



Effectiveness of various phytochemicals as quorum sensing and microbial biofilm inhibitors: An evidence-based study

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Abstract

In nature, microbial biofilms easily grow on a variety of substrates, even vegetation. Microorganisms have been using a cell-to-cell signalling technique called quorum sensing (QS) to regulate the production of these biofilms. Since this production of biofilms on vascular plants may still be harmful to the recipients, plants develop antagonists to prevent this. The goal of this brief review is to determine the biosynthetic families of bioactive components, their pharmacological effectiveness in experiments, and their relatives of appearance and range from published studies on plant-derived chemicals that suppress microbiological biofilm or quorum sensing regulation. Plant phenolics, such as benzoates, phenyl propanoids, stilbenes, flavonoids, gallotannins, proanthocyanidins, and coumarins, have been identified as major regulators of both microbiological biofilm or quorum sensing. Anti-QS and anti-biofilm characteristics are found in monoterpenes, sesquiterpenes, diterpenes, and triterpenes, for example. There were only a few alkaloids so far reported. Quinones and other phytochemicals, particularly garlic components, have also been effective. The polar character of these substances is an universal trait. Angiosperms in subtropical and tropical locations have a lot of compounds with these actions, whereas gymnosperms, bryophytes, and pteridophytes didn't ever have.

Keywords: Quorum sensing; bioactive compounds; terpenes; quinones; organosulfur compounds

Introduction

Biofilms are microbial communities that are anchored in a matrix and adhere to a surface. Living in the organised environment of a biofilm community benefits organisms much ^[1]. Living in a house keeps us safe from the elements (desiccation, cold, UV damage, and so on); residing in biofilms protects bacteria from the same risks, along with stress unique to microbes, including pH variations ^[2]. Toxic metals, salts, antibiotics, and predators can all kill microorganisms, but biofilms can protect them from all. The plague bacterium, *Yersinia pestis*, is transferred by infected fleas whose digestive systems are clogged by bacterial biofilms; the biofilms aid in propagation while also protecting the bacteria against hunting ^[3]. Biofilms are defined by three key ideas: organized populations of one or more species of microorganisms, the creation of a gel network by key stakeholders, and attachment to a biologic or a biological base or interaction. The architecture of biofilms has been classified as colonies or pellicles (free-floating). Planktonic cells stick reversibly to a surface of a solid, cells proliferate and create an irreversible organ attachment, the biofilm community expands, adding to the community's three-dimensionality, and as the biofilm matures, sections disconnect in a dissemination stage that generates new biofilms ^[4]. Species recruit potential mates through communicating during the attachment and adhesion stages. In a process known as coaggregation, the partner species cluster tightly ^[5]. In contrary to coadhesion, which occurs when free-floating species adhere to a species that has been attached to a surface as component of a biofilm, coadhesion occurs when free-floating species adhere to a species that has already been affixed to a substrate as part of

a biofilm. The extent to which genetics and the environment affect bacterial colonization and the architectural features that form is a hotly debated topic ^[6]. Cell-cell signalling, stickiness, and the production of pili, flagella, and extra-polymeric substances are all regulated by genes (EPSs) ^[4, 7]. Nevertheless, when investigators use mutants to knock out specific genes, they discover that biofilm production is not inhibited, showing that the genes are redundant ^[7]. Biofilms can arise in a variety of ways depending on the environment (Fig 1). Water shear and the degree and just kind of nutrients accessible are two important environmental elements that influence the type of biofilm that forms. Scientists and biologists collaborated to enlighten on the viscoelastic and hydrogel properties of biofilms. The biofilm polymer is hydrated with water to produce a viscous jelly-like structure in biofilms classified as hydrogels ^[8]. Water flow can stretch the viscous matrix, and if the biofilm's tensile properties are surpassed, it will disintegrate under the shear forces. Many human pathogens develop biofilms, which are responsible for numerous sustained nosocomial infections, particularly in immunocompromised patients. *Pseudomonas aeruginosa* ^[9], *Burkholderia cepacia* ^[10], *Listeria monocytogenes* ^[11], *Staphylococcus aureus* ^[8], and *Candida albicans* ^[12] are just a handful of the pathogens involved. Many chemical compounds that prevent microbial biofilm formation are described in a recent thorough study gathered from medicinal chemistry and biodiversity literature ^[13]. Inside this broad field of research, the occurrence of quorum signalling and biofilm inhibition in plants, which has now been reported in a variety of plant species using several modelling microorganism biocontrol agents is addressed.

To use quorum sensing spontaneously, microorganisms must have three abilities: secretion of a chemical messenger, secretion of a transcription factor (to detect changes in signalling molecule quantity), and response control of gene transcription [6]. This procedure is extremely reliant on the signalling molecules' diffusion mechanism. Organisms normally release only a small number of QS signalling molecules. The chemicals may just diffuse aside at low cell density. The regional quantity of signal transduction may surpass its threshold value at high cell density, causing gene regulation alterations [8]. The production of a biofilm is a complicated process that necessitates the synchronised expression of several genes [12]. Quorum sensing (QS), a cell-to-cell communication system, regulates the transition between planktonic and biofilm formation. Autoinducers, which are signal molecules secreted by bacteria, are involved in QS [9,11]. Autoinducing peptides (AIPs) are used by Gram-positive bacteria for signalling, while N-acyl-

homoserine lactones (AHLs) are used by Gram-negative bacteria [1-5]. Farnesol and tyrosol are quorum sensing chemicals in fungus like *Candida albicans* [6,7]. When communities reach one certain density or threshold, genes like virulence determinants and attachment proteins can be expressed [12]. QS activation is essential for biofilm formation in many types of bacteria; however, inhibition of QS is needed in others, such as *Staphylococcus aureus* [8]. Diverse bacterial populations and materials have been used to create a variety of biofilm prototypes. Growing a biofilm in a microtiter surface in the presence and absence of the target compound, and then evaluating the labelled biofilm mass at the ideal wavelength is the easiest and most used experiment [14]. Fungi, Gram-positive, and Gram-negative bacteria can all be tested using this approach. With dual mixed-species biofilms have also been researched on a range of surfaces [15].

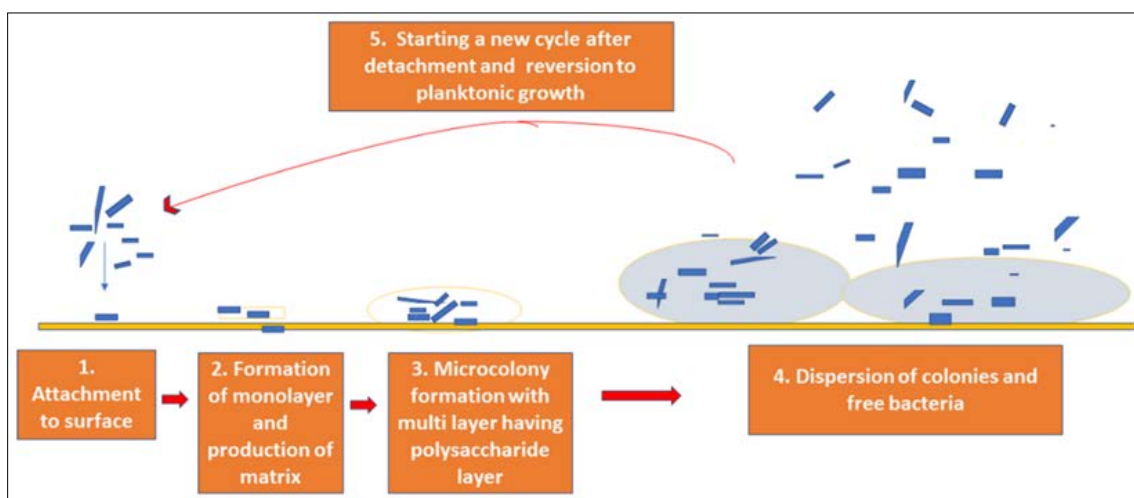


Fig 1: Mechanism of formation of biofilm

The occurrence of biofilms in plants is important for both a basic picture of the concepts in the environment and its operational use [10]. To begin with, the finding of these chemicals in plants provides chemical ecological principles with insights into potential new survival instincts in plants for future research, including which species and ecosystems are concerned. It also sheds new light on the historic usage of these chemicals and plants in therapeutics [13]. The classification of bioactive molecules (phytochemicals) formed from plants provides data on their origin, as well as molecules of relevance for physiological research and key constituents for analoging, structure - function studies, and drug discovery [16].

The goal of this study is to explore phytochemicals that can alter microbial quorum sensing and biofilm development in land plant species.

Materials and Methods

At the very beginning, we used major electronic databases such as PubMed, MEDLINE and SCOPUS. Google Scholar was also included for additional literature search related to the topic using the specific words strings like 'phytochemicals and microorganism', 'antibacterial activity of phytochemicals', 'potential bioactive compounds from plant', 'medicinal plant', 'inhibition of biofilm formation' 'inhibition of quorum sensing' etc. Obtained literatures were saved based on the content. Those articles, which did not

associate to the specific topic according to our search words of current topic, were not included. Even many papers were dropped for not being in English language. Each and every article that discussed only any inhibitory activity against any microorganism were kept back. Those articles reporting only effectiveness as inhibitor molecule on biofilm and quorum sensing published before the year 2010 have also been excluded based on content. Moreover, some articles which were published earlier than this used according to their aptness to the current review.

Results

Plant species and their natural habitat produce bioactive chemicals

In the literature, bioactive compounds have been identified from a large number of plant groups. They are angiosperms, and if the re-occurrence of compounds in other families is taken into account, many more might be included. Unfortunately, out of the 642 plant families recorded in the plant list [15], this is a minor percentage. Monocots and eudicots are both documented in the literature, while gymnosperms, bryophytes, and pteridophytes are either nonexistent or undervalued. *Zingiberaceae*, *Rubiaceae*, *Lauraceae*, and *Theaceae* are tropical or subtropical plant families, while *Asteraceae*, *Lamiaceae*, *Ericaceae*, *Berberidaceae*, and *Apiaceae* are temperate plant families [16]. Minimal data exists for plant groups found in arid or dry

environments [5, 17]. There have been no evidence of the common family *Cactaceae*, *Poaceae*, or *Crassulaceae*, for example. This could be due to sampling bias, and there aren't enough studies focused to draw any conclusive results at this moment, and it could be an interesting subject to research. Tropical plants, according to our studies, are a great source of biofilm and QS opponents [18]. The extreme humidity and constant wetness of rainforests are favourable for bacterial biofilm formation [7, 19]. When they occur on leaves, the exopolysaccharide layer provides an ideal environment for bryophyte spores to germinate, resulting in leaf fouling and a reduction in plant nutrition as fouled leaves are unable to complete respiration (Sunlight is blocked from entering the mesophyll region by the bryophyte mat) [20].

Discussion

Quorum sensing and Biofilm Inhibitors using Phenylpropanoids

When comparing to all other types of active compounds, phenolics have the greatest number of active compounds

documented in terms of its effects on quorum sensing and biofilm development (Table1). Eugenol, a phenylpropene found in many plants, has been demonstrated to reduce QS-mediated traumatic memories synthesis in *Chromobacterium violaceum* and pathogenicity factors in *Pseudomonas aeruginosa* PAO1 by 32 to 56 percent at doses of 50 to 200 μ M, respectively [21, 22]. *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Klebsiella pneumoniae* clinical isolates were likewise resistant to this drug [23-25]. Brackman *et al.*, [26] found that cinnamaldehyde, another phenylpropene, inhibits the AI-2-mediated QS mechanism in *Vibrio* spp. (65% suppression at 100 μ M). Cinnamaldehyde has been demonstrated to be efficient both against Gram-positive and Gram-negative bacteria in respect of biofilm development, including *Listeria monocytogenes* [24], *Staphylococcus epidermidis* [27], and *Cronobacter sakazakii* [28]. The active concentrations in these investigations spanned from 946 μ M to 38 mM, and they were found to inhibit the development of spontaneous and preformed biofilms as well as suppress the transcription of biofilm-related genes [24, 27, 28].

Table1: Phenylpropanoids and derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Phenylpropanoids	References
Name of the compounds	Experimental evidences	
Eugenol	Reduce QS-mediated traumatic memories synthesis in <i>Chromobacterium violaceum</i> and pathogenicity factors in <i>Pseudomonas aeruginosa</i> PAO1 by 32 to 56 percent at doses of 50 to 200 μ M, respectively	23
	<i>Pseudomonas aeruginosa</i> , <i>Listeria monocytogenes</i> , and <i>Klebsiella pneumoniae</i> clinical isolates were likewise resistant to this drug.	23-25
Cinnamomum	Inhibits the AI-2-mediated QS mechanism in <i>Vibrio</i> spp. (65% suppression at 100 μ M).	26
	Efficient both against Gram-positive and Gram-negative bacteria in respect of biofilm development, including <i>Listeria monocytogenes</i> , <i>Staphylococcus epidermidis</i> , and <i>Cronobacter sakazakii</i> .	24, 27, 28

Quorum sensing and biofilm inhibition with benzoic acid derivatives

Benzoic acid derivatives like vanillin and gallic acid had a range of impacts according on the organism and degree studied (Table2). Vanillin at 250 μ g/mL suppressed QS in *Chromobacterium violaceum* and biofilm in *Aeromonas hydrophila* in a research by Ponnusamy *et al.*, [29]. Kappachery *et al.*, [30] used diverse abiotic substrates to confirm vanillin's anti-biofilm efficacy. Post with vanillin at a dosage of 0.18 mg/mL inhibited *Aeromonas hydrophila* biofilm growth on filter media by 90% [30]. Vanillin increased N-acyl-homoserine lactones generation in *E. coli* JDL271/pAL105 and biofilm in *Pseudomonas aeruginosa* PAO1 and *Agrobacterium tumefaciens* C58 along with at least two-fold at 200 μ g/mL in that other research [31]. Gallic acid at 200 μ g/mL showed no effect on *P. aeruginosa* PA14 biofilms [32], but it did decrease *P. aeruginosa* PAO1 biofilm production by 30% [31] and three-fold boosted *Staphylococcus epidermidis* biofilms at the same dose [33].

Gallic acid was found to suppress *Eikenella corrodens* biofilm development by 80% at a significantly higher dose of 1 mM [34]. Borges *et al.*, [35] used *Chromobacterium violaceum* and reported a 59 percent drop in violacein production at 1 mg/mL. Anti-QS and anti-biofilm properties have also been observed for other benzoic acid derivatives. When comparing to the positive control *Delisea pulchra* (Greville) Montagne extract, ellagic acid at 36 μ g/disc inhibited QS more in *C. violaceum* [32]. Huber *et al* [17], detected QS suppression in *E. coli* MT102 and *Pseudomonas putida* at doses of 40 μ g/mL and 30 μ g/mL, correspondingly, confirming this functionality. Around 10 μ g/mL, this chemical proved ineffective against *P. aeruginosa* PA14 biofilms [32]. Additional study found that at 4 μ g/mL, ellagic acid reduced biofilm in several *Streptococcus dysgalactiae* strains by 70% [36]. *E. coli*, *Burkholderia cepacia*, *Staphylococcus aureus*, and *Candida albicans* biofilms were all inhibited by ellagic acid at greater doses of 15 to 40 μ g/mL [37].

Table2: Benzoic Acid derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Benzoic acid derivatives	References
Name of the compounds	Experimental evidences	
Vanillin	Vanillin at 250 μ g/mL suppressed QS in <i>Chromobacterium violaceum</i> and biofilm in <i>Aeromonas hydrophila</i>	29
	At a dosage of 0.18 mg/mL inhibited <i>Aeromonas hydrophila</i> biofilm growth on filter media by 90%	30

	Increased N-acyl-homoserine lactones generation in <i>E. coli</i> JDL271/pAL105 and biofilm in <i>Pseudomonas aeruginosa</i> PAO1 and <i>Agrobacterium tumefaciens</i> C58 along with at least two-fold at 200 µg/mL	31
Gallic acid	Gallic acid at 200 µg/mL showed no effect on <i>P. aeruginosa</i> PA14 biofilms, but it did decrease <i>P. aeruginosa</i> PAO1 biofilm production by 30% and three-fold boosted <i>Staphylococcus epidermidis</i> biofilms at the same dose.	31- 33
	Gallic acid was found to suppress <i>Eikenella corrodens</i> biofilm development by 80% at a significantly higher dose of 1 mM.	34
Ellagic acid	Inhibited biofilm formation (50% at 40 µg/mL) and swarming motility (100% at 20 µg/mL) in <i>B. cepacian</i>	17
	Inhibited QS in <i>E. coli</i> MT102 (pSB403) by 40% at 40 µg/mL and <i>P. putida</i> (pKR-C12) by 40% at 30 µg/mL	17
	Reduced biofilm formation in <i>S. dysgalactiae</i> strains by 70% at 4 µg/mL	32
	Inhibited biofilm formation in <i>S. aureus</i> ATCC 11632 (60% at 15 µg/mL), MRSA ATCC 33591 (70% at 20 µg/mL), <i>E. coli</i> ATCC 10536 (60% at 15 µg/mL), and <i>C. albicans</i> ATCC 90028 (50% at 20 µg/mL)	36,37

Tannins as quorum sensing and biofilm inhibitors

Tannins, such as ellagitannins and proanthocyanidins, are also a type of phenolic that has been shown to decrease biofilm growth and quorum sensing (Table 3). When applied at a concentration of 4 M, 1, 2, 3, 4, 6-penta-O-galloyl-D-glucopyranose (a frequent progenitor of gallotannins) lowered biofilm growth in *Staphylococcus aureus* by 50% [38]. Additional tannins that really can disrupt with QS in Gram-negative and Gram-positive bacteria are punicalagin [39] and hamamelitannin [40]. Punicalagin (an ellagitannin present in pomegranate and Combretaceae species) suppressed violacein synthesis in *C. violaceum* along with gliding and flocking motility in *Salmonella typhimurium* SL1344 at 15.6 µg/mL in a research by Li *et al* [39]. These researchers found that at the same dose, QS and motility-related genes were downregulated in *S. typhimurium*. Similarly, at 4 µg/mL, hamamelitannin (a gallotannin derived from American witch-hazel) was demonstrated to inhibit cell adhesion of methicillin-resistant

Staphylococcus aureus in culture [40]. Hamamelitannin suppressed-hemolysin synthesis and the QS controller RNAlII in *S. aureus* at a dose of 50 µg/mL. This reduction in aggressiveness was also shown *In vivo* utilising a transplant approach that incorporates in rats. Within a week of 7 days, transplanted grafts displayed no measurable methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus epidermidis* (MRSE) burdens at a well before concentration of 30 mg/mL [40]. Tannic acid has been shown to be effective towards Gram-positive and Gram-negative microorganisms. Huber *et al.*, [17] found an increase in biofilm development in *Pseudomonas aeruginosa* PA14 at 100 µg/mL; yet, similar investigation [32] found a 72 percent suppression of PA14 biofilms at 200 µg/mL. Anti-biofilm actions against *S. aureus* have been demonstrated in several experiments at lower doses ranging from 3.4 to 20 µg/mL [41, 42]. In *Pseudomonas putida* at 30 µg/mL [17] and *S. aureus* at 20 µg/mL [42], suppression of QS was found.

Table 3: Tannins and derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Tannins	References
Name of the compounds	Experimental evidences	
Proanthocyanidins	Inhibited biofilm formation in <i>S. aureus</i> (IC ₅₀ = 3.6 µM).	38
Punicalagin	Inhibited violacein production in <i>C. violaceum</i> and swimming/swarming motility in <i>S. typhimurium</i> SL1344 at 15.6 µg/mL. Downregulate expression of motility and QS related genes in <i>S. typhimurium</i> at 15.6 µg/mL.	39
Hamamelitannin	Act as QS regulator as well as inhibitors for RNAlII and δ-hemolysin production at 50 µg/mL. Reduced cell attachment of MRSA to polystyrene plate at 4 µg/mL. In mice infection model, treatment of grafts with 30 mg/mL showed no detectable MRSA and MRSE load after one week.	40
Tannic acid	Tannic acid has been shown to be effective towards Gram-positive and Gram-negative microorganisms. Found an increase in biofilm development in <i>Pseudomonas aeruginosa</i> PA14 at 100 µg/mL; yet, similar investigation found a 72 percent suppression of PA14 biofilms at 200 µg/mL.	17,32
	Anti-biofilm actions against <i>S. aureus</i> have been demonstrated in several experiments at lower doses ranging from 3.4 to 20 µg/mL. In <i>Pseudomonas putida</i> at 30 µg/mL and <i>S. aureus</i> at 20 µg/mL, suppression of QS was found.	17,41,42

Quorum sensing as well as biofilm suppression with stilbenes plus flavonoids

Two very different fungal and bacterial biofilms were shown to be inhibited by stilbenes like resveratrol and pterostilbene (Table 4). Cho *et al.* [42], found that 50 µg/mL of resveratrol reduced biofilm formation in *P. aeruginosa* PA14 and *E. coli* O157:H7 bacteria. Coenye *et al.* [43], found that 0.32 percent resveratrol prevented microbial activity in *Propionibacterium acnes* in another investigation. This chemical was antagonistic to *S. aureus* biofilms at a dosage of 100 µg/mL, while it made progress biofilm development in *S. epidermidis* [33]. Pterostilbene, at a dose of 4 µg/mL, reduced hyphal development in the same pathogenic fungi in the same investigation. The number of genes associated in morphological metamorphosis, ergosterol production, filamentation, and cell surface proteins was affected by this chemical, according to transcriptomic analysis. Additionally, pterostilbene therapy displayed anti-biofilm properties in a dose-dependent fashion in a rat central line model system [44]. Flavonoids are a kind of phenolic compound that has been shown to suppress quorum signalling and biofilm. Vikram *et al.*, [45], found that at a dosage of 6.25 µg/mL, quercetin inhibited bioluminescence in *Vibrio harveyi* strains by 75%. At the same dose, Lee *et al.*, [46], exhibited anti-biofilm activity against *E. coli* O157:H7 and *V. harveyi* BB120, along with suppression of

S. aureus biofilms at 1 µg/mL. The same team found that quercetin lowered the transcription of genes encoding in QS and *S. aureus* pathogenicity in microarray studies. Catechins from green tea (*Camellia sinensis* L.) and other flavonoids have similar effects.

At µg/mL, gallicocatechin, epigallocatechin, catechin gallate, epicatechin gallate, gallicocatechin gallate, epigallocatechin gallate, gallicocatechin gallate, and epigallocatechin gallate all prevented biofilm development in *Eikenella corrodens*, according to Matsunaga *et al.* A similar chemical, catechin, reduced the synthesis of violacein and pathogenicity factors in *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1, correspondingly [47]. Conversely, the action of epicatechin (another similar chemical) varies based on the microorganism studied. Epicatechin increased biofilm formation in *P. aeruginosa* PAO1 and *Agrobacterium tumefaciens* C58 at a concentration of 200 µg/mL [31]. This chemical prevented *Escherichia coli* JM109 biofilms by 40% when used at larger doses of 1 mg/mL [35]. Epicatechin suppressed violacein formation in *C. violaceum* at 1 mg/L [35], but boosted AHL generation in *E. coli* DL271/pAL105 at 40 to 200 µg/mL [31]. At 40 µg/mL, epigallocatechin gallate inhibited swarming movement in *Burkholderia cepacia* along with *E. coli* MPT102 and *Pseudomonas putida* [17].

Table 4: Stilbenes and Flavonoids affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents Name of the compounds	Stilbenes and Flavonoids Experimental evidences	References
Resveratrol	50 µg/mL of resveratrol reduced biofilm formation in <i>P. aeruginosa</i> PA14 and <i>E. coli</i> O157:H7 bacteria and 0.32 percent resveratrol prevented microbial activity in <i>Propionibacterium acnes</i> .	42, 43
	Antagonistic to <i>S. aureus</i> biofilms at a dosage of 100 µg/mL, while it made progress biofilm development in <i>S. epidermidis</i>	33
Pterostilbene	Inhibits formation of new and mature <i>C. albicans</i> SC5314, Y0109, 0304103, and 01010 biofilms at 16 µg/mL. Inhibits hyphal formation in <i>C. albicans</i> at 4 µg/mL. Treatment of 16 µg/mL altered expression of genes involved in morphological transition, ergosterol biosynthesis.	44
Quercetin	Inhibits bioluminescence in <i>Vibrio harveyi</i> strains by 75%. At the same dose exhibited anti-biofilm activity against <i>E. coli</i> O157:H7 and <i>V. harveyi</i> BB120, along with suppression of <i>S. aureus</i> biofilms at 1 µg/mL.	46
Catechin	Inhibits violacein production in <i>C. violaceum</i> CV026 (50% at 2 mM), pyocyanin (50% at 0.25 mM) and elastase production (30% at 4 mM) in <i>P. aeruginosa</i> PAO1. Reduced biofilm formation and downregulated QS genes expression in <i>P. aeruginosa</i> PAO1 by at 4 mM	47
Gallicocatechin, Epigallocatechin, Catechin gallate, Epicatechin gallate, Gallicocatechin gallate, Epigallocatechin gallate, Gallicocatechin gallate, and Epigallocatechin gallate	At µg/mL all prevented biofilm development in <i>Eikenella corrodens</i>	47
	Epicatechin increased biofilm formation in <i>P. aeruginosa</i> PAO1 and <i>Agrobacterium tumefaciens</i> C58 at a concentration of 200 µg/mL	31
	Epicatechin prevents <i>Escherichia coli</i> JM109 biofilms by 40% when used at larger doses of 1 mg/mL. Suppresses violacein formation in <i>C. violaceum</i> at 1 mg/L, but boosted AHL generation in <i>E. coli</i> DL271/pAL105 at 40 to 200 µg/mL.	31, 35
	At 40 µg/mL, epigallocatechin gallate inhibited swarming movement in <i>Burkholderia cepacia</i> along with <i>E. coli</i> MPT102 and <i>Pseudomonas putida</i> .	17

Diarylheptanoids as biofilm modulators and quorum sensing agents

Curcumin is yet another interesting phenolic in regards of QS and biofilm activity (a diarylheptanoid found in turmeric). This chemical has been thoroughly investigated and has been shown to have a variety of pleiotropic physiological effects (Table 5). Karaman *et al.*,^[47] demonstrated that this chemical improved biofilm formation in *Staphylococcus aureus* at a concentration of 16 µg/mL. Curcumin decreased AHL generation and biofilm expansion in *P. aeruginosa* PAO1 at 1 µg/mL and fully prevented biofilm in *Helicobacter pylori* strains isolated at 8 µg/mL,

according to Rudrappa and Bais^[48] and Pattiyathane *et al.*,^[49] correspondingly.

This compound showed anti-biofilm activity against *Staphylococcus epidermidis*, *Vibrio sp.*, *Escherichia coli*, *Pseudomonas aeruginosa* PAO1, *Proteus mirabilis*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Streptococcus mutans*, and *Candida albicans* at elevated doses ranging from 25-625 µg/mL^[25, 27, 50-54]. Curcumin suppressed the formation of violacein and pathogenicity factors in *Vibrio sp.*, *P. aeruginosa* PAO1, and *S. marcescens* at quantities ranges from 3 µg/mL to 100 µg/mL^[48, 52, 53].

Table 5: Diarylheptanoids and derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Diarylheptanoids	
Name of the compounds	Experimental evidences	References
Curcumin	Alters expression of QS-related genes, reduced virulence factors production (60% to 80%) and mortality in infection models (28% to 80%) with treatment of 3 µg/mL in <i>P. aeruginosa</i> PAO1	27
	Completely inhibits biofilm formation in <i>H. pylori</i> ATCC 43504 and other clinical isolates at 8 µg/mL for up to 10 days. Inhibits QS activity (IC ₅₀ = 10 µM) and biofilm formation in <i>S. mutans</i> UA159 (at 15 µM).	48
	Completely eradicated 48-h and 14-day biofilms and reduced biomass of 8-week biofilm in <i>E. faecalis</i> ATCC 29212 (625 µg/mL). Inhibits biofilm formation (50%), alginate production (20% to 70%), and motility in <i>V. harveyi</i> , <i>V. parahaemolyticus</i> , and <i>V. vulnificus</i> at 75 µg/mL.	49
	Inhibits violacein production in <i>C. violaceum</i> CV026 and virulence factors production in <i>P. aeruginosa</i> PAO1 and <i>S. marcescens</i> FJ584421 at 100 µg/mL (56%–63%).	50
	Inhibits swimming motility by 50% in <i>E. coli</i> ATCC 10536 (50 µg/mL), <i>P. aeruginosa</i> PAO1 (50 µg/mL), <i>P. mirabilis</i> ATCC 7002 (75 µg/mL), and <i>S. marcescens</i> FJ584421 (75 µg/mL).	51
	Inhibits biofilm formation in <i>E. coli</i> (52%), <i>P. aeruginosa</i> PAO1 (89%), <i>P. mirabilis</i> (52%), and <i>S. marcescens</i> (76%) at 100 µg/mL. Inhibits biofilm formation (50%) and surface adhesion (15%) in <i>C. albicans</i> at 50 µg/mL. Inhibits biofilm formation in <i>S. epidermidis</i> (MIC = 25 µg/mL)	52
	Inhibits biofilm formation in <i>S. epidermidis</i> (MIC = 25 µg/mL). Inhibits biofilm formation and AHLs production in <i>P. aeruginosa</i> at 1 µg/mL. Altered expression of QS-related genes, reduced virulence factors production (60% to 80%) and mortality in infection models (28% to 80%) with treatment of 3µg/mL in <i>P. aeruginosa</i> PAO1. Completely inhibited biofilm formation in <i>H. pylori</i> ATCC 43504 and other clinical isolates at 8 µg/mL for up to 10 days.	53,54

Terpenoids as biofilm antagonists and quorum sensing agents

Anti-biofilm and/or anti-QS characteristics have been found in monoterpenes, limonoids, and triterpenes, among other terpenes (Table 6). The monoterpenes thymol and carvacrol were shown to be beneficial both Gram-positive and Gram-negative bacteria biofilms^[24, 56, 57]. At 0.5 mM and 5 mM, thymol prevented the production of new *Listeria monocytogenes* biofilms and downregulated existing ones in a research by Upadhyay *et al*^[24]. Genes important for *L. monocytogenes* biofilm growth were significantly suppressed at lower concentrations^[24]. The authors used the same model organism to demonstrate that carvacrol is beneficial against nascent and existing biofilms at doses of 0.65 mM and 10 mM, accordingly. At 0.65mM, this drug inhibited biofilm-associated genes in *L. monocytogenes*^[24]. Soumya *et al.*,^[56] found antagonistic effect of these monoterpenes against a few strains of *Pseudomonas*

aeruginosa in some other investigation. Thymol suppressed the biofilm density of *P. aeruginosa* isolates ATCC 27853, CIP A22, and IL5 by 86%, 54%, and 70%, respectively, at a concentration of 0.1 percent. Likewise, 0.04 percent carvacrol suppressed biofilm formation in the very same strains by over 90%^[56]. The limiting action of thymol had also been validated by Qiu *et al*^[57], who found that exposure with 64µg/mL reduced the production of enterotoxin genes in *Staphylococcus aureus* by almost 5-fold. Biofilm accumulation in *P. aeruginosa*, *S. aureus*, and *Staphylococcus epidermidis* has been found to be inhibited by sesquiterpenoids such as salvipisone and acanthospermolides^[58-60]. *S. alvipesone* (derived from the roots of *Salvia sclarea* L.) suppressed pre-existing *S. epidermidis* biofilms by at least 85% and preformed *S. aureus* biofilms along with at least 85% at a dose of 37.5 µg/mL^[58, 59]. A couple of acanthospermolides derived from *Acanthospermum hispidum* DC. were found to have at least

70% inhibitory activity against *P. aeruginosa* biofilms at 2.5 µg/mL in a further investigation [60]. In low quantities, limonoids (sourced from triterpenes) extracted from *Citrus aurantium* L. (bitter orange) displayed anti-QS activity [61]. Isolimononic acid, ichangin, and a number of many other chemicals were shown to decrease bioluminescence in *Vibrio harveyi* by 17 to 83 percent at 6.25 µg/mL, with dose-dependent action for all tested compounds [61]. Different actions have been described for other triterpenoids. In a test with *P. aeruginosa* PA14, betulinic acid, a lupane triterpene, exhibited little action at 5 µg/mL [32], while biofilm production is considerably improved at 100 µg/mL [42]. From the other hand, ursane triterpenoids have been demonstrated to be efficient antagonists. Ursolic acid was shown to inhibit *P. aeruginosa* PAO1, *E. coli* JM109, and *V. harveyi* BB120 biofilms by 35% to 87% at

10 µg/mL [21, 62]. At quite a dosage of 5 µg/mL, this drug proved ineffective against *P. aeruginosa* PA14 biofilm [32]. Additionally, VEDIYAPPAN *et al.* [63] showed that gymnemic acids (ursane triterpene glycosides) derived from *Gymnema sylvestre* prevent yeast-to-hypha transformation in *Candida albicans* SC5314. At even a concentration of 40 µg/mL, the four-compound combination inhibited hyphal transit and forced hyphal transcription to yeast form; following 11 hours, 100 percent metamorphosis has been identified. At the very same dose, these chemicals suppressed conidial hatching and hyphal proliferation in *Aspergillus fumigates*. Additionally, administration with a gymnemic acid cocktail at a concentration of 40 µg/mL enhanced the survivability of *Candida albicans*-infected *Caenorhabditis* worms and concluded in 100% cure [63, 64].

Table 6: Terpenoids and derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Terpenoids	
Name of the compounds	Experimental evidences	References
Monoterpenes: Thymol and Carvacrol	Beneficial both Gram-positive and Gram-negative bacteria biofilms. At 0.5 mM and 5 mM, thymol prevented the production of new <i>Listeria monocytogenes</i> biofilms and downregulated existing ones.	24,56,57
	Beneficial against nascent and existing biofilms at doses of 0.65 mM and 10 mM, accordingly. At 0.65mM, this drug inhibited biofilm-associated genes in <i>L. monocytogenes</i>	24
	Thymol suppressed the biofilm density of <i>P. aeruginosa</i> isolates ATCC 27853, CIP A22, and IL5 by 86%, 54%, and 70%, respectively, at a concentration of 0.1 percent. Likewise, 0.04 percent carvacrol suppressed biofilm formation in the very same strains by over 90%. With 64 µg/mL reduced the production of enterotoxin genes in <i>Staphylococcus aureus</i> by almost 5-fold.	56,57
Salvipisone and Acanthospermolides	Biofilm accumulation in <i>P. aeruginosa</i> , <i>S. aureus</i> , and <i>Staphylococcus epidermidis</i> has been found to be inhibited by sesquiterpenoids such as salvipisone and acanthospermolides. Salvipisone suppresses preexisting <i>S. epidermidis</i> biofilms by at least 85% and preformed <i>S. aureus</i> biofilms along with at least 85% at a dose of 37.5 µg/mL.	58-60
Gymnemic acid	Cocktail at a concentration of 40 µg/mL enhances the survivability of <i>Candida albicans</i> -infected <i>Caenorhabditis</i> worms and concluded in 100% cure.	63,64

Quorum sensing and biofilm inhibition with sulfur-containing phytochemical compounds

Sulfur-containing agents like as allicin, ajoene, and thiocyanates have been shown to suppress biofilm and quorum sensing in Gram-positive and Gram-negative microorganisms (Table 7) [32, 64-69]. Allicin (from garlic) inhibited biofilm adhesion in *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* PAO1 as well as the triggering of QS-regulated pathogenicity mechanisms in *P. aeruginosa* PAO1 [64, 70]. Regarding *P. aeruginosa* PA14, a 74 percent sharp decrease in biofilm density was found at a dosage of 1.1 mg/mL [32]. Exposure of 128 µg/mL allicin with GFP-transformed *P. aeruginosa* PAO1 culminated in a 50% reduction in biofilm density and a 70% reduction in EPS generation in another investigation [64, 71]. 4 mg/mL allicin suppressed biofilm by much more nearly 90% in *S. epidermidis* strains [65]. Ajoene (even from garlic) reduced QS in the sensor bacteria *P. aeruginosa* lasB-gfp, *P. aeruginosa* rhlA-gfp, and *E. coli* luxI-gfp at 50 percent effective doses (ED₅₀) varying from 15 to 100 M [72]. At 80

mg/mL, ajoene reduced the expression of specific genes (elastase, rhamnolipid, and enterotoxins) by quadrupling [66]. In a murine model, subcutaneous injection of ajoene at 25 mg/kg enhanced bacteria evacuation after three days [73]. Thiocyanates from *Brassicaceae* species, such as sulforaphane and allyl isothiocyanate, were shown to suppress QS in *Escherichia coli* and *Chromobacterium violaceum*, along with diminish biofilm in *Pseudomonas aeruginosa* and *Listeria monocytogenes* [35, 67, 68, 74]. At 100 µM of sulforaphane, Ganin *et al.*, [67] found full suppression of QS in *E. coli* DH5 (pJN105L) (pSC11). At doses of 37 µM and 100 µM, this drug reduced biofilm generation and pyocyanin synthesis (QS-mediated) in *P. aeruginosa* PAO1 by 60% and 70%, accordingly [67]. Furthermore, at 5 µg/mL, allyl isothiocyanate was demonstrated to inhibit violacein synthesis in *C. violaceum* by 70% (QS-controlled) [66, 75]. The anti-biofilm potency of one such drug was demonstrated in *L. monocytogenes*, *P. aeruginosa*, and *E. coli*, with 1 mg/mL exposure resulting in 61 to 100% inhibition [76].

Table 7: Sulfur-Containing Phytochemicals affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Sulfur-containing phytochemical compounds	References
Name of the compounds	Experimental evidences	
Allicin	Inhibits biofilm adhesion in <i>Staphylococcus epidermidis</i> and <i>Pseudomonas aeruginosa</i> PAO1 as well as the triggering of QS-regulated pathogenicity mechanisms in <i>P. aeruginosa</i> PAO1.	64
	4 mg/mL allicin suppressed biofilm by much more nearly 90% in <i>S. epidermidis</i> strains.	65
	Regarding <i>P. aeruginosa</i> PA14, a 74 percent sharp decrease in biofilm density was found at a dosage of 1.1 mg/mL.	32
	Exposure of 128 µg/mL allicin with GFP-transformed <i>P. aeruginosa</i> PAO1 culminated in a 50% reduction in biofilm density and a 70% reduction.	64
Ajoene	Reduces QS in the sensor bacteria <i>P. aeruginosa</i> lasB-gfp, <i>P. aeruginosa</i> rhlA-gfp, and <i>E. coli</i> luxI-gfp at 50 percent effective doses (ED ₅₀) varying from 15 to 100 M. At 80 mg/mL, ajoene reduced the expression of specific genes (elastase, rhamnolipid, and enterotoxins) by quadrupling.	66,72
Sulforaphane and Allyl isothiocyanate	Both suppress QS in <i>Escherichia coli</i> and <i>Chromobacterium violaceum</i> , along with diminish biofilm in <i>Pseudomonas aeruginosa</i> and <i>Listeria monocytogenes</i> .	35,67,68
	At 100 µM of sulforaphane founds full suppression of QS in <i>E. coli</i> DH5 (pJN105L) (pSC11). At doses of 37 µM and 100 µM, this drug reduces biofilm generation and pyocyanin synthesis (QS-mediated) in <i>P. aeruginosa</i> PAO1 by 60% and 70%, accordingly.	67
Isothiocyanate	At 5 µg/mL, allyl isothiocyanate was demonstrated to inhibit violacein synthesis in <i>C. violaceum</i> by 70% (QS-controlled). The anti-biofilm potency of one such drug was demonstrated in <i>L. monocytogenes</i> , <i>P. aeruginosa</i> , and <i>E. coli</i> , with 1 mg/mL exposure resulting in 61 to 100% inhibition.	66,75

Coumarins as biofilm modulators and quorum sensing agents

Coumarins have been proven to be anti-QS and anti-biofilm (Table 8). At 500 micrograms, aesculetin inhibited QS in *C. violaceum*, *P. aeruginosa*, and *E. coli* JB523 by 30 to 78 percent [18]. In additional investigations, Dürig *et al.*, [36] and Lee *et al.*, [69] found that aesculetin inhibited *S. aureus* biofilms (>50 percent reduction at 128 µg/mL) as well as altered biofilm-related gene function (at 50 µg/mL) in *E. coli* O157:H7, significantly. Aesculetin also diminished

virulence in a *C. elegans* model system and suppressed Shiga-like toxin synthesis in *E. coli* O157:H7 [69]. At such a concentration of 50 µg/mL, Lee *et al.*, [69] found that umbelliferone (additional coumarin) reduced biofilm (by 90%) and regulation of motility and attachment genes in *E. coli* O157:H7. Monte *et al.*, [71] verified the anti-biofilm action of umbelliferone in *S. aureus* CECT976, where dosing with 800 µg/mL resulted in a 50% reduction in biofilm production.

Table 8: Coumarins and derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Coumarins	References
Name of the compounds	Experimental evidences	
Aesculetin	At 500 micrograms, aesculetin inhibited QS in <i>C. violaceum</i> , <i>P. aeruginosa</i> , and <i>E. coli</i> JB523 by 30 to 78 percent.	18
	Inhibits <i>S. aureus</i> biofilms (>50 percent reduction at 128 µg/mL) as well as altered biofilm-related gene function (at 50 µg/mL) in <i>E. coli</i> O157:H7, significantly.	36,69
	Diminishes virulence in a <i>C. elegans</i> model system and suppressed Shiga-like toxin synthesis in <i>E. coli</i> O157:H7.	69
Umbelliferone	Anti-biofilm action of umbelliferone in <i>S. aureus</i> CECT976, where dosing with 800 µg/mL resulted in a 50% reduction in biofilm production.	71
	50 µg/mL, found that umbelliferone (additional coumarin) reduces biofilm (by 90%) and regulation of motility and attachment genes in <i>E. coli</i> O157:H7.	69

Biofilm inhibitors and quorum sensing quinones

Quinones have already been shown to have anti-biofilm properties towards microbes (Table 9) [72]. Quinones notably chrysophanol, emodin, and shikonin all demonstrated antagonistic effects against biofilm communities of *Pseudomonas aeruginosa* PAO1 and *Stenotrophomonas*

maltophilia in an experiment by Ding *et al* [72]. Emodin has been 10 times more potent than all of chrysophanol and shikonin, causing a 75 point drop in biofilm development for *P. aeruginosa* PAO1 and a 43 percent significant decline in biofilm development for *S. maltophilia* after exposure with 20 µM [73]. Across both bacterium, chrysophanol and

shikonin demanded a high proportion of 200 μM to evoke the same number of responses. At 3 $\mu\text{g}/\text{mL}$, additional quinone, purpurin, has been found to inhibit the yeast-to-hypha transition in *Candida albicans* SC5314 [74]. This chemical proven successful against new and current *C.*

albicans biofilms at higher doses of 5 to 10 $\mu\text{g}/\text{mL}$. (30 percent to 50 percent inhibition). Purpurin inhibited the transcription of hypha-specific genes, according to even further research [75].

Table 9: Quinones and derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Quinones	
Name of the compounds	Experimental evidences	References
Chrysophanol, Emodin, and Shikonin	Exhibits antagonistic effects against biofilm communities of <i>Pseudomonas aeruginosa</i> PAO1 and <i>Stenotrophomonas maltophilia</i>	72
	Emodin has been 10 times more potent than all of chrysophanol and shikonin, causing a 75 point drop in biofilm development for <i>P. aeruginosa</i> PAO1 and a 43 percent significant decline in biofilm development for <i>S. maltophilia</i> after exposure with 20 μM .	72
	Chrysophanol and Shikonin reveals a high proportion of 200 μM to evoke the same number of responses on <i>P. aeruginosa</i> PAO1 and <i>S. maltophilia</i> .	74
	At 3 $\mu\text{g}/\text{mL}$, additional quinone, purpurin, has been found to inhibit the yeast-to-hypha transition in <i>Candida albicans</i> SC5314.	75

Biofilm antagonists and quorum sensing with alkaloids

In the case of alkaloids, only a few substances have now been documented to have antibacterial biofilm inhibitory action (Table 10). Berberine prevented the production of biofilms in *Staphylococcus epidermidis* and *Klebsiella pneumoniae*, according to Jakobsen *et al.*, [76] Borges *et al.*, [77] and Magesh *et al.* [25]. Berberine inhibited biofilm development in various *K. pneumoniae* clinical specimens at a dose of 63.5 $\mu\text{g}/\text{mL}$ [25]. *S. epidermidis* biofilms were

inhibited by berberine at concentrations of 30 to 45 $\mu\text{g}/\text{mL}$ [77]. Likewise, micromolar doses of chelerythrine and sanguinarine appeared potent towards Gram-positive biofilms of *S. aureus* and *S. epidermidis* [74, 75], with 50 percent antagonistic values ranging between 15 to 25 μM for *S. aureus* and 5 to 9 μM for *S. epidermidis* [74]. With perhaps an inhibitory activity of 15.6 $\mu\text{g}/\text{mL}$, reserpine, an alkaloid from *Rauwolfia sp.* (Apocynaceae), exhibited significant efficacy over *Candida albicans* biofilms [25, 78].

Table 10: Alkaloids and derivatives affecting microbial quorum sensing (QS) and/or biofilm formation.

Active constituents	Alkaloids	
Name of the compounds	Experimental evidences	References
Berberine	Prevents the production of biofilms in <i>Staphylococcus epidermidis</i> and <i>Klebsiella pneumoniae</i>	76,77, 25
	inhibits biofilm development in various <i>K. pneumoniae</i> clinical specimens at a dose of 63.5 $\mu\text{g}/\text{mL}$.	25
Chelerythrine and Sanguinarine	Micromolar doses of chelerythrine and sanguinarine appeared potent towards Gram-positive biofilms of <i>S. aureus</i> and <i>S. epidermidis</i> , with 50 percent antagonistic values ranging between 15 to 25 μM for <i>S. aureus</i> and 5 to 9 μM for <i>S. epidermidis</i> .	74,75
Reserpine	15.6 $\mu\text{g}/\text{mL}$ dose exhibits significant efficacy over <i>Candida albicans</i> biofilms on biofilm development.	25, 78

Conclusions

Altogether, the research reveals a wide range of biochemical families of chemicals that suppress biofilms and QS. To present, the released literature has consistently indicated effectiveness with phenolics, terpenoids, organosulfur compounds, and quinones, with just some few alkaloids and other forms of bioactive molecules. The overall

effectiveness of various phytochemicals as inhibitors of microbial biofilm and quorum sensing activity have been depicted in fig 2.

The observed antagonists could be a gold mine of lead chemicals for developing medications to combat antimicrobial resistance or immunotherapy to hinder pathogen survival.

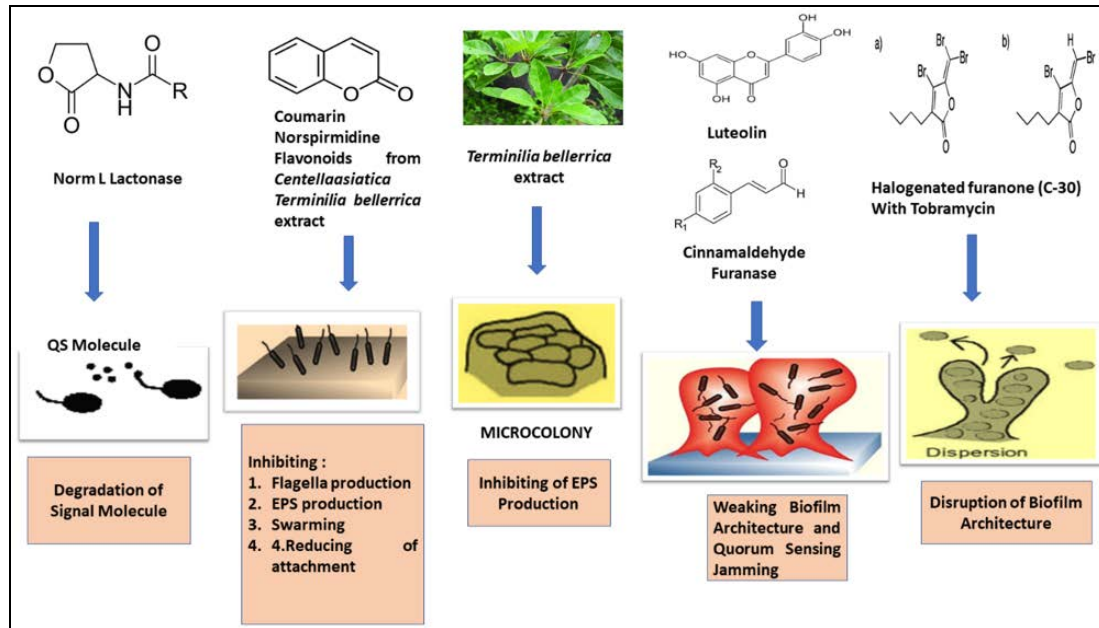


Fig 2: The overall effectiveness of various phytochemicals as inhibitors of microbial biofilm and quorum sensing activity.

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