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Antibacterial and Antibiofilm Activity of Ethanolic and Methanolic *Nigella sativa* L. Seed Extracts Against *Pseudomonas aeruginosa* A TCC 27853

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Abstract
Biofilm associated infection is responsible for majority of chronic bacterial diseases. The conventional antimicrobial has not been able to cope with the increasing level of resistance. *Nigella sativa* L. (commonly called black cumin or black seed) has demonstrated diverse pharmacological properties, but standardized quantitative phenotypic assessment of its crude solvent extracts against *Pseudomonas aeruginosa* biofilms at subinhibitory concentrations remains limited. This study evaluated the in vitro antibacterial and antibiofilm effects of ethanolic and methanolic *N. sativa* seed extracts against *P. aeruginosa* ATCC 27853. Antibacterial activity was determined by agar well diffusion, yielding inhibition zones of 26.50 ± 0.87 mm (ethanolic) and 28.00 ± 1.15 mm (methanolic). Broth microdilution established identical MIC and MBC values of 250 µg/mL and 500 µg/mL, respectively, for both extracts. Biofilm inhibition was assessed by the tissue culture plate method with crystal violet staining at sub-MIC concentrations (125.00 to 7.81 µg/mL). The untreated control exhibited strong adherence. Following treatment, biofilm categories decreased to weak or moderate adherence. Percentage inhibition ranged from 68.34% to 89.45%, with the methanolic extract achieving 89.45% inhibition at 125.00 µg/mL. These findings indicate that N. sativa seed extracts possess antibiofilm potential against *P. aeruginosa* at subinhibitory concentrations. However, as this was a purely phenotypic study, the mechanisms underlying the observed inhibition remain speculative and require direct experimental investigation.

Keywords: Antibiofilm activity, Biofilm inhibition, *Nigella sativa*, *Pseudomonas aeruginosa*, Subinhibitory concentrations, Natural products

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Introduction
Biofilms make infections incredibly difficult to treat. Bacteria living in these structured communities tolerate antibiotics at concentrations 1000× higher than free-floating cells partly because the biofilm matrix blocks drug penetration, and partly because some cells enter a dormant state [1]. Stewart [2] showed this isn't just about physical barriers; metabolic changes inside biofilms fundamentally alter how bacteria respond to drugs. The matrix physically blocks drugs, and some cells enter a dormant state where antibiotics simply don't work. In the US, multidrug-resistant *P. aeruginosa* causes roughly 32,000 infections and 2,700 deaths yearly [3]. World Health Organization [4] still classifies carbapenem-resistant strains as high priority in their 2024 list, reflecting its substantial clinical burden [4].

P. aeruginosa is a versatile gram-negative pathogen. It is found everywhere, from soil, hospital sinks and water to the lungs of cystic fibrosis patients [5]. Its virulence toolkit includes type III secretion effectors, siderophores, exotoxins, and most relevant here an exceptional ability to build biofilms [6].The process is fairly well mapped out: reversible attachment first, then irreversible adhesion via type IV pili and flagella, microcolony formation, 3D structuring, and finally dispersal of free-swimming cells [7]. Quorum sensing (QS) coordinates both biofilm development and virulence factor production [8]. Disruption of biofilm formation or QS signalling represents a promising therapeutic strategy.

As conventional antibiotics lose efficacy, natural products have regained attention as alternative therapies. Plant secondary metabolites polyphenols, alkaloids, terpenoids, flavonoids, have shown biofilms inhibition through multiple pathways: quenching QS signals, breaking up extracellular polymeric substances, or damaging membranes [9]. Recent examples include baicalin from *Scutellaria baicalensis* [9], sesquiterpene lactones from Inula species [10], and various flavonoids [9].

Nigella sativa L. (Ranunculaceae), or black cumin, has been used in traditional medicine across South Asia, the Middle East, and the Mediterranean for over two thousand years [11]. Its seeds are packed with bioactive compounds thymoquinone, thymohydroquinone,

dithymoquinone, p-cymene, carvacrol, and longifolene, to name the main ones [11, 12]. Extracts and essential oils from this plant have shown antioxidant, anti-inflammatory, antidiabetic, antineoplastic, and antimicrobial effects [12, 13].

Antibacterial investigations have confirmed the efficacy of *N. sativa* constituents against a spectrum of pathogens, including *Escherichia coli, Pseudomonas aeruginosa Staphylococcus, Salmonella typhi, Klebsiella pneumoniae*, and *Bacillus subtilis* [14, 15, 16]. Chaieb et al. [17] reported that thymoquinone inhibited *P. aeruginosa* growth with an MIC of 450 µg/mL. More recently, Al-Rabia et al. [18] demonstrated that thymoquinone attenuated *P. aeruginosa* biofilm formation by over 80% at concentrations of 25–50 µg/mL, implicating quorum-sensing interference as the underlying mechanism though this was supported by gene expression analysis not performed in the present study. While antibacterial and antibiofilm effects of *N. sativa* constituents have been reported [17, 18], standardized quantitative phenotypic assessment of biofilm inhibition categories at subinhibitory concentrations using the tissue culture plate method remains limited.

The present investigation was conceived to contribute phenotypic screening data on *N. sativa* extracts against *P. aeruginosa* biofilms. Specifically, we aimed to: (i) characterise the antibacterial activity of ethanolic and methanolic *N. sativa* seed extracts against *P. aeruginosa* ATCC 27853 through standardized diffusion and microdilution assays; (ii) determine MIC and MBC values to establish activity thresholds; and (iii) evaluate antibiofilm efficacy at sub-inhibitory concentrations using the tissue culture plate methodology. We explicitly did not aim to investigate genetic regulatory mechanisms, as no molecular experiments were conducted. We hypothesised that both extracts would inhibit planktonic growth and attenuate biofilm formation at concentrations below their MIC.

Materials and Methods

Plant Material and Extract Preparation

Seeds of *Nigella sativa* L. (Ranunculaceae) were obtained from Ethiopian commercial supplier. Taxonomic verification was done at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Akoka, Lagos State, Nigeria, with voucher specimen (UNILAG/PHCO/2023/047) archived in the institutional herbarium. Seeds were ground to fine powder with a mechanical grinder. Extraction used an ultrasonic bath (Sonorex Digitec, DT 31H; 40 kHz, 40°C) for 30-minute cycles, following parameters optimized by Moghimi et al. [17] for *N. sativa* seed extraction. Two separate runs were done: one with absolute ethanol (Sigma-Aldrich, St. Louis, MO, USA) and one with methanol (Sigma-Aldrich). After extraction, we filtered and rotary-evaporated to dryness. Recoveries were 8.4% for the ethanolic extract and 12.3% for the methanolic extract, calculated relative to starting biomass. Dried extracts were dissolved in DMSO (Sigma-Aldrich) to make 1000 µg/mL stock solutions, stored at 4°C in light-protected containers. Final DMSO concentration in assay wells never exceeded 1.0% (v/v). We picked this cutoff because Balouiri et al. [19] showed DMSO at ≤1% has no measurable antibacterial effect against *P. aeruginosa*. We confirmed this ourselves: DMSO-only controls at 1.0% produced no inhibition zones or growth suppression in any assay.

Bacterial Strain and Culture Conditions

Pseudomonas aeruginosa ATCC 27853 (American Type Culture Collection, Manassas, VA, USA) was used as the test organism. The stain was maintained by periodic subculture on Mueller-Hinton Agar (MHA; Sigma-Aldrich). For experiments, inocula were prepared in either Mueller-Hinton Broth (MHB; Sigma-Aldrich) or Luria-Bertani (LB) broth (Sigma-Aldrich), depending on the assay and were incubated at 37°C for 18-24 hours to obtain optimal growth. Turbidity-adjusted suspensions matching 0.5 McFarland standard (~1.5×10^8 CFU/mL) were prepared in sterile saline. For broth microdilution, this was diluted 1:150 in cation-adjusted Mueller-Hinton broth (CAMHB) to reach ~1.0×10^6 CFU/mL. Then 100 µL of this working inoculum went into each well already containing 100 µL of serially diluted extract, giving a final inoculum of ~5.0×10^5 CFU/mL per CLSI M07 guidelines (CLSI, 2022). For agar diffusion, the 0.5 McFarland Standard suspension was used directly as the inoculum.

Agar Well Diffusion Assay

The well diffusion setup followed Balouiri et al. [19] for the well diffusion setup. Bacterial lawns were made by spreading McFarland-adjusted inocula evenly across MHA plates with sterile cotton swabs, then letting the surface dry for 15 minutes at room temperature. Wells were punched with a sterile cork borer: 8 mm diameter, 5 mm depth. This size sits within the 6–8 mm range Balouiri et al. [19] recommend, and it accommodates 50 µL extract volume while keeping enough agar wall thickness to prevent leakage. Each well received 50 µL of extract solution. This volume fits the 20–100 µL standard for wells this size and gives adequate radial diffusion of bioactive compounds into the agar. Ciprofloxacin discs (5 µg) and pure DMSO (1.0% v/v) served as positive and

solvent controls. Plates incubated at 37°C for 16–18 hours, after which we measured inhibition zones with calipers. Triplicate independent experiments were conducted on three separate days (biological replicates), with three technical replicates (wells per plate) each time. Reported values represent mean ± SD of nine measurements total (3 biological × 3 technical).

Broth Microdilution and MBC Determination

Broth microdilution followed CLSI [20] protocols. Two-fold serial dilutions from 1000 down to 1.95 µg/mL were prepared in CAMHB. We dispensed 100 µL aliquots into microplate wells and inoculated with equal volumes of bacterial suspension (final density: 5.0×10^5 CFU/mL). Uninoculated MHB and DMSO-supplemented MHB (1.0% v/v) were negative and solvent controls. After overnight incubation (35 ± 1°C, 18–24 hours), we read optical density at 600 nm on a Biotek Epoch Microplate Reader. MIC was the lowest concentration showing visible growth suppression. For MBC, we streaked 10 µL from MIC wells and the next few higher concentrations onto fresh MHA plates, incubated at 37°C for 24 hours, and checked for colony growth. MBC was the lowest concentration killing ≥99.9% of bacteria. Quadruplicate determinations were done across two independent experimental runs on separate days (biological replicates), with four technical replicates (wells per concentration) per run. MIC was taken as the modal value across runs. MBC determination used triplicate plating from MIC-confirmed wells across two independent runs on separate days.

Biofilm Inhibition Assay

Biofilm attenuation was assessed by an adherent-cell staining protocol adapted from Merritt et al. [21], using 96-well polystyrene tissue culture plates. Extract dilutions (1000–1.95 µg/mL, two-fold steps) were prepared in LB broth (Sigma-Aldrich). We chose LB because it supports robust, reproducible biofilm formation in *P. aeruginosa* ATCC 27853 under static conditions, whereas MHB gives comparatively weak biomass [21, 22]. This medium choice is standard practice in biofilm inhibition studies. Yes, it creates a medium inconsistency between the antibacterial (MHB) and antibiofilm (LB) assays, but MIC/MBC and biofilm inhibition endpoints are independently standardized and not directly compared numerically.

Sub-Mic concentration were selected as a standard two-fold dilution series (125.00 to 7.81 µg/mL) to systematically bracket the subinhibitory range below the established MIC of 250 µg/mL. Aliquots of 100 µL of each dilution were transferred to individual wells and inoculated with 10 µL of standardized bacterial suspension. Extracts remained present throughout the 24-hour incubation. Negative controls had sterile LB only; positive controls had bacterial suspension without extract. After 24 hours at 37°C, we decanted non-adherent cells and gently washed sessile biofilms with PBS (Sigma-Aldrich) to remove loosely attached material. Fixation and staining with 0.1% crystal violet (10 minutes) visualized adherent biomass. Excess dye was rinsed off, and 96% ethanol solubilized the bound stain for spectrophotometric reading at 595 nm.

Biofilm density categories followed Stepanović et al. [22]: non-adherent (OD ≤ ODc), weakly adherent (ODc < OD ≤ 2×ODc), moderately adherent (2×ODc < OD ≤ 4×ODc), and strongly adherent (OD > 4×ODc), where ODc = mean OD of negative control + 3 × SD. Percentage inhibition was computed as:

$$\text{Inhibition (\%)} = [(OD_{control} - OD_{extract}) / OD_{control}] \times 100$$

Quadruplicate determinations were done across three independent experimental runs on separate days (biological replicates), with four technical replicates (wells per concentration) per run. Reported OD_{595} values are mean ± SD of twelve measurements (3 biological × 4 technical).

Phytochemical Characterisation

Qualitative phytochemical screening was performed on both extracts using standard colorimetric assays for alkaloids (Dragendorff's test), flavonoids (Shinoda test), terpenoids (Salkowski test), tannins (ferric chloride test), saponins (froth test), and phenolic compounds (Folin-Ciocalteu reagent). Quantitative profiling of specific marker compounds (e.g., thymoquinone by HPLC-DAD, HPLC-MS, or GC-MS) was not performed in this study. This constitutes a significant methodological limitation, as the chemical composition of the extracts remains uncharacterised. Consequently, comparisons of bioactivity with other studies, attribution of effects to specific constituents, and batch-to-batch reproducibility cannot be established. Future investigations must prioritise quantitative phytochemical fingerprinting

Statistical Analysis

Microsoft Excel 2019 generated descriptive statistics (means, standard deviations, inhibition percentages). Inferential comparisons employed one-way ANOVA for agar diffusion zone diameters (independent variable: treatment group; dependent variable: inhibition zone diameter in mm) and two-way ANOVA for biofilm inhibition data (independent variables: extract type and concentration; dependent variable: OD_{595}). Post-hoc analyses used Fisher's Least Significant Difference (LSD)

test (SPSS version 23). Statistical significance was set at $p < 0.05$. For biofilm data, the following specific comparisons were tested: Main effect of extract type (ethanolic vs. methanolic) across all concentrations and concentration (each sub-inhibitory concentration vs. positive control); Interaction effect (extract type × concentration). Effect sizes were estimated using Cohen's d for pairwise comparisons.

Results

Antibacterial Activity by Agar Diffusion

Table 1:Antibacterial Activity of *N. sativa* Extracts Against *P. aeruginosa* ATCC 27853 by Agar Well Diffusion Method

Test Sample	Zone of Inhibition (mm)	Activity
Seed ethanolic extract	26.50 ± 0.87	Active
Seed methanolic extract	28.00 ± 1.15	Active
Ciprofloxacin (5 µg)	25.00 ± 0.50	Active
DMSO (negative control)	0.00 ± 0.00	Inactive

Note. Values are expressed as mean ± standard deviation (n = 9; 3 biological replicates × 3 technical replicates). One-way ANOVA: $F(3,8) = 412.6$, $p < 0.001$. Fisher's LSD post-hoc: methanolic vs. ethanolic, $p = 0.042$; methanolic vs. ciprofloxacin, $p = 0.008$; ethanolic vs. ciprofloxacin, $p = 0.031$. DMSO = dimethyl sulfoxide.

Both Nigella sativa seed extracts exhibited discernible antibacterial activity against *P. aeruginosa* ATCC 27853 (**Table 1**). The methanolic extract produced the largest inhibition zones (28.00 ± 1.15 mm), followed closely by the ethanolic extract (26.50 ± 0.87 mm). Ciprofloxacin, the positive control, yielded zones of 25.00 ± 0.50 mm, while DMSO (1.0% v/v) produce no measurable inhibition, confirming the absence of solvent-mediated antibacterial effects. It is important to note that zone diameter comparisons between crude extracts as pure antibiotic discs are not indicative of relative potency. The crude methanolic extract was applied at 50 µL of 1000 µg/mL stock (50 µg total mass per well), whereas ciprofloxacin was a standardised 5 µg commercial disc. The larger zone for the extract reflects high-concentration diffusion of multiple compounds, not superior intrinsic activity.

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *N. sativa* Extracts Against *P. aeruginosa* ATCC 27853

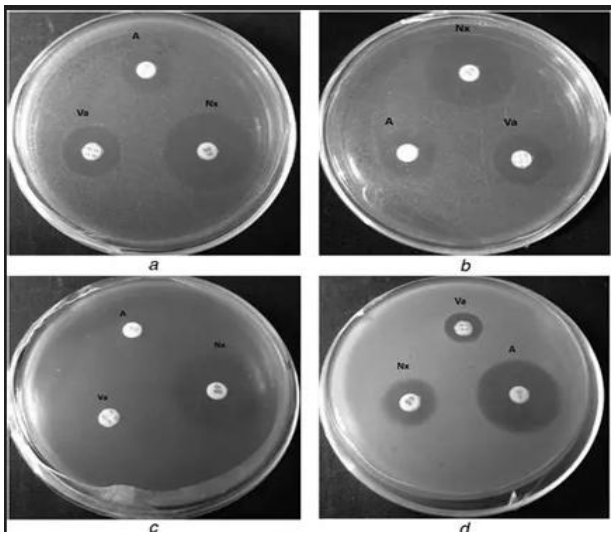
Extract	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC
Seed ethanolic extract	250	500	2
Seed methanolic extract	250	500	2
Ciprofloxacin (positive)	0.5	1.0	2
DMSO (negative control)	>1000	>1000	–

Note. MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration. Values represent modal MIC across two independent runs (4 technical replicates per run)

MIC and MBC Determinations

Broth microdilution gave identical MIC values of 250 µg/mL for both extracts. MBC was 500 µg/mL for each, giving a bactericidal ratio (MBC/MIC) of 2 (**Table 2**). These MICs are lower than the 450 µg/mL reported for pure thymoquinone by Chaieb et al. [17], which hints at synergistic action among multiple constituents in the crude extracts.

Figure1: Growth inhibition of *P. aeruginosa* achieved through the activity of black seed extracts. (A) Seed ethanolic extract (S1); (B) Seed methanolic extract (S2); (C) Positive control (ciprofloxacin); (D) Negative control (DMSO). Optical density



values represent mean ± standard deviation of quadruplicate determinations at 600 nm.

Biofilm-Forming Phenotype of the Test Strain

In the absence of extract, *P. aeruginosa* ATCC 27853 demonstrated robust biofilm formation, classifying as strongly adherent according to Stepanović et al. [22] criteria. This phenotype aligns with the well-documented proficiency of this species in biofilm elaboration and validates the strain's suitability for antibiofilm screening.

Sub-Inhibitory Biofilm Modulation

Below the MIC, both extracts markedly changed biofilm architecture (**Table 3**; **Figure 3**). The ethanolic extract dropped the biofilm category from strongly adherent to moderately adherent at 62.50–7.81 µg/mL, with inhibition from 68.34% to 79.21%. The methanolic extract had stronger effects: at 125.00 µg/mL it reduced biofilm to weakly adherent, and this persisted down to 31.25 µg/mL. Inhibition for the methanolic extract ranged from 72.18% to 89.45%, peaking at 89.45% at 125.00 µg/mL. At 15.62 µg/mL, the methanolic extract still showed moderate adherence with 72.18% inhibition.

Table 3: Effect of Sub-Inhibitory Concentrations of *N. sativa* Extracts on *P. aeruginosa* ATCC 27853 Biofilm Formation

Concentration (µg/mL)	Ethanolic Extract OD_{595} ± SD	Ethanolic Extract % Inhibition	Methanolic Extract OD_{595} ± SD	Methanolic Extract % Inhibition	Biofilm Category (Methanolic)	Biofilm Category (Ethanolic)
125.00	–	–	0.085 ± 0.012	89.45	Weakly adherent	–
62.50	0.142 ± 0.018	68.34	0.092 ± 0.015	88.59	Weakly adherent	Moderately adherent
31.25	–	–	0.098 ± 0.011	85.67	Weakly adherent	–
15.62	–	–	0.245 ± 0.032	72.18	Moderately adherent	–
7.81	0.098 ± 0.014	79.21	–	–	–	Weakly adherent
Positive control	0.880 ± 0.065	–	0.880 ± 0.065	–	Strongly adherent	Strongly adherent
Negative control	0.045 ± 0.008	–	0.045 ± 0.008	–	Non-adherent	Non-adherent

Note. OD_{595} = optical density at 595 nm; SD = standard deviation; % Inh. = percentage inhibition; NT = indicates concentrations that were no evaluated for the respective extract during the antibiofilm assay, therefore, OD values, percentage inhibition, and biofilm category could not be determined. Biofilm categories determined per Stepanović et al. (2007): non-adherent (OD ≤ ODc), weakly adherent (ODc < OD ≤ 2×ODc), moderately adherent (2×ODc < OD ≤ 4×ODc), strongly adherent (OD > 4×ODc), where ODc = 0.069. Two-way ANOVA (extract × concentration): $F(1,30) = 24.7$, $p < 0.001$ for extract type; $F(4,30) = 156.3$, $p < 0.001$ for concentration; $F(4,30) = 8.9$, $p < 0.001$ for interaction. Values represent mean ± SD of 12 measurements (3 biological replicates × 4 technical replicates).

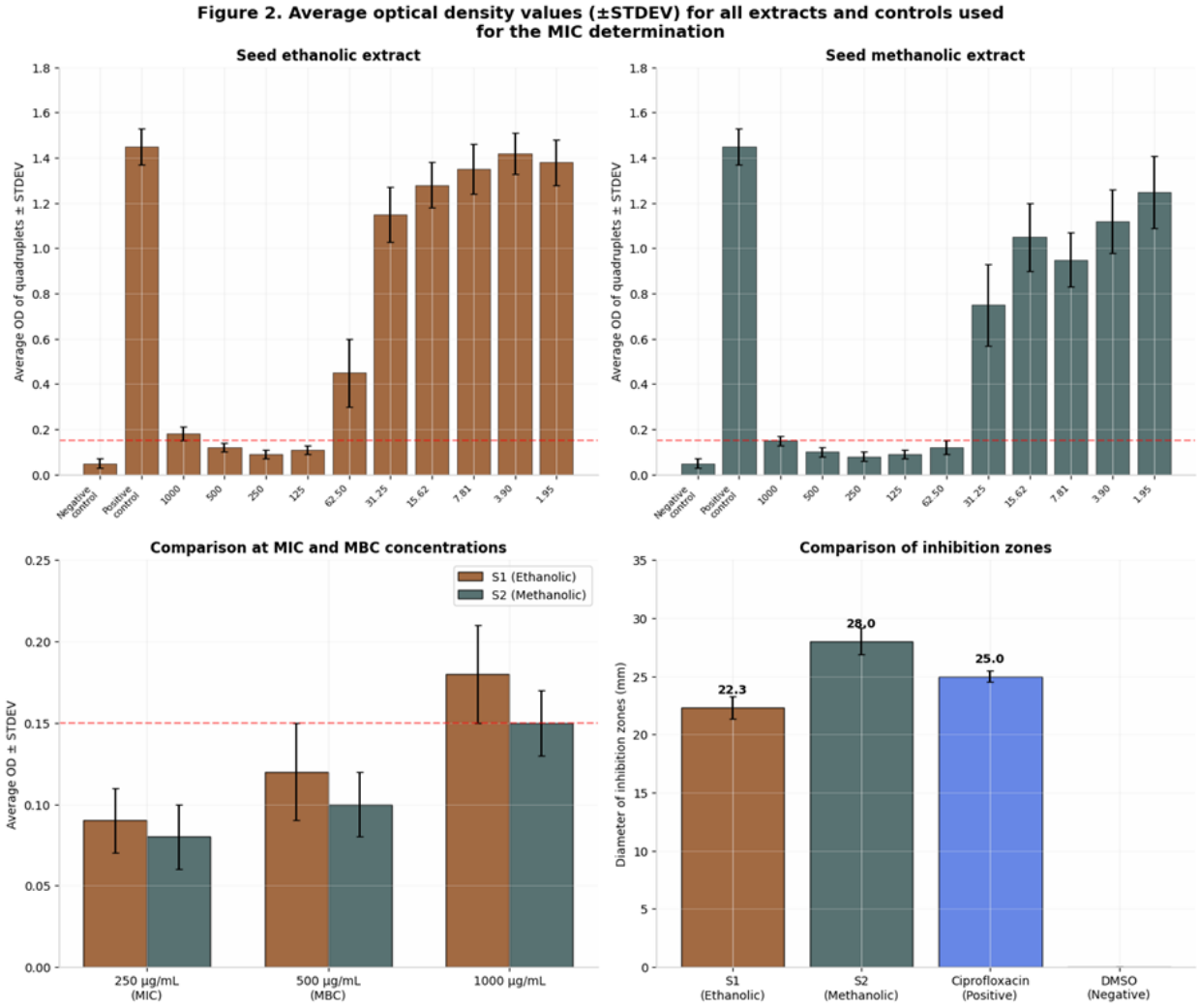


Figure 2: Average optical density values (± standard deviation) for all extracts and controls used for MIC determination. (A) Seed ethanolic extract; (B) Seed methanolic extract; (C) Comparison at MIC and MBC concentrations; (D) Comparison of inhibition zones from agar diffusion assay.

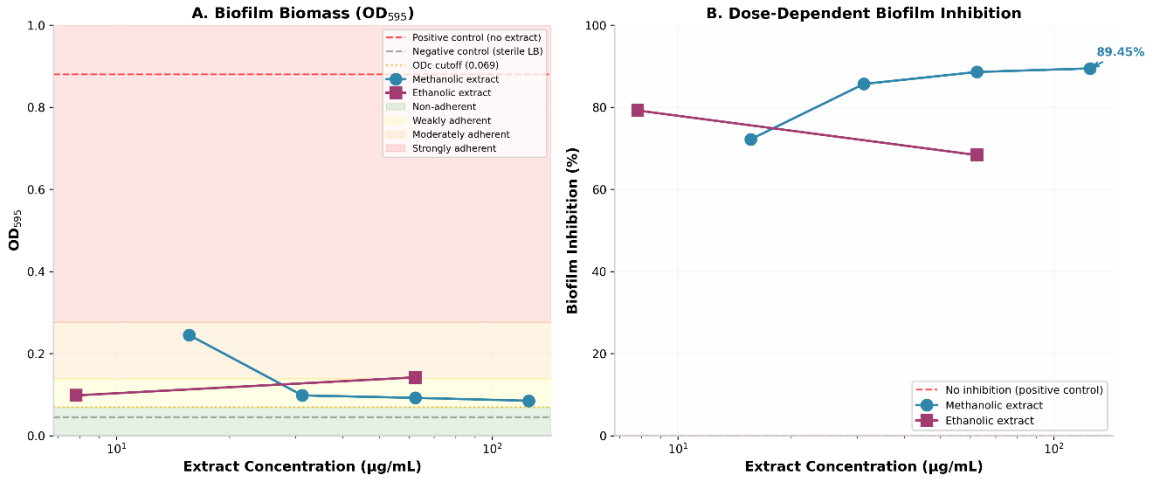


Figure 3: Dose-dependent biofilm inhibition by *N. sativa* seed extracts against *P. aeruginosa* ATCC 27853. (A) Biofilm biomass (OD_{595}) at sub-inhibitory concentrations, with adherence category boundaries indicated (ODc = 0.069; 2×ODc = 0.138; 4×ODc = 0.276). (B) Percentage biofilm inhibition relative to positive control. Data represent mean ± SD of 12 replicates (3 biological × 4 technical).

Not all concentrations were tested for both extracts. Preliminary screening indicated comparable activity trends and concentrations were selected to bracket the sub-MIC range while avoiding redundant measurement.

Discussion

The present findings are consistent with previous reports on *N. sativa* preparations against *P. aeruginosa*. The methanolic extract produced slightly larger inhibition zones than the ethanolic which may reflect the higher polarity of methanol and its capacity to extract thymoquinone and related phenolics more effectively [11, 12]. Both extracts MICs of 250 µg/mL substantially lower than the 450 µg/mL reported for pure thymoquinone [17]. This gap probably comes from synergy among multiple bioactive compounds in the crude extracts, a common story in phytomedicine, where whole extracts often beat isolated compounds [12].

The zone diameter comparison between the crude methanolic extract and ciprofloxacin requires careful interpretation. The crude methanolic extract was loaded at 50 µL mass per well, whereas ciprofloxacin was delivered as a standardized 5 µg disc. The MIC data demonstrate the substantial difference in intrinsic activity: ciprofloxacin at 0.5 µg/mL versus 250 µg/mL for the extracts, representing a 500-fold difference potency. Zone diameter in diffusion assays depends on concentration and diffusion coefficient rather than therapeutic value; therefore, crude extracts and pure drugs cannot be directly compared using this endpoint.

The most significant finding is biofilm attenuation at concentrations substantially below the MIC. This subinhibitory activity indicates mechanisms other than direct bactericidal action. Al-Rabia et al. [18] showed that thymoquinone suppresses *lasI, lasR, rhlI, rhlR*, and *pqsA-E/H* expression, and binds *LasR* and *RhlR* receptors. However, these mechanistic insights derive from their molecular study, not the present phenotypic investigation. The strong antibiofilm activity observed with the methanolic extract may well relate to higher thymoquinone content, given that methanol extracts more efficient than ethanol [12], though this was not quantified in the present study.

The reduction of *P. aeruginosa* from strongly adherent to weakly adherent status by sub-MIC concentrations of the methanolic extract carries implications for biofilm structural integrity. Strong biofilm formation is contingent upon robust expression of the *pel*, *psl*, and *alg* exopolysaccharide operons, as well as functional QS circuits [23]. The observed attenuation in this study is purely phenotypic; gene expression, protein levels, and signaling molecules were not measured.

Our 89% inhibition places *N. sativa* among the stronger plant-derived antibiofilm agents in phenotypic assays. Paunova-Krasteva et al. [26] reported 61–71% inhibition with *Inula* sesquiterpene lactones against PAO1. Namratha et al. [9] showed baicalin disrupting QS-controlled virulence in mice. Direct comparisons across studies are constrained by differences in strains, methodologies, and extract standardization.

Several limitations must be acknowledged. First, all experiments were conducted in vitro, without host immune responses, nutrient gradients, or polymicrobial communities. Second, quantitative phytochemical profiling; HPLC-MS or GC-MS is essential future work. Third, genetic regulatory effects were guessed from phenotypes, not shown with molecular techniques. Fourth, only a single reference strain was evaluated.

Clinical *P. aeruginosa* isolates vary considerably in biofilm capacity, QS function, resistance profiles, and mucoid phenotype [28; 29]. A diverse panel including multidrug-resistant and mucoid strains is required to assess generalizability.

Conclusions

Ethanol and methanol extracts of *N. sativa* seeds kill *P. aeruginosa* ATCC 27853 planktonic cells and block biofilm formation at sub-MIC concentrations. Methanol extraction yielded higher antibiofilm result (89.45% inhibition at 125 µg/mL, dropping strong biofilms to weakly adherent status) in this experimental model, however, this conclusion is limited to single reference strain and uncharacterized extracts. The MIC/MBC baseline (250/500 µg/mL) provides a reference point for crude preparations against strain. These findings are purely phenotypic and lack mechanistic or in vivo validation. Future studies should address HPLC-MC fingerprinting, gene expression analysis, clinical applications can be considered.

Ethics Statement

This study involved exclusively in vitro experiments using a commercially obtained reference bacterial strain (*Pseudomonas aeruginosa* ATCC 27853, American Type Culture Collection) and commercially purchased *Nigella sativa* seeds. No human participants, animal subjects, or primary clinical isolates were used

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request

Authors' Contributions

KS Conceptualization, methodology, investigation, formal analysis, writing-original draft. **MOI** Methodology, investigation, data curation, writing-review and editing. **OEO** Resources, supervision, project administration. **AAL** Validation, visualization, writing-review and editing.

Conflict of Interest

No competing interests were disclosed.

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References

1. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nat Rev Microbiol. 2016;14(9):563–575. https://doi.org/10.1038/nrmicro.2016.94
2. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. Int J Med Microbiol. 2002;292(2):107–113. https://doi.org/10.1078/1438-4221-00206
3. Centers for Disease Control and Prevention, 2019. cdc.gov/nchs/data/hus/hus19-508.pdf
4. World Health Organization. WHO bacterial priority pathogens list 2024. Geneva: WHO; 2024. https://www.who.int/publications/i/item/9789240093461
5. Moradali MF, Ghods S, Rehm BHA. *Pseudomonas aeruginosa* lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol. 2017;7:39. https://doi.org/10.3389/fcimb.2017.00039
6. Pugazhendhi AS, Wei F, Hughes M, Coathup M. Bacterial adhesion, virulence, and biofilm formation. In: Musculoskeletal infection. Cham: Springer; 2022. p. 19–64. https://doi.org/10.1007/978-3-030-83251-3_2
7. Stoodley P, Sauer K, Davies DG, Costerton JW. Biofilms as complex differentiated communities. Annu Rev Microbiol. 2002;56:187–209. https://doi.org/10.1146/annurev.micro.56.012302.160705
8. Lee J, Zhang L. The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. Protein Cell. 2015;6(1):26–41. https://doi.org/10.1007/s13238-014-0100-x
9. Namratha B, Martis GJ, Gaonkar SL, Hakkimane SS, et al. Exploration of natural and synthetic small molecules as anti biofilm agents for infection management. Discover Appl Sci. 2025;8(2):125. https://doi.org/10.1007/s42452-025-08124-5
10. Paunova Krasteva T, Dimitrova PD, Damyanova T, Borisova D, et al. Interference of *Pseudomonas aeruginosa* virulence factors by extracts from Inula species. Pharmaceuticals. 2025;18(12):1824. https://doi.org/10.3390/ph18121824
11. Bourgou S, Pichette A, Marzouk B, Legault J. Bioactivities of black cumin essential oil and its main terpenes from Tunisia. S Afr J Bot. 2017;112:270–275. https://doi.org/10.1016/j.sajb.2017.06.004
12. Kooti W, Hasanzadeh-Noohi Z, Sharafi-Ahvazi N, Asadi-Samani M, Ashtary-Larky D. Phytochemistry, pharmacology, and therapeutic uses of black seed (*Nigella sativa*). Chin J Nat Med. 2016;14(10):732–745. https://doi.org/10.1016/S1875-5364(16)30088-7
13. Sowunmi K, Kaka Z. Antioxidant activity of *Nigella sativa* essential oil. In: Biochemistry. IntechOpen; 2023. https://doi.org/10.5772/intechopen.109086
14. Shafodino FS, Lusilao JM, Mwapagha LM. Phytochemical characterization and antimicrobial activity of *Nigella sativa* seeds. PLoS One. 2022;17(8):e0272457. https://doi.org/10.1371/journal.pone.0272457
15. Bakal SN, Bereswill S, Heimesaat MM. Finding novel antibiotic substances from medicinal plants: antimicrobial properties of *Nigella sativa*. Eur J Microbiol Immunol. 2017;7(1):92–98. https://doi.org/10.1556/1886.2017.00001
16. Abbas M, Gururani MA, Ali A, Bajwa S, et al. Antimicrobial properties and therapeutic potential of bioactive compounds in *Nigella sativa*: a review. Molecules. 2024;29(20):4914. https://doi.org/10.3390/molecules29204914
17. Chaieb K, Kouidhi B, Jrah H, Mahdouani K, Bakhrouf A. Antibacterial activity of thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation. BMC Complement Altern Med. 2011;11:29. https://doi.org/10.1186/1472-6882-11-29
18. Al Rabia MW, Asfour HZ, Alhakamy NA, Abdulaal WH, Ibrahim TS, Abbas HA, et al. Thymoquinone is a natural antibiofilm and pathogenicity attenuating agent in *Pseudomonas aeruginosa*. Front Cell Infect Microbiol. 2024;14:1382289. https://doi.org/10.3389/fcimb.2024.1382289
19. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal. 2016;6(2):71–79. https://doi.org/10.1016/j.jpha.2016.02.002

20. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI supplement M100. Wayne (PA): CLSI; 2022.
21. Merritt JH, Kadouri DE, O'Toole GA. Growing and analyzing static biofilms. Curr Protoc Microbiol. 2005;1:1B.1.1–1B.1.17. https://doi.org/10.1002/9780471729259.mc01b01s01
22. Stepanović S, Vuković D, Hola V, Di Bonaventura G, et al. Quantification of biofilm in microtiter plates. APMIS. 2007;115(8):891–899. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x
23. Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. Curr Opin Microbiol. 2007;10(6):644–648. https://doi.org/10.1016/j.mib.2007.09.010
24. Lee J, Zhang L. The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. Protein Cell. 2015;6(1):26–41. https://doi.org/10.1007/s13238-014-0100-x
25. Hengge R. Principles of c-di-GMP signalling in bacteria. Nat Rev Microbiol. 2009;7(4):263–273. https://doi.org/10.1038/nrmicro2109
26. Paunova Krasteva T, Dimitrova PD, Damyanova T, Borisova D, et al. Interference of *Pseudomonas aeruginosa* virulence factors by extracts from Inula species. Pharmaceuticals. 2025;18(12):1824. https://doi.org/10.3390/ph18121824
27. Moghimi S, Ghiasi F, Bahrani S. Optimization of ultrasound-assisted extraction of *Nigella sativa* seed oil. Discover Appl Sci. 2024;6:257. https://doi.org/10.1007/s42452-024-05714-7
28. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019;37(1):177-192. https://doi.org/10.1016/j.biotechadv.2018.11.013
29. Colquhoun DJ, Duodu S, Vagnes Ø. A polyphasic approach for phenotypic and genetic characterization of the fastidious aquatic pathogen *Francisella noatunensis* subsp. orientalis. Front Microbiol. 2017;8:2324. https://doi.org/10.3389/fmicb.2017.02324