extracts tested against the antibiotic resistant *Pseudomonas aeruginosa* isolates ranged from 100.00±89.44 – 63.64±50.45 mg/ml, and showed no significant difference (P> 0.05), with acetone stem bark extracts having higher MBC (63.64±50.45 mg/ml), and aqueous and ethanol root bark extracts having the lower MBC values of 100.00±89/44 mg/ml and 100.00±77.46 mg/ml respectively. These values represent the in vitro bacteriostatic and bactericidal concentrations of these crude extracts against the multi drug resistant *Pseudomonas aeruginosa* strains. The high concentrations of the secondary metabolites such as tannins, alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, among others in this plant extracts could be attributed to the high antimicrobial activity recorded in this study. The findings are indicative of the various efficacy levels of *Terminilia avecennoides* extracts that can be enhanced by further separation, purification and concentration of the bioactive compounds of the plants.

This study revealed all the *Terminalia avicennioides* extracts derived AgNPs (NPs1 to NPs9) as brown - like in colour. The final colour of AgNPs solution; NPs1, NPs2 and NPs3 were light-brown, greenish-brown and light brown respectively. NPs4, NPs5 and NPs6 were redish- brown, coffee brown and redish-brown respectively. NPs7, NPs8 and NPs9 were dark brown, coffee-brown and dark-brown respectively. Similar findings have been reported by; Balashanmugam and Kalaichelvan, Henry *etal.*, Suresh *etal.*, and Qwidwai *etal.* [23, 22, 24, 52]. The brown color formation after 24 hours incubations as well as the significant increases in zone of growth inhibition from that of the extracts alone helps in establishing the success of the biogenic synthesis of the *Terminalia avicennioides* extracts derived Silver nanoparticles in this study. Brown colour formation have been reported as one of the visual inspection characteristics of the formation of Ag+ ions complex due to the compound reduction under mild heat at 60 ^{o}C for 1hour, and then subsequent production of AgNPs under incubation at dark condition for 24 hours [23], [24]. Production of AgNPs final brown colour is mostly related to the reaction of silver nitrate with plant extract with subsequent reduction of $Ag^{+}$to Ag^{o}. Many types of plant bioactive compounds acts as reducing agents to form nanoparticles from metal salts with consequence of any toxic by-product [53].

The findings of this study showed the antimicrobial activity of biologically synthesized silver nanoparticles

(AgNPs) of *Terminalia avicenode* extracts on antibiotic resistant *Pseudomonas aeruginosa* isolates. The diameter zones of growth inhibition produced by the biologically sythesised silver nanoparticles tested against the antibiotic resistant *Pseudomonas aeruginosa* isolates ranged from 28.00±13.51 – 53.00±76.97 mm and showed no significant diferrence (P> 0.05) with the acetone root bark extracts derived AgNPs (NP7) having larger zones of growth inhibition (53.00±76.97 mm) compared to all other AgNPs including the standard antibitiotic zone of growth inhibition.

Suresh *et al.* reported similar findings using biosynthesised silver nanoparticles derived from ethanolic extracts of *Coccinia indica* leaves against *Pseudomonas aeruginosa* isolates [24]. Several studies including studies conducted by Skandalis *et al.* and Henry *et al*. also reported similar findings on the activity of silver nanoparticles derived from plant extracts against antibiotic resistant *Pseudomonas aeruginosa* and *Escherichia coli* [54, 23]. The mechanisms of action of silver nanoparticles against bacterial pathogens has been reported by different researchers. The efficatious properties attributed to AgNPs have been reported to include; its independancy of cell wall structure, and ability to disrupt the integrity of cell membrane and cell wall of Gram - negative and Gram-positive bacteria [54]. Another property is its small size nature which makes it easier to penetrate the outer cell wall of bacteria, enter the respiratory chain and thus inhibit cell respiration and bacterial death [23]. In the same manner, plant extracts derived silver nanoparticles has been reported to exert damage to bacterial cell membrane by damaging it membrane thereby affecting the bacterial cell shape, and consequently resulting to shrinkage of bacterial membrane and formation of holes causing damage to the cell membrane [54].

This current research findings showed clear zone of growth inhibition by both the antimicrobial activity of *Terminalia avicennioides* extracts and their respective biologically synthesised silver nanoparticles (AgNPs) against the antibiotic resistant *Pseudomonas aeruginosa* isolates. There are however, a significant difference (P<0.05) between their antimicrobial activity against these wound pathogens, with the biologically synthesised plant extract derived nanoparticles having larger zone of growth inhibition (28.39±2.98 mm) than that of the plant extracts alone (16.83±12.70 mm). Many findings have reported High antimicrobial activity of biologically synthesised silver nanoparticles derived from medicinal plants extracts [55]. The capacity of these medicinal plants extracts derived nanoparticles to penetrate the flexible cell walls of bacteria is said to be responsible to their high antimicrobial activity even against antibiotic resistant bacterial pathogens [56]. The sizes of zones of growth inhibition formed by the antimicrobial activity of the various plant extracts derived biological synthesised silver nanoparticles in this study worth categorising them into "strong inhibitory activity" according to Davis and Stout [57]. It is however, evident from this study findings that the*Terminalia avicennioides* extracts derived silver nanoparticles exerted effective and broad spectrum antimicrobial activity against the studied antibiotic resistant *Pseudomonas aeruginosa* isolates, hence, can further be study and develop for therapeutic applications on bacteria wound pathogens.

4. Conclusion

Pseudomonas aeruginosa were isolated from wounds in clinics at Barau Dikko teaching hospital Kaduna, Nigeria. Susceptibility profile revealed that all the *Pseudomonas aeruginosa* were multidrug resistant strains. Findings showed the antibiotic; imipenem to be 100% effective against all these strains of wound pathogens. The extracts from*Terminalia avicennioides* plant parts contain important bioactive compounds capable of exerting antimicrobial effect, hence, exhibited significant antimicrobial activity against the antibiotic resistant wound bacteria pathogens. The biological synthesised silver nanoparticles exhibited higher antimicrobial activity against the antibiotic resistant *Pseudomonas aeruginosa strains* than the plant extracts. However, both the *Terminalia avicennioides* extracts and their respective derived silver nanoparticles efficacy against the antibiotic resistant *Pseudomonas aeruginosa* strains indicated that an effective wound therapy nano material can be produced from the*Terminalia avicennioides* plant extracts.

Declarations

Funding Source

None (self-funding).

Competing Interests

All authors have no conflict of interest to disclosed.

Study Limitations

Insufficient funds and standard equipment to explored characterisation on nano particles.

Ethical Approval

Permission to collect patients' wound samples (swabs) for isolation of *Pseudomonas aeruginosa* was obtained from the Health Research Ethics Committee of the Barau Dikko Teaching Hospital, Kaduna State University, Kaduna, Nigeria (Reference number: HREC - 20-0004).

Informed Consent

Informed consents were obtained from the patients or their guidance. Patients were also informed of their liberty to consent or decline participation. Guardian or parents of children with wound infection were requested to give assent for the children.

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