

Research Article

Melia azedarach Phenolic and Terpenoid Leaf Extracts Impair Biofilm Production and Quorum Sensing in Clinical *Escherichia coli* Isolates

Iman Jawad Kadhim[✉], Alaa Akeel Jasim[✉] and Hadeel Abbas Hassan^{✉*}

Biological Control Technologies Department, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.

Abstract | To address the escalating drug resistance of uropathogenic *Escherichia coli* (UPEC), this study aimed to examine the phytochemical profile and biological potential of *Melia azedarach* leaf extracts. Hexane and ethanol fractions were obtained *via* ultrasound-assisted extraction and analyzed using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), followed by standard phenotypic assays to evaluate the antimicrobial, anti-biofilm, and anti-quorum-sensing activities. Among the total collection, 24% (n = 72) of the isolates were identified as *E. coli*. The phenotypic screening for biofilm development revealed that 46 isolates (63.8%) exhibited a strong biofilm production. The remaining isolates demonstrated a moderate (19.4%; n = 14), weak (6.9%; n = 5), or no biofilm-forming ability (9.7%; n = 7). HPLC analysis identified flavonoids and phenolic acids, specifically chlorogenic acid and catechin. These two compounds were the main constituents of the plant leaf, recording 93.48 µg/ml and 86.42 µg/ml, respectively. Additionally, rutin was the main constituent of the flavonol fractions. GC-MS screening revealed the presence of 11 terpenoids in the hexane fraction, including caryophyllene-β, germacrene D, and phytol. These two tested extracts demonstrated a remarkable antibacterial activity, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranging from 25-50 mg/ml to 50-100 mg/ml, respectively. The ethanolic leaf extract was more effective in its anti-biofilm activity, showing 90.9% inhibition at MIC/2, compared to 66.7% for the hexane fraction. Furthermore, MIC/2 treatment with the ethanolic extract substantially reduced *E. coli* swarming and swimming motility by 89.4% and 65.3%, respectively. Ethanolic extracts of *M. azedarach* inhibited quorum sensing (QS), demonstrating an 85.19% inhibition of violacein production at MIC/2, while hexane extracts displayed a 76.35% inhibition at the same concentration. The QS-inhibiting properties of both extracts and their bioactive constituents suggested their suitability for treating bacterial diseases at sub-MIC levels, promoting their biomedical use against multidrug-resistant (MDR) infections.

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***Correspondence** | Hadeel Abbas Hassan, Biological Control Technologies Department, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq; **Email:** h.alhayli@atu.edu.iq

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Introduction

Escherichia coli can cause diseases such as urinary tract infections (UTIs), bloodstream infections (sepsis), middle ear infections, wound sepsis, newborn meningitis, peritonitis, and respiratory tract infections (Naqid *et al.*, 2020; Gagaletsios *et al.*, 2025). The most common causative agent of UTIs in humans is *E. coli* (Rahman *et al.*, 2019; Naqid *et al.*, 2020; Mayer *et al.*, 2025). Biofilm formation on multiple surface types substantially contributes to the prominence of uropathogenic *Escherichia coli* (UPEC) as a leading cause of healthcare-associated complications in humans (Mayer *et al.*, 2025). Cases of multidrug-resistant (MDR) *E. coli* have increased notably, showing the highest prevalence observed in UTIs (Batubara *et al.*, 2025). Globally, *E. coli* ranks as the second critical MAR pathogen that urgently requires new therapies, heavily driving an infection-related mortality (Mayer *et al.*, 2025).

Numerous studies have shown that constituents extracted from various medicinal plants possess a range of biological activities, including antibacterial and antifungal properties (Touzout *et al.*, 2023; Naveed *et al.*, 2024). *M. azedarach*, a *Meliaceae* tree commonly known as chinaberry or Persian lilac, provides well-established medicinal benefits with documented antifungal and antibacterial effects (Touzout *et al.*, 2023; Hemdan *et al.*, 2023). *M. azedarach* contains numerous secondary metabolites, including glycosides, tannins, phenols, and flavonoids, which are responsible for its various biological activities (Touzout *et al.*, 2023; Naveed *et al.*, 2024; Hassan *et al.*, 2025). The leaf extract of this plant contains phytochemicals with an antibacterial activity against Gram-positive and Gram-negative bacteria (Naveed *et al.*, 2024; Batubara *et al.*, 2025).

The capacity to form biofilms, swarming motility, exopolysaccharide production, and the inhibition of violacein production represent critical QS-dependent factors regulated by the QS mechanism (Alrashidi *et al.*, 2022; Chaichana *et al.*, 2024). Biofilms constitute a critical threat to healthcare systems because of their elevated tolerance to conventional interventions. This resistance stems from multifactorial mechanisms, including restricted antimicrobial diffusion across the biofilm matrix and the upregulation of resistance mechanisms within the colonizing *E. coli* cells (Moradi *et al.*, 2020; Mayer *et al.*, 2025). Therefore,

it is necessary to investigate and explore new anti-biofilm compounds (Ismail *et al.*, 2024; David *et al.*, 2026).

Quorum sensing (QS) crucially regulates target gene expression, particularly virulence genes such as those involved in antibiotic resistance, motility, pigment production, biofilm formation, and other virulence factors (Dimitrova *et al.*, 2023). In addition, several protein studies have shown that downregulation of the proteins associated with QS influences the virulence-associated phenotypes, such as motility and biofilm formation in *E. coli* (Chaichana *et al.*, 2024; Mayer *et al.*, 2025). The use of quorum-sensing inhibitors (QSIs) offers a key advantage of an anti-virulence strategy relative to the standard antibiotic therapies (Maura *et al.*, 2016; Saeki *et al.*, 2020; Santos *et al.*, 2021). Emerging research indicated that phenolic-rich plant extracts possess potent QSI properties (Nazir *et al.*, 2020).

Given the scarcity of studies on the application of ultrasound-assisted extraction, we selected this technique for the present study. To the best of our knowledge, no previous studies have examined the effect of this extraction technique on the antibacterial, anti-biofilm, and anti-QS activity against *E. coli* isolated from various clinical samples in Babylon City, Iraq. Hence, this study aimed to assess the phytochemical composition of the ethanol and hexane leaf extracts of *M. azedarach* using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), respectively. The nature of the anti-QS properties of *M. azedarach* remains unknown. Therefore, the anti-QS activity of sub-MICs of these extracts was investigated by assessing the inhibition of motility (swimming and swarming) in *E. coli*, as well as the inhibition of violacein pigment production by *Chromobacterium violaceum*, representing an initial baseline evaluation.

Materials and Methods

Bacterial isolation and identification

Approximately 300 clinical samples, including 100 from each of urine, wound, and sputum were collected from patients, which attended to a Hospital in Babylon, Iraq, between October 2025 and December 2025. The suspected bacterial isolates obtained from these clinical samples were streaked on

eosin methylene blue (EMB) agar and MacConkey agar (Oxoid, Basingstoke, UK), and then incubated for 24 h at 37°C. After incubation, to identify the developed *E. coli* isolates, conventional biochemical assays were conducted, including indole, carbohydrate fermentation, catalase, oxidase, H₂S production, Voges–Proskauer, methyl red test, Kligler’s Iron Agar (KIA), urea hydrolysis, and citrate utilization, following the methods described by [Abdul Hamid and Khoshaba \(2024\)](#); [Getahun et al. \(2025\)](#). The Vitek 2 system (bioMérieux, France) and the API 20E identification kit (bioMérieux, France) further verified the identity of the recovered *E. coli* isolates. For the QS inhibition assay, stocks of *C. violaceum* ATCC 12472 strain obtained from the Microbiology Department, University of Babylon, were used.

Assessment of biofilm-forming capacity

Escherichia coli biofilm formation was quantified using the 96-well microtiter plate method described by [Fink \(2023\)](#); [David et al. \(2026\)](#). Briefly, overnight bacterial cultures in Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) were adjusted to 0.5 McFarland standard, and 200 µl aliquots were inoculated into BHI agar plates, followed by incubation at 37 °C for 48 h. Afterward, the grown cells were washed with phosphate-buffered saline and the developed biofilm cells were stained with 0.1% crystal violet for 15 min. The bound dye was solubilized using 96% ethanol, followed by absorbance measurement at 600 nm. Uninoculated BHI broth served as the negative control. Biofilm formation was categorized according to the following criteria: strong biofilm producer = OD > 4 × OD_{cut}; moderate biofilm producer = 2 × OD_{cut} < OD ≤ 4 × OD_{cut}; weak biofilm producer = OD_{cut} < OD ≤ 2 × OD_{cut}; and non-biofilm producer = OD ≤ OD_{cut}, where OD_{cut} = OD of the negative control ([Alshammari et al., 2023](#)).

Plant material collection and preparation

In November 2025, *M. azedarach* leaves were collected from the Babylon Tourist Resort, Iraq. An expert in plant taxonomy at the University of Babylon authenticated the plant identity. Following a water wash, the leaves were dried in the dark at room temperature before being ground into a fine powder using an electric laboratory mill and stored at 4°C until required ([Kadhim et al., 2021](#)).

Extracts preparation

Bioactive compounds were extracted from the dried

leaf powder *via* ultrasound-assisted extraction using an ultrasonic bath (Bandelin Electronic, Berlin, Germany) for 60 min at 40°C following the methodology described by [Batubara et al. \(2025\)](#). Specifically, hexane was employed as the solvent for terpenoid extraction, while 70% ethanol was utilized for phenolic extraction. Quantification of total phenolics was performed spectrophotometrically *via* the Folin–Ciocalteu method against a standard curve of gallic acid (Sigma-Aldrich, St. Louis, MO, USA) ([Mykhailenko et al., 2025](#); [Kadhim et al., 2026](#)). Moreover, total terpenoids were determined spectrophotometrically as per the procedure described by [Biswas et al. \(2021\)](#).

In vitro antibacterial efficacy of M. azedarach extracts

Stock solutions of *M. azedarach* leaf terpenoids extract (MLTE) and *M. azedarach* leaf phenolic extract (MLPE) were prepared at concentrations of 10, 25, 50, and 75 mg/ml ([Al-Aboudi and Al-Azawi, 2025](#)). The disc diffusion technique was performed to determine the growth inhibition zones according to [Touzout et al. \(2023\)](#); [Getahun et al. \(2025\)](#). Briefly, bacterial suspensions adjusted to 10⁸ cfu/ml were inoculated onto Mueller-Hinton Agar (MHA) plates. Sterile 6 mm paper discs were loaded with 50 µl of each extract dilutions and placed aseptically onto the agar surface. Ciprofloxacin (5 µg/ml) and Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) served as the positive and negative controls, respectively. Following incubation at 37 °C for 24 h, the inhibitory activity of the extracts was determined by measuring the diameter of the zone of inhibition (mm) using a calibrated ruler ([Touzout et al., 2023](#); [Getahun et al., 2025](#)). The MIC values were determined *via* the resazurin microtiter assay following the methodology described by [Fink \(2023\)](#). Briefly, 100 µl of each extract dilution were individually mixed with 100 µl of Nutrient Broth (NB) and 5 µl of *E. coli* inoculum (10⁶ cfu/mL) in 96-well microplates. Following incubation at 37°C for 24 h, each well received 10 µl of 0.015% resazurin solution. The plates were incubated further under identical conditions for 4 h to evaluate the colorimetric changes. The MIC represented the lowest extract concentration that prevented conversion of the blue dye to pink. The MBC was determined by subculturing a 10 µl aliquot from each microtiter well showing no visible turbidity onto MHA (Oxoid, Basingstoke, UK) plates, before maintaining them at 37°C for 24 h ([Getahun et al., 2025](#)).

Antibiofilm effects of M. azedarach extracts

The anti-biofilm activity of *M. azedarach* extracts was tested at the MIC, MIC/2, MIC/4, and MIC/8, where all of them represented the sub-MIC concentrations. The biofilm assay was performed as described above (Al-Aboudi and AL-Azawi, 2025).

The percentage of biofilm inhibition = [(OD control–OD extract)/ OD control] ×100 (David *et al.*, 2026)

Chemical composition of the extracts

The chemical composition of the hexane extract (terpenoid extract, MLTE) was determined *via* GC-MS (Agilent Technologies, Santa Clara, CA, USA) system equipped with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness), using helium as a carrier gas at a flow rate of 1.0 ml/min. Aliquots of 100 µL of extract were analyzed with an inlet temperature of 300°C. The mass spectrometer operated in electron ionization mode, and identification of compound involved spectral comparison with library databases and authentic standards. Relative abundances were quantified from the peak areas in the total ion chromatograms (Naveed *et al.*, 2024; Batubara *et al.*, 2025). The chemical composition of the ethanol extract (phenolic extract, MLPE) was characterized using a HPLC system (Shimadzu, Kyoto, Japan). Analyses were performed according to Touzout *et al.* (2023); Mykhailenko *et al.* (2025). By using an Agilent Technologies 1100 series liquid chromatograph equipped with a Kinetex Evo C18 analytical column (250 × 4.6 mm, 3 µm) maintained at 40°C. The mobile phase consisted of 1% formic acid in water (A) and acetonitrile plus 1% formic acid (B), with the elution rate set to 0.8 ml /min. Sample aliquots of 20 µl were injected, and peak monitoring was achieved at 254 nm. Identification of phenolic compounds involved matching their retention times and UV spectra against authentic reference standards analyzed under matching conditions.

Effect of extracts on the growth curves of Escherichia coli

The broth microdilution technique in 96-well microtiter plates was employed to monitor the inhibitory influence of the extracts on *E. coli* growth kinetics. 100 µl of a bacterial suspension adjusted to 10⁶ cfu/ml were mixed with 100 µl of graded plant extract concentrations (*i.e.*, MIC, MIC/2, MIC/4, and MIC/8). Wells containing inoculated broth without extracts served as the growth control. The plates were incubated at 37°C for 24 h, and optical density readings were recorded at 600 nm every 1 h

using a microplate reader (BMG LabTech, Offenburg, Germany) to construct continuous kinetic curves. (Chaichana *et al.*, 2024).

Anti- quorum sensing activity

To ensure that the extracts did not affect the bacterial growth, two QS assays were conducted at sub-inhibitory concentrations.

Evaluation of bacterial swimming and swarming motilities

Following minor optimizations to the established protocol, swarming migration was evaluated using semi-solid LB agar matrices (0.5%) supplemented with 0.5% filter-sterilized D (+)-glucose (Santos *et al.*, 2021). To evaluate the anti-motility effects, the plant extracts were incorporated individually into the agar at graded concentrations, specifically ranging from MIC down to MIC/16, including MIC/2, MIC/4, and MIC/8 (corresponding to 50, 25, 12.5, 6.25, and 3.12 mg/ml for MLTE, and 30, 15, 7.5, 3.75, and 1.87 mg/ml for MLPE, respectively). Swimming translocation profiles were monitored using a semi-solid LB agar (Oxoid, Basingstoke, UK) medium (0.25% agar) containing the extracts at sub-MICs. Approximately 2 µl aliquot of a bacterial culture inoculum containing 10⁸ cfu/ml of *E. coli* were spotted at the center of the plate, followed by incubation at 37°C for 12 h (swarming motility) or 20 h (swimming motility). The control plate consisted of a semi-solid agar prepared without the extracts. Both types of motility were assessed by measuring the diameter of the circular growth spread around the inoculation point by using a calibrated ruler (Bose *et al.*, 2020).

Determination of violacein production

To evaluate the potential of the extracts to inhibit the QS system at sub-MICs, suppression of violacein pigment synthesis by *C. violaceum* ATCC 12472 was assessed. An overnight Luria-Bertani (LB) broth culture of *C. violaceum* ATCC 12472 strain was prepared. The assay was performed according to Moradi *et al.* (2020). Violacein production inhibition was quantified in 96-well microtiter plates using *C. violaceum* ATCC 12472 strain. Briefly, 100 µl of LB broth containing sub-MICs of extracts were inoculated with 10 µl of a bacterial suspension 10⁶ cfu/ml and incubated at 30°C for 24 h in a shaking incubator. Untreated *C. violaceum* cultures served as the positive control, while wells containing broth and extracts alone acted as the negative control. Following incubation, the density of the extracted violacein pigment was measured

colorimetrically at 585 nm using a microplate reader. The absorbance of each well was measured at 585 nm and 600 nm to determine pigment yields and host bacterial proliferation, respectively. Violacein pigment inhibition (%) was calculated using the following equation (Alrashidi *et al.*, 2022):

$$\text{Inhibition (\%)} = \left[\frac{(OD_{585nm} C - OD_{585nm} T)}{OD_{585nm} C} \right] \times 100$$

Where $OD_{585nm} C$ is the OD_{585nm} of violacein production in control wells (without extracts) and $OD_{585nm} T$ is the OD_{585nm} of violacein production in wells containing *C. violaceum* ATCC 12472 strain treated with the extracts.

Statistical analysis

All treatment data were obtained from three replicate, and results are expressed as mean $\pm$ standard deviation ($\pm$ SD). Data were subjected to analysis of variance (ANOVA) by Tukey's post-hoc test using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA) and the Chi-square test. A completely randomized design was used, with significance evaluated at $p \leq 0.05$.

Results

Identification of *Escherichia coli*

Escherichia coli accounted for 24% of all isolates

recovered during the study period. Of the 300 samples, 72 isolates (24%) were identified as *E. coli*: 14 *E. coli* isolates were from wound samples (WEC), nine isolates from sputum samples of pneumonia patients (SEC), and 49 isolates from urine samples of UTI patients (UEC). Identification of the isolates was further confirmed using the standard analytical profile index (API 20E) identification kits and the Vitek 2 system.

Investigating biofilm production

Biofilm production and density were measured using the microtiter plate assay technique, with absorbance readings recorded at 600 nm. The results of biofilm production are presented in Figure 1. All UEC isolates produced biofilms, but with varying degrees: 71.4% were strong producers, 20.4% moderate, and 8.2% weak. Among the WEC isolates, 71.4% produced biofilms, with 57.1% being strong producers and 14.3% moderate producers, whereas 28.6% were non-producers. For SEC isolates, 66.6% produced biofilms; 33.3% were strong, 22.2% moderate, and 11.1% weak, and 33.3% were being non-producers. Across all the combined clinical sources (*i.e.* urine, wound, and sputum), strong biofilm expression reached 63.8%, followed by moderate (19.4%) and weak (6.9%) production, whereas 9.7% of the isolates were non-biofilm-producers.

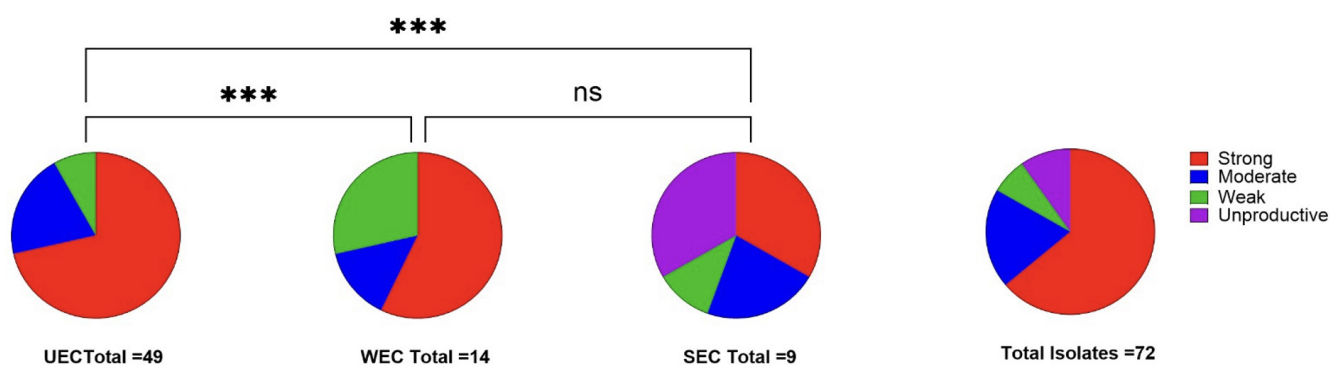


Figure 1: Biofilms formation by *E.coli* isolates (UEC: *E. coli* from urine, SEC: *E. coli* from sputum, and WEC: *E. coli* from wound). Classification of biofilm production in urine, wound, and sputum isolates. Proportions of different biofilm production levels among all the tested isolates. *** indicates a statistically significant difference ($p < 0.001$) based on the Chi-square test of independence. ns indicates a non-significant difference.

Antibacterial susceptibility screening

The antibacterial activities of MLPE and MLTE were evaluated through an *in vitro* inhibition zone assay using *E. coli* isolates. Antibacterial activities were observed for both MLPE and MLTE with concentrations ranging from 10 to 75 mg/ml, whereby strong inhibition of microbial growth was observed compared to ciprofloxacin (positive control) and DMSO (negative control), respectively. Compared to the control, the microbial proliferation was inhibited by both extracts, demonstrating high antibacterial efficacy with relative inhibition percentages ranging from 21.4% to 75.0% (Table 1). The highest relative inhibition percentage (75.0%) was observed for MLPE (75 mg/ml) against SEC isolates. The highest percentage efficacy for the hexane extract (75 mg/ml) was recorded as 46.4% against SEC isolates. Based on the established MIC and MBC parameters, both tested extracts demonstrated clear bactericidal properties against the isolates. However, MLPE showed stronger bacteriostatic effects against the isolates. The MIC values for both extracts ranged from 25 mg/ml to 50 mg/ml. Both extracts demonstrated a remarkable antibacterial potency, with the lowest MIC concentration of 25 mg/ml recorded for the MLPE extract against SEC isolates. The MIC value of MLTE was higher for UEC isolates (50 mg/ml) than for MLPE. The MBC of both extracts ranged from 50 to 100 mg/ml. The lowest MBC (50 mg/ml) was observed for MLPE against SEC isolates. Compared to the control, these MIC obtained results indicated

that MLPE was more effective than MLTE against the *E. coli* isolates. Both UEC and WEC isolates had MIC values of 30 mg/ml for MLPE, while SEC isolates had an MIC of 25 mg/ml for MLPE. The MBC values required to completely inhibit growth of the tested *E. coli* isolates were approximately 50–60 mg/ml for MLPE and 80–100 mg/ml for MLTE.

Anti-biofilm activity of extracts

To determine the anti-biofilm efficacy of both extracts, crystal violet staining biomass assays were conducted using *E. coli* isolates, which were incubated with sub-MIC levels (MIC, MIC/2, MIC/4, and MIC/8), as shown in Figure 2. Both tested extracts showed different effects on the development of the preformed biofilms. The analysis displayed that the MIC of both extracts substantially reduced biofilm formation in all isolates. SEC isolates demonstrated a greater sensitivity to MLPE, followed by UEC isolates, whereas WEC isolates showed a lower sensitivity to the extracts at MIC/2. In contrast, WEC isolates demonstrated a greater sensitivity to MLTE, followed by SEC isolates, whereas UEC isolates showed a lower sensitivity to MLTE at MIC/2. Maximal anti-biofilm activity was achieved with MLPE, which disrupted the SEC isolates biofilm matrices by up to 90.9% at MIC/2, while MLTE reduced WEC isolate's biofilm by up to 66.7% at MIC/2. Our data confirmed a concentration-dependent effect of the extracts, with a peak biofilm reduction recorded at the highest tested extract concentrations.

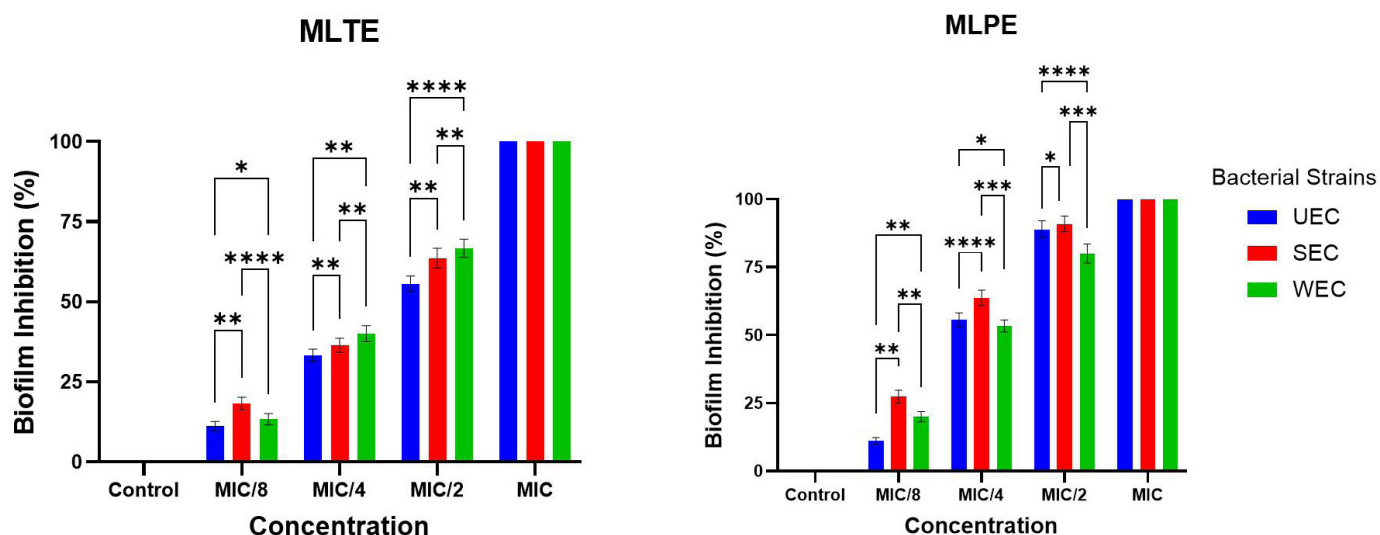


Figure 2: Percentages of biofilm inhibition of *E. coli* isolates (UEC: *E. coli* from urine, SEC: *E. coli* from sputum, and WEC: *E. coli* from wound), in the presence of *M. azedarach* leaf phenolic extract (MLPE) and *M. azedarach* leaf terpenoid extract (MLTE) at sub-minimal inhibitory concentrations. Error bars represent the standard deviation (SD) calculated from three independent experimental replicate. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$) indicate statistically significant differences.

Table 1: Antibacterial activity and its relative percentage for both *Melia azedarach* phenolic leaf extract (MLPE) and *Melia azedarach* terpenoids leaf extract (MLTE) against *E. coli* isolates.

Extract	Concen- tration (mg/ ml)	Bacterial isolates						p-value (isolate)
		UEC (NO. 49)		SEC (NO. 9)		WEC (NO. 14)		
		Inhibi- tion zone (mm±SD)	Relative inhibition (%)	Inhibition zone (mm±SD)	Relative inhibition (%)	Inhibi- tion zone (mm±SD)	Relative inhibition (%)	
MLTE	10	7±1	25	6±0.95	21.4	7±0.8	25	< 0.001
	25	9±0.54	32.1	8±0.47	28.6	10±0.22	35.7	< 0.001
	50	10±0.47	35.7	9±0.55	32.1	11±0.47	39.3	< 0.001
	75	12±0.72	42.9	13±0.63	46.4	11±0.28	39.3	< 0.001
MLPE	10	12±0.36	42.9	11±0.37	39.3	12±0.94	42.9	< 0.001
	25	15±0.38	53.6	14±0.28	50	16±0.38	57.1	< 0.001
	50	16±0.47	57.1	16±0.38	57.1	18±0.62	64.3	< 0.001
	75	19±0.66	67.9	21±0.66	75	20±0.44	71.4	< 0.001
Control (-) DMSO	10 %	0	0	0	0	0	0	
Control (+) ciprofloxacin	5 µg/ml	28	100	28	100	28	100	
MIC for hexane extract		50 (mg/ ml)		40 (mg/ ml)		40 (mg/ ml)		<0.001
MIC for ethanol extract		30 (mg/ ml)		25 (mg/ ml)		30 (mg/ ml)		<0.001
MBC for hexane extract		100 (mg/ ml)		80 (mg/ ml)		80 (mg/ ml)		<0.001
MBC for ethanol extract		60 (mg/ ml)		50 (mg/ ml)		60 (mg/ ml)		<0.001

Where: UEC: *E. coli* isolate from urine, SEC: *E. coli* isolate from sputum, WEC: *E. coli* isolate from wound, SD: standard deviation, DMSO: dimethyl sulfoxide, MIC: minimal inhibitory concentration, MBC: minimal bactericidal concentration. Data mean ± standard deviation (±SD) of triplicate independent experiments. $p < 0.001$ indicates a statistically significant difference among the bacterial isolates within the same concentration. NO. indicates the isolates number.

GC-MS Chromatogram Profile (Realistic Baseline)

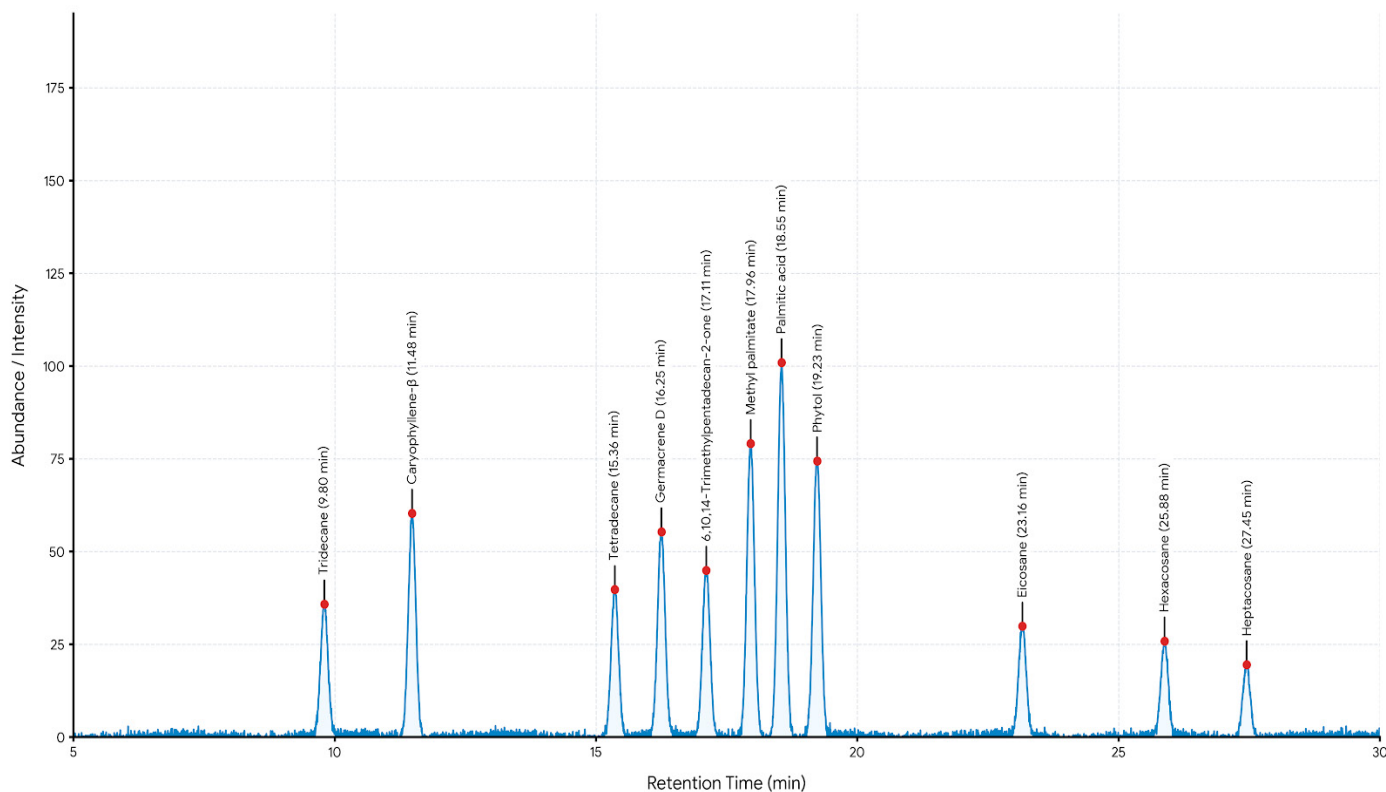


Figure 3: GC-MS chromatogram profile of *Melia azedarach* leaf terpenoid extract (MLTE), demonstrating the separation peaks of the prominent bioactive compounds.

The GC-MS analysis of hexane extract (terpenoid extract)
Chemical profiling of the MLTE fraction by GC-MS analysis identified 11 compounds and their corresponding retention times (Table 2, Figure 3). The prominent terpenoids in the MLTE fraction were caryophyllene- β (Peak 2; RT: 11.48 min), germacrene D (Peak 4; RT: 16.25 min), and phytol (Peak 8; RT: 19.23 min). Furthermore, the MLTE extract comprised several alkanes, including tridecane, eicosane, and heptacosane.

HPLC analysis of ethanol extract (phenolic extract)
Phenolics in the *M. azedarach* MLPE fraction were fingerprinted using HPLC. These bioactive phenolics were detected based on their retention times and elution sequence (Table 3, Figure 4). The first three peaks were identified as the phenolic acids: gallic, chlorogenic, and caffeic acids. Other peaks were observed to be flavonoids, including catechin, catechin-7-O-glycoside, hyperoside, rutin, isoquercetin, quercetin, isorhamnetin, and chrysin. Chlorogenic acid (93.48 $\mu\text{g}/\text{ml}$) and catechin (86.42 $\mu\text{g}/\text{ml}$) were the most abundant constituents, with rutin being identified as the predominant flavonol.

Effect of extracts on the growth curves of Escherichia coli
To examine the effect of *M. azedarach* leaf extracts on the QS system, the growth of *E. coli* isolates was

determined in the presence of various concentrations of MLPE and MLTE. The MIC values varied among the bacterial isolates: MLTE showed values of 50 mg/ml for UEC isolates and 40 mg/ml for WEC and SEC isolates. Meanwhile, MLPE showed MIC values of 30 mg/ml for UEC and WEC isolates and 25 mg/ml for SEC isolates. As shown in Figures 5 and 6, the growth curve of the control *E. coli* isolates entered the

Table 2: Identification of biologically active compounds in the *M. azedarach* hexane leaf extract (terpenoids extract) detected by Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Peak	Retention time (min.)	Chemical Compounds	Nature of the compound
1	9.80	Tridecane	Alkanes
2	11.48	Caryophyllene- β	Terpenes
3	15.36	Tetradecane	Alkanes
4	16.25	Germacrene D	Terpenes
5	17.11	6,10,14-Trimethylpentadecan-2-one	Phytone
6	17.96	Methyl palmitate	Esters
7	18.55	Palmitic acid	Fatty acid
8	19.23	Phytol	Terpenes
9	23.16	Eicosane	Alkanes
10	25.88	Hexacosane	Alkanes
11	27.45	Heptacosane	Alkanes

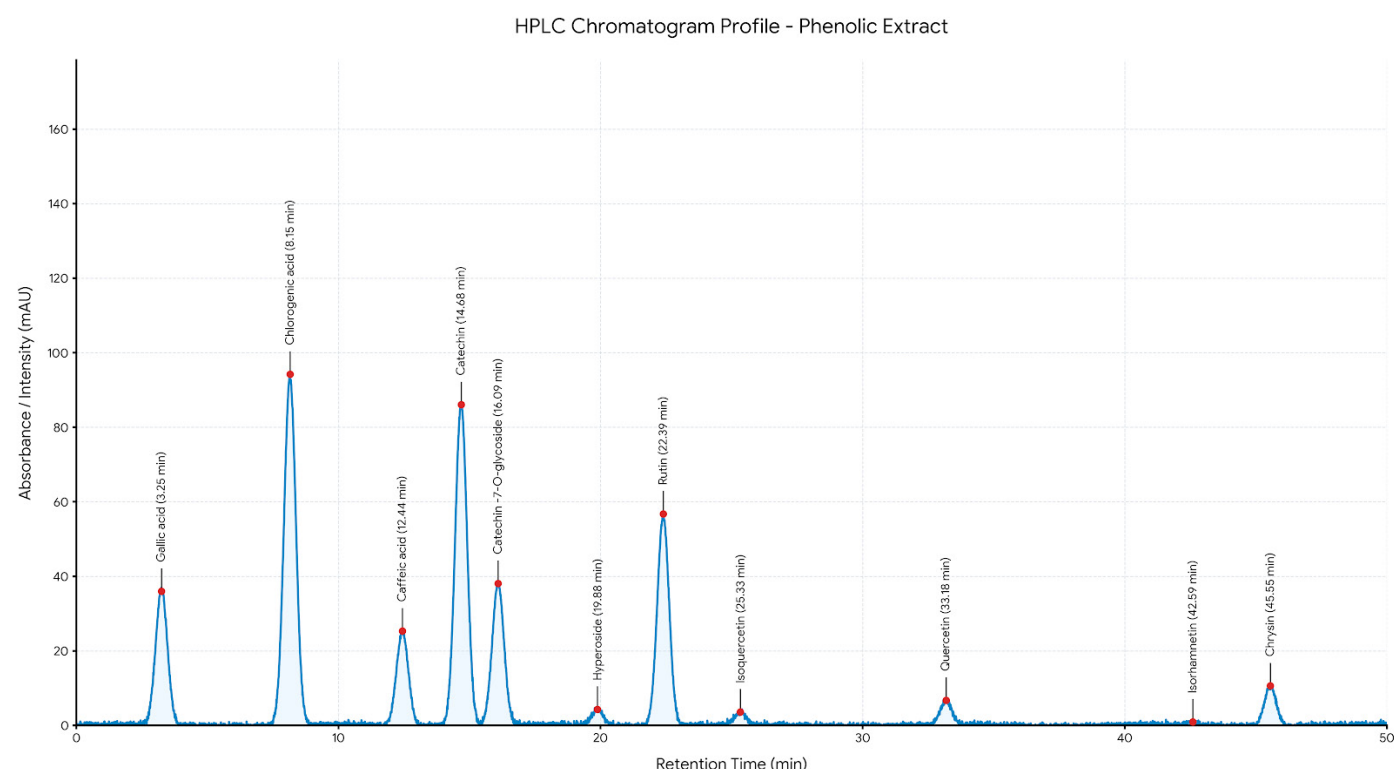


Figure 4: HPLC-UV chromatogram profile of *Melia azedarach* leaf phenolic extract (MLPE), demonstrating the separation peaks of the prominent bioactive compounds evaluated at 254 nm.

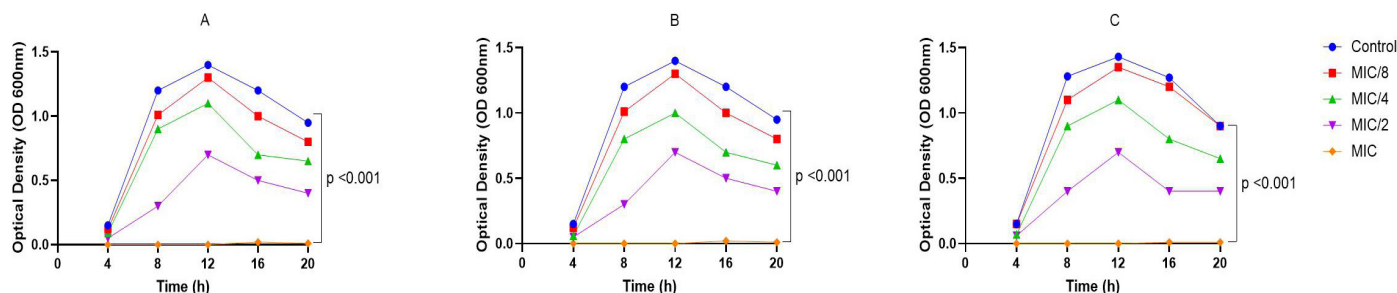


Figure 5: Growth curves of *E. coli* isolates: (A) *E. coli* from urine, (B) *E. coli* from sputum, (C) *E. coli* from wound, treated with *M. azedarach* leaf phenolic extract (MLPE) at sub- minimal inhibitory concentrations. $p < 0.001$ indicates statistically significant differences.

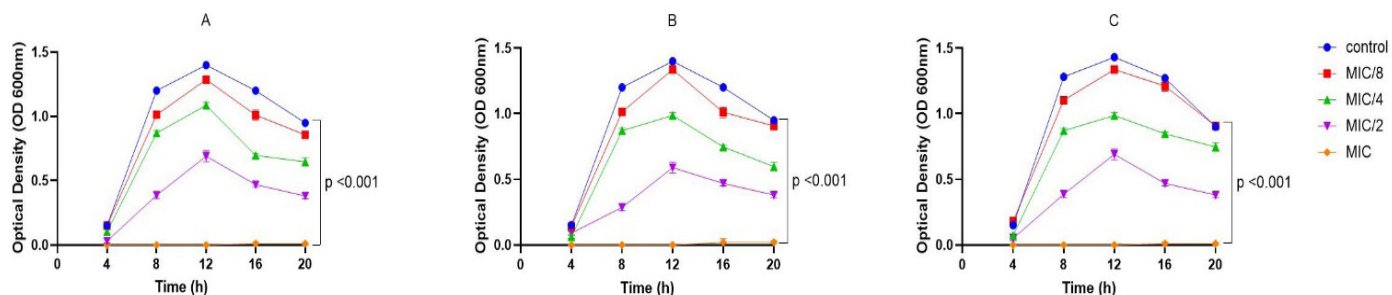


Figure 6: Growth curves of *E. coli* isolates: (A) *E. coli* from urine, (B) *E. coli* from sputum, (C) *E. coli* from wound, treated with *M. azedarach* leaf terpenoid extract (MLTE) at sub- minimal inhibitory concentrations. $p < 0.001$ indicates statistically significant differences.

Table 3: Identification of biologically active compounds in the *M. azedarach* ethanol leaf extract (phenolic extract) detected by High-Performance Liquid Chromatography (HPLC) analysis.

Peak	Reten- tion time (min.)	Chemical compounds	Nature of the compound	µg/ ml
1	3.25	Gallic acid	Phenolic acids	36.35
2	8.15	Chlorogenic acid	Phenolic acids	93.48
3	12.44	Caffeic acid	Phenolic acids	25.34
4	14.68	Catechin	Flavonoids (flavan- 3-ols)	86.42
5	16.09	Catechin -7-O-glycoside	Flavonoids (flavonols)	37.88
6	19.88	Hyperoside	Flavonoids (flavonols)	4.26
7	22.39	Rutin	Flavonoids (flavonols)	56.33
8	25.33	Isoquercetin	Flavonoids (flavonols)	3.27
9	33.18	Quercetin	Flavonoids (flavonols)	6.36
10	42.59	Isorhamnetin	Flavonoids (flavonols)	0.67
11	45.55	Chrysin	Flavonoids (flavones)	10.68

exponential phase within 10–15 h of incubation and reached the stationary phase after 15 h. Both MLPE and MLTE completely inhibited *E. coli* growth at the MIC, whereas bacterial growth was slower at concentrations below the MIC, eventually reaching

the logarithmic and stationary phases. Sub-MIC concentrations were determined through growth curve analysis. The MIC/8 concentration showed no inhibitory effect on *E. coli* growth, and all subsequent experiments were conducted at concentrations below sub-MICs.

Anti-QS Activity of extracts

Evaluation of anti-motility (Swimming and swarming assay)

The influence of both extracts on the motility of *E. coli* isolates was also evaluated, given the pivotal role that motility plays in biofilm formation by these bacteria. In this study, the anti-QS potential of both extracts at sub-MICs on the swarming and swimming motility of *E. coli* isolates was examined. The obtained results displayed that both extracts inhibited the swarming and swimming motility of *E. coli* isolates at various sub-MIC concentrations. At MIC/2, swarming was inhibited by 79.4% and 89.4% for MLTE and MLPE, respectively (Figure 7), with SEC isolates showing the highest percentage of inhibition. At MIC/2, swimming was inhibited by 53.3% and 65.3% for MLTE and MLPE, respectively (Figure 8), with UEC isolates demonstrating the highest percentage of inhibition. For MLPE-treated SEC isolates, the diameters of the swarming and

swimming motility zones were decreased to 55.1% and 89.4%, respectively, compared to the control samples (Table 4). The motility of *E. coli* isolates decreased following exposure to both extracts at sub-MICs, in a concentration-dependent manner. At MIC/2, both extracts caused a considerably greater

inhibition of swimming motility than at MIC/4. At MIC/2 for MLTE, the swimming motility of the UEC, SEC, and WEC *E. coli* isolates was reduced by 53.3%, 49.4%, and 50.1%, respectively. While at MIC/2 for MLPE, the motility was decreased by 65.3%, 55.1%, and 57.8%, respectively.

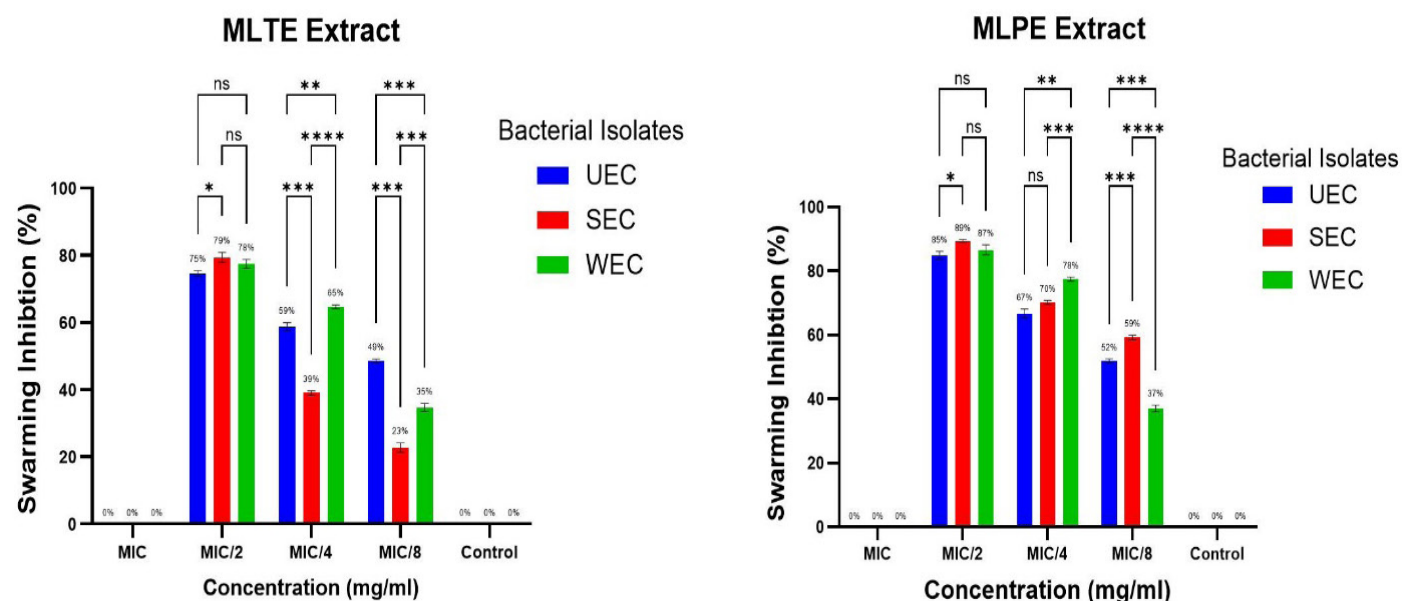


Figure 7: Swarming motility inhibition of *E. coli* isolates (UEC: *E. coli* from urine, SEC: *E. coli* from sputum, and WEC: *E. coli* from wound) upon exposure to sub-minimal inhibitory concentrations levels of the *M. azedarach* leaf phenolic extract (MLPE) and *M. azedarach* leaf terpenoid extract (MLTE). Error bars represent the standard deviation (SD) calculated from three independent experimental replicate. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$) indicate statistically significant differences. ns indicates a non-significant difference.

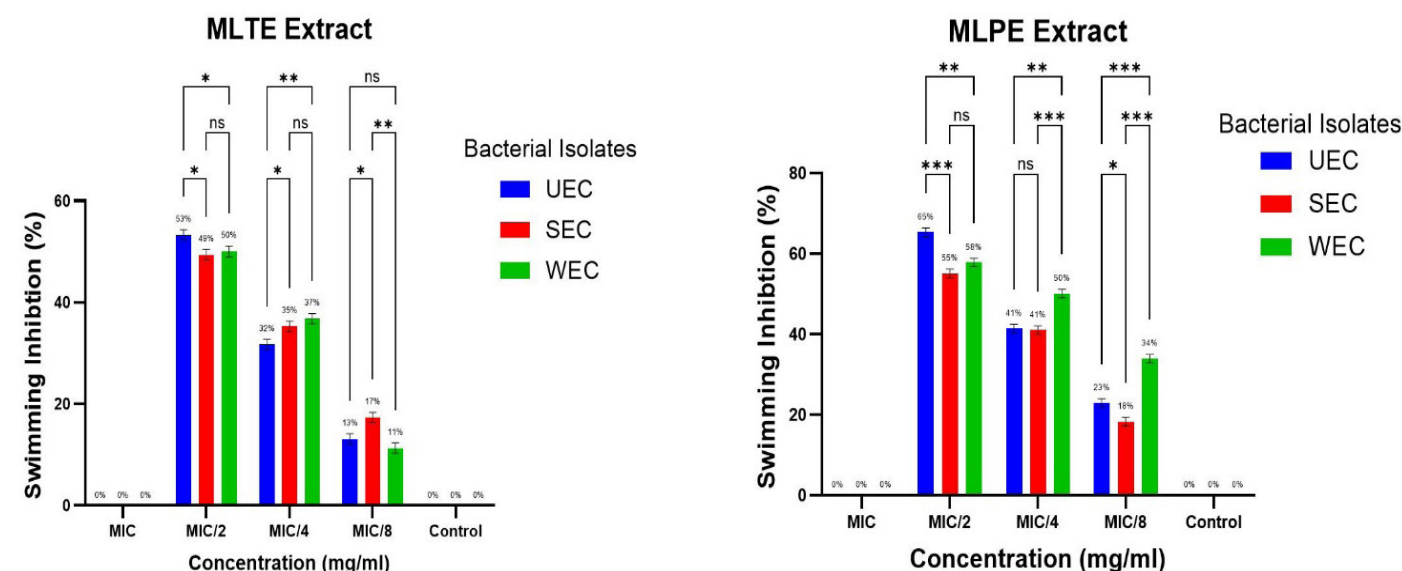


Figure 8: Swimming motility inhibition of *E. coli* isolates (UEC: *E. coli* from urine, SEC: *E. coli* from sputum, and WEC: *E. coli* from wound) upon exposure to sub-minimal inhibitory concentrations levels of the *M. azedarach* leaf phenolic extract (MLPE) and *M. azedarach* leaf terpenoid extract (MLTE). Error bars represent the standard deviation (SD) calculated from three independent experimental replicate. (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) indicate statistically significant differences. ns indicates a non-significant difference.

Table 4: *Swimming and swarming motility zone diameter (mm) of E. coli isolates treated with sub- minimal inhibitory concentrations of the two extracts.*

Extract	Concentration (mg/ ml)	Diameter of swimming zone (mm±SD)			Diameter of swarming zone (mm±SD)			p-value vs control
		UEC	SEC	WEC	UEC	SEC	WEC	
Hexane extract	MIC	0	0	0	0	0	0	<0.001
	MIC/2	20.3±0.7	15.8±0.63	19.4±0.85	10.3±0.35	3.8±0.1	7.4±0.2	<0.001
	MIC/4	29.7±1.1	20.2±0.82	24.6±1.09	16.7±0.5	17.2±0.7	11.6±0.4	<0.001
	MIC/8	37.8±1.4	25.8±1.2	34.5±1.3	20.8±0.96	18.8±0.8	21.5±1.1	<0.001
Ethanol extract	MIC	0	0	0	0	0	0	<0.001
	MIC/2	15.1±0.7	14.0±0.4	16.4±0.8	6.1±0.3	3.0±0.1	4.4±0.2	<0.001
	MIC/4	25.5±1.4	18.4±0.9	19.4±0.9	13.5±0.7	8.4±0.5	7.4±0.21	<0.001
	MIC/8	33.5±1.3	25.5±1.2	25.7±1.3	19.5±	11.5±0.4	20.7±1.2	<0.001
Control		43.5±1.5	31.2±1.3	38.9±1.7	40.5±1.8	28.2±1.2	32.9±1.4	-

Where: UEC: *E. coli* isolate from urine, SEC: *E. coli* isolate from sputum, and WEC: *E. coli* isolate from wound, SD: standard deviation, MIC: minimal inhibitory concentration. Data mean ± standard deviation (±SD) of triplicate independent experiments. $p < 0.001$ indicates a statistically significant difference among the bacterial isolates within the same concentration.

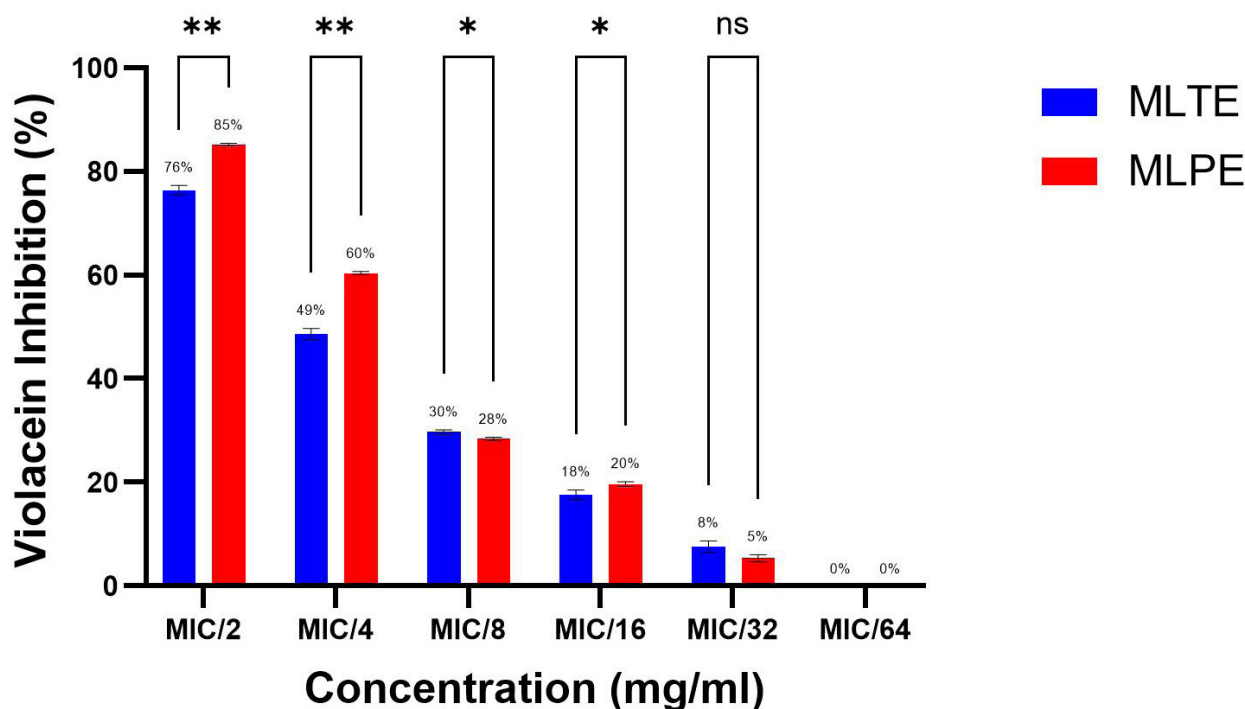


Figure 9: *Effect of sub- minimal inhibitory concentration levels of the M. azedarach leaf phenolic extract (MLPE) and M. azedarach leaf terpenoid extract (MLTE) on violacein production by C. violaceum ATCC 12472 strain. (* $p < 0.05$, ** $p < 0.01$) indicate statistically significant differences . ns indicates a non-significant difference.*

Violacein inhibition assay in chromobacterium violaceum
Figure 9 illustrates the effect of both extracts on violacein production by *C. violaceum* ATCC 12472 strain. Pigment production was reduced by 85.19% following treatment with MLPE (MIC/2), while MLTE achieved 76.35% inhibition at the same tested concentration. Sub-MIC concentrations did not affect the growth of *C. violaceum* ATCC 12472. Inhibition of violacein production (measured at 585 nm) was increased with increasing the extract concentrations

below the MIC, relative to the extract-free control plates. Cell counts ($OD_{600nm} = 1$) obtained results confirmed that growth of *C. violaceum* ATCC 12472 was not inhibited by the MLTE and MLPE extract's treatment, where the results were compared to the untreated control group. Statistical analysis confirmed that both extracts elicited a peak suppression of violacein pigment synthesis at MIC/2. At lower concentrations of both extracts, such as MIC/32, no anti-QS efficacy was observed.

Discussion

Regarding the comparative epidemiological prevalence, nearly 90% of community-acquired UTIs and up to half of nosocomial UTIs are caused by UPEC (Soujanya and Banashankari, 2023; Gagaletsios *et al.*, 2025). The most common causative agent of UTIs is *E. coli* (91%) (Rahman *et al.*, 2019). Consistent with previous literature, *E. coli* was the predominant isolate in this study, aligning with prevalence rates ranging from 36.8% to 49.1%, which were reported by Bharti *et al.* (2016); Nazmeen *et al.* (2018); Rafalskiy *et al.* (2020). Al-Taie *et al.* (2014) revealed that the percentage of *E. coli* isolated from wounds was 15.73%. Gagaletsios *et al.* (2025) reported that *E. coli* isolates were recovered from 65 urine samples, one swab, and one sputum sample. *E. coli* isolates were most frequently obtained from urine samples (92.2%), followed by wound samples (3.9%) (Naqid *et al.*, 2020). Another study demonstrated that 35% of *E. coli* were isolated from urine samples, followed by 5% and 17% from wound and sputum, respectively (Tewari *et al.*, 2018).

In terms of the phytochemical distribution trend, several compounds have been currently identified from *M. azedarach* extracts, including unique products belonging to the terpenoids, specifically limonoids and tirucallane-type triterpenoids. The obtained results showed higher levels of flavonoids and phenolic compounds in more polar fractions, especially in the ethanol fraction. In general, the absorption spectra reflected a trend related to polarity, with hexane extract containing non-polar components and ethanol extract containing highly polar compounds, as previously demonstrated by Batubara *et al.* (2025).

Regarding the chromatographic and phytochemical profiling, GC-MS analysis revealed 11 compounds, including β -caryophyllene, neophytadiene, and a phytol isomer, as major constituents. These chemical profiles align with previous findings reported by Hassan *et al.* (2025), confirming the consistent presence of diverse bioactive terpenoids and other phenolic compounds in *M. azedarach* leaves. Similarly, Batubara *et al.* (2025) revealed the presence of extended-chain hydrocarbons in the leaf extracts of the same plant. The current notable abundance of both heptacosane and eicosane within leaf extracts further supported this finding. HPLC analysis was used to identify and quantify flavonoids and phenolic compounds

in the ethanolic extract of *M. azedarach*, confirming the findings of Hemdan *et al.* (2023), who reported the presence of numerous phenolic compounds in *M. azedarach*. Catechin and chlorogenic acid were observed to be the major constituents of the phenolic extract.

Concerning the comparative antibacterial efficacy, the inhibition zones diameters measured in this study (with corresponding relative inhibition percentages detailed in the results) agree with the previous reported values of 15–13 mm recorded at 195 mg/ml of the *M. azedarach* methanolic leaf extract against *E. coli* (Getahun *et al.*, 2025). Similarly, the methanolic leaf extract suppressed the proliferation of MDR *E. coli* K1-1, recording a 10.93 mm inhibition zone diameter, whereas hexane leaf extract generated a 7.4 mm zone diameter (Batubara *et al.*, 2025). This finding is also consistent with the reported ranges of 12.0–16.7 mm (at 300 mg/ml) and 10.0–13.0 mm (at 150 mg/ml) for methanol leaf extract of *M. azedarach* against *E. coli* (Touzout *et al.*, 2023). Likewise, the ethanolic extract of *M. azedarach* has been shown to exert bactericidal activity against *E. coli*, with a recorded inhibition zone diameter of 16 mm (Hemdan *et al.*, 2023).

In terms of the minimum inhibitory parameters, the MIC of MLPE against *E. coli* ranged from 25 to 30 mg/ml, and the MBC values ranged from 50 to 60 mg/ml. Meanwhile, these recorded values were higher than those reported by Getahun *et al.* (2025) for *M. azedarach* methanolic leaf extract against *E. coli*, where the MIC and MBC were 6.09 mg/ml and 12.18 mg/ml, respectively. This discrepancy is directly related to the different extraction solvents and the configurations evaluated across the studies. This is consistent with the findings of Hemdan *et al.* (2023), who reported that *M. azedarach* extract was effective against *E. coli* at an MIC of 50 mg/ml. The methanol leaf extract exhibited a superior antibacterial efficacy against *E. coli* compared to the hexane extract, demonstrating lower MIC (1 vs. 20 mg/ml) and MBC (2 vs. 20 mg/ml) values (Batubara *et al.*, 2025).

Regarding the underlying molecular mechanisms of action, *M. azedarach* contained abundant polyphenols, which may express an antibiotic activity. Its antibacterial activity was attributed to the disruption of the cell wall's integrity and cohesion, leading to the leakage of cell contents and cell lysis (Hemdan *et al.*, 2023). Gallic acid, a phenolic acid known

for its antibacterial efficacy, triggers irreversible permeability changes, membrane rupture, and pore formation (Sorrentino *et al.*, 2018; Silva *et al.*, 2018; Santos *et al.*, 2021). The phenolic compounds induce microbial death by blocking enzyme active sites and acting as hydrogen ion carriers to disrupt ATP energy production (Hemdan *et al.*, 2023; Al-Aboudi and Al-Azawi, 2025). The antibacterial activity of the hexane extract of *M. azedarach* leaves was attributed to its high proportion of sesquiterpenes, most notably β -caryophyllene, which is known to penetrate the microbial cell membranes and inhibit its growth (Dahham *et al.*, 2015). The terpenoids induce membrane damage in bacteria through direct contact with the cells, increasing membrane permeability, interfering with ion transport and membrane proteins, affecting cellular enzymes, and inhibiting DNA synthesis (Fink, 2023).

With respect to biofilm formation dynamics, biofilm formation was observed in 90.1% of the evaluated isolates. This finding corroborates that of Gagaletsios *et al.* (2025), who reported that approximately 87% of *E. coli* strains exhibited a pronounced ability to form biofilms. In a similar study performed by Gagaletsios *et al.* (2025), *E. coli* strains were categorized based on their biofilm capabilities, where 71 isolates exhibited a moderate production, 16 showed high production, and 13 displayed a weak biofilm production. Bacterial biofilms facilitate adherence to host cells (Al-Aboudi and Al-Azawi, 2025) and act as a protective barrier against the immune system, thereby increasing antibiotic resistance and complicating the bacterial treatment (Fink, 2023).

Regarding the specific anti-biofilm mechanisms, the *M. azedarach* methanol leaf extract exhibited a peak anti-biofilm efficacy, achieving up to 60.26% inhibition of *E. coli* biofilm formation (Touzout *et al.*, 2023). Fink (2023) demonstrated that eugenol can remove up to 67% of *E. coli* biofilm. A similar trend was observed for limonene (46%), while terpineol removed up to 44% of *E. coli* biofilm. Flavonoid compounds have likewise demonstrated an anti-biofilm efficacy by inhibiting biofilm production (Al-Aboudi and Al-Azawi, 2025). Flavonoids possess potent antibacterial and anti-biofilm properties that enable them to combat a wide range of pathogenic bacteria (Al-Aboudi and Al-Azawi, 2024). The biological activity of these extracts was influenced by several parameters, including the type of extraction

solvent, geographic origin, harvesting period, storage climate, and dehydration techniques (Getahun *et al.*, 2025).

Regarding the inhibition of QS and bacterial motility, the obtained results showed that both extracts suppressed the swimming and swarming motility, along with biofilm formation, across all *E. coli* isolates at sub-MIC levels without affecting the bacterial growth. However, these concentrations had a lesser inhibitory effect on swimming motility. Swimming, unlike swarming, results from rotation of the bacterial flagellar rotor and is not a multicellular behavior (Ren *et al.*, 2001). Our study revealed the anti-QS activity of both extracts against the tested strain *C. violaceum* ATCC 12472, which synthesizes violacein under the control of the QS system (Moradi *et al.*, 2020). Inhibition of QS-dependent violacein formation by sub-MIC concentrations of both extracts yielded favorable results. Bacterial locomotion and various virulence factors are heavily dependent on QS mechanisms (Chaichana *et al.*, 2024). Through motility, *E. coli* strains can migrate and colonize new territories, which ultimately promotes biofilm proliferation (Benyoussef *et al.*, 2022).

Regarding the surface migration and swarming dynamics, the swarming motility represents a QS-dependent, coordinated surface migration that drives biofilm assembly and infection by adapting the bacteria to environmental challenges, virulence, and drug resistance (Rütschlin and Böttcher, 2019; Santos *et al.*, 2021). Natural phytochemicals are extensively documented for their anti-motility properties, particularly in weakening the migratory capabilities of *E. coli* (Garcia-Heredia *et al.*, 2016; Sheng *et al.*, 2016; Chaichana *et al.*, 2024). Disrupting the QS pathways using botanical agents represents a viable strategy against MDR strains (Moradi *et al.*, 2020). The phenolic phytochemicals can competitively inhibit QS receptors by blocking N-acyl homoserine lactones (AHL) signaling molecules; thus, disrupting the swarming motility, representing a remarkable alternative for limiting or preventing biofilm-associated infections (Santos *et al.*, 2021). Decreased production of extracellular polysaccharides may explain this inhibition, negatively affecting the migratory capabilities of the microbial pathogens (Zheng *et al.*, 2020; Santos *et al.*, 2021). Multiple studies have also reported an inhibition of bacterial movement by phenolic compounds, which completely

blocked the swarming motility (O'May and Tufenkji, 2011; Santos *et al.*, 2021).

Conclusions and Recommendations

The results of the present investigation indicated that *Melia azedarach* contains potential antibacterial components, which may be of considerable value to the pharmaceutical industry as a therapy against infectious diseases. Specifically, the experimental findings demonstrated the distinct effectiveness of the extracts. Notably, the maximum relative growth inhibition reached 75.0% for MLPE and 46.4% for the hexane extract at a concentration of 75 mg/ml. Additionally, peak biofilm inhibition reached 90.9% for MLPE and 66.7% for MLTE at their respective MIC/2 values. Furthermore, at the same concentration (MIC/2), swarming motility was suppressed by up to 89.4% for MLPE and 79.4% for MLTE, while swimming motility was reduced by up to 65.3% and 53.3%, respectively. Further studies are recommended to identify and characterize the biologically active constituents responsible for this antibacterial effectiveness. Furthermore, a future validation using an expanded clinical sample size is recommended.

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Novelty Statement

This study utilized the ultrasound-assisted extraction (UAE) method, which serves as a pioneering investigation confirming the multi-targeted efficacy of these green botanical fractions, demonstrating up to 75.0% relative growth inhibition, 90.9% biofilm inhibition, and profound restrictions in motilities (up to 89.4% for swarming and 65.3% for swimming). Notably, within the available literature, there is a distinct lack of studies evaluating the targeted inhibitory effect of *Melia azedarach* extracts against bacterial violacein pigment production, where our findings demonstrated a remarkable 85.19% inhibition at sub-inhibitory concentrations.

Author's Contributions

IJK: Conceptualization, methodology, validation,

investigation, resources, writing original draft, writing review and editing, supervision, funding acquisition, visualization, and project administration. **AAJ:** Methodology, formal analysis, investigation, data curation, writing original draft, writing review, editing, funding acquisition, and resources.

HAH: Conceptualization, methodology, validation, investigation, Software, resources, writing original draft, writing review, editing, and project administration.

All authors have read and agreed to the published version of this manuscript.

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Ethical approval

This study was conducted in accordance with the ethical approval granted by Al-Furat Al-Awsat Technical University, under Protocol No. 95/ ALMS dated August 16, 2025. Written consent of the participants was obtained.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interests

The authors have declared no conflicts of interest.

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