Antibiofilm and Antibacterial Properties of Herbal Extracts as Alternatives to Current Treatment Approaches: A Narrative Review

ABSTRACT

Bacterial biofilm formation poses significant challenges in the healthcare sector due to increased antibiotic resistance and persistent infections. This literature review explores the potential of some herbs and their extracts as alternative approaches to combat biofilm formation and multidrug-resistant bacteria. A detailed literature search was conducted across databases for published studies till 2023, to identify studies on medicinal plants' anti-biofilm and antibacterial properties. Key compounds within plant extracts showing anti-biofilm activity and their mechanisms of action were highlighted. A combination of keywords, MeSH terms, and Boolean operators were used to formulate the search strategy. Numerous studies demonstrated the efficacy of medicinal plants in inhibiting biofilm formation and combating multidrug-resistant bacteria. Active compounds such as benzyl (6Z,9Z,12Z)-6,9,12-octadecatrienoate, 3-benzyloxy-1-nitro-butan-2-ol, Pyridine, 3-(1-methyl-2-pyrrolidinyl)-(S), and others exhibited anti-biofilm and antibacterial potential. Extracts from *Berginia ciliata, Clematis grata*, and *Clematis viticella* showed over 80% inhibition of biofilm formation, while mango leaf extracts interfered with quorum sensing mechanisms in *Pseudomonas aeruginosa* PAO1. *Salvadora persica* extracts displayed significant biofilm inhibition against cariogenic *Streptococcus mutans* isolates. Medicinal plants and their extracts hold promise as alternative strategies to combat bacterial biofilms and multidrug-resistant bacteria. The identification of active compounds provides opportunities for further research and drug development. Molecular docking studies are crucial for understanding the molecular interactions between these compounds and bacterial targets, guiding the design of effective antibacterial agents based on natural compounds. Further research, including preclinical and clinical trials, is essential to validate the safety and efficacy of these extracts and their compounds for practical application in healthcare.

***Keywords**: Bacterial biofilm, Clinical isolates, Herbal extract, Phytochemical analysis, Anti-biofilm properties, Anti-bacterial, Quorum sensing, Multidrug-resistant bacteria.*

1. INTRODUCTION

Biofilms are self-produced matrices of diverse organic compounds, which present a formidable challenge.[1] These microbial communities anchor to surfaces, manifesting distinct traits influenced by factors like quorum sensing.[2][3] Biofilms develop on varied surfaces, including medical devices,

incurring significant healthcare costs.[4]Prominent biofilm-forming bacteria like *Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli* thrive within clinical settings.[5][6][7] Their resistance to antibiotics and capacity to colonize medical devices compound the limitations of treatment.[8][9]This resistance to conventional treatments necessitates innovative strategies that are still in the early development stages. In this scenario, herbal derivatives emerge as an alternative [10][11][12]. This narrative review explores the potential of herbal extracts against biofilm-related infections, aiming to shed light on their efficacy and mechanisms of action. A detailed literature search was conducted across databases for published literaturetill 2023, to identify studies on medicinal plants' anti-biofilm and antibacterial properties. Key compounds within plant extracts showing anti-biofilm activity and their mechanisms of action were identified. A combination of keywords, MeSH terms, and Boolean operators were used to formulate this search strategy.

2. MATERIALS AND METHODS

Search Strategy:

We conducted a detailedliterature search to identify relevant studies. Databases searched included Google Scholar, PubMed/MEDLINE, and Scopus. We used a combination of keywords, MeSH terms, and Boolean operators to formulate our search strategy. The search was conducted on published studies till 2023 and focus was given to studies published within last 15 years to ensure the inclusion of the most relevant studies. We also reviewed the reference lists of included studies for additional sources.

3. RESULTS

3.1. Biofilm Formation: Mechanisms and Clinical Implications:

Biofilms on medical devices can be formed by a wide range of bacteria, encompassing both gram-positive and gram-negative strains. Among these, some of the most frequently encountered biofilm-forming bacteria include *Enterococcus faecalis, Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus viridans, E. coli, Klebsiella pneumoniae, Proteus mirabilis*, and *Pseudomonas aeruginosa.* The mechanism of biofilm formation is similar amongst the species of bacteria but there can be slight differences among them based on species and habitat.[13] The process begins as bacterial cells interact with surfaces or each other. Initially, they weakly adhere via van der Waals forces and hydrophobic effects, followed by aggregation and the development of an extracellular matrix that fosters communication through biochemical signals and genetic exchange.[14][15]

The complex process of formation of bacterial biofilms is characterized by distinct stages, each involving specific mechanisms and interactions. Four steps are involved; Initial Attachment (Reversible and Irreversible), Maturation of Microcolonies, and Dispersion or Detachment.[16][17] Attachment within biofilm formation involves several key processes. Initially, bacterial adhesins are produced, facilitating the binding of bacterial cells to a surface. As biofilms mature, cell-cell adhesion mechanisms come into play, mediating the cohesion among these cells. Additionally, enzymes that degrade the biofilm matrix play a role in dispersal. In the context of biofilm attachment, it is important to distinguish between "adhesion" and "cohesion." Adhesion pertains to the attachment of bacterial cells to a surface, while cohesion refers to the attachment among bacterial cells within the biofilm. Multiple factors, including hydrophobic interactions, steric interactions, protein adhesion, electrostatic interactions, and Van der Waal forces, influence the adherence of biofilms to surfaces. These interactions collectively contribute to the stability of biofilm attachment to surfaces.[18][19]

In the maturation phase, adhered cells undergo growth and development through intercellular interactions driven by the production of autoinducer signals. These signals activate biofilm-specific genes, ultimately promoting biofilm formation and influencing virulence factors and gene regulation.[17]

This final phase of biofilm development, known as dispersion, carries significant clinical implications. It involves releasingindividual cells or small microcolonies from the biofilm structure, allowing biofilm-producing bacteria to detach and potentially establish new biofilm microcolonies in the surrounding environment. This process, often referred to as metastatic seeding, can lead to chronic infections and severe complications, including embolic events, demanding prompt and effective treatment strategies. Understanding the complexities of biofilm dispersion is critical for comprehending the dynamics of biofilm-associated infections and developing targeted prevention and control measures in clinical settings.[3][20]

These biofilms, like a protective shield, help microorganisms resist the host's immune system, make them more harmful, and contribute to the development of antibiotic resistance.[21]

3.2. Conventional Treatment Approaches and Challenges:

The occurrence of multidrug-resistant bacteria among biofilm-forming strains adds to the complications in managing such cases within clinical settings.[22]

Current medical approaches involve physically removing biofilms and administering localized, high-dose antimicrobial treatments like antibiotics.[23] For instance, intravenous catheters are often treated with "lock therapy," where a concentrated antibiotic solution is introduced into the catheter's lumen for

an extended duration. However, despite these efforts, biofilm-related challenges are rising across healthcare, the food industry, and various sectors. Over a decade, the pharmaceutical industry's lack of new antibiotic development adds to the problem. Additionally, most biofilm bacteria exhibit antibiotic tolerance. Consequently, there is a pressing need to explore alternative treatments for biofilm-related infections beyond antibiotics.[24][25] Some new approaches to address these complications have been made, like the synthetic retinoid antibiotic CD437, which targets and eliminates methicillin-resistant S. aureus (MRSA) persister cells by disrupting their lipid bilayer. Additionally, it exhibits a synergistic antibacterial effect when used alongside gentamicin. A novel antibiotic, V-r8, combines vancomycin with a guanidine-rich cell-penetrating molecular transport protein known as D-octaarginine (r8).[26][27] There is a need for innovative approaches to combat biofilm-associated bacteria, as no single or current treatment appears to be sufficient, because conventional antibiotic treatment is ineffective in fully eliminating bacterial cells located within the core of biofilms, contributing to the escalating global challenge.[28][29]

3.3. Herbal Extracts: Potential against Biofilm Formation

This exploration shows how various plant natural compounds exhibit potent antimicrobial and anti-biofilm properties in vitro. These biofilm-disrupting effects primarily involve inhibiting polymer matrix formation, reducing cell adhesion, interrupting extracellular matrix generation, decreasing virulence factor production, and ultimately impeding the quorum sensing network, thereby curtailing biofilm development.[30]

Several established studies substantiate the ethnopharmacological claim regarding the anti-biofilm activity of herbal extracts and their active compounds. There is an interesting quote, to begin with-: “While the endeavor for drug discovery from herbal medicines is experience-driven, the search for a therapeutically useful synthetic drug, like looking for a needle in a haystack, is a daunting task”.[31]

3.4. Indian medicinal plants:

(*Cinnamomum glaucescens*, *Smilax zeylanica*, *Syzygium praecox*, *Trema orientalis*, *Bischofiajavanica*, *Beilschmiediaroxburghiana* and *Mikania micrantha*)

A study by Panda et al.(2020), aimed to assess the antibacterial effectiveness of selected Indian medicinal plants against multidrug-resistant (MDR) and biofilm-forming Staphylococcus strains. They tested 20 traditional Indian medicinal plants against 17 clinical strains, all resistant to five classes of antibiotics. The study identified several plants, including *Cinnamomum glaucescens, Smilax zeylanica, Syzygium praecox, Trema orientalis, Bischofiajavanica*, and others, that exhibited anti-staphylococcal activity not previously reported. These plants showed potential in controlling the

formation of *S. aureus* biofilms and could be a source of active compounds for novel drug development. Additionally, *Beilschmiediaroxburghiana* and *Mikania micrantha*inhibitedt the growth of *S. aureus* resistant to all five antibiotic groups tested. Their study highlighted the value of exploring the mechanisms of action of these plants. While developing new drugs from these compounds may take time, the extracts could potentially be introduced into clinical practice, particularly for topical treatments, aligning with traditional medicine practices. The study emphasized the significance of traditional herbal knowledge in the search for new antibacterial solutions, with plant-based preparations offering promising avenues for future research and drug development.[32]

3.5. *Acalypha wilkesiana*:

The University of Nottingham Malaysia Campus researchers, investigated the potential of a bioactive fraction, isolated from *Acalypha wilkesiana* Müll. Arg. Which is a shrub or tree that grows primarily in the wet tropical biome, in combating biofilm formation by methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA biofilms are known to enhance bacterial virulence and are associated with persistent hospital infections. The study employed various assays to assess the anti-biofilm activity of the fraction (9EA-FC-B). They found that this fraction exhibited an inhibitory effect on MRSA biofilm production, particularly by preventing the initial cell-surface attachment. Interestingly, 9EA-FC-B also reduced the presence of the antibiotic-resistant protein, penicillin-binding protein 2a (PBP2a), within the biofilm matrix. This protein is known to contribute to MRSA's virulence. Chemical analysis revealed that 9EA-FC-B is a complex mixture containing various compounds, including tannins, saponins, sterol/steroids, and glycosides.[33]

3.6. *Vitexin*:

A study byresearchers from the Central University of Himachal Pradesh, India, explored the potential of *vitexin*, a polyphenolic phytochemical with antimicrobial properties, in combating biofilm formation by *Pseudomonas aeruginosa*, a model biofilm-forming pathogenic bacterium. *Vitexin* demonstrated a minimum inhibitory concentration (MIC) of 260 µg/ml. Their study assessed its antibiofilm activity through various methods, including safranin staining, protein extraction, microscopy, extracellular polymeric substances (EPS) quantification, and in vivo models, using sub-MIC doses. Additionally, the impact of *vitexin* on quorum sensing (QS) mediated phenomena, such as swarming motility, protease activity, pigment production, and enzyme activity, was evaluated. The results revealed a significant reduction in biofilm formation and QS-mediated phenotypes of *Pseudomonas aeruginosa* when exposed to 110 µg/ml vitexin in combination with azithromycin and gentamicin. Molecular docking studies also indicated a strong binding affinity between vitexin and proteins associated with quorum sensing and motility in the bacterium.[34]

3.7. *Nicotiana tabacum* L:

A study conducted by researchers from Arba Minch University in Ethiopia investigated the antimicrobial properties and phytochemical constituents of *Nicotiana tabacum* L. extracted using various organic solvents. The aim was to assess the plant's antibacterial activity against different types of bacteria. *Nicotiana tabacum* L. samples were collected from Western Ethiopia and subjected to extraction using seven different organic solvents. The researchers conducted in vitro antibacterial assays, including agar well diffusion tests, against various bacteria, including culture collection strains, clinical bacterial isolates, and biofilm-forming bacteria. Gas chromatographic and mass spectroscopic (GC-MS) analysis was employed to identify the phytochemical constituents of the plant extracts. The study's findings revealed that the antimicrobial activity of the plant extracts varied depending on the solvent used, with ethyl acetate-based extracts exhibiting the most potent antimicrobial activity. Among the tested organisms, biofilm-forming uropathogenswere the most susceptible, while clinical isolates displayed the greater resistance. GC-MS analysis identified Pyridine, 3-(1-methyl-2-pyrrolidinyl)-(S) as the major compound in the active ethyl acetate extract. Their study demonstrated that *Nicotiana tabacum* L. extracts, particularly those obtained using ethyl acetate, possessed strong antimicrobial activity against biofilm-forming uropathogens. However, clinically isolated bacteria were more resistant. This antibacterial effect may be attributed to the presence of Pyridine, 3-(1-methyl-2-pyrrolidinyl)-(S), and suggests the potential of *Nicotiana tabacum* L. as a source of antimicrobial agents.[35]

3.8. *Allium sativum*:

The most unexpected natural products hold several potential compounds that have therapeutic effects, even our daily consumables. A collaboration study conducted by researchers from institutions in China, Saudi Arabia, and South Korea, explored the potential of *Allium* (garlic) bulb extract in treating biofilm-forming clinical pathogens isolated from periodontal and dental caries samples. The researchers identified various biofilm-producing bacteria, including *Lactobacillus acidophilus, Streptococcus sanguis, Streptococcus salivarius, Streptococcus mutans,* and *Staphylococcus aureus*, from periodontal and dental caries samples. Among these, *S. aureus* and *S. mutans* exhibited strong biofilm-forming capabilities, while *Streptococcus sanguis* and *S. salivarius* showed moderate biofilm formation. The study also investigated the production of extracellular polysaccharides by these pathogens, with S. aureus synthesizing higher amounts of EPS than*S. sanguis* and *S. salivarius*. The researchers extracted phytochemicals from the *Allium sativum* bulb, revealing the presence of carbohydrates, total protein, alkaloids, saponins, flavonoids, tannins, and steroids. These phytochemicals demonstrated a broad range of antibacterial activity against the selected dental pathogens, with ethanol extract showing high activity against *S. aureus*. Minimum Inhibitory Concentration (MIC) values for the crude garlic bulb extract varied across the bacterial strains,

highlighting differences in susceptibility to secondary metabolites. The MIC values ranged from 20 ± 2 mg/ml to 120 ± 6 mg/ml, while Minimum Bactericidal Concentration (MBC) values ranged from 60 ± 5 mg/l to 215 ± 7 mg/ml. Their study suggests that *Allium sativum* bulb extract, due to its antibacterial properties, could effectively treatinfections associated with periodontal and dental caries, particularly those caused by biofilm-forming pathogens.[36]

3.9. *Acacia nilotica*:

Another study conducted by Elamary et al.(2020) aimed to investigate the effectiveness of *Acacia nilotica* aqueous extract in treating biofilm-forming and multidrug-resistant uropathogens isolated from patients with urinary tract infections (UTIs). A total of 170 urine samples were collected from patients in Luxor, Egypt, and analyzed for the presence of uropathogens. *Escherichia coli* was identified as the most prevalent causative agent, followed by other bacterial species. These isolates were found to be multidrug-resistant, carrying various antibiotic-resistant genes. The study assessed the impact of *Acacia nilotica* aqueous extract on these uropathogens and found that the extract was effective against all isolates at concentrations of 15-16.7 mg/ml. Time-killing assays confirmed the bactericidal effect of the extract over a 20-24 hour period. Phytochemical analysis of the extract revealed the presence of various bioactive compounds. Furthermore, the extract significantly reduced the biofilm-forming ability of *E. coli, K. pneumoniae, P. mirabilis*, and *P. aeuroginosa*, demonstrating its potential in combating biofilm-associated infections caused by these pathogens.[37]

3.10. *Annona muricata*:

A study by Neglo et al.(2021) investigated the potential influence of *Annona muricata* extracts on the activity of selected antibiotics against biofilm-forming Methicillin-resistant *Staphylococcus aureus* (MRSA). Various parts of the Annona muricata plant were processed into powder and extracted using either ethanol or hot water. These extracts were then screened for the presence of phytochemicals. The study found that different parts of the *Annona muricata* plant contained varying proportions of secondary metabolites. When tested against MRSA at a concentration of 100 mg/mL, the stem extract exhibited the highest inhibitory activity, which was comparable to that of the control antibiotic tetracycline. Additionally, the study explored the modulatory effect of *Annona muricata* extracts on certain antibiotics when combined with MRSA. Four out of the ten extracts antagonized the activity of ampicillin against MRSA, reducing its effectiveness by a factor of 0.5 folds. In contrast, the remaining extracts potentiated the drug, enhancing its efficacy by 1-4 folds. Furthermore, the extracts significantly potentiated the effectiveness of streptomycin and tetracycline against MRSA by a range of 1-32 folds, with the aqueous root extract showing the highest synergistic effect.[38]

3.11. *Sclerocaryabirrea*(Marula):

Marula is a significant African plant with wide-ranging socio-economic importance, especially in southern Africa. Traditionally, various plant parts, including the bark, have been used for medicinal purposes. In a study conducted by Sarkar et al. (2014), researchers aimed to investigate the anti-biofilm properties of methanol extract from Marula bark, focusing on its potential to combat antimicrobial resistance associated with bacterial biofilms. The study began by evaluating the extract's antimicrobial properties, finding that it did not inhibit bacterial growthat 200 µg/ml concentrations. However, the extract demonstrated significant anti-biofilm activity at sub-lethal concentrations (100 µg/ml), reducing biofilm formation by approximately 75%. To understand the mechanism of this anti-biofilm activity, the researchers examined its impact on quorum sensing (QS)-mediated processes known to be associated with biofilm formation and pathogenicity. The extract inhibited quorum-sensing mediated swarming motility and reduced virulent factors such as protease and pyoverdine release.[39]

3.12. *Chamaemelum nobile*:

Chamomile, known for its therapeutic anti-inflammatory and antimicrobial effects, was investigated for its potential to inhibit biofilm formation by *Pseudomonas aeruginosa*. The study found that Chamomile extract displayed anti-quorum sensing (QS) properties, inhibiting biofilm formation in *P. aeruginosa*. The *Chamaemelum nobile* extract exhibited biofilm inhibition within1.6 to 100 mg/ml concentration range. Effective concentrations for preventing biofilm formation ranged from 6.25 to 25 mg/ml, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were in the ranges of 12.5-50 mg/ml and 25 mg/l, respectively This suggests that Chamomile could offer an alternative strategy in combating bacterial infections, particularly those involving biofilm formation, although further research is needed to understand its precise antibacterial mechanism.[40]

3.13. *Salvadora persica*:

A study by Al-Sohaibani. (2020) investigated the growth inhibition and anti-biofilm effects of various extracts from *Salvadora persica* sticks, commonly used for oral hygiene, on cariogenic *Streptococcus mutans* isolates. The results showed that all *Salvadora persica* extracts exhibited significant inhibitory activity against *Streptococcus mutans*, with varying susceptibility among the cariogenic strains. The methanol and ethanol extracts demonstrated the highest biofilm inhibition, reducing biofilm formation by 87.92% and 85.75%, respectively. Gas chromatography-mass spectrometry (GC-MS) analysis identified more than 28 compounds in the extracts. Notably, compounds such as benzyl (6Z,9Z,12Z)-6,9,12-octadecatrienoate, 3-benzyloxy-1-nitro-butan-2-ol, and 1,3-cyclohexane dicarbohydrazide were found to interact efficiently with bacterial communication quorum-sensing (QS) regulators, suggesting a dual-function role as anti-biofilm agents that not only inhibit bacterial growth but also control the colonization and accumulation of caries-causing *Streptococcus mutans.*[41]

3.14. *Sesbania grandiflora*:

Gandhi et al. (2017) conducted a study aimed to explore the anti-biofilm and antibacterial properties of *Sesbania grandiflora* against *Staphylococcus aureus.* Various analyses, including UV-Vis (Ultraviolet-visible) spectroscopy, Fourier transform infrared spectroscopy, and Dynamic light scattering, were conducted on *Sesbania grandiflora* extracts. Biofilm-forming pathogens were identified using the congo-red assay, and the quantification of extracellular polymeric substances (EPS), particularly protein and carbohydrate, was performed. The results demonstrated that *Sesbania grandiflora* effectively reduced protein and carbohydrate content in the EPS of *S. aureus*, indicating its potential to inhibit biofilm formation. Moreover, *Sesbania grandiflora* exhibited significant antibacterial activity against *S. aureus*, suggesting its efficacy in controlling microbial populations.[42]

3.15. *Berginiaciliata, Clematis grata, Clematis viticella*:

A study conducted by Alam et al. (2020) aimed to investigate the antibiofilm potential of different solvent-based extracts from medicinal plants, including *Berginiaciliata, Clematis grata*, and *Clematis viticella*, traditionally used in the Himalayan region of Pakistan. *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen known for its biofilm-forming ability, was chosen as the model pathogen due to its involvement in various infections, particularly in immunocompromised patients. Various organic solvents and aqueous solutions were used to extract plant components, and their ability to inhibit biofilm formation was assessed. The results showed that the choice of solvent significantly influenced the plant extracts' activity against PAO1 biofilm. Notably, the 1% methanolic extract of *Berginiaciliata* (rhizome with skin) demonstrated over 80% inhibition of biofilm formation without affecting bacterial growth. The study also revealed a significant correlation between flavonoid content and antibiofilm activity in the methanolic extract, highlighting the role of secondary metabolites in inhibiting *Pseudomonas aeruginosa* PAO1 biofilm formation.[43]

3.16. *Mangifera indica*:

A study by Husain et al.(2017) explored the potential of *Mangifera indica* L. (mango) leaf extracts as anti-infective agents by targeting bacterial quorum sensing (QS), a global gene regulatory mechanism associated with various virulence factors. The research investigated the impact of leaf extracts on QS-regulated virulence factors and biofilm formation in Gram-negative pathogens, focusing on *Pseudomonas aeruginosa* PAO1. The results demonstrated that mango leaf extracts, particularly the methanol extract, exhibit dose-dependent interference with QS, leading to a reduction in the production of virulence factors such as elastase, total protease, pyocyanin, chitinase, exopolysaccharides, and swarming motility in *P. aeruginosa* PAO1. Additionally, mango leaf extracts significantly inhibit biofilm formation by *P. aeruginosa* PAO1 and *Aeromonas hydrophila* WAF38. The study also includes evidence from scanning electron microscopy, confirming the observed inhibition of

biofilm formation. Furthermore, mango leaf extracts*Caenorhabditis elegan'*ssurvival pre-infected with *P. aeruginosa*PAO1. Phytochemical analysis of the active extracts revealed a high phenolic content in the methanol extract and the identification of 14 compounds through GC-MS and UPLC (Gas Chromatography-Mass Spectrometry and Ultra-Performance Liquid Chromatography analyses). These findings suggest that phytochemicals from mango leaves have promising anti-infective properties, warranting further investigation for potential therapeutic applications.[44]

3.17. *Boerhaviadiffusa*:

Boerhaviadiffusa L. (*B. diffusa*), a medicinal herb often considered a weed, holds significant potential for pharmaceutical applications. A study conducted by Kaviya et al. (2022) delves into the phytochemical analysis of different parts of *B. diffusa*, including leaves, stems, and roots, using various extraction solvents and methods. Notably, the decoction method yielded promising results in qualitative and quantitative tests and in antioxidant assays like DPPH, FRAP, and ABTS. The antibacterial activity of B. diffusa root ethanol extract is particularly interesting, which demonstrated inhibition against the growth of *Pseudomonas aeruginosa* and Staphylococcus aureus. This finding highlights the plant's potential in combating bacterial infections. Molecular docking analysis identified specific molecules within the plant extract that exhibited a high affinity for inhibiting the pathogenic bacterium *P. aeruginosa*growth. Identified molecules includes-:2-(1,2-dihydroxyethyl)-5-[[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-3, 4-dihydrochromen-6-yl]oxy]oxolane-3, 4-diol, amodiaquine TMS derivative, amodiaquine, and 2-propen-1-one, 3-hydroxy-1,3-diphenyl, which were subsequently evaluated using GLIDE docking. Their results underscore the need for further research to explore and unlock the pharmaceutical applications and commercialization potential of *B. diffusa*, especially in the context of its anti-biofilm properties.[45]

4. DISCUSSION

The reviewed studies highlight the potential of various medicinal plants and their extracts in combating biofilm formation and multidrug-resistant bacteria, offering promising avenues for both pharmaceutical and clinical applications.

Various studies identified specific compounds within plant extracts that exhibited anti-biofilm activity or inhibitory effects on multidrug-resistant bacteria. Notable compounds include benzyl (6Z,9Z,12Z)-6,9,12-octadecatrienoate, 3-benzyloxy-1-nitro-butan-2-ol, 2-(1,2-dihydroxyethyl)-5-[[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydrochromen-6-yl]oxy]oxolane-3,4-diol, Pyridine, 3-(1-methyl-2-pyrrolidinyl)-(S), and others. These compounds exhibit potential antibacterial and anti-biofilm properties.

While multiple herbs and plant extracts demonstrated anti-biofilm activity, some exhibited stronger inhibitory effects than others. For example, *Berginiaciliata, Clematis grata*, and *Clematis viticella* extracts showed over 80% inhibition of biofilm formation against *Pseudomonas aeruginosa* PAO1. Mango leaf extracts, particularly the methanol extract, effectively interfered with quorum sensing mechanisms and reduced virulence factors in *Pseudomonas aeruginosa* PAO1. *Salvadora persica* extracts displayed significant biofilm inhibition against cariogenic *Streptococcus mutans* isolates.

The studies collectively underscore the potential of medicinal plants and plant extracts as alternative strategies for combating bacterial biofilms and multidrug-resistant bacteria. The identified active compounds present opportunities for further research and drug development. Additionally, exploring the mechanisms of action of these plant-based treatments and conducting clinical trials are essential steps toward their practical application.

While these studies provide valuable insights, there is a clear need for more extensive research in this area. Further investigations should focus on isolating and characterizing active compounds, elucidating their mechanisms of action, and conducting preclinical and clinical trials to assess their safety and efficacy in humans. Additionally, the synergistic effects of plant extracts in combination with existing antibiotics warrant exploration.

5. CONCLUSION

The potential of medicinal plants and their extracts in addressing the challenges associated with bacterial biofilms and multidrug-resistant bacteria is diverse. The discovery of active compounds within these natural resources opensexciting opportunities for further research and the development of novel antibacterialand antibiofilm solutions. Harnessing the anti-biofilm properties of medicinal plants offers a promising avenue for tackling antibiotic resistance and biofilm-related infections in both medical and commercial contexts.

Moreover, these studies underscore the critical need for molecular docking and homology modeling approaches to better understandthe specific molecular-level interactions between the active compounds and antigen-binding sites in bacteria. Molecular docking studies can provide insights into how these compounds bind to bacterial targets, disrupting biofilm formation and inhibiting bacterial growth.

ACKNOWLEDGEMENTS

Our sincere thanks to the faculty members of Srinivas Institute of Medical Sciences and Research Center, for providing us with the necessary support and guidance.

COMPETING INTERESTS

No competing interests exist

AUTHORS' CONTRIBUTIONS

Rasmi T R[1]designed the study, managed the literature searches, and wrote the first draft of the manuscript. Dr. Pavan Chand Attavar[2]and M Shashidhar Kotian[4] managed the analyses of the study. Sona P H[1]and Delna N S[5] managed the literature searches. All authors read and approved the final manuscript.

REFERENCES

1. Simões, Manuel, Lúcia C. Simões, and Maria J. Vieira. "A review of current and emergent biofilm control strategies." LWT-Food Science and Technology 43.4 (2010): 573-583. https://www.sciencedirect.com/science/article/pii/S0023643809003430
2. O'Toole, George, Heidi B. Kaplan, and Roberto Kolter. "Biofilm formation as microbial development." Annual Reviews in Microbiology 54.1 (2000): 49-79. https://www.annualreviews.org/doi/abs/10.1146/annurev.micro.54.1.49
3. Costerton, J. William, Philip S. Stewart, and E. Peter Greenberg. "Bacterial biofilms: a common cause of persistent infections." science 284.5418 (1999): 1318-1322. https://www.science.org/doi/abs/10.1126/science.284.5418.1318
4. Beech, I. B., et al. "Microbially-influenced corrosion: damage to prostheses, delight for bacteria." The International journal of artificial organs 29.4 (2006): 443-452. https://journals.sagepub.com/doi/abs/10.1177/039139880602900415
5. Wolcott, R. D., et al. "Chronic wounds and the medical biofilm paradigm." Journal of wound care 19.2 (2010): 45-53. https://www.magonlinelibrary.com/doi/abs/10.12968/jowc.2010.19.2.46966
6. Sanchez, Carlos J., et al. "Biofilm formation by clinical isolates and the implications in chronic infections." BMC infectious diseases 13.1 (2013): 1-12. https://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-2334-13-47
7. Donlan, Rodney M., and J. William Costerton. "Biofilms: survival mechanisms of clinically relevant microorganisms." Clinical microbiology reviews 15.2 (2002): 167-193. https://journals.asm.org/doi/abs/10.1128/CMR.15.2.167-193.2002

8. Donlan, Rodney M. "Biofilms: microbial life on surfaces." Emerging infectious diseases 8.9 (2002): 881. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2732559/
9. Gebreyohannes, Gebreselema, et al. "Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms." Heliyon 5.8 (2019). https://www.cell.com/heliyon/pdf/S2405-8440(19)35852-9.pdf
10. Das, P. K., & Samal, S. (2018). Microbial biofilms: pathogenicity and treatment strategies. PharmaTutor, 6(1), 16-22. https://www.researchgate.net/profile/Smrutipragnya-Samal/publication/322204505_Microbial_Biofilms_Pathogenicity_and_Treatment_Strategies/links/5ae01ad10f7e9b285945ec42/Microbial-Biofilms-Pathogenicity-and-Treatment-Strategies.pdf
11. Del Pozo, J. L., and R. Patel. "The challenge of treating biofilm-associated bacterial infections." Clinical Pharmacology & Therapeutics 82.2 (2007): 204-209. https://ascpt.onlinelibrary.wiley.com/doi/abs/10.1038/sj.clpt.6100247
12. Pereira, José Alberto, et al. "Walnut (Juglans regia L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars." Food and chemical toxicology 45.11 (2007): 2287-2295. https://www.sciencedirect.com/science/article/pii/S0278691507001949
13. Chen, Meng, Qingsong Yu, and Hongmin Sun. "Novel strategies for the prevention and treatment of biofilm-related infections." International journal of molecular sciences 14.9 (2013): 18488-18501. https://www.mdpi.com/1422-0067/14/9/18488
14. Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., &Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563-575.
15. Sutherland, Ian W. "The biofilm matrix–an immobilized but dynamic microbial environment." Trends in Microbiology 9.5 (2001): 222-227. https://www.cell.com/trends/microbiology/fulltext/S0966-842X(01)02012-1
16. Khatoon, Zohra, et al. "Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention." Heliyon 4.12 (2018). https://www.cell.com/heliyon/pdf/S2405-8440(18)36901-9.pdf
17. Gu, Huan, et al. "Patterned biofilm formation reveals a mechanism for structural heterogeneity in bacterial biofilms." Langmuir 29.35 (2013): 11145-11153. https://pubs.acs.org/doi/abs/10.1021/la402608z

18. Joo, Hwang-Soo, and Michael Otto. "Molecular basis of in vivo biofilm formation by bacterial pathogens." Chemistry &Biology 19.12 (2012): 1503-1513. https://www.cell.com/ccbio/pdf/S1074-5521(12)00423-1.pdf

19. Kaplan, J. áB. "Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses." Journal of Dental Research 89.3 (2010): 205-218. https://journals.sagepub.com/doi/abs/10.1177/0022034509359403

20. Gupta, Priya, et al. "Biofilm, pathogenesis and prevention—a journey to break the wall: a review." Archives of Microbiology 198 (2016): 1-15. https://link.springer.com/article/10.1007/s00203-015-1148-6

21. Veerachamy, Suganthan, et al. "Bacterial adherence and biofilm formation on medical implants: a review." Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine 228.10 (2014): 1083-1099. https://journals.sagepub.com/doi/abs/10.1177/0954411914556137

22. Mittal, Seema, Madhu Sharma, and Uma Chaudhary. "Biofilm and multidrug resistance in uropathogenic Escherichia coli." Pathogens and global health 109.1 (2015): 26-29. https://www.tandfonline.com/doi/abs/10.1179/2047773215Y.0000000001

23. Koo, Hyun, et al. "Targeting microbial biofilms: current and prospective therapeutic strategies." Nature Reviews Microbiology 15.12 (2017): 740-755. https://www.nature.com/articles/nrmicro.2017.99

24. Li, X., Sun, L., Zhang, P., & Wang, Y. (2021). Novel approaches to combat medical device-associated biofilms. Coatings, 11(3), 294.

25. WC Soo, Valerie, et al. "Repurposing of anticancer drugs for the treatment of bacterial infections." Current topics in medicinal chemistry 17.10 (2017): 1157-1176. https://www.ingentaconnect.com/content/ben/ctmc/2017/00000017/00000010/art00004

26. Kim, Wooseong, et al. "A new class of synthetic retinoid antibiotics effective against bacterial persisters." Nature 556.7699 (2018): 103-107. https://www.nature.com/articles/nature26157

27. Antonoplis, Alexandra, et al. "A dual-function antibiotic-transporter conjugate exhibits superior activity in sterilizing MRSA biofilms and killing persister cells." Journal of the American Chemical Society 140.47 (2018): 16140-16151. https://pubs.acs.org/doi/abs/10.1021/jacs.8b08711

28. Kulshrestha, Shatavari, et al. "A graphene/zinc oxide nanocomposite film protects dental implant surfaces against cariogenic Streptococcus mutans." Biofouling 30.10 (2014): 1281-1294. https://www.tandfonline.com/doi/abs/10.1080/08927014.2014.983093

29. Sun, Fengjun, et al. "Biofilm-associated infections: antibiotic resistance and novel therapeutic strategies." Future Microbiology 8.7 (2013): 877-886. https://www.futuremedicine.com/doi/abs/10.2217/fmb.13.58

30. Lu, Lan, et al. "Developing natural products as potential anti-biofilm agents." Chinese Medicine 14.1 (2019): 1-17. https://cmjournal.biomedcentral.com/articles/10.1186/s13020-019-0232-2

31. Pan, Si-Yuan, et al. "New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics." Evidence-Based Complementary and Alternative Medicine 2013 (2013). https://www.hindawi.com/journals/ecam/2013/627375/

32. Panda, Sujogya Kumar, et al. "Indian medicinal plant extracts to control multidrug-resistant S. aureus, including in biofilms." South African Journal of Botany 128 (2020): 283-291. https://www.sciencedirect.com/science/article/pii/S0254629919314723

33. Santiago, Carolina, et al. "Inhibition of penicillin-binding protein 2a (PBP2a) in methicillin-resistant Staphylococcus aureus (MRSA) by combination of ampicillin and a bioactive fraction from Duabanga grandiflora." BMC complementary and alternative medicine 15 (2015): 1-7. https://link.springer.com/article/10.1186/s12906-015-0699-z

34. Das, Manash C., et al. "Attenuation of Pseudomonas aeruginosa biofilm formation by Vitexin: a combinatorial study with azithromycin and gentamicin." Scientific Reports 6.1 (2016): 23347. https://www.nature.com/articles/srep23347

35. Ameya, Gemechu, Aseer Manilal, and Behailu Merdekios. "In vitro antibacterial activity and phytochemical analysis of Nicotiana tabacum L. extracted in different organic solvents." The Open Microbiology Journal 11 (2017): 352. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5759108/

36. Bin, Chen, et al. "Potential effect of Allium sativum bulb for the treatment of biofilm forming clinical pathogens recovered from periodontal and dental caries." Saudi journal of biological sciences 27.6 (2020): 1428-1434. https://www.sciencedirect.com/science/article/pii/S1319562X2030111X

37. Elamary, Rokaia B., Fawziah M. Albarakaty, and Wesam M. Salem. "Efficacy of Acacia nilotica aqueous extract in treating biofilm-forming and multidrug resistant uropathogens isolated from patients with UTI syndrome." Scientific Reports 10.1 (2020): 11125. https://www.nature.com/articles/s41598-020-67732-w

38. Neglo, David, et al. "Evaluation of the modulatory effect of Annona muricata extracts on the activity of some selected antibiotics against biofilm-forming MRSA." Evidence-Based

Complementary and Alternative Medicine 2021 (2021). https://www.hindawi.com/journals/ecam/2021/9342110/

39. Sarkar, Ratul, et al. "Anti-biofilm activity of Marula–a study with the standardized bark extract." Journal of Ethnopharmacology 154.1 (2014): 170-175. https://www.sciencedirect.com/science/article/pii/S0378874114002712

40. Kazemian, Hossein, et al. "Antibacterial, anti-swarming and anti-biofilm formation activities of Chamaemelum nobile against Pseudomonas aeruginosa." Revista da Sociedade Brasileira de Medicina Tropical 48 (2015): 432-436. https://www.scielo.br/j/rsbmt/a/NLjLRQ4xYQZXdNRfvdnV39J/?format=html&lang=en

41. Al-Sohaibani, Saleh, and Kasi Murugan. "Anti-biofilm activity of Salvadora persica on cariogenic isolates of Streptococcus mutans: in vitro and molecular docking studies." Biofouling 28.1 (2012): 29-38. https://www.tandfonline.com/doi/abs/10.1080/08927014.2011.647308

42. Gandhi, Arumugam Dhanesh, et al. "In vitro anti-biofilm and anti-bacterial activity of Sesbania grandiflora extract against Staphylococcus aureus." Biochemistry and biophysics reports 12 (2017): 193-197. https://www.sciencedirect.com/science/article/pii/S2405580817301838

43. Alam, Khan, et al. "Anti-biofilm activity of plant-derived extracts against infectious pathogen-Pseudomonas aeruginosa PAO1." Journal of Infection and Public Health 13.11 (2020): 1734-1741. https://www.sciencedirect.com/science/article/pii/S1876034120305736

44. Husain, Fohad M., et al. "Leaf extracts of Mangifera indica L. inhibit quorum sensing–regulated production of virulence factors and biofilm in test bacteria." Frontiers in Microbiology 8 (2017): 727. https://www.frontiersin.org/articles/10.3389/fmicb.2017.00727/full

45. Kaviya, Mohandass, et al. "Evaluation of Nutritional Substances and Investigation of Antioxidant and Antimicrobial Potentials of Boerhaviadiffusa with in Silico Molecular Docking." Molecules 27.4 (2022): 1280. https://www.mdpi.com/1420-3049/27/4/1280