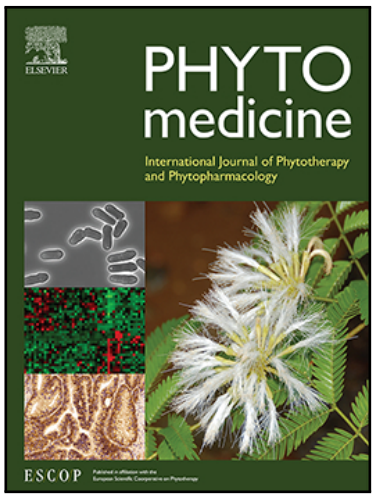

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Evolving biofilm inhibition and eradication in clinical settings through plant-based antibiofilm agents

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Abstract

Background: After almost 100 years since evidence of biofilm mode of growth and decades of intensive investigation about their formation, regulatory pathways and mechanisms of antimicrobial tolerance, nowadays there are still no therapeutic solutions to eradicate bacterial biofilms and their biomedical related issues.

Purpose: This review intends to provide a comprehensive summary of the recent and most relevant published studies on plant-based products, or their isolated compounds with antibiofilm activity mechanisms of action or identified molecular targets against bacterial biofilms. The objective is to offer a new perspective of most recent data for clinical researchers aiming to prevent or eliminate biofilm-associated infections caused by bacterial pathogens.

Methods: The search was performed considering original research articles published on PubMed, Web of Science and Scopus from 2015 to April 2023, using keywords such as "antibiofilm", "antivirulence", "phytochemicals" and "plant extracts".

Results: Over 180 articles were considered for this review with a focus on the priority human pathogens listed by World Health Organization, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. Inhibition and detachment or dismantling of biofilms formed by these pathogens were found using plant-based extract/products or derivative compounds. Although combination of plant-based products and antibiotics were recorded and discussed, this topic is currently poorly explored and only for a reduced number of bacterial species.

Conclusions: This review clearly demonstrates that plant-based products or derivative compounds may be a promising therapeutic strategy to eliminate bacterial biofilms and their associated infections. After thoroughly reviewing the vast amount of research carried out over years, it was concluded that plant-based products are mostly able to prevent biofilm formation through inhibition of quorum sensing signals, but also to disrupt mature biofilms developed by multidrug resistant bacteria targeting the biofilm extracellular polymeric substance. Flavonoids and phenolic compounds seemed the most effective against bacterial biofilms.

Keywords:

Plant extract, plant-based compounds, biofilm, antibiofilm activity, antivirulence activity, synergism

Abbreviations

AHL - N-acyl homoserine lactone

AMB - Activity on mature/preformed biofilms

AQSV - anti-quorum sensing/anti-virulence

CF - cystic fibrosis

CviR - cytoplasmic DNA binding transcription factor

DSF - diffusible signal factos

EPS - Extracellular polymeric substance

IBF - Inhibition of biofilm formation

MIC - minimum inhibitory concentration

MRSA - methicillin resistant *Staphylococcus aureus*

MSSA - methicillin sensitive *Staphylococcus aureus*

QS – quorum sensing

QSI - quorum sensing inhibitors

WHO – World Health Organization

1. Introduction

Chronic microbial infections have become one of the major health care problems worldwide due to the rise of multidrug resistance of pathogens. Bacterial infections by multidrug resistant pathogens are especially problematic to immunocompromised and hospitalized patients since they are most at risk to develop chronic infections, leading to significant morbidity and mortality (Christaki et al., 2020; McEwen and Collignon, 2018). Over the years theoretical chemistry and bioinformatics have delivered tools to design specific molecules with target functionalities. However, modern technology appears to be reaching its limit and it has not resulted in the expected drug productivity and efficiency to combat antibiotic resistance. In contrast, nature has millions of years of evolution in creating solutions for survival and adaptation to different environments and stressful conditions and have become one of the most important resources for developing new lead compounds and scaffolds. The interest of the scientific community on plant products in the healthcare field, in particular the investigation of plant-based products for the treatment of infectious diseases is clearly growing. This trend will continue or even increase in the next years because the plant kingdom is still a reservoir of unknown bioactive compounds. Regarding infectious diseases, the scientific community has essentially turned to plants to find new products with antimicrobial activity in an attempt to efficiently treat microbial chronic infections. However, clinicians and researchers understood that the global crisis of antibiotic resistance will not be solved only by seeking antimicrobial compounds. Much of the literature generally agrees that the major cause of chronic microbial infections is the presence of biofilms and according to the U.S. National Institute of Health (NIH), 80% of chronic infections are associated with biofilm formation (Jamal et al., 2018). These microbial communities are structured consortia of microorganisms, embedded in a self-produced matrix able to tolerate up to 1,000-fold higher concentrations of antimicrobial agents than those required to inhibit their planktonic (free living) counterparts (Römling and Balsalobre, 2012; Vestby et al., 2020).

The substantial clinical impact of biofilms has led researchers in the last decades to intensively investigate the biofilm mode of growth, their regulatory pathways, and their antimicrobial mechanisms of resistance to get closer to the ultimate goal, biofilm eradication. Researchers understood that the current pharmacopeia lack compounds able to eradicate biofilm-associated infections, including antibiotics that forces community to seek alternative therapeutic strategies, and nature continues to be considered as an origin of transformative drugs including antibiofilm agents. Therefore, the aim of this review is to indicate promising

plant-based antibiofilm compounds, and provide inspiration, or new starting points for the development of antibiofilm agents for main problematic human pathogens. Once bacterial pathogenic biofilms are currently a remarkable challenge in diverse human activities such as in veterinary, food, environmental and clinical field, there are an increased number of reviews (Ćirić et al., 2019; Nuță et al., 2021; Slobodníková et al., 2016), but this review intends to offer a distinct perspective of the use of plant products on clinical biofilms. Typically reviews of antibiofilm plant-based agents present a quite similar organization and discussion of the information by, for instance, plant or extract (Lu et al., 2019), phytochemicals (Lu et al., 2019; Melander et al., 2020; Shamim et al., 2023; Song et al., 2018), or mechanism of action (Rossi et al., 2022) lacking a discussion from a clinical point of view. In clinical field researchers deal with a specific infectious condition (e.g. skin, urinary, vaginal infections, indwelling infections) caused by a specific pathogen or a limited number of pathogens and they aim to easily access to the information about the antibiofilm plant products against their specific target species. The lack of a review with a clinical comprehensive overview of the recent findings of antibiofilm activity of plant-based compounds and the potential synergy with antibiotics for the most relevant human health threatening bacterial species implies that clinical researchers (and others) have to read a significant amount of information not related to their target species to identify possible antibiofilm products. Therefore, this review aims to provide a balanced high-yield resource covering the biofilm thematic and discussing the most relevant and sound science of antibiofilm plant products or compounds to advance drug discovery targeting the most difficult bacterial biofilm-associated infections to treat. Moreover, this review discusses the limitations and challenges posed by biofilms and the use of plant-based products that have contributed for few plant-based candidates in clinical trial testing and regulatory approval.

The first and second section of this review summarizes the biofilm development stages and the different biofilm targeting approaches, respectively. Examples of successful plant-based products with antibiofilm activity will be reviewed for the priority bacterial species determined by World Health Organization (WHO), including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* (WHO, 2017). In the third section, the potential synergy of combining plant-based antibiofilm products with antibiotics are discussed despite the quite reduced number of studies addressing this topic. In the last section, the limitations, challenges, difficulties of handling natural products are debated as well as the opportunities to advance drug discovery and development from plants.

2. Biofilms

Bacteria are capable to develop biofilms in the human tissues, such as infections of skin and soft tissues, pneumonia in cystic fibrosis (CF) patients, chronic otitis media, recurrent urinary tract infections, meningitis, endocarditis, periodontitis and dental caries; and in medical devices including catheters, ventilator tubes or organ replacements (prothesis, pacemakers and others) (Høiby et al., 2010b; Römling and Balsalobre, 2012; Taylor et al., 2014; Vestby et al., 2020). Biofilm formation has been described for several bacterial species (O'Toole et al., 2000; Rabin et al., 2015; Vetrivel et al., 2021) and, in general, the development of biofilms occurs in three main steps: attachment, maturation, and dispersion/detachment. It starts with the attachment of planktonic cells to a biotic or abiotic surface (reversible attachment) and progress to irreversible attachment to the surface and to each other as a result of extracellular matrix production. The continued growth and division of cells within the biofilm leads to the formation of a more mature biofilm with full developed three-dimensional structure. Eventually, some cells can detach from the biofilm becoming motile and capable of spreading the infection, establishing new biofilms in other infection sites, worsening the clinical outcome (de la Fuente-Núñez et al., 2013; Vestby et al., 2020). Although the multi-step process of biofilm formation is understood, the expression and regulation of the mechanisms during biofilm formation stages of the distinct bacterial species is diverse and still unclear.

The major challenge of biofilms is their drug tolerance resulting from the combination of several mechanisms, including restricted penetration of antimicrobials through the matrix, bacterial slow growth and phenotypic diversity caused by microscale chemical gradients, and quorum sensing molecules (de la Fuente-Núñez et al., 2013; Høiby et al., 2010a; Mah, 2012; Rabin et al., 2015; Sousa et al., 2013). Among these mechanisms, it is uncontested the importance of the extracellular matrix in biofilm tolerance. The extracellular matrix produced by bacteria is composed of exopolysaccharides, proteins, extracellular deoxyribonucleic acid (DNA) and lipids, and acts as a protective barrier that prevents the penetration of antibiotics, resulting in an increased tolerance (Chen et al., 2013; Mah, 2012; Roy et al., 2018). Moreover, biofilm matrix can also be a fundamental mechanism of antibiotic tolerance in an "indirect way". This barrier also limits the diffusion and distribution of nutrients and oxygen along the biofilm depth, sparking microbial organization into a wide range of distinct subpopulations within the biofilm strata with diversified metabolic profiles, genetic programs, spatial segregation, and differential stress responses (Mah, 2012; Penesyan et al.,

2021; Roy et al., 2018; Walters et al., 2003; Yan and Bassler, 2019). Decreased metabolic activity of the cells within the biofilm is one of the most impacting features on the efficacy of antibiotics. Even if antibiotic molecules penetrate into biofilm matrix, biofilm subpopulations displaying differential growth and reduced metabolic rates, antagonize the action of the antimicrobials since most of them target biological processes during aerobic respiration (e.g. DNA replication, translation, cell wall synthesis) (Jensen et al., 2017; Ryall et al., 2012; Stewart and Franklin, 2008; Walters et al., 2003). Therefore, antibiotics typically are able of clearing the outer layers of biofilms where active growing cells are located (considering the most common top-to-bottom gradient of decreasing metabolic activity), and present reduced cell killing into the middle and inner layers (Soares et al., 2019; Walters et al., 2003; Williamson et al., 2012). The nutritional and oxygen constraints within the biofilm, and the cellular exposure to antimicrobials can also be a driving force for a small fraction of the population enter into a dormant state. This fraction of the population called persister cells or persisters or dormant cells comprise around 1% of the cells within the biofilm and are highly tolerant to antibiotics without undergoing genetic changes (Conlon et al., 2015; Hall and Mah, 2017). They can survive and remain viable even if the biofilm population is eradicated during an antibiotic treatment and after antibiotic level drops, persister cells can repopulate and originate a biofilm (Grassi et al., 2017; Soares et al., 2019). By this reason, the persister cells are frequently associate to the relapse of infection (Lewis, 2008; Soares et al., 2019).

Bacteria within biofilms have a regulatory mechanism that acts as a communication system between the cells, named quorum sensing (QS) (Al-Wrafy et al., 2017). This system, mediated by small signalling molecules known as auto-inducers, responds to changes in the cell-population density to synchronize gene expression and control cellular behaviour. QS controls several processes and phenotypic behaviours, including stress resistance, expression of virulence factors, and biofilm formation. The relevance of QS leads researchers to put great efforts on understanding how biofilm development and QS are interconnected and consequently several studies have been published disclosing the regulatory mechanisms for several species, especially on *P. aeruginosa* (Balestrino et al., 2005; Li et al., 2007; Solano et al., 2014; Yarwood et al., 2004). *P. aeruginosa* is one of the most threatening pathogens to human health, and its virulence potential is widely regulated by the QS system. It is constituted by the lasI/R system which plays the leading role in regulating the activity of the rhlI/R. The las system controls the production of virulence factors such as LasB elastase, LasA protease, alkaline protease, exotoxin A and biofilm formation, while the rhl controls the production of virulent pigments, such as pyocyanin and pyoverdine, and rhamnolipids

(Balestrino et al., 2005; Li et al., 2007; Solano et al., 2014; Yarwood et al., 2004). These virulence factors are involved in cellular toxicity and acute infection development. Also, QS has been intensively studied on gram-positive bacteria. In *S. aureus*, a leading cause of nosocomial infections worldwide, the accessory gene regulator system (agr) mediates the QS system through the production and sensing of a secreted cyclic peptide signal (autoinducing peptide) (Abisado et al., 2018; Solano et al., 2014; Yarwood et al., 2004). The role of agr in biofilm formation is controversial. Some evidence has pointed that agr is need for biofilm formation while others demonstrated that dysfunction of agr might promote enhanced ability to form biofilms (Traber et al., 2008; Vuong et al., 2000; Yarwood et al., 2004). This kind of contradictory evidence well demonstrate the complexity of studying and combating biofilms.

Human infections including biofilm-associated infections are frequently polymicrobial and this feature can complicate the design of antibiotic therapies. Interspecies interaction within biofilms is frequently achieved through QS signals (Abisado et al., 2018; Federle and Bassler, 2003). For instance, *P. aeruginosa* and *S. aureus* are common etiological agents of several polymicrobial infections, including wounds, otitis media and oral infections and they are frequently isolated together from CF lungs (Reece et al., 2021). Their interspecies interactions can influence the pathogenesis of *P. aeruginosa*, contributing for its persistence and worsening of the patient's condition (Magalhães et al., 2017). The cohabitation with *S. aureus* hinders the host immune response against *P. aeruginosa* and increases its virulence and tolerance to antibiotics (Beaudoin et al., 2017). In turn, several studies have shown that some molecules produced by *P. aeruginosa*, such as 4-hydroxy-2-heptylquinoline N-oxide (HQNO), a molecule pertaining to the *Pseudomonas* quinolone signal (PQS) QS system pathway, is able to suppress *S. aureus* planktonic growth, while protecting it from aminoglycosides by inhibition of electron transport through cytochrome b in *S. aureus* (Hoffman et al., 2006; Orazi and O'Toole, 2017; Painter et al., 2015). Moreover, it was showed that HQNO increased *S. aureus* biofilm formation, and long-term exposure was shown to induce formation of small colony variants known to be highly resistant to several antibiotics (Mitchell et al., 2010). Also, interspecies communications between *Stenotrophomonas maltophilia* and *P. aeruginosa* can be achieved through the action of some fatty acids produced by *S. maltophilia*, named the diffusible signal factors (DSF: cis-11-methyl-2-dodecenoic acid). The formation of *S. maltophilia* and *P. aeruginosa* mixed biofilms is likely to occur in CF lungs in which several types of cis-2-unsaturated fatty acids were found in CF sputum, supporting the hypothesis that both species can communicate and interact (Twomey et al., 2012) . Communication by DSF can modulate the architecture of *P.*

aeruginosa in vitro biofilms from flat to filamentous structure when *S. maltophilia* is present (Ryan et al., 2008). Moreover, perception of DSF can also promote on *P. aeruginosa* the abundance of proteins that contribute to stress tolerance and increase virulence, including FliC (flagellin), AhpC (alkyl hydroperoxide reductase) and Adk (adenylate kinase) (Ryan et al., 2008). These alterations could contribute for *P. aeruginosa* chronic infection development in CF lungs.

Overall, compelling evidence obtained in different bacterial species coincides that biofilm eradication is a tricky process due to its multifactorial antimicrobial tolerance that antibiotics in general are not able to eliminate. To be successful implies a shift of the current therapeutic paradigm making imperative to find compounds able to inhibit, disrupt and/or dismantle biofilms. Biofilm-associated infections could be successfully eradicated if synergies between antibiotics and natural or plant-based antibiofilm agents were found. In the next sections, it will be provided a comprehensive assessment of the latest data and representative number of the plant-based compounds studied as antibiofilm agents and evidences on their potential adjuvant effect when combined with antibiotics against the most health-threatening antibiotic-resistant bacteria following the priority list divulgated by WHO (WHO, 2017). For this review, the research was performed on three databases, PubMed, Web of Science and Scopus, considering only original research articles published from 2015 to April 2023 and using the keywords “antibiofilm”, “antivirulence”, “phytochemicals” and “plant extracts”.

3. Antibiofilm activity of plant-based fractions/compounds

Antibiofilm agents can be considered the molecules or compounds that can inhibit the formation of biofilms mainly by tackling the initial adhesion, extracellular polymeric substance (EPS) production, or disrupt or dismantle pre-formed and mature biofilms by targeting maturation and dispersal or detachment stages. Interestingly, plant-based products proved to be effective in both antibiofilm approaches (Figure 1 and Table 1).

The benefits of preventing biofilm formation rather than eradicating are obvious and include decreased risk of the emergence of multidrug resistant phenotypes, and non-development of chronic infections which are much more difficult to eradicate and harmful to the host cells (Chen et al., 2013; Roy et al., 2018; Solano et al., 2014). Inhibition of the bacterial adhesion to a surface is mainly achieved by surface-coatings altering the superficial properties of the surface (e.g. hydrophobicity) or impregnating the surface with antimicrobial molecules. For instance, Trentin et al. (2015) showed that proanthocyanidins, a compound

isolated from the leaves of *Pityrocarpa moniliformis* (Benth.) Luckow & R.W.Jobson were capable of prevent the *Staphylococcus epidermidis* initial attachment to the surface by changing the characteristics of the surface, which in turn reduced the biofilm formation without inhibiting the planktonic growth.

Another interesting strategy for inhibition of biofilm formation is targeting the virulence potential of bacteria (Figure 1). This is considered one of the most promising and effective approaches with the advantage to better control the emergence and dissemination of antibiotic resistant phenotypes. Several virulence factors are involved in the initial steps of formation and maturation of biofilms and controlled by the QS. Consequently, QS constitutes one of the most exploited targets for the development of anti-virulence and antibiofilm drugs (Paluch et al., 2020; Rasmussen and Givskov, 2006; Zhou et al., 2020) and numerous QS inhibitors (QSI) have been found in plants (Figure 1) (Burt et al., 2014; Cheng et al., 2020; Ćirić et al., 2019; Das and Mehta, 2018; Noumi et al., 2018; Rama Devi et al., 2016; Wang et al., 2019). Carvacrol reduced the expression of two virulence factors, violacein and chitinase activity regulated by the QS in *Chromobacterium violaceum* (Burt et al., 2014). Likewise, an essential oil of *Melaleuca bracteata* F.Muell (golden tea tree*)* rich in methyleugenol inhibited violacein production, and suppressed the production of C6-HSL, a signalling molecule involved in the QS of *C. violaceum* (Wang et al., 2019). Rosmarinic acid also supressed several virulence factors, such as the production of hemolysin, lipase and elastase in *Aeromonas hydrophila* (Rama Devi et al., 2016).

EPS-targeting strategies are also the most antibiofilm approaches studied because EPS is responsible for the adherence of cells to the surfaces and to each other, as well as for structural stability and protection to the biofilm against external aggressions or stresses (de la Fuente-Núñez et al., 2013; Mah, 2012; Rabin et al., 2015). Therefore, products that destabilize the EPS matrix are an effective way of attacking biofilms. EPS targeting can occur in the early stages of biofilm formation by inhibiting the EPS production, which will interrupt the biofilm cycle averting maturation; or in pre-formed biofilms by disrupting or degrading the matrix, allowing biofilm-cells exposure to antibiotics and increasing their cellular uptake, while also promoting bacterial dispersal of the cells within the biofilm (Figure 1) (Chen et al., 2013; Roy et al., 2018). For example, a cranberry extract rich in polyphenols was capable of reducing the biofilm matrix production in *Vibrio cholerae*, which in turn inhibited the biofilm formation during the initial development (Pederson et al., 2018).

At first sight, it seems very straightforward to eliminate biofilms, but there are several hitches. For example, the disassembly of biofilms by dispersal or disruption can cause a

massive release of bacteria, which can represent a serious risk to patients. For instance, bacteria can enter into the bloodstream leading to sepsis if this release is not combined with antibiotics to eliminate the released cells (Minasyan, 2019). Moreover, fractions of biofilm-cells might not exhibit antibiotic susceptibilities identical to planktonic counterparts because biofilms encompass quite different phenotypes that may be resistant to several antibiotics unlike the planktonic cells. Even if an antibiofilm compound was able to significantly reduce biofilm biomass (cells and matrix), biofilms can contain persister cells that, once the treatment has stopped, are capable of generate a new biofilm with the same characteristics as the original one, restarting the infection (Lewis, 2008). These cells remain viable and keep persisting after each treatment turning this infection in a vicious cycle that can only be truly stopped if the persister cells are completely eradicated (Conlon et al., 2015; Hall and Mah, 2017; Soares et al., 2019; Wood et al., 2013). Therefore, it is reasonable to assume that to effectively inhibit biofilm formation or disassemble pre-formed biofilms there is the need to master knowledge about biofilm formation, physiology, matrix composition, microbial composition and stratification. It is also clear that inhibition of biofilm formation is more feasible than disassemble pre-formed biofilms in which the complex three-dimensional structure and the intricated mechanisms of antibiotic tolerance (e.g. distinct physiological state of cells and dense extracellular matrix) are well established. The lower potential activity for eradication or at least reduction of preformed biofilms than for inhibition of biofilm formation is verified for all kind of drugs, including antibiotics and plant-derived products (Galvão et al., 2020; Hengzhuang et al., 2011; Silva et al., 2020). Accordingly, literature has accumulated a wide range of evidence that demonstrated the main antibiofilm activity of plant-based products relies on inhibition of bacterial adhesion to surfaces, reduction of matrix production, attenuation of virulence factors expression and blocking QS as demonstrated in Table 1. The plant extracts or their isolated compounds with antibiofilm activity against most human life-threatening bacteria will be discussed in more detailed in the next sections.

3.1. *Pseudomonas aeruginosa*

P. aeruginosa is a gram-negative opportunistic pathogen in hospitalized or immune-compromised patients causing a wide range of infections, including lung infections in people with CF and chronic obstructive lung disease, infections in burns, and wounds, as well as it causes urinary tract and gastrointestinal infections, otitis media, keratitis and ventilator-associated pneumonia in intubated patients (Høiby et al., 2010b; Taylor et al., 2014; Thi et al., 2020; Vetrivel et al., 2021). Since biofilms are the responsible for the majority of *P. aeruginosa* chronic infections, they are one of the most well studied biofilms worldwide (Thi et al., 2020; Vetrivel et al., 2021) and, consequently, there are countless studies trying to tackle *P. aeruginosa* biofilms, including through the application of plant-based products (Table 1).

One of the most studied plants against biofilms is *Centella asiatica* (L.) Urb., commonly known as Indian Pennyworth, whose leaves have been used in African and Chinese medicine to treat skin problems and heal wounds (Hamid et al., 2002). Vasavi et al. (2016) reported that ethyl acetate fraction of *C. asiatica* exhibited anti-QS activity against *P. aeruginosa*. The leaves extract inhibited some QS-related traits such as pyocyanin production, elastolytic and proteolytic activities, swarming motility and it also reduced the initial formation of biofilm. The ethyl acetate fraction of *C. asiatica* also exhibited anti-QS activity against *C. violaceum* which led to consider that this extract somehow modulated the interaction of N-acyl homoserine lactone (AHL) with the cytoplasmic DNA binding transcription factor (CviR) that activates gene expression (Stauff and Bassler, 2011). This anti-QS activity of *C. asiatica* against both species might indicate a broad spectrum of action and it might be the result of the presence in the extract of kaempferol, a flavonoid. Nevertheless, the anti-QS activity may also be a result of synergism with other flavonoid compounds such as quercetin, apigenin, rutin, and naringin (Vasavi et al., 2016).

Herba patriniae another plant from Chinese medicine also showed impressive results against *P. aeruginosa* biofilms (Fu et al., 2017). A water extract of *H. patriniae* prevented the formation of mature biofilms only allowing the formation of smaller cell clusters. The impairment of biofilm maturation resulted from a significant reduction of EPS production and fostering swarming motility (reducing adhesion and favouring the planktonic state of growth). Moreover, a significant decreased of virulence genes expression including *algU*, *algA*, *pslM*, *bdlA*, *pelA* was observed after the application of the *H. patriniae* extract (Fu et al., 2017). These genes have been associated to the different stages of biofilm development

since surface sensing, exopolysaccharide biosynthetic functions and dispersion (Bazire et al., 2010; Jackson et al., 2004; Morgan et al., 2006).

The methanolic extract of *Iris pallida* Lam (Dalmatian iris) and *Iris versicolor* L. (northern blue flag) showed anti-adhesion activity reducing the biomass adhered when applied before the initial stage of biofilm development. This antibiofilm potential might be significantly correlated with myristic acid content that inactivates bacterial adhesins and enzymes hindering the bacteria-surface interaction and, consequently, the adhesion and biofilm formation (Hoang et al., 2020). However, myristic acid can have other mechanisms of action including as QSI (Abd-Alla and Bashandy, 2012). Additionally, *I. pallida* was able to disrupt 4h-old mature biofilms which might resulted from the interaction of several compounds including 7-beta-hydroxystigmast-4-en-3-one content with QS system but the underlying mechanisms are not clearly understood (Hoang et al., 2020). Attenuation of the QS-related traits of *P. aeruginosa* was also observed using methanolic extract and essential oils from *Terminalia bellerica* leaves. They inhibited the production of pyocyanin and EPS, while also reducing the biofilm formation up to 78% at 0.5 mg/mL. This antibiofilm activity might have resulted in the inhibition of the AHL molecule caused by the active compounds present in the extract (Sankar Ganesh and Ravishankar Rai, 2018).

The therapeutic activity of *Melaleuca alternifolia* (Maiden & Betche) Cheel (tea tree) and *Camellia sinensis* (L.) Kuntze (green tea), namely their anti-inflammatory activity has been studied for years (Zhao et al., 2013), but recently a new biological activity showed up. Noumi et al. (2018) demonstrated that the essential oil of *M. alternifolia* inhibited the swarming motility in *P. aeruginosa* and Qais et al. (2019) reported that the leaves of green tea extracted with ethyl acetate inhibited *P. aeruginosa* QS-related phenotypes including swimming motility, the production of pyocyanin, pyoverdine, exoprotease, elastase and rhamnolipids. As reported previously by Vasavi et al. (2016), the authors verified that the compounds of the green tea extracts compete for ligand binding domain of CviR (Qais et al., 2019). Identical *P. aeruginosa* targets were used by chloroform and methanol extracts of *Andrographis paniculate* (Banerjee et al., 2017) and ethanol extracts of leaves of *Cinnamomum verum* J.Presl (the cinnamon tree) (Alva et al., 2021).

Overall, it was clear that targeting initial adhesion or interfering with the QS are the major mechanism of action that plant-based products use to hinder *P. aeruginosa* biofilm formation. In contrast, the compounds responsible for tackling *P. aeruginosa* biofilms are poorly known but flavonoids and other phenolic compounds seemed the most active.

3.2. *Staphylococcus aureus* and MRSA

S. aureus is a gram-positive bacterium that causes a wide range of infections such as skin infections, pneumonia, meningitis, endocarditis and device-related infections (Archer et al., 2011; Lister and Horswill, 2014). Infections are further complicated by the development of resistant strains such as methicillin resistant *Staphylococcus aureus* (MRSA), one of the most common source of hospital acquired infections, and, more recently by vancomycin resistant *Staphylococcus aureus* (VRSA) representing a significant burden on the healthcare system (Archer et al., 2011; Chen et al., 2015; Muhs et al., 2017). Planktonic *S. aureus* are generally responsible for acute infections such as bacteraemia and skin abscesses through the production of secreted toxins and exo-enzymes, while biofilms are responsible for chronic infections such as osteomyelitis and endocarditis but mainly for device-related infections associated to the use of catheters, prosthetic joints, and pacemakers (Muhs et al., 2017). Similarly to other bacterial species, the formation of biofilms further contributes to the persistence of *S. aureus* infections because bacteria are protected by the matrix that reduces the efficacy of host defences and antibiotic activity (Kahl et al., 2016; Wolter et al., 2013).

Several authors have found plant extracts that inhibited biofilm formation in *S. aureus.* For instance, Tamfu et al. (2020) reported that N-cerotoyltryptamine isolated from seed extract of *Annona senegalensis* Pers. (Annonaceae) showed the highest biofilm inhibition among asimicin (2) and ent-19-carbomethoxykauran-17-oic acid achieving a reduction of 27 and 44% of biofilm biomass after the application of 4-fold lower minimum inhibitory concentration (MIC) and MIC, respectively. Muhs et al. (2017) reported that a flavone rich extract of *Schinus terebinthifolia* Raddi (Brazilian peppertree) fruits were able to inhibit the agr QS system and prevented biofilm formation by *S. aureus* without killing or inhibiting bacterial growth. The agr system can be necessary for *S. aureus* biofilm formation and its downregulation decrease gene expression of cell wall-associated adherence factors needed for initial biofilm formation stages (Archer et al., 2011; Lister and Horswill, 2014). Recently, Tang et al. (2020) isolated and identified three triterpenoid acids (3-oxo-olean-12-en-28-oic acid, 3-oxotirucalla-7,24Z-dien-26-oic acid, 3α-hydroxytirucalla-7,24Z-dien-26-oic acid) from methanolic extract of *S. terebinthifolia* that might be the responsible for the inhibition of the expression of *S. aureus* agr types.

Plant-based products has also proved to be a promising strategy to prevent the formation of antibiotic resistant biofilms including MRSA. Extract of *Duabanga grandiflora* (Roxb. Ex DC.) Walp. leaves reduced cell-surface attachment of MRSA by decreasing expression levels of PBP2a which led to the formation of weaker biofilm structures (Santiago et al., 2015).

PBP2a is a protein that facilitates the cell-to-cell interactions during biofilm development (Mack et al., 2004; Pozzi et al., 2012; Santiago et al., 2015). A water extract of *Artemisia princeps* Pamp. also showed activity against MRSA inhibiting not only biofilm formation but also proliferation and expression of some virulence genes (mecA, sea, agrA, and sarA) involved in the biofilm formation (Choi et al., 2015). The phytochemical analysis of *A. princeps* indicated that its activity may be related to organic acids and glycosides, the major components of the extract (Choi et al., 2015). Moreover, phenols such as gallic acid of the water extract of *Cochlospermum regium* (Schrank) Pilg. leaves might also be the responsible for the complete inhibition of both MRSA and methicillin sensitive *Staphylococcus aureus* (MSSA) biofilm formation (Galvão et al., 2020), possibly by inhibiting the polysaccharide synthesis (glucose and fructose), as previously reported by Sendamangalam et al. (2011) and Liu et al. (2017).

Eucalyptus globulus Labill. (eucalyptus) leaves have been used as conventional medicine for years with different purposes and recently was demonstrated its antibiofilm activity (Merghni et al., 2018). The essential oil obtained from the leaves of *E. globulus* and its isolated compound 1,8-cineole were capable of reducing the initial attachment and subsequent biofilm formation by MRSA and it was even capable of disrupt pre-formed biofilms (Merghni et al., 2018). Likewise, the essential oil from the leaves of *M. alternifolia*, commonly known as the tea tree, and its isolated compound terpinene-4-ol inhibited the formation of biofilm in MRSA by reducing the initial cell adhesion (Noumi et al., 2018).

Dismantling of pre-formed biofilms is a harder task than inhibition but even so plant-derived products has proven to be effective. Leaves of *Allium stipitatum* Regel (Karunanidhi et al., 2018) and *Syagrus coronata* (Martius) Beccari (Souza dos Santos et al., 2019) can cause alterations in the *S. aureus* biofilm structure leading to its disintegration. These plant extracts, or their isolated compounds penetrated in deep layers of the biofilms and affected mature biofilms formed by antibiotic resistant bacteria, which might represent a great advance in the fighting of antibiotic resistance global crisis. Also, methylene chloride-methanol extract of *Callistemon citrinus* (Curtis) Skeels (a synonym of *Melaleuca citrina* (Curtis) Dum.Cours.) leaves and the isolated pulverulentone A dismantled MRSA and MSSA biofilms reducing the production of staphyloxanthin, a hallmark virulence factor of *S. aureus* that mostly acts as an antioxidant against host immune response (Clauditz et al., 2006) and causing significant alterations in the biofilm structure (Shehabeldine et al., 2020).

Overall, it is verified that plant extracts can reduce *S. aureus* biofilms presenting promising results against the antibiotic resistant strains such as MRSA. The compounds

responsible for the antibiofilm activity are diverse but flavonoids, organic acids and phenolic acids seemed to be the most active. They can exert distinct mechanisms of action but hinder the initial stages of biofilm formation, such as the attachment to the surface and reduction of virulence potential, is the most common mechanism.

3.3. *Klebsiella pneumoniae*

K. pneumoniae is a leading cause of nosocomial infections and of a large spectrum of community-acquired infections. Typically, it infects immunocompromised patients or patients with indwelling devices such as urinary catheters on which bacteria are able to form biofilms (Guerra et al., 2022). *K. pneumoniae* antibiotic resistance is currently a hot topic in clinical community worldwide, since it often shows a high resistance to a broad spectrum of drugs including β-lactam antibiotics, fluoroquinolones and aminoglycosides, and frequently multi-drug resistance and even extremely drug resistance (Navon-Venezia et al., 2017). Bacteria growing in biofilms formed in living or abiotic surfaces critically exacerbate this scenario. Therefore, the inhibition of biofilm formation on surfaces has been the most used approach by researchers to avoid an escalation of drug resistance.

Virulence factors facilitates *K. pneumoniae* colonization, evasion to immune system and infection development in the human host and biofilm formation. The capsule, type 1 and type 3 pili and lipopolysaccharides in *K. pneumoniae* contribute to the formation of biofilm being responsible for a proper initial coverage of substrate and construction of mature biofilm architecture and the initial adhesion on abiotic surfaces (Guerra et al., 2022; Vuotto et al., 2014). Extract of *Arctium lappa* L. (burdock) root showed promising results against *K. pneumoniae* reaching up to 80% of inhibition of biofilm formation at the maximum concentration tested (100 μg/ml), without affecting planktonic bacteria. Moreover, this extract was also capable of disrupting pre-formed biofilms (Rajasekharan et al., 2017). *In vitro* experiments and *in silico* docking support the hypothesis that the chlorogenic acid present in the extract might bind the active sites of sulfhydryl-variable-1 β-lactamase and downregulated biofilm-associated genes including type 3 fimbriae mrkD and trehalose-6-phosphate hydrolase treC.

A chloroform extract of *Fagonia indica* Burm.fil. inhibited biofilm formation in *K. pneumoniae* by reducing the initial bacterial attachment to the surface, while also causing disintegration of the bacterial cell wall (Aslam et al., 2022). Also, a methanolic extract of *Pulicaria crispa* (Forssk.) Oliv. (a synonym of *Pulicaria undulata*), a Saharan plant that local population used for antimicrobial and antiseptic purposes was capable of inhibiting biofilm

formation by *K. pneumoniae* (Thinina et al., 2020). Its antibiofilm action might be attributed to its chemical composition of quercetin, the major component, ellagic acid, gallic acid, rosmarinic acid, proanthocyanidin dimer and rutin produced in response to the arid environmental conditions. Indeed, the different components of this extract have different mechanisms of action: proanthocyanidin dímer might contribute for reduced EPS production (Blanco et al., 2005); ellagic acid might damage cell membrane while also inhibit biofilm formation (Bakkiyaraj et al., 2013; Fontaine et al., 2017); gallic acid might inhibit the activity of enzymes involved in glucose and fructose synthesis leading to biofilm inhibition (Sendamangalam et al., 2011; Sowndarya et al., 2020); rosmarinic acid causes inhibition of the early stages of biofilm formation (Corral-Lugo et al., 2016; Slobodníková et al., 2013); and the flavonoid rutin might inhibit nucleic acid synthesis (Mirzoeva et al., 1997; Z. Wang et al., 2021). So far it is unclear if the antibiofilm activity results from the synergy of two or more compounds or only from quercetin.

Anthocyanins isolated from *Syzygium cumini* (L.) Skeels (commonly known as black plum or Indian blackberry) well known for its antimicrobial activity against food-borne pathogens, were capable of inhibiting the biofilm formation and EPS production up to 70%, through a pronounced inhibitory effect on QS (Gopu et al., 2015). Similarly to other bacterial species, QS also plays a significant role in *K. pneumoniae* biofilm formation in the initial adhesion to surfaces and maturation, and regulation of the production of EPS that protects cells from external aggressions (Guerra et al., 2022). The anti-QS activity of the anthocyanin of *S. cumini* might be attributed to malvidin that exhibited high binding rate with LasR receptor (Gopu et al., 2015).

Extracts of *Hyptis suaveolens* (L.) Poit. (a synonym of *Mesosphaerum suaveolens* (L.) Kuntze) exhibited various effects on biofilms (Salini et al., 2015). In general, methanol, hexane, ethyl acetate and aqueous extracts of *H. suaveolens* exhibited anti-QS activities in *K. pneumoniae*. Among them, hexane extract exhibited a notorious antibiofilm activity reducing the biomass in *K. pneumoniae* biofilms while also reducing the bacterial motility (disabling bacteria to reach and adhere to surfaces) and the production of several QS-related virulence factors important in the development of the infection, including protease, hemolysin, prodigiosin. In this study, it was shown that this extract had effect not only on *K. pneumoniae* but also on other pathogens involved in urinary tract infections including *Proteus mirabilis*, *Proteus vulgaris*, *Serratia marcescens* and *E. coli* (Salini et al., 2015). This could be a relevant feature since biofilm-associated infections are frequently polymicrobial.

Although the great impact of *K. pneumoniae* in the global crisis of antibiotic resistance and be a serious threat to the patients, there are scares studies of plant-based products for the inhibition of biofilms. Comparing with other species such *P. aeruginosa*, *S. aureus*, *S. epidermidis*, *E. coli*, the understanding about the ecology and physiology of *K. pneumoniae* biofilms is still limited which impact on the number of studies addressing antibiofilm strategies. Therefore, a significant effort must be put on providing insights about *K. pneumoniae* biofilm physiology in order to design effective antibiofilm strategies.

3.4. *Escherichia coli*

E. coli is a gram-negative bacterium mainly responsible for urinary tract infections, one of the most common bacterial infections in humans. Living commonly in the urinary tract, it frequently forms biofilms on the surface of catheter materials and on the bladder epithelial cells (Sharma et al., 2016). The ability to form biofilm is highly dependent on the expression of virulence factors such as Type 1 fimbriae, curli fimbriae and adhesins that allows it to move and colonize various sites in the urinary system and overcoming host defences (Eberly et al., 2017; Lüthje and Brauner, 2014). The clinical importance of *E. coli* biofilms was clearly stated with its correlation with antibiotic resistance in catheter-associated urinary tract infections (CAUTI) (Sharma et al., 2016).

A. senegalensis is widely used in West and Central Africa due to its antimicrobial and antioxidant activities, but recently it was also reported antibiofilm activity against *E. coli* and *S. aureus*. Tamfu et al. (2020) reported that N-cerotoyltryptamine, asimicin and ent-19-carbomethoxykauran-17-oic acid isolated from seed extract of *A. senegalensis* inhibited biofilm formation, being asimicin the most active compounds against *E. coli*. It provoked a reduction of 19 and 43% of biofilm biomass using 4-fold lower MIC and MIC, respectively.

Clinopodium bolivianum (Benth.) Kuntze, a South American aromatic herb known for its anti-infective and immunomodulatory activities and frequently used to treat gastrointestinal disorders was found to reduce the adherence ability of *E. coli* to uroepithelial cells and, consequently, reduce the biofilm formation without inhibiting bacterial growth (Mohanty et al., 2017). The authors considered that polyphenols and fructose present in the extract are the suppressors of biofilm formation.

A methanolic extract of *Citrus limon* (L.) Burm fruits was also capable of reducing the biofilm formation while also decreasing the maturation of the biofilm matrix (Singha et al., 2023). The authors found the two major components of this extract, cyclobarbital and 3-methylsalicylhydrazid, to be responsible for the antibiofilm activity. Likewise, an aqueous

extract of *Polypodium vulgare* L. rhizome was capable of altering the structure of uropathogenic *E. coli* biofilms, producing a much weaker biofilm than the untreated cultures (Gleńsk et al., 2019). The authors found that the compound osladin was responsible for this activity. However, the rhizome extract was more effective against biofilms than the isolated compound, meaning that other compounds present in the extract might also have antibiofilm activity or several compounds might act synergistically.

Essential oil of *Rosmarinus officinalis* L. (a synonym of *Salvia rosmarinus* Spenn.) (rosemary), *Thymus zygis* L. (thyme) and *Origanum majorana* L. (sweet marjoram) were studied on *E. coli* cells isolated from urinary tract infections and these essential oils were capable of inhibiting the initial attachment. *R. officinalis* demonstrated the highest inhibition rate of biofilm formation (Lagha et al., 2019). According to the biochemical composition, 1.8-cineole present in *R. officinalis* might be the biofilm suppressor compared to the linalool and terpinen-4-ol present in the other essential oils. Interestingly, in this work, the essential oil with the highest antibiofilm activity corresponded the lowest antibacterial effect. Also, Abu El-Wafa et al. (2020) and Zhang et al. (2014) verified that antibiofilm activity was not related to antibacterial action. For instance, polyphenolic extract of *Rosa rugosa* Thunb. tea blocked QS system affecting the QS-controlled traits including swarming motility and biofilm formation of *E. coli* and *P. aeruginosa* at sub-MIC concentrations, without having an antibacterial effect on the planktonic cells (Zhang et al., 2014). This evidence reinforce that plant-based products must be evaluated independently of the previous reported activities, in particular antimicrobial activity.

The leaves of *Symplocos racemosa* Roxb., commonly known as Lodh tree in India, displayed a significant antibiofilm potential against several bacterial species including *E. coli* (Sood et al., 2020). Diverse compounds were obtained from this plant, but flavonoids were the most effective against *E. coli*. They caused inhibition of the initial cell attachment and disruption of pre-formed biofilms, and also reduced the biofilm-cells metabolic activity (Sood et al., 2020). Nevertheless, cardiac glycosides also provoked a reduction in biofilm-cells viability but less efficiently than the flavonoids. The antibiofilm potential of flavonoids and cardiac glycosides were also verified for other bacterial species, including *K. pneumoniae* and *S. aureus* (Arora and Mahajan, 2019; Sood et al., 2020).

Aqueous extract of *Acacia nilotica* (L.) Willd. ex Delile (a synonym of *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb.), in particular 3-cyclohexane-1-carboxaldehyde, 2,6,6-trimethyl; á-selinene; oleic acid; globulol and isochiapin detected in the its composition demonstrated a promising ability to reduce biofilm biomass of *E. coli* as well as *K. pneumoniae*, *P. mirabilis*

and *P. aeruginosa* (Elamary et al., 2020). Several other phytochemicals have shown activity against *E. coli*. For instance, the compound ginkgolic acid isolated from *Ginkgo biloba* L. caused downregulation of the curli and prophage genes which inhibited the biofilm formation (Lee et al., 2014); and a maple syrup extract rich in phenolic compounds caused a reduction in adhesion and biofilm formation, while also repressed drug resistance genes in *E. coli* (Maisuria et al., 2015).

In summary, it is clear the activity of plant extracts on *E. coli* biofilms predominantly acting on their inhibition. Polyphenols and flavonoids seemed the most active compounds in the inhibition of bacterial adhesion and blocking QS. It is also noted that there are few studies of plant products against *E. coli* biofilms, despite their relevance in community and hospital-acquired infections.

4. Synergism of plant extract/compounds and antibiotics

The current international, national and local approaches used for control of biofilm-associated infections included the administration of dual or combinatorial therapies, either by administrating two antibiotics simultaneously or by combining an antibiotic with a 'helper' or adjuvant agent/drug/compound. For instance, to manage *P. aeruginosa* biofilm-associated infections, combinations of antibiotics such as ceftolozane and tazobactam are used to treat urinary tract infections (Bassetti et al., 2018); and combinations of sulfamethoxazole and trimethoprim are used treat *S. aureus* and MRSA infections (Hodille et al., 2017). There is no doubt about the value of antibiotics to control or eliminate infections and the lives that they can save, but antibiotics are designed to target and kill bacteria and not to disrupt or dismantle biofilms. Consequently, most of the biofilm-associated infections persist after antibiotic treatments (Conlon et al., 2015; Römling and Balsalobre, 2012; Vestby et al., 2020). An antibiofilm compound acting alone towards pre-existing biofilms will release an overload burden of 'single' cells or produce small aggregates of biofilm-cells that most of the antibiofilm compounds cannot eradicate. If the released biofilm-cells were not eliminated, bacteria can initiate a new cycle of biofilm formation perpetuating the infection (Chen et al., 2013; Roy et al., 2018; Yan and Bassler, 2019). Therefore, it seems beneficial the combination of two kind of compounds, antibiotic and antibiofilm drug in order to achieve the eradication of the biofilm-associated infections. Although therapies using more than one molecule increase the risk of negative interactions between drugs or increased the toxicity to the host, the benefit of increased efficacy of antibiotics can outweigh the risk. The clinical

community have understood this benefit long time ago against planktonic bacteria. A polyphenolic flavonoid (glabridin) isolated from *Glycyrrhiza glabra* L. has shown to potentiate the activity of oxacillin, vancomycin and norfloxacin against planktonic *S. aureus* (Singh et al., 2015). The authors found that the oxacillin and vancomycin MIC were reduced by 2-fold when combined with glabridin and the norfloxacin MIC reduced by 4-fold, demonstrating the potential of glabirin as antibiotic adjuvant. The polyphenol epigallocatechin gallate (EGCG) isolated from *C. sinensis* has shown to improve gentamicin activity against both *E. coli* and *S. aureus*, probably by the cell membrane disruption caused by the EGCG (Parvez et al., 2019). Likewise, the use of antibiofilm plant extracts or isolated compounds with antibiotics holds great expectation for fighting biofilms. Liu et al. (2015) reported that four essential oils components (thymol, eugenol, and cinnamaldehyde) potentiated the activity of streptomycin against *Listeria monocytogenes* and *Salmonella* Typhimurium. The authors showed that these components facilitate the antibiotic penetration into the biofilm, allowing the antibiotic to access and kill the biofilm cells. Similarly, it has also been reported that thyme oil increased the activity of ciprofloxacin in *K. pneumoniae* biofilms, reducing the biofilm cells viability (Mohamed et al., 2018).

Abu El-Wafa et al. (2020) showed that the combination of both extracts of pomegranate and rosemary with piperacillin, ceftazidime, imipenem, gentamycin, or levofloxacin provoked a higher biofilm eradication activity compared with plant extracts and antibiotics acting alone. Microscopic evidence indicated that the pre-formed biofilms of *P. aeruginosa* were destroyed and removed from the surface and bacteria eliminated after this combinatorial treatment (Abu El-Wafa et al., 2020). Dey et al. (2020) reported that the flavonoid naringin in combination with ciprofloxacin and tobramycin had a stronger antibiofilm activity against *P. aeruginosa* than the antibiotics or the flavonoid alone. Both combinations caused a significant disintegration of mature biofilms, with loss of EPS and reduced biofilm thickness and cell density. Vitexin, a flavone glycoside found in the passion flower, bamboo leaves and pearl millet, combined with azithromycin and gentamicin was also capable of reducing the initial attachment of biofilm forming cells to the surface and the production of EPS in *P. aeruginosa* (Das et al., 2016). Both combinations had anti-QS activity, hindering the swarming motility and the production of virulence factors such as pyoverdine and pyocyanin while also reducing the activity of LasA protease and LasB elastase (Das et al., 2016). Proanthocyanidins isolated from *Vaccinium macrocarpon* Aiton known as American cranberry was combined with ciprofloxacin against biofilm of *P. aeruginosa* and surprisingly the activity of the proanthocyanidins did not reduce the biofilm but, instead, the isolated

compound acted on the QS system reducing the expression of some virulence factors which improved the activity of ciprofloxacin (Vadekeetil et al., 2016). A polyphenolic compound, hordenine, isolated from barley was combined with the antibiotic netilmicin and they significantly reduced biofilm formation by interfering with the lasR gene expression (Zhou et al., 2018).

The potential of plant extracts as antibiotic adjuvants was also reported for *S. aureus*. Abreu et al. (2016) showed that methanolic extract of *Buxus sempervirens* L. combined with erythromycin, ciprofloxacin and tetracycline (at MIC level) provoked 88, 81, 79% of biofilm reduction, respectively, that antibiotics alone did not achieve. After extract fractionation for the identification of the bioactive compounds, oleanolic acid seemed to be the main responsible for the improved antibiotic performance. Endo et al. (2018) tested the activity of hydroalcoholic extracts of leaves of *R. officinalis* and *Tetradenia riparia* (Hochst.) Codd, and peel of *Punica granatum* L. (pomegranate) and the three extracts were capable of destroying 24h preformed biofilms. Moreover, extracts had synergistic interactions with penicillin against *S. aureus* and MRSA (Endo et al., 2018).

Wojnicz et al. (2015) found that the pentacyclic triterpenes asiatic acid and ursolic acid enhanced the activity of ciprofloxacin against *E. coli*. In that study, the combination of ciprofloxacin with ursolic acid was capable of reducing the formation of biofilm in microtiter plates. Also, when combined with each of these compounds, ciprofloxacin was able to penetrate the biofilm structure. Likewise, an extract of *Myrtus communis* L., rich in ursolic acid, was found to increase the activity of erythromycin and clindamycin against biofilms of *Propionibacterium acnes* (Feuillolay et al., 2016).

Although it seems noticeable how antibiofilm compounds extracted from plants can function together with antibiotics and the benefits of this combination or synergy, there are few studies addressing the combination of antibiofilm plant-based products with antibiotics against biofilms. The issues behind the limited knowledge about the interactions between antibiofilm plant-based products and antibiotics and the development hurdles of a therapy are discussed in the next section.

5. Current challenges

It is undoubtable the potential of plant-derived products as therapeutic agents in particular to inhibit biofilm formation or disrupt/disassemble pre-formed biofilms. However, there are some challenges associated with the use of plant compounds at different levels.

Environmental factors

One of the first issues about the development of drugs from natural products, including plants, is the wide variability in the composition of the matrix due to the diversity of factors involved in the growth of the plant. Environmental factors such humidity, soil change, light and temperature can cause a drastic variation in the final plant composition. These factors can change the final composition, varying the content of water, lipids, proteins and secondary metabolites, which will obviously affect the drug performance that is being developed (Batista and Oliveira, 2010). Further, other factors like the plant age and harvesting procedures are also determinant in the final composition. Therefore, standardization procedures have to be carefully thought to overcome this high variability.

Technical factors: extraction

The recovery procedure to obtain an active plant extract is also decisive to the feasibility of the final application. The extraction parameters, for instance, such as temperature, time and solvent, may have a critical impact on the final extract obtained (Butler, 2004). Many bioactive compounds are susceptible to the extraction conditions and may be degraded or changed by the extraction and recovery process (Koehn and Carter, 2005). For instance, it has been shown that the solvent used in the extraction has a key role in the bioactivity of the extract. Alam et al (2020) has reported that the same matrix extracted with different solvents had distinct activity against biofilms of *P. aeruginosa*. Methanolic extract of *Bergenia ciliata* (Haw.) Sternb achieved over 70% of inhibition of biofilm formation, probably through inhibition of some virulence factors preserving *P. aeruginosa* growth, while ethanolic extract also inhibited biofilm formation (also at around 70%) but provoked by its antimicrobial effect on the bacterial cells. In turn, extractions performed with acetone, ethyl acetate, hexane and water, produced extracts with less than 20% of biofilm inhibition, and the extraction with chloroform resulted in a product that increased the biofilm formation (Alam et al., 2020). These various activities arise due to solvents have distinct affinity for different compounds and, therefore, the extract (relative) chemical composition depends mainly on the solvent and compound's polarities (Cowan, 1999). Consequently, a plant that has been studied with one solvent with unsuccessful results against biofilms (or planktonic bacteria) does not

necessarily imply that it will not have activity when extracted with a different solvent. The same can be said about the activity against different bacteria species and the interactions between plant extracts and antibiotics. For instance, the activity of *Anacardium microcarpum* Ducke (a synonym of *Anacardium occidentale L.*) , a type of cashew nut, exhibited synergy with different antibiotics against planktonic bacteria, but the final activity was dependent on the solvent used for the extraction (Coutinho et al., 2015). Ethyl acetate fraction (EAF) of *A. microcarpum* showed synergy with imipenem and gentamicin against planktonic *S. aureus*, while ethanolic extract (CEE) only exhibited synergy with imipenem and methanolic fraction (MF) with gentamicin. Moreover, an antagonism effect was found when combining gentamicin with CEE. Also, for *P. aeruginosa* synergies were dependent on the solvent used during extraction. Synergies were found combining CEE with amikacin, EAF with gentamicin and MF with ciprofloxacin, but EAF combined with amikacin had antagonistic effects (Coutinho et al., 2015). These findings lead to another issue. Biofilm infections are frequently polymicrobial making the design of antibiofilm therapies much more difficult. Since extracts have distinct activity against different species, higher concentrations of extract or even combinations of natural products can be needed to inhibit or eradicate the polymicrobial biofilms. For instance, methanol extracts of leaves of *Iris pallida* were capable of disrupting mature single species biofilms of *S. aureus* and *P. aeruginosa*, achieving a disruption of 60% in biofilm viability (Hoang et al., 2020). However, when tested against multi-species biofilms of dental plaque (*Streptococcus gordonii*, *Veillonella parvula*, *Fusobacterium nucleatum*, and *Actinomyces naeslundii*), the biofilm viability was only reduced 20% (Hoang et al., 2020).

Plant extract stability

Natural medicines or drugs for human use need to be stored for long periods of time without losing their effect. Most of the antibiofilm plant-derived extracts or products are a multi-component formulations, i.e., are constitute by a wide range of different molecules which raises concerns about their stability for clinical trial testing and for regulatory approval (Beutler, 2009; Butler, 2004). Several factors can perturb the stability of a plant-based product including temperature, light, air, humidity and the presence of various compounds in the product that might interact with each other under specific conditions which could reduce the efficacy of the final product or promote undesired or unexpected effects. This drawback can be overcome by fractioning and purifying the extract in order to find the compound that has the specific activity and specifically address its stability issues; however, besides loosing

possible synergies between different compounds responsible for the bioactivity, this also brings the next issue.

Quantity available and chemical composition

After fractioning and purifying the extract, there is a chance that the final compound obtained will not be in enough quantity, either to continuing the tests or to develop a feasible final drug. This gets even more problematic for some extracts or products that after fractioning and purifying may not maintain the level of activity of the initial extract because the compound present in higher concentrations is not the one responsible for the extract activity, but the activity result from one or more compounds in lower concentrations. For instance, Vasavi *et al* (2016) found that the major compound of the extract, asiatic acid (a triterpene compound) had no anti-QS activity, but others in minor concentrations, including kaempferol showed an anti-QS activity similar to the extract. Merghni *et al* (2018) also reported the essential oil of eucalyptus had anti-QS activity, whereas its main component the 1,8-cineole showed a much lower activity even at higher concentrations. Similarly, the extracts of *Bistorta officinalis* Delarbre and *Persicaria maculosa* Gray showed higher inhibition of virulence factors in *P. aeruginosa* than the pure compounds present in each extract (Jovanović et al., 2020). Therefore, there is a chance that the activity of some plant products may be a result from synergism amongst several compounds present in the extract, which makes the fractioning and purification much harder. Thus, huge quantities of the source need to be collected and used which in turn may cause over-exploration of the environment (Butler, 2004; Mahidol et al., 1998). Integrated biorefinery strategies should be designed to use the side-streams and by-products generated in order to decrease this impact and allow efficient exploitation of the entire resource (Beutler, 2009; Koehn and Carter, 2005).

Drug delivery systems like micro and nanoparticles may be a solution for several of the issues associated with the use natural products here discussed, such as low availability, compound stability and others such as cytotoxicity. For instance, these particles are typically designed to deliver very low quantities of product to a specific target solving thus the low availability of compounds. At the same time, it would be needed less product to achieve the same effect while also avoiding possible toxic side effects (cytotoxicity) of the bioactivity compounds. Encapsulation of the natural products can also reduce degradation of the

compounds by efficient storage, and increase the drug stability and shelf life (Armendáriz-Barragán et al., 2016).

Web-based resources

This wide range of factors with influence in the expressed bioactivity creates another important hurdle in the development of new drugs: screening the available information and comparing results. Different plants, parts of plants, growth conditions, harvesting procedures, solvents, extraction protocols, fractioning, purification, synergies, etc. creates an unmanageable range of possible combinations. There should be a worldwide database and searching tools for the activity of natural or plant products and extracts, constituted not only by those that are bioactive but also by those which do not. Currently, this information is so spread out on various studies that makes difficult for the researchers and organizations to find and use it and thus moving forward in testing. The creation of a universal web-knowledgebase for easy access would boost the discovery of the natural antibiofilm products against the different species. For instance, a web-based central resource that allows storage and annotation of information, comparison and analysis of results can be a crucial step for the development of new natural product-based therapies.

6. Conclusions and Perspectives

Bacterial biofilms are of great clinical concern since the existing antibiotics are not capable of eliminating their causative infections and there are no expectations that the new generation of antibiotics in the horizon will be able to do it. Plant-based products may be a solution or part of it. In this review it was studied the vast research that has been carried out to find plants with potential to inhibit biofilm formation or disrupt the complex three-dimensional structure of mature biofilms. A considerable effort was made in this review to provide details about the bioactive compounds of the plant extracts responsible for the antibiofilm activity and their underlying mechanism, but few data is described in literature which limited the discussion. It is imperative to identify the antibiofilm phytochemical(s) further unlocking the drug discovery and development processes. New methodologies and approaches must be implemented to boost compound identification, isolation and testing. High-throughput screenings and multivariate data analysis coupled to metabolomics (eventually coupled with artificial intelligence) may be relevant tools to allow broadening the search and identification of the active compounds. To help disclosing the associated

mechanism of action Attenuated Total Reflectance-Fourier Transform InfraRed (ATR-FTIR) spectroscopy may be further explored. The application of these and other methods can be helpful in speed up the formulation of new antibiofilm therapies.

The results presented in this review demonstrated that mostly plant-based products show antivirulence activity affecting the expression of virulence determinants essential to initiate biofilm formation. In this regard, inhibition of QS seems the preferential mechanism of action of plant-based products that avoid the QS signal molecules accumulation in the environment or within immature biofilms manipulating thus the gene expression of virulence factors such as exopolysaccharide production. The most active anti-QS plant-derived compounds are flavonoids, phenols, terpenoids, and steroids. An interesting feature of their bioactivity against biofilms reported by some studies is their reduced antimicrobial activity. More future studies must be oriented towards the investigation of plant extracts or products regardless their antimicrobial activity in order to have a wider range of possible antibiofilm drug candidates.

The eradication of an already formed biofilm is clinically more relevant but more difficult to achieve including by plant-based products. The main mechanism of action against mature or pre-formed biofilms by plant-based products seems to be EPS targeting. Plant extracts or -derived products or compounds mainly act on the various structural components of EPS (e.g. extracellular DNA, exopolysacharides) weakening the cohesiveness of biofilm and favouring the detachment of cells or small aggregates. Even the gathered information allowed to understand this common mechanism of action, much efforts are still need to verify the action of several antibiofilm extracts and compounds on mature biofilms.

Although the promising potential of plant extracts or derivative products, most of them are not able to kill bacteria, meaning that eradication of infection is not achieved. Therefore, co-administration of antibiofilm plant products with antibiotics are a fruitful strategy, however there are a limited number of studies focused on the combination of these two distinct agents. The limitations might be mainly related to the ‘nature’ of plant products that can alter the final plant composition, the wide range of different extraction and recovery processes producing distinct extracts with distinct outcomes; poor extraction yield; the lack of stability of the product; poor identification of effector compounds because activity is frequently a result of complex synergism amongst several compounds of the extract making fractioning and purification harder; and product cytotoxicity at effective dosages.

Biofilms also represent a significant barrier to the development of an effective therapy. Biofilm formation is a complex and dynamic process influenced by numerous factors of

different kind (e.g. chemical, biological) making each biofilm a unique entity to target. Most of the studies are conducted in laboratories and although few of them used the most advanced *in vitro* models, the interplay between biofilms and host tissues and cells can influence the antibiofilm activity. More research needs to be done for elucidating therapy formulation (composition and dosages), efficacy, frequency and duration of therapy, safety issues using adequate *in vivo* models to better mimic the clinical condition.

Moreover, in clinical field, biofilms are frequently polymicrobial rather than single species and the efficacy of antibiofilm plant-derived products might not be identical when other bacterial species are present in the biofilm. Interspecies interactions have been increasingly pointed out as one of the factors that can influence the pathogenesis of organisms and augmenting the tenacity and recalcitrance of biofilms. Therefore, it would be of utmost importance that studies include plant product activity against polymicrobial biofilms. It may be that in this way the *in vitro* results would be more similar to the *in vivo* performance.

Another limitation of the review is data discussion centred on the application of plant-based products on surface-attached biofilms. These studies certainly cover an important part of biofilm-associated infections detected for instance in endotracheal tubes, vascular catheters, urinary catheters, prosthetic joints and orthopaedic implants, but there is a significant part of infections that biofilms are formed on human tissues or fluids including cystic fibrosis, burn and chronic wounds, urinary tract, genital tract (e.g. vaginosis). It is vital to conduct *in vitro* and *in vivo* studies to assess antibiofilm activity of plant products on biofilms formed on biotic surfaces.

Although the limitations and development hurdles, research on biofilm inhibition and eradication using plant (and also natural) products remains a booming field. However, focus must put on the identified challenges in order to put product candidates in clinical trial testing.

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Declaration of Competing Interest

The authors confirm that there are no conflicts of interest associated with this publication.

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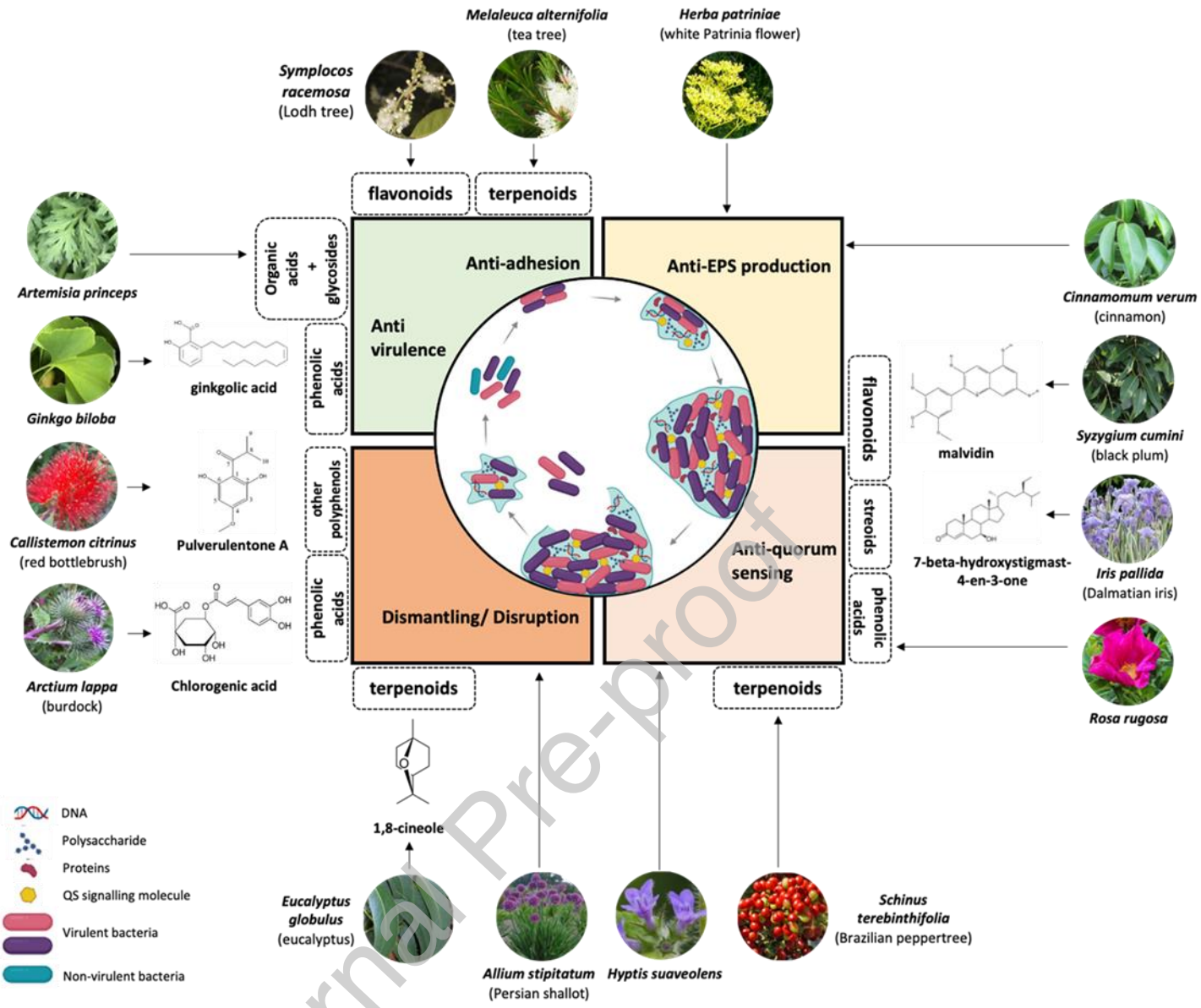

Figure 1

Legend of Figures

Figure 1 – Representation of the different stages of biofilm formation and the most common mechanisms of antibiofilm activity found in plant extracts. In this scheme, examples of plant extracts or phytochemicals (if identified) are associated to each mechanism of action.

Table 1 List of the most relevant studies reporting extracts of plants with antibiofilm activity (published in 2015-2023).

Plant	Plant family	Extract/compound[a]	Bacterial species tested	Mode of action	Reference
Acacia macrostachya Rchb. Ex DC. (syn. *Senegalia macrostachya* (Rchb. Ex DC.) Kyal. & Boatwr.)	Fabaceae Juss.	Methanol extract of stem bark	*S. aureus* *P. aeruginosa* *E. coli*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited efflux pump activity	Barfour et al. (2021)
Acacia nilotica (L.) Willd. ex Delile (a synonym of *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb.)	Fabaceae Juss.	Aqueous extract (N/S)	*E. coli* *K. pneumoniae* *P. mirabilis* *P. aeruginosa*	**AMB**: reduced biofilm activity	Elamary et al. (2020)
Acca sellowiana (O.Berg) Burret	Myrtaceae Juss.	Acetone extract of fruit	*S. aureus* MRSA	**IBF**: reduced biofilm biomass adhered by inhibition of the initial attachment and without inhibiting bacterial growth **AMB**: disrupted biofilm structure	Dell'Olmo et al. (2021)
Adiantum philippense L. (a synonym of *A. lunulatum* Burm. f.)	Pteridaceae E.D.M.Kirchn.	Methanol extract of whole plant	*E. coli* *S. aureus* *P. aeruginosa* *S. flexneri*	**IBF:** inhibited the initial attachment and reduced the EPS production **AMB**: disrupted biofilm structure	Adnan et al. (2020)
Agrimonia pilosa Ledeb.	Rosaceae Juss	Wogonin	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: downregulating the expression of QS-related genes and reducing the production of virulence factors, including the EPS production, twitching, swimming, and swarming motilities	S. Wang et al. (2021)
Allium stipitatum Regel	Amaryllidaceae J.St.-Hil.	Hexane and dichloromethane extracts of bulb	*S. aureus* MRSA *A. baumannii* *S. maltophilia*	**AMB**: reduction in biofilm viability and disruption of biofilm structure	Karunanidhi et al. (2018)
Andrographis paniculata (Burm.f.) Wall. ex Nees	Acanthaceae Juss.	Chloroform and methanol extracts of whole plant	*P. aeruginosa*	**IBF**: reduced swarming motility without affecting its planktonic growth; inhibited the adherence to surface affecting the initial step of biofilm formation **AQSV**: reduced production of pyocyanin, elastase,	Banerjee et al. (2017)

				protease, rhamnolipid and hemolysin	
		Ethyl acetate extract of leaves	*E. coli*	**AQSV:** reduced EPS production and downregulated the expression of AmpC gene	Sah et al. (2019)
Anethum graveolens L.	Apiaceae Lindl.	Methanol extract of seeds	*S. marcescens*	**IBF**: reduced biofilm biomass adhered and microcolony formation **AQSV**: reduced biosynthesis of prodigiosin, downregulated bsmA, fimC and flhD gene; reduced motility and adherence	Salini and Pandian (2015)
Anthemis stiparum subsp. sabulicola (Pomel) Oberpr	Asteraceae Giseke	Methanol extract and essential oil of aerial parts	*S. aureus* *S. epidermidis* *B. subtilis*	**IBF**: reduced biofilm biomass adhered	Chemsa et al. (2018)
Arisaema sinii K.Krause	Araceae Juss.	Ethanol extract of whole plant	*M. tuberculosis*	**IBF**: reduced biofilm formation, without inhibiting the bacterial growth **AMB**: promoted dispersion on 4h-old biofilms and disruption or dissolution on 35 day-old biofilms	Jiang et al. (2019)
Artemisia herba-alba Asso *Artemisia campestris* Pursh *Artemisia absinthium* L.	Asteraceae Giseke	Essential oils of aerial parts	*E. coli*	**IBF**: reduced biofilm biomass adhered, by reducing EPS and forming scattered microcolonies	Mathlouthi et al. (2021)
Artemisia princeps Pamp.	Asteraceae Giseke	Ethanol extract of leaves	MRSA	**IBF**: Inhibited bacterial proliferation **AQSV**: inhibited acid production, and decreased gene expression of mecA, sea, agrA and sarA	Choi et al. (2015)
Citrus ×bergamia (Risso) Risso & Poit. (a synonym of *Citrus ×limon* (L.) Burm.fil.) *Aspidosperma quebracho-blanco* Schltdl.	Rutaceae Juss. Apocynaceae Juss.	Essential oils	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited swarming motility and inhibited the production of protease and pyocyanin.	Ahmed et al. (2021)
Bergenia ciliata (Haw.) Sternb *Clematis grata* O.Hoffm. ex Baker (a synonym of *Clematis wightiana* Wall. ex	Saxifragaceae Juss. Ranunculaceae Juss.	Methanol extract of rhizome with skin (*B. ciliata*) Ethanol extract of leaves (*C. grata*)	*P. aeruginosa*	**IBF**: inhibited formation of biofilm without affecting bacterial growth	Alam et al. (2020)

Wight & Arn.)					
Callistemon citrinus (Curtis) Skeels (a synonym of *Melaleuca citrina* (Curtis) Dum.Cours.)	Myrtaceae Juss.	Dichloromethane - methanol extract of leaves/ pulverulentone A	*S. aureus* MRSA	**AMB**: reduced biofilm biomass adhered and thickness and destroyed the architecture without affecting the planktonic growth **AQSV**: lowered staphyloxanthin biosynthesis	Shehabeldine et al. (2020)
Calpurnia aurea (Aiton) Benth.	Fabaceae Juss.	Acetone, ethanol and ethyl acetate extracts of leaves	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered by cell membrane damages (ethanolic and ethyl acetate extracts showing only mild interferences) **AMB**: biofilm biomass adhered by disruption of biofilm structure (acetone extracts showed a moderate eradication) **AQSV**: inhibited violacein production in *C. violaceum*; reduced swimming and swarming motility in *P. aeruginosa*	Cosa et al. (2020)
Camellia sinensis (L.) Kuntze	Theaceae Mirb.	Ethyl acetate fraction of methanol extract of leaves	*P. aeruginosa* *S. marcescens*	**IBF**: reduced biofilm biomass and caused weaker structure **AQSV**: Inhibited violacein production in *C. violaceum*; reduced virulence factors expression of *P. aeruginosa* PAO1: pyocyanin, pyoverdine, exoprotease, elastase, rhamnolipid production, and swimming motility; decreased prodigiosin, protease activity, cell surface hydrophobicity, and swimming of *S. marcescens*	Qais et al. (2019)
		Tea polyphenols/ epigallocatechin-3-gallate (EGCG)	*F. nucleatum*	**IBF**: reduced biofilm biomass adhered **AMB**: time-dependent decrease in biofilm viability (ATP measure) **AQSV**: damaged the integrity of bacterial cell membrane, inhibited hemolysis, and decreased adherence to epithelial cells	Ben Lagha et al. (2017)
Capsicum baccatum var. *pendulum* (Willd.) Eshbaugh	Solanaceae Adans.	Aqueous extract of seeds	*P. aeruginosa* *S. epidermidis*	**IBF:** prevented bacterial adhesion to the surface, only allowed the formation of smaller cell clusters or cells	Von Borowski et al. (2019)

(a synonym of *Capsicum frutescens* L.)				without biofilm matrix	
Carissa spinarum L.	Apocynaceae Juss.	Methanol extract of unripe and ripe fruits	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited violacein production in *C. violaceum* and swimming motility	Nazareth et al. (2021)
Carum copticum (L.) Benth. & Hook.f. ex Hiern	Apiaceae Lindl.	Ethanol and methanol extracts	*A. baumannii*	**IBF**: disruption of biofilm structure and reduced biofilm metabolic activity	Mohammadi et al. (2019)
Centella asiatica (L.) Urb.	Apiaceae Lindl.	Ethyl acetate extract of leaves	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited production of pyocyanin and pyoverdine and violacein production in *C. violaceum*	Khan et al. (2022)
		Ethyl acetate fraction of ethanol extract of leaves	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: decreased production of pyocyanin and elastolytic and proteolytic activities, decreased swarming motility	Vasavi et al. (2016)
Cinnamomum verum J.Preslce	Lauraceae Juss.	Ethanol extract of leaves	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: reduced the production of elastase and pyocyanin, reduced the swarming motility; lower expression of virulence genes	Alva et al. (2021)
		Ethanol extract of bark powder	*E. coli* *V. parahaemolyticus*	**IBF**: reduced biofilm biomass adhered, by reducing EPS and forming scattered microcolonies **AMB**: disrupted biofilm structure	Lu et al. (2021)
Citrus limon (L.) Burm	Rutaceae Juss.	Methanol extract of fruits	*E. coli*	**IBF**: reduced biofilm biomass adhered by inhibiting the formation and maturation of the biofilm matrix	Singha et al. (2023)
Clinacanthus nutans (Burm.f.) Lindau	Acanthaceae Juss.	Chloroform extract of leaves/ purpurin-18 phytyl ester	*S. mutans*	**IBF**: reduced biofilm biomass without inhibiting planktonic growth **AMB**: purpurin-18 phytyl ester penetrates biofilm structure and kill bacteria.	Roeslan et al. (2019)
Clinopodium bolivianum (Benth.) Kuntze	Lamiaceae Martinov	Hydro-ethanolic extract of leaves and stems	*E. coli*	**IBF**: reduced adhesion by bacterial and reduced biofilm formation, without inhibiting the bacterial growth	Mohanty et al. (2017)
Cochlospermum regium (Schrank) Pilg.	Bixaceae Kunth	Aqueous and ethanol extracts of leaves	MRSA	**IBF**: reduced biofilm biomass adhered; altered cell morphology and provoke loss of cell wall integrity;	Galvão et al. (2020)

				decrease carbohydrate and protein content in the matrix	
Coriandrum sativum L. *Mentha × piperita L.* *Pimpinella anisum L.*	Apiaceae Lindl. Lamiaceae Martinov Apiaceae Lindl.	Essential oils of seeds (*C. sativum* and *P. anisum*) and leaves (*M. piperita*)	*E. coli* *S. aureus*	**IBF:** reduced initial bacterial attachment to surface	Bazargani and Rohloff (2016)
Cuphea carthagenensis (Jacq.) J.F.Macbr.	Lythraceae J.St.-Hil.	Methanol extract of leaves	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered without affecting bacterial growth and reduced EPS production **AQSV**: inhibited swimming and production of pyocyanin and pyoverdine	Rather et al. (2021)
Drosera rotundifolia L. *Drosera intermedia* Hayne	Droseraceae Salisb.	Ethanol extract of whole plants	*E. coli*	**IBF**: reduced biofilm biomass adhered	Gerschler et al. (2022)
Duabanga grandiflora (Roxb. Ex DC.) Walp.	Lythraceae J.St.-Hil.	Ethyl acetate extract of leaves	MRSA	**IBF**: inhibited cell-surface attachment leading to weaker biofilm structure, and attenuation of PBP2a level	Santiago et al. (2015)
Eruca sativa Miller	Brassicaceae Burnett	Ethanol extract of whole plants	*E. coli* *S. aureus*	**IBF:** reduced bacterial adhesion to surface and inhibited the EPS production, weaker biofilm structure **AMB:** reduced viability of cells within the biofilm	Awadelkareem et al. (2022)
Eucalyptus globulus Labill.	Myrtaceae Juss.	Essential oil of leaves and isolated 1,8-cineole	*S. aureus* *P. aeruginosa*	**IBF**: anti-attachment effect and reduced biofilm biomass adhered **AMB**: decreased biofilm viability **AQSV**: inhibited violacein production in *C. violaceum* and reduced swarming motility in *P. aeruginosa*	Merghni et al. (2018)
Fagonia indica Burm.f. (a synonym of *Zygophyllum indicum* (Burm.f.) Christenh. & Byng)	Zygophyllaceae R.Br.	Chloroform extract of aerial parts	*S. aureus* *K. pneumoniae*	**IBF:** reduced the initial attachment to surface and caused disintegration of bacterial cell wall	Aslam et al. (2022)
Ginkgo biloba L.	Ginkgoaceae Engl.	Ethanol extract of exocarp	*S. aureus* MRSA	**IBF**: reduced biofilm biomass adhered **AQSV**: downregulation of virulence factirs icaA, sarA and sigB **AMB**: disrupted mature biofilms	B. Wang et al. (2021)
Glycyrrhiza glabra L.	Fabaceae Juss.	Aqua-alcoholic extract of stem and glycyrrhizic acid	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: reduced efflux pump activity and altered the membrane permeability.	Chakotiya et al. (2016)

		/glabridin isolated			
Gutierrezia microcephala (DC.) A.Gray *Prosopis laevigata* (Humb. et Bonpl. ex Willd) M.C. *Opuntia ficus-indica* (L.) Mill.	Asteraceae Giseke Fabaceae Juss. Cactaceae Juss.	Methanol extracts of aerial parts (*G. microcephala*), bark and leaves (*P. laevigata*) and cladode (*O. fucus-indica*)	*E. coli*	**IBF:** reduced biofilm biomass adhered and caused weaker biofilm structure	Sánchez et al. (2016)
Himatanthus drasticus (Mart.) Plumel	Apocynaceae Juss.	Hydroalcoholic extract of leaves	*K. pneumoniae*	**IBF**: reduced biofilm biomass adhered and caused destabilization of cell membrane	Figueiredo et al. (2017)
Humulus lupulus L.	Cannabaceae Martinov	Humulone, lupulone and xanthohumol	*S. epidermidis* *S. aureus* MRSA	**AMB**: inhibited the release of cells from the biofilm; penetrated into biofilms and kill bacteria.	Bogdanova et al. (2018)
Iris pallida Lam *Iris versicolor* L.	Iridaceae Juss.	Methanol extract of leaves, roots and rhizomes	*P. aeruginosa* *S. aureus* multi-species biofilm	**IBF**: reduced the initial adhesion of cells to the surface (mono and multispecies) **AMB**: disruption of mature *P. aeruginosa* biofilms.	Hoang et al. (2020)
Juglans regia L.	Juglandaceae DC. ex Perleb	Methanol extract of leaves	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered	Dolatabadi et al. (2018)
		Ethanolic extract of pellicle	*S. epidermidis*	**IBF**: reduced biofilm biomass adhered and cell viability	Acquaviva et al. (2021)
Kalanchoe blossfeldiana Poelln.	Crassulaceae J.St.-Hil.	Methanol extracts of leaves	*P. aeruginosa*	**IBF:** reduced biofilm biomass adhered **AMB:** disrupted mature biofilms **AQSV:** secretion of virulence factors (protease and pyoverdin) along with generation of acyl homoserine lactone (AHL)	Sarkar et al. (2015)
Kalanchoe laxiflora Baker	Crassulaceae J.St.-Hil.	Methanol extract of flowers	*E. coli*	**IBF:** reduced biofilm biomass adhered	Osman et al. (2022)
Melaleuca alternifolia (Maiden & Betche) Cheel	Myrtaceae Juss.	Essential oil of leaves/ terpinene-4-ol	*S. aureus* MRSA *P. aeruginosa*	**IBF**: reduction of cell adhesion in *S. aureus* **AMB**: bacterial killing and degradation of extracellular matrix in *S. aureus*	Noumi et al. (2018)

				AQSV: reduced violacein production in *C. violaceum*, inhibited swarming motility in *P. aeruginosa*	
Melianthus comosus Vahl	Francoaceae A.Juss.	Water, methanol and dichloromethane extracts of leaves/ guanosine	*S. aureus* *P. aeruginosa*	**IBF**: reduced biofilm biomass adhered by inhibition of the initial attachment **AQSV**: inhibited swimming and swarming motility in *P. aeruginosa*; disrupt violacein production in *C. violaceum*	Baloyi et al. (2021)
Musa acuminata Colla	Musaceae Juss.	Methanol extract of fruit peel	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered, initial attachment and EPS and protein production **AQSV**: interfered with Lasl and Rhll gene expression and decreased the production of pyocyanin, protease, elastase, rhamnolipid and alginate.	Vijayakumar and Ramanathan (2020)
Musa paradisiaca L.	Musaceae Juss.	Methanol extract of fruit/ 1, 8-cineole	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered without affecting bacterial growth **AQSV**: reduced production of virulence factors (protease, elastase, pyocyanin, alginate and rhamnolipid) **AMB**: disrupted biofilm structure turning it into microcolonies	Karuppiah et al. (2021)
Myrsine umbellata Mart.	Primulaceae Batsch ex Borkh.	Ethanol extract and essential oil of leaves	*E. coli* *S. aureus* *S. enteritidis*	**AMB**: reduced the biomass in the biofilm	Laskoski et al. (2022)
Nigella sativa L.	Ranunculaceae Juss.	Supercritical CO_2 extraction of seeds/ thymoquinone (isolated from the extract)	*S. aureus* MRSA	**IBF**: reduced biofilm biomass adhered dependent on thymoquinone content	Gawron et al. (2019)
Notopterygium incisum K.C.Ting ex H.T.Chang	Apiaceae Lindl.	Isolated compound falcarindiol	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: reduced production of virulence factors (elastase, pyocyanin, and rhamnolipid)	Zhao et al. (2021)
Origanum majorana L.	Lamiaceae Martinov	Essential oil of leaves/ sabinene	*E. coli*	**IBF**: reduced biofilm biomass adhered **AQSV**: reduced efflux pump ativity	Ghazal et al. (2022)
Origanum vulgare L.	Lamiaceae	Ethanol extracts	*E. coli*	**IBF**: reduced biofilm biomass adhered without affecting	Panayi et al.

Rosmarinus officinalis L. (a synonym of *Salvia rosmarinus* Spenn.) *Salvia officinalis* L.	Martinov	(N/S)		bacterial growth **AQSV**: inhibited swimming and swarming motility	(2022)
Patrinia villosa Juss.	Caprifoliaceae Juss.	Water extract (N/S)	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered, formation of smaller cell clusters, altered the structure of biofilms, and inhibited exopolysaccharide production **AQSV**: affected the expression of algU, algA, pslM, bdlA, pelA genes associated to biofilm formation	Fu et al. (2017)
Peganum harmala L.	Nitrariaceae Lindl.	*n*-butanol extract of seeds/ harmaline	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered, formation of smaller cell clusters **AMB**: disruption of mature biofilm and reduction in cell viability	Khadraoui et al. (2022)
Persea americana Mill.	Lauraceae Juss.	Ethanol extract of seeds	*E. coli*	**IBF**: reduced biofilm biomass adhered by inhibition of the initial attachment	Molina Bertrán et al. (2022)
Persicaria maculosa Gray *Bistorta officinalis* Delarbre	Polygonaceae Juss.	Ethanol extract of aerial parts (*P. maculosa*) and rhizome (*B. officinalis*)	*P. aeruginosa* *S. enteritidis* *S. aureus*	**IBF**: both extracts reduced biofilm biomass adhered of *P. aeruginosa* and *S. enteritidis*; *B. officinalis* only reduced biofilm biomass adhered of *S. aureus* **AQSV**: reduced violacein production in *C. violaceum* and reduced pyocyanin production, altered swarming motility and reduced the activity of LasR receptor in *P. aeruginosa*.	Jovanović et al. (2020)
Piper betle L.	Piperaceae Giseke	Ethyl acetate extract of leaves	*S. marcescens*	**IBF**: reduce total biomass adhered and microcolony formation **AQSV**: reduction of prodigiosin production, interrupted EPS production, reduction in swarming motility, and downregulation of QS regulated genes fimA, fimC, flhD, bsmA and bsmB	Srinivasan et al. (2016)
		Ethanol extract of leaves	*E. coli* *S. aureus*	**IBF**: reduced biofilm biomass adhered **AMB**: disrupted mature biofilms	Saeloh and Visutthi (2021)
Plectranthus barbatus Andrews (a synonym of	Lamiaceae Martinov	Essential oil of root	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited swarming and twitching motility in *P.*	Chatterjee and Vittal (2021)

Coleus barbatus (Andrews) Benth. ex G.Don)				*aeruginosa*.	
Plumbago zeylanica L.	Plumbaginaceae Juss.	Methanol extract of root	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited motility and inhibited the production of pyocyanin, pyoverdine, rhamnolipid.	Qais et al. (2021)
Podocarpus lambertii Klotzsch ex Endl.	Podocarpaceae Endl.	Methanol extract of leaves	*E. coli*	**AMB:** reduced biomass of 24h preformed biofilms	Bandeira et al. (2022)
Polypodium vulgare L.	Polypodiaceae J.Presl & C.Presl	Aqueous extract of rhizome/ compound osladin	*E. coli*	**IBF:** reduced biofilm biomass adhered and caused weaker biofilm structure	Gleńsk et al. (2019)
Pongamia pinnata (L.) Pierre	Fabaceae Juss.	Ethyl acetate extract of seeds	*S. epidermidis*	**IBF**: reduced biofilm biomass adhered;	Rajput et al. (2021)
Prunus avium L.	Rosaceae Juss.	Methanol extracts of cherry stalks	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited swarming motility	Önem et al. (2021)
Pulicaria crispa (Forssk.) Oliv. (a synonym of *Pulicaria undulata*)	Asteraceae Giseke	Methanol extract (N/S)	*K. pneumoniae*	**IBF**: reduced biofilm biomass adhered	Thinina et al. (2020)
Rhamnus prinoides L'Hér.	Rhamnaceae Juss.	Ethanol extract of leaf and stem	*S. aureus* *B. subtilis* *S. mutans*	**IBF**: inhibited biofilm formation by biocidal or bacteriostatic mechanism	Campbell et al. (2019)
Rosa canina L.	Rosaceae Juss.	Methanol extract of leaves	*E. coli* *P. aeruginosa*	**IBF**: reduced biofilm biomass adhered	Živković et al. (2015)
Rosmarinus officinalis L. (a synonym of *Salvia rosmarinus* Spenn.) *Thymus zygis* L. *Origanum majorana* L.	Lamiaceae Martinov	Essential oils (N/S)	*E. coli*	**IBF**: reduced the attachment of cells to the abiotic surface	Lagha et al. (2019)
Rosmarinus officinalis L. (a synonym of *Salvia rosmarinus* Spenn.)	Lamiaceae Martinov	1,8-cineole	*E. coli*	**AMB**: disruption of mature biofilm and reduction in cell viability	Vazquez et al. (2020)
Russula integra (L.) Fr.	Russulaceae	Methanol and ethanol	*S. aureus*	**AMB**: disruption of 24h biofilms	Kostić et al.

Russula rosea Pers. *Russula nigricans* (Bull.) Fr.	Lotsy	extracts (N/S)			(2020)
Salacia crassifolia (Mart.) G.Don	Celastraceae R.Br.	Hexane extract of root/ pristimerin	*S. aureus*	**AMB**: disrupted biofilms and altered the membrane stability	Nizer et al. (2021)
Salvadora persica L.	Salvadoraceae Lindl.	Methanol extracts of fruit, stem and leaves	*S. aureus* *P. aeruginosa*	**IBF**: reduced biofilm biomass adhered in *S. aureus* **AQSV**: inhibited violacein production in *C. violaceum*, and inhibited swarming motility in *P. aeruginosa*	Noumi et al. (2017)
Sanguisorba officinalis L.	Rosaceae Juss.	Ethanol extract of root	MRSA	**IBF**: reduced biofilm biomass adhered and caused weaker structure **AQSV**: increase transcript level icaR leading to strong inhibitory effect of icaADBC operon (adhesin synthesis)	Chen et al. (2015)
Sapindus mukorossi Gaertn.	Sapindaceae Juss.	Methanol extract of seeds	MRSA	**IBF**: reduced biofilm biomass adhered without affecting bacterial growth **AQSV**: in cell surface hydrophobicity, slime and production of EPS and extracellular DNA, downregulation of virulence genes (icaA, ciaD,fnbA, fnbB, clfA, can and altA)	Selvaraj et al. (2021)
Sarcochlamys pulcherrima (Roxb.) Gaudich.	Urticaceae Juss.	Tormentic acid, 23-hydroxycorosolic acid	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered and reduction of EPS formation **AQSV**: suppressed the production of pyoverdine, protease and swarming motility	Ghosh et al. (2020)
Schinus terebinthifolia Raddi	Anacardiaceae R.Br.	Triterpenoid acids (3-oxo-olean-12-en-28-oic acid, 3-oxotirucalla-7,24Z-dien-26-oic acid, 3α-hydroxytirucalla-7,24Z-dien-26-oic acid)	*S. aureus*	**IBF**: reduced biofilm biomass adhered without inhibiting growth **AQSV**: Inhibited agr types and d-toxin production.	Tang et al. (2020)
		Methanol extract of fruits	*S. aureus*	**IBF**: inhibition of the initial attachment for biofilm formation without affecting the planktonic growth **AQSV**: inhibition of agr system	Muhs et al. (2017)
Syagrus coronata (Martius)	Arecaceae	Essential oil of seeds	*S. aureus*	**AMB**: decrease cell viability in biofilm, caused	Souza dos

Beccari	Bercht. & J.Presl			structural alterations, and loss of roughness in biofilm structure	Santos et al. (2019)
Symplocos racemose Roxb.	Symplocaceae Desf.	Ethyl acetate extract of bark	*E. coli* *K. pneumoniae* *S. aureus*	**IBF**: reduced biofilm biomass adhered by inhibition of the initial attachment **AMB**: disruption of biofilms and reduced metabolically active cells	Sood et al. (2020)
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae R.Br.	Methanol extract and essential oil of leaves	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: inhibited violacein production in *C. violaceum*; inhibited pyocyanin and EPS production	Sankar Ganesh and Ravishankar Rai (2018)
Thymus daenensis Čelak. *Satureja hortensis* L.	Lamiaceae Martinov	Essential oils (N/S)	*E. coli*	**IBF**: reduced biofilm biomass adhered **AQSV**: downregulation of luxS and pfs	Sharifi and Nayeri Fasaei (2022)
Torilis japonica (Houtt.) DC.	Apiaceae Lindl.	Ethanol extract of fruits	*S. aureus* MRSA	**IBF**: reduced biofilm biomass adhered **AQSV**: expression of agrA, sarA, icaA, hla, and RNAIII	Kim et al. (2022)
Trigonella foenum-graecum L.	Fabaceae Juss.	Methanol extract (N/S)	*E. coli* *S. aureus*	**IBF**: reduced biofilm biomass	Alenazy (2023)
Vaccinium corymbosum L.	Ericaceae Juss.	Water extract of fruit	*E. coli* *S. aureus*	**IBF:** reduced bacterial adhesion without affecting the planktonic growth	Silva et al. (2016)
		Saline extract of fruit	*K. pneumoniae*	**IBF**: altered the bacterial adherence capacity inhibiting the initial stage of biofilm formation without affecting growth	Gato et al. (2020)
Vernonia adoensis Sch.Bip. ex Walp. (a synonym of *Baccharoides adoensis* (Sch.Bip. ex Walp.) H.Rob.)	Asteraceae Giseke	Chondrillasterol isolated from acetone extract of leaves	*S. aureus* *K. pneumoniae* *P. aeruginosa*	**IBF**: biofilm formation by supressing bacterial growth **AMB**: disrupted mature 72h-old biofilms of *P. aeruginosa*	Mozirandi et al. (2019)
Vitex gardneriana Schauer	Lamiaceae Martinov	Essential oil of leaves	*S. aureus* *P. aeruginosa*	**IBF**: reduced biofilm biomass and number of viable cells	Vale et al. (2019)
Warburgia ugandensis Sprague	Canellaceae Mart.	Ethanol and acetone extract of leaves/ alpha-linolenic acid (ALA), warburganal	*S. epidermidis* *S. aureus*	**IBF**: extracts and ALA reduced biofilm biomass adhered **AMB**: warburganal reduced biofilm biomass adhered	Kipanga et al. (2020)

Zingiber officinale Roscoe	Zingiberaceae Martinov	Aqua-alcoholic extract of rhizome	*P. aeruginosa*	**IBF**: reduced biofilm biomass adhered **AQSV**: affected the permeability and efflux activity of bacteria.	Chakotiya et al. (2017)
Zingiber zerumbet (L.) Roscoe ex Sm.	Zingiberaceae Martinov	zerumbone	*S. aureus*	**IBF**: inhibited initial adhesion, reduced biofilm biomass adhered **AMB**: reduced biofilm amount determined by cell metabolic activity	Shin and Eom (2019)

[a] N/S: plant part not specified

A. baumannii: *Acinetobacter baumannii*; *B. subtilis*: *Bacillus subtilis*; *C. violaceum*: *Chromobacterium violaceum*; *E. coli*: *Escherichia coli*; *F. nucleatum*: *Fusobacterium nucleatum*; *K. pneumoniae*: *Klebsiella pneumoniae*; MRSA: methicillin-resistant *Staphylococcus aureus*; *M. tuberculosis*: *Mycobacterium tuberculosis*; *P. mirabilis*: *Proteus mirabilis*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. enteritidis*: *Salmonella enteritidis*; *S. marcescens*: *Serratia marcescens*; *S. flexneri*: *Shigella flexneri*; *S. aureus*: *Staphylococcus aureus*; *S. epidermidis*: *Staphylococcus epidermidis*; *S. maltophilia*: *Stenotrophomonas maltophilia*; *S. mutans: Streptococcus mutans; V. parahaemolyticus*: *Vibrio parahaemolyticus*

EPS: Extracellular polymeric substance QS: Quorum sensing

IBF: Inhibition of biofilm formation; **AMB**: Activity on mature/preformed biofilms; **AQSV**: anti-quorum sensing/antivirulence

Declaration of Competing Interest

The authors confirm that there are no conflicts of interest associated with this publication.

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Graphical Abstract