Review Article

Anti-biofilm Activity of Plant Bioactive Substances

ABSTRACT

Biofilms are a group of microorganisms that exist in living or non-living surfaces, within extracellular matrices produced by microbial cells.Their recalcitrant nature and ability to resist antimicrobial and chemical agents make them a serious threat and an area of research focus. Antimicrobial resistance and treatment failure are primarily caused by them in clinical settings. Plants are effective and promising sources of anti-biofilm agents.Increased knowledge of plant anti-biofilm agents such as alkaloids, tannins, indole, terpenes, and flavonoids, as well as their mechanisms of action—which include quorum sensing disruption, adhesion inhibition, extracellular polymeric substance modulation, and damage to the integrity of the cell membrane—is the goal of this review. Furthermore, we talked about the present and the future research perspectives of these phytochemicals, including synergism with conventional antibiotics, advanced drug delivery systems like nanocomposites and microencapsulation, and metal complexation in treating and eradicating biofilm-associated infections.

Keywords: Anti-biofilms, plant anti-biofilms, phytochemicals, biofilm inhibition.

1. INTRODUCTION

Microorganisms form biofilms by aggregating and embedding themselves inside a matrix of extracellular polymeric substances (EPS) created by the organism itself, adhering to surfaces and each other [1]. They may consist of a single microorganism or a combination of different species, including yeasts, bacteria, fungus, archaea, and protozoa.The presence of biofilms is a significant obstacle to treating bacterial infections and contributes significantly to the long-lasting nature of these infections [2].Due to their tolerance to external stimuli, the body's immune system, and antibiotics, bacterial biofilms are now a significant contributing factor to worldwide health crises. Biofilms are frequently found on medical devices, human tissue, a variety of industrial surfaces, food processing facilities, and natural environments [3]. Biofilms are thought to be the cause of 80% of persistent microbial illnesses in humans, resulting in higher rates of hospitalization, increased healthcare expenses, and greater mortality and morbidity rates [4]. Biofilms form on non-living surfaces, among other medical gadgets, like cochlear implants, dentures, orthopedic implants, coronary stents, prosthetic heart valves, catheters, neurosurgical implants, and breast implants [5]. Biofilms have gained global recognition in the scientific literature, and continued research has resulted in exploring new questions.Because of the mechanical, physicochemical, microbiological, and medicinal elements of biofilms, different disciplines provide distinct insights: chemists focus on organized molecules, while physicists study thermodynamics,and biologists investigate microbial physiology influencing formation of biofilms and uncovering resistance patterns. But the question of how these elements work together to create the threat posed by biofilms remains a challenge for all. The ongoing need for innovative approaches to combat biofilms and the study of their structure and behavior arises from the distinct characteristics of biofilm colonies in relation to infection[6].

Bacteria's ability to form surface biofilms enables them to evade innate immune responses and undergo metabolic changes within the biofilm. This results in reduced antibiotic penetration and the release of bacterial byproducts or toxins [7,8]. Because of the increased susceptibility to antibiotics and diminishing effectiveness of traditional medications in treating biofilm-related infections, the pharmaceutical and scientific community has

shifted focus to new therapies and anti-biofilm agents [9]. Historically, natural products have provided a wide range of chemical compounds with various biological properties and have been crucial in drug discovery for conditions such as biofilm-associated infections. The ability of bacteria to develop and maintain biofilms can be disrupted by small molecules, which can assist overcome the antibiotic tolerance that biofilms are linked to. These small molecules also hold potential for being used in combination therapies with traditional antibiotics [10]. Drug discovery is greatly interested in natural chemicals produced by bacteria, fungi, plants, and other organisms because of their diverse mechanisms and low drug resistance profiles [11].The existence of bioactive compounds in plant extracts accounts for their antibiofilm activity. These substances are secondary metabolites found in minute quantities in plants, and they can impact the cellular and physiological processes of the animals and people who eat them [12]. Bioactive compounds exhibit antibiofilm effects through various mechanisms based on their physical or chemical structure. These mechanisms may involve targeting quorum sensing (QS), breaking down the extracellular matrix, preventing microbial attachment, and eradicating persister cells [13].This review offers a synopsis of the processes connected to the anti-biofilm properties of plant bioactive compounds, alkaloids, tannins, indole, terpenes, and flavonoids.

2. BIOFILM DEVELOPMENT AND ANTI-BIOFILMS

Biofilms evolve gradually over time, just like other communities. Regardless of the phenotypic of the organism, biofilm development follows a universal five-stage growth cycle that demonstrates shared traits.The attachment phase, or stage 1, is brought on by external cues and can be started in a matter of seconds. These signals differ amongst organisms and include iron, pH, temperature, oxygen levels, osmolality, and changes in nutrition availability and concentration. Because rough surfaces have more surface area and less shear pressures, biofilms are more likely to grow on them. Research suggests that hydrophobic materials, such as Teflon and other plastics, are more conducive to the formation of biofilms than are glass and metal. Some cells separate from the substrate during the first stage of reversible binding. The growth rates of the bacterial cells are logarithmic at stage I. stage II begins soon after Stage 1, and is characterized by irreversible binding. Once attached to the surface of the epithelium, bacteria begin to grow and release signals that allow "intercommunication" between individual cells. The genetic mechanisms that produce exopolysaccharide (EPS) are triggered when the signal intensity exceeds a specific threshold, which allows nutrients and planktonic bacteria to be trapped[14].Cell aggregates start to develop during Stage II, and as the aggregates get progressively stacked, motility starts to decline. The biofilm enters Stage III, often referred to as Maturation I, when its thickness exceeds 10 μm. The biofilm is in Stage IV, or maturation II, when it reaches its maximum thickness, which is often greater than 100 mm.Cell dispersion, which occurs when certain bacteria take on a planktonic phenotype and exit the biofilm, is what defines stage V. This procedure starts a few days following Stage IV[15]. Anti-biofilm agents target any of the biofilm formation stages to prevent biofilm development.

3. PLANT ANTI-BIOFILM AGENTS, TYPES AND MECHANISMS OF ACTION

3.1 Alkaloids

Alkaloids are phyto-secondary metabolites. These natural products are thought to be attractive prospects for drug discovery since they mostly consist of nitrogen-containing basic and heterocyclic molecules. Different bacteria respond differently to alkaloids. 1,3,4-oxadiazole prevents Pseudomonas aeruginosa from producing the toxins pyocyanin and QS signal precursor HHQ [16, 17], 7-hydroxyindoloe alters virulence genes expression and stops swarming motility [17], and solenopsin A prevents the virulence genes transcription process and and they enzyme elastase B synthesis [18]. Alkaloids also have the ability to break down fimbriae and other adhesions that support biofilms production and cell adhesion.

Quinoline- or quinolone-based compounds work against bacteria by undermining the integral conformation of their cell membranes. The antibacterial chemical HT61, which is generated from quinolines, has the ability to depolarize and release the intercellular components at concentrations below and above the minimum inhibitory concentration (MIC) of the drug[19]. Electrostatic interactions link the cationic molecule to negatively charged bilayers, enters the membrane, and induces conformational changes, thereby enhancingcationic molecules and membrane interaction and ultimately causing the cell membrane to depolarize and the loss of cytoplasmic components.

Hordenin, a dietary phyto-substance found in barley, is locally recognized for its antimicrobial effects, inhibition of mono-amine oxidase B, stimulation of gastrin production, and its vaso-constrictive effects [20]. The effects of hordenine had been explored in another to act as both a quorum sensing inhibitor and a catalyst for aminoglycoside antibiotics against *Pseudomonas aeruginosa* PAO1. Their findings revealed that hordenine effectively decreased

the production of acyl-homoserine lactones (AHLs), which in turn led to a reduction in biofilm formation, motility, and various virulence factors like elastase, protease, rhamnolipids, pyoverdine, and pyocyanin. These factors are critical markers of the QS system in *P. aeruginosa*. The research team specifically examined the impact of hordenine on the expression levels of QS-associated genes (lasI, lasR, rhlI, and rhlR) within *P. aeruginosa* PAO1. They realized a notable suppression of all these genes following treatment with hordenine. The significance of these findings lies in its potential as a competitive QS inhibitor, which may finely regulate major virulence determinants in the microorganism studied, so potentially mitigating infections [21].

Rhamno-lipids is a form of glycolipid mediated by the rhl system, they are crucial for surface movement and the initial formation of biofilms. These compounds serve as significant surfactants in bacteria and are a major virulence factor in *P. aeruginosa* [22]. Rhamno-lipids aid in the breakdown of the matrix of biofilm and enhance motility, facilitating the colonization of new areas by the bacteria. Furthermore,rhamnolipids production by *P. aeruginosa* in patients with endotracheal tubes has been linked to the onset of pneumonia [23].

Additionally,alkaloids of other forms have been documented for their anti-biofilm effects, such as caffeine [24] and 7-fluoro indole [25]. Both substances greatly hindered biofilm formation in *P. aeruginosa* and disrupted QS mechanisms by targeting motility, swarming, and multiple virulence factors.

3.2 Tannins

Tannins are complex molecules with relatively high molecular weights, due to their characteristic complex formation with alkaloids, polysaccharides, and polypeptides. They are classified into two primary categories: the hydrolyzable tannins, which are the gallic acid esters, and the condensed tannins, commonly as proanthocyanidins; polymers composed of monomers of polyhydroxyflavan-3-ol [26]. Interaction to cell adhesion receptors is an attributable feature of the tannins. These tannin-cell adhesion receptors interactions can sometimes result in the formation of ion channels within cell membranes, disrupting the electric potential [12].

Proanthocyanidins (PACs), complex molecules predominantly made up of pro-fisetinidin and pro-robinetinidininin *Anadenanthera colubrina* and *Caesalpinia leptophloeos*respectively, are known for inhibiting biofilm adhesions. Likewise, the hydrolyzable tannins, as seen in *Myracrodruonurundeuva*, have been shown to have bacteriostatic and anti-adhesive effects on *Pseudomonas aeruginosa* [27]. Specific tannins such as hamamelitannin also have quorum sensing inhibition effect, specifically by inhibiting RNAIII quorum sensing regulator [28, 29]. Another tannin compound, punicalagin, has demonstrated α-hemolysin inhibition and significantly inhibits biofilm formation [30, 31]. Further research on punicalagin has revealed its action against *Staphylococcus aureus*, where it caused cell membrane damage and induced the efflux of potassium ions. Additionally, it was found that tannic acids can act against the formation of biofilm in *S. aureus* by downregulating the genes responsible for bacterial adhesion, such as agrA, icaA, and icaD.

3.3 Indoles

Indole is a complex aromatic compound, composed of a benzene ring combined with a pyrrole ring. Lee et al., [33] have noted that derivatives of indole are widespread in prokaryotes and eukaryotes, yet the precise mechanisms by which these compounds operate remain unclear [33]. Indole is produced by as many as 85 species or more of gram-positive and gram-negative bacteria, utilizing it for various signaling purposes [34]. Beyond its recognized roles in fighting cancer, inflammation, and microbial infections, indole also plays a part in biofilm formation, stress adaptation, pathogenicity, the shift from growth to stationary phases, and interaction between gut bacteria and their animal hosts [35, 36].

Numerous bacterial species, including the gram-positive and gram-negative, like the *Escherichia coli*, generate indoles that function as signaling molecules for communication within and across species. These indoles have significant effects on various bacterial behaviors and immune responses in eukaryotes [33]. In particular, indole has been observed to influence the formation of biofilms and persister cells in *Escherichia coli* [33, 37]. In a study by Monte et al. [38], the antibacterial properties of some selected phytochemicals were tested against both planktonic and biofilm forms of *S. aureus* and *E. coli*. The study also explored the possible synergistic effects of these phytochemicals when combined with three different antibiotics. The results indicated that 7-HC and 13C were particularly potent against *S. aureus* and *E. coli*, significantly interfering with cell communication and biofilm regulation by altering motility and quorum sensing [38]. However, none of the phytochemicals managed to completely eliminate the biofilms.

In a similar study, various derivatives of indole were screened to identify new compounds capable of inhibiting persister cell and biofilm formation in *S. aureus* and *E. coli*. They found that halogenated indoles were effective in eliminating persister formation of cells in both bacterial species. Of all the halogenated indoles, 5-iodoindole was the most effective inhibiting formation of biofilms. It not only prevented the formation of persister cells but also reduced the production of staphyloxanthin, a carotenoid that helps *S. aureus* evade the immune system. This reduction in staphyloxanthin production yielded a decrease in the strain's virulence factor production [33].

Kemp *et al.* [39] validated the potential of indole in quorum-sensing inhibition. They specifically examined the use of indolederivatives , such as indole-3-carboxaldehyde (ICA), in QS inhibition in *E. coli*. Their study explored the use of bromination to enhance the QSI activity of indole carboxaldehyde, demonstrating a novel approach to modulate quorum-sensing-mediated behaviors in bacteria.

3.4 Terpenes

Terpenes, or terpenoids, are the most abundant and diverse natural substances in plants and animals [40, 41]. Terpenes are hydrocarbon secondary metabolites made up of 5-carbon isoprene units linked together [42]. They are a significant constituent of plant essential oils and are found mainly in tea plants. Based on the number of carbon atoms and isoprene units, they could be classified as hemiterpenes (5 carbons), monoterpenes (10 carbons), sesquiterpenes (15 carbons), diterpenes (20 carbons), triterpenes (30 carbons), tetraterpenes (40 carbons), and polyterpenes (more than 40 carbons) [43]. Linalool, nerol, isopulegol, menthol, carvone, α-thujone, farnesol, citral, eucalyptol, and limonene are some examples of terpenes [44]. Terpenes have been the subject of numerous studies, revealing their diverse applications as anti-tubercular, anti-diabetic, anti-malarial, antiviral, and antibacterial agents, among others [44-49]. Cox-Georgian *et al.*, [41] found that terpenes' antimicrobial activity depends on their oxygenated status rather than their hydrogen atoms. Several studies on terpenes and their antibiofilm activity exist with various mechanisms [50, 52]. Terpenes are reported to interfere with biofilm formation through anti-quorum sensing, cell adhesion inhibition, and cell membrane disruption [44, 53].

Quorum sensing is a cell-to-cell communication that occurs in biofilms, and it enables them to coordinate their behavior and function as a collective. This communication system plays a crucial role in biofilm formation and disrupting it can prevent or reduce biofilm growth [54, 55]. Terpenes have been found to interfere with quorum sensing, thereby inhibiting biofilm formation. The triterpenoids of Inula extract inhibited the formation of *C. violaceum* biofilm through anti-quorum sensing [53]. Carvacrol downregulated speB, srtB, luxS, covS, dltA, ciaH, and hasA genes that play a role in quorum sensing [56]. In Staph aureus, 4 mg mL−1 of carvacrol downregulated the Quorum sensing Agr genes in the quorum system [57]. Farnesol, a sesquiterpene, interfered with the quorum sensing of *Candida spp* and *Pseudomonasaeruginosa*, thereby reducing biofilm formation. Terpinen-4-ol reduced the expression of lasI, lasR, rhlI, rhlR, rhlAB, lasB, aprA, toxA, and plcH in *Pseudomonas aeruginosa* [58].

Terpenes reduce the formation of extra polymeric substances in *Salmonella* biofilms. Cellulose is a major component of *Salmonella* EPS, and terpenes prevent cellulose synthesis by inhibiting the enzyme, glycosyltransferase responsible for cellulose synthesis [49].

The Extracellular Polymeric Substances (EPS) of *Escherichia coli* biofilms aid their maintenance and persistence. They comprise glucans, cellulose, colonic acid, and poly-B-1,6-N-acetyl-glucosamine, with cellulose as the primary component [59]. Gao *et al.* [47] studied the efficacy of lemongrass essential oil and citral, its bioactive component against mixed species biofilms of *Staphylococcus aureus* and *Candida species.* They reported that the extracted citral and geraniol inhibited glucan formation of Escherichia coli biofilms, by inhibiting the enzyme glucosyltransferase responsible for glucan formation, ultimately reducing biofilm formation and growth.

The adhesion phase of biofilm formation requires metabolic activity and could serve as a target for anti-biofilm agents. The metabolic (respiratory) activity of biofilms was inhibited by essential oil from Oregano and thyme comprising majorly thymol and carvacrol, thereby reducing *Salmonellaenteritidis* biofilm adhesion and growth [60]. Carvacrol, cinnamaldehyde, and thymol inhibited the metabolic activity of the adhesion phase of *Candida* biofilms and reduced their biomass. [61].

Salinas *et al.* [62], in their investigation into the effect of individual and combined terpenes on *Staphylococcus aureus* biofilms, found that, while all terpene combinations could disrupt biofilm formation, the combination of (--)-trans-Caryophyllene and Linalool at 500 µg/mL produced an 88%inhibition. This combination notably interfered with

the initial adhesion and quorum sensing processes of *Staphylococcus aureus* by reducingsdr, spa, agr, and hld gene expressions.

In a study by Khammassi*et al*.[63], sesquiterpenes and oxygenated terpenes were the major constituents of *Eucalyptus occidentalis, E. striaticalyx and E. stricklandii*, and Eucalyptol was the major terpene present. These terpenes inhibited the adhesion of biofilms of *Acinetobacterbaumanni* and *Staphylococcus aureus*.

3.5 Flavonoids

Flavonoids are natural products widely found in various plant-based foods, including vegetables, fruits, and commonly consumed drinks. They are usually constituents of flower pigments but are also found in other parts of the plant [64]. They are responsible for plant colour, fragrance, and flavor. They comprise a 15-carbon skeleton in a three-membered ring comprising two benzene rings (A and B) connected by a pyran ring (C). [65]. They are classified into isoflavones, neoflavones, flavones, flavonols, flavanones, flavanonols, flavanols (catechins), anthocyanins, and chalcones based on the C-ring carbon that attaches to the B ring, and unsaturation and oxidation of the C ring [64]. Apigenin, propolis, quercetin, and kaempferol are examples of flavonoids. Flavonoids possess antioxidant, anti-inflammatory, anticancer, and antimicrobial effects, among others [66-68].

Flavonoids have been reported to possess antibiofilm activity [69, 70]. They are reported to have anti-amyloid effects, degrade extracellular matrices, and disrupt cell membrane integrity [71]. In a study by Bouchelaghem *et al*. [69], the ethanolic extracts of Hungarian propolis degraded the biofilm of MRSA clinical studies. The study also showed that the extract inactivated *S. aureus* metabolism within strains, ultimately leading to cell death. Flavonoids also exhibit antibiofilm activity by interfering with the extracellular matrix. Matilla-Cuenca *et al*. [72] determined the antibiofilm activity of quercetin, myricetin, and scutellarein on the amyloid protein Bap, which is a component of coagulase-negative staphylococci and certain*S.aureus* strains. The matrix of *S. aureus* biofilms is composed of a diverse array of molecules, including exopolysaccharides, proteins that attach to the surface, extracellular DNA and amyloid fibers all of which interact to provide structural integrity [73]. This study revealed that flavonoids suppressed the ability of *S. aureus* to form biofilms via the Bap pathway, without affecting the genes involved in this process.

Raorane*et al.* [74], investigated the antibiofilm activities of 12 flavonoids against *Acinetobacter boumanni* biofilm, and found that Fisetin, phloretin, and curcumin efficiently reduced the biofilm formation. Curcumin was the most active and inhibited biofilm formation at low concentrations by blocking the biofilm response regulator, BfmR of *Acinetobacter boumanni*.

Flavonoids also cause membrane disruption. Flavonoids extracted from the jujube fruit reduced the thickness of *S. aureus* biofilms, damaging their 3D structures and eventually interfering with biofilm maturation. The exopolysaccharides were cleared, leaving the bacteria exposed [75].

Pruteanu *et al.* [76], explored the activity of major plant flavonoids on macrocolonies and submerged biofilms of *E. coli, P. aeruginosa,* and *B. subtilis*; they found that while the submerged biofilm of *Escherichia coli* was unaffected, the extracellular matrix of the macrocolonieswas strongly reduced by luteolin, myricetin, morin, and quercetin. This interference was reported to occur through inhibiting amyloid curli fibres assembly and cellulose production, which happened through unknown mechanisms. Interestingly, the same flavonoids had the opposite effect on *Pseudomonas aeruginosa*, enhancing the formation of both macrocolonies and submerged biofilms, suggesting that the anti-biofilm properties of plant flavonoids are species- specific and not general.

In some bacteria, for example, *Escherichia coli,* cellulose exists a phosphoethanolaminederivative,pEtN-Cellulose, which could be a target for antibiofilm agents [77]. Hengge [71] reports that the catechin epigallocatechin-3-gallate inhibits bacteria biofilms that utilize amyloid fibers and pEtN-cellulose as major extracellular matrix components, which has been studied extensively in *Escherichia coli*. It was found that epigallocatechin-3-gallate eliminated the entire curli fibers and pEtN-cellulose of the extracellular matrix and activated the cell stress response pathway mediated by RpoE, inducing small perforations on the cell surface, ultimately leading to cell envelope damage.

4. CONCLUSION AND FUTURE PERSPECTIVES

Plant phytochemicals hold great promise as anti-biofilm agents, as several studies have found that they inhibit biofilm formation; some have also been effective against mature biofilms. Unlike conventional anti-biofilm agents, they have the advantage of low resistance occurrence [78]. Some studies even found that plant-based anti-biofilms reversed the resistance profile of certain bacterial biofilms. Future studies should focus on determining the optimal

solvent for bioactive extraction as some solvents corresponded with a higher volume of bioactive. Additionally, standardization of plant bioactive should be determined as some extracts exhibited varying activities against biofilms depending on their concentrations; at low concentrations, biofilm formation was induced, whereas a higher extract concentration inhibited biofilm concentration [62]. While many studies focused on their effectiveness, less is known about the pharmacokinetics, safety, and toxicological profiles of plant bioactives.

Advanced delivery systems such as nanoparticles, hydrogels, microencapsulation, and coating are current research areas and should be explored further. Microencapsulation of bioactive substances from plants is another promising area; this helps to improve their stability, reduce toxicity, and improve antibiofilm activity. The encapsulation of carvacrol and thymol improved solubility and antibacterial activity against pathogenic bacteria while reducing the amount of carvacrol and thymol needed [79, 80].

Furthermore, the synergistic effect of conventional antimicrobials and plant bioactives is a vital focus. At concentrations that do not affect bacterial growth or survival, plant anti-biofilm agents target and disrupt biofilm-associated components or regulatory mechanisms, preventing biofilm formation or eradicating existing biofilms [81, 82]. Therefore, they can be combined with antibiotics. Some studies found an enhanced antibiofilm activity with a combination of conventional antimicrobial and plant bioactives compared to when used alone. Focusing on identifying synergistic pairs, determining optimal dosing regimens, and understanding their mechanisms could lead to developing combination therapies that are more effective against biofilm-associated infections.

REFERENCES

1.Jiang Y, Geng M, Bai L. Targeting biofilms therapy: current research strategies and development hurdles. Microorganisms. 2020 Aug 11;8(8):1222.

2.Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. Frontiers in cellular and infection microbiology. 2023 Apr 6;13:1137947.

3.Schulze A, Mitterer F, Pombo JP, Schild S. Biofilms by bacterial human pathogens: Clinical relevance-development, composition and regulation-therapeutical strategies. Microbial Cell. 2021 Feb 2;8(2):28.

4.Römling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. Journal of internal medicine. 2012 Dec;272(6):541-61.

5. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. Journal of medical microbiology. 2015 Apr;64(4):323-34.

6.Battin TJ, Sloan WT, Kjelleberg S, Daims H, Head IM, Curtis TP, Eberl L. Microbial landscapes: new paths to biofilm research. Nature Reviews Microbiology. 2007 Jan 1;5(1):76-81.

7.Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., &Aalinkeel, R. (2023). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. *Microorganisms*, *11*(6), 1614.https://doi.org/10.3390/microorganisms11061614

8.Rather, M. A., Gupta, K., & Mandal, M. (2021). Microbial biofilm: Formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology: [Publication of the Brazilian Society for Microbiology]*, *52*(4), 1701–1718.https://doi.org/10.1007/s42770-021-00624-x

9. Singh, A., Amod, A., Pandey, P., Bose, P., Pingali, M. S., Shivalkar, S., Varadwaj, P. K., Sahoo, A. K., & Samanta, S. K. (2022). Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies. *Biomedical Materials*, *17*(2), 022003.https://doi.org/10.1088/1748-605X/ac50f6

10. Melander RJ, Basak AK, Melander C. Natural products as inspiration for the development of bacterial antibiofilm agents. Natural product reports. 2020;37(11):1454-77.

11.Álvarez-Martínez, F. J., Barrajón-Catalán, E., & Micol, V. (2020). Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. *Biomedicines*, *8*(10), 405.https://doi.org/10.3390/biomedicines8100405

12.Lahiri D, Dash S, Dutta R, Nag M. Elucidating the effect of anti-biofilm activity of bioactive compounds extracted from plants. Journal of biosciences. 2019 Jun;44(2):52.

13.Mishra, R., Panda, A. K., De Mandal, S., Shakeel, M., Bisht, S. S., & Khan, J. (2020). Natural Anti-biofilm Agents: Strategies to Control Biofilm-Forming Pathogens. *Frontiers in Microbiology*, *11*, 566325.https://doi.org/10.3389/fmicb.2020.566325

14.Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. science. 1999 May 21;284(5418):1318-22.

15.John GT, Donale CL. Biofilms: architects of disease. Textbook of diagnostic microbiology. 3rd ed. Saunders. 2007:884-95.

16.Morkunas B, Gal B, Galloway WR, Hodgkinson JT, Ibbeson BM, Tan YS, Welch M, Spring DR. Discovery of an inhibitor of the production of the Pseudomonas aeruginosa virulence factor pyocyanin in wild-type cells. Beilstein journal of organic chemistry. 2016 Jul 11;12(1):1428-33.

17.Lee J, Attila C, Cirillo SL, Cirillo JD, Wood TK. Indole and 7- hydroxyindole diminish Pseudomonas aeruginosa virulence. Microbial biotechnology. 2009 Jan;2(1):75-90.

18.Park J, Kaufmann GF, Bowen JP, Arbiser JL, Janda KD. Solenopsin A, a venom alkaloid from the fire ant Solenopsisinvicta, inhibits quorum-sensing signaling in Pseudomonas aeruginosa. Journal of Infectious Diseases. 2008 Oct 15;198(8):1198-201.

19.Hubbard AT, Barker R, Rehal R, Vandera KK, Harvey RD, Coates AR. Mechanism of action of a membrane-active quinoline-based antimicrobial on natural and model bacterial membranes. Biochemistry. 2017 Feb 28;56(8):1163-74.

20.Hapke HJ, Strathmann W. Pharmacological effects of hordenine. DTW. Deutsche TierarztlicheWochenschrift. 1995 Jun 1;102(6):228-32.

21.Zhou, J.W.; Luo, H.Z.; Jiang, H.; Jian, T.K.; Chen, Z.Q.; Jia, A.Q. Hordenine: A Novel Quorum Sensing Inhibitor and Anti-biofilm Agent against Pseudomonas aeruginosa. J. Agric. Food Chem. 2018, 66, 1620–1628.

22.O'May C, Tufenkji N. The swarming motility of Pseudomonas aeruginosa is blocked by cranberry proanthocyanidins and other tannin-containing materials. Applied and environmental microbiology. 2011 May 1;77(9):3061-7.

23.Köhler T, Guanella R, Carlet J, van Delden C. Quorum sensing-dependent virulence during Pseudomonas aeruginosa colonisation and pneumonia in mechanically ventilated patients. Thorax. 2010 Aug 1;65(8):703-10.

24.Chakraborty P, Dastidar DG, Paul P, Dutta S, Basu D, Sharma SR, Basu S, Sarker RK, Sen A, Sarkar A, Tribedi P. Inhibition of biofilm formation of Pseudomonas aeruginosa by caffeine: a potential approach for sustainable management of biofilm. Archives of microbiology. 2020 Apr;202:623-35.

25.Lee JH, Kim YG, Cho MH, Kim JA, Lee J. 7-fluoroindole as an antivirulence compound against Pseudomonas aeruginosa. FEMS microbiology letters. 2012 Apr 1;329(1):36-44.

26.Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. International journal of molecular sciences. 2007 Sep 12;8(9):950-88.

27.Mierziak J, Kostyn K, Kulma A. Flavonoids as important molecules of plant interactions with the environment. Molecules. 2014 Oct 10;19(10):16240-65.

28.Vermote A, Brackman G, Risseeuw MD, Vanhoutte B, Cos P, Van Hecke K, Breyne K, Meyer E, Coenye T, Van Calenbergh S. Hamamelitannin analogues that modulate quorum sensing as potentiators of antibiotics against Staphylococcus aureus. AngewandteChemie International Edition. 2016 May 23;55(22):6551-5.

29.Kiran MD, Adikesavan NV, Cirioni O, Giacometti A, Silvestri C, Scalise G, Ghiselli R, Saba V, Orlando F, Shoham M, Balaban N. Discovery of a quorum-sensing inhibitor of drug-resistant staphylococcal infections by structure-based virtual screening. Molecular pharmacology. 2008 May 1;73(5):1578-86.

30.Mun SH, Kong R, Seo YS, Zhou T, Kang OH, Shin DW, Kwon DY. Subinhibitory concentrations of punicalagin reduces expression of virulence-related exoproteins by Staphylococcus aureus. FEMS microbiology letters. 2016 Nov;363(22):fnw253.

31.Xu Y, Shi C, Wu Q, Zheng Z, Liu P, Li G, Peng X, Xia X. Antimicrobial activity of punicalagin against Staphylococcus aureus and its effect on biofilm formation. Foodborne pathogens and disease. 2017 May 1;14(5):282-7.

32.Lee BH, Cole S, Badel-Berchoux S, Guillier L, Felix B, Krezdorn N, Hébraud M, Bernardi T, Sultan I, Piveteau P. Biofilm formation of Listeria monocytogenes strains under food processing environments and pan-genome-wide association study. Frontiers in microbiology. 2019 Nov 21;10:2698.

33.Lee JH, Kim YG, Gwon G, Wood TK, Lee J. Halogenated indoles eradicate bacterial persister cells and biofilms. Amb Express. 2016 Dec;6:1-2.

34.Chimerel C, Field CM, Piñero-Fernandez S, Keyser UF, Summers DK. Indole prevents Escherichia coli cell division by modulating membrane potential. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2012 Jul 1;1818(7):1590-4.

35.El-Sawy ER, Bassyouni FA, Abu-Bakr SH, Rady HM, Abdlla MM. Synthesis and biological activity of some new 1-benzyl and 1-benzoyl 3-heterocyclic indole derivatives. Acta pharmaceutica. 2010 Mar 1;60(1):55-71.

36.Fiester SE, Actis LA. Stress responses in the opportunistic pathogen Acinetobacter baumannii. Future microbiology. 2013 Mar 1;8(3):353-65.

37.Kim SK, Park HY, Lee JH. Anthranilate deteriorates the structure of Pseudomonas aeruginosa biofilms and antagonizes the biofilm-enhancing indole effect. Applied and environmental microbiology. 2015 Apr 1;81(7):2328-38.

38.Monte J, Abreu AC, Borges A, Simões LC, Simões M. Antimicrobial activity of selected phytochemicals against Escherichia coli and Staphylococcus aureus and their biofilms. Pathogens. 2014 Jun 18;3(2):473-98.

39.Kemp CA, McCullough DK, Bialonska D, Johnson PJ. Effect of bromination on the quorum sensing-inhibiting properties of indole-3-carboxaldehydes in Chromobacteriumviolaceum AHL system. Microbiology Research. 2021 Apr 12;12(2):376-82.

40.Cappiello F, Loffredo MR, Del Plato C, Cammarone S, Casciaro B, Quaglio D, *et al.* The Revaluation of Plant-Derived Terpenes to Fight Antibiotic-Resistant Infections. Antibiotics. 2020 Jun 13;9(6):325.

41.Cox-Georgian, Ramadoss N, Dona C, Basu C. Therapeutic and Medicinal Uses of Terpenes. Medicinal Plants. 2019;333.

42.Mabou FD, Yossa IBN. TERPENES : structural classification and biological activities. 2021;

43.Perveen S, Mohammad Al-Taweel A, editors. Terpenes and Terpenoids - Recent Advances [Internet]. IntechOpen; 2021 [cited 2024 Jul 29]. (Biochemistry; vol. 21). Available from:https://www.intechopen.com/books/terpenes-and-terpenoids-recent-advances

44.Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, *et al.* Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. Molecules. 2019 Jul 5;24(13):2471.

45.Aelenei P, Rimbu CM, Guguianu E, Dimitriu G, Aprotosoaie AC, Brebu M, *et al.* Coriander essential oil and linalool – interactions with antibiotics against Gram-positive and Gram-negative bacteria. Letters in Applied Microbiology. 2019;68(2):156–64.

46.Cáceres M, Hidalgo W, Stashenko E, Torres R, Ortiz C. Essential Oils of Aromatic Plants with Antibacterial, Anti-Biofilm and Anti-Quorum Sensing Activities against Pathogenic Bacteria. Antibiotics. 2020 Mar 30;9(4):147.

47.Gao S, Liu G, Li J, Chen J, Li L, Li Z, *et al.* Antimicrobial Activity of Lemongrass Essential Oil (Cymbopogon flexuosus) and Its Active Component Citral Against Dual-Species Biofilms of Staphylococcus aureus and Candida Species. Frontiers in Cellular and Infection Microbiology [Internet]. 2020;10. Available from:https://www.scopus.com/inward/record.uri?eid=2-s2.0-85099023148&doi=10.3389%2ffcimb.2020.603858&partnerID=40&md5=967d1f1a7e4a891833daf17c4f7b9648

48.Kumar Dash D, Kishore Tyagi C, Kumar Sahu A, Tripathi V. Revisiting the Medicinal Value of Terpenes and Terpenoids. In: Singh Meena V, Prasad Parewa H, Kumari Meena S, editors. Revisiting Plant Biostimulants [Internet]. IntechOpen; 2022 [cited 2024 Jul 29]. Available from:https://www.intechopen.com/chapters/80481

49.Palomares-Navarro JJ, Bernal-Mercado AT, González-Aguilar GA, Ortega-Ramirez LA, Martínez-Téllez MA, Ayala-Zavala JF. Antibiofilm Action of Plant Terpenes in Salmonella Strains: Potential Inhibitors of the Synthesis of Extracellular Polymeric Substances. Pathogens. 2022 Dec 26;12(1):35.

50.Centorbi HJ, Aliendro OE, Mattana CM. Effect of thymol and environmental factors on growth and biofilm formation by Listeria monocytogenes. BoletinLatinoamericano y del Caribe de PlantasMedicinales y Aromaticas. 2019;18(4):411–24.

51.Cordeiro L, Figueiredo P, Souza H, Sousa A, Andrade-Júnior F, Medeiros D, *et al.* Terpinen-4-ol as an antibacterial and antibiofilm agent against staphylococcus aureus. International Journal of Molecular Sciences. 2020;21(12):1–14.

52.Sá Firmino NC, Alexandre FSO, de Vasconcelos MA, Pinheiro AA, Arruda FVS, Guedes MLS, *et al.* Diterpenes isolated from Croton blanchetianusBaill: Potential compounds in prevention and control of the oral Streptococci biofilms. Industrial Crops and Products. 2019;131:371–7.

53.Dimitrova PD, Ivanova V, Trendafilova A, Paunova-Krasteva T. Anti-Biofilm and Anti-Quorum-Sensing Activity of Inula Extracts: A Strategy for Modulating Chromobacteriumviolaceum Virulence Factors. Pharmaceuticals. 2024 Apr 30;17(5):573.

54.Annous BA, Fratamico PM, Smith JL. Scientific Status Summary: Quorum Sensing in Biofilms: Why Bacteria Behave the Way They Do. Journal of Food Science [Internet]. 2009 Jan [cited 2024 Jul 31];74(1). Available from:https://ift.onlinelibrary.wiley.com/doi/10.1111/j.1750-3841.2008.01022.x

55.Parsek MR, Greenberg EP. Sociomicrobiology: the connections between quorum sensing and biofilms. Trends in Microbiology. 2005 Jan;13(1):27–33.

56.Wijesundara NM, Lee SF, Rupasinghe HPV. Carvacrol inhibits Streptococcus pyogenes biofilms by suppressing the expression of genes associated with quorum-sensing and reducing cell surface hydrophobicity. Microbial Pathogenesis. 2022 Aug;169:105684.

57.Peng Q, Tang X, Dong W, Zhi Z, Zhong T, Lin S, *et al.* Carvacrol inhibits bacterial polysaccharide intracellular adhesin synthesis and biofilm formation of mucoid *Staphylococcus aureus* : an *in vitro* and *in vivo* study. RSC Adv. 2023;13(41):28743–52.

58.Bose SK, Chauhan M, Dhingra N, Chhibber S, Harjai K. Terpinen-4-ol attenuates quorum sensing regulated virulence factors and biofilm formation in Pseudomonas aeruginosa. Future Microbiology. 2020;15(2):127–42.

59.Beloin C, Roux A, Ghigo JM. Escherichia coli Biofilms. In: Romeo T, editor. Bacterial Biofilms [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2008 [cited 2024 Jul 29]. p. 249–89. (Compans RW, Cooper MD, Honjo T, Koprowski H, Melchers F, Oldstone MBA, *et al.*, editors. Current Topics in Microbiology and Immunology; vol. 322). Available from:http://link.springer.com/10.1007/978-3-540-75418-3_12

60.Čabarkapa I, Čolović R, Đuragić O, Popović S, Kokić B, Milanov D, *et al.* Anti-biofilm activities of essential oils rich in carvacrol and thymol against Salmonella Enteritidis. Biofouling. 2019;35(3):361–75.

61.Miranda-Cadena K, Marcos-Arias C, Mateo E, Aguirre-Urizar JM, Quindós G, Eraso E. In vitro activities of carvacrol, cinnamaldehyde and thymol against Candida biofilms. Biomedicine & Pharmacotherapy. 2021 Nov;143:112218.

62.Salinas C, Florentín G, Rodríguez F, Alvarenga N, Guillén R. Terpenes Combinations Inhibit Biofilm Formation in Staphyloccocus aureus by Interfering with Initial Adhesion. Microorganisms. 2022 Jul 28;10(8):1527.

63.Khammassi M, Polito F, Amri I, Khedhri S, Hamrouni L, Nazzaro F, *et al.* Chemical Composition and Phytotoxic, Antibacterial and Antibiofilm Activity of the Essential Oils of Eucalyptus occidentalis, E. striaticalyx and E. stricklandii. Molecules [Internet]. 2022;27(18). Available from:https://www.scopus.com/inward/record.uri?eid=2-s2.0-85138433014&doi=10.3390%2fmolecules27185820&partnerID=40&md5=b61a590192c7fdfc4e61feb807569de7

64.Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.

65.Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chemistry. 2022 Jul;383:132531.

66.Bylka W, Matławska I, Pilewski N. Natural flavonoids as antimicrobial agents. JANA. 2003 Nov 30;7.

67.Ekalu A, Habila JD. Flavonoids: isolation, characterization, and health benefits. Beni-Suef Univ J Basic Appl Sci. 2020 Dec;9(1):45.

68.Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019 Feb;18(1):241–72.

69.Bouchelaghem S, Das S, Naorem RS, Czuni L, Papp G, Kocsis M. Evaluation of Total Phenolic and Flavonoid Contents, Antibacterial and Antibiofilm Activities of Hungarian Propolis Ethanolic Extract against Staphylococcus aureus. Molecules. 2022 Jan 17;27(2):574.

70.Faleye OS, Lee JH, Lee J. Selected flavonoids exhibit antibiofilm and antibacterial effects against Vibrio by disrupting membrane integrity, virulence and metabolic activities. Biofilm. 2023 Dec;6:100165.

71.Hengge R. Targeting Bacterial Biofilms by the Green Tea Polyphenol EGCG. Molecules. 2019 Jun 29;24(13):2403.

72.Matilla-Cuenca, L., Gil, C., Cuesta, S., Rapún-Araiz, B., Žiemytė, M., Mira, A., Lasa, I., & Valle, J. (2020). Antibiofilm activity of flavonoids on staphylococcal biofilms through targeting BAP amyloids. *Scientific Reports*, *10*(1), 18968.https://doi.org/10.1038/s41598-020-75929-2

73.Karygianni L, Ren Z, Koo H, Thurnheer T. Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. Trends in Microbiology. 2020 Aug;28(8):668–81.

74.Raorane CJ, Lee JH, Kim YG, Rajasekharan SK, García-Contreras R, Lee J. Antibiofilm and Antivirulence Efficacies of Flavonoids and Curcumin Against Acinetobacter baumannii. Front Microbiol. 2019 May 8;10:990.

75.Miao W, Sheng L, Yang T, Wu G, Zhang M, Sun J, *et al.* The impact of flavonoids-rich Ziziphus jujuba Mill. Extract on Staphylococcus aureus biofilm formation. BMC Complement Med Ther. 2020 Dec;20(1):187.

76.Pruteanu M, Hernández Lobato JI, Stach T, Hengge R. Common plant flavonoids prevent the assembly of amyloid curli fibres and can interfere with bacterial biofilm formation. Environmental Microbiology. 2020 Dec;22(12):5280–99.

77.Thongsomboon W, Serra DO, Possling A, Hadjineophytou C, Hengge R, Cegelski L. Phosphoethanolamine cellulose: A naturally produced chemically modified cellulose. Science. 2018 Jan 19;359(6373):334–8.

78.Borges A, Abreu A, Dias C, Saavedra M, Borges F, Simões M. New Perspectives on the Use of Phytochemicals as an Emergent Strategy to Control Bacterial Infections Including Biofilms. Molecules. 2016 Jul 5;21(7):877.

79.Hajibonabi A, Yekani M, Sharifi S, Nahad JS, Dizaj SM, Memar MY. Antimicrobial activity of nanoformulations of carvacrol and thymol: New trend and applications. OpenNano. 2023 Sep;13:100170.

80.Mechmechani S, Gharsallaoui A, Fadel A, El Omari K, Khelissa S, Hamze M, *et al.* Microencapsulation of carvacrol as an efficient tool to fight Pseudomonas aeruginosa and Enterococcus faecalis biofilms. Pileggi M, editor. PLoS ONE. 2022 Jul 1;17(7):e0270200.

81.Richards JJ, Melander C. Controlling bacterial biofilms. Chembiochem. 2009 Sep 21;10(14):2287–94.

82.Stewart PS. Prospects for Anti-Biofilm Pharmaceuticals. Pharmaceuticals (Basel). 2015 Aug 27;8(3):504–11.