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Plant-derived Bioactive Compounds and Their Mechanistic Roles in Combating Microbial Biofilms

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Biofilms are a group of microorganisms that exist on living or non-living surfaces, embedded within extracellular matrices produced by microbial cells. They primarily cause antimicrobial resistance and treatment failure in clinical settings. Biofilms resist conventional antimicrobials because of their polymicrobial nature, ability to evade host immune detection, and increased tolerance to antimicrobial agents. Consequently, considerable attention is given to finding alternative anti-biofilm agents. Medicinal plants contain diverse biologically active compounds reported to possess antimicrobial and anti-biofilm activities. This review elucidates the mechanisms of action of plant-derived bioactive compounds (alkaloids, tannins, indoles, terpenes, and flavonoids) on *in vitro* microbial biofilms, shedding light on their ability to disrupt and prevent biofilm formation. Additionally, the review emphasizes current and future research directions for these phytochemicals, including synergism with conventional antibiotics and advanced drug delivery systems for treating and eradicating biofilm-associated infections.

Keywords: Microbial biofilms; 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 organisms, adhering to surfaces and each other [1]. They may consist of a single microorganism or a combination of different species, including yeasts, bacteria, fungi, 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]. Owing 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 cause 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 the formation of biofilms and uncovering resistance patterns. However, the question of how these agents 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 assist in overcoming the antibiotic tolerance that biofilms are linked to.

These small molecules could be used in combination therapies with traditional antibiotics [10]. Drug discovery scientists are 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 organism's phenotype, 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 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 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 I, and is characterized by irreversible binding. Once attached to the surface of the epithelium, the 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

The antibiofilm activity of plant extracts is due to the presence of bioactive compounds, which are plant secondary metabolites present in minute amounts, and can influence the physiological and cellular activities of animals and humans that consume them. These bioactive compounds are classified into various types depending on their various functions and structures [83].

Alkaloids are basic plant secondary metabolites mainly consisting of nitrogen-containing heterocyclic molecules. They are attractive prospects for drug discovery because different organisms respond differently to them. 1,3,4-oxadiazole prevents *Pseudomonas aeruginosa* from producing the toxin pyocyanin and quorum-sensing (QS) signal precursor HHQ [16,17]. 7-hydroxyindole alters virulence genes expressions and stops swarming motility [17], and solenopsin A prevents the virulence gene transcription process and the synthesis of the enzyme elastase B [18]. Alkaloids can break down fimbriae and other adhesion molecules that support biofilm 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, can 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, enter the membrane, and induce conformational changes, thereby enhancing cationic molecules and membrane interaction. Consequently, this interaction causes depolarization of the cell membrane and loss of cytoplasmic components.

Hordenine, a dietary phyto-substance found in barley, is locally recognized for its antimicrobial effects, inhibition of monoamine oxidase B, stimulation of gastrin production, and vaso-constrictive effects [20]. Hordenine acted as both a quorum sensing inhibitor and a catalyst for aminoglycoside antibiotics against *Pseudomonas*

aeruginosa PAO1. It 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, potentially mitigating infections [21].

Rhamnolipids are a form of glycolipids mediated by the rhl system, and are crucial for surface movement and initial formation of biofilms. These compounds serve as significant surfactants in bacteria and are a major virulence factor in *P. aeruginosa* [22]. Rhamnolipids aid in the breakdown of the biofilm matrix 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, such as caffeine [24] and 7-fluoro indole [25], have been documented for their anti-biofilm effects. 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 because they form complexes 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 interactions can sometimes form ion channels within cell membranes, disrupting the electric potential [12].

Proanthocyanidins (PACs), complex molecules predominantly made up of pro-fisetinidin and pro-robinetinidinin in *Anadenanthera colubrina* and *Caesalpinia leptophloeos* respectively, are known for inhibiting biofilm adhesions. Likewise, the hydrolyzable tannins, as seen in *Myracrodruon urundeuva*, have been shown to have bacteriostatic and anti-adhesive effects on *Pseudomonas aeruginosa* [27]. Specific tannins such as hamamelitannin also have a 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 *S. aureus* biofilms by downregulating the genes responsible for bacterial adhesion, such as agrA, icaA, and icaD [32].

3.3 Indoles

Indole is a complex aromatic compound composed of a benzene ring combined with a pyrrole ring. Lee et al., [33] noted that indole derivatives 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 [35,36].

Numerous bacterial species, including the gram-positive and gram-negative, like *Escherichia coli*, generate indoles that function as signaling molecules for communication within and across species. These indoles have considerable effects on various bacterial behaviors and immune responses in eukaryotes [33]. 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 eliminated 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 biofilm formation. It prevented the formation of persister cells and reduced the production of staphyloxanthin, a carotenoid that helps *S. aureus* evade the immune system. This reduction in staphyloxanthin production decreased the strain's virulence factor production [33].

Kemp et al. [39] validated the potential of indole in quorum-sensing inhibition. They examined indole derivatives, 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 major constituent of plant essential oils mainly found 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 [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 *S. 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 *Pseudomonas aeruginosa*, 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 mainly thymol and carvacrol, thereby reducing *Salmonella enteritidis* 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 reducing sdr, 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 *Acinetobacter baumanni* 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 [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. The matrix of *S. aureus* biofilms is composed of diverse molecules, including exopolysaccharides, proteins that attach to the surface, extracellular DNA and amyloid fibers, all of which interact to provide structural integrity [72]. Matilla-Cuenca et al. [73] determined the antibiofilm activity of quercetin, myricetin, and scutellarin on the amyloid protein Bap, which is a component of coagulase-negative staphylococci and certain *S. aureus* strains. 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 baumannii* biofilm, and found that Fisetin, phloretin, and curcumin efficiently reduced the biofilm formation. Curcumin was the most active, inhibiting biofilm formation at low concentrations and blocking the biofilm response regulator, BfmR of *Acinetobacter baumannii*.

Flavonoids also cause membrane disruption. Flavonoids extracted from the jujube fruit reduced the thickness of *S. aureus* biofilms by damaging their 3D structures and interfering with biofilm maturation [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 macrocolonies was strongly reduced by luteolin, myricetin, morin, and quercetin. This interference occurred by inhibiting amyloid curli fibres assembly and cellulose production through unknown mechanisms. The same flavonoids had the opposite effect on *Pseudomonas aeruginosa*, enhancing the formation of macrocolonies and submerged biofilms, suggesting that the anti-biofilm properties of plant flavonoids are species-specific.

In some bacteria, for example, *Escherichia coli,* cellulose exists as a phosphoethanolamine derivative, 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

Natural plant bioactive compounds, including alkaloids, indoles, tannins, terpenes, and flavonoids show great potential in inhibiting biofilm formation and disrupting already established microbial biofilms. They could interfere with the quorum sensing pathways, prevent the formation of extra polymeric substances, inhibit metabolic activity, or disrupt the structure of biofilms. Unlike conventional anti-biofilm agents, these compounds offer the additional benefit of a reduced likelihood of resistance development [78]. For instance, phytochemicals like phenolics reversed the resistance profiles of certain bacterial biofilms [84].

Future research should prioritize optimizing the extraction process for bioactive compounds, as the choice of solvent impacts the yield of these compounds. Additionally, standardization of plant bioactive is crucial, given that different concentrations of their extracts exhibit varying effects on biofilm activity; lower concentrations may induce biofilm formation, while higher concentrations may inhibit it [62]. While many studies focused on the effectiveness of plant bioactive substances as anti-biofilm agents, less is known about their pharmacokinetics, safety, and toxicological profiles. This review discussed the anti-biofilm activity of plant bioactive compounds in vitro; studies on their in vivo effects are lacking.

Advanced delivery systems such as nanoparticles, hydrogels, microencapsulation, and coating, are promising research areas for enhancing the effectiveness of plant bioactive compounds as anti-biofilms. Microencapsulation of bioactive substances from plants is another promising area; this helps to improve their stability, reduce toxicity, and improves antibiofilm activity of these compounds. The encapsulation of carvacrol and thymol improved solubility and antibacterial activity against pathogenic bacteria while concurrently reducing the amount of these compounds required for optimal activity [79,80].

Furthermore, the synergistic effect of conventional antimicrobials and plant bioactives is a vital area of focus. At concentrations that do not affect bacterial growth or survival, plant bioactive compounds 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 their use alone. Identifying synergistic combinations, optimizing dosing regimens, and elucidating their mechanisms of action could pave the way for more effective combination therapies against biofilm-associated infections.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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