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# GREEN ANTI-BIOFILM AGENTS: A MINI-REVIEW

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**ABSTRACT** Microbial biofilm is the polymeric matrix formed by coordinated assembly of species of similar or different microorganisms. The bacteria in form of biofilms are more resistant to drugs than compared to their planktonic counterparts, hence making it difficult to deal with biofilm-associated nosocomial and medical device infections. Around $11 billion is annual expenditure reported to be employed in healthcare to deal with biofilm infections but the success rates are very low. Studies on natural anti-biofilm agents from the last two decades notably demonstrated their biofilm modulation properties. Therefore, present state of affairs demands active pursual for non-toxic natural anti-biofilm agents. In this mini-review article, we summarize green anti-biofilm agents: antimicrobial peptides (AMPs), phytochemicals, biosurfactants, nanoparticles, and weak organic acids (WOA) with a brief note on their mode of action.

**KEYWORDS :** Antibiofilm agents; antimicrobial peptides; weak organic acids; biosurfactants; phytochemicals; nanoparticles

## INTRODUCTION:

The term "biofilm" was first coined by Costerton et al. 1978[1] to denote bacterial colonization. This is an ancient trait of bacteria that they employed first to protect themselves from predators like amoebae grazing and antimicrobial action, then on body sites of higher organisms like humans followed by colonization on medical devices[2]. Biofilm is a polymeric matrix constituting 2-5% microbial cells, <1-2% DNA and RNA, 1-2% Proteins, 97% water[3] which is why it is complex macromolecular assembly leading to multi-drug resistance.

### *Biofilm Formation:*

The steps in biofilm formation are 1) initial reversible attachment of bacteria on biotic or abiotic surfaces 2) irreversible cell-cell adhesion and development of microcolonies by division of bacterial cell 3) maturation of biofilms. After maturation, cells disperse to new surfaces to form a fresh biofilm. Along with this step-by-step progression of biofilm, there is the involvement of components like cell surface proteins, DNA, etc followed by extracellular matrix thereby increasing resistance of these entities towards drugs, which leads to emergence of multi-drug resistant strains.

The drug resistance of biofilm is declared as a threat by World Health Organisation (WHO) and challenge for scientific coterie[4]. It is an important virulent pathogenic mechanism of clinical pathogens like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* in causing infections like vaginitis, colitis, conjunctivitis, gingivitis, urethritis, otitis[5] periodontitis, and microbial colonization on devices like catheters, heart valves, orthopedic devices and on contact lenses[6]. National Institutes of Health (NIH) reports that 80% of human infections are due to pathogenic biofilm[7]. Previous reports demonstrate microbial consortia in biofilm exhibits resistance to antibiotic, biocidal, and chemical or physical agents compared to their planktonic counterparts[6]. Hence, studies and exploration of anti-biofilm agents are in high demand.

### *Biofilm Resistance Mechanisms:*

Biofilms exhibit varied resistance responses which differ from microorganisms to microorganism, between different species and within species. However, the most common responses observed are given in Figure 1.[8] Anti-biofilm agents work either by interfering with the initiation of biofilm formation or by disruption or disassembly of a mature biofilm.

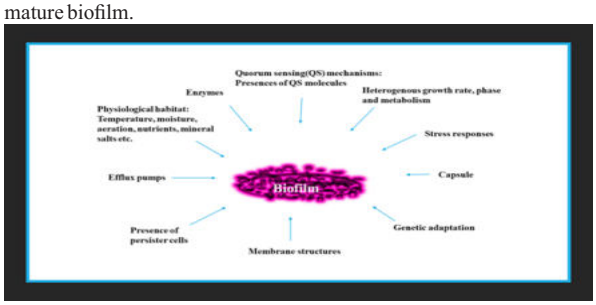


**Figure 1:** The various resistance mechanisms employed by biofilms against anti-microbial agents.

### *Anti-adhesive Property:*

The very first step in biofilm formation is reversible adhesion of planktonic cells on biotic and abiotic surfaces. Interaction of the suspended micro-organisms (co-aggregation) and that between already adhered microorganisms (co-adhesion) of same or different species is observed[9,10]. Therefore, a good anti-biofilm agent should have anti-adhesive activity (cell permeation, hydrophobicity activity which is the modification of cell membrane, anti-microbial, etc) to prevent initial attachment of live cells onto different surfaces.

### *Mature Biofilm Disruption:*

The irreversible adhesion of the microbial cell along with extracellular polymeric substances (EPS) is called mature biofilm. It is vigorously hydrated consortia [enzymes, carbohydrates, fibers, peptides, quorum sensing (QS) molecules i.e., autoinducers such as peptides and acyl-homoserine lactones (AHLs), species-specific polysaccharides, concentrated minerals, etc] are responsible for multidrug resistance of biofilm. Therefore, a good anti-biofilm agent should have properties like proteolytic, amylolytic, dehydration, anti-microbial, anti-oxidant, etc to break open the assemblage.

### *Quorum-sensing Inhibitors:*

QS mechanism too is observed during biofilm formation which is cross-talk between bacterial cells, that involves density dependant extracellular production of autoinducers are sole reason for pathogenic nature of biofilm[11] and their uptake by neighboring cells. Therefore, bioactive compound inhibiting QS forms a part of the innovative strategy in anti-biofilm agent's exploration.

aBiofilm' (http://bioinfo.imtech.res.in/manojk/abiofilm/) is a Quantitative structure-activity relationship-based database (reporting 1988-2017 anti-biofilm agents) which predicts and visualize module of anti-biofilm agents? 'aBiofilm' banks 1720 unique biochemical and chemical structural details of 5027 anti-biofilm agents, targeting more than 140 bacteria (Gram-positive and Gram-negative) and fungi. These agents constitute bacterial and plant secondary metabolites, nano-particles, etc which act on a polymeric matrix and signalling molecules[12].

### *Green Antibiofilm Agents*

The present review aims to summarize different green antibiofilm agents like antimicrobial peptides, phytochemicals, nanoparticles, biosurfactants, weak organic acids, and their mode of action towards clinical pathogens.

### *Antimicrobial Peptides (AMPs):*

Antimicrobial peptides (AMPs) have demonstrated properties like antimicrobial, anti-adhesive, and anti-biofilm towards biofilm-associated chronic infections caused by bacterial pathogens. AMPs are essential components of first-line defence against any infection[13]. These AMPs are classified into four major classes: β-sheet, α-helical, loop, and extended peptides[14]. Therapeutic application of these

molecules is interesting due to their amino-acid sequence, amphipathicity, net positive charge, and small size whose mode of actions are membrane disruption and inhibition of biosynthesis of proteins, nucleic acid and cell wall[15].

***Mode Of Action:***

AMPs generally target cytoplasmic membrane and cause cell lysis by permeation. This mode of action specifically depends on its amino-acid sequences, small size, hydrophobicity, and amphipathicity[16]. They are broad-spectrum, their mode of action is specific, less bacterial resistant incidences, stable at wide pH and temperature range[17] found to be disrupting mature biofilms at low concentration, anti-adhesive property and leads to downregulation of QS genes[18]. Table.1 gives a detailed insight to the AMPs.

**Table 1: Different Types Of Antimicrobial Peptides With Anti-biofilm Activities:**

| S. No | AMPs | Broad or narrow spectrum | Treatment | References |
|---|---|---|---|---|
| 1. | Pexiganan synthetic variant of magainin 2 | Broad-spectrum antibiotic | diabetic foot ulcer infections | 19 |
| 2. | Omiganan | Broad-spectrum antibiotic | Topical gel used in prevention of catheter-associated infections. | 20 |
| 3. | Selectively Targeted Antimicrobial Peptides (STAMPs) | Narrow spectrum | killing potency, selectivity, and kinetics against targeted bacteria | 21 |
| 4. | Oritavancin, a semi-synthetic lipoglycopeptide | Narrow Spectrum | methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *S. aureus* (VRSA) | 22 |
| 5. | Polymyxins | Narrow Spectrum | Gram-negative bacterial infections. | 23 |
| 6. | SMAP-29, a cathelicidin-derived peptide from sheep myeloid mRNA, | Broad spectrum | methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VREF), and mucoid *P. aeruginosa*. | 24 |
| 7. | Antibacterial peptides isolated from *Enterococcus mundtii* ST4V. | Broad spectrum | Gram-positive (+ve) and Gram-negative(-ve) bacterium | 25 |
| 8. | Synthetic antimicrobial peptide (AMP) NA-CATH:ATRA1-ATRA1 and natural AMP LL-37 from the cathelicidin family | Narrow spectrum | *S. aureus* | 26 |
| 9. | Lactoferrin, conjugated lactoferricin, melimine and citropin along with rifampicin and minocycline | Broad spectrum | *S. aureus* and *P. aeruginosa* infections | 27 |
| 10. | LL-37 a Human cationic host defence peptide. | Broad spectrum | *P. aeruginosa, Staphylococcus epidermidis*. | 28,29 |

***Phytochemicals:***

Since time immemorial, plants are known as an excellent source of many bioactive compounds. Rainforests plants are partly explored for extraction of new compounds and thereby estimated that around 6% of the approximately 300,000 species are investigated for pharmaceutical purposes, and around 15% for phytochemicals[30]. Plant bioactive compounds (Table 2) are simply their secondary metabolites that can be extracted from different parts of the root and shoot system[31].

**Table 2: Different Types Of Phytochemicals With Anti-biofilm Activities**

| S. No | Phytochemical | Broad or narrow spectrum | Treatment | References |
|---|---|---|---|---|
| 1. | Alkaloid | | | |
| | Berberine | Broad Spectrum | Mixed culture bacterial suspension: *Fusobacterium nucleatum, Prevotella intermedia* and *Enterococcus faecalis, Staphylococcus epidermidis* strains ATCC 35984 and *S. epidermidis* 243 | 32,33 |
| | Reserpine | Narrow Spectrum | *Klebsiella pneumoniae* | 34 |
| | Piperine | Narrow Spectrum | *Staphylococcus mutants* | 35 |
| | Embelin Harmaline | Narrow Spectrum | *Staphylococcus aureus* | 36 |
| 2. | Polyphenols: | | | |
| | Artocarpin and Artocarpesin | Narrow Spectrum | *S. mutants* | 37 |
| | Flavanoids | Broad Spectrum | *S. mutants, actinomycetes,* and *lactobacilli* | 38 |
| | Guaijaverin | Narrow Spectrum | *S. mutants* | 39 |
| 3. | Terpenes | | | |
| | Bakuchiol | Broad Spectrum | Oral Bacterial Species | 40 |
| | Sagittines A-E | Broad Spectrum | *S. mutans* ATCC 25175, *Actinomyces naeslundii* ATCC 12104, and *Actinobacillus actinomycetemcomitans* ATCC 43717 | 41 |
| | Macrocarpal A, B, C | Narrow Spectrum | *Pseudomonas gingivalis* | 42 |
| | Simplexene A, B, E, D | Narrow Spectrum | *Candida. albicans* | 43 |
| 4. | Essential Oils | | | |
| | Manuka, Tea tree, Eucalyptus, Lavandula, and Romarinus | Broad Spectrum | *P. gingivalis* *S. mutants* | 44 |

***Mode Of Action:***

The phytochemicals are effective broad-spectrum, multi-targeted, biofilm inhibitors and thus draws the attention of the scientific community, however the exact mode of action of these phytochemicals on biofilms is not very clear, previous reports suggest that they have anti-adhesive properties which are still under trials[7].

***Biosurfactant:***

Biosurfactants are surface-active amphipathic molecules that are produced by microbial cells. They reduce liquid surface tensions. Biosurfactants have wide applications in industrial, environmental, and biomedical fields. Four main types of biosurfactants are (1) glycolipids, (2) phospholipids, (3) lipoproteins or lipopeptides, (4) polymeric. Despite the versatile application of biosurfactants, they exhibit anti-adhesive properties and have attracted researchers in exploring them for antibiofilm activities[45] (Table. 3).

***Mode Of Action:***

Biosurfactants have an important property that is surface modification / selective hydrophobicity and can interfere with microbial

colonization and biofilm formation. They selectively reduce hydrophobicity of bacterial cell wall. Biosurfactants are also reported for disruption of mature biofilms and downregulation of virulence and biofilm genes[45].

**Table 3: Different Types Of Biosurfactants With Anti-biofilm Activities.**

| S. No | Biosurfactant | Broad or narrow spectrum | Treatment | References |
|---|---|---|---|---|
| 1. | Glycolipids rhamnolipids, trehalolipids, and sophorolipids | Broad spectrum | Gram +ve, Gram -ve and Candida sps | 46,47,48 |
| 2. | Phospholipids Miltefosine | Narrow spectrum | *Candida albicans* | 49 |
| 3. | Lipoprotiens or Lipopeptides Surfactin Iturin Fengycin | Broad spectrum | Gram +ve, Gram -ve and Fungal spp | 50,51,52 |
| 4. | Polymeric emulsan, liposan, alasan, lipomanan, and other polysaccharide-protein complexes | Broad spectrum | Control of dental plaque and caries US 4737359 A. Patent | 53 |

***Nanoparticles:***

Nanotechnology is an upcoming and promising field[54]. The word nano is a Greek word which means small. Nanoparticles are two or more dimensional entities whose size ranges between 1 to 100nm (ASTMInternational)[55]. They possess unprecedented physical, chemical, and unique electronic properties hence find applications in photochemical, electrochemistry, and biomedical research[56]. They exhibit broad-spectrum bactericidal properties and non-toxic to mammalian cells. Currently, they are widely utilized in drug determination and drug delivery studies[57]. For example, silver nanoparticles are reported to exhibit anti-biofilm property in synergy with phytomedicine against *E. coli* ECDCM1[58].

***Mode Of Action:***

The nanoparticles have commendable anti-bacterial and anti-oxidant properties. The mode of action of nanoparticles as anti-biofilm agents is not clearly described yet, however, they are known to cause DNA damage by the production of oxidative/free radical formation[59]. Table 4 lists different types of nanoparticles.

**Table 4: Different Types Of Nanoparticles With Anti-biofilm Activities.**

| S. No | Nanoparticles | Broad or narrow spectrum | Treatment | References |
|---|---|---|---|---|
| 1. | Zinc oxide | Broad spectrum | Gram-positive (+ve), Gram-negative (-ve). | 61 |
| 2. | Titanium dioxide | Broad spectrum | Gram +ve, Gram -ve | 61 |
| 3. | Copper oxide | Broad spectrum | Gram +ve, Gram -ve | 61 |
| 4. | Carbon nanotubes | Broad spectrum | Gram +ve, Gram -ve | 61 |
| 5. | Chitosan | Broad spectrum | Gram +ve, Gram -ve | 61 |
| 6. | Gold | Broad spectrum | Gram +ve, Gram -ve | 61 |
| 7. | Silver | Broad spectrum | Gram +ve, Gram -ve | 61 |
| 8. | Quarternary ammonia | Broad spectrum | Gram +ve, Gram -ve | 61 |

***Weak Organic Acids (WOA):***

The documentation for use of WOA in medicine as disinfectant or antiseptic is 6000 years ago, however, there are no pieces of evidence for their clinical trials. One of the earliest documentations for use of organic acids as antibiofilm agents is 200 years ago it was reported by John Hunter (the surgeon) that urinary catheter patency can be prolonged by dipping in acetic acid before bladder insertion[60].

The most popular example of WOA is N-acetyl-L-cysteine (NAC) and acetic acid as antibiofilm agents against *P. aeruginosa* and *S. epidermidis* respectively. The concentration and pH of WOA are known for the variation in their ability to serve as anti-adhesive or mature biofilm disruption agents[62].

***Mode Of Action:***

WOA is hydrophobic and causes cell membrane diffusion leading to ion trapping which involves dissociation and lowered internal cytoplasmic pH of the bacteria[60]. Table 5 lists the WOA.

**Table 5: Different Types Of WOA With Anti-biofilm Activities.**

| S. No | WOA | Broad or narrow spectrum | Treatment | References. |
|---|---|---|---|---|
| 1. | Acetic acid | Broad Spectrum | Gynecology Hepatology/oncology Plastics/dermatology | 60 |
| 2 | N-acetyl-L-cysteine (NAC) | Narrow Spectrum | *P. aeruginosa* | 63 |
| 3 | Citric acid | Broad Spectrum | Urology Haematology/ Oncology/ renal | 60 |
| 4. | Triprotic Acid | Broad Spectrum | *K. pneumoniae*, *P. putida*, *S. aureus*, as well as antibiotic-resistant and cystic fibrosis isolates | 63 |

***CONCLUSION:***

Biofilm are the social beings that are notorious, stringent, and multi-drug resistant establishment of microbial communities. They have created havoc in the medical research, around 60-70% of deaths are reported due to biofilm-related infections annually according to NIH and expenditure involved in eradicating these dreadful actualities is very high. Bacterial biofilm are found to be exhibiting resistance to synthetic drugs like daptomycin and vancomycin (popularly used anti-biofilm agents). Therefore, it is important to search for anti-biofilm agents and needs urgent inputs. In our review, we have documented five types of green antibiofilm agents i.e., antimicrobial peptides, phytochemicals, nanoparticles, biosurfactants, weak organic acids and their mode of action against clinical pathogens which are from the cheap source, cost-effective and promising and proposed strategies to deal with these stringent entities invitro and invivo. (Figure 2).


**Figure 2:** Proposed strategies (Invitro and Invivo) to combat the antimicrobial resistance of biofilms.

Many of the antibiofilm agents are reported to be working well in concurrence with other antibiotic drugs, per se phytochemicals, which works best in synergy with synthetic drugs, however, they still lack clinical trials at invitro and in vivo levels. The dosage, formulation, and working concentrations, and techniques employed for different anti-biofilm agents need to be researched extensively.

Likewise, several reports on anti-biofilm studies state that the anti-oxidant activity of a compound is directly proportional to its anti-biofilm activity. Higher the anti-oxidant activity, higher will be its anti-biofilm activity and vice-versa.

Even though uncountable antibiofilm agents are discovered annually, their efficacy differs due to an ignored factor i.e., geographic location of the biofilm-forming pathogen. Undoubtedly genetic studies unveil mode of action and efficacy of a bioactive agent at molecular level against biofilm, but the fact that genetic makeup is vulnerable to environmental stress, physical, and chemical mutagens cannot be neglected. A lacuna, which has to be filled are 1) increasing sample size of these pathogens collected from multifarious locations 2) assessing/exploring antibiofilm activity of bioactive compounds considering a) different physiological factors like temperature, pH, moisture and aeration, b) the toxicity levels (high antibiofilm activity at low concentration is less toxic to tissues than vice versa) c) employing different laboratory techniques on individual biofilm-forming pathogens and their consortia, invitro, and invivo studies should be taken up because the compounds behave differently in different environments. d) singly and in synergy with synthetic drugs and non- antibiotic drugs, etc is likely to give an all-rounded picture of the performance of these agents which would be an interesting approach for future research.

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