Special Conference Edition, November, 2019

http://dx.doi.org/10.4314/bajopas.v12i1.93S

Bayero Journal of Pure and Applied Sciences, 12(1): 619 - 627
ISSN 2006 – 6996

NATURAL AND SYNTHETIC ANTIBIOFILM COMPOUNDS: A REVIEW

Usman, N.A[a*] , Adeshina, G.O[b] , Tytler, B.A[b] ,
[a] Department of pharmaceutics & pharmaceutical microbiology,
Faculty of pharmacy, University of Maiduguri, Nigeria.
[b] Department of pharmaceutics & pharmaceutical microbiology,
Faculty of pharmaceutical science, Ahmadu Bello University, Zaria, Nigeria.
*Corresponding author: nastradoy@gmail.com.

ABSTRACT
Biofilm is a community of microorganisms adhering to biotic or abiotic surfaces embedded by a self-produced extra-polymeric matrix facilitating the survival in an adverse environment. Quorum sensing systems provide for the bacteria an access to nutrients and favourable environmental niches as well as enhanced action against competing bacteria and environmental stresses. Quorum sensing has also been identified as a mode of resistance to antibacterial treatment. The aim of this review is to give a synopsis which will serve as a knowledge base for researchers on antibiofilms. Provided in this work are naturally occurring and synthetic antibiofilms which are at various stages of development. The mechanisms of action of these compounds are yet to be fully understood. While some antibiofilm compounds prevent the formation of biofilms, others disrupt already formed biofilms. Notable among compounds which prevent the formation of biofilms are quorum sensing inhibitor, ajoene obtained from garlic, proanthocyanidins from cranberry and others. The chemical structures of some of these compounds were highlighted with a view to identifying the moieties responsible for their antibiofilm activities. Among the synthetic antibiofilm compounds, marine products, like dihydrooroidin (DHO) have been identified to have both antibiofilm and antifouling activities. It is pleasing that, not only do we have in the pipeline many biological compounds to be used for prevention and disruption of already-formed biofilms, we also have synthetic antibiofilms largely from aquatic sources
Keywords : Biofilm, quorum sensing, natural antibiofilm compounds, synthetic antibiofilm compounds, resistance.

INTRODUCTION

Biofilms are a biologically active matrix of cells and extra-cellular substances in association with a solid surface, frequently embedded in a matrix of extracellular polymeric substances (Heydorn *et al*., 2000). Over the past few decades, biofilm growth has been observed in many industrial and domestic domains. Unfortunately, in most cases the growth of biofilms has been detrimental. Examples of industries that suffer the ill-effects of biofilm include the maritime, dairy , food , water systems, oil, paper, opticians, dentistry and hospitals (Dunne, 2002). Perhaps the environment where people are exposed to biofilms most frequently is the domestic environment (Klahre *et al*., 2000).

Product spoilage, reduced production efficiency, corrosion, unpleasant odours (malodours), unsightliness, infection, pipe blockages and equipment failure are examples of the detrimental effects of biofilms (Espeland et al., 2001).

Quorum sensing is a cell-to-cell communication mechanism, which plays an important role in biofilm development and balances the environment when the bacteria density becomes high (Lan lu *et al*., 2019). It is a signaling system through which sessile cells in a biofilm "talk" to each other in order to build microcolonies and to keep water channels open. A variety of different molecules can be used as signals. Karbasizade *et al*. (2017) reported that extracts from plants have been shown to regulate biofilm formation and inhibit quorum sensing. Authors of this review therefore, found it rational to give a collection of plants and marine products with antibiofilm activities currently undergoing clinical trials. This review will offer biofilm researchers opportunity to further investigate the activities of these antibiofilm agents or validate the claims on them.

Here, the naturally-occurring antibiofilm compounds are highlighted, stating their mechanisms of action as well as their molecular structures stating the moieties involved in their chemical interaction with the biofilm matrices. Next, we discussed the synthetically-derived

antibiofilm compounds, their sources and their potential of seeing the light of the day in terms of industrial production.

Mechanism of biofilm infections

The pathogenesis of biofilm-related infections involve a) detachment of cells or biofilm aggregates may result in bloodstream or urinary tract infections or in the production of emboli, b) cells may exchange resistance plasmids within biofilms, c) cells in biofilms have dramatically reduced susceptibility to antimicrobial agents, d) biofilm-associated gram-negative bacteria may produce endotoxins, and e) biofilms are resistant to host immune system clearance (Characklis, 1973).

Quorum Sensing

Cell-to-cell signaling, known as quorum sensing, has been shown to play a role in biofilm formation in foodborne pathogens. Bacterial gene expression in some bacterial species may be regulated by quorum sensing, a cell density-dependent signaling system mediated by chemical autoinducer molecules produced by bacteria (Miller *et al.*, 2001). The autoinducer molecules bind to the appropriate transcription regulator(s) when the bacterial population reaches the quorum level (that is, the signal concentration reaches a threshold concentration sufficient to facilitate binding to the receptor) (Bassler 2001). Binding of the autoinducers is followed by activation or repression of target genes. Thus, quorum sensing allows bacteria to display a unified response that benefits the population (Smith *et al.*, 2004). Bacterial quorum sensing systems enhance access to nutrients and more favorable environmental niches, and they enhance action against competing bacteria and environmental stresses. Examples of cellular processes modulated by quorum sensing are symbiosis, transfer of conjugative plasmids, sporulation, antimicrobial peptide synthesis, regulation of virulence, and biofilm formation (Bassler 2001).

There are according to Bassler. (2001), several different quorum sensing autoinducer systems in bacteria. Three main QS systems can be distinguished: the acylhomoserine lactone (AHL) QS system in Gram-negative bacteria, the autoinducing peptide (AIP) QS system in Gram-positive bacteria and the autoinducer-2 (AI-2) QS system in both Gram-negative and -positive bacteria. For example, in Gram-negative bacteria, the quorum sensing system is dependent on homologues of the *Vibrio fischeri* LuxI-LuxR regulatory proteins (Miller *et al.*, 2001). Synthesized by the LuxI-like proteins, the autoinducer compounds are acylated homoserine lactones (AHLs), which are also known as autoinducer 1 (AI-1) . The AHLs consist of a homoserine lactone ring with a variable length acyl side chain. The AHL is synthesized inside the cell and is either diffused or secreted outside to the external environment. The concentration of AHLs increases as the bacterial population increases. When the AHLs reach a critical threshold level, they re-enter the bacterial cell to bind to the LuxR-like protein receptors. The LuxR-AHL complexes activate or repress target gene transcription (Miller *et al.*, 2001).

Organizational Structure of Biofilms

Understanding the structure of medically significant biofilms, and how to prevent them, are important sectors of current research in the microbiological field.

In an aqueous environment, bacteria may form an organized community that is attached to a solid surface at the liquid interface; this is known as a biofilm (Zheng *et al.*, 1994). Bacteria that form biofilms must be able to adhere to a surface, as well as accumulate to form multilayered cell clusters through intracellular adhesion (Rijnaarts *et al.*, 1993 ; Zheng *et al.*, 1994)

In this diagram below, reversible attachment and irreversible attachment are two different steps.

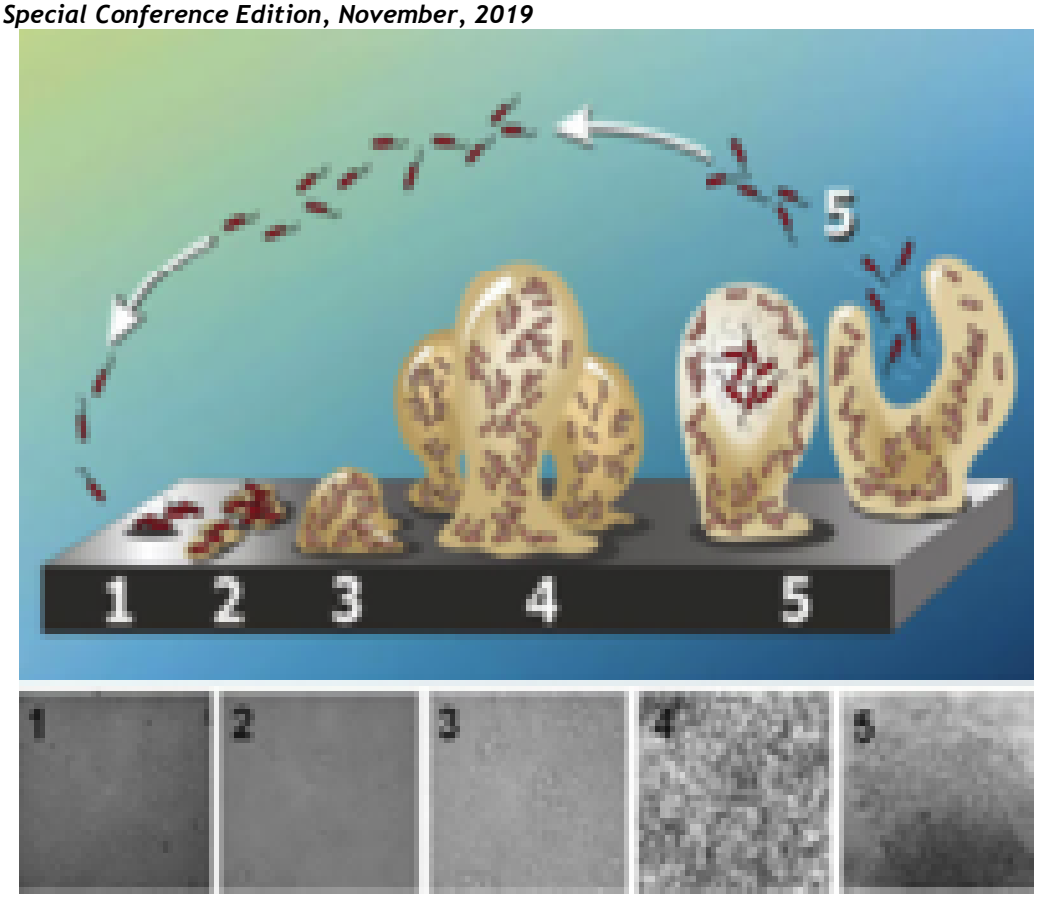


Figure 1: Stages of Development of Biofilm
Lab Medicine, 2015

The formation of a biofilm involves a four step process: attachment of bacterial cells to a surface, accumulation of bacterial cells forming multiple layers, maturation of the biofilm, and release of some bacteria in the planktonic state, which can attach to a new surface and form another biofilm (Zheng *et al.*, 1994). These sessile communities offer protected growth from the environment (Costerton *et al.*, 1999), and have a heightened resistance to antimicrobials as well as to the host immune responses (Stewart *et al.*, 2001).

Mode of action of antibiofilm agents

About 60 synthesized compounds were screened for antimicrobial and anti-biofilm activity, ¼ of which were interesting for further investigation and recommended for follow-up studies (Mitchenko *et al.*, 2015).

Membranolytic mode of action in Gram-positive bacteria was revealed for several compounds

Antibiofilm substances can inhibit biofilm formation (preventive effect) or alternatively act on biofilms already formed (therapeutic effect). The mechanism of action against established biofilms may be through disruption of biofilm biomass and/or direct killing of the biofilm bacteria. (Brazil oral research., 2013).

Naturally-occurring antibiofilm compounds

The following is a concise review of some of the natural anti-biofilm agents currently under study.

Biofilm-Disrupting Enzymes

Enzymes, like DNase I, α-amylase and DspB are biofilm-dispersing agents that degrade the biofilm matrix, permitting increased penetration of antibiotics. DNase I cleavage of extracellular DNA leads to alterations in biofilm architecture, which permits increased antibiotic penetration (Tetz *et al.*, 2009). A DNA-dissolving drug (Pulmozyme) has been used in cystic fibrosis patients to help disrupt the biofilm. α-amylase is a proven anti-biofilm agent against *Staphylococcus aureus*, *Vibrio cholerae* and *Pseudomonas aeruginosa*, not only inhibiting biofilm formation, but also degrading preformed mature biofilms (Kalpana *et al.*, 2012). DspB is a soluble β-*N*-acetylglucosaminidase with broad-spectrum activity to dissolve the biofilm matrix, and shows synergy with other antimicrobials (Darouiche *et al.*, 2009).

Proteolytic enzymes like serrapeptase help the body break down protein involved in inflammation and mucous. It may also help

disrupt the outer layers of biofilms and uncover hidden microbes.

Unfortunately, the high cost of industrial enzyme production makes their large-scale application as anti-biofilm agents unfeasible (Sun *et al.*, 2013).

Bacteriophages are viruses that produce a number of enzymes that negate the protection afforded by biofilms. Phages degrade the biofilm matrix and lyse bacteria, while leaving friendly bacteria unharmed. Phage modification of biofilm architecture also increases susceptibility to antibiotics. However, phage-resistant bacteria can evolve rapidly. (Sun *et al.*, 2013)

Quorum-Sensing Inhibitors

Quorum-Sensing (QS) is a form of communication bacteria use to cooperatively build biofilm communities. Most bacteria produce QS signals, as well as QS inhibitors. Usnic acid, a lichen metabolite, possesses inhibitory activity against bacterial and fungal biofilms via QS interference. QS inhibitors can increase the susceptibility of biofilms to antibiotics. QS Inhibitors are generally regarded as safe in humans. (Sun *et al.*, 2013)

Garlic inhibits the expression of several genes that control bacterial QS. The star in garlic's arsenal is ajoene, the sulfur-containing compound produced when garlic is crushed. Ajoene inhibits production of rhamnolipid, which shields biofilms from white blood cells. Over 90% of biofilm bacteria were killed with a combination of ajoene and the antibiotic tobramycin. Garlic also has anti-viral, anti-fungal, and anti-protozoal properties, and benefits the cardiovascular and immune systems. (Jakobsen *et al.*, 2012) These sulfur compounds from garlic quickly lose their activity upon exposure to oxygen. A willow bark extract, hamamelitannin, also inhibits QS. (Morgan., 2015).

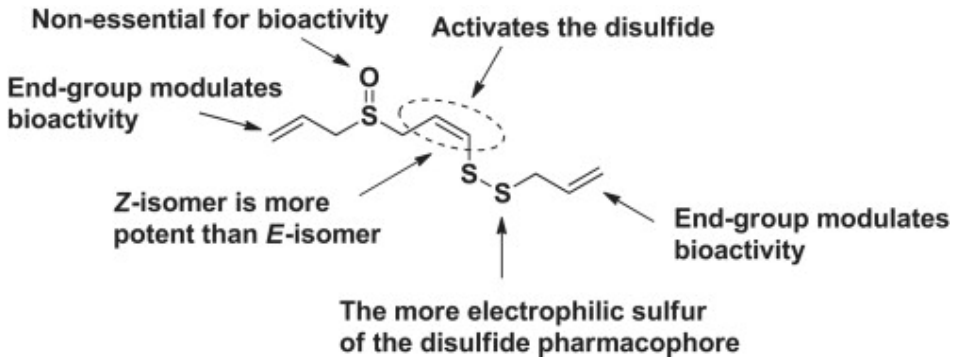


Figure. 2: Chemical structure of ajoene functional group

Antioxidants

According to Lee *et al* (2011), the anticancer, antioxidant, and anti-inflammatory effects of flavonoids are well established. Yet, their biofilm disrupting function is practically unknown. Flavonoids appear to suppress the formation of biofilms via a non-specific QS inhibition (Vikram *et al.*, 2010). The flavonoid phloretin inhibited biofilm formation in *E. coli* O157:H7, and ameliorated colon inflammation in rats without harming beneficial biofilms (Lee *et.al.*, 2011). Naturally occurring flavanols in cocoa may reverse memory decline significantly. (Brickman, *et al.,* 2014) Their ability to inhibit QS might provide a clue for their action.

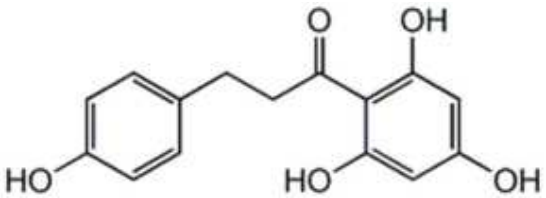


Figure 3 : Flavanol moiety

Cranberry has a reputation for keeping bacteria from sticking to surfaces. The red pigments in cranberries have been shown to inhibit biofilm formation. These proanthocyanidins (PACs) have been reported to possess antimicrobial, anti-adhesion, antioxidant, and anti-inflammatory properties (Bodet *et al.*, 2006).

They prevent the attachment of pathogens to host tissues, and can inhibit the formation of biofilms in the mouth and urinary tract. (Labrecque *et al.*, 2006) Cranberry PACs stopped the gum disease pathogen, *Porphyromonas gingivitis*, from adhering and forming biofilm, which markedly reduced its invasiveness. [La *et al.*, 2010] These unique PACs also prevented adherence and biofilm formation by *Candida albicans*, the causative agent of thrush and yeast infections. (Feldman *et al.*, 2012) .Cranberry juice extract, at low micromolar levels, inhibited tissue-destroying enzymes made by bacteria (La *et al.*, 2010) and humans. (Bodet *et al.*, 2007) Cranberry PACs also prevented dental plaque, by inhibiting biofilm-forming enzymes, (Steinberg *et al.,* 2004) and keeping bacteria from aggregating. (Weiss *et al.*, 1998; Yamanaka *et al.,* 2004) Daily use of a cranberry-containing mouthwash for 6 weeks significantly reduced levels of mutans *streptococci* in human saliva. (Weiss *et al.,* 2004) The anti-adhesive benefits of cranberry for urinary tract infections may be substantially increased by increasing the alkalinity of urine.

Chemical structure of proanthocyanidins

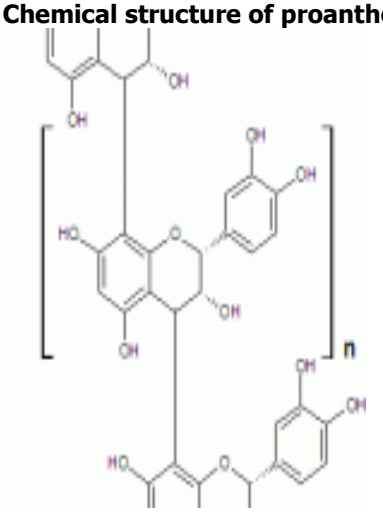


Figure 4: Procyanidin Skeleton

Chlorogenic acids (CGA), largely from coffee, are cinnamic acid derivatives with important antioxidant and anti-inflammatory activities. (Farah *et al.,* 2008) In vitro antibacterial and anti-biofilm activities of chlorogenic acid against clinical isolates of *Stenotrophomonas maltophilia* resistant to trimethoprim/sulfamethoxazole (TMP/SMX) was investigated. The MIC and MBC values ranged from 8 to 32 µg/mL. In vitro antibiofilm testing showed a 4-fold reduction in biofilm viability at 4x MIC. (Karunanidhi *et al.,* 2012)

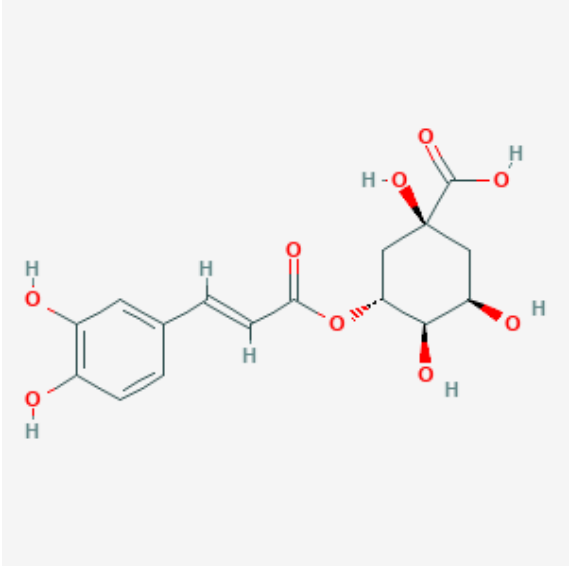
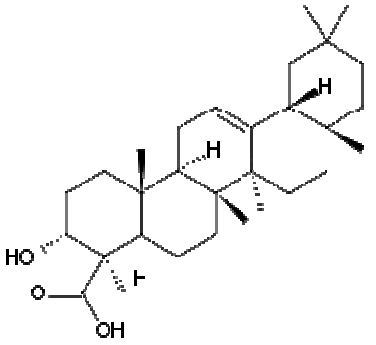


Figure 5 : Chlorogenic acid skeleton

Boswellic acids are pentacyclic triterpenes, produced in plants belonging to the genus Boswellia, with potent anti-biofilm properties. Acetyl-11-keto-β-boswellic acid, which exhibited the most potent antibacterial activity, was effective against all 112 pathogenic gram positive bacteria tested (MIC range, 2-8 µg/ml). It inhibited biofilms formed by *S. aureus and S. epidermidis*, and could also disrupt preexisting biofilms. Disruption of bacterial membranes is the likely mode of action (Raja *et al.,* 2011).



Structure of α-boswellic acid

Boswellic acids are a series of pentacyclic triterpene molecules that are produced by plants in the genus *Boswellia* like *Boswellia odorata*

The leaf extract of *Pongamia pinnata* showed significant antibiofilm activity (Karlapudi *et al*., 2012). The antimicrobial activity of the plant extract is attributed to the presence of phenolic compounds, such as alkaloids, flavonoids, terpenoids and polyacetylenes. (Shan *et al.,* 2007)

Five Indonesian medical plant extracts were shown to inhibit *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilm formation at concentrations as low as 0.12 mg/mL. (Pratiwi *et al*., 2015)

Wheat bran extract exhibits anti-biofilm activity, inhibiting biofilm formation and destroying pre-formed *S. aureus* biofilm in dairy cows with mastitis (González-Ortiz *et al.,* 2014).

Farnesol and xylitol were shown to possess antibiofilm and antibacterial effects when used in root canal irrigants (Alves *et al*., 2013). Xylitol is a low-carb sweetener found in toothpaste and diet sodas. When bacteria incorporate xylitol into the biofilm, it makes for a flimsy structure (Morgan., 2015).

Aspirin and many other naturally-occurring salicylates have been shown to inhibit the macromolecules that make up the biofilm matrix (Domenico *et al.,* 1990; Muller *et al*., 1998). Salicylates are produced by many plants in response to infection.

Pro-oxidants can also be effective against biofilms. Oxidative agents are microbicidal, and offer possibilities for reducing the pathogenic activities of biofilms, especially those with an anaerobic component.

Fatty Acid Inhibitors

Several *Salvia* (Sage) species widely used as spices were evaluated for their antimicrobial activities, including their anti-adhesive and anti-biofilm effects. *Salvia triloba* extract demonstrated significant bacteriocidal activity against MRSA. Its volatile oil was active against all tested microorganisms except *P. aeruginosa*. *S. triloba* extract and volatile oil were active against biofilms, demonstrating anti-adhesion and anti-biofilm activities, respectively. The antimicrobial activities of other *Salvia* species were negligible (Al-Bakri *et al*., 2010).

Synthetically-derived antibiofilm compounds

Anti-Biofilm Agents Derived from Marine Sponges

Marine sponges can be likened to little factories for bioactive secondary metabolites. These benthic organisms are some of the simplest multicellular animals with little differentiation and long lives, relying on the water around them to supply all their essential needs. Therefore, the generation of chemical defenses is a key element of their survival, whether they need to ward off predators (Callow *et al*., 2006), fight off competition for space and resources (Beech *et al*., 2004) or control surface fouling (Musk *et al*., 2006). Sponges utilize a plethora of chemical classes to protect themselves and even to communicate with symbiotic organisms that can provide nutrients and additional protection (Callow *et al*., 2006). Many of these chemicals have been found to have antifouling and anti-biofilm properties, but very few have been shown to modulate biofilm formation without killing the bacteria or disrupting their growth.

To date, only two classes of marine sponge metabolites house non-bactericidal biofilm modulators, the terpenoids (Musk *et al.*, 2006) and the pyrrole-imidazoles (Beech *et al.,* 2004).

Although not unique to sponges, one of the most potent and diverse groups of molecules is the terpenes and their derivatives. Terpenes have a high degree of structural diversity stemming from the modification of isoprene subunits and are valued for their broad range of biological activity, from antifouling agents to antiproliferative cancer therapeutics (Callow *et al.*, 2006 ; Davies *et al.*, 2003). Within the sponge terpenoids, only ageloxime-D, manoalide, and two manoalide congeners have been reported to have the ability to infer with bacterial biofilm formation without disrupting cellular growth.

In addition, the Melander group has been able to construct from marine sponges, a series of very successful anti-biofilm libraries that utilize the 2-aminoimidazole moiety found within a class of potent molecules, the pyrrole-imidazole alkaloids (PIAs).

CONCLUSION

Many biological compounds of plant or animal origin have emerged some capable of preventing the formation of biofilms, while others are capable of prevention and disruption of already formed biofilms. More intriguing is the fact that we now have synthetic antibiofilms coming from marine (aquatic) sources widening the array of biofilm-inhibiting substances that can be used synergistically with the conventional antimicrobial agents in use.

RECOMMENDATION

As we advance our knowledge of how to design potent anti-biofilm molecules, a few issues have become clear. First, we need to understand the basic mechanistic aspects of how these molecules exert their activity. These molecules have the ability to provide tools to deconvolute bacterial signaling pathways that are both conserved and unique to Gram-positive and Gram-negative bacteria. Second, these compounds need to be evaluated in various animal models of infection. As a therapeutic strategy, these compounds will mostly likely serve as adjuvants to conventional antibiotics, and there are dosing and pharmacokinetic/pharmacodynamic (PK/PD) issues that must be optimized between the antibiotic and anti-biofilm agent. Third, new molecular classes should be investigated to expand our repertoire of anti-biofilm agents.

REFERENCES

Al-Bakri A.G.; Othman, G, and Afifi, F.U. (2010). Determination of the antibiofilm, antiadhesive, and anti-MRSA activities of seven *Salvia* species. *Pharmacognosy Magazine* 6(1):264-70.

Alves F.R.F.; Neves M.A.S., and Silva M.G. (2013). Antibiofilm and antibacterial activities of farnesol and xylitol as potential endodontic irrigants. *Journal of Biomolecules*

Beech I.B, Sunner J. Biocorrosion: Towards understanding interactions between biofilms and metals. Current Opinion on Biotechnology. 2004;15:181–186.

Bodet C, Chandad F, Grenier D. Anti-inflammatory activity of a high-molecular weightcranberry fraction on macrophages stimulated by lipopolysaccharides from periodontopathogens. Journal of Dental Research 2006;85:235-9.

Bodet C, Chandad F, Grenier D. Inhibition of host extracellular matrix destructive enzyme production and activity by a high molecular-weight cranberry fraction. J Periodontal Res 2007;42:159–68

Brazil Oral Research (2013). *Brazilian Dent Journal* Jan-Feb;27(1):20-5.

Brickman AM, et.al. Enhancing dentate gyrus function with dietary flavanols improves cognition in older adults. Nature Neurosci 2014;17**:**1798-803

Callow M.E. and Callow J.A. (2000). Substratum location and zoospore behaviour in the fouling alga enteromorpha. *Biofouling*. 15:49–56.

Callow J.A. and Callow M.E. (2006). Biofilms And Antifouling Compounds. *Springer*; Berlin, Germany, Vol 42: pp. 141–169.

Characklis WG, McFeters GA, Marshall KC Physiological ecology in biofilm systems. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons; 1990. p. 341–94

Costerton, J.W. (1999). Introduction to biofilm. *International Journal of Antimicrobial* Agents, 11, pp. 217–221.

Darouiche RO, Mansouri MD, Gawande PV, Madhyastha S. (2009). Antimicrobial and antibiofilm efficacy of triclosan and DispersinB¯ combination. J Antimicrob Chemother 64:88-93

Davies, D.2003.Understanding biofilm resistance to antibacterial agents. National Review of Drug Discovery .2:1118

Domenico P, Hopkins T, Schoch PE, Cunha BA. Potentiation of aminoglycoside inhibition and reduction of capsular polysaccharide in *Klebsiella pneumoniae* with sodium salicylate. J Antimicrob Chemother 1990;25:903-14.

Dunne, W.M. (2002). Bacterial adhesion: seen any good biofilms lately? *Clinical Microbiology Review*, 15, pp. 155–166.

Espeland, E.M. and Wetzel, R.G. (2001). Complexation, stabilization, and UV photolysis of extracellular and surface-bound glucosidase and alkaline phosphatase: implications for biofilm

microbiota. *Microbiology and Ecology*, 42(9):572–585. 10.1007/s00248-001-1023-7.

Farah A, Monteiro M, Donangelo CM, Lafay S. Chlorogenic acids from green coffee extract are highly bioavailable in humans. J Nutr 2008;138:2309-15

Feldman M, Tanabe S, Howell A, Grenier D. Cranberry proanthocyanidins inhibit the adherence properties of *Candida albicans* and cytokine secretion by oral epithelial cells. *BMC Comp Alt Med* 2012;12:6

González-Ortiz G, Quarles Van Ufford H C, Halkes S Bart A, et al. New properties of wheat bran – anti-biofilm activity and interference with bacteria quorum-sensing systems. *Envir Microbiol* 2014;16:1346-53

Heydorn, B. Ersboll, M. and Hentzer (2000). Experimental reproducibility in flow-chamber biofilms. *Microbiology,* 146 , pp. 2409–2415.

Jakobsen TH, van Gennip M, Phipps RK, Shanmugham MS, Christensen LD, Alhede M, Skindersoe ME, Rasmussen TB, Friedrich K, Uthe F, Jensen PØ, Moser C, Nielsen KF, Eberl L, Larsen TO, Tanner D, Høiby N, Bjarnsholt T, Givskov M. Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. Antimicrob Agents Chemother. 2012;56(5):2314–25

Kalpana, VN, Devi Rajeswari, V. A review on green synthesis, biomedical applications, and toxicity studies of ZnO NPs. Bioinorganic Chemical Application. 2018.

Karbasizade V., Dehghan P., Sichani, M.M., Shahanipoor, K., Jafari, R. and Yousefian R. (2017). Evaluation of three plant extracts against biofilm formation and expression of quorum sensing regulated virulence factors in *Pseudomonas aeruginosa*. *Pakistan Journal of pharmaceutical sciences*, 30(4)585-589.

Karunanidhi A, Thomas R, van Belkum A, Neela V. In vitro antibacterial and antibiofilm activities of chlorogenic acid against clinical isolates of *Stenotrophomonas maltophilia* including the trimethoprim/sulfamethoxazole resistant strain. Biomed Res Int. 2013;2013:392058

Klahre, J and Flemming, H.C. (2000). Monitoring of biofouling in paper mill process waters Water Resources, 34 pp. 3657–3665.

La VD, Howell AB, Grenier D. Anti-*Porphyromonas gingivalis* and anti-inflammatory activities of A-type cranberry proanthocyanidins. Antimicrob Agents Chemother 2010;54:1778–84

Labrecque J, Bodet C, Chandad F, Grenier D. Effects of a high-molecular-weight cranberry fraction on growth, biofilm formation and adherence of *Porphyromonas gingivalis*. J Antimicrob Chemother 2006;58:439–43.

Lan L., Wei H. and Mingxing, L. (2019). Developing natural products as potential antibiofilm agents. *Chinese medicine*. 6(3): pp 34-6.

Lee JH, et.al. Apple flavonoid phloretin inhibits *Escherichia coli* O157:H7 biofilm formation and ameliorates colon inflammation in rats. Infect Immun 2011;79:4819-27.

Morgan B. Microbial gangs are organised killers. https://cosmosmagazine.com/life-sciences/microbial-gangs-are-organised-killers. Cosmos, 4 May 2015

Miller C, Thomsen LE, Gaggero C, Mosseri R, Ingmer H, Cohen SN. 2004. SOS response induction by beta-lactams and bacterial defense against antibiotic lethality. Science 305:1629–1631. 10.1126/science.1101630

Morgan S.D, D. Rigby, and D. J. Stickler (2015). A study of the structure of the crystalline bacterial biofilms that can encrust and block silver Foley catheters, *Urological Research,* vol. 37, no. 2, pp. 89–93.

Muller E, Al-Attar J, Wolff AG, Farber BF. Mechanism of salicylate-mediated inhibition of biofilm in *Staphylococcus epidermidis*, J Infect Dis 1998;177:501-3.

Musk, D., and D. Banko. "Iron Salts Perturb Biofilm Formation and Disrupt Existing Bio- films of Pseudomonas Aeruginosa." Chemical Biology 12 (2005): 789-96

Partiwi SU, Lagendijk EL, Hertiani T. Antimicrobial effects of Indonesian medicinal plants extracts on planktonic and biofilm growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Intl J Pharm Pharmaceut Sci 2015;7:183-91

Raja AF, Ali F, Khan IA, et al. Antistaphylococcal and biofilm inhibitory activities of acetyl-11-keto-β-boswellic acid from *Boswellia serrata*. BMC Microbiology 2011

Rijnaarts, H.H.M., Bendinger B., Altendorf, K. and Zehnder, A.J.B (1993). Physicochemical cell surface and adhesive properties of coryneform bacteria related to the presence and chain length of mycolic acids. *Application of Environmental Microbiology*.59:3973–7.

Rijnaarts H.H, Norde, W, Bouwer, E.J, Lyklema, and Zehnder, J. (1993). Bacterial adhesion under static and dynamic conditions. *Application of Environmental Microbiology* 59(3)3255–65.

Shan B, Cai Y, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol 2007;117:112–9.

Smith K, Perez A, Ramage G, Gemmell CG, Lang S. 2009. Comparison of biofilm-associated cell survival following in vitro exposure of meticillin-resistant Staphylococcus aureus biofilms to the antibiotics clindamycin, daptomycin, linezolid, tigecycline and vancomycin. Int. J. Antimicrob. Agents 33:374–378. 10.1016/j.ijantimicag.2008.08.029

Steinberg PD, De Nys R, Kjelleberg S. *2002.* Chemical cues for surface colonization. *Journal of Chemical Ecology 28:1935–1951. doi:10.1023/A:1020789625989*

Stewart, P., and Consterton, J. (2001). "Antibiotic Resistance of Bacteria in Biofilms." *The Lancet*, DOI:10.1016/S0140-6736(01)05321-1.

Sun D,. Berberine sulfate blocks adherence of Streptococcus pyogenes to epithelial cells, fibronectin, and hexadecane. Antimicrobial Agents Chemotherapy 1988; 32: 1370-1374

Tetz GV, Artemenko NK, Tetz VV (2009) Effect of DNase and Antibiotics on Biofilm Characteristics. Antimicrob Agents Chemother 53: 1204–1209

Vikram A, et.al. Suppression of bacterial cell–cell signalling, biofilm formation and type III secretion system by citrus flavonoids, J Microbiol 2010;109:515–27.

Weiss EI, Lev-Dor R, Kashamn Y et al. Inhibiting interspecies coaggregation of plaque bacteria with a cranberry juice constituent. J Am Dent Assoc 1998

Yamanaka A, Kimizuka R, Kato T, Okuda K. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbiol Immunol 2004;19:150–4.

ZhengZ and Stewart P.S.2002. Penetration of rifampin through Staphylococcus epidermidis biofilms. Antimicrobial Agents Chemotherapeutics. 46:900–903. 10.1128/AAC.46.3.900 903.2002