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Review article

Use of phytochemicals as emerging strategy for control of biofilm formed by pathogens

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Abstract

Most of infectious diseases these days are not treated by regular antibiotics therapy, due to evolution of multiple drug resistant bacteria. The development of resistance towards commonly used antibiotics is a huge problem in the health sector. The formation of microbial biofilms by pathogenic microorganisms is an important reason for failure of antimicrobial therapy. The biofilm generally cannot be treated by antibiotic therapy as the microorganisms in it remain unaffected. Pathogenic bacteria in biofilms are resistant to current therapeutic regimes and efficient removal of biofilm is a big challenge in healthcare sector, especially in living system where harsh treatments cannot be given. Instead of that, milder and natural reagents which are highly selective and capable of disrupting the structural stability of the biofilm matrix can be of great importance. These problems create the requirement to find new sources of antimicrobial activity. In order to find new antimicrobial agents, plant products or phytochemicals were studied as alternate or complementary products to antibiotics for which bacteria already acquired resistance. Phytochemicals have clearly shown to be best as antibiofilm, antimicrobial and quorum sensing inhibition agents. They exert their antibacterial effect through a different mechanism of action, such as damage to the bacterial cell membrane, suppression of virulence factors like inhibition of enzyme activity, toxins and biofilm formation. The phytochemicals represent a possible source of effective, inexpensive and safe antimicrobial agent due to its antibiofilm and quorum sensing inhibition properties.

Key words: Biofilm, phytochemicals, pathogens, antibiotic resistance

Abbreviations: I-3-C:-indole-3-carbinol; SA:Salicylic acid; 7-HC:7-Hydroxycoumarin; SP: Saponin; MIC:Minimum inhibitory concentration; MBC:Minimum bactericidal concentration

1. Introduction

Biofilms are surface associated microorganisms, secreting an extra polymeric matrix around them (Kumar *et al.*, 2017). Biofilm cells differ from other free living cells in their surroundings by having a reduced growth rate, up and down regulation of genes and extra polymeric substance (EPS) formation. The matrices contain polysaccharides, proteins, and extracellular microbial DNA. The biofilm can consist of one or more microbial (bacterial or fungal) species (Aleksandra *et al.*, 2012). EPS helps in protecting the micro-organism from immune response and antimicrobial agents. It helps microorganisms to survive in unfavorable conditions (Parsek and Singh, 2003). Biofilm offers cell-to-cell communication and horizontal gene transfer (Keller and Surette, 2006; West *et al.*, 2006), hence they develop antibiotic resistance. These are the main reason for the failure of clinical therapy associated with biofilms (Parsek and Singh, 2003; Donlan and Costerton, 2002; Hall *et al.*, 2004). Biofilms comprise multiple microorganisms that are found to be associated with the biotic and abiotic surfaces. Biofilms can be either single or multilayered and can have either homogenous or heterogeneous populations of bacteria which remain in the matrix, made up of extracellular polymeric substances, secreted by constituent population of the biofilm (Gupta *et al.*, 2016). Different biofilms differ from their free-living counterparts in their growth rate, constitution, structure and increased resistance to biocides, antibiotics and antibodies by virtue of up-regulation and/or down regulation of approximately 40% of their genes. This makes them highly difficult to eradicate with therapeutic doses of antimicrobial agents (Prakash *et al.*, 2003).

Most of the biofilm cells and planktonic cells normally killed by drug treatment. However, drug tolerant persisters disseminate into single microbial cell and start a new cycle of biofilm development (Lewis, 2010; Keren *et al.*, 2011; Zhang, 2014) which subsequently increases the duration of treatment of diseases, caused by biofilm forming pathogenic microorganisms. The structure and physiological characteristics of the formed biofilms are mainly responsible for antimicrobial resistance (Garg and Azmi, 2017). The bacteria residing within biofilms are generally antibiotic tolerant and susceptible to antibiotics or other chemicals upon dispersal from biofilm. This suggests that microbial capacity of survival against antibiotics is due to phenotypic adaptability and not essentially due to genetic adaptability (Anwar *et al.*, 1989). Factors such as mechanical stress,

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enzymatic digestion, pH, oxygen availability, temperature and limiting nutrition trigger dispersal of cells from the biofilm. Biofilms induced due to low oxygen condition whereas normoxia decreases biofilm formation (Totani *et al.*, 2017). Further, enhanced bacterial respiration reduces the persisters in bacterial population (Vilcheze *et al.*, 2017). Quorum sensing (QS) is the mode of cell-to-cell communication in biofilms. QS is operated by autoinducing peptides (AIPs) in Gram-positive bacteria and N-acly-homoserine lactones (lipids molecules) in Gram-negavtive bacteria. Biofilms can develop on both animate and non-animate substances. The 65-80% of human clinical infections are associated with biofilm formation (Pletzer and Honcock, 2016). Biofilms can be formed on all types of materials including medical implants living cells and instruments (Donelli and Francolini, 2001). Public health is facing a biggest threat due to the development of antibiotic resistant varieties of pathogens (Byarugaba, 2004; Okeke *et al.*, 2005). These bacteria can even survive the treatments of UV lights, heavy metal, acidity, changes in hydration or salinity (Espeland and Wetzel, 2001; Le *et al.*, 2000; Leid *et al.*, 2002; McNeill and Hamilton, 2003; Teitzel and Parsek, 2003). Biofilm degradation by antibiotics requires high MIC and MBC value, which can be fatal when used *in vitro* (Wu *et al.*, 2015; Hengzhuang *et al.*, 2011; Hoiby *et al.*, 2011).

Further, the ability of pathogens to cause infection is depend on the secretion of agents, termed as virulence factors, such as toxins and adhesion molecules, that actively cause damage to host tissues. The increasing attention has been given in recent years to 'disarm' the pathogenicity of bacteria rather than killing them. This can be done by targeting virulence using anti-infective or anti-virulence drugs. Search for more antimicrobial compounds is continuously going on due to limitations of present therapy regimens and phytochemicals are now considered as an important source of antimicrobial agents for biofilm degradation (Rasooli *et al.*, 2008; Koo *et al.*, 2010; Shayegh *et al.*, 2008). Majority of the phytochemicals can act synergistically with antibiotics and some of them are very effective alone too. Phytochemicals have a broad spectrum of action including bacteria, insects, nematodes, fungi and yeast (Abreu *et al.*, 2013). Phytochemicals work by damaging the microbial membrane structure, inhibiting peptidogylcan synthesis, modifying bacterial surface hydrophobicity and modulating quorum-sensing (QS) (Rasooli *et al.*, 2008). Phyto-chemicals have reported to be used as QS inhibitors and help to overcome the selective pressure created by antibiotic use (Borges *et al.*, 2014).

2. Types of biofilm

Biofilms can be formed on both animate and inanimate things. Biofilms can easily develop on the inert surfaces of medical devices, contact lenses and catheters or living tissues, as on epithelium of the lungs (particularly in cystic fibrosis patients), on the endocardium and wounds (Aleksandra *et al.*, 2012). Biofilm can also found to be associated with diseases like endocarditis, periodontitis, rhinosinusitis and osteomyelitis (Figure 1), but more commonly seen in medical implants and urinary catheters. These infections can often only be treated by removal of the implant which increase the trauma to the patient and the cost of treatment.

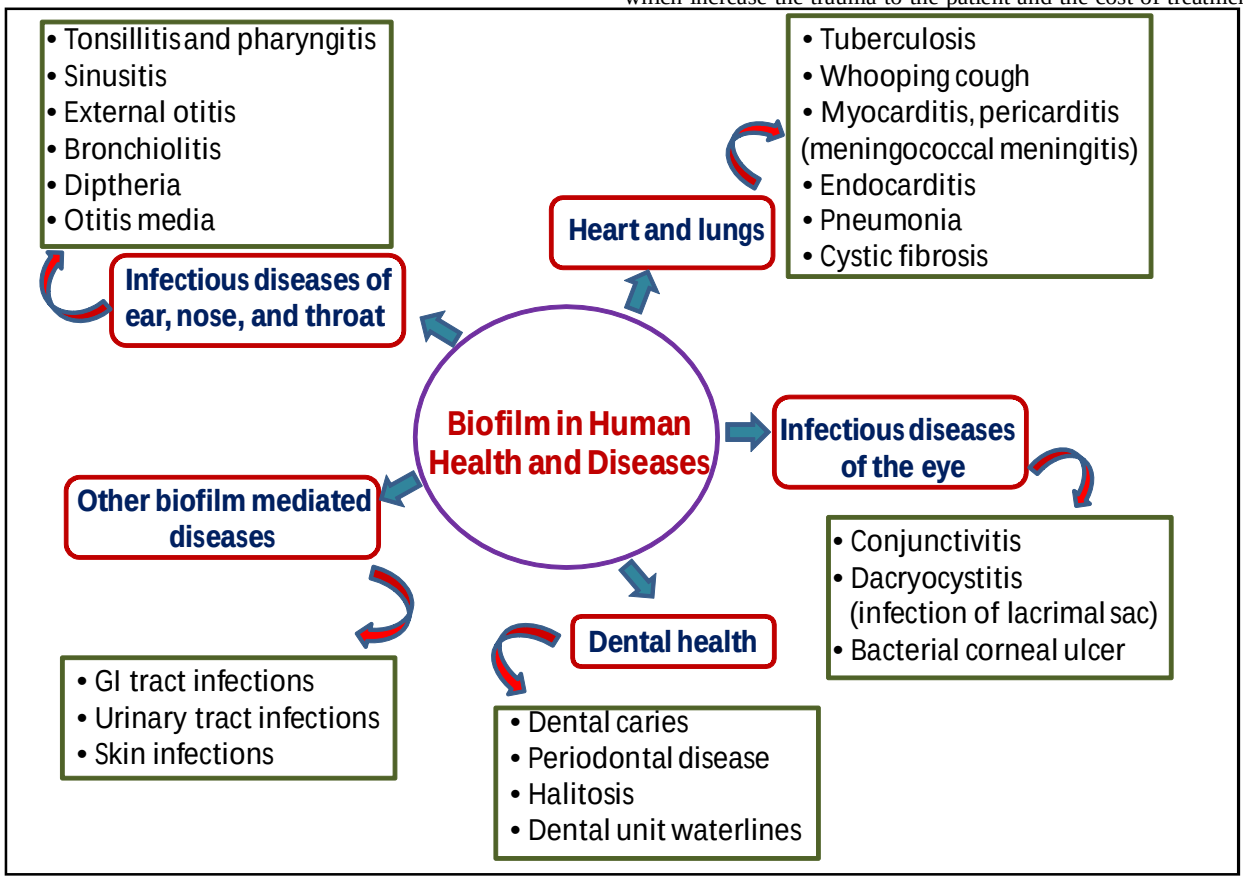

Figure 1: Association of biofilm with various diseases.

2.1 Biofilms on medical devices

A list of medical devices having biofilms colonization was provided by Costerton *et al.* (1999).

2.1.1 Prosthetic heart valves (PHV)

The surgical implantation of prosthetic valve damages the tissue which results in the accumulation of platelets and fibrin at the suture site and on the device.

Sewing cuff fabric of PHV gets colonized by microorganism (Illingworth *et al.*, 1998). Coagulase negative *Staphylococcus* is the main inhabitants in early stage of prosthetic valve endocarditis (PVE) due to initial contamination of the surroundings (Hancock *et al.*, 1994; Karchmer and Gibbons, 1994). In the later stage of PVE (after 12 months of valve replacement), infection is mainly caused by *Streptococci*, Coagulase negative *Staphylococcus*, Enterococci, *Staphylococcus aureus*, Gram-negative Coccobacilli, or fungi (Karchmer and Gibbons, 1994). However, despite major advances in cardiovascular surgical protocols and use of antimicrobial drugs, PVE continues to complicate the course of 1.4 and 3.1% of patients after cardiac valve replacement within 12 months of valve replacement (Douglas and Cobb, 1992).

2.1.2 Central venous catheters (CVC)

Catheters are medical devices that can be inserted in the body to treat disease or perform a surgical procedure. Among indwelling medical device, CVCs accounts for the maximum device related infection ranging from 3-5% (Maki, 1994). The device becomes coated with platelets, plasma and tissue proteins such as albumin, fibrinogen, fibronectin and laminin as it comes in direct contact with the bloodstream (Raad, 1998). The *S. aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis* and *Candida albicans* are the main CVC infection causing organisms (Elliott *et al.*, 1997; Raad, 1998**)**

2.1.3 Contact lenses

Bacteria adhere rapidly to both soft and hard types of contact lenses (Miller and Ahearn, 1987; Stapleton *et al.*, 1993; Stapleton and Dart, 1995). *P. aeruginosa, S. aureus, S. epidermidis, Serratia* spp., *E. coli, Proteus* spp. and *Candida* spp and protozoan *Acanthamoeba* are the main inhabitants (Dart,1996; McLaughlin *et al.*, 1998). The development of biofilms has even been observed on the storage cases of the lenses (Dart, 1996; McLaughlin *et al.*, 1998; Wilson *et al.*, 1991).

2.1.4 Intra uterine devices (IUD)

The IUDs play an important role in cause and spread of pelvic inflammatory disease (Wolf and Kreiger, 1986; Chesney, 1994; Lewis, 1998). The species of *Corynebacterium, Micrococcus* and *Enterococcus* along with *Lactobacillus plantarum*, group B *streptococci, Streotococcus epidermidis, Candida albicans* and *S. aureus* have been isolated from IUDs (Marrie and Costerton, 1983). The heavy contamination of IUDs with *S. epidermidis, enterococci* and anaerobic *lactobacilli* has also been reported (Wolf and Kreiger, 1986).

2.1.5 Urinary catheters

The urinary catheters are silicon and latex tubular devices which are used in treatment of urinary system related problems (Kaye and Hessen, 1994). The 10-50% of catheterization for short periods of time (6-7 d) causes infection whereas almost all long term catheterization (>28 d) leads to bacteriuria (Stickler, 1996). Micro-organism enters into the urethra or bladder directly with the insertion of the catheter, through its tubes and collecting bags or through the exudates sheath that surrounds the catheters (Kaye and Hessen, 1994). The *S. epidermidis, Enterococcus faecalis, E. coli, Proteus mirabilis, Providencia stuartii, P. aeruginosa* and *Klebsiella pneumoniae* are the initial inhabitants of these devices (Stickler, 1996). Other organisms like *Morganella morganii, Acinetobacter calcoaceticus* and *Enterobacter aerogenes* were also detected in the biofilm (Stickler *et al.*, 1993).

2.2 Biofilm on various organs

Biofilm can also very frequently reside in various organs and cause infections.

2.2.1 Middle ear

Otitis media is a common children disease which occurs due to the inflammation of the mucoperiosteal lining of middle ear. Tympanostomy tubes are used in such conditions to prevent the pressure and hearing loss. These tubes can develop biofilm on their inner surfaces (Biedlingmaier *et al.*, 1998). *P. aeruginosa, S. aureus, S. epidermidis* and *S. aureus* biofims were observed in armstrong-style silicone tubes (Biedlingmaier *et al.*, 1998; Saidi *et al.*, 1999). Along with antibiotic resistance of biofilm, middle ear fluid is less penetrated by antibiotics due to the formation of biofilm (Krause *et al.*, 1982).

2.2.2 Prostate gland

Bacteria from urethra and infected urine can ascend into the prostate gland and cause chronic bacterial prostatitis. The *E. coli* was found to be most common isolate, however, *Klebsiella*, enterobacteria, *Proteus, Serratia, P. aeruginosa, Staphylococcus*, coryneforms, and *E. faecalis* were also isolated from an infected prostate gland. In another study conducted by Nickel and Costerton (1993), *E. coli, P. aeruginosa, Bacteroides* spp., *Gardnerella* spp., *Coryne bacterium* spp. and coagulase negative *Staphylococcus* were observed to inhabit the prostate gland. Bacteria get a hostile environment in prostate gland, develop glycocalyx covering around them and become inactive. This inactivation makes it more difficult for antibiotics to kill these bacteria and that's why prostate gland infection is generally difficult to treat (Domingue and Hellstrom, 1998)

2.2.3 Teeth

Moore *et al.* (1983) observed several types of bacteria which were present on the teeth of the patient of a periodontal disease. These were *Fusobacterium nucleatum, Peptostreptococcus micros, Eubacterium timidum, Eubacterium brachy, Lactobacillus* spp., *Actinomyces naeslundii, Pseudomonas anaerobius, Eubacterium* spp strain D8, *Bacteroide sintermedius, Fusobacterium* spp, *Selenomonas sputigena, Eubacterium* spp strain D6, *Bacteroide spneumosintes* and *Haemophilus aphrophilus* (Moore *et al.*, 1983). A protein layer called pellicle develops around the teeth right after

it was cleaned and within hours of pellicle formation, it gets surrounded with a layer of Gram-positive cocci and rod shaped bacteria mainly streptococci, actinomycetes, and smaller numbers of *Haemophilus* (Marsh, 1995). These cells develop the extra polymeric matrix around them after few days and now onwards actinomycetes were found to be in dominant numbers (Marsh, 1995). A layer of plaque is formed 2-3 weeks later and mineralized plaque with calcium and phosphate is called calculus or tartar (Shapiro and Stallard, 1997; Lamont and Jenkinson, 1998).

2.2.4 Heart valve

Bacteria and fungi can infect various heart valves and cause valve endocarditis (Livornese and Karzeniowski, 1992). *Streptococci, Enterococci, Pneumococci, Streptococcus bovis, Staphylococci*, Gram-negative bacteria and fungi (*Candida* and *Aspergillus spp*.) were found as the infecting microorganism (Tunkel and Mandell, 1992).

2.2.5 Cystic fibrosis (CF)

It is a genetically transferred respiratory disorder in which a viscous mucus secretion covers the respiratory epithelium (Koch and Hoiby, 1993). This mucus increases the chances of bacterial and fungal lung infections (May *et al*., 1991). The lungs of nearly all CF patients are chronically colonized by *P. aeruginosa*, which significantly reduces life expectancy of individual. It is the leading cause of morbidity and mortality for CF patients. At the initial stage of infection, the microorganisms are non-mucoid type but with their prolonged and demanding stay in the lungs, they become mucoid. The biofilm formed by *P. aeruginosa* protects them from immune system defense actions and effect of antibiotics (Koch and Hoiby, 1993). This mucoid secretion is of a polysaccharide material called as alginate (Lam *et al.*, 1980). Microorganisms can adopt other defense methods to get protected. One of such ways has been studied by Cochrane *et al.* (1988). They found that bacteria can produce an iron rich protein in order to survive in the low level of iron in blood of the host. The *S. aureus* and *Haemophilus inûuenzae* makes lungs susceptible to colonization of *P. aeruginosa* (Govan and Deretic, 1996). Pyocyanin produced by *P. aeruginosa* act as both a virulence factor and a quorum sensing signaling molecule for *P. aeruginosa* (Lau *et al.*, 2004; Karatuna and Yagci, 2010). It has been identified that pathogen-associated proteins have homology only with pathogenic bacteria and not with non-pathogens (Ho Sui *et al.*, 2009). Such types of proteins are more likely to have virulence-related functions. The identified pathogen-associated proteins have been included in components of the phenazine biosynthesis pathway and, hence pyocyanin biosynthesis is an attractive target for anti-infective drug intervention. The time period of infection affects its chances to getting cured and it has been reported that early infection can be controlled easily as compared to an old one (Anwar *et al*., 1992).

3. Target areas of phytochemicals

The phytochemicals represent the richest available reservoir of novel therapeutics (Manoharachary and Nagaraju, 2016; Nooreen *et al*., 2018; Dang, 2018) (Table 1). The antimicrobial activities of plant extracts are beyond doubt, in many instances; however, their exact mechanism of antimicrobial functionality is not well understood. Volatile oils plant origin are frequently used as antimicrobial agents because of their feasibility and safety (Fahim *et al*., 2017). Prior to the commercial use of phytochemicals, various antibiotics and others chemicals have been involved in removal of biofilms. In *P. aeruginosa*, clarithromycin blocks biofilm matrix formation (Yasuda *et al*., 1993). The overall thickness of the biofilm reduces by ciprofloxacin and exposes the immature biofilm to phagocytosis by polymorphonuclear neutrophils and the matrix polymer of biofilm in *S. aureus* was dissolved by streptokinase (Nemoto *et al*., 2000). The acyl-homoserine lactone interferes with cellular signalling mechanisms which have been used for QS adversely affects normal biofilm formation (Parsek and Greenberg, 2000). However, due to the antibiotic resistance of biofilm-associated bacteria, alternate and efficient tools are needed to overcome these limitations and the use of different enzyme is one of the most promising approaches.

The composition of the EPS matrix has been studied in bacteria such as *P. aeruginosa*, *Bacillus spp.*, *staphylococcus spp.* and *streptococcus spp.* The constituent of extracellular matrix depends on the environment and the type of bacteria present within the biofilms. The main component of biofilms is DNA, polysaccharides, proteins, and EPSs and the degradation of matrix components can weaken or disperse biofilms. The use of various reagents can leads to complete an effective disruption of the biofilms architecture (Fleming *et al.*, 2017). The EPS matrix also acts as a diffusion barrier to delay the infiltration of some antimicrobial agents (Xu *et al*., 2000). The reactive chlorine species in a number of these agents deactivated at the surface layers of the biofilm before they are not able to disseminate into the interior of the biofilm (de-Beer *et al.*, 1994). A study showed that oxacillin, cefotaxime, and vancomycin had reduced the penetration throughout *S. aureus* and *S. epidermidis* biofilms (Singh *et al.*, 2010). However, with the emergence of multidrug resistant of *S. aureus*, the need for more effective treatments of biofilm-associated infections becomes imperative (Kalia and Purohit, 2011; Pooi and Yien, 2014). The biofilm matrix is composed of a variety of diverse components and its resistance to antibiotics indicates that the disruption of the biofilm structure could be achieved *via* the degradation of individual biofilm compounds by various therapeutic molecules (Aleksandra *et al*., 2012) and this phenomenon creates an opportunity for use of different phytochemicals as alternate for the disruption of biofilm integrity. The target areas for different reagents for control and management of biofilms have been summarized in Figure 2.

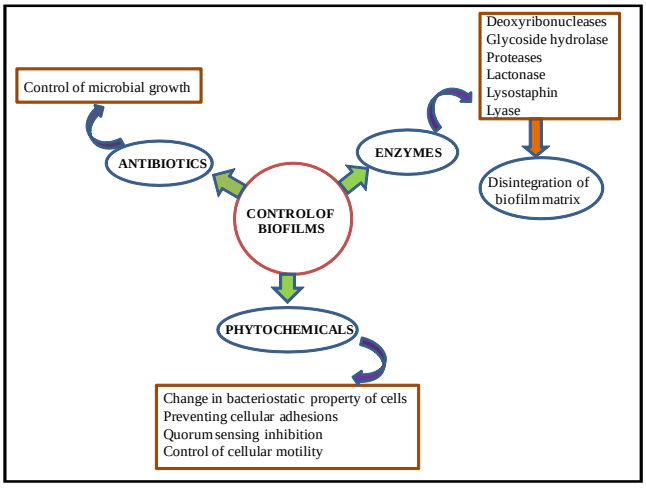

Figure 2: Target areas of different reagents for control of biofilms.

Table 1: Various groups of phytochemicals and their antimicrobial activity

Phytochemicals	Plant source	Microorganisms	References
Phenylpropenoids **General mechanism of action:** Inhibition of energy generation by inhibiting glucose uptake or utilization of glucose and affects on membrane permeability.			
Eugenol	Various species	*P. aeruginosa* PAO1 *K. pneumoniae* *L. monocytogenes*	Zhou *et al.*, 2013 Magesh *et al.*, 2013 Upadhyay *et al*., 2013
Cinnamaldehyde	*Cinnamomum* sp.	*L. monocytogenes* *Vibrio* spp. *S. epidermidis* *C. sakazakii*	Upadhyay *et al*., 2013 Brackman *et al*., 2008 Sharma *et al*., 2014 Amalaradjou and Venkitanarayanan, 2011
Benzoic acid derivatives **General mechanism of action:** Enzyme inhibition and non specific interaction with proteins.			
Vanillin	Vanilla planifolia Jacks	*C. violaceum* CV026, *A. hydrophila* *P. aeruginosa* PAO1 *A. tumefaciens* C58	Ponnusamy *et al.*, 2009 Kappachery *et al.*, 2010 Plyuta *et al.*, 2013
Gallic acid	Various species	*P.aeruginosa* PAO1 *A. tumefaciens* C58 *S. epidermidis* *E. corrodens* *C. violaceum* ATCC 12472	Plyuta *et al.*, 2013 Plyuta *et al.*, 2013 Moran *et al.*, 2014 Matsunaga *et al.*, 2010 Borges *et al.*, 2014
Ellagic acid	Various species	*B. cepacia*, *P. putida* pKR–C1 *C. violaceum, S. dysgalactiae* *S. aureus* ATCC 11632 *C. albicans* ATCC 90028 *E. coli* ATCC 10536	Huber *et al.*, 2003 Huber *et al.*, 2003 Ta *et al.*, 2014 Durig *et al.*, 2010 Bakkiyaraj *et al.*, 2013
Tannins **General mechanism of action:** Binds to proteins, enzyme inhibition and substrate deprivation.			
Punicalagin	Punicagranatum and Combretaceae species	*C. violaceum, S. typhimurium* SL 1344	Li *et al.*, 2014
Tannic acid	Various species	*P. aeruginosa* PA14, *P. putida* pKR–C12), *E. coli* MT102 *S. aureus*	Huber *et al.*, 2003 Cho *et al.*, 2013
Stilbenes **General mechanism of action:** DNA damage, cell division impairment, oxidative membrane damage, and metabolic enzymes inhibition.			
Resveratrol	Vitaceae and Ericaceae species	*S. epidermidis, S. aureus* *P. aeruginosa* PA14, *E. coli* O157:H7 *P. acnes*	Moran *et al.*, 2014 Cho *et al.*, 2013 Coenye *et al.*, 2012
Pterostilbene	Various species	*C. albicans* SC5314, *C. albicans* Y0109, *C. albicans* 0304103, *C. albicans* 01010	Li *et al.*, 2014

Flavonoids **General mechanism of action:** Binds to adhesions, complex with cell wall, inactivate enzymes.			
Quercetin	Various species	*E. coli* O157:H7, *V. harveyi* BB120	Vikram *et al.*, 2011
Epicatechin	*Camellia sinensis*	*E. coli* JDL271/Pal105, *P. aeruginosa* PAOI, *C. violaceum* ATCC 12472	Plyuta *et al.*, 2013 Borges *et al.*, 2014
Gallocatechin	*Camellia sinensis*	*E. corrodens*	Matsunaga *et al.*, 2010
Epigallactechin	*Camellia sinensis*	*E. corrodens*	Matsunaga *et al.*, 2010
Diarylheptanoids **General mechanism of action:** Membrane permeabilization and membrane leakage in Gram-negative and Gram-positive bacteria.			
Curcumin	*Curcuma longa*	*S. epidermidis*, *P. aeruginosa* *S. mutans* UA159, *V. harveyi* *V. parahaemolyticus*, *V. vulnificus* *E. coli*, *P. aeruginosa* *P. mirabilis* *C. albicans*	Sharma *et al.*, 2014 Rudrappa and Bias, 2008 Hu and Chen, 2013 Packiavathy *et al.*, 2013 Packiavathy *et al.*, 2014 Shahzad *et al.*, 2014
Monoterpenes **General mechanism of action:** Change in the transmembrane potential and membrane perforation.			
Thymol	*Thymus vulgaris*	*L. monocytogenes* *P. aeruginosa* ATCC 27853, *P. aeruginosa* CIP A22 *S. aureus*	Upadhyay *et al.*, 2013 Soumya *et al.*, 2011 Qiu *et al.*, 2010
Carvacrol	*Thymus vulgaris*	*L. monocytogenes* *P. aeruginosa* ATCC27853, *P. aeruginosa* CIPA22, *P. aeruginosa* IL5	Upadhyay *et al.*, 2013 Soumya *et al.*, 2011
Sesquiterpenes **General mechanism of action:** Strong inhibitors of biofilm formation and attachment			
Salvipisone	*Salvia sclarea*	*S. epidermidis* RP12 *S. aureus* 1474	Kuzma *et al.*, 2007 Walencka *et al.*, 2007
Acanthospermolide	*Acanthospermum hispidum*	*P. aeruginosa*	Cartagena *et al.*, 2007
Triterpenoids **General mechanism of action:** Strong inhibitors of biofilm formation and attachment, repressing flagellar operon, interfere with the DNA binding activities and phosphorylation events.			
Isolimonic acid	*Citrus aurantium* L.	*V. harveyi* BB170	Vikram *et al.*, 2011
Ichangin	*Citrus aurantium* L.	*V. harveyi* BB120	Vikram *et al.*, 2011
Betulinic acids	Various species	*P. aeruginosa* PA14	Cho *et al.*, 2013
Ursolic acid	Various species	*P. aeruginosa* PAO1, *E. coli* JM109 *V. harveyi* BB120	Ren *et al.*, 2005
Gymnemic acid	Gymnemasylvestre	*C. albicans* SC5314, *A. fumigates*	Vediyappan *et al.*, 2013
Sulfur-containing compounds **General mechanism of action:** Reacts with accessible cysteines in proteins and can inactivate essential enzymes, react with glutathione, shifts the cell redox potential to a more oxidized state and causes disulfide stress			
Allicin	*Alum sativum*	*P. aeruginosa* PA14, *S. epidermidis*	Ta *et al.*, 2014 Pérez-Giraldo *et al.*, 2003
Ajoene	*Alum sativum*	*P. aeruginosa* lasB-gfp, *E. coli* luxI-gfp	Jakobsen *et al.*, 2012
Sulforaphane	Brassicaceae species	*P. aeruginosa* PA01, *E. coli* DH5	Ganin *et al.*, 2012
Ally isothiocyanate	Brassicaceae species	*L. monocytogenes*, *E. coli* *C. violaceum* ATCC 1247	Borges, *et al.*, 2013 Borges *et al.*, 2014

3.1 Preventing microbial adhesion

Various factors like pH, ionic strength, temperature, nutrients, genotype and phenotype of microorganism influence the process of adhesion. The bacterial adhesion mainly depends on the charge, hydrophobicity, presence of adhesion components (*e.g.,* fimbriae, flagella and pili) and the EPS structure of microorganism (Donlan, 2002). The surface property of the material on which biofilm is formed, also plays an important role in its formation (Grossner *et al*., 2009). The hydrophobicity determines adhesion rate and experimentally the hydrophobicity or surface charge of micro-organism is calculated as the zeta potential. It is defined as the mobility of the cell in the presence of an electric field under standard pH and temperature conditions (Ferreira *et al*., 2010; Palmer *et al*., 2007). The surface charge of the cells is often determined as its zeta potential, has been measured from the mobility of cells (Pratt and Kolter, 1998; Verstraeten *et al.,* 2008). Hydrophobic surfaces have more negative value of hydrophobic attraction and hydrophilic surface tend to have positive hydrophobic attraction (Chaves and Da, 2004; Araújo *et al.*, 2010). In most studies, it is found that hydrophobic, nonpolar surface like teflon and other plastics, harbor more microbial adhesion than hydrophilic, polar surfaces like glass or metal (Fletcher and Loeb, 1979; Pringle and Fletcher, 1983; Bendinger *et al*., 1993). Stainless steel showed less bacterial load as compared to sandblast steel (Arnold and Bailey, 2000). Researchers have studied the effects of phenolic compounds on the change of cell surface charge with the some bacteria. Interaction of *E. coli* and *S. aureus* with phenolics (gallic and ferulic acids) reduces their negative charge (Abreu *et al*., 2013). The bacterial cells were treated with phenyl isothiocyanate and a significant change was observed in their hydrophobicity. The surface was made more hydrophilic (Abreu *et al.,* 2013).

3.2 Control of cellular motility

Bacteria show various types of movements like swimming, swarming, gliding, *etc*., and these movements play an important role in biofilm formations. In case of swarming movement, the force generated by the motion overcomes the electrostatic force between the substratum and bacteria which help them in the initial attachments (Pratt and Kolter, 1998). Studies showed that a mutation in swarming controlling gene made it difficult for bacteria to form biofilm (Verstraeten *et al*., 2008; Inoue *et al*., 2008). The phytochemical, I-3-C decreased sliding and swimming movement whereas no effect was observed on bacterial swarming movement (Table 2). Varying results were observed by different phyto-chemicals on cellular motility during different duration of time. The swimming and swarming motility of *P. aeruginosa*, *P. mirabilis* and *Serratia marcescens* were decreased by methanolic extracts of *Cuminum cyminum* (Sybiya *et al*., 2012*)*. However, cinnamaldehyde and eugenol from *Cinnamomum cassia* decreased the swimming motility of *E. coli* (Niu and Gilbert, 2004).

Table 2: Effect of various phytochemicals on modes of movement of microbes

Phytochemicals	Micro-organisms	Movements affected	References
Indole-3-carbinol	*E. coli* *S. aureus*	Swimming Sliding	Joana *et al.*, 2014
Salicylic acid	*E. coli*	Swimming	Joana *et al.*, 2014
Gallic acid and ferulic acid	*E. coli, P. aeruginosa, S. aureus, L. monocytogenes*	Swimming, sliding	Borges *et al.*, 2012
Ferulic acid and Salicylic acid	*Bacillus cereus, P.fluorescens*	Swimming	Borges *et al.*, 2012; Lemos *et al.*, 2014
Allylisothiocyanate and 2phenylethylisotiocyanate	*E. coli, P. aeruginosa, S. aureus, L. monocytogenes*	Swimming, sliding	Borges *et al.*, 2012
Methyl eugeno (*Cuminumcyminum)*	*P. aeruginosa, P. mirabilis, Serratiamarcescens*	swimming and swarming	Sybiya *et al.*, 2012
Cinnamaldehyde and eugenol (*Cinnamomum cassia)*	*E. coli*	Swimming	Niu and Gilbert, 2004

3.3 Quorum sensing

QS plays an important role in the formation of biofilm (Xie *et al*., 2000). Cell-to-cell communication is dependent on synthesis of the inducer and their proper exchange and binding (Khan *et al*., 2009). Davies *et al.* (1998) performed an experiment on *P. aeruginosa* having two signaling pathways (lasR-lasI and rhlR-rhlI). The double mutants were used which did not produce any of the signal through which the biofilm was formed. This formed biofilm, lacked the typical biofilm architecture of a wild type, were thinner and cells were densely packed. Moreover, on simple surface treatment, these biofilms were easily removed (Davies *et al.,* 1998). Quorum sensing inhibition (QSI) was performed on a biosensor strain *Chromobacterium violaceum* (CV12472), using the disc diffusion method (Borges *et al.,* 2014). QSI was found to be dependent on phytochemical concentration. A clove oil compound, cinnamon, peppermint and lavender were identified having QS inhibitory

properties against *C. violaceum* (CV12472) (Khan *et al.*, 2009; Zahin *et al.*, 2010; Borges *et al.*, 2014). *Tecoma capensis, Sonchus oleraceus, Pityriasis alba, Pinusnigra, Jasminum sambac, Rosmarinus officinalis, Lavandula angustifolia* and *Laurus nobilis* also act as a source of antimicrobial and QS inhibitors (Al-Hussaini and Mahasneh, 2009). Phytochemicals act on various target areas in order to bring out inhibitory effect on QS like inhibiting signal biosynthesis and acyl homoserine lactone synthase enzyme production and inhibiting the reception of signal molecules.

3.4 Change in bacterial static properties

The bacterial static property against phytochemicals proves to be helpful in controlling their effects when bacteria were found successful in forming a biofilm. The MIC and MBI values of phytochemicals were needed to be established (Chieu and John, 2015). The MIC and MBI values for Gram-negative bacteria is always greater than for Gram-positive bacteria (Vaara, 1992**;** Simões *et al.*, 2008). The morphology of the *E. coli* and *S. aureus* cells in biofilm changed when observed after treatment with phytochemicals (essentials oils). The reduction in cell size, length and diameter was observed and the peptidoglycan structure of cell wall gets disrupted, cell contents leaks out and eventually leads to cell death (Chieu and John, 2015). Gallic (hydroxybenzoic acid), ferulic acids (hydroxy-cinnamic acid), hydroxycinnamic acid and hydroxybenzoic acid were also tested for their antimicrobial activities against *E. coli* and *S. aureus* (Borges *et al.*, 2013).

4. Combined effects of phytochemicals and antibiotics

Phytochemicals act synergistically with antibiotics to overcome the problem posed by resistance strain. This combination even reduces the chances of side effects which are usually caused by use of antibiotics (Table 3). Many phytochemicals have been studied as resistance-modifying-agents (Abreu *et al.*, 2013). The combinations of antibiotics (ciprofloxacin, tetracycline and erythromycin) with phytochemical (I-3-C, SP, SA and 7-HC) were tested for four different strains of *S. aureus* and three types of effects; synergistic, additive and antagonistic effect with antibiotics was observed (Joana *et al.*, 2014). Many studies have been done on the combined effect of antibiotics and phytochemicals (LeBel, 1988; Simões *et al.*, 2008; Saavedra *et al.*, 2010; Biswas and Roymon, 2012; Abreu *et al.*, 2013). The use of sesquiterpenoid, a phytochemical in combination of four antibiotics (ciprofloxacin, erythromycin, gentamicin and vancomycin), was found to increase the overall antimicrobial activity against *E. coli* and *S. aureus* (Simões *et al.*, 2008). Further, an additive effect was observed when isothiocyanate and phenyl isothiocyanate were used with ciprofloxacin and erythromycin against *S. aureus* (Abreu *et al.*, 2013). However, saponin with chloramphenicol showed synergistic behavior against *E. coli* (Biswas and Roymon, 2012).

Table 3: Effect of combinations of phytochemicals and antibiotics on *S. aureus* biofilm

S. No.	Bacterial strains	Phytochemicals + Antibiotics
Synergistic effect		
1.	*S. aureus* CECT 976	Indole-3-carbinol + Tetracycline, Erythromycin, Ciprofloxacin
2.	*S. aureus* XU212	Indole-3-carbinol + Tetracycline Saponin + Tetracycline Salicylic acid + Tetracycline
3.	*S. aureus* RN4220	Indole-3-carbinol + Erythromycin Saponin + Erythromycin Salicylic acid + Erythromycin
4.	*S. aureus* SA1199B	Indole-3-carbinol + Ciprofloxacin Saponin + Ciprofloxacin Salicylic acid + Ciprofloxacin
Additive effect		
1.	*S. aureus* CECT 976	Saponin + Erythromycin
2.	*S. aureus* XU212	7-Hydroxycoumarin + Tetracycline
Antagonistic effect		
1.	*S. aureus* CECT 976	7-Hydroxycoumarin + Erythromycin Saponin + Tetracycline Saponin + Ciprofloxacin
2.	*S. aureus* RN4220	7-Hydroxycoumarin + Erythromycin

5. Conclusion

Biofilm formation enables microorganism to endure situations such as immune defenses and conventional antimicrobial therapies. The biofilms are the dominant lifestyle of microorganisms in all environments, either natural or manmade and remain a serious concern in the healthcare, food and marine industries. This ability has challenged the treatment of infections caused by such microorganism. The development of effective strategies to combat biofilms is a challenging task. The rise of antibiotic resistance has led to a decrease in the efficacy of treatments for the elimination of biofilm infections. The researchers and clinicians have begun concentrating their efforts on coupling biofilm destruction with antimicrobial therapy as the increased tolerance of biofilm-embedded pathogens to antibiotics.

Phytochemicals represent a possible alternate for effective, inexpensive and safe antimicrobial agents. With the evolution of multiple drug resistant bacteria, there is always a need for new strategies to control them. The use of plant extract is very common in medicine since ancient times. The phytochemicals can be used in adjuvant or alone for control of infections as they are side-effect free. Phytochemicals have great ability to inhibit the bacterial quorum sensing system, therefore, reduce the bacterial pathogenesis. In recent time, the pharmacological effects of phytochemicals have been considered as a promising future antimicrobial drug for the management of infectious diseases. In the future, the active ingredients of more plants should be identified, purified and their antimicrobial role and the mechanism of action should be studied. Though, the phytochemicals have been considered as side-effect free but there are any adverse effects of these phtytochemicals then it should also be studied on long term basis. The phtyochemicals has a good future in treating deadly infectious diseases and may one day emerge as good adjuvant or substitute for conventional antibiotic therapies.

Future prospective

The major role of biofilm is in developing antimicrobial resistance, in chronic diseases and biofilm itself as a reservoir for pathogenic organism. Microbial biofilm research is proceeding on many fronts with particular emphasis on elucidation of the genes specifically expressed by biofilm-associated organism. More research is needed that should focus on the development of new methods of degradation of biofilms. The new approaches such as phytochemical treatments gaining more attentions that weaken the structure of the biofilm, and target every important component of biofilm. These seems to be better strategies for biofilm dispersal as it can more effectively release biofilm-associated microbes from the protection of the EPS. The reagents that can target the EPS on a molecular scale, or cause the microbes themselves to actively degrade their own biofilms, may represent the next logical step towards total eradication of biofilm-afforded protection to infectious microorganisms. The phytochemicals demonstrated significant potential to reverse antibiotic resistance. However, in order to apply these phytochemicals with therapeutic/clinical purposes, further studies are required to ascertain their toxicity against mammalian cells and potential side effects.

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Conflict of interest

We declare that we have no conflict of interest.

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