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Antimicrobial and anti-biofilm activities of plant extracts against *Pseudomonas aeruginosa* – a review

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ABSTRACT

Antimicrobial resistance among bacterial pathogens, including *Pseudomonas aeruginosa*, is a global problem that has led to research on naturally occurring compounds as an alternative source of antibacterial and anti-biofilm agents. This review focuses on determining plant extracts' antimicrobial and anti-biofilm activities against *P. aeruginosa*, an opportunistic pathogen contributing to microbial and biofilm-associated infections in humans. Medicinal plants are being widely researched as they are rich sources of phytochemicals, including flavonoids, alkaloids, tannins and terpenoids. These phytochemicals have been well known for their antibacterial activity, which contributes to the effectiveness of certain plants, including *Punica granatum* and *Triumfetta welwitschia*, against *P. aeruginosa*. *Hypericum perforatum* and *Berginia ciliata* contains phytochemicals that directly inhibit the quorum sensing mechanism, inhibiting the direct cell-to-cell communication, thereby preventing or reducing biofilm formation by *P. aeruginosa*. Plant extracts also inhibit bacterial growth and should be considered an alternative to antibiotics. Furthermore, plant extracts can be used with antibiotics for better efficacy against *P. aeruginosa*. However, more research must be carried out to select plants with a broad spectrum of activity against not only *P. aeruginosa* infections but other gram-negative bacteria in general. It would be economically viable to develop as a therapeutic drug. This would align with the third United Nations sustainable development goals on good health and well-being and is a significant step forward in the battle against antibiotic resistance.

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1 Introduction

Pseudomonas aeruginosa is a common species of the genus *Pseudomonas*, which causes human infections. Unfortunately, antipseudomonal drug resistance is increasing, compromising suitable antimicrobial therapy selection. As a result, there is an increase in morbidity and death in patients infected with multi-drug resistant strains of *P. aeruginosa* (Montero et al. 2020). This bacterium has been observed to trigger severe clinical manifestations in humans, such as severe neutropenia and septic shock (Recio et al. 2020). Furthermore, *P. aeruginosa* also causes nosocomial infections such as urinary tract infections (UTI) and ventilator-associated pneumonia (VAP), particularly in immunocompromised patients, which increases the mortality rates of *P. aeruginosa* infections (Ramirez-Estrada et al. 2016).

Further, *P. aeruginosa* can produce biofilms as part of its virulence mechanisms, contributing to its persistence in healthcare (Das et al. 2017; Labovska 2021). These biofilms occur on a variety of abiotic (plastic, metal, minerals, and glass) and biotic (humans, animals, and plants) surfaces and have complex structure and dynamic architecture (Labovska 2021).

A crucial aspect of biofilm-related infections is increased antibiotic resistance among the strains that cause biofilm development, making these infections long-lasting and challenging to eliminate (Dincer et al. 2020). Hence, antimicrobial resistance remains a crucial global health issue for humans and animals, compounded by the problems in developing new antimicrobials.

Plant extracts have significant antimicrobial activity and are a source of effective antimicrobial compounds that can work against planktonic and biofilms of bacterium (Famuyide et al. 2019). Much research is being carried out on the antimicrobial activity of plant extracts, essential oils, and phytochemicals, including secondary metabolites that have been extracted from various parts of the plants, including leaves, roots, and seeds using solvents which are then tested against multiple strains of bacteria including *P. aeruginosa* (Famuyide et al. 2019).

Antibiotics are routinely utilized to control Gram-positive and Gram-negative infections by inhibiting their growth and survival. However, biofilm-forming bacterial isolates have been shown to acquire antibiotic resistance very swiftly (Fair and Tor 2014). Hence, treating infections with microbial biofilms with standard antibiotics is rendered unmanageable. Consequently, new-found antibacterial and anti-biofilm compounds from plant extracts have elicited new attention to counteract microbial growth and biofilm formation (Abdel-Bar et al. 2022). Thus, due to their significant antibacterial and anti-biofilm properties, this study aims to review if plant-derived extracts are potential alternatives to antibiotics to treat infections caused by *P. aeruginosa*.

The methodology used to obtain information for this systematic review utilized keywords such as *P. aeruginosa*, antibacterial activity and antibiofilm activity of medicinal plants. Research articles published between 2012 and 2022 from SCOPUS and Web of Science (WoS) journals were evaluated to extract the information related to this issue.

2 Mechanisms of Resistance in *P. aeruginosa*

Due to the extraordinary ability of this pathogen to withstand antibiotics, treatment *of P. aeruginosa* infections has become increasingly challenging. Most of the commonly used antibiotics, such as imipenem, meropenem, aztreonam, and chloramphenicol, are found to be ineffective in the treatment of some of the *P. aeruginosa* infections due to the high levels of inherent, acquired, and adaptive resistance mechanisms (Meng et al. 2020).

Intrinsic antibiotic resistance is the bacteria's ability to decrease antibiotics' effectiveness because of fundamental structure or functionality features (Blair et al. 2015). *P. aeruginosa* exhibits a high intrinsic resistance to most antibiotics due to reduced outer membrane permeability, efflux pumps which pump out medicines from the cell, and the presence of enzymes such as β-lactamases, which destroy antibiotics (Pachori et al. 2019). By mutational alterations or horizontal transfer of antibiotic resistance genes, bacteria can also develop antibiotic resistance. Acquired resistance helps create multidrug-resistant strains, which, together with the high degree of inherent antibiotic resistance in *P. aeruginosa*, make eradication more challenging and increase the incidence of chronic infections (Munita and Arias 2016). By causing reversible modifications in gene and protein expression in response to stimulation from the environment, this acquired resistance increases a bacterium's capacity to resist antibiotic attack (Papaleo et al. 2022). The formation of biofilms by *P. aeruginosa* is characterized by adaptive resistance mechanisms that cause an infection to last longer and can lead to more severe conditions of existing diseases (Taylor et al. 2014).

3 Quorum Sensing and Biofilm Development in *P. aeruginosa*

Biofilms are microbial communities that are encased in an extracellular matrix (or extracellular polymeric substances, EPS) made up of proteins, extracellular DNA (eDNA), lipids, and exopolysaccharides (Jennings et al. 2015). One of the most essential aspects of microbial biofilms is that bacteria stay within the protected microenvironment of the biofilm while conditions outside the biofilm are harmful. Bacteria typically make up less than 10% of the dry mass of biofilms, while matrix can make up more than 90% (Mishra et al. 2023). The EPS is the matrix formed by microbes comprised of several biopolymers, allowing bacteria to live in proximity and interact and behave differently than their planktonic counterparts (Roy et al. 2018). The EPS offer an extra

layer of protection around the cells, shielding them from numerous pressures, enabling the bacteria within the biofilm to resist antibiotics and environmental challenges and even escape host immunological responses (Gonzalez et al. 2018). Pathogens can, therefore, hide within a biofilm, undetected by the immune system and tolerant of antibiotics. This is the basis for many chronic and relapsing infections. *P. aeruginosa*, an effective biofilm-producing Gram-negative bacteria, habitually forms biofilm throughout virulence exhibition and disease development (Vetrivel et al. 2021). Quorum sensing (QS) is a cell density-based signalling system that aids bacterial cell-to-cell communication and regulates virulence factors, including pigment and biofilm formation, contributing to chronic infection development (Lin and Cheng 2019). Specifically, QS regulates the expression of several genes involved in various biological functions such as virulence determinants, bioluminescence, motility, plasmid transfer, enzyme and toxin secretion, bacteriocin synthesis, efflux pump, and biofilm formation (Sionov and Steinberg 2022). The opportunistic pathogen *P. aeruginosa* has two well-defined QS systems, LasI and RhlI. N-(3-oxododecanoyl) homoserine lactone (3O-C12-HSL) is synthesized by LasI, while N-butyryl homoserine lactone (3O-C12-HSL) is synthesized by RhlIR (C4-HSL) (Lin and Cheng 2019). In *P. aeruginosa*, the las and rhl QS systems regulate various genes and gene products implicated in virulence. Furthermore, it was discovered that the las system controls Rhl at two levels: transcriptional and posttranslational. These two QS systems are vital during the early phases of biofilm formation, particularly when cells adhere to the surface and produce microcolonies (Lin and Cheng 2019).

The resistance mechanisms in *P. aeruginosa* have led to the development of multidrug-resistant strains, which cause difficult-to-treat infections and an increase in the mortality rate due to infections with this bacterium. The currently available antibiotics are losing their therapeutic ability due to the multiple resistance mechanisms employed by *P. aeruginosa.* Hence, there is a need to seek alternative therapeutic options, and one good avenue being explored is using plant extracts.

4 Plants as Potential Sources of Antimicrobial agents

Traditional antibiotic discovery strategies have fallen behind the evolution of resistance. As a result, improved antimicrobial agents are urgently required. Many medicinal plants such as *Punica granatum, Zingiber officinales, Cuminum cyminum, T. impetiginosa*, and *T. welwitschia* are being widely researched for their antimicrobial and anti-biofilm activity against *P. aeruginosa* (Ulloa-Uriza et al. 2015; Mostafa et al. 2018; Mombeshora et al. 2021). The findings from these studies have shown the potential of using plant extracts as antimicrobial agents against *P. aeruginosa* due to the strong inhibitory effects that seem comparable to antibiotics such as ciprofloxacin (Karuppiah and Rajaram 2012). The ability of plant extracts to inhibit microbial growth is based on the presence of plant secondary metabolites (phytochemicals), which are biologically active compounds that are not involved in essential plant processes but play crucial roles in plant defences against pathogens, pests, and environmental stresses (Khare et al. 2021).

Phytochemicals have already been shown to exhibit antibacterial properties when applied alone or synergistically with other antibacterial drugs (Abreu et al. 2012). Using phytochemical products and plant extracts as resistance-modifying agents (RMAs) is becoming a popular issue in science. RMAs slow down the process of resistance development in bacteria against plant phytochemicals. This makes them more suitable for developing antimicrobial agents as an alternative to antibiotics. Table 1 shows a few RMA's from plants (Patel and Patel 2016; Phitaktim et al. 2016; Siriwong et al. 2016; Owusu et al. 2021; Walczak et al. 2021).

Phytochemicals often work through different pathways than traditional antibiotics, making them potentially valuable for treating antibiotic-resistant microorganisms (Abreu et al. 2012). The discovery and development of new active compounds capable of partially or inhibiting bacterial resistance mechanisms is a promising technique for combating the resistance problem (Patel and Patel 2016).

Table 1 Examples of antibiotic resistance modifying compounds from plants

Compound	Plant source	Antibiotics potentiated	Reference
Ferruginol 5-Epipisiferol	*Chamaecyparis lawsoniana*	Oxacillin, Tetracycline, Norfloxacin, Tetracycline,	Patel and Patel 2016
Carnosic acid carnosol	*Rosmarinus officinalis*	Erythromycin	Patel and Patel 2016
Ethyl gallate	*Caesalpinia spinosa*	β-lactams	Patel and Patel 2016
Epicatechin gallate	*Camellia sinensis*	Norfloxacin, Imipenem, Panipenem, β –Lactams	Patel and Patel 2016
Epigallocatechin gallate		Oxacillin	Phitaktim et al. 2016
a-mangostin	*Garcinia mangostana* L	Amoxicillin	Siriwong et al. 2016
Quercetin	*Allium cepa L; Solanaceae lycopersicum*	Gentamicin	Walczak et al. 2021
Carvacrol/thymol	*Thymus maroccanus*		

5 Antibacterial Activity of Plant Extracts Against *Pseudomonas aeruginosa*

Table 2 is a compilation of the antibacterial activity of ethanolic extracts from various plants against common bacterial pathogens, including *P. aeruginosa*, obtained from studies that tested various plant extracts against *P. aeruginosa*, *E. coli* and *S. aureus*. Based on the data summarized in Table 2, the plant that exhibited the most significant antibacterial activity against *P. aeruginosa* was *Triumfetta welwitschii*, followed by *Punica granatum* and *Thymus vulgaris* (Phitakin et al. 2016; Lin and Cheng 2019; Mombeshora et al. 2021).

Of the three bacterial strains tested, *P. aeruginosa* was more susceptible to most plant extracts than *Staphylococcus aureus* and *Escherichia coli*. These findings were interesting as Gram-positive bacteria, including *S. aureus*, are generally more susceptible to antibacterial agents, including plant extracts (Sakha et al. 2018).

Mombeshora and Mukanganyama (2019) demonstrated the antibacterial activity of *T. welwitschii* leaf extracts against *P. aeruginosa* and other bacteria; most of the extracts inhibited *P. aeruginosa* compared to other bacterial species tested. It is interesting to note that *E. coli* remained resistant to the plant extracts, which is expected as Gram-negative bacteria have two cellular membranes, one of which consists of lipopolysaccharides (LPS), which serves as a protective barrier to external molecules (Onivogui et al. 2016). However, the results indicated that the LPS layer did not confer the expected protection to *P. aeruginosa* against these plant extracts.

T. welwitschii was found to be capable of significantly increasing membrane permeability and causing nucleic acid leaking from *P. aeruginosa* cells. The loss of membrane integrity (membrane disruption) was found to be the mechanism of action of the plant extracts against the bacteria tested in this study, which could have been contributed by the common phytochemicals, phenols, flavonoids and coumarins (Onivogui et al. 2016).

In a study by Rahman et al. (2017), *Punica granatum* was also shown to have significant antibacterial activity against *P. aeruginosa*. Some researchers suggested that phytochemical components of *P. granatum*, such as ellagitannins, phenols, tannins, punicic acid, flavonoids and flavones, exhibit antibacterial activity. *P. granatum* has tannin-rich ellagitannins that may have antimicrobial properties. It has also been proposed that phenolic compounds are important and are active antibacterial substances whereby they interact with enzymes and other proteins, causing cell membrane disruption and inducing a flow of protons out of the cell, resulting in cell death or blocking the amino acid biosynthesis-related enzymes (Mostafa et al. 2018).

As per data in several published studies, different plant extracts vary in phytochemical compositions, which subsequently can contribute to these plants' antibacterial and anti-biofilm activities. Due to the additional outer layer membrane of LPS in the cell membrane and periplasmic space of Gram-negative bacteria, they are often more resistant to antibacterial agents compared to Gram-positive bacteria. However, from the data in the literature, with *P. aeruginosa*, there seems to be significantly more susceptibility against plant extracts than other Gram-negative bacteria (Ahmed et al. 2021; Mombeshora et al. 2021). Some studies have been conducted to elucidate the mechanisms of action of plant extracts against *P. aeruginosa* and have generalized the action of phytochemicals and their effectiveness against bacterial species. There are resistant mechanisms unique to multi-drug resistant *P. aeruginosa*, for example, the synergism between the efflux system or the AmpC β-lactamase and low outer membrane permeability (Zeb et al. 2017). Antibiotic efflux is one of the most common mechanisms in this opportunistic pathogen, where the antibacterial

Table 2 Antimicrobial activity of selected plants against *S. aureus*, *E. coli* and *P. aeruginosa*

Name of plant	Inhibition zones (mm)*			Reference
	S. aureus	*E. coli*	*P. aeruginosa*	
Thymus vulgaris	17.6 ± 0.31	0.0 ± 0.0	14.7 ± 0.25	Mostafa et al. 2018
Zingiber officinales	15.4±0.23	0.0±0.0	11.2±0.17	Mostafa et al. 2018
Punica granatum	16.3 ± 0.57	14.2 ± 0.61	16.1 ± 0.46	Phitaktim et al. 2016
Triumfetta welwitschia	20.0 ± 1.00	-	46 ± 1.00	Mombeshora et al. 2021
Olea Europaea	8.0±1.00	8.00±1.00	10.00±1.00	Siriwong et al. 2016
Artemisia vulgaris	8±1.00	9±1.00	11±1.00	Walczak et al. 2021
Azadirachta indica	7±1.00	10±1.00	11±1.00	Walczak et al. 2021
Momordica charantia	13±1.00	0±0.0	10±1.00	Rahman et al. 2017
Thymus algeriensis	15.5±1.00	10±1.00	14±1.00	Shah et al. 2017

agent is expelled through the cell wall (Issa et al. 2018). Efflux pump expression from the resistance-nodulation-cell division (RND) family, which is tightly controlled by regulator genes, is closely linked with this resistance mechanism. In response to an extracellular signal, such as the presence of antibiotics or other environmental variables, gene activators and repressors that control the expression of these pumps have been discovered (Issa et al. 2018). Inhibition of these pumps by efficient EPIs (efflux pump inhibitors) would almost certainly reverse MDR's growth. As a result, the efflux pump regulators of *P. aeruginosa* are also a potential therapeutic target of plant phytochemicals. Plants are a rich source of bioactive compounds, including potential EPIs such as alkaloids, and hence play an important role in developing new antimicrobial agents (Shriram et al. 2018; Seukep et al. 2020).

6 Anti-biofilm Activity of Plant Extracts Against *P. aeruginosa*

Several plant extracts can prevent the implantation of sessile groups of microbial cells on the surface, resulting in biofilm formation, inhibiting and preventing biofilm-associated infection (Table 3). In previous studies, plant extracts such as *Berginia ciliata*, *Clematis grata*, *Eucalyptus globulus* and *Triumfetta welwitschia* have demonstrated significant antibiofilm activities against *P. aeruginosa* (Sambyal et al. 2017; Alam et al. 2020; Mombeshora et al. 2021).

Plant-derived anti-biofilm extracts identified against *P. aeruginosa* consist of phytochemicals alkaloids, organosulfur compounds, flavonoids, phenolic compounds, and terpenoids (Guzzo et al. 2020). These are plant secondary metabolites, which are extra nutritional constituents found in meagre amounts in plants that can affect the physiological and cellular activity of patients who consume them.

Alam et al. (2020) studied three plant extracts, of which *B. ciliata* and *C. grata* were found to efficiently inhibit biofilm formation by *P. aeruginosa* at 81% and 80% inhibition, respectively (Alam et al. 2020). Consequently, phytochemical analysis was conducted to provide a basic understanding of the active compounds in these extracts involved in biofilm inhibition. The correlation studies showed that flavonoids, particularly catechin, can potentially inhibit quorum sensing-controlled virulence factors in *P. aeruginosa*, including biofilm production (Aliyu et al. 2016; Gorniak et al. 2019). In similar findings, several types of flavonoids have also been found to have the ability to suppress virulence factors such as pyocyanin and elastase synthesis, as well as biofilm inhibition, without affecting the growth of *P. aeruginosa* (Vandeputte et al. 2011; Kalia et al. 2015).

In another study by Sambyal et al. (2017), the essential oils from plant extracts of *M. alternifolia, S. aromatic, C. zeylanicum* and *E. globulus* oil were investigated to explore their potential as anti-biofilm agents against *P. aeruginosa* and *S. aureus*. In the study, *P. aeruginosa*, producing biofilm, showed 65.43% sensitivity to eucalyptus oil, which was the highest among all the plant oils used, compared to *S. aureus*, which showed 54.16% sensitivity (Sambyal et al. 2017). The findings indicate that certain plant essential oils have anti-biofilm properties against human pathogenic bacteria *P. aeruginosa*. The presence of essential oils is reported in most plant parts; along with this, extracts prepared using ethanol are composed of a variety of chemical components, and their antibacterial activity can be attributed to several distinct mechanisms. The interaction of major essential oils with the cell wall and membrane is the most elucidated action of their function. Compounds such as carvacrol, thymol, and others can disrupt the membrane integrity of the Gram-negative bacteria's envelope and prevent them from growing (Nazzaro et al. 2013).

Furthermore, Mombeshora et al. (2021) investigated the properties of *Triumfetta welwitschia*. They found that this plant is a rich source of phytochemicals that have anti-biofilm and antibacterial properties, including catechin, umbelliferone, and a luteolin derivative, which were shown to reduce the content of capsular polysaccharide in *P. aeruginosa* biofilms by 65% and biofilms' extracellular DNA content by 72%. The eDNA is a structural component of biofilm that binds to the biofilm during formation, protects it against antimicrobials, and is part of *P. aeruginosa* resistance mechanisms. Hence, the release of eDNA is

Table 3 Examples of plants exhibiting antibiofilm activity against *P. aeruginosa*

Plant extract	Main phytochemicals involved	Reference
Berginia ciliata	Flavonoids (cathecin)	Alam et al. 2020
Clematis grata	Flavonoids (cathecin)	
Eucalyptus globulus	Carvacrol, thymol	Sambyal et al. 2017
Triumfetta welwitschia	Catechin, umbelliferone, luteolin derivative	Mombeshora et al. 2021
Hypercium perforatum	Naphthodianthrones, phloroglucinols	Dogan et al. 2019
Terminalia chebula	Ellagic acid derivatives	Munir et al. 2020
Lavandula coronopifolia	Flavones (glucuronides), to triterpenes	Emam et al. 2021

an important part of anti-biofilm agents' mode of action (Tahrioui et al. 2019). Recent studies have shown that coumarins exhibit potent antioxidant, antibacterial, and anti-biofilm activities on Gram-positive and Gram-negative bacteria, particularly Gram-negative bacteria, because of their strong bioactive properties. Although the exact mechanism of coumarins' antibacterial effect is unknown, studies have revealed that they can destroy bacteria's cellular membranes (Yang et al. 2016). Hence, coumarins might be one of the phytochemicals that decrease the amount of eDNA, which may reduce biofilm formation. In addition, a study by Emam et al. (2021) demonstrated that the extracts from *Lavandula coronopifolia* inhibited the biofilm formation in *P. aeruginosa*, which was attributed to the flavons and triterpenes contained in this plant.

Microbial pathogens have developed biofilms, which have enhanced antibiotic resistance. As a result, finding new techniques to reduce biofilms is critical. Plant extracts (leaves, barks, flowers, and roots) include anti-adherence present in flavonoids and anti-QS chemicals present in phenolic compounds aid in increasing bacteria susceptibility and, therefore, eliminating biofilms (Sikic Pogakar et al. 2016; Husain et al. 2017).

Besides, another plant extract from *Hypericum perforatum L.* (HP), a well-known wound healer, was evaluated to determine its anti-biofilm activity against *P. aeruginosa*. Doğan et al. (2019) extracted aerial parts of HP in ethanol solvents, which were found to inhibit the QS systems of *P. aeruginosa*, specifically the LasIR and Rh1IR signalling pathways up to 65.43% and 28.80%, respectively (Dogan et al. 2019). QS regulatory system proteins regulate virulence factor transcription and biofilm formation (Alonso et al., 2020). Quorum inhibitory action has already been discovered in several plant-derived phytochemicals. When employed in high concentrations, phenolic compounds derived from plants have been shown to suppress biofilm production in the PAO1 strain of *P. aeruginosa*. In addition, ellagic acid derivatives from *Terminalia chebula*, as well as aqueous extracts from *Callistemon viminalis*, *Bucida buceras*, and *Conocarpus erectus*, have been shown to inhibit QS of *P. aeruginosa* QS via Rh1R and LasR, while also reducing virulence factors production and enhancing biofilm sensitivity in the PAO1 strain of *P. aeruginosa* (Munir et al. 2020).

Multiple flavonoids in the plant extracts have recently been shown to prevent *P. aeruginosa* biofilm formation, suggesting that they work by disrupting QS signalling (Paczkowski et al. 2017). Their mechanisms of action, however, were not studied. It is recently discovered that novel flavonoids with dihydroxyl moieties in the flavone A-ring backbone, as well as the previously described flavonoids baicalein and quercetin, bind to the QS receptors LasR and RhlR, reducing their ability to bind to DNA encoding QS-regulated promoters. The presence of two hydroxyl groups in the flavone A-ring is required for LasR and RhlR inhibition, according to structure-activity relationship (SAR) analysis (Paczkowski et al. 2017). The flavonoids work via an allosteric mechanism, according to LasR, the representative receptor. In a LasR/RhlR-dependent mechanism, the flavonoids decrease virulence factor synthesis and swarming. These are the first noncompetitive QS inhibitors to target LasR/RhlR and impede DNA binding. They antagonize the autoinducer-binding receptors, LasR and RhlR, which impair quorum sensing. According to the structure-activity relationship analysis, including two hydroxyl moieties in the flavone A-ring backbone is necessary to inhibit LasR/RhlR strongly. According to biochemical analysis, the flavonoids block LasR/RhlR DNA binding non-competitively. The flavonoids decrease virulence factor synthesis and modify the transcription of quorum sensing-controlled target promoters (Paczkowski et al. 2017).

To date, most of the research has been focused on the action of phytochemicals on the down-regulation of certain quorum-sensing genes, which impact the QS ability of *P. aeruginosa*. This has significantly contributed to the antibiofilm activity of plant extracts against the bacterium. However, there could be more mechanisms of action, but they have not been elucidated yet.

Another significant contribution of plant extracts towards effective antimicrobial and anti-biofilm activity against *P. aeruginosa* infections could be in combination with currently used antibiotics. When combined with antibiotics, the plant extracts may boost its effectiveness against harmful microorganisms.

7 Combination of Plant Extracts with Antibiotics as an Optional Treatment Against *P. aeruginosa*

Using plant extracts as RMAs is an increasingly researched topic to combat bacterial drug resistance. In the case of RMAs, multiple studies have demonstrated that plant-derived compounds can be employed to boost antibiotic treatment efficacy (Patel and Patel 2016). Self-medication with various plant items from herbal suppliers and natural food stores is becoming increasingly popular as people become more aware of antibiotic resistance issues (Patel and Patel 2016).

Hence, another focus could be on combining these plants with antibiotics. From the data reviewed on the antimicrobial and antibiofilm activity of plant extracts (Table 4), plants such as *E. globulus*, *E. camaldulensis*, *T. maroccanus*, and *R. oficinalis* exhibit antibiofilm and antimicrobial activities against *P. aeruginosa* (Reda et al. 2017; Cheesman et al. 2017; Sagar et el. 2020; Abu El-Wafa et al., 2020). Therefore, it would be interesting to see if, when combined with antibiotics, these plant extracts can enhance the activity of antibiotics against *P. aeruginosa* while reducing the possibility of resistance development against antibiotics. Several studies have already been conducted

Table 4 Examples of plant-based antimicrobials used in combination with antibiotics demonstrate successful antimicrobial activity

Plant extract	Antibiotic	Comments	Reference
Eucalyptus globulus	Hyperoside + ceftazidime	Inhibition of quorum sensing-dependent factors and biofilm formation	Sagar et al. 2020
Eucalyptus camaldulensis	Proanthocyanidine/ ellagitannin + ceftriaxone	Cytoplasmic membrane rupture observed in *P. aeruginosa*	Reda et al. 2017
Thymus maroccanus	Caravacrol/thymol + ciprofloxacin	Synergy up to 98% demonstrated against *P. aeruginosa*.	Cheeseman et al. 2017
Rosmarinus officinalis	Rosmaridiphenol + piperacillin	Decrease in biofilm formation	Abu El-Wafa et al. 2020

combining different plants with antibiotics to treat *P. aeruginosa* infections. A study was conducted by Dzotam and Kuete (2017) to determine the antimicrobial activity of several Cameroonian plant extracts in combination with antibiotics against *P. aeruginosa*. The research findings indicated that the tested plants could be used alone or with antibiotics to treat bacterial infections, including the multidrug-resistant bacteria *P. aeruginosa*, as they showed 75% antibacterial activity against the bacterium (Dzotam and Kuete 2017). This suggests that the combination of the two substances may then aid in preventing resistant mutants and restoring antibiotic action. In another study, a combination of antibiotics and extracts of clove, jambolan, pomegranate, and thyme demonstrated significant synergistic activity against a multidrug-resistant isolate of *P. aeruginosa* (Cheesman et al. 2017).

Certain plants have significant antibacterial activity against *P. aeruginosa*, and it is interesting to know that there are natural solutions for developing a drug which could be an alternative to antibiotics against *P. aeruginosa* using plant extracts. Plant-based antimicrobials have powerful antimicrobial properties and can be used alone or in combination with antibiotics to combat the current antibiotic resistance challenge, which aligns with the third United Nations sustainable development goal on good health and well-being.

Detailed research on plant extracts is required as not all show significant activity against *P. aeruginosa*. Some studies show *Justicia flava* and *Myrianthus arboreu* are the plants to which *P. aeruginosa* was not susceptible compared to *S. aureus* or other bacterial species (Rahman et al. 2017). Plant extracts with significant antimicrobial activity against Gram-positive bacteria do not appear to have the same antimicrobial activity against other Gram negative bacteria (Elisha et al. 2017). This might challenge drug development companies as the plants to develop antimicrobial agents against this need to be carefully sourced. Furthermore, finding plants effective against *P. aeruginosa* alone might be economically unviable as drug companies look to produce effective antimicrobial agents against various bacteria. In addition, the quantity of extracted phytochemicals used should be considered to ensure that drug development companies determine the toxicity levels for these compounds before developing them into consumable therapeutic agents. Hence, although plants hold considerable promise as the future of antimicrobial and anti-biofilm agents, much research must be carried out before they can become a commercially viable entity.

Conclusion and Future Prospects

This review showed that plant extracts have significant antimicrobial and anti-biofilm activity and thus have the potential to become alternatives to antibacterial agents. Another advantage of plant extracts over antibiotics is resistance modifying agents that slow the development of bacterial resistance to plant phytochemicals. However, from the drug development perspective, there are certain limitations because all the research that has been done has pointed out a few things. Firstly, plant extracts that are effective against *P. aeruginosa* are not as effective against other gram-negative bacteria. This would make drug development very challenging economically because antibiotics cannot just be developed for *P. aeruginosa* only. The antibacterial is needed to be set against gram-negative pathogens in general. Secondly, not all plants are effective against *P. aeruginosa;* hence, finding the correct plant extract can be challenging. Another reason is that most gram-negative bacteria are not susceptible to the plant extracts. However, *P. aeruginosa* seems unusual in that specific plant extracts that can be used against a broad range of clinically critical gram-negative bacteria must be identified. Also, drug-developing companies take up to ten years to develop a new antibiotic. But it only takes two years for a bacterium to become resistant to that antibiotic. So, there are less new antimicrobials available against resistance pathogens. Thus, alternatives must be sourced. Phytochemicals can be made into therapeutic agents or along with currently used antibiotics. However, to do this, the drug companies need to test toxicity and determine the dosage patients could consume. Plant extracts seem to be the best alternative if scientists can do more research and overcome the issues involved in plant extracts. If suitable plant extracts are extracted, they can be developed as potential drug targets with a broad spectrum of antimicrobial and anti-biofilm activities against gram-negative bacteria, including *P. aeruginosa.* Given the global dimension of this epidemic and its significant health and economic costs, we urgently need to find new solutions and implement new policies to prevent antibiotic resistance. Plants are the safest and most effective solution to these concerns due to the presence of natural

compounds, which have shown phenomenal antimicrobial and anti-biofilm activity on *P. aeruginosa.*

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Conflict of Interest

None of the authors have a conflict of interest to report. This paper has not been submitted for publication in any other journal.

References

Abdel Bar, F. M., Alossaimi, M. A., Elekhnawy, E., Alzeer, M. A. A., Abo Kamer, A., Moglad, E., & ElNaggar, M. H. (2022). Anti-Quorum Sensing and Anti-Biofilm Activity of Pelargonium × hortorum Root Extract against *Pseudomonas aeruginosa*: Combinatorial Effect of Catechin and Gallic Acid. *Molecules*, *27*(22), 7841.

Abreu, A.C., McBain, A.J., & Simoes, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. *Natural Product Reports, 29*(9), 1007-1021.

Abu El-Wafa, W. M., Ahmed, R. H., & Ramadan, M.A. (2020). Synergistic effects of pomegranate and rosemary extracts in combination with antibiotics against antibiotic resistance and biofilm formation of *Pseudomonas aeruginosa*. *Brazilian Journal of Microbiology*, *51*(3),1079-1092.

Ahmed, O.M., Mohamed, H., Salem, W., Afifi, M.M., & Song, Y. (2021). Efficacy of Ethanolic Extract of Syzygium aromaticum in the Treatment of Multidrug-Resistant *Pseudomonas aeruginosa* Clinical Isolates Associated with Urinary Tract Infections. *Evidence-based Complementary and Alternative Medicine : eCAM, 2021*.. doi: 10.1155/2021/6612058.

Alam, K., Farraj, D. A. A., Mah-E-Fatima, S., Yameen, M. A., Elshikh, M. S., et al. (2020). Anti-biofilm activity of plant derived extracts against infectious pathogen- *Pseudomonas aeruginosa* PAO1. *Journal of infection and public health*, *13*(11), 1734–1741. https://doi.org/10.1016/j.jiph.2020.07.007.

Aliyu, A.B., Koorbanally, N.A., Moodley, B., Singh, P., & Chenia, H.Y. (2016). Quorum sensing inhibitory potential and molecular docking studies of sesquiterpene lactones from *Vernonia blumeoides*. *Phytochemistry*, *126*, 23-33

Alonso, B., Fernández-Barat, L., Di Domenico, E. G., Marín, M., Cercenado, E., Merino, I., de Pablos, M., Muñoz, P., & Guembe, M. (2020). Characterization of the virulence of Pseudomonas aeruginosa strains causing ventilator-associated pneumonia. *BMC infectious diseases*, *20*(1), 909. https://doi.org/10.1186/s12879-020-05534-1

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature reviews. Microbiology*, *13*(1), 42–51. https://doi.org/10.1038/nrmicro3380.

Cheesman, M. J., Ilanko, A., Blonk, B., & Cock, I. E. (2017). Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution?. *Pharmacognosy reviews*, *11*(22), 57–72. https://doi.org/10.4103/phrev.phrev_21_17.

Das, A., Das, M.C., Sandhu, P., Tribedi, P., De, U.C., et al. (2017). Antibiofilm activity of *Parkia javanica* against *Pseudomonas aeruginosa*: a study with fruit extract. *RSC advances*, *7*(9), 5497-5513.

Dincer, S., Uslu, F.M., & Delik, A. (2020). Antibiotic resistance in biofilm. *In* Bacterial biofilms. *IntechOpen*. 2020. https://doi:10.5772/intechopen.92388

Doğan, Ş., Gökalsın, B., Şenkardeş, İ., Doğan, A., & Sesal, N. C. (2019). Anti-quorum sensing and anti-biofilm activities of Hypericum perforatum extracts against *Pseudomonas aeruginosa*. *Journal of ethnopharmacology*, *235*, 293–300. https://doi.org/10.1016/j.jep.2019.02.020.

Dzotam, J. K., & Kuete, V. (2017). Antibacterial and antibiotic-modifying activity of methanol extracts from six Cameroonian food plants against multidrug-resistant enteric bacteria. *BioMed Research International*. 1583510. doi: 10.1155/2017/1583510. Epub 2017 Aug 20.

Elisha, I.L., Botha, F.S., McGaw, L.J., & Eloff, J.N. (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complementary and Alternative Medicine*, *17*(1), 1-10.

Emam, M., Abdel-Haleem, D.R., Salem, M.M., Abdel-Hafez, L.J.M., Latif, R.R.A., et al. (2021). Phytochemical Profiling of *Lavandula coronopifolia* Poir. Aerial Parts Extract and Its Larvicidal, Antibacterial, and Antibiofilm Activity Against *Pseudomonas aeruginosa*. *Molecules*, *26*, 1710. https://doi.org/10.3390/molecules26061710

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry*, *6*, 25–64. https://doi.org/10.4137/PMC.S14459.

Famuyide, I.M., Aro, A.O., Fasina, F.O., Eloff, J.N., & McGaw, L.J. (2019). Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African Eugenia and Syzygium (Myrtaceae) species and their selectivity indices. *BMC Complementary and Alternative Medicine, 19*(1), 1-13.

González, J.F., Hahn, M.M., & Gunn, J.S. (2018). Chronic biofilm-based infections: skewing of the immune response. *Pathogens and Disease, 76*(3):fty023. doi: 10.1093/femspd/fty023.

Górniak, I., Bartoszewski, R., & Króliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews, 18*(1), 241-272.

Guzzo, F., Scognamiglio, M., Fiorentino, A., Buommino, E., & D'Abrosca, B. (2020). Plant Derived Natural Products against *Pseudomonas aeruginosa* and *Staphylococcus aureus*: Antibiofilm Activity and Molecular Mechanisms. *Molecules (Basel, Switzerland), 25*(21), 5024. https://doi.org/10.3390/molecules25215024.

Husain, F. M., Ahmad, I., Al-Thubiani, A. S., Abulreesh, H. H., AlHazza, I. M., & Aqil, F. (2017). Leaf Extracts of *Mangifera indica* L. Inhibit Quorum Sensing - Regulated Production of Virulence Factors and Biofilm in Test Bacteria. *Frontiers in microbiology, 8*, 727. https://doi.org/10.3389/fmicb.2017.00727

Issa, H.B., Phan, G., & Broutin, I. (2018). Functional mechanism of the efflux pumps transcription regulators from *Pseudomonas aeruginosa* based on 3D structures. *Frontiers in Molecular Biosciences, 5*, 57.

Jennings, L. K., Storek, K. M., Ledvina, H. E., Coulon, C., Marmont, L. S., et al. (2015). Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(36), 11353–11358. https://doi.org/10.1073/pnas.1503058112.

Kalia, M., Yadav, V. K., Singh, P. K., Sharma, D., Pandey, H., Narvi, S. S., & Agarwal, V. (2015). Effect of Cinnamon Oil on Quorum Sensing-Controlled Virulence Factors and Biofilm Formation in *Pseudomonas aeruginosa*. *PloS one*, *10*(8), e0135495. https://doi.org/10.1371/journal.pone.0135495.

Karuppiah, P., & Rajaram, S. (2012). Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. *Asian Pacific Journal of Tropical Biomedicine, 2*(8), 597-601.

Khare, T., Anand, U., Dey, A., Assaraf, Y. G., Chen, Z. S., Liu, Z., & Kumar, V. (2021). Exploring Phytochemicals for Combating Antibiotic Resistance in Microbial Pathogens. *Frontiers in pharmacology*, *12*, 720726. https://doi.org/10.3389/fphar.2021.720726.

Labovska, S. (2021). *Pseudomonas aeruginosa* as a cause of nosocomial infections. In Das, T. (Ed.), *Pseudomonas aeruginosa - Biofilm Formation, Infections and Treatments*. *IntechOpen*. https://doi.org/10.5772/intechopen.95908

Lin, J., & Cheng, J. (2019). Quorum sensing in *Pseudomonas aeruginosa* and its relationship to biofilm development. In *Introduction to Biofilm Engineering, ACS Symposium Series, Vol. 1323* (pp. 1-16). American Chemical Society, DOI: 10.1021/bk-2019-1323.ch001.

Meng, L., Liu, H., Lan, T., Dong, L., Hu, H., Zhao, S., Zhang, Y., Zheng, N., & Wang, J. (2020). Antibiotic Resistance Patterns of *Pseudomonas* spp. Isolated From Raw Milk Revealed by Whole Genome Sequencing. *Frontiers in microbiology*, *11*, 1005. https://doi.org/10.3389/fmicb.2020.01005.

Mishra, S., Gupta, A., Upadhye, V., Singh, S. C., Sinha, R. P., & Häder, D. P. (2023). Therapeutic Strategies against Biofilm Infections. *Life (Basel), 13*(1):172, doi: 10.3390/life13010172.

Mombeshora, M., Chi, G.F., & Mukanganyama, S. (2021). Antibiofilm activity of extract and a compound isolated from *Triumfetta welwitschii* against *Pseudomonas aeruginosa*. *Biochemistry Research International, 2021*. https://doi.org/10.1155/2021/9946183

Mombeshora, M., & Mukanganyama, S. (2019). Antibacterial activities, proposed mode of action and cytotoxicity of leaf extracts from *Triumfetta welwitschii* against *Pseudomonas aeruginosa*. *BMC complementary and alternative medicine*, *19*(1), 315. https://doi.org/10.1186/s12906-019-2713-3

Montero, M.M., Lopez Montesinos, I., Knobel, H. et al. (2020). Risk factors for mortality among patients with *Pseudomonas aeruginosa* bloodstream infections, *Journal of Clinical Medicine* ,*9*,(2), 514.

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi journal of biological sciences*, *25*(2), 361–366. https://doi.org/10.1016/j.sjbs.2017.02.004.

Munir, S., Shah, A. A., Shahid, M., & Manzoor, I. (2020). Quorum sensing interfering strategies and their implications in the management of biofilm-associated bacterial infections. *Brazilian Archives of Biology and Technology, 63*, e20190555.

Munita, J.M., & Arias, C.A. (2016). Mechanisms of antibiotic resistance. Virulence mechanisms of bacterial pathogens. *Microbiology Spectrum, 4*(2), 10.1128/microbiolspec.VMBF-0016-2015 481-511.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals (Basel, Switzerland), 6*(12), 1451–1474. https://doi.org/10.3390/ph6121451

Onivogui, G., Letsididi, R., Diaby, M., Wang, L., & Song, Y. (2016). Influence of extraction solvents on antioxidant and antimicrobial activities of the pulp and seed of *Anisophyllea laurina* R. Br. ex Sabine fruits. *Asian Pacific Journal of Tropical Biomedicine, 6*(1), 20-25.

Owusu, E., Ahorlu, M. M., Afutu, E., Akumwena, A., & Asare, G. A. (2021). Antimicrobial Activity of Selected Medicinal Plants from a Sub-Saharan African Country against Bacterial Pathogens from Post-Operative Wound Infections. *Medical sciences (Basel, Switzerland), 9*(2), 23. https://doi.org/10.3390/medsci9020023.

Pachori, P., Gothalwal, R., & Gandhi, P. (2019). Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes and Diseases, 6*(2),109-119. doi: 10.1016/j.gendis.2019.04.001.

Paczkowski, J. E., Mukherjee, S., McCready, A. R., Cong, J. P., Aquino, C. J., et al. (2017). Flavonoids Suppress *Pseudomonas aeruginosa* Virulence through Allosteric Inhibition of Quorum-sensing Receptors. *The Journal of biological chemistry, 292*(10), 4064–4076. https://doi.org/10.1074/jbc.M116.770552.

Papaleo, S., Alvaro, A., Nodari, R., Panelli, S., Bitar, I., & Comandatore, F. (2022). The red thread between methylation and mutation in bacterial antibiotic resistance: How third-generation sequencing can help to unravel this relationship. *Frontiers in microbiology, 13*, 957901. https://doi.org/10.3389/fmicb.2022.957901

Patel, V., & Patel, R. (2016). Plants as latent sources of new antimicrobials and resistance modifying agents against multi drug resistant (MDR) strains. *International Journal of Biochemistry and Molecular Biology, 4*, 27-42.

Phitaktim, S., Chomnawang, M., Sirichaiwetchakoon, K., Dunkhunthod, B., Hobbs, G., & Eumkeb, G. (2016). Synergism and the mechanism of action of the combination of α-mangostin isolated from Garcinia mangostana L. and oxacillin against an oxacillin-resistant Staphylococcus saprophyticus. *BMC microbiology, 16*(1), 195. https://doi.org/10.1186/s12866-016-0814-4

Rahman, M.M., Shahriar, M.R., Sultana, N., & Ishika, T. (2017). Antimicrobial activity of some medicinal plant extracts against Gram positive and 38 Gram negative bacteria in Bangladesh. *Asian Journal of Medical and Biological Research, 3*(4), 405-411.

Ramírez-Estrada, S., Borgatta, B., & Rello, J. (2016). *Pseudomonas aeruginosa* ventilator-associated pneumonia management. *Infection and drug resistance, 9*, 7–18.

Recio, R., Mancheño, M., Viedma, E., Villa, J., Orellana, M. Á., Lora-Tamayo, J., & Chaves, F. (2020). Predictors of Mortality in Bloodstream Infections Caused by *Pseudomonas aeruginosa* and Impact of Antimicrobial Resistance and Bacterial Virulence. *Antimicrobial agents and chemotherapy, 64*(2), e01759-19. https://doi.org/10.1128/AAC.01759-19.

Reda, F.M., El-Zawahry, Y.A, & Omar, A.R. (2017). Synergistic effect of combined antibiotic and methanol extract of *Eucalyptus camaldulensis* leaf against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *International Journal of Applied Sciences and Biotechnology, 5*(4), 486-497.

Roy, R., Tiwari, M., Donelli, G., & Tiwari, V. (2018). Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. *Virulence, 9*(1), 522–554. https://doi.org/10.1080/21505594.2017.1313372.

Sagar, P. K., Sharma, P., & Singh, R. (2020). Antibacterial efficacy of different combinations of clove, eucalyptus, ginger, and selected antibiotics against clinical isolates of *Pseudomonas aeruginosa*. *Ayu, 41*(2), 123–129. https://doi.org/10.4103/ayu.AYU_101_19.

Sakha, H., Hora, R., Shrestha, S., Acharya, S., Dhakal, D., Thapaliya, S., & Prajapati, K. (2018). Antimicrobial Activity of Ethanolic Extract of Medicinal Plants against Human Pathogenic Bacteria. *Tribhuvan University Journal of Microbiology, 5*, 1–6. https://doi.org/10.3126/tujm.v5i0.22292.

Sambyal, S. S., Sharma, P., & Shrivastava, D. (2017). Anti-biofilm activity of selected plant essential oils against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *International Journal of Current Microbiology and Applied Sciences, 6*(3), 444-450.

Seukep, A. J., Kuete, V., Nahar, L., Sarker, S. D., & Guo, M. (2020). Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *Journal of pharmaceutical analysis, 10*(4), 277–290. https://doi.org/10.1016/j.jpha.2019.11.002.

Shah, F., Hasan, Z., & Zaidi, K.U. (2017). Phytochemical constituents and synergistic activity of *Olea europaea* plant

extracts against some human disease-causing species. *Journal of Microbiology and Experimentation*, *4*(5), 00127.

Shriram, V., Khare, T., Bhagwat, R., Shukla, R., & Kumar, V. (2018). Inhibiting Bacterial Drug Efflux Pumps via Phyto-Therapeutics to Combat Threatening Antimicrobial Resistance. *Frontiers in microbiology*, *9*, 2990. https://doi.org/10.3389/fmicb.2018.02990

Šikić Pogačar, M., Klančnik, A., Bucar, F., Langerholc, T., & Smole Možina, S. (2016). Anti-adhesion activity of thyme (*Thymus vulgaris* L.) extract, thyme post-distillation waste, and olive (*Olea europea* L.) leaf extract against Campylobacter jejuni on polystyrene and intestine epithelial cells. *Journal of the science of food and agriculture*, *96*(8), 2723–2730. https://doi.org/10.1002/jsfa.7391.

Sionov, R.V., & Steinberg, D. (2022). Targeting the Holy Triangle of Quorum Sensing, Biofilm Formation, and Antibiotic Resistance in Pathogenic Bacteria. *Microorganisms*, *10*, 1239. https://doi.org/10.3390/microorganisms10061239

Siriwong, S., Teethaisong, Y., Thumanu, K., Dunkhunthod, B., & Eumkeb, G. (2016). The synergy and mode of action of quercetin plus amoxicillin against amoxicillin-resistant Staphylococcus epidermidis. *BMC pharmacology & toxicology*, *17*(1), 39. https://doi.org/10.1186/s40360-016-0083-8.

Tahrioui, A., Duchesne, R., Bouffartigues, E., Rodrigues, S., Maillot, O., Tortuel, D., et al.. (2019). Extracellular DNA release, quorum sensing, and PrrF1/F2 small RNAs are key players in *Pseudomonas aeruginosa* tobramycin-enhanced biofilm formation. *NPJ biofilms and microbiomes*, *5*(1), 15. https://doi.org/10.1038/s41522-019-0088-3

Taylor, P.K., Yeung, A.T., & Hancock, R. E. (2014). Antibiotic resistance in *Pseudomonas aeruginosa* biofilms: towards the development of novel anti-biofilm therapies. *Journal of Biotechnology*, *191*, 121-130

Ulloa-Urizar, G., Aguilar-Luis, M. A., De Lama-OdrÝa, M.D.C., Camarena-Lizarzaburu, J., & del Valle Mendoza, J. (2015). Antibacterial activity of five Peruvian medicinal plants against *Pseudomonas aeruginosa*. *Asian Pacific Journal of Tropical Biomedicine*, *5*(11), 928-931.

Vandeputte, O. M., Kiendrebeogo, M., Rasamiravaka, T., Stévigny, C., Duez, P., et al. (2011). The flavanone naringenin reduces the production of quorum sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. *Microbiology (Reading, England)*, *157*(Pt 7), 2120–2132. https://doi.org/10.1099/mic.0.049338-0.

Vetrivel, A., Ramasamy, M., Vetrivel, P., Natchimuthu, S., Arunachalam, S., Kim, G.S., & Murugesan, R. (2021). *Pseudomonas aeruginosa* Biofilm Formation and Its Control. *Biologics*, *1*(3), 312–336. http://dx.doi.org/10.3390/biologics1030019

Walczak, M., Michalska-Sionkowska, M., Olkiewicz, D., Tarnawska, P., & Warżyńska, O. (2021). Potential of Carvacrol and Thymol in Reducing Biofilm Formation on Technical Surfaces. *Molecules (Basel, Switzerland)*, *26*(9), 2723. https://doi.org/10.3390/molecules26092723.

Yang, L., Ding, W., Xu, Y., Wu, D., Li, S., Chen, J., & Guo, B. (2016). New Insights into the Antibacterial Activity of Hydroxycoumarins against Ralstonia solanacearum. *Molecules (Basel, Switzerland)*, *21*(4), 468. https://doi.org/10.3390/molecules21040468.

Zeb, A., Ullah, I., Rehman, H.U., et al. (2017). Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* in tertiary care hospital. *Journal of Entomology and Zoology Studies*, *20*, 50.