TIU Transactions on Human sciences

The possibility of phytochemicals in reducing indiscriminate use of broad spectrum antibiotics in humans

Pia Dey[a*]

[a]*Techno India University, EM-4, EM Block, Sector V, Bidhannagar, Kolkata, West Bengal 700091, India.*

Abstract

Antibiotic resistance has been proven to be one of the incessant hurdles in the progress of healthcare industry. One of the major reasons that enhance the rise in the cases of antibiotic resistance is the indiscriminate use of antibiotics in various sectors such as human health, livestock feed and agricultural usages. Biofilms are a specialized lifestyle exhibited by many microorganisms including bacteria that directly escalates the incidences of antibiotic resistance. With no new novel antibiotic in the clinical pipeline and indiscriminate use of broad spectrum antibiotics, phytochemicals are considered to be an alternative novel therapeutant, which can treat resistant pathogenic diseases. With various reports on the antibacterial and antibiofilm effectiveness of phytochemicals, they are an extreme point of contention in recent medical research. The diverse nature of phytochemicals along with their scanty resistance reports makes phytochemicals a potential candidate for treating resistant diseases. Thus, immediate action along with detailed research is the need of the hour to mitigate emerging and re-emerging microbial diseases having new resistance mechanisms and to manage the rapid spread of such diseases among the human population by means of novel alternate phytochemicals.

Keywords: Biofilm; antibiotic resistance; phytochemicals; human health; combinatorial therapy

1. Introduction

Biofilms are preciously organized three-dimensional assemblages of various microorganisms, enclosed in a sheath of self-produced, complex extra polymeric substance (EPS). Biofilms are often composed of homogenous or heterogenous populations of bacteria, fungi or other organisms attached to a biotic or abiotic substratum in a single or multilayered orientation [1]. Biofilms are so versatile that they have even established their existence on liquid surfaces where they occur as floating mat or remain suspended in submerged condition [2]. Biofilms are a predominant cause of persistent bacteremia. It is the ubiquitous nature of occurrence, systematic communication, and minimized penetrance of antibiotics through inert EPS make, which makes biofilm more resistant and fatal. Biofilms are reported to accelerate the pathogenicity of bacterial infection, which eventually heightens the mortality rate in nosocomial infections. The extracellular matrix of the biofilm is reported to be composed of polysaccharides, lipids, various extracellular enzymes, nucleic acids (DNA, RNA), and water where ions and other required nutrients are sequestered from the surrounding environment. Bacterial species namely *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus epidermidis, Escherichia coli, Bacillus cereus*, *B. licheniformis*, *Candida albicans, Arthrobacter species* are known to affect the human by the capability of their vivid biofilm formation [3,4,5]. Though bacteria have immense evolutionary advantage in planktonic stage in terms of high cell growth and reproduction rates but some prefer to convert to biofilm state due to the following reasons. The primary reason lies with the survival advantage in harsh, non-bearable environment followed by resistance from antibiotic treatment. Ordered orchestration of cells with varied physical and physiological properties, along with the presence of eDNA, decreased mobility, increased cell density, increased horizontal gene transfer (HGT) are some of the main reasons behind the survival advantage of

bacterial biofilms in hostile conditions [6]. Some of the notable studies reported that distinct proteins, like BslA of *Bacillus subtilis* and RbmA of *V. cholerae* are crucial for the foundation, survival, maturation and transmission of biofilms to human hosts. These proteins maintain two inter-convertible configurations (hydrophobic/hydrophilic or oxidized/reduced state) depending on the requirement of the biofilm lifestyle [7,8].Thus planktonic and biofilm lifestyles maintain two distinct set of protein systems, which are directly influenced by changing environmental conditions and cellular densities. According to World Health Organization (WHO), with global rise in antimicrobial resistance, recalcitrant nature of biofilm is becoming a major concern affecting the healthcare set up, endangering sustainable pharmacological progress, threatening medical procedures, thereby causing inflammations in the overall economy [9]. A few of the documented biofilm mediated diseases are endocarditis, periodontitis, infection in chronic wounds, cystic fibrosis, osteomyelitis, rhinosinusitis, prosthetic device infections like central venous catheter, prosthetic heart valve, artificial voice prosthesis, urinary catheter, artificial hip prosthesis, intrauterine device [1,10]. Since 2011, WHO and European Union have been working to implement several strategies that can combat antibacterial drug resistance. It is predicted that the world might be on the inception of post antibiotic era. On the verge of this critical standpoint, several novel natural compounds are screened for possessing either antimicrobial or antibiofilm property. Since ancient ages, phytochemicals are reported to exhibit therapeutic properties with relatively different target sites, less toxicity, and a wide range of structural variations. Moreover, WHO reports the customary use of traditional medicines as a primary medication in both developing and developed countries. The statistics show that about 40% of the total health care in China, 71% in Chile, 40% in Columbia and 65% in India, with developed countries such as 48% in Australia, 31% in Belgium, 70% in Canada, 49% in France and 42% in the United States of America are using phytochemicals as an alternative or complementary source of treatment [11]. Such a surveillance report enforces the need to explore more natural compounds and phytochemicals with probable antimicrobial and anti-biofilm efficacy. This review explores the effect of biofilm formation on overall antimicrobial resistance, factors triggering transition and reversal between planktonic and biofilm state, phytochemical-antibiotic adjunctive therapy for existing medical exigencies, phytochemical as an anti-biofilm therapeutant, along with unique target sites of phytochemicals, which entitle phytochemicals to be treated as a novel approach in medicinal research.

2. Effect of biofilm formation on overall antimicrobial resistance:

Multi-drug resistance (MDR) has been reported as one of the major annoying hurdles in the progress of healthcare industry during the last two decades. With course of time, MDR is not only affecting healthcare organizations but also food and agricultural industry. Indiscriminate uses of broad spectrum antibiotics, reckless use of antibiotics in animal feed, unsafe disposal of medicine are some of the vital reasons for transmission of resistance at an alarming rate [12]. The growing interference of MDR on human health and several industries are compelling researchers to study the cause cure meticulously. Increasing incidents of MDR are due to the simultaneous effect of improper use of broad spectrum antibiotics along with poor infection control practices [13]. Antimicrobial resistance (AMR) may be attributed either as an acquired trait or an inherent characteristic. The inherent resistance is purely an intrinsic property of bacteria while the acquired one may be the outcome of horizontal gene transfer (HGT) or DNA mutations (chromosomal and/or plasmid) [14]. It is the presence of biofilm mediated infections in the population that worsens the scenario of resistance. The menacing nature of biofilm is attributed by its escaping nature from antibiotic treatment and host immune attack. Host defense systems are not as much active in sessile form as in their planktonic state [15]. Several mechanisms have been proposed to explain the elevated resistance in biofilms such as restricted antibiotic penetration, horizontal gene transfer, decreased growth rates and metabolism, altered chemical microenvironment different from their planktonic counterpart and induction of altered cellular phenotypes known as “persister” cells [16,17]. The degree of resistance for biofilm of a particular species is a multifactorial event, driven by several environmental aspects [18]. Although the exact mechanisms of resistance cannot be schematically concluded but a few of the mechanisms that are adapted by different species to adjust in unfavorable conditions are discussed. Adsorption of antibiotics on the EPS of biofilm restricts the complete penetration of antibiotics into the biofilm [19]. The restrained activity of antibiotic penetrance is further enhanced by the inactivation or diluted effect of certain antibiotics on the biofilm. For example, different physiological aspects like oxygen concentration, optimum pH and osmotic stress influence the efficacy of antibiotics in a biofilm. Due to altered microenvironment, the challenge for antibiotic penetration strengthens, along with the presence of an altered phenotype persister cells. These dominant variant of cells pose a major antibiotic resistance in the biofilm and are also the major cause of recalcitrance of biofilm-mediated infections of pathogenic bacteria. The

resistance of bacteria in biofilm state is established by its incredible adaptability whereby it can survive in dormant, non-growing form for long periods of time in partial or complete lack of nutrition.

3. Factors triggering transition and reversal between planktonic and biofilm state:

Under favourable environmental conditions such ample nutrient and water availability, optimum environmental conditions along with requisite spatial capacity, the bacteria retains planktonic form of lifestyle. The primary factor that triggers transition of planktonic bacteria to sessile form is harsh, intolerable environment threatening the survival of the bacteria. Additionally, the bacteria protect itself from antimicrobial agents, mechanical wash off and host immune attack [20]. Bacteria transform itself to a more adaptable life form of biofilm formation as a defense mechanism. Pre-conditioning of the substratum is an essential requirement for bacterial biofilm formation, where the bacterial cells are transported for initial adsorption [21]. Initiation of biofilm formation is stimulated by the attachment of bacterial consortium to biotic or abiotic substratum. The features of substratum have substantial effect on the degree of biofilm formation. The substratum may vary from hydrophobic depending on material such as teflon, plastic, latex, silicon to hydrophilic material *viz.* glass and various metals with smooth or rough texture [22]. Several physical forces like van der Waals forces, steric and electrostatic interactions influence the cohesion of bacteria to different substratum [23]. The presence of cell appendages like flagella, pili, fimbriae, or glycocalyx also enhances biofilm formation, by their additional retention property to the substratum. Cell surface hydrophobicity is another important virtue of cell attachment [24].

Reversibly bound cells are mechanically washed off leaving behind irreversible consortium of bacteria capable of microcolony formation. Irreversible cell attachment and microcolony formation are followed by biofilm growth and maturation. During this phase, the aggregation of cell encloses themselves in a sheath of complex, self-produced extra polymeric substance (EPS). This extracellular covering not only protects bacteria but also contains several minute water channels, transporting water, nutrients and oxygen to the cell and maintaining required hydration within biofilm. For example, *P. aeruginosa* produces three polysaccharides, namely alginate, Pel (glucose rich polysaccharide) and Psl (pentasaccharide), where alginate interacts with nutrients and water, supplying nutrients to the biofilm [25]. Various quorum sensing molecules like oligopeptides, N-acylhomoserine lactones (AHL), autoinducing peptides (AIP) of Agr system in *Staphylococcus* [26], autoinducer-2 (AI 2) are produced in density dependent response. Numerous signals transmitted by these quorum sensing molecules which are required to control individual cells in heterogeneous cell population or regulate the biofilm as a whole. During this stage the biofilm forms a heterogeneous three-dimensional structure. On perceiving favourable conditions, the population retracts back from its sessile form from indwelling medical devices or other host sites via lymph and blood stream in a QS-mediated manner. There are several theories on the dispersal mechanism of biofilm. This includes terminating the synthesis of the biofilm matrix components, degrading the extracellular matrix followed by disruption of non-covalent interactions between matrix constituents [26]. The extrinsic environmental factors trigger intrinsic QS regulatory networks, involving several genes, whose products act in an extremely ordered fashion to bring out the transition [27]. Mechanisms of different phases of biofilm, that is, formation, maturation and dispersal may greatly vary with different species of bacteria (mainly among the pathogenic micro-organisms) depending on their particular infectious niche and mode of infection.

4. Phytochemical as an anti-biofilm therapeutant:

Although a number of in vitro strategies are available in combating biofilm mediated infections ranging from prevention of biofilm adhesion to iontophoresis in medical prosthetic devices, but in vivo cure of such infections still remains a challenge [28]. This is mainly because of the reduced/altered antimicrobial property in vivo condition along with toxicity imposed by therapeutant against normal host cells. On the other hand, abundant chemical diversity, advanced fractionation techniques, unique target sites different are promoting the use of phytochemicals as an alternative or collateral constituent in combinational therapy against biofilm mediated chronic infections. Moreover there is no published report of bacterial resitance to phytochemicals [29]. Furthermore, many of the phytochemicals can be effortlessly included in regular diet manifesting their easy administration among patients. Recently, Noumi et al. reported antiquorum sensing (anti-QS) and antibiofilm potential of *Salvadora persica* L. methanolic against *Staphylococcus* infection, as an alternative to antibiotics. The methanolic extracts from fruit, leaves and stems inhibited QS-depedent phenomenon such as violacein pigment production in *Chromobacterium violaceum*, swarming motility of *Pseudomonas aeruginosa* PAO1 and biofilm formation in oral *Staphylococcus* strains [30]. Srinivasan et al. published

that *Piper betle* ethyl acetate extract showed antibiofilm activity targeting the initial attachment as well as microcolony formation stage of *V. harveyi* biofilm [31]. These natural products are mainly secondary metabolites possessing evolutionary benefit for overcoming different environmental challenges [32]. Such properties of phytochemicals are exploited to control insistent biofilm mediated infections, which are evolving continually with changing environment. Phytochemicals that have shown substantial effect as anti-biofilm therapeutant can be grouped according to their chemical nature as alkaloids, phenolics, terpenoids and essential oils, organosulfur compounds and isothiocyanates [29,33].

4.1 *Alkaloids:*

Alkaloids are heterocyclic nitrogen compounds with extreme chemical variability. They are produced by a large number of organisms such as plants, bacteria, fungi and animals. They have important physiological impact on human and other animals and have been used in medicine since ancient times.[34] They have reported antibacterial activity and are hence explored for antibiofilm prospects. Alkaloids mainly act by impairing FtsZ Z-ring formation, thereby interfering with bacterial cytokinesis.[3536] They also intercalate bacterial DNA, sequentially resulting in cell death [33]. They first came into the limelight with discovery of morphine from *P. somniferum,* of clinical importance [29]. Berberine is reported to have antibiofilm effect against heterogenous biofilm of *E. faecalis, Fusobacterium nucleatum*, and *Prevotella intermedia* [34,35,36]. Chelerythrine and sanguinarine inhibit biofilm of strains of *S. aureus* and *S. epidermidis* [37].

4.2 *Terpenoids and Essential Oils:*

Terpenoids are the largest group of natural compounds possessing antibacterial property. They are compounds based on isoprene structure and can have additional elements such as oxygen, when they are further categorized as terpenes. They are composed of two to five-carbon building blocks and are classified as monoterpenoids (C10), sesquiterpenoids (C15), diterpenoids (C20), and sesterterpenoids (C25) accordingly. Monoterpenoids and/or sesquiterpenoids constitute essential oils, derived from plants, which have additional antioxidant property along with antimicrobial feature. Although their mode of action is not fully understood but it is presumed that they act by membrane disruption by the lipophilic compounds and their activity largely depends on the structure of the compound [38,39,40,41]. This disruption interferes with membrane permeability thereby hindering ion transport processes [39.40,41]. Some of the reported antibiofilm impacts are observed in methyl eugenol against *P. aeruginosa*, *P. mirabilis* and *S. marcescens,* diterpenoid salvipisone *against S. aureus* and *S. epidermidis* [42,43]. Several familiar dietary supplements such as clove, cinnamon, peppermint and lavender act as quorum sensing inhibitors [44]. While others act on irremovable dual-species biofilm such as carvacrol against *Salmonella* spp. and *S. aureus* [45] thyme, carvacrol and oregano against *S. aureus* and *S. epidermidis* [46].

4.3 *Phenolics:*

Phenolics are widely distributed aromatic compounds, sub-classed into flavones, flavanoids and flavanols containing one carbonyl group, quinones with two carbonyl groups, polymeric phenolic compounds like tannins, coumarins, phenolic compounds with fused benzene and pyrone groups [47,48]. Like most of the plant secondary metabolites, phenolics also exhibit antibacterial, anti-infective and antioxidant property [49]. They disable bacterial cells by distorting the cell envelope, inactivating crucial bacterial proteins and enzymes, forming stable free radicals, finally strangling the cell to death [48,49,50]. Curcumin, well known phenol found in turmeric, has strong antibiofilm activity against *E. coli*, *P. aeruginosa*, *P. mirabilis* and *S. marcescens* [51]. 6-gingerol reduces the biofilm formatiom and virulence in *P. aeruginosa* [52].

4.4 *Isothiocyanate:*

Isothiocyanates are organosulfur compounds, derived from enzymatic conversion of glucosinolates. Natural isothiocyanates are found in a large number of edible vegetables such as wasabi, mustard, horseradish, radish, Brussels sprouts, cabbage, broccoli, papaya seeds. Though isothiocyanates in their naïve form does not show any antimicrobial activity but their hydrolyzed product (glucosinolate hydrolysis products or GHP) are effective against wide number of clinically important organisms in their planktonic form [53,54,56,57]. Allylisothiocyanate (AITC) was reported to inhibit the swimming motility in *E. coli*, *P. aeruginosa* and *L. monocytogenes* while 2-phenylethylisothiocyanate (PEITC) hindered the swarming motility in *E. coli* and *P. aeruginosa* along with interference in their adhesion properties [58]. Iberin acts as quorum sensing inhibitor (QSI) in *P. aeruginosa* [59]. Therefore GHP act by interfering with the genes responsible for adhesion and quorum sensing abilites. Hence less reported but GHP have antibiofilm effect along with antimicrobial efficacy. Organosulfur compounds such as allicin and ajoene, derived from garlic, also show QSI property, but more effectively acting as an adjuvant overcoming the resistance barrier posed against commercial antibiotics. For example other garlic derived organosulfur compounds is reported to inhibit biofilm formation by *P. aeruginosa* and *E. coli* through QS inhibition [60].

5. Phytochemical-Antibiotic combinatorial therapy:

Plant extracts have exhibited immense restorative solution to several types of wounds, infections and acted as prophylaxis ever since ancient times. Initial usage of natural products was instinctive and the plant products acted as panacea when the exact cause and cure of the diseases were unknown. With the advent of antibiotic era, the natural products lost their prominence and were replaced by commercial antibiotics, with higher efficacy and distinctively lower MIC value. As disturbed nature followed its own transition rule, the world is now on the verge of post antibiotic era. Hence, natural drugs are regaining their importance on the virtue of their immense chemical diversity and unique target sites, dissimilar from available antibiotics. Moreover phyto-products are much more sustainable, cost effective and show less cytotoxicity towards human host than most of the conventional antibiotics.

With increased resistance and supplementary threat posed by biofilm-mediated infections, requirement for the search of new phytochemicals with antimicrobial and antibiofilm trait began. Since then, the antibiofilm activity of phytochemicals was reported against several chronic infections such as urinary and pulmonary infection along with several prosthetic device infections [61]. The only drawback that withheld phytochemicals from emerging as an outstanding remedy was their high MIC value (even >1000µg/mL) [62]. This imperfection was resolved by combinational therapy of phytochemical with antibiotic, where the phytochemical acted as an adjuvant making the biofilm more susceptible to antibiotic treatment. As biofilm infections posed multifactorial hindrance in the operation of antibiotics, synergistic action of phytochemical and antibiotic resulted in enhanced inhibition or complete eradication [63]. Synergism revealed an added advantage of low dosage use of both phytochemicals and antibiotics, than when used individually. This reduced the cytotoxicity on human host [64]. Furthermore phytochemicals act on target sites (such as matrix components, resistant persister cell population or altered physico-chemical environment) different from available antibiotics, thus widening the abolition spectrum and narrowing the blooming of resistant mutants [65]. The combination can be immensely diverse ranging from antibiotic-antibiotic, antibiotic-phytochemical, phytochemical-phytochemical or integrating antibiotic with any kind of natural products such as peptides, peptidomimetics, enzymes (e.g., DNases), bacteriophages, essential oils, secondary metabolites [66]. It can also be combined with non-antimicrobial compounds, only showing collaborative antimicrobial and/or antibiofilm activity [67]. Abreu et al. observed antibiofilm activity against selected phytochemicals namely reserpine, pyrrolidine, quinine, morin and quercetin combining with antibiotics ciprofloxacin, tetracycline and erythromycin against *S. aureus* biofilms [68]. Betoni and coworkers reported synergistic interactions between extracts of Brazilian medicinal plants and eight antibiotics on *S. aureus* [69]. Ahmad et al. showed synergistic communication of crude extracts of Indian medicinal plants with tetracycline and ciprofloxacin against ESBL-producing MDR-enteric bacteria [70]. Aumeeruddy-Elalfi et al. documented synergistic effect of essential oils of *Pimenta dioica*, *Psidia arguta* and *Piper betle* with gentamicin against *E. coli* and *S. epidermis* [71]. As explained earlier, many natural products also manifest synergistic reaction on combining with commercial antibiotics. Extracts of wild mushroom *Mycena rosea* and *Fistulina hepatica*, revealed synergistic activities with a handful of commercial antibiotics such as penicillin, ampicillin, amoxicillin/clavulanic acid, cefoxitin, ciprofloxacin, cotrimoxazol, levofloxacin against *E. coli*, ESBL *E. coli* and MRSA [72, 73, 74, 75].

6. Unique Target Sites of Phytochemicals:

The demolition of biofilm by phytochemicals can occur at several steps of biofilm formation targeting its various stages ranging from blocking its formation by material optimization and/or surface modification [76,77,78,79,80]. Certain mechanical methods of washing off reversibly bound cells, hindering irreversible attachment by targeting adhesin production and its interaction with substratum, inhibiting nucleotide signalling biosynthesis such as cyclic diguanosine monophosphate (c-di-GMP), suppressing maturation focusing on various quorum sensing inhibitions and stimulating dispersal with the aid of enzymes, metal chelators, QS inhibitors and other molecules such as D-amino acids, norspermidine, dispersin B, N-acetylcysteine, cis-2-decenoic acid and nitric oxide [81,82,83]. Among these various mechanisms aiming biofilm inhibition or its reversal to planktonic state, phytochemicals target the irreversible adhesion, maturation and dispersal phase of sessile state [84]. Some of the reported distinctive target sites acted upon by phytochemicals are listed below:

6.1 *Biofilm Metal Chelators:*

Bacteria employ metals such as calcium, magnesium, copper, manganese, zinc and iron primarily for their growth and survival which is succeeded by their infectious activities invading host tissue [84]. The tussle between bacteria and host system is determined by the balance of metal ions in both systems. On one hand where optimal metal ion concentration promotes their defensive mechanisms, higher concentrations may be toxic against each other and lower concentrations threatening their own survival. Hence maintenance of optimum metal ion concentration, according to the requirement of the cell is very essential. Many experiments had reported the contrasting roles played

by iron ions in both promotion and inhibition of sessile lifestyle. On one hand, iron serves as signalling factor in biofilm formation [74], followed by its notable role in cell growth and adherence, aiding in cell attachment and microcolony formation [76]. In *Pseudomonas aeruginosa*, it even plays avid roles in biofilm formation, pathogenesis and virulence in QS controlled sequential orchestration. On the contrary, iron promotes biofilm formation in *Staphylococcus aureus* under diluted concentration, with few iron salts showing toxicity for bacteria and disrupting preformed biofilms. Along with iron, calcium is required for cross-linking properties among components of biofilm matrix, thus maintaining the extracellular matrix [78]. Hence ideal metal concentration according to the need of the bacteria is very important. This property empowered researchers to use metal chelators in altering the necessary metal concentration and disrupting the overall biofilm integrity. Initially, synthetic metal chelators such as diethylenetriaminepentacetic acid, ethylenediaminetetraacetic acid, ethylenediamine-N,N9 diacetic acid, deferoxamine mesylate, 2,21-dipyridyl, citrate and acetohydroxamic acid were used to destabilize the bacteria along with complete biofilm demolition in certain cases. Consequently many plant derived natural metal chelators in the chemical classes of polyphenols, phenolic acids and flavonoids were discovered. Alizarin, reported as a calcium specific chelating agent, inhibited biofilm formation by sequestering Ca^{2+} ions in three *Staphylococcus aureus* strains and *Staphylococcus epidermidis* strain, hence exhibiting antibiofilm and anti-hemolytic activities [85]. Raad et al. designed chelator-based catheter lock solutions with methylene blue-citrate-parabens targeting immature Gram-positive, Gram-negative, and fungal biofilms and minocycline-EDTA-25% ethanol targeting both mature and immature biofilms [86]. Advancement in controlling catheter-related bloodstream infection was achieved by adjuvant therapy of cation chelator EDTA in combination with several antibiotics like gentamicin, amikacin and vancomycin, whereby metal chelation enhanced the activity of the known antibiotics [87]. *Vaccinium macrocarpon* (cranberry) was observed to attenuate *E. coli* biofilm formation encompassing its various aspects. Cranberry juice impacted bacterial growth by interfering with the doubling time along with altered gene expression associated with iron transport and essential metabolic enzymes. Proanthocyanidins, a major constituent of cranberry juice, possessed significant iron chelating property [88]. Small quinoline molecules exhibited biofilm dispersal and antibacterial activity against methicillin resistant *S. aureus* and *S. epidermidis*, using a scaffold hopping mechanism. These quinolines displayed metal-chelator property, as they shared similar structure as nitroxoline, reported metal chelator antibiotic with antibiofilm property [89].

6.2 *Biofilm Quorum Sensing Inhibitors:*

The whole event of biofilm formation particularly following irreversible adsorption is mediated by an array of intracellular communicators known as quorum sensing inducers. Regulating the gene expression of these molecules directly enables researchers to manipulate the virulence of the organism thereby, affecting its pathogenesis. This finding lead to the search of novel quorum sensing inhibitors or QSIs, targeting the virulence and/or biofilm forming ability of organisms without affecting their cellular growth. This in turn will not exert any selective pressure thereby restricting the chances of resistance. Intracellular communication is an essential feature both in planktonic and sessile form thereby holding the flexibility to arrest different phases of lifecycle. QSIs are reported to prevent initial biofilm formation and altering its progression by the secretion of adhesin proteins, targeting irreversible surface adhesion. It can also aim cellular appendages (which affect bacterial motility), cell formation of microcolonies and inhibition of the EPS production [29]. Some QSIs and manipulated QS signals can also be used to induce biofilm dispersal [28]. QSIs may not always result in complete eradication of biofilm but like other plant based inhibitors; act as a promoter increasing the susceptibility of resistant biofilm towards available antibiotics.

7. Conclusion:

Phytochemicals and phytopeptides (plant peptides with antimicrobial property) hold promising prospect in the progressing remediation of biofilms in prosthetic devices and/or biofilm mediated infections in other sites. Plants being renewable, with no report of resistance and being able to target bacteria via unique mechanisms, are the emerging warrior against biofilm and biofilm mediated resistance. But what holds them back from thriving as one of the principle contender of antibiofilm therapy are their laborious fractionation techniques, exceptional reports of biofilm promotion instead of inhibition and doubtful optimum in vivo efficacy. Although till date approximately 3500 compounds have been examined for their antibacterial property but that is only a small percentage of the available resources explored. Therefore rigorous screening is required to gather explicit knowledge about the wide diversity of phytochemicals available, their side effects and storage procedures, which might translate itself in an applicative form. Optimum dosage of phytochemicals is another public concern that is directly correlated with its in vivo pharmacokinetics, as in some cases low concentration may promote biofilm

development whereas in others higher doses might be cytotoxic. Synergistic activity of phytochemicals and antibiotics are a retort to few of the drawbacks exhibited by phytochemicals. The most affirming aspect of phytochemical lies in their innate nature as resistant modifying agents. Upcoming advanced technique such as molecular docking aids in intricate comprehension of predominant binding of phytochemicals with bacterial target sites. Novel phytochemical loaded bio-nanocomposites are synthesized for enhanced efficacy such as curcumin entrapped in Carboxymethyl Starch (CMS)-Chitosan (CS)-Montmorillonite (MMT) [90]. Phytochemicals thus hold latent, substantial prospective not only in chronic biofilm infections but also altering the overall resistance scenario consequently. Although phytochemicals have entirely established themselves in *in vitro* antimicrobial assays but their proper use requires preclinical trials on animal systems followed by clinical human trials. Some of the preclinical trials with synergistic combinations of phytochemical and antibiotic are common but clinical trials are uncommon, so are trials with their resistance modifying properties.

Phytochemicals thus hold latent, substantial prospective not only in chronic biofilm infections but also altering the overall resistance scenario consequently. Although phytochemicals have entirely established themselves in *in vitro* antimicrobial assays but their proper use requires preclinical trials on animal systems followed by clinical human trials. Some of the preclinical trials with synergistic combinations of phytochemical and antibiotic are common but clinical trials are uncommon, so are trials with their resistance modifying properties.

Acknowledgments

The author acknowledges the support received from Techno India University, West Bengal, India.

References

[1] Microbiol, A., Gupta, P., Sarkar, S., Das, B. & Bhattacharjee, S. Biofilm , pathogenesis and prevention — a journey to break the wall : a review. *Arch. Microbiol.* (2015). doi:10.1007/s00203-015-1148-6

[2] Vasudevan, R. Biofilms: Microbial Cities of Scientific Significance. *J. Microbiol. Exp.* **1,** 1–16 (2014).

[3] Naves, P. *et al.* Effects of human serum albumin, ibuprofen and N-acetyl-l-cysteine against biofilm formation by pathogenic Escherichia coli strains. *J. Hosp. Infect.* **76,** 165–170 (2010).

[4] Al-Fattani, M. A. & Douglas, L. J. Biofilm matrix of Candida albicans and Candida tropicalis: Chemical composition and role in drug resistance. *J. Med. Microbiol.* **55,** 999–1008 (2006).

[5] Gayathri, K. V. & Vasudevan, N. Enrichment of Phenol Degrading Moderately Halophilic Bacterial Consortium from Saline Environment. *J. Bioremediation Biodegrad.* **1,** 1–6 (2010).

[6] Hausner, M. & Wuertz, S. High Rates of Conjugation in Bacterial Biofilms as Determined by Quantitative In Situ Analysis High Rates of Conjugation in Bacterial Biofilms as Determined by Quantitative In Situ Analysis. **65,** 3710–3713 (1999).

[7] Fong, J. C. *et al.* Structural dynamics of RbmA governs plasticity of *Vibrio cholerae* biofilms. *Elife* **6,** 1–22 (2017).

[8] Arnaouteli, S. *et al.* Bifunctionality of a biofilm matrix protein controlled by redox state. *Proc. Natl. Acad. Sci.* **114,** E6184–E6191 (2017).

[9] Donlan, R. M. Biofilm Formation : A Clinically Relevant Microbiological Process. **33,** (2001).

[10] WORLD HEALTH ORGANIZATION. A report on Traditional medicines. *Fifty-Sixth World Heal. Assem.* 14–17 (2003).

[11] Khameneh, B., Diab, R., Ghazvini, K. & Fazly Bazzaz, B. S. Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. *Microbial Pathogenesis* (2016). doi:10.1016/j.micpath.2016.02.009

[12] Akova, M. Epidemiology of antimicrobial resistance in bloodstream infections. *Virulence* **7,** 252–266 (2016).

[13] Chen, C. W. *et al.* Metal nanobullets for multidrug resistant bacteria and biofilms. *Advanced Drug Delivery Reviews* (2014). doi:10.1016/j.addr.2014.08.004

[14] Wolcott, R. D. *et al.* Biofilm maturity studies indicate sharp debridement opens a time- dependent therapeutic window. *J. Wound Care* **19,** 320–328 (2010).

[15] Stewart, P. S. & Costerton, J. W. Antibiotic resistance of bacteria in biofilms. *Lancet (London, England)* **358,** 135–8 (2001).

[16] Stewart, P. S. Mechanisms of antibiotic resistance in bacterial biofilms. *Int. J. Med. Microbiol.* **292,** 107–113 (2002).

[17] Donlan, R. M. & Costerton, J. W. Biofilms : Survival Mechanisms of Clinically Relevant Microorganisms. **15,** 167–193 (2002).

[18] Shigeta, M. *et al.* Permeation of Antimicrobial Agents through Pseudomonas aeruginosa Biofilms: A simple Method. *Exp. Chemother.* **43,** 340–345 (1997).

[19] Jones, L. H. Medicinal Chemistry. *Future Med. Chem.* **7,** 2131–2141 (2015).

[20] Bennett, R. N., Rosa, E. A. S. & Simo, M. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. 746–757 (2009). doi:10.1039/b821648g

[21] Donlan, R. M. Biofilm Formation: A Clinically Relevant Microbiological Process. *Clin. Infect. Dis.* **33,** 1387–1392 (2001).

[22] Garrett, T. R., Bhakoo, M. & Zhang, Z. Bacterial adhesion and biofilms on surfaces. *Prog. Nat. Sci.* **18,** 1049–1056 (2008).

[23] Götz, F. & Peters, G. Colonization of medical devices by coagulase-negative staphylococci. *Infections associated with indwelling medical devices* 55–88 (2000). doi:10.1128/9781555818067.ch4

[24] Rasamiravaka, T., Labtani, Q., Duez, P. & Jaziri, M. El. The Formation of Biofilms by Pseudomonas aeruginosa : A Review of the Natural and Synthetic Compounds Interfering with Control Mechanisms. **2015,** (2015).

[25] Otto, M. Staphylococcal Infections: Mechanisms of Biofilm Maturation and Detachment as Critical Determinants of Pathogenicity. *Annu. Rev. Med.* **64,** 175–188 (2013).

[26] Solano, C., Echeverz, M. & Lasa, I. Biofilm dispersion and quorum sensing. *Curr. Opin. Microbiol.* **18,** 96–104 (2014).

[27] Lebeaux, D., Ghigo, J.-M. & Beloin, C. Biofilm-Related Infections: Bridging the Gap between Clinical Management and Fundamental Aspects of Recalcitrance toward Antibiotics. *Microbiol. Mol. Biol. Rev.* **78,** 510–543 (2014).

[28] Borges, A., Saavedra, M. & Simoes, M. Insights on Antimicrobial Resistance, Biofilms and the Use of

Phytochemicals as New Antimicrobial Agents. *Curr. Med. Chem.* **22,** 2590–2614 (2015).
[29] Noumi, E. *et al.* Phytochemical composition, anti-biofilm and anti-quorum sensing potential of fruit, stem and leaves of Salvadora persica L. methanolic extracts. *Microb. Pathog.* (2017). doi:10.1016/j.micpath.2017.05.036
[30] Srinivasan, R., Santhakumari, S. & Ravi, A. V. In vitro antibiofilm efficacy of Piper betle against quorum sensing mediated biofilm formation of luminescent Vibrio harveyi. *Microb. Pathog.* (2017). doi:10.1016/j.micpath.2017.07.001
[31] Kennedy, D. O. Do & Wightman, E. E. L. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Adv. Nutr. An ...* **2,** 32–50 (2011).
[32] Savoia, D. Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol.* **7,** 979–990 (2012).
[33] Barbieri, R. *et al.* Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological Research* (2017). doi:10.1016/j.micres.2016.12.003
[34] Beuria, T. K., Santra, M. K. & Panda, D. Sanguinarine blocks cytokinesis in bacteria by inhibiting FtsZ assembly a bundling. *Biochemistry* **44,** 16584–16593 (2005).
[35] Kelley, C. *et al.* 3-Phenyl substituted 6,7-dimethoxyisoquinoline derivatives as FtsZ-targeting antibacterial agents. *Bioorganic Med. Chem.* **20,** 7012–7029 (2012).
[36] Artini, M. *et al.* Bacterial biofilm formation inhibitory activity revealed for plant derived natural compounds. *Bioorganic Med. Chem.* **20,** 920–926 (2012).
[37] Termentzi, A., Fokialakis, N. & Skaltsounis, A. L. Natural resins and bioactive natural products thereof as potential anitimicrobial agents. *Curr. Pharm. Des.* **17,** 1267–1290 (2011).
[38] Trombetta, D. *et al.* Mechanisms of Antibacterial Action of Three Monoterpenes. **49,** 2474–2478 (2005).
[39] Daisy, P., Mathew, S., Suveena, S. & Rayan, N. A. A novel terpenoid from Elephantopus Scaber - Antibacterial activity on Staphylococcus aureus: A substantiate computational approach. *Int. J. Biomed. Sci.* **4,** 196–203 (2008).
[40] Brehm-Stecher, B. F. & Johnson, E. A. Sensitization of Staphylococcus aureus and Escherichia coli to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone. *Antimicrob. Agents Chemother.* **47,** 3357–3360 (2003).
[41] Sybiya Vasantha Packiavathy, I. A., Agilandeswari, P., Musthafa, K. S., Karutha Pandian, S. & Veera Ravi, A. Antibiofilm and quorum sensing inhibitory potential of Cuminum cyminum and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. *Food Res. Int.* **45,** 85–92 (2012).
[42] Ro, M. Antimicrobial activity of diterpenoids from hairy roots of Salvia sclarea L .: Salvipisone as a potential anti-biofilm agent active against antibiotic resistant Staphylococci. **14,** 31–35 (2007).
[43] Khan, M. S. A., Zahin, M., Hasan, S., Husain, F. M. & Ahmad, I. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett. Appl. Microbiol.* **49,** 354–360 (2009).
[44] Knowles, J. R. & Roller, S. Antimicrobial Action of Carvacrol at Different Stages of Dual-Species Bio lm Development by. *Society* **71,** 797–803 (2005).
[45] Nostro, A. *et al.* Effects of oregano, carvacrol and thymol on Staphylococcus aureus and Staphylococcus epidermidis biofilms. *J. Med. Microbiol.* **56,** 519–523 (2007).
[46] Cowan, M. M. Plant Products as Antimicrobial Agents. **12,** 564–582 (1999).
[47] Kurek, A., Grudniak, A. M., Kraczkiewicz-Dowjat, A. & Wolska, K. I. New antibacterial therapeutics and strategies. *Polish J. Microbiol.* **60,** 3–12 (2011).
[48] Saleem, M. *et al.* Antimicrobial natural products: an update on future antibioticdrug candidates. *Nat. Prod. Rep.* **27,** 238–254 (2010).
[49] Cazarolli, L. *et al.* Flavonoids: Prospective Drug Candidates. *Mini-Reviews Med. Chem.* **8,** 1429–1440 (2008).
[50] Packiavathy, I. A. S. V., Priya, S., Pandian, S. K. & Ravi, A. V. Inhibition of biofilm development of uropathogens by curcumin - An anti-quorum sensing agent from Curcuma longa. *Food Chem.* **148,** 453–460 (2014).
[51] Kim, H., Lee, S., Byun, Y. & Park, H. virulence via quorum sensing inhibition. (2015). doi:10.1038/srep08656
[52] Saavedra, M. J. *et al.* Antimicrobial activity of phenolics and glucosinolate hydrolysis products and their synergy with streptomycin against pathogenic bacteria. *Med. Chem.* **6,** 174–183 (2010).
[53] Jang, M., Hong, E. & Kim, G. H. Evaluation of antibacterial activity of 3-butenyl, 4-pentenyl, 2-phenylethyl, and benzyl isothiocyanate in Brassica vegetables. *J. Food Sci.* **75,** 412–416 (2010).
[54] Chen, H., Wang, C., Ye, J., Zhou, H. & Chen, X. Antimicrobial activities of phenethyl isothiocyanate isolated from horseradish. *Nat. Prod. Res.* **26,** 1016–1021 (2012).
[55] Vu, B., Chen, M., Crawford, R. J. & Ivanova, E. P. Bacterial Extracellular Polysaccharides Involved in Biofilm Formation. 2535–2554 (2009). doi:10.3390/molecules14072535
[56] Lin, C. M., Kim, J., Du, W. X. & Wei, C. I. Bactericidal activity of isothiocyanate against pathogens on fresh produce. *J. Food Prot.* **63,** 25–30 (2000).
[57] Borges, A., Simoes, L. C., Saavedra, M. J. & Simoes, M. The action of selected isothiocyanates on bacterial biofilm prevention and control. *Int. Biodeterior. Biodegrad.* (2014). doi:10.1016/j.ibiod.2013.01.015
[58] Jakobsen, T. H. *et al.* Food as a source for quorum sensing inhibitors: Iberin from horseradish revealed as a quorum sensing inhibitor of Pseudomonas aeruginosa. *Appl. Environ. Microbiol.* **78,** 2410–2421 (2012).
[59] Cady, N. C. *et al.* Inhibition of biofilm formation, quorum sensing and infection in pseudomonas aeruginosa by natural products-inspired organosulfur compounds. *PLoS One* **7,** (2012).
[60] Römling, U. *et al.* Microbial biofilm formation: A need to act. *J. Intern. Med.* **276,** 98–110 (2014).
[61] Kyaw, B. M., Arora, S. & Lim, C. S. Bactericidal antibiotic-phytochemical combinations against methicillin resistant Staphylococcus aureus. *Brazilian J. Microbiol.* **43,** 938–945 (2012).
[62] Wu, H., Moser, C., Wang, H.-Z., Høiby, N. & Song, Z.-J. Strategies for combating bacterial biofilm infections. *Int. J. Oral Sci.* **7,** 1–7 (2014).
[63] de la Fuente-Núñez, C., Reffuveille, F., Haney, E. F., Straus, S. K. & Hancock, R. E. W. Broad-Spectrum Anti-biofilm Peptide That Targets a Cellular Stress Response. *PLoS Pathog.* **10,** (2014).
[64] Ribeiro, S. M. *et al.* New frontiers for anti-biofilm drug development. *Pharmacology and Therapeutics* (2016). doi:10.1016/j.pharmthera.2016.02.006
[65] Kunze, B. *et al.* Damage of Streptococcus mutans biofilms by carolacton, a secondary metabolite from the myxobacterium Sorangium cellulosum. *BMC Microbiol.* **10,** 199 (2010).
[66] Wagner, H. Synergy research: Approaching a new generation of phytopharmaceuticals. *Fitoterapia* **82,** 34–

37 (2011).

[67] Abreu, A. C., Saavedra, M. J., Simões, L. C. & Simões, M. Combinatorial approaches with selected phytochemicals to increase antibiotic efficacy against Staphylococcus aureus biofilms. *Biofouling* (2016). doi:10.1080/08927014.2016.1232402

[68] Betoni, J. E. C., Mantovani, R. P., Barbosa, L. N., Di Stasi, L. C. & Fernandes, A. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. *Mem. Inst. Oswaldo Cruz* **101,** 387–390 (2006).

[69] .Ahmad, I. & Aqil, F. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiol. Res.* **162,** 264–275 (2007).

[70] Aumeeruddy-Elalfi, Z., Gurib-Fakim, A. & Mahomoodally, F. Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. *Ind. Crops Prod.* **71,** 197–204 (2015).

[71] Alves, M. J. *et al.* Wild mushroom extracts potentiate the action of standard antibiotics against multiresistant bacteria. *J. Appl. Microbiol.* **116,** 32–38 (2014).

[72] Miladi, H. *et al.* Synergistic effect of eugenol, carvacrol, thymol, p-cymene and γ-terpinene on inhibition of drug resistance and biofilm formation of oral bacteria. *Microb. Pathog.* (2017). doi:10.1016/j.micpath.2017.09.057

[73] Fankam, A. G., Kuiate, J. R. & Kuete, V. Antibacterial and antibiotic resistance modifying activity of the extracts from allanblackia gabonensis, combretum molle and gladiolus quartinianus against Gram-negative bacteria including multi-drug resistant phenotypes. *BMC Complement. Altern. Med.* **15,** 206 (2015).

[74] Barreto, H. M. *et al.* Enhancement of the antibiotic activity of aminoglycosides by extracts from Anadenanthera colubrine (Vell.) Brenan var. cebil against multi-drug resistant bacteria. *Nat. Prod. Res.* **30,** 1289–1292 (2016).

[75] Tankeo, S. B., Tane, P. & Kuete, V. In vitro antibacterial and antibiotic-potentiation activities of the methanol extracts from Beilschmiedia acuta, Clausena anisata, Newbouldia laevis and Polyscias fulva against multidrug-resistant Gram-negative bacteria. *BMC Complement. Altern. Med.* **15,** 412 (2015).

[76] Touani, F. K. *et al.* Antibiotic-potentiation activities of four Cameroonian dietary plants against multidrug-resistant Gram-negative bacteria expressing efflux pumps. *BMC Complement. Altern. Med.* **14,** 258 (2014).

[77] Barreto, H. M. *et al.* Effect of Lippia origanoides H.B.K. essential oil in the resistance to aminoglycosides in methicillin resistant Staphylococcus aureus. *Eur. J. Integr. Med.* **6,** 560–564 (2014).

[78] Hwang, J. H. *et al.* Synergistic antibacterial and antibiofilm effect between (+)-medioresinol and antibiotics in vitro. *Appl. Biochem. Biotechnol.* **170,** 1934–1941 (2013).

[79] Myint, K. B., Sing, L. C. & Wei, Z. Tannic Acid as Phytochemical Potentiator for Antibiotic Resistance Adaptation. *APCBEE Procedia* **7,** 175–181 (2013).

[80] Zeng, X. *et al.* Synergistic effect of 14-alpha-lipoyl andrographolide and various antibiotics on the formation of biofilms and production of exopolysaccharide and pyocyanin by Pseudomonas aeruginosa. *Antimicrob. Agents Chemother.* (2011). doi:10.1128/AAC.00575-10

[81] Leite, B. *et al.* Staphylococcus epidermidis biofilms control by N-acetylcysteine and rifampicin. *Am J Ther* **0,** 1–7 (2011).

[82] Mansouri, M. D., Hull, R. A., Stager, C. E., Cadle, R. M. & Darouiche, R. O. In vitro activity and durability of a combination of an antibiofilm and an antibiotic against vascular catheter colonization. *Antimicrob. Agents Chemother.* **57,** 621–625 (2013).

[83] Borges, A. *et al.* New perspectives on the use of phytochemicals as an emergent strategy to control bacterial infections including biofilms. *Molecules* (2016). doi:10.3390/molecules21070877

[84] Lee, J. H., Kim, Y. G., Yong Ryu, S. & Lee, J. Calcium-chelating alizarin and other anthraquinones inhibit biofilm formation and the hemolytic activity of Staphylococcus aureus. *Sci. Rep.* **6,** 1–11 (2016).

[85] Raad, I. *et al.* Chelator-based catheter lock solutions in eradicating organisms in biofilm. *Antimicrob. Agents Chemother.* **57,** 586–588 (2013).

[86] Lebeaux, D., Leflon-Guibout, V., Ghigo, J. M. & Beloin, C. In vitro activity of gentamicin, vancomycin or amikacin combined with EDTA or L-arginine as lock therapy against a wide spectrum of biofilm-forming clinical strains isolated from catheter-related infections. *J. Antimicrob. Chemother.* **70,** 1704–1712 (2014).

[87] Lin, B., Johnson, B. J., Rubin, R. A., Malanoski, A. P. & Ligler, F. S. Iron chelation by cranberry juice and its impact on Escherichia coli growth. *BioFactors* **37,** 121–130 (2011).

[88] Abouelhassan, Y. *et al.* Discovery of quinoline small molecules with potent dispersal activity against methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis biofilms using a scaffold hopping strategy. *Bioorganic Med. Chem. Lett.* **24,** 5076–5080 (2014).

[89] Jahanizadeh, S., Yazdian, F., Marjani, A., Omidi, M. & Rashedi, H. Curcumin-loaded chitosan/carboxymethyl starch/montmorillonite bio-nanocomposite for reduction of dental bacterial biofilm formation. *Int. J. Biol. Macromol.* **105,** 757–763 (2017).