



Antibacterial Activity of *Avicennia Germinans* L. Extract and Its Green Synthesized-Gold Nanoparticles Against Drug-Resistant Uropathogenic *Escherichia Coli*

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ABSTRACT:

The emergence of antimicrobial resistance in Uropathogenic Escherichia coli (UPEC) is a major public health concern in the management of urinary tract infections. This study assessed the antibacterial activity of the crude aqueous extract and green-synthesized gold nanoparticles (AuNPs) of Avicennia germinans L. as potential alternative antibacterial strategies against multidrug-resistant (MDR) UPECs. This involved phytochemical analysis, green synthesis and characterization of AuNPs, and comparative antimicrobial analysis of crude extract and AuNPs. The plant extract contained major phytochemicals, and the successful synthesis of the nanoparticles were confirmed by evidence provided by the spectroscopic and microscopic analysis. The crude aqueous extract showed a dose-dependent antibacterial effect against the test isolates, with isolate E8 showing the strongest susceptibility (15.7 ± 1.0 mm) and E16 exhibiting the lowest (12.7 ± 1.2 mm) at a concentration of 100 mg/mL. In contrast, AuNPs exhibited variable responses across different test concentrations and isolates, with E3 showing the strongest susceptibility (15.7 ± 0.8 mm) at 25 μ g/mL. There was statistical significance between antibacterial agents and their concentrations ($p < 0.05$), with the lowest MIC and MBC at 30 mg/mL for the crude aqueous extract and 60 μ g/mL for the AuNPs. Considering the difference in concentration units, these findings revealed that the AuNPs were more potent against the MDR UPECs at significantly lower concentrations.

KEYWORDS:

Avicennia germinans; Escherichia coli; Phytochemicals; Urinary tract infection; Multidrug-resistant bacteria; Antibacterial activity; Gold nanoparticle

1. Introduction

Urinary Tract Infections (UTIs) remain one of the commonest bacterial infections, posing severe health and economic burdens. This infection, affecting over 150 million individuals yearly, often causes short-term morbidity, including lower abdominal pain, dysuria, fever, and even permanent kidney scarring [1–3]. Studies have highlighted uropathogenic *Escherichia coli* (UPEC) as a major aetiological agent associated with community-acquired urinary tract infections (UTIs) [4]. This bacterial species has been extensively studied for its virulence factors, which are responsible for evading the host's immune system and exacerbating infections [5]. Antibiotics, including fluoroquinolone, aminoglycosides, and beta-lactams, are the primary therapeutic options for treating UTIs, but the emergence and spread of multidrug-resistant (MDR) organisms, including MDR UPECs, pose a major challenge. Thus, this has resulted in a growing need for alternative therapeutic approaches, including the application of nanotechnologies in the development of drugs from medicinal plants for the management of drug-resistant infections [6,7].

Nanoparticles of noble metals like gold nanoparticles (AuNPs) have been of significant interest due to their biocompatibility [8,9]. Their predominant antimicrobial activity is often attributed to their cytotoxicity effects against various microbes, resulting from several interactions with the functional groups exposed on the surface of bacterial cells [10]. While physical and chemical synthesis of nanoparticles is possible, green synthesis using medicinal plants is an efficient, less toxic, and more affordable

approach [1,11,12]. Unlike traditional physical and chemical synthesis methods, green synthesis of nanoparticles is an eco-friendly, efficient, and cost-effective approach [13]. Studies have demonstrated the successful application of plant extracts, such as *Syzygium cumini*, *Benincasa hispida*, and *Zingiber officinale*, as reducing agents for the synthesis of gold nanoparticles and the optimization of their reaction parameters. The nanoparticles synthesized using these plants were effective against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [7,14,15].

Several phytoextracts from plants such as *Hibiscus pedunculatus*, *Hibiscus pusillus*, *Helichrysum patulum*, and *Avicennia marina* have been found as potential cures for UTIs [16–18]. *Avicennia germinans* L. (black mangrove) has also been highlighted for its antibacterial potential against *E. coli*, *S. aureus*, and *Bacillus* sp. [19]. Thus, this plant and its synthesized gold nanoparticle may serve as a promising therapeutic agent against multidrug-resistant uropathogens, including *S. aureus*, *K. pneumoniae*, and *E. coli*. Although a recent report indicates that *A. germinans* contains alkaloids, flavonoids, phenols, and tannins [20], there are no reports on its antibacterial activity specifically against MDR uropathogens. Hence, this study assessed the antibacterial activity of crude aqueous extract and green-synthesized gold nanoparticles of *Avicennia germinans* L. against drug-resistant uropathogenic *Escherichia coli* isolates from selected hospitals in Kaduna, Nigeria.

2. Materials and Methods

Collection, Identification, and Preparation of the Plant Sample

The leaves of *Avicennia germinans* were collected in December 2024 from Ogbogoro River in Obio/Akpor LGA (4° 50' 48"N and 6° 55' 50"E), Rivers State, Nigeria. The plant was identified using standard vegetative morphological identification (voucher code: UPH/P/481). This was done at the herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The identified plant leaves were rinsed and dried in a shaded area at 25°C for 45 days, after which they were pulverized into a fine powder using a clean blender [7]. To prepare the crude aqueous extract, 100 g of the plant powder was extracted in 1,000 mL of distilled water and kept at 25°C for 24 hours. The plant mixture was filtered and centrifuged at

5,500 rpm for 20 minutes. The supernatant was collected and filtered twice, using an 11 µm pore size Whatman filter paper (Grade 1). The pH of the plant aqueous extract was measured using a pH meter, and the extract was stored at 4°C for further use [21,22].

Phytochemical Analysis

A qualitative screening was performed using standard procedures for the identification of major phytochemicals present. The tests included Dragendorff's (alkaloids), Ferric chloride (phenols), Shinoda (flavonoids), Liebermann–Burchard (terpenoids), Salkowski (steroids), and the Foam test (saponins). Then, major phytochemicals (alkaloids, saponins, terpenoids, flavonoid, tannins, phenols, and coumarins) were further quantified using standard colorimetric procedures and reported as equivalents (mg/g dry weight). Phytochemical screening and quantification were conducted in triplicate at the Biochemistry Laboratory of Bayero University, Kano [23–27].

Identification of Plant Bioactive Compounds Using Gas Chromatography–Mass Spectrometry (GC–MS)

The crude aqueous extract of *A. germinans* leaves was analysed using GC–MS (Agilent GC, USA) at the multi-user laboratory of Ahmadu Bello University, Zaria, Nigeria. The aqueous extract was lyophilized and later dissolved in ethyl acetate (at 1 mg/mL). Then, the concentrated extract was filtered using a syringe (0.45 µm). Afterwards, 2 µL of the filtered extract was injected into the GC–MS system using an auto-injector. The GC–MS was equipped with an Rtx–5 capillary column (30 m × 250 µm, with 0.25 µm film thickness), and helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. The oven temperature was set to rise from 60 °C to 280 °C at a rate of 10°C every 2 minutes. A post-run at 50°C (6 min hold), and the MS transfer line was maintained at 300°C. Scans were done at *m/z* 50 to 450. The MS fragmentation patterns of the compounds were compared and identified using the National Institute of Standards and Technology library, with the mass spectrometer functioning at 70 eV [28].

Green synthesis and Characterization of *A. germinans* Gold Nanoparticles

The plant extract and a 3 mM gold (III) chloride trihydrate ($\text{AuCl}_3 \cdot 3\text{H}_2\text{O}$) solution were mixed in a

1:3 ratio to achieve a final volume of 400 mL. The mixture exhibited a colour change from yellow to ruby red and was incubated at 40°C for a 24-hour period, monitored for a colour change (ruby red colour) indicating nanoparticle synthesis. Afterwards, the nanoparticles were purified using distilled water and dried at 25°C for 2 minutes [7,14]. AuNPs were characterized using UV-visible spectroscopy (UV-Vis) (T70 UV-Vis Spectrophotometer, PG Instruments Ltd, UK). The morphology was evaluated using scanning electron microscopy (SEM) (Phenom ProX Desktop SEM, Thermo Fisher Scientific), coupled with Energy-Dispersive X-ray (EDX) analysis for elemental characterization. Then, the crystalline nature was accessed through X-ray diffraction (XRD) patterns using a Rigaku X-ray diffractometer (Tokyo, Japan). The average crystallite size of the nanoparticles was estimated using the Debye–Scherrer equation ($D = k\lambda / \beta \cos\theta$). Finally, Fourier transform infrared spectroscopy (FTIR) (Shimadzu IRTracer-100, Kyoto, JA) with a wavenumber range of 400–4000 cm^{-1} was used to detect the functional groups on the surface of the AuNPs [29–31]. These analyses were conducted at the National Research Institute for Chemical Technology and the National Steel Raw Materials Exploration Agency, Kaduna, Nigeria.

Antibacterial Activity of Crude Aqueous Extract and Green-Synthesized AuNPs of *A. germinans*

The antibacterial effect of the crude aqueous extract and green-synthesized AuNPs of *A. germinans* was assessed against five UPEC isolates. These isolates were obtained from Barau Dikko Teaching Hospital (E1 and E3), Kawo General Hospital (E8), Jowako Specialist Hospital (E14), and Dan Musa Diagnostic Centre (E16), Kaduna, Nigeria. Their identity was confirmed through Gram staining, biochemical (IMViC) tests, and their MDR status was confirmed through antimicrobial susceptibility testing [32–34]. For the antibacterial activity, concentrations of 10 $\mu\text{g}/\text{mL}$, 25 $\mu\text{g}/\text{mL}$, 50 $\mu\text{g}/\text{mL}$, and 100 $\mu\text{g}/\text{mL}$ were prepared for the green-synthesized AuNPs using 3% dimethyl sulfoxide (DMSO), as well as for the aqueous *A. germinans* leaf extract. Ciprofloxacin (10 mg/mL) was the positive control. 0.5 McFarland standardized suspensions of UPEC inoculum (1.5×10^8 CFU/ml) were streaked on Mueller-Hinton (MH) agar in triplicate, and 9 mm wells were created [35]. Then, 50 μL of each solution and concentration were introduced into

the designated wells. The plates were incubated at 37°C for 24 hours, and clear inhibition zones were measured (millimetres) [36,37].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The MIC of the crude aqueous extract and green-synthesized AuNPs of *A. germinans* was evaluated using the macro-broth diluted method using bacterial culture in tryptone soy broth adjusted to 0.5 McFarland standard. Subsequently, 50 μL of bacterial suspension was introduced to each test tube containing MH broth. Then, 50 μL (each) of the green synthesized gold nanoparticles and the aqueous plant extract were introduced separately into test tubes, with final concentrations ranging from 0.1 to 120 $\mu\text{g}/\text{mL}$ and 0.1 to 120 $\mu\text{g}/\text{mL}$, respectively. The positive control for both the green-synthesized AuNPs and the plant extract was ciprofloxacin, while the negative control was sterile 3% DMSO and sterile distilled water, respectively. The tubes were incubated at 37°C for 20 hours [14,38]. For the MBC, 50 μL from MIC tubes without growth were subcultured onto Mueller-Hinton (MH) agar plates and incubated at 37°C for 24 hours, after which they were observed for growth [39].

Statistical Analysis

All tests were conducted in triplicate and expressed as mean and standard deviation. The efficacy of the aqueous leaf extract and AuNPs against the UPEC isolates was analyzed for statistical significance ($p < 0.05$) using ANOVA and Tukey's post hoc test with the Statistical Package for the Social Sciences (SPSS) software version 23 (SPSS Inc., Chicago, IL, USA).

3. Results

Phytochemical Composition of *A. germinans* Leaf Aqueous Extract

The major phytochemicals present in the leaves of *A. germinans* included phenols (7.24 ± 1.32 mg/g), flavonoids (3.14 ± 1.20 mg/g), tannins (2.68 ± 0.94 mg/g), saponins (2.56 ± 1.14 mg/g), and alkaloids (2.42 ± 1.12 mg/g). While coumarins and terpenoids were 1.14 ± 0.89 mg/g and 1.03 ± 0.12 mg/g , respectively (Table 1).

Table 1: Phytochemical Composition of the Crude Aqueous Extract of *Avicennia germinans* Leaves

S/N	Phytochemicals	Leaf Extract (mg/g dry wt)	
		Qualitative	Quantitative
1	Alkaloids	+	2.42 ± 1.12
2	Saponins	+	2.56 ± 1.14
3	Terpenoids	+	1.03 ± 0.12
4	Flavonoids	+	3.14 ± 1.20
5	Tannins	+	2.68 ± 0.94
6	Phenols	+	7.24 ± 1.32
7	Coumarins	+	1.14 ± 0.89
8	Triterpenoids	-	ND
9	Phytosterols	-	ND
10	Cardiac Glycoside	-	ND
11	Antraquinones	-	ND
12	Anthocyanins	-	ND

“+” = present, “-” = absent, “ND” = Not Detected. Values are presented as means ± Standard deviation.

Bioactive Constituents *Avicennia germinans* Leaf Aqueous Extract.

The GC-MS analysis revealed the presence of

thirty-four compounds in the aqueous extract of *Avicennia germinans* leaf (Fig. 1) and (Table 2). These include phenolic compounds, fatty acids (and their esters), hydrocarbons/terpenoids, as well as halogen and nitrogen-containing molecules with Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl) as the most abundant compound (10.93%).

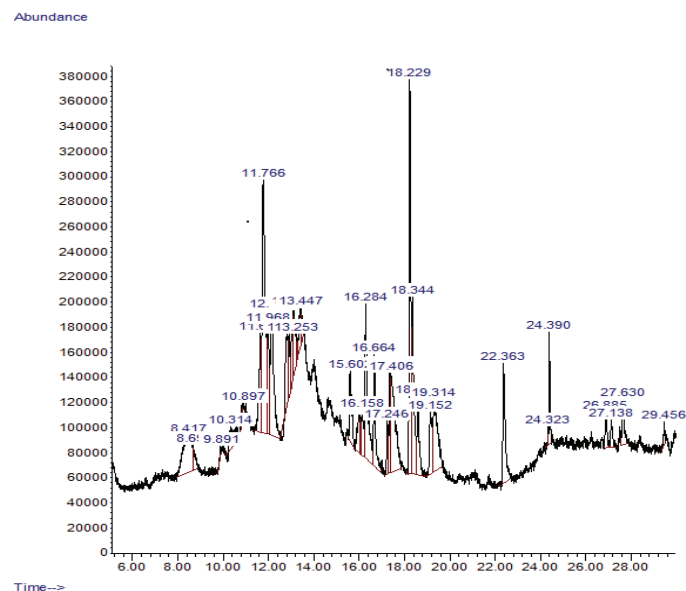


Figure 1: GC-MS Chromatogram of the Crude Aqueous Extract of *Avicennia germinans* Leaves

Table 2: GC-MS Analysis of *A. germinans* Aqueous Leaf Extract

S/N	Compound Name	Area (%)	RT	M.W (g/mol)	M.F
1	Benzenemethanol, 4-hydroxy-.alpha.-[1-(methylamino)ethyl]-, (R*,S*)-	4.8057	8.417	181.23	C ₁₀ H ₁₅ NO ₂
2	1-Hydroxysulfonyl-3,4,4-trimethyl-2-azetidinone	0.6863	8.6954	193.22	C ₆ H ₁₁ NO ₄ S
3	Benzenamine, N- (1-methylethyl)-	0.1583	9.8906	135.21	C ₉ H ₁₃ N
4	4-Hydroxy-3-methylacetophenone	0.7589	10.3144	150.17	C ₉ H ₁₀ O ₂
5	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-	0.5814	10.8586	313.4	C ₂₀ H ₂₇ NO ₂
6	Benzene, 1- (1,5-dimethyl-4-hexenyl)-4-methyl-	3.3109	11.6558	202.33	C ₁₅ H ₂₂
7	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	10.932	11.7663	204.35	C ₁₅ H ₂₄
8	Caryophyllene	3.4835	11.9683	204.35	C ₁₅ H ₂₄
9	Cyclohexene, 3- (1,5-dimethyl-4-hexenyl)-6-methylene-, [S- (R*,S*)]-	6.0523	12.1559	204.35	C ₁₅ H ₂₄
10	5-Undecanol, 2-methyl-	2.7007	12.8381	186.33	C ₁₂ H ₂₆ O
11	1-Octene, 6-methyl-	0.9354	12.9068	126.24	C ₉ H ₁₈
12	2-Octenoic acid, 4,5,7-trihydroxy	2.3805	13.0844	190.19	C ₈ H ₁₄ O ₅
13	3-Cyclohexen-1-ol, 3-methyl-	1.8003	13.1172	112.17	C ₇ H ₁₂ O
14	1-Hexyl-2-nitrocyclohexane	0.4752	13.2528	213.32	C ₁₂ H ₂₃ NO ₂

15	Cyclohexane, (2,2-dimethylcyclopentyl)-	0.9966	13.3966	180.33	C ₁₃ H ₂₄
16	Tetradecanoic acid	2.308	15.6018	228.37	C ₁₄ H ₂₈ O ₂
17	Phenol, 2,6-diamino-	1.1451	15.981	124.14	C ₆ H ₈ N ₂ O
18	Trimethylsilylpyrazole	0.9149	16.0261	140.26	C ₆ H ₁₂ N ₂ Si
19	1,4-Benzenediol, 2-methyl-	1.3989	16.1576	124.14	C ₇ H ₈ O ₂
20	Cyclohexadecane	6.115	16.2836	224.42	C ₁₆ H ₃₂
21	Pentadecanoic acid, 14-methyl-, methyl ester	3.0711	16.6641	270.5	C ₁₇ H ₃₄ O ₂
22	Didodecyl phthalate	1.0983	17.246	502.8	C ₃₂ H ₅₄ O ₄
23	n-Hexadecanoic acid	2.258	17.3745	256.42	C ₁₆ H ₃₂ O ₂
24	1-Octadecene	9.5566	18.229	350.5	C ₂₂ H ₃₈ O ₃
25	Heptadecanoic acid, 16-methyl-, methyl ester	2.5109	18.5887	298.5	C ₁₉ H ₃₈ O ₂
26	cis-11-Hexadecenal	2.4328	19.1517	238.41	C ₁₆ H ₃₀ O
27	Undec-10-ynoic acid, dodecyl ester	4.7637	19.3138	350.6	C ₂₃ H ₄₂ O ₂
28	Bis (2-ethylhexyl) phthalate	4.2297	22.3629	390.6	C ₂₄ H ₃₈ O ₄
29	Oxirane, 2,2-dimethyl-3- (3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	0.1926	24.3226	426.7	C ₃₀ H ₅₀ O
30	1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	1.8139	24.3905	192.34	C ₁₄ H ₂₄
31	N-Methyl-1-adamantaneacetamide	1	26.8848	207.31	C ₁₃ H ₂₁ NO
32	6-Chloro-1-ethyl-4-oxoquinoline-3-carboxylic acid	0.7226	27.1378	251.66	C ₁₂ H ₁₀ ClNO ₃
33	dl- α -Tocopherol	1.467	27.6299	430.7	C ₂₉ H ₅₀ O ₂
34	i-Propyl 9-tetradecenoate	0.4994	29.4562	268.4	C ₁₇ H ₃₂ O ₂

Characterization of *A. germinans* AuNPs

The synthesized *A. germinans* AuNPs produced a characteristic peak at 590 nm from the UV-Vis spectra (Fig. 2a) and the presence of differently shaped nanoparticles, with nanoprisms and nanotriangles being predominant in the SEM micrograph (Fig. 2b). The AuNPs exhibited a significant degree of aggregation and agglomeration, characterized by irregular spaces between them. EDX spectra for the AuNPs revealed gold molecule signals at 2.12–2.22 keV (Fig. 2c), and an atomic concentration of 97.69% (Table 3). Additionally, there were weak signals, indicating the presence of stromeyerite (zirconium, sulfur, iron, silver, and aluminum). The XRD analysis (Fig. 2d) depicts the crystalline nature of *A. germinans* AuNPs, with well-defined diffraction peaks at $2\theta = 38.08^\circ$, 44.29° , and 64.56° , corresponding to the 111, 200, and 220 crystallographic planes of face-centered cubic (FCC) gold (Au), respectively. Two weak

reflections of the stromeyerite (Ag, Cu, and S) were also revealed at $2\theta = 34.12^\circ$ and 44.29° . The calculated size of the AuNPs was 29.23nm, and the average lattice spacing was 4.075Å. Then, the FTIR spectrum (Fig. 2e) revealed three major peaks at 1635.69 cm^{-1} , 2139.13 cm^{-1} , and 3342.75 cm^{-1} .

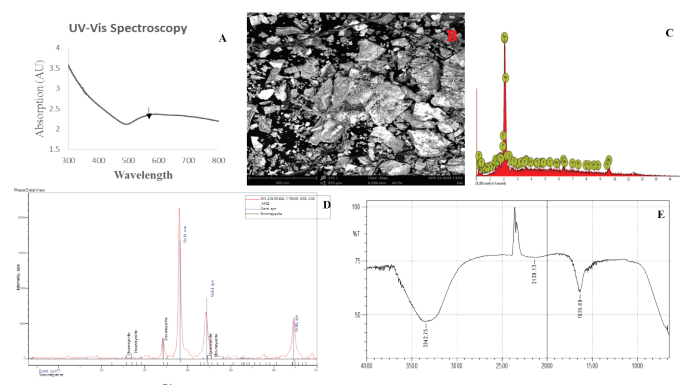


Figure 2: UV-Vis (A); SEM (B); EDX (C); XRD (D); FTIR (E) Characterization of *A. germinans* AuNPs

Table 3: EDX Elemental Microanalysis of the Biosynthesized Gold Nanoparticles

Element	Atomic Concentration (%)	Weight Concentration (%)
Gold (Au)	97.69	99.16
Zirconium (Zr)	1.19	0.56
Sulfur (S)	0.35	0.06
Iron (Fe)	0.31	0.09
Aluminum (Al)	0.12	0.02
Calcium (Ca)	0.08	0.02
Copper (Cu)	0.07	0.02
Tin (Sn)	0.05	0.03
Silver (Ag)	0.04	0.02
Chromium (Cr)	0.04	0.01
Potassium (K)	0.03	0.01
Magnesium (Mg)	0.03	0.00
Total	100	100

Antibiotic Susceptibility Pattern of MDR Uropathogenic *E. coli* Isolates

The antibiogram of the uropathogenic *E. coli* isolates showed a MAR index ranging from 0.7 to 1, and these isolates were confirmed to be multidrug-resistant to different classes of antibiotics, such as aminoglycosides, cephalosporins, penicillins, and fluoroquinolones (Table 4).

Table 4: MAR Index and MDR profile of the Uropathogenic *E. coli* Isolates

Isolates	MAR Index	MDR status
E1	1	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E3	0.9	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E8	0.7	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E14	1	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides
E16	0.8	fluoroquinolones, penicillins, cephalosporins, and aminoglycosides

Antibacterial Activity of Crude Aqueous Extract and Green-synthesized AuNPs of *A. germinans*

The antibacterial activity of the crude aqueous extract and green-synthesized AuNPs of *A. germinans* indicated significant inhibitory effects against MDR UPECs (Table 5). At the highest concentration of the plant extract (100mg/mL), isolate E8 showed the strongest susceptibility (15.7 ± 1.0 mm), while isolate E16 had the lowest susceptibility (12.7 ± 1.2 mm) recorded. However, the AuNPs showed an inconsistent dose-dependent result, with isolate E3 exhibiting a high ZOI (15.7 ± 0.8 mm) at 25 μ g/mL. Then, the control antibacterial drug (ciprofloxacin) exhibited limited antibacterial activity, with a zone of inhibition (ZOI) of 10.0 ± 0.0 mm recorded for only isolates E1 and E8. Statistical analysis showed a significant difference between the concentrations and isolates ($P < 0.05$). Tukey HSD revealed that treatments with varying superscripts (a vs b) were significantly different from each other. Considering the difference in concentration units, the nanoparticles indicated greater potency, based on their lower unit concentration. The least MIC and MBC values for the plant extract were recorded at 30 mg/mL and 60 μ g/mL for the AuNPs (Table 6).

Table 5: Average Zone of Inhibition of *A. germinans* Crude Aqueous Extract, Gold Nanoparticle, and Control (Ciprofloxacin)

Antibacterial Agent	Concentrations	Mean Zone of Inhibition (mm) of the UPEC Samples				
		E1	E3	E8	E14	E16
Plant Extract	100 (mg/mL) ^a	13.7±1.3	13.7±1.6	15.7±1.0	13.7±1.5	12.7±1.2
	50 (mg/mL) ^a	13.7±1.5	12.2±1.2	12.0±0.0	12.5±1.3	12.5±0.5
	25 (mg/mL) ^a	12.8±1.4	0.0±0.0	10.2±0.3	12.0±1.7	12.3±1.2
	10 (mg/mL) ^b	11.8±1.1	0.0±0.0	0.0±0.0	0.0±0.0	9.7±0.3
Gold Nanoparticle	100 (µg/mL) ^a	11.0±1.0	12.0±0.9	13.7±0.3	0.0±0.0	0.0±0.0
	50 (µg/mL) ^b	10.5±0.5	11.0±0.9	14.7±1.2	0.0±0.0	0.0±0.0
	25 (µg/mL) ^b	11.0±0.5	15.7±0.8	11.0±0.9	0.0±0.0	0.0±0.0
	10 (µg/mL) ^b	0.0±0.0	11.0±0.0	10.0±0.9	0.0±0.0	0.0±0.0
Control	10 (mg/mL) ^b	10.0±0.0	0.0±0.0	10.0±0.0	0.0±0.0	0.0±0.0

According to Tukey's HSD, concentrations with the same alphabet indicate no significant statistical difference ($P > 0.05$), while those with different alphabets indicate a significant statistical difference ($P < 0.05$).

Table 6: MIC and MBC of *A. germinans* Crude Aqueous Extract and Green Synthesized Gold Nanoparticles

Antibacterial Agent	MIC					MBC				
	E1	E3	E8	E14	E16	E1	E3	E8	E14	E16
Plant Extract (mg/mL)	-	30	120	120	-	-	30	120	120	-
Gold Nanoparticle (µg/mL)	60	60	60	120	-	120	60	-	-	-
Ciprofloxacin (mg/mL)	-	-	-	-	-	-	-	-	-	-
Distilled Water	-	-	-	-	-	-	-	-	-	-
3% DMSO	-	-	-	-	-	-	-	-	-	-

Values are triplicate, represented as the mean. "-" = no inhibitory/no bactericidal activity

4. Discussion

Urinary tract infections remain one of the common bacterial infections, and the spread of MDR uropathogens further exacerbates the burden of this infection. As conventional antibiotics are gradually waning, there is a need for more effective and safer alternative treatment options. Plant extracts and green-synthesized nanoparticles have been a major focus for researchers as alternatives to conventional antibiotics [7,17]. In this study, both the crude aqueous extract and green synthesized AuNPs of *A. germinans* were tested for their antibacterial properties against UPECs. This study revealed the presence of major phytochemicals, such as alkaloids, flavonoids, tannins, and phenols.

Also, the AuNPs synthesis was successful with the presence of heterogenous nanoparticles. Furthermore, the study highlighted a dose-dependent antibacterial activity for the plant extract and an inconsistent dose-dependent antibacterial activity for the AuNPs against selected UPECs.

The presence of the phytochemicals in *A. germinans* aligns with the findings of Rajeswari et al. [19], conducted on the methanolic, hexane, ethyl acetate, petroleum ether, and dichloromethanolic extracts of this plant. Several studies have highlighted the antibacterial potential of a majority of the bioactive compounds found in *A. germinans* leaves, especially phenolic compounds, fatty acids

(and their esters), and hydrocarbon/terpenoid molecules of plant-derived compounds [40–42]. The most abundant compound, Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl) (10.93%), belongs to a group of compounds known as sesquiterpenoids. These have been known for their antibacterial activities, even against *E. coli* [43]. Other compounds, such as methyl 14-methylpentadecanoate (3.0711%), 4-Hydroxy-3-methylacetophenone (0.7589%), n-Hexadecanoic acid (2.258%), and Oxirane (0.1926%) derivatives have also been found present in the leaf extract of *A. alba* [44]. The formation of *A. germinans* AuNPs was further confirmed by the UV-Vis peak at 590nm. Based on previous studies, AuNPs exhibit characteristic peaks within the range of 500nm to 600nm [14,15,45]. The SEM micrograph showed heterogeneous nanoparticles with evidence of aggregates and agglomerates. Geometrical shapes are prevalent among nanoparticles and have been described as capable of influencing their biological activities [46–48] gold nanoparticles (AuNPs). The gold molecule signals (2.12–2.22 keV) and atomic concentration of (97.69%) from the EDX spectra highlight the purity and yield of gold present in the nanoparticles [49]. The XRD peaks at $2\theta = 38.08^\circ$, 44.29° , and 64.56° closely align with those recorded by Clarence et al. [50].

Also, the AuNPs measured 29.23nm in size; Fouda et al. [15] reported a calculated size of 18nm. The lattice value 4.075Å, aligns with the finding Karuppiah et al. [51]. From the FTIR spectra, the peak observed at 1635.69 cm^{-1} corresponds to the N-H vibrations of the primary amines in proteins. Then, the absorption band at 2139.13 cm^{-1} reveals the presence of C≡C internal alkyne stretching. Finally, the peak at 3342.75 cm^{-1} suggests the presence of the hydroxyl (OH) group [52–54]. This implies that the AuNPs contain important metabolites and proteins, featuring amines, alkynes, and alcohols, which are initially present in the *A. germinans* leaves [19].

While the preliminary quantitative analysis highlighted phenols to be the most abundant phytochemicals, the GC-MS analysis revealed a sesquiterpenoid to be the most abundant compound. This may be attributed to the selective extraction of mostly volatile, non-polar constituents by ethyl acetate used in the GC-MS analysis, whereas aqueous extraction retains more polar phenolics [61,62] human beings have traditionally employed many folkloric herbal resources as complementary and alternative remedies, and these remedies

have played a pivotal role in modern medicines for many decades, as scientists have used them to develop drugs. We studied the effects of employing solvents with varying polarity on the yields of phytochemical components extracted from the plant *Rhazya stricta*. We used chloroform-methanol (1:1). The colour change to ruby red, indicating the synthesis of AuNPs, is attributed to surface plasmon resonance [14]. Aggregation and agglomeration of AuNPs occur due to electrostatic forces and are influenced by higher metal salt concentrations, longer reaction or incubation times, and post-synthesis drying [63,64]. The presence of stromeyerite can be attributed to the natural elements present in *A. germinans* [65–67]. Then, the functional groups highlighted by the FTIR spectra are responsible for the gold salt reduction (i.e., Au^{3+} to Au^0) [52]. Now, the high MAR index and MDR of the obtained UPEC isolates align with the findings of Nkene et al. [55] on *E. coli* isolated from suspected UTI patients in Keffi, Nigeria, and other reports on the MDR status of UPEC isolates [56] this cross-sectional study was conducted to predict the association of phenotypic and genotypic resistance traits in uropathogenic *Escherichia coli* (UPEC). The dose (concentration) dependent activity of the aqueous plant extract agrees with the findings in previous studies, reported for *Piper betle* and *Solanum khasianum* [57,58]. The least MIC and MBC recorded were at 30 mg/mL for the plant aqueous extract and 60 µg/mL for the nanoparticles are different from the report of Arsene et al. [59], who highlighted lower MICs and MBCs for methanolic extracts of the leaves of *Amaranthus tricolor*, *Mentha piperita*, and *Anthocephalus cadamba*, with MICs ranging from 0.36 mg/mL to 125 mg/mL, and MBCs of 21.67 mg/mL when tested against UPECs.

Compared to the plant extract, the inconsistent dose-dependent (non-linear) antibacterial activity recorded for the gold nanoparticles may be due to the formation of aggregates or agglomerates and particle heterogeneity in such nanoparticle solutions. The presence of aggregates could prevent the AuNPs from interacting with the bacterial cells [60]. Nevertheless, Diksha et al. [7] recorded a higher MIC value of 450 µg/mL for an MDR UPEC using *Syzygium cumini* gold nanoparticles.

This is the first report on the antibacterial activity of the crude aqueous extracts and green-synthesized AuNPs of *A. germinans* against MDR UPEC. It also provides evidence that supports the pharmacological potentials of *A. germinans*

leaves and their suitability as reducing and capping agents for the synthesis of antibacterial AuNPs. However, this study did not include the formation of AuNPs with controlled shapes and sizes, nor did it assess their cytotoxic effects. In addition to this, future studies should also involve dynamic light scattering (DLS) and zeta potential measurements for the nanoparticles, time–kill kinetics, growth curve inhibition studies, anti-biofilm or quorum–sensing inhibition assays, and mechanistic investigations.

5. Conclusion

The crude aqueous extract of *A. germinans* leaves was confirmed to contain significant amounts of alkaloids, saponins, terpenoids, flavonoids, tannins, phenols, and coumarins. The plant extract contained 34 bioactive compounds, with Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl) (10.93%) being the most abundant. The green synthesis of AuNPs using *A. germinans* leaf extract was successful, as further confirmed by standard spectrophotometric and microscopic analysis. This study confirms

the antibacterial activities of both the crude aqueous extract and green-synthesized AuNPs of *A. germinans* against MDR UPECs. Further studies should investigate the antibacterial activity of different bioactive compounds in the leaves and other toxic effects.

Conflicts of Interest

The authors declares no conflict of interest.

Author Contribution

Ewofere, E.E.: Conceptualisation, Methodology, Investigation, Data Analysis, Original Draft Writing, and Curation.

Oaikhena, E.E: Supervision, Validation, Review and Editing.

Egbe, N.E.: Review and Editing.

Afegbua, S.L. : Conceptualisation, Review and Editing, Data Curation and Visualisation, Validation, and Supervision.

Data Availability

Further data supporting this study are available at zenodo.org

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