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Review article

Role of naturally occurring phytochemicals in overcoming the pathogenicity of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is one of the most common pathogenic bacteria with ability to act as both planktonic and biofilm, causing various disease conditions like cystic fibrosis, bacteremia, meningitis, urinary tract infections, as well as skin and soft-tissue infections. The incidence of infection also increases due to the genomic size that is 5567 genes encoded in 6.26 Mbp of DNA which also is one of the causes of multiple drug resistance (MDR) in this specie. The MDR in *P. aeruginosa*is due to various factors like the mutations in the genes encoding the porins, efflux pumps, penicillin-binding proteins, and chromosomal β-lactamase. These factors also cause resistance against potent drugs like β-lactams, quinolones and aminoglycosides. To overcome this resistance, extensive researches have been done which led to the development of various molecular level strategies. Apart from these chemicals, various phytochemicals have also shown to exhibit antimicrobial and antibiofilm activity against many pathogenic bacteria. Therefore, this review mainly focuses on the role of phytochemicals in controlling pathogenic microbe, *P. aeruginosa*.

Key words: *Pseudomonas aeruginosa*, MDR, molecular strategies, phytochemicals

Abbreviations

EDTA	:	Ethylenediaminetetraacetic acid
K^+	:	Potassium
OprD	:	Outer membrane porins
TOC	:	Translocon on the outer chloroplast membrane
PA01 strain	:	*P. aeruginosa* strain1
GTP	:	Guanosine tri-phosphate
β-lactam	:	Beta lactam
BTC	:	Bacterial inhibition concentration
MIC	:	Minimum inhibitory concentration
LPS	:	Lipopolysaccharide
mg/ml	:	milligram/millilitre
RND	:	Resistance-nodulation-division
ATP	:	Adenine tri phosphate
EPI	:	Efflux pump inhibitor
EOs	:	Essential oils

1. Introduction

Pseudomonas aeruginosa (PA) is an opportunistic pathogenic bacterium which is responsible for many health issues among the humans worldwide such as cystic fibrosis and nosocomial infections (Bentzmann and Plésiat, 2011). Other predominant infections caused by PA are pneumonia, bacteremia, meningitis, urinary tract infections, as well as skin and soft-tissue infections (Porras-Gómez *et al*., 2012). Although, many antibiotics exist for combating the effect of this microorganism like β-lactams, quinolones and aminoglycosides but in due to course of time, antibiotics over exploitation has led to the development of resistance in PA for many antibiotics which make the infection difficult to treat. The incidence of the infections caused by PA is increasing by alarming rate and subsequent use of drugs; therefore, makes them more resistant towards most of the antimicrobials. The multidrug resistance (MDR) in this gram negative bacteria are said to be due to various factors like the mutations in the genes encoding the porins, efflux pumps, penicillin-binding proteins, and chromosomal β-lactamase which make them resistant to antibiotics such as β-lactams, quinolones and aminoglycosides. Low permeability of the drugs to outer membrane, PA also contributes to MDR. Further, biofilms formed by this specie is also a major issue. The secretion of alginate and other exoproducts like lipopolysaccharides and elastase induce harmful pathogenesis, resulting in tissue destruction (Verma and Rampal, 2007). Due to these phenomena, the control of PA is rather challenging (Table 1). This article tries to present the strategies involved at molecular level to combat the pathogen and would also discuss the natural sources that could help in inhibiting both the planktonic as well as the biofilms formed by PA.

Table 1: Antimicrobial categories for control of *Pseudomonas aeruginosa*

Antipseudomonal penicillin + β-lactamase inhibitors	**Ticarcillin - clavulanic acid Piperacillin-tazobactam**	**Reference**
Antipseudomonal cephalosporins	Ceftazidime, Cefepime	Magiorakos *et al*., 2012
Antipseudomonas carbapenems	Imipenem, Meropenem, Doripenem	

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2. Mechanism involved in multidrug resistance

PA has a natural mechanism for being resistant to the antimicrobials and also has the ability to acquire resistance based on the mutational changes in genetic material (Araque and Velazco, 1998). Bacteria mainly rely on the peptidoglycan layer present in cell wall for their structural integrity (Wilke *et al*., 2005). This layer is formed as a result of a catalytic activity of an enzyme, known as transpeptidase that uses the active site serine and complete the cycle through an acylation/deacylation pathway. This particular activity of the transpeptidase is inhibited by β-lactam antibiotics which subsequently lead to cell death. Further, the activity of the β-lactam is inhibited by β-lactamases by causing the hydrolysis of amide bonds in the four membered β-lactam ring (Estiu *et al*., 2005). The major mechanisms related to development of multidrug resistance in microorganisms have been depicted in Figure 1.

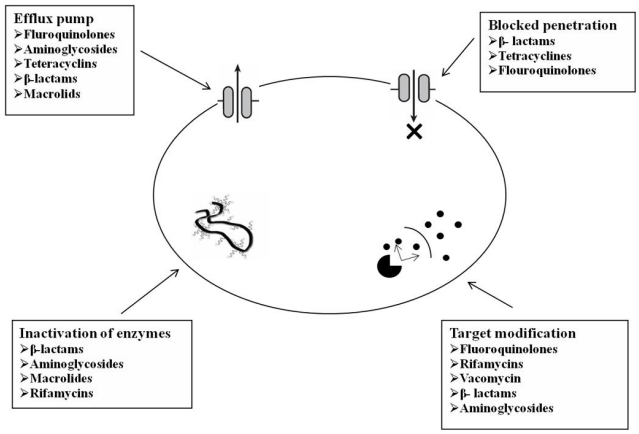

Figure 1: Multiple strategies used by microorganisms to develop resistance to antimicrobial drug.

2.1 Efflux pumps

Efflux pump is another mechanism through which the resistance can be developed by the active transport of the antibiotics out of the bacterial cells (Kadry, 2003). The studies conducted at the genomic levels have shown that around 5-10% of the total genes encodes for the efflux pumps and can be organized in five major families, namely; small multidrug resistance, major facilitator superfamily, multidrug endosomal transporter, multiantimicrobial resistance and resistance nodulation division (Bambeke *et al*., 2000; Chen *et al*., 2004).

2.2 Other resistance mechanisms

Other factors that enable PA to be resistant towards antimicrobials is the development of barriers in the permeability as a result of loss in the expression of specific outer membrane proteins which in turn cause the loss of the penetration of certain molecules through the outer membrane (Kadry, 2003). It has been shown that outer membrane permeabilizers such as EDTA increase susceptibility to antibiotics, indicating that the lack of OprD protein leads to a reduction of active antibiotic molecules capable of reaching the target penicillin-binding-proteins.

The two-component system is also a common mechanism that imparts bacteria to adopt regulation in presence of complex environment, generally involving a sensor histidine kinase and a response regulator (Chen *et al*., 2004). This sensor kinase consists of a signal recognition domain together with autokinase domain. Two hypotheses have been proposed to explain the two-component system; according to one, the genes of this system is a result of duplication and then differentiation of these in bacterial gene while other model explains that this system has evolved as a result of sensor gene and regulatory gene assembly from heterologous two-component system genes. All these theories have been observed and shown in PA PA01 strain.

It has also been shown that cytotoxicity is an important mechanism that contributes to high morbidity and mortality in PA infections, particularly in cystic fibrosais (Guepin-Michel *et al*., 2004). Along with mucoids resultant from the release of alginate, PA synthesizes a secretory apparatus (Type III) that allows it to inject toxins from their cytoplasm into the target cell. The later mechanism allows mucoid bacteria to lyse the host's macrophages and overcome various defense such as in the case of cystic fibrosis lung infection.

3. Biofilm resistance to antimicrobials

The structure and physiological characteristics of the formed biofilms are mainly responsible for antimicrobial resistance. This resistance may involve certain possible mechanisms like: (1) late diffusion of the antimicrobials through the biofilm matrix like in case of PA and *S. epidermidis* where they are susceptible to ciprofloxacin and tobramycin; (2) alteration in the growth of the biofilm forming organism; (3) physiological changes which are the result of manner in which the biofilm grows. These problems associated with biofilm formation have led to the strategies for either reducing the quorum sensing mechanism or biofilm formation.

3.1 Late diffusion of the antimicrobials

The antimicrobial molecules need to penetrate deep into the biofilm structure in order to repress the microbial growth but the presence of extracellular polymeric substances creates a barrier which does not allow the penetration either by changing the rate of transport of molecules or due to certain reactions between the molecule and matrix material. A study observed the delayed penetration of ciprofloxacin into PA (Suci *et al*., 1994). Further, it has been demonstrated that the dispersed bacterial cells are much more affected by the tobramycin than the biofilm (Hoyle *et al*., 1992).

3.2 Alteration in the growth of the biofilm forming organism

It has been proposed that the cells that are associated to biofilm grow slowly as compared to the planktonic species; therefore, this may be one of the reasons why biofilms are resistant to antimicrobials. It was observed that the slowest growing *E. coli* are more resistant to an antiseptic compound cetrimide (Evans *et al*., 1990). Another study showed that the growth rate of the *Streptococcus epidermidis* biofilm strongly influences the viability, *i.e*., more the specific growth rate, more will be the inactivation by ciprofloxacin (Duguid *et al*., 1992). The older (around 10 d old) culture of PA biofilm showed more resistant to tobramycin and piperacillin as compared to the younger biofilm (Anwar *et al*., 1992).

4. Strategies to overcome MDR

4.1 Increasing the antibiotic influx

The bacterial membrane plays a major role in the development of resistance in gram-negative bacteria and, therefore, certain strategies

should be developed in order to reduce the resistance. These strategies include increase in the influx of the antibiotics or reducing the expression of efflux system proteins. The impermeability of the membranes can be reduced by using certain chemicals like detergents, surfactants, polymyxin. Colistin, a polymyxin has been shown to give good results when combined with meropenem (Humphries *et al*., 2010). The bactericidal activity of polymyxin B in combination with doripene and rifampin against *Klebsiella pneumoniae, Acenatobacter. baumanmii* and PA was also observed (Hirsch and Tam, 2010). The destabilization of the LPS barrier is another strategy to overcome the resistance wherein the chaotropic agents and the detergents helps in the transportation of the hydrophobic molecules across the membrane.

4.2 Blocking the efflux

The involvement of the RND transporters has attracted many researchers to develop efflux pump inhibitors along with the antibiotics where these inhibitors just help in increasing the internal concentration of the antibiotics (Drawz and Bonomo, 2010). The molecular basis was described through a pharmacophore model with the involvement of MexAB-OprM in PA in the group of pyrido-pyrimidine derivatives as the inhibitors of OprM (Seeger *et al*., 2008). Peptidomimatic is the first synthetic EPI recognized that work against PA by overexpressing the MexAB-OprM efflux pump (Renau *et al*., 1999).

5. Plant-derived antimicrobial agents

The antibiotics played a major role in reducing the threat of infectious diseases. These antibiotics were discovered by screening for their ability to inhibit or kill the pathogens logarithmically. The antibiotics may affect the microbe by targeting the biosynthesis of proteins, peptidoglycan, folic acid, DNA and RNA but with time and excessive use, the microbes have become resistant to these drugs. In the recent years, the researchers have been seeking for new alternative agents to kill the pathogenic microorganisms (Fischbach and Walsh, 2009; Wise, 2011).

5.1 Essential oil

One such alternative which has attracted the pharmacologists and researchers are the plant-derived compounds to control the pathogens. The plant-derived compounds have been used for the treatment of many diseases since centuries and there have been many studies conducted that show the antimicrobial capabilities of these compounds (Table 2). Essential oils are one of the widely researched plant-derived aromatic compounds which show bactericidal, virucidal and fungicidal activity against a number of microorganisms. Studies have shown the antimicrobial efficacy of essential oils towards *L. monocytogenes, S. typhimurium, E. coli* O157:H7, *S. dysentery, B. cereus, S. aureus* and PA (Burt, 2004). Essential oils show a wide range of antimicrobial activity and these activities vary according to their chemical composition (Assob *et al*., 2011).

Table 2: Most active efflux pump blockers, with antibiotics and bacterial species in which their activity has been demonstrated

Compounds		Bacterial species	Antibiotics	Reference
Synthetic products	Peptidomimetics	*P. aeruginosa* *E. coli* *K. pneumoniae*	Quinolones	Pagès *et al*., 2009 Yoshida *et al*., 2007 Renau *et al*., 1999
	Quinolines	*E. aerogenes*	Quinolones, Phenicols, Cyclines	Pagès *et al*., 2009 Chevalier *et al*., 2010 Mahamoud *et al*., 2011
	Arylpiperazines	*E. coli* *Acenetobacter baumanii*	Flouroquinolones	Bohnert and Kern, 2005 Hannula and Hänninen, 2008
	Phenolthiazines	*B. pseudomallei* *S. enterica*	Ethidium bromide Levofloxacin	Martins *et al*., 2008 Viveiros *et al*., 2008
Natural products	Lupulone Humulone	*P. mirabilis* *S. marcescens*	Polymyxin B	Natarajan *et al*., 2008
	Eugenol	*E. coli* *P. aeruginosa*.	β-lactams Erythromycin	Hemaiswarya and Doble, 2009

The essential oils are one such group of compound which has been shown to implicate antimicrobial properties against various pathogens (Bakkali *et al*., 2008; Hennebelle, 2008). These EOs are volatile, natural and aromatic compounds usually extracted in liquid form from any part of the plant are in the stem, roots or leaves but most commonly from the leaves and the flowers (Belay *et al*., 2011). EOs are the derivatives of the complex metabolic pathways which helps the plant to fight against various pathogens, insects and grazing animals, by either repelling them or reducing the desire of hunger by conferring unpleased taste or aroma to the plant (Shaaban *et al*., 2012). These EOs also helps in the dispersion of pollen and may help in keeping the undesired insects away from the plant (Vigan, 2010). Several reports have implicated the antibacterial, antiviral, antioxidant and anti-inflammatory properties of EOs (Dagli *et al*., 2015).

The EOs are the mixture of various components among which terpenoid-like mono-terpenes (C10), sesqui-terpenes (C15) and diterpenes (C20) are the primary components. Apart from these, EOs may consist of acids, alcohols, aldehydes, aliphatic hydrocarbons, *etc*., produced through the acetate-mevalonic acid pathway, shikimic-phenylpropanoid route and other miscellaneous pathways. These EOs are generally soluble in lipid and organic solvent with density lower than water and are generally found in the plants of temperate and warm climate. These EOs consist of secondary metabolites which either inhibit or slows down the

growth of bacteria, yeast and molds by targeting the membrane and cytoplasm and sometime also by destructing the complete morphology of the organism (Martino *et al*., 2009; Trombetta *et al*., 2005; Tiwari *et al*., 2009). The small size facilitates their easy diffusion through the cell membrane and the skin layer which make them useful in enhancing the permeation of active pharmaceutical agents (Nikaido, 1994).

The EOs effect on the microorganisms depend on the chemical composition of the compound as in the case of phytochemicals, thymol and carvacrol. Both these compounds have same antimicrobial profile against pathogenic microbes but with a different mechanism. This may be due the location of functional groups in these compounds, for example, the position of the hydroxyl group in thymol and carvacrol is different. The EOs can affect single or multiple target organisms. For example, trans-cinnamaldehyde can inhibit the growth of *Salmonella typhimurium*, *E. coli*, and PA (Helander *et al*., 1998; Martino *et al*., 2009). The major chemical component of EOs can be the terpenoid and phenylpropenes which help in killing the microbes. Terpenoid like thymol, carvacrol and piperitone are the terpenes, having additional oxygen molecule with their antimicrobial activity primarily lying on the functional group and the hydroxyl group (Ultee *et al*., 2002; Arfa *et al*., 2006). Carvacrol is considered to be more effective than other essential oils and the activity of this compound is not affected by the position of the hydroxyl group. It has been reported that carvacrol has similar antimicrobial activity against *B. cereus*, *S. aureus*, and PA, irrespective of the position of hydroxyl group (Lambert *et al*., 2001). The thymol and carvacrol have proved to increase the permeability of the cytoplasmic membrane for the K^+ and ATP. Further, the thymol has also been proved to induce the release of the lipopolysaccharide (Walsh *et al*., 2003; Xu *et al*., 2008). Carvacrol has been considered one of the major EO that effect the outer membrane of gram-negative bacteria (La Storia *et al*., 2011).

Phenylpropane is the other group of EOs, having six-carbon aromatic phenyl groups and three-carbon propene tail. The examples of phenylpropenes include eugenol, vanillin, safrole and cinnamaldehyde and their activity depends upon the free hydroxyl group and the type and the number of substitutions that are present on the aromatic rings (Pauli and Kubeczka, 2010). Eugenol and isoeugenol have been proved to show strong antimicrobial activity against gram-negative bacteria as compared to gram-positive bacteria (Hyldgaard *et al*., 2012). The cinnamaldehyde has believed to affect the transportation of ATP and ions, in changing the fatty acid profile of the bacteria and also affect the enzymes like histidine carboxylase, ATPase, amylase and protease (Thoroski, 1989). Further, the cinnamaldehyde is said to have different effects at different concentrations. At low concentration, it affects the enzymes involved in cytokine interaction while at higher concentration, it can act as an ATPase inhibitor and finally, at lethal concentration, it disrupts the cytoplasmic membrane and capable of affecting the lipid profile of the microbes (Wendakoon and Sakaguchi,1995).

5.2 Mechanism of action

The antimicrobial properties of the EOs mainly depends on their structural composition and also on the amount of certain specific component present which acts as an active compounds (Rauha *et al*., 2000). These active compounds are generally the natural constituents of the plants or can be synthesized by the activity of certain enzymes and their amount affect the antimicrobial properties (Lis-Balchin *et al*., 1998) like cinnamaldehyde and eugenol at higher concentration show more antimicrobial effect (Davidson, 2001). The mechanism of action of EOs also depends upon their chemical constituents. Generally, the EOs are responsible for the inhibition of the growth of microorganism and for the reduction of toxic metabolites produced by bacteria. The mechanism of action include the disruption of membrane leading to the increase in its permeability (Trumpower and Gennis, 1994), degeneration of cell wall (Gill and Holley, 2006), coagulation of cytoplasm (Gustafson *et al*., 1996), damage to the membrane proteins and increased permeability leading to the leakage of cell components and reduction in the production of ATP (Burt, 2004). The effect on the fatty acid profile of the microorganism has also been studied and it has been hypothesised that the EOs may alter the fatty acid composition of the cell membrane of the microbes due to their hydrophobic nature which subsequently leads to the change in the percentage as well as structure of the unsaturated fatty acids (Heath and Rock, 2004; Heath *et al*., 2001). Other reports also suggest that fatty acids are the prominent target of the EOs in the microorganisms (Campbell and Ronan, 2001; Heath and Rock, 2004). The change in the number of fatty acids is also said to be one of the effects of EOs. The thymol, carvacrol and eugenol may result in the increase in the number of saturated C16 and C18 fatty acids and the decrease in the number of unsaturated C18 fatty acids. EOs can also affect the activity of the cis-trans isomerase which is mainly involved in the conversion of cis fatty acids to trans-isomers. These trans-isomers help the microorganisms to adapt to a particular environment (Heipieper *et al*., 2003). Another perspective of EOs is in their effect on the proteins which interfere with the cell division process. The cinnamaldehyde has been shown to affect FtsZ (filamenting temperature sensitive mutant Z) protein which is mainly involved in the regulation of cell division and also inhibit the GTP-dependent FtsZ polymerization *in vitro* (Domandia *et al*., 2007). The mapping of the site where the cinnamaldehyde binds to the FtsZ was successfully done and it was found that the binding pockets of cinnamaldehyde at C-terminal consists of a T7 loop of the FtsZ protein. Therefore, it leads to their binding and interrupts in the formation of cytokinetic Z-ring.

The thymol affects the proteins by upregulating TOC protein, present in the outer membrane and accumulating the misfolded proteins (Baucheron *et al*., 2005) and can also affect the expression of proteins like enolase which are involved in the energy metabolism (Pasqua *et al*., 2013). Thymol can lead to the down-regulation of certain proteins like DNA-binding protein H-NS and the ribosomal proteins L7/L12 that led to the stability of DNA which in turn inhibit the process of transcription (Miesel *et al*., 2003). EOs effect on ATP production and the activity of ATPase enzyme has also been studied. One such study showed that the on treatment with mustard EOs the *E. coli* 0157:H7 showed reduced levels of ATP (Cailet *et al*., 2009). Similarly, cinnamaldehyde also resulted in the inhibition of ATP and disruption of the cell membrane in *E. coli* and *Listeria monocytogens* (Gill and Holley, 2004). Further, the *E. coli* BL21 when treated with cinnamaldehyde lead to the increased production of metabolites like indol, alkanes, alcohols, acids and esters which can cause increased cell stress, depending on the level of cinnamaldehyde treatment (Hossain *et al*., 2015). The exopolysaccharide present on the outer membrane gets detached

when treated with cefodizime or may lead to disruption of cytoplasmic membrane and peptidoglycan layer (Shavik *et al*., 1995).

5.3 Phytochemicals for biofilms

These phytochemicals have also been proved to work against biofilms. Chamomile (*Chamaemelum nobile*), a small aromatic plant has been shown to have anti-inflammatory, antimicrobial and antiseptic properties against the biofilm formation by PA at BIC of 6.25-25 mg/ml and MIC of 12.5-50 mg/ml (Kazemian *et al*., 2013). Synthetic cationic peptides like human peptide LL-37 and 1037 inhibit the biofilm formation in PA and *Burkholderia cenocepacia* and in gram-positive *L. monocytogenes* (Fuente-Nunez *et al*., 2012). The use of camelliagenin from *Camellia olivera*, an evergreen plant from China with antioxidant and analgesic properties due to presence saponin was found to inhibit biofilm formation of *E. coli* (amoxicillin resistant) and *S. aurus* (erythromycin resistant) which cause the infections to poultry (Yong *et al*., 2015). *Lactobacillus* isolates like *L. plantarum* PA21 have the ability to form strong biofilm with pathogenic and food spoilage bacteria's which subsequently inhibit the growth of *P. fluorescent, A. hydrophila* and *B. cereus* in the biofilm (Jalilsood *et al*., 2015). The ethanol extracts and the fraction of extracts (AqF, HaF and WsF) from *Terminalia fagifolia* stem bark belonging to Combretaceae family, was used as folk medicine, have the ability to inhibit biofilm formation in *Staphylococcus* sp. (Araunjo *et al*., 2015). *Trigonella foenum-graecum* L. (Fenugreek), an important medicinal plant belonging to Leguminosa, was found to inhibit quorum sensing and biofilm formation. The methanol fraction of the seed extract inhibits AHL-regulated virulence factors, pyocyanin production, chitinase, EPS, and swarming motility in PA PAO1 and PAF79 (Husain *et al*., 2015). The combination of Curcumin from *Curcuma longa* and epigallocatechin, a constituent of green tea has been shown to interfere with AHL quorum, sensing signals mediated biofilm formation by gram-negative bacteria in membrane bioreactors (Lade *et al*., 2017). Bacterial contamination issues are also related to the various paper products like paper towels, filter paper in water and air purifying system. Therefore, the use of selenium microparticles coated paper towel surfaces by quick precipitation method helps in preventing biofilm formation by *S. aurus, E. coli, S. epidermidis* and PA on paper towels (Wang *et al*., 2015). Further, the individual components of EOs like carvacrol, citral, or (+)-limonene have the ability to inhibit biofilm formation (Dastjerdi *et al*., 2014). The EOs from *M. spicata* has shown to inhibit the biofilm formation of *Vibrio sp*. (Espina *et al*., 2015). It has been demonstrated the antibiofilm effects of cinnamon aldehyde, an essential oil from a cinnamon tree, acts by affecting on transcription of quorum sensing promoters at low concentration (Snoussi *et al*., 2015; Nin *et al*., 2006). Zinc oxide nanoparticles are very well known to affect many bacterial species and have the ability to reduce biofilm formation (Kalia *et al*., 2015). Microencapsulation of plant extract has also been reported to reduce the biofilm formation at low concentration. The synthesis of micro-particles of aqueous leaf extract of *Eucalyptus globules* by using microwave irradiation at 2450 MHz has been reported and their effect on biofilm of *S. aurus* and PA was investigated (Hsueh *et al*., 2015).

The antibiofilm activity of the EOs from three sources, namely; cinnamon, Tea tree and Palmarosa were evaluated against PA. The synergistic effect of these EOs with chemical antibiotics was also evaluated and very encouraging results were obtained (Coelho and Pereira, 2013). Similar results were obtained with combinations of gentamicin and caffeic acid, sulfadiazine and protocatechuic acid, quercetin and caffeic acid and reduction in the MIC value were also observed (Sakharkar *et al*., 2009). Apart from this, certain enzymes produced by the bacteria also help in controlling of the basic mechanism of biofilm formation. These enzymes can be divided into three main groups; (1) Acyle homoserine lactone (AHL) acylase, (2) AHL lactonase and (3) oxidoreductase (Lade *et al*., 2014). AHL acylase irreversibly hydrolyses amide linkages between the acyl chain and the homoserine moiety which subsequently form homoserine lactone and corresponding fatty acid. This enzyme was first reported in *Variovorax paradoxus* VAI-C strain (Leadbetter and Greenberg, 2000), thereafter, many other strains like *AiiC* in *Anabaena sp*., PCC7120, QuiP in PA PAO1, AiiD in *Ralstonia sp.* XJ12B (Lin *et al*.,2003) with ability to synthesize this enzyme were discovered. AHL-lactonase acts by cleaving homoserine lactone ring in hydrolytic and reversible manner and was first reported in *aiiA* gene in *Bacillus sp*. 240B1. *Ochrobactrum sp*. T63, *A. tumefacines* c58, PA PAO1, and *Bacillus sp*. 240B1 encode AHL-acylase enzyme which degrade the AHLs and subsequently inhibit biofilm formation (Dong *et al*., 2000). Oxidoreductase target acyl side chain in oxidative and reductive manner and catalysis structural modification without degradation of AHL signal as in case of *B. megaterium* (Chan *et al*., 2011).

6. Conclusion

A novel and promising approach to deal with multidrug resistance is to improve the clinical performance of various antibiotics by employing active molecules, capable of restoring antibiotic susceptibility in MDR pathogens. The design of such a combination is a promising alternative that takes into account the scarcity of new and effective therapeutic antibacterials, especially against gram-negative bacteria. Phytochemicals provide an alternative which work as antibiotic adjuvant by inhibiting the efflux pump of drug. The capacity of biofilm-embedded cells to resist to antibacterial compounds increased the interest in the search of new agents that are effective against bacteria in this mode of growth. In this context, many species of plants provide an enormous diversity of phytochemicals with a range of biological effects, namely; antimicrobial properties against clinically relevant microorganisms. Moreover, it is a known fact that phytochemicals act through different mechanisms from those of synthetic drugs, which make these compounds ideal candidates to reduce bacterial infections. Bacterial adhesion, motility and QS are the major targets to restrict the biofilm formation by the use of novel drugs. Overall, it is possible to conclude from the research findings that dietary phytochemicals, such as essential oils, phenolics and isothiocyanates, have potential to become antimicrobial agents for the treatment of biofilm infection. These compounds as dietary supplements could be used as prophylactic treatment and may leads to decreased risk of developing infections. The phytochemicals, being the natural as well as safe becomes one of the novel ways in overcoming the pathogenicity of the PA. Although, these phytochemicals have advantages still they face some issues due to their volatile nature and also low bioavailability. Therefore, advancement in the field of nanotechnology opened may doors for overcoming these issues. Many studies have been done and are still going on nanoencapuslation of these natural compounds that would overcome the limitations related to use of phytochemicals as therapeutics.

Conflict of interest

We declare that we have no conflict of interest.

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