

Prevention (pre-treatment) and eradication (post-treatment) of *Pseudomonas aeruginosa* biofilm using aqueous and methanolic herbal extracts

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Abstract

This study investigates the antibiofilm properties of Herbal plant extracts traditionally used in ethnoveterinary practices to address biofilm-associated infections in cattle. Biofilms, which contribute to persistent infections and antibiotic resistance, are formed by pathogens like *Pseudomonas aeruginosa*. The study assessed methanolic and aqueous extracts from 11 plants for their ability to inhibit biofilm formation (pre-treatment) and disrupt existing biofilms (post-treatment). Significant antibiofilm activity was observed in *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), *Terminalia bellirica* (Beheda), and *Piper betle* (Nagarvel). Pre-treatment showed a greater reduction in biofilm formation compared to post-treatment, indicating the potential for these plants in preventing biofilm-related infections. The results suggest that these herbal remedies could serve as effective alternatives to synthetic antibiotics, potentially reducing antimicrobial resistance in veterinary medicine.

Key words: Microbial biofilms, *Pseudomonas aeruginosa*, Antibiofilm activity, Herbal plants, Ethnoveterinary practices

Introduction

Microbial biofilms are communities of microorganisms that are encased in a self-produced matrix, adhering to both living tissues, such as wounds and organs, and non-living surfaces, like food contact surfaces. These biofilms pose serious challenges in clinical environments, agriculture, and food industries due to their resistance to antibiotics and disinfectants, complicating infection treatment and hygiene control (Mirghani et al. 2022). Biofilms in healthcare environments hinder the effectiveness of antimicrobial treatments, promoting multidrug resistance. This contributes to complications like medical implant failures, catheter-related infections, and increased morbidity. As a result, these infections lead to higher healthcare costs due to extended hospital stays, longer treatment durations, and additional strain on healthcare personnel (S. Sharma et al. 2023). In animal husbandry, biofilms also

have a detrimental effect on milk production. The excessive use of antibiotics and biocides to control biofilms further accelerates the development of antimicrobial resistance, creating a vicious cycle of worsening problems (Manyi-Loh et al. 2018).

In cattle, biofilm-forming pathogens like *E. coli*, *Klebsiella*, *P. aeruginosa*, *S. aureus* and others contribute to persistent infections. Biofilms enhance microbial resistance through intrinsic factors such as persister cells, oxidative stress, and quorum sensing, alongside extrinsic factors like horizontal gene transfer, antibiotic-degrading enzymes, and active efflux pumps. These mechanisms make biofilm-associated infections difficult to treat, highlighting the need for targeted antimicrobial strategies. (Dutt et al. 2022; S. Sharma et al. 2023).

Biofilm-mediated multidrug resistance challenges healthcare and veterinary medicine, increasing interest in herbal medicines as antibiotic alternatives. Herbal treatments are valued for promoting health, long-lasting effects, and minimal side effects, offering potential solutions for biofilm-associated infections and antimicrobial resistance. Previous studies highlight the antimicrobial efficacy of plant extracts, with alternative methods needed to evaluate biofilm-forming bacteria, which are significantly more resistant than planktonic cells (Muteeb et al. 2023) (Jain et al. 2015; Vaou et al. 2021).

Previous research has investigated the antibiofilm activity of aqueous and organic extracts from various herbal plants against pathogenic microorganisms. Notably, methanolic extracts of Amla (*Phyllanthus emblica*) have demonstrated 60-70% inhibition of *Klebsiella pneumoniae* biofilm formation. Similarly, Neem (*Azadirachta indica*) extracts have shown a 50-65% reduction in biofilm formation by both *Klebsiella pneumoniae* and *Staphylococcus aureus*. These findings suggest the potential of herbal extracts as effective agents against biofilm-associated infections (Olawuwo, Famuyide, and McGaw 2022). Haldi (*Curcuma longa*) extracts demonstrated antibiofilm activity against *Klebsiella pneumoniae* (Dai et al. 2022) and Organic extracts from Beheda (*Terminalia bellirica*) effectively reduce biofilm formation in *E. coli* and *Klebsiella pneumoniae*, suggesting their potential in controlling bacterial biofilm-related infections (Tiwana, Cock, and Cheesman 2024).

These findings emphasize the potential of herbal extracts in managing biofilm-associated infections, with possibilities for enhanced efficacy through synergistic combinations or optimized extraction methods. Also, *Pseudomonas aeruginosa*, a common target in biofilm research due to its high biofilm-forming capacity and multidrug resistance, has shown significant response to various plant extracts. Methanolic extracts of different plant extracts exhibited a reduction. These results highlight the effectiveness of these plant extracts as promising alternatives for managing *P. aeruginosa*-related biofilm infections (Damyanova et al. 2024; Vaou et al. 2021).

While most studies focus on preventing *P. aeruginosa* biofilm formation, some highlight the potential of herbal extracts in removing pre-formed biofilms. For example, zingerone has been shown to reduce biofilm mass by 70% on 3-day-old *P. aeruginosa* biofilms. This suggests that certain herbal extracts could not only prevent biofilm formation but also disrupt existing biofilms, offering valuable therapeutic options. Our research evaluates plant extracts traditionally used in treating cattle diseases,

aiming to assess their antibiofilm efficacy against *P. aeruginosa*. Given *P. aeruginosa* role as a biofilm indicator organism, this study seeks to explore herbal remedies' potential in managing biofilm-associated infections in cattle (Guzzo et al. 2020a; Kim and Park 2013).

Material and Methods

Study Area: The study aims to survey medicinal plants used in ethnoveterinary practices in the Anand area. Field surveys were conducted periodically between October 2023 and 2024 to collect plant samples and associated ethnoveterinary data. The plant parts used in ethnoveterinary formulations were gathered from the ICAR-Directorate of Medicinal and Aromatic Plants Research in Anand, a renowned institution specializing in medicinal and aromatic plants.

Data Collection, plant collection and identification: Data on medicinal plants used in ethnoveterinary practices were collected from informants in the Anand area using questionnaire surveys, semi-structured interviews, discussions, and field observations. Informants were selected through purposive sampling based on their knowledge of traditional veterinary medicine. The questions focused on the types of medicinal plants used, their preparation methods, administration, and effectiveness. A field data sheet was developed to document information on the plant species, including local names, parts used, preparation methods, and modes of administration. The plants were then collected and identified with the assistance of taxonomic experts to ensure accurate identification for further study (Prakash et al. 2021; Tariq et al. 2014).

Drying method for plant parts: The oven drying method for plant materials used in ethnoveterinary formulations begins with collecting parts like leaves, roots, or flowers. These materials are washed with water to remove dirt, insects, or debris and gently dried with a cloth or paper towel. Larger plant parts are cut into smaller pieces for even drying. The oven is preheated to a temperature between 40-60°C (104-140°F) to preserve bioactive compounds. The plant material is placed on a clean tray or paper in a single layer for uniform drying. It is checked periodically to prevent overheating or burning. Once the material becomes crisp and brittle, it is removed from the oven. The dried material is stored in airtight containers, away from light, heat, and moisture to maintain its medicinal properties (Krakowska-Sieprawska et al. 2022; Müller and Heindl 2007).

Preparation of Plant Extract (Organic and Aqueous): Bioactive compounds were extracted from plant material using the Soxhlet extraction method. The powdered plant material was packed into a porous thimble and placed in the extraction chamber. Methanol was used for organic extraction, while distilled water was used for aqueous extraction. The Soxhlet apparatus was assembled, with the solvent added to the boiling flask and connected to a condenser. Heating was maintained at 70°C for organic solvents and 100°C for aqueous solvents. The extraction lasted 3–4 hours. Afterward, solvents were removed using a rotary evaporator or gentle heating for aqueous extracts. The final extract was stored at 4°C for further analysis (Bitwell et al. 2023; Kasiramar and Gopalasatheeskumar 2019).

Biofilm development Assay: The biofilm formation assay was performed using a microtiter plate following the method of (K. Sharma and Pagedar Singh 2018). An overnight-activated culture with OD adjustment was inoculated at 10% in TSB, and 200 µL/well was dispensed in triplicate into a polystyrene microtiter plate (Axiva Biotech, Mumbai). After 24 hours of incubation at 37°C, wells were washed with PBS (2–3 times) to remove loosely adhered cells, blotted on a paper towel, and air-dried. The biofilm was stained with 1% crystal violet and destained with 33% glacial acetic acid. Optical density was measured at 595 nm using a microplate reader (Epoch2C, Biotek, USA). Negative, positive, and methanol test controls were included to ensure assay reliability.

Calculation percentage inhibition,

$$\% \text{ Inhibition} = \frac{(\text{control } OD_{595nm} - \text{test } OD_{595nm})}{\text{control } OD_{595nm}} \times 100$$

Pre-treatment of biofilm with extracts: Briefly, extract was added to obtain final concentration of 2, 5, 10% of Herbal extract in TSB containing 10% inoculum in a volume of 200 µL/well. Plates were incubated at 37°C for 24 hrs., decanted, rinsed, air dried, stained, destained and quantified as described in previous section.

Post-treatment of biofilms with extracts: Biofilm was prepared by inoculating TSB with 10% inoculum and incubating the plate at 37°C for 24 hrs. Subsequently, the content of the plate was decanted aseptically, rinsed with sterile distilled water to remove any loosely adhered cells, air dried and Herbal extract of final concentration of 2, 5, 10% v/v in sterile water was added to each well in triplicate. The contact time of herbal extract with preformed biofilm was maintained for 6 hours at 37°C (K. Sharma and Pagedar Singh 2018). The plate was decanted, rinsed, air dried, stained, destained and quantified as described in previous section.

Data Analysis: The percentage inhibition of plant extracts against *Pseudomonas aeruginosa* was analysed in Excel 2021 using descriptive statistics to summarize results. This helped in identifying the most effective plant extracts with antibacterial potential.

Result and Discussion

Biofilm-mediated infections in cattle pose a significant challenge due to their resistance to conventional antimicrobials. Such infections often persist despite treatment, leading to recurrent disease and prolonged recovery. Frequent antibiotic use in these cases increases the risk of antimicrobial resistance through adaptive or cross-resistance mechanisms (Uruén et al. 2020).

The concept of using herbal plants as therapeutic agents is well established in traditional veterinary medicine. Regular supplementation with specific herbal extracts may help prevent biofilm-associated infections, similar to the "pre-treatment" approach in this study. Pre-treatment refers to evaluating herbal extracts' ability to inhibit biofilm formation when present during bacterial colonization. On the other hand, "post-treatment" involves applying herbal extracts to pre-existing biofilms, mimicking their

therapeutic role(Araújo et al. 2024; Parham et al. 2020). This dual approach highlights the prophylactic and curative potential of herbal medicines in managing biofilm-associated infections in cattle, reducing dependency on synthetic antibiotics, and mitigating antimicrobial resistance(Shariati et al. 2022).

The effectiveness of herbal extracts in preventing biofilm formation during pre-treatment may depend on their impact on bacterial cell viability within the inoculum. In some cases, the inoculum might not reach the necessary cell density to initiate biofilm formation. Various antibiofilm mechanisms of herbal extracts have been proposed, contributing to biofilm reduction. These mechanisms include antiadhesive properties rather than direct antibacterial action, formation of weakly adhered biofilms that detach during quantification, increased bacterial motility, decreased exopolymer matrix production, and reduced bacterial communication(Lebeloane et al. 2024; S., A., and Grace 2020). The preventive potential of herbal extracts against biofilm formation was assessed by incorporating the extract at the time of bacterial inoculation. For cattle experiencing recurrent infections and frequent antibiotic use, regular supplementation with herbal products may offer a natural strategy to prevent biofilm-related diseases and mitigate antimicrobial resistance risks(Saeloh and Visutthi 2021).

In the present study, methanolic and aqueous extracts from 11 medicinal plants were evaluated for their potential to disrupt *Pseudomonas aeruginosa* biofilms. The selected plants included Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*), Amla (*Phyllanthus emblica*), Shatavari (*Asparagus racemosus*), Mulethi (*Glycyrrhiza glabra*), Moringa (*Moringa oleifera*), Ashwagandha (*Withania somnifera*), Beheda (*Terminalia bellirica*), Tulsi (*Ocimum sanctum*), and Nagarvel (*Piper betle L*). *P. aeruginosa* was chosen as the test organism due to its well-established role as a biofilm model and its inherent multidrug resistance, making it a suitable indicator for evaluating antibiofilm efficacy.

The pre-treatment results indicate reduction in *P. aeruginosa* biofilm formation across all tested herbal extracts. Among the extracts, *Curcuma longa* (Turmeric) showed the highest reduction at 10% concentration (88.19%), followed closely by *Terminalia bellirica* (Beheda) (87.56%) and *Azadirachta indica* (Neem) (85.42%). Similarly, *Piper betle L* (Nagarvel) (86.03%) and *Phyllanthus emblica* (Amla) (84.95%) demonstrated strong antibiofilm potential. Lower concentrations (2%) showed relatively moderate reductions, with values ranging from 48.29% (*Asparagus racemosus* - Shatavari) to 60.45% (*Curcuma longa* - Turmeric). At 5% concentration, reductions increased across all extracts, with *Curcuma longa* (79.62%), *Terminalia bellirica* (78.34%), and *Piper betle L* (76.58%) displaying significant inhibition. The post-treatment analysis revealed that methanolic extracts retained their antibiofilm efficacy, with *Curcuma longa* (Turmeric) (83.41%), *Terminalia bellirica* (Beheda) (81.89%), and *Piper betle L* (Nagarvel) (80.62%) demonstrating the highest reductions at 10% concentration. In the case of methanolic re-extracts, a similar trend was observed, with *Curcuma longa* (Turmeric) (72.65%), *Terminalia bellirica* (Beheda) (71.02%), and *Azadirachta indica* (Neem) (70.43%) showing the highest reduction in biofilm formation at 10% concentration. In the methanolic

re-extracts, biofilm reduction did not always show a strong concentration-dependent increase. While some extracts, like *Zingiber officinale* (Ginger), exhibited a clear rise in inhibition (36.65% at 2% to 68.23% at 10%), others, such as *Piper betle* L (Nagarvel) and *Asparagus racemosus* (Shatavari), showed minimal increase or plateaued effects. Extracts like *Curcuma longa* (Turmeric) and *Azadirachta indica* (Neem) displayed a moderate rise, suggesting variable bioavailability or saturation of bioactive compounds at higher concentrations. These variations indicate that the effectiveness of herbal extracts depends on their chemical composition, extraction efficiency, and interaction with *P. aeruginosa* biofilms Fig (1).

Previous research indicates that *Azadirachta indica* (Neem) exhibits significant inhibitory effects on *Pseudomonas aeruginosa* biofilm formation, with inhibition levels varying based on extract concentration. A study on the antibiofilm activity of neem leaf methanolic extract tested concentrations ranging from 0.1 to 25 mg/mL. The extract demonstrated complete biofilm inhibition ($\geq 90\%$) at concentrations between 1.56 and 25 mg/mL. At 0.78 mg/mL, inhibition reached 75.9%, while at 0.1 mg/mL, it was 9.2%. The minimum biofilm inhibitory concentration (MBIC₅₀) required for 50% reduction was approximately 0.39 mg/mL (Qaralleh 2024). The study indicates that pre-treatment with herbal extracts leads to higher biofilm reduction compared to post-treatment, suggesting that early exposure disrupts bacterial adhesion and the initial stages of biofilm formation. Additionally, methanolic extracts were found to exhibit slightly higher biofilm inhibition than methanolic re-extracts, implying that primary extraction yields more potent bioactive compounds. Furthermore, biofilm inhibition was shown to be concentration-dependent, with higher concentrations of herbal extracts leading to greater inhibition, reinforcing the dose-dependent nature of their antibiofilm activity. These findings highlight the importance of early exposure, the superior efficacy of primary extracts, and the concentration-dependent effectiveness of herbal antibiofilm agents. Another study on neem seed oil (NSO) assessed its effects on *P. aeruginosa*, revealing that NSO concentrations between 0.1% and 50% (v/v) inhibited planktonic growth. Biofilm inhibition was observed only at concentrations above 25% (v/v), with significant reduction occurring at concentrations exceeding 12.5% (v/v). A 90% reduction (MIC₉₀) was achieved at a 50% (v/v) concentration (Katiyar, Khare, and Kaistha 2023).

Previous research has shown that curcumin in livestock enhances growth performance, improves disease resistance. Its antimicrobial properties resulting in increased weight gain and improved feed efficiency. Additionally, curcumin's anti-inflammatory and antioxidant effects help mitigate stress responses and support overall animal well-being. These findings highlight curcumin's potential as a natural feed additive for improving livestock productivity and health (Balasubramanian et al. n.d.). Several medicinal plants, including *Zingiber officinale* (Ginger), *Phyllanthus emblica* (Amla), *Asparagus racemosus* (Shatavari), *Glycyrrhiza glabra* (Mulethi), *Moringa oleifera* (Moringa), *Withania somnifera* (Ashwagandha), *Terminalia bellirica* (Beheda), *Ocimum sanctum* (Tulsi), and

Piper betle L. (Nagarvel), possess antimicrobial properties. However, specific studies detailing their effects against *Pseudomonas aeruginosa* remain limited. While Ginger, Amla, Mulethi, Moringa, Ashwagandha, Tulsi, and betel leaf extracts have exhibited broad-spectrum antimicrobial activity, their direct impact on *P. aeruginosa* is not well-documented. Similarly, Shatavari and Beheda have shown potential antimicrobial effects, but data against *P. aeruginosa* are scarce. Further research is needed to explore the efficacy of these herbal plants in combating *P. aeruginosa*-related infections.

The aqueous extract data presented shows the effects of pre-treatment and post-treatment with various herbal extracts on biofilm reduction across different concentrations (2%, 5%, and 10%). Among the herbal extracts tested, *Azadirachta indica* (Neem) exhibited the highest biofilm reduction, with pre-treatment showing 75.12%, 80.25%, and 85.32% inhibition at 2%, 5%, and 10% concentrations, respectively, and post-treatment showing a slightly lower reduction at 68.45%, 72.63%, and 76.34%. Similarly, *Curcuma longa* (Turmeric) demonstrated high biofilm inhibition, with pre-treatment values reaching 80.25%, 83.72%, and 88.90%, and post-treatment showing lower inhibition at 72.34%, 76.45%, and 79.12%. *Phyllanthus emblica* (Amla) also showed notable inhibition, particularly at higher concentrations, with pre-treatment showing up to 89.93% inhibition at 10%, while post-treatment inhibition was significantly lower at all concentrations. Other herbs such as *Zingiber officinale* (Ginger), *Moringa oleifera* (Moringa), and *Withania somnifera* (Ashwagandha) exhibited moderate biofilm reduction, with pre-treatment consistently outperforming post-treatment. Herbs like *Asparagus racemosus* (Shatavari), *Glycyrrhiza glabra* (Mulethi), *Terminalia bellirica* (Beheda), *Ocimum sanctum* (Tulsi), and *Piper betle* L. (Nagarvel) showed varying levels of biofilm inhibition, with *Shatavari* and *Mulethi* demonstrating slightly higher pre-treatment biofilm reduction at 10%, but the overall trend indicates that pre-treatment with herbal extracts tends to be more effective in reducing biofilm formation compared to post-treatment Fig (2).

A comprehensive review published in 2020 explored the anti-biofilm properties of plant-derived chemicals, focusing on compounds like terpenes, flavonoids, alkaloids, and phenolic compounds. These bioactive substances have shown promising results in disrupting biofilm formation by *Pseudomonas aeruginosa*, a major pathogen in livestock, particularly dairy cattle. Biofilm formation by *P. aeruginosa* contributes to its persistent infection in cattle, particularly in conditions such as mastitis, which is a costly and prevalent disease in dairy herds. Biofilms protect bacteria from antibiotics and immune responses, making infections harder to treat. The plant-derived compounds discussed in the review inhibit biofilm formation by suppressing cell adhesion, reducing virulence factor production, and interfering with the quorum sensing system of the bacteria. Quorum sensing is a critical regulatory mechanism used by bacteria to coordinate group behaviours, such as biofilm formation, and disrupt this system could render *P. aeruginosa* less virulent and easier to manage. These findings are particularly relevant to the ongoing challenge of multidrug-resistant (MDR) *P. aeruginosa* strains, which have

become increasingly prevalent in veterinary settings. A study focusing on dairy cattle investigated the prevalence of MDR *P. aeruginosa* strains isolated from various sources, including rectal swabs, udder skin swabs, milk, and environmental samples. The study revealed that MDR strains of *P. aeruginosa* were widely distributed, underlining the need for alternative antimicrobial approaches. Conventional antibiotic treatments are becoming less effective against these resistant strains, highlighting the urgency of exploring plant-based alternatives as adjunct or replacement therapies. The natural antimicrobial properties of plant extracts, including their ability to inhibit biofilm formation, present a potential solution to controlling *P. aeruginosa* infections in cattle, reducing the reliance on antibiotics and mitigating the risk of resistance development. Overall, plant-derived compounds show significant promise in managing *P. aeruginosa* infections in cattle, offering a potential pathway for safer, more sustainable treatments in veterinary medicine (Badawy et al. 2023; Guzzo et al. 2020b).

While these studies underscore the potential of plant-derived natural products in combating *P. aeruginosa* infections in cattle, further research is essential to identify specific plant extracts and their active compounds. Additionally, determining optimal concentrations and assessing their safety and efficacy in veterinary applications are crucial steps toward developing effective treatments for *P. aeruginosa* infections in cattle.

Conclusion

The study highlights the promising potential of herbal extracts in managing biofilm-associated infections caused by *Pseudomonas aeruginosa* in cattle, with significant implications for both veterinary and healthcare settings. The results demonstrate that various herbal plants, including *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), *Terminalia bellirica* (Beheda), *Piper betle* (Nagarvel), and *Phyllanthus emblica* (Amla), possess substantial antibiofilm activity. Notably, pre-treatment with herbal extracts showed higher biofilm reduction compared to post-treatment, emphasizing the importance of early intervention in disrupting bacterial adhesion and biofilm formation. The study also revealed a dose-dependent response, with higher concentrations of herbal extracts leading to more significant inhibition of biofilm formation. While methanolic extracts generally demonstrated more potent activity than aqueous extracts, both types of extracts displayed promising results in inhibiting biofilm formation and disrupting pre-existing biofilms. The findings suggest that these plant-based treatments could serve as natural, sustainable alternatives to conventional antibiotics, offering a dual approach—prophylactic and therapeutic—in combating biofilm-associated infections and mitigating antimicrobial resistance. This study supports the idea of integrating herbal medicine into livestock management, potentially reducing the reliance on synthetic antibiotics and minimizing the risk of multidrug resistance.

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Conflicts of interest

The corresponding author confirms that there is no conflict of interest. The results of the study do not affect any associated parties.

References

1. Araújo, Daniela, Ana Rita Silva, Rúben Fernandes, Patrícia Serra, Maria Margarida Barros, Ana Maria Campos, Ricardo Oliveira, et al. 2024. "Emerging Approaches for Mitigating Biofilm-Formation-Associated Infections in Farm, Wild, and Companion Animals." *Pathogens* 13(4). doi:10.3390/pathogens13040320.
2. Badawy, Basma, Samar Moustafa, Radwa Shata, Mohamed Z. Sayed-Ahmed, Saad S. Alqahtani, Md Sajid Ali, Nawazish Alam, et al. 2023. "Prevalence of Multidrug-Resistant *Pseudomonas Aeruginosa* Isolated from Dairy Cattle, Milk, Environment, and Workers' Hands." *Microorganisms* 11(11). doi:10.3390/microorganisms11112775.
3. Balasubramanian, Balamuralikrishnan, Ruben Merino-Guzman, Mohammed Fouad, El Basuini, Matteo Dell'anno, and Taesun Min. *The Impact of Curcumin on Livestock and Poultry Animal's Performance and Management of Insect Pests*.
4. Bitwell, Chibuye, Singh Sen Indra, Chimuka Luke, and Maseka Kenneth Kakoma. 2023. "A Review of Modern and Conventional Extraction Techniques and Their Applications for Extracting Phytochemicals from Plants." *Scientific African* 19. doi:10.1016/j.sciaf.2023.e01585.
5. Dai, Chongshan, Jiahao Lin, Hui Li, Jianzhong Shen, Zhangqi Shen, Yang Wang, and Tony Velkov. 2022. "The Natural Product Curcumin as an Antibacterial Agent: Current Achievements and Problems." *Antioxidants* 11(3). doi:10.3390/antiox11030459.
6. Damyanova, Tsvetozara, Petya D. Dimitrova, Dayana Borisova, Tanya Topouzova-Hristova, Emi Haladjova, and Tsvetelina Paunova-Krasteva. 2024. "An Overview of Biofilm-Associated Infections and the Role of Phytochemicals and Nanomaterials in Their Control and Prevention." *Pharmaceutics* 16(2). doi:10.3390/pharmaceutics16020162.
7. Dutt, Yogesh, Ruby Dhiman, Tanya Singh, Arpana Vibhuti, Archana Gupta, Ramendra Pati Pandey, V. Samuel Raj, Chung Ming Chang, and Anjali Priyadarshini. 2022. "The Association between Biofilm Formation and Antimicrobial Resistance with Possible Ingenious Bio-Remedial Approaches." *Antibiotics* 11(7). doi:10.3390/antibiotics11070930.
8. Guzzo, Francesca, Monica Scognamiglio, Antonio Fiorentino, Elisabetta Buommino, and Brigida D'abrosca. 2020a. "Plant Derived Natural Products against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*: Antibiofilm Activity and Molecular Mechanisms." *Molecules* 25(21). doi:10.3390/molecules25215024.

9. Guzzo, Francesca, Monica Scognamiglio, Antonio Fiorentino, Elisabetta Buommino, and Brigida D'abrosca. 2020b. "Plant Derived Natural Products against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*: Antibiofilm Activity and Molecular Mechanisms." *Molecules* 25(21). doi:10.3390/molecules25215024.
10. Jain, Isha, Pankaj Jain, Dakshina Bisht, Alosha Sharma, Binita Srivastava, and Nidhi Gupta. 2015. "Comparative Evaluation of Antibacterial Efficacy of Six Indian Plant Extracts against *Streptococcus Mutans*." *Journal of Clinical and Diagnostic Research* 9(2): ZC50–53. doi:10.7860/JCDR/2015/11526.5599.
11. Kasiramar, Gopalasatheeskumar, and K Gopalasatheeskumar. 2019. *SIGNIFICANT ROLE OF SOXHLET EXTRACTION PROCESS IN PHYTOCHEMICAL RESEARCH*. www.mintagejournals.com43.
12. Katiyar, Ravish, Ekta Khare, and Shilpa Deshpande Kaistha. 2023. "Concentration Dependent Effect of *Azadirachta Indica* (Neem) Seed Oil and Neem Bark Extract on Planktonic and Established Biofilm Growth of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*." *Journal of Pure and Applied Microbiology* 17(3): 1669–78. doi:10.22207/JPAM.17.3.30.
13. Kim, Han Shin, and Hee Deung Park. 2013. "Ginger Extract Inhibits Biofilm Formation by *Pseudomonas Aeruginosa* PA14." *PLoS ONE* 8(9). doi:10.1371/journal.pone.0076106.
14. Krakowska-Sieprawska, Aneta, Anna Kielbasa, Katarzyna Rafińska, Magdalena Ligor, and Bogusław Buszewski. 2022. "Modern Methods of Pre-Treatment of Plant Material for the Extraction of Bioactive Compounds." *Molecules* 27(3). doi:10.3390/molecules27030730.
15. Lebeloane, M. M., I. M. Famuyide, J. P. Dzoyem, R. O. Adeyemo, F. N. Makhubu, E. E. Elgorashi, K. G. Kgosana, and L. J. McGaw. 2024. "Influence of Selected Plant Extracts on Bacterial Motility, Aggregation, Hydrophobicity, Exopolysaccharide Production and Quorum Sensing during Biofilm Formation of Enterohaemorrhagic *Escherichia Coli* O157:H7." *South African Journal of Botany* 167: 197–208. doi:10.1016/j.sajb.2024.02.022.
16. Manyi-Loh, Christy, Sampson Mamphweli, Edson Meyer, and Anthony Okoh. 2018. "Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications." *Molecules* 23(4). doi:10.3390/molecules23040795.
17. Mirghani, Ruba, Tania Saba, Hebba Khaliq, Jennifer Mitchell, Lan Do, Liz Chambi, Kelly Diaz, et al. 2022. "Biofilms: Formation, Drug Resistance and Alternatives to Conventional Approaches." *AIMS Microbiology* 8(3): 240–78. doi:10.3934/microbiol.2022019.
18. Müller, Joachim, and Albert Heindl. 2007. "Drying of Medicinal Plants." In *Medicinal and Aromatic Plants*, Springer Netherlands, 237–52. doi:10.1007/1-4020-5449-1_17.
19. Muteeb, Ghazala, Md Tabish Rehman, Moayad Shahwan, and Mohammad Aatif. 2023. "Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review." *Pharmaceuticals* 16(11). doi:10.3390/ph16111615.
20. Olawuwo, Olasunkanmi S., Ibukun M. Famuyide, and Lyndy J. McGaw. 2022. "Antibacterial and Antibiofilm Activity of Selected Medicinal Plant Leaf Extracts Against Pathogens Implicated in Poultry Diseases." *Frontiers in Veterinary Science* 9. doi:10.3389/fvets.2022.820304.
21. Parham, Shokoh, Anousheh Zargar Kharazi, Hamid Reza Bakhsheshi-Rad, Hadi Nur, Ahmad Fauzi Ismail, Safian Sharif, Seeram Ramakrishna, and Filippo Berto. 2020. "Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials." *Antioxidants* 9(12): 1–36. doi:10.3390/antiox9121309.
22. Prakash, Pramod, Radha, Manoj Kumar, Ashok Pundir, Sunil Puri, Suraj Prakash, Neeraj Kumari, et al. 2021. "Documentation of Commonly Used Ethnoveterinary Medicines from Wild Plants of the High Mountains in Shimla District, Himachal Pradesh, India." *Horticulturae* 7(10). doi:10.3390/horticulturae7100351.

23. Qaralleh, Haitham. 2024. "The Inhibitory Effect of Azadirachta Indica Extract on Pseudomonas Aeruginosa Biofilm, Virulence Factors, and Quorum Sensing." *Microbial Biosystems* 9(2): 155–65. doi:10.21608/mb.2024.312958.1151.
24. S., Lakshmana Prabu, Umamaheswari A., and Grace Felciya Grace. 2020. "Investigation on the Biofilm Eradication Potential of Selected Medicinal Plants against Methicillin-Resistant Staphylococcus Aureus." *Biotechnology Reports* 28. doi:10.1016/j.btre.2020.e00523.
25. Saeloh, Dennapa, and Monton Visutthi. 2021. "Efficacy of Thai Plant Extracts for Antibacterial and Anti-Biofilm Activities against Pathogenic Bacteria." *Antibiotics* 10(12). doi:10.3390/antibiotics10121470.
26. Shariati, Aref, Mojtaba Didehdar, Shabnam Razavi, Mohsen Heidary, Fatemeh Soroush, and Zahra Chegini. 2022. "Natural Compounds: A Hopeful Promise as an Antibiofilm Agent Against Candida Species." *Frontiers in Pharmacology* 13. doi:10.3389/fphar.2022.917787.
27. Sharma, Komal, and Ankita Pagedar Singh. 2018. "Antibiofilm Effect of DNase against Single and Mixed Species Biofilm." *Foods* 7(3). doi:10.3390/foods7030042.
28. Sharma, Satish, James Mohler, Supriya D. Mahajan, Stanley A. Schwartz, Liana Bruggemann, and Ravikumar Aalinkeel. 2023. "Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment." *Microorganisms* 11(6). doi:10.3390/microorganisms11061614.
29. Tariq, Akash, Sakina Mussarat, Muhammad Adnan, Naser M. Abdelsalam, Riaz Ullah, and Abdul Latif Khan. 2014. "Ethnoveterinary Study of Medicinal Plants in a Tribal Society of Sulaiman Range." *Scientific World Journal* 2014. doi:10.1155/2014/127526.
30. Tiwana, Gagan, Ian Edwin Cock, and Matthew James Cheesman. 2024. "Combinations of Terminalia Bellirica (Gaertn.) Roxb. and Terminalia Chebula Retz. Extracts with Selected Antibiotics Against Antibiotic-Resistant Bacteria: Bioactivity and Phytochemistry." *Antibiotics* 13(10). doi:10.3390/antibiotics13100994.
31. Uruén, Cristina, Gema Chopo-Escuin, Jan Tommassen, Raúl C Mainar-Jaime, and Jesús Arenas. 2020. "Antibiotics Biofilms as Promoters of Bacterial Antibiotic Resistance and Tolerance." doi:10.3390/antibiotics.
32. Vaou, Natalia, Elisavet Stavropoulou, Chrysa Voidarou, Christina Tsigalou, and Eugenia Bezirtzoglou. 2021. "Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives." *Microorganisms* 9(10). doi:10.3390/microorganisms9102041.

Fig 1: Reduction of *P. aeruginosa* PAO1 biofilm in percentage due to pre-treatment and post-treatment using methanolic extracts and re-extracts of Herbal Extracts

Botanical Name	Common name	Methanolic			Methanolic re-extract			Methanolic			Methanolic re-extract		
		2%	5%	10%	2%	5%	10%	2%	5%	10%	2%	5%	10%
<i>Azadirachta indica</i>	Neem	58.12	77.93	85.42	46.78	50.12	82.74	59.38	71.27	79.34	65.21	69.58	70.43
<i>Curcuma longa</i>	Turmeric	60.45	79.62	88.19	48.56	52.89	83.41	61.72	73.54	81.67	67.89	71.82	72.65
<i>Zingiber officinale</i>	Ginger	56.35	57.91	94.49	73.24	73.32	77.76	61.16	62.01	63.56	36.65	52.64	68.23
<i>Phyllanthus emblica</i>	Amla	55.86	74.15	84.95	44.24	46.99	81.31	56.09	68.4	76.16	62.51	67.34	68.19
<i>Asparagus racemosus</i>	Shatavari	48.29	65.42	78.23	41.27	44.85	71.39	52.76	60.43	66.78	58.12	62.98	63.74
<i>Glycyrrhiza glabra</i>	Mulethi	50.13	69.74	80.92	43.56	46.73	73.48	54.89	63.12	68.35	59.42	64.21	65.17
<i>Moringa oleifera</i>	Moringa	52.87	71.23	83.76	45.32	48.91	76.54	57.34	65.89	70.92	61.78	66.48	67.34
<i>Withania somnifera</i>	Ashwagan dha	53.21	72.48	84.89	46.78	50.12	78.21	58.97	67.21	72.38	63.25	68.32	69.11
<i>Terminalia bellirica</i>	Beheda	59.78	78.34	87.56	47.89	51.34	81.89	60.94	72.89	80.12	66.45	70.21	71.02
<i>Ocimum sanctum</i>	Tulsi	51.65	70.12	81.45	44.98	47.89	74.63	56.89	64.45	69.18	60.78	65.21	66.07
<i>Piper betle</i> L.	Nagarvel	57.32	76.58	86.03	45.92	49.78	80.62	58.74	70.12	78.43	64.78	68.97	69.54

Fig (2): Reduction of *P. aeruginosa* PAO1 biofilm in percentage due to pre-treatment and post-treatment using aqueous extracts of Herbal extract

Herbal extracts		Pre-treatment			Post-treatment		
Botanical names	Common names	2%	5%	10%	2%	5%	10%
<i>Azadirachta indica</i>	Neem	75.12	80.25	85.32	68.45	72.63	76.34
<i>Curcuma longa</i>	Turmeric	80.25	83.72	88.90	72.34	76.45	79.12
<i>Zingiber officinale</i>	Ginger	63.58	65.06	77.81	69.69	71.62	74.27
<i>Phyllanthus emblica</i>	Amla	82.36	86.26	89.93	55.45	56.85	62.22
<i>Asparagus racemosus</i>	Shatavari	60.11	63.47	70.12	57.02	60.34	64.50
<i>Glycyrrhiza glabra</i>	Mulethi	58.34	62.13	65.98	55.23	58.67	61.52
<i>Moringa oleifera</i>	Moringa	65.84	68.50	73.61	61.70	64.12	67.49
<i>Withania somnifera</i>	Ashwagandha	67.42	70.68	75.34	60.50	62.84	65.21
<i>Terminalia bellirica</i>	Beheda	59.14	62.79	68.23	63.92	66.08	68.10
<i>Ocimum sanctum</i>	Tulsi	55.92	59.45	63.78	51.14	53.32	56.87
<i>Piper betle L.</i>	Nagarvel	60.03	64.45	69.02	58.91	61.25	64.03