Received: 14 March 2018 | Revised: 24 April 2018 | Accepted: 28 April 2018
DOI: 10.1002/jobm.201800131

REVIEW

Journal of Basic Microbiology

Proanthocyanidins—Will they effectively restrain conspicuous bacterial strains devolving on urinary tract infection?

Venkataseshan Jagannathan | **Pragasam Viswanathan**

Renal Research Lab, School of Bio Sciences and Technology, Vellore Institute of Technology, Centre for Bio Medical Research, Vellore, Tamil Nadu, India

Correspondence
Pragasam Viswanathan, Renal Research Lab, School of Biosciences and Technology, Vellore Institute of Technology, Centre for Biomedical Research, Vellore 632 014, Tamil Nadu, India.
Email: pragasam.v@vit.ac.in

Struvite or infection stones are one of the major clinical burdens among urinary tract infection, which occur due to the interaction between microbes and urine mineral components. Numerous urinary tract infection (UTI) causing microbes regulate through biofilm formation for survival from host defense, it is often found difficult in its eradication with simple anti-microbial agents and also the chance of recurrence and resistance development is significantly high. Cranberry consumption and maintenance of urinary tract health have been supported by clinical, epidemiological, and mechanistic studies. It predominantly contains proanthocyanidins that belong to the class of polyphenols with repeating catechin and epicatechin monomeric units. Numerous studies have correlated proanthocyanidin consumption and prevention of bacterial adhesion to uroepithelial cells. Quorum sensing (QS) is the prime mechanism that drives bacteria to coordinate biofilm development and virulence expression. Reports have shown that proanthocyanidins are effective in disrupting cell–cell communication by quenching signal molecules. Overall, this review assesses the merits of proanthocyanidins and its effective oppression on adherence, motility, QS, and biofilm formation of major UTI strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* by comparing and evaluating results from many significant findings.

KEYWORDS
biofilm, proanthocyanidins, quorum sensing, struvite stones, urinary tract infection

1 | INTRODUCTION

Biofilm is a sessile community which is characterized by the same or different group of bacteria clogging to a substratum each other irreversibly. They are all embedded in a hydrated exopolymeric matrix (EPM) which is mostly negative in charge [1]. Biofilms result in a chronic persistent infection that is difficult to eradicate with simple antimicrobial agents [2]. Initially, many microbiologists concerned biofilm only in connection with bioprocess engineering and microbial ecology. It was the work on *Pseudomonas aeruginosa* that led to an alternate perspective, in which correlation between biofilm and cell–cell signaling was demonstrated [3]. Microbial community development on a surface is a stepwise process involving adhesion, growth, motility, and EPM formation through

Abbreviations: acyl-HSL, acyl-homoserine lactone; BHL, N-butanoyl-L-homoserine lactone; CA, contact angle; EPM, exo-polymeric matrix; HHL, N-hexanoyl-L-homoserine lactone; HSL, homoserine lactone; IUD, intrauterine device; MR/P, mannose resistant Proteus-like fimbriae; Od-DHL, N-3-(oxododecanoyl)-L-homoserine lactone; OOHL, N-3-(oxooctanoyl)-L-homoserine lactone; PAC, proanthocyanidins; PMF, Proteus mirabilis fimbriae; QS, Quorum sensing; UCA, uroepithelial cell adhesion.

quorum sensing (QS) mediated signal [4]. Adhesion of bacteria to uroepithelium is the initial stage in mammalian urinary tract infection (UTI) [5]. It is more common in women because of the shorter urethral length which facilitates easier invasion of microbe into the bladder. 40–50 % of adult women are reported to have UTI related problem at some point of their life with recurrence up to 27–48 % [6]. Biofilm is not only relevant to prime bodily part, but also to the foreign body such as indwelling urinary catheters. Both UTI and catheter associated urinary tract infection (CAUTI) are serious obsession in the field of medical microbiology, due to its prominent recurrence. *Escherichia coli*, *P. aeruginosa*, and *Proteus* spp., are the most common microbes associated with UTI and CAUTI [7]. They have gained significant clinical importance as they are associated with various nosocomial infections such as ventilator associated pneumonia, cystic fibrosis, etc.

They have a high tendency of biofilm formation through QS which eventually stimulates its virulence factors and cause serious illness. More than 60% of microbes that are currently being treated by the physicians are capable of forming biofilms (Table 1). Cranberries (*Vaccinium macrocarpon*) have long been used as a prophylaxis for urinary tract related infections. The principal active compound present in the fruit is proanthocyandins (PAC) a form of condensed tannin which comprises around 65% of the total active metabolites. Clinical studies have found that PAC is effective against bacterial adhesion and biofilm formation [8–10]. With such relevant findings, this review matriculates the effective oppression of PAC on UTI strains and discusses the possibilities of preventing struvite stone recurrence. In addition we identify a question that, if experimentally addressed, this provides a guide to explore and synthesize similar compounds to control biofilm-related infection.

2 | BIOFILM FORMATION

The biofilm formation has been classified into five stages as follows (Fig. 1) [11]:

1. Stage I—Attachment Phase (reversible, log phase, conditioning).
2. Stage II—Irreversible Binding Phase (QS, EPM).
3. Stage III—Maturation Phase I (width >10 µm, micro-colonies, QS).
4. Stage IV—Phase II (width >100 mm, pedestal-like structures.
5. water channels, QS).
6. Stage V—Dispersion Phase (several days after stage IV, QS).

The deposition of host urine components such as organic salts, electrolytes, and proteins either in bladder mucosa or catheter is the first step in biofilm formation related to UTI [1,2]. In general bacteria possess several surface adhesins such as fibrinogen and fibronectin-binding proteins that establish contact with the host. Overall, this forms a conditioning film to which the free swimming bacteria can easily adhere through hydrophobic and electrostatic interaction that offers stability to the encased microbe [2]. This is followed by cell division and secretion of EPM that favors other planktonic bacteria to adhere over one another. Cell–cell communication (QS) recruits other bacteria and directs the loosely bound matrix to become rigid up to few millimeter thicknesses and the biofilm architecture becomes highly complex. This affects the distribution of chemical gradients potentially against anti-microbial agents and host immune cells from the clearance of the microbe [3]. *S*everal factors are shown to have effect on biofilm architecture such as motility, rhamnolipid production, EPM, etc. This is followed by the formation of fluid channels between the biofilm matrix for

TABLE 1 Microbial infections in human regulated by biofilm formation

S. No	Common diseases/infection	Associated microbe
1	Struvite Stones	*Proteus* spp., *Pseudomonas* spp.
2	Biliary tract infection	Enteric bacteria
3	Prostatitis	*E. coli* and other gram -ve bacteria
4	Necrotizing fasciitis	Group A Streptococci
5	Endocartitis	Streptococci spp., Staphlyococci spp.
	Infections associated with foreign materials	
6	Urinary catheters	*E. coli*, *Pseudomonas* spp.
7	Intrauterine device (IUD)	*Actinomyces israelii*
8	Endovascular catheter infection	Staphylococci
9	Contact lens	*P. aeruginosa*
10	Sutures	Staphylococci spp.

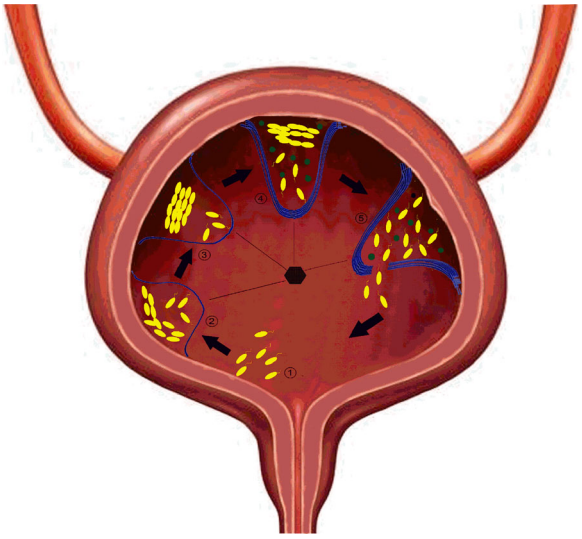

FIGURE 1 Stages in biofilm formation on urinary bladder. 1, Attachment phase (reversible, log phase); 2, irreversible binding phase (quorum sensing, exopolymeric matrix); 3, maturation phase I (>10 µm, micro-colonies, quorum sensing); 4, maturation phase II (>100 mm, pedestal-like structures, water channels, quorum sensing); 5, dispersion phase (several days after stage IV, quorum sensing); ● N-acyl homoserine lactone (signal molecule)

nutrient and waste exchange. Finally, when the resource becomes limited, the fully matured microbial cell gets detached from the biofilm matrix under the regulation of QS signal molecule and colonizes a new surface to repeat the cycle thus, provoking further infection [4].

3 | QUORUM SENSING THE DRIVING FORCE OF BIOFILM

QS is a response or stimuli corresponding to the bacterial community. It is a phenomenon in which the accumulation of signaling molecule from one bacterium enable it to sense the minimal population required to achieve combined action [12]. With increase in population the production of signal molecule reaches a threshold and results in its recognition by cognate receptor that eventually activate and express the virulence factor related genes [13]. Although a number of signaling molecules exists, acyl-homoserine lactone (acyl-HSL) mediated QS mechanism is perhaps the best understood one that has been extensively studied on *P. aeruginosa*.

P. aeruginosa has two important QS systems namely: (i) Las(R-I) system and (ii) Rhl(R-I) system. Both the systems have specific autoinducer (LasI, RhlI) and receptor (LasR, RhlR) for regulation. One more incomplete system, QscR which has no cognate I-protein was also discovered [14]. In HSL mediated QS, HSL synthase (I-protein) encoded by lasI gene synthesizes a signal molecule N-(3-oxododecanoyl)-L-homoserine lactone ($3OC_{12}$-HSL) using S-adenosylmethionine and acyl chains derived from common fatty acid biosynthesis pathway. This short chain HSL can readily diffuse through the bacterial membrane and accumulates in the environment. The HSL receptor (R-protein) of LasR family acts as the receptor and regulates the transcription of associated genes. The mechanism is similar to Rhl system with the only difference in the signal molecule. Rhl-I protein directs the synthesis of N-butyryl-L-homoserine lactone (C_4-HSL) which is recognized by Rhl-R receptor and triggers the transcription of associated virulence genes such as *lasB* (elastase), *lasA* (staphylolysin) [3]. The QS circuit in *P. aerugionosa* is well regulated and arranged in hierarchal cascade. LasR is a dimer and binds to a conserved palindromic sequence of QS promoters including Rhl(R-I) for its activation, and LasI product ($3OC_{12}$) activates QscR that further controls many genes induced by LasR and RhlR [15] (Fig. 2). Although this mechanism is similar in most of the gram negative bacteria, the differences lies only in synthase and receptor gene and the type of HSL molecule that the bacteria employs for biofilm and virulence expression [16]. Some other form of HSL molecule and its phenotypic regulations are given (Table 2).

4 | BIOFILM AND ITS PATHOGENESIS ON URINARY TRACT

Pathogenesis refers to the invasion and multiplication of bacteria or other microorganisms which are not present in the body and can lead to a disease state. Infectious diseases are major health issue being faced by humankind due to their high rate of incidence, diversified causative agents, complex mechanism of infection, and lack of proper diagnostic techniques. UTIs are mostly caused by bacteria which results in serious clinical burden [17]. One such is struvite/infection stones that form due to interaction between pathogenic bacteria and mineral components present in the urine. Infection stones makeup approximately 15% among other urinary stone disease (oxalate stones, uric acid stones, and cysteine stones), thus been an important category of nephrolithiasis [18].

4.1 | Struvite/infection stones

Struvite is a crystalline mineral compound contains magnesium, ammonium, and phosphate [19]. Chemically they are called as magnesium ammonium phosphate stones ($MgNH_4PO_4 . 6H_2O$). Modern crystallographic analyses have demonstrated that human "struvite" stones are a mixture

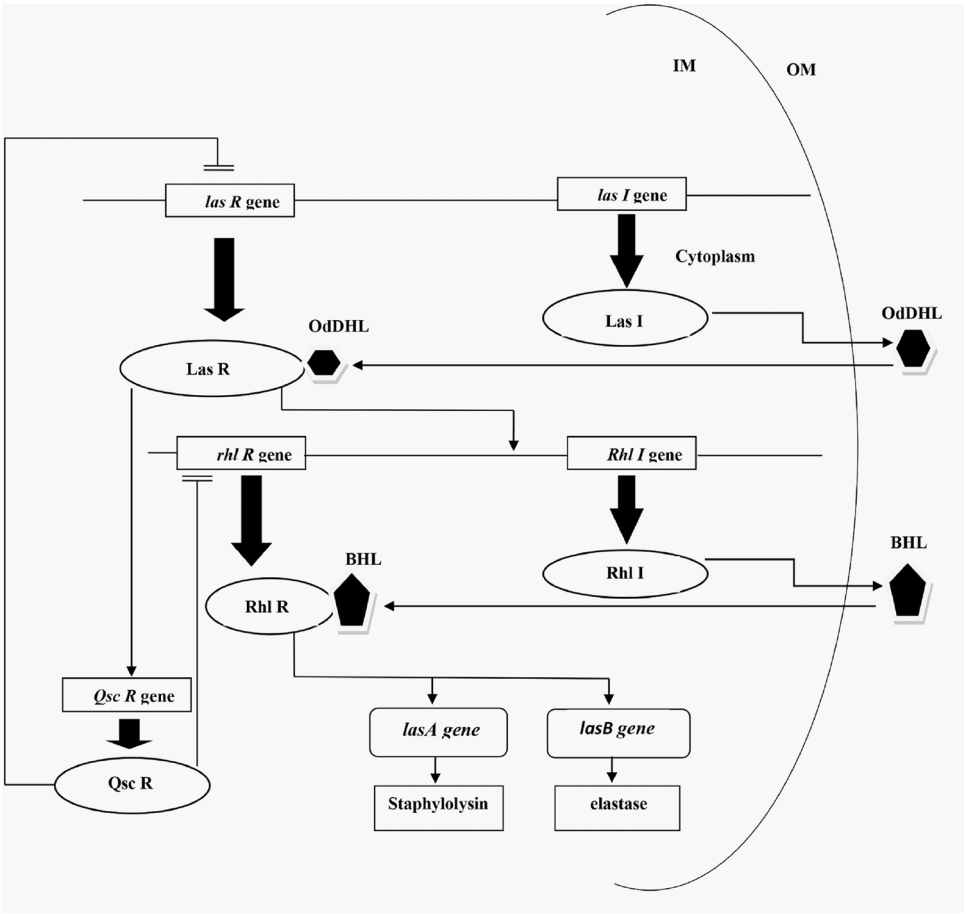

FIGURE 2 Schematic representation of quorum sensing mechanism of P. aeruginosa. lasI/R, rhlI/R gene translation is shown as ⬇. Regulation of LasI/R, RhlI/R protein is shown as ➔ and control (activation/suppression) by QscR is shown as ⊤. IM, inner membrane; OM, outer membrane; Od-DHL, N-3-(oxododecanoyl)-L-homoserine lactone; BHL, N-butanoyl-L-homoserine lactone

of struvite ($MgNH_4PO_4$ $6H_2O$) and carbonate apatite (Ca_{10} $[PO_4]_{45}$ CO_3) [20]. Bacteria such as *Klebsiella* spp., *Proteus* spp., *E. coli*, and *Pseudomonas* spp., are responsible for struvite stone formation. The ureolytic activities of these pathogenic bacteria break down urea present in urine into ammonia and CO_2. The ammonia thus produced enhances the urine pH (7.4–8.2) and promotes precipitation of mineral salts and destroys the glycosaminoglycan layer of uroepithelium. Bacteria invade the uroepithelium inciting inflammatory responses which result in the secretion of muco-substances. The bacteria form micro-colonies as they keep on adhering to the uroepithelium and then secrete extracellular polysaccharides. Meanwhile, the ammonium ions produced due to the cleavage of urea start participating in the formation of struvite crystals, associating with the already existed magnesium and phosphate ions in the urine. The phosphate, carbonate, and calcium ions associate to produce calcium carbonate appatite. Eventually, magnesium ammonium phosphate (struvite) and calcium carbonate appatite crystals combine to form the struvite_appetite dust and this crystallization takes place in the polysaccharide matrix formed by the bacterial biofilm [21]. Now, the planktonic bacteria present in the

TABLE 2 Different form of bacterial acyl-HSL signal molecules and its phenotype regulation

Organism	Phenotype regulation	Signal molecule
P. aeruginosa	Virulence factor associated with rhamnolipid, hemolysin	BHL & OdDHL
Pseudomonas aureofaciens	Phenazine synthesis	HHL
Serratia liquefacians	Swarming motility	BHL
Aeromonas hydrophila	Biofilms, exoprotease	BHL & HHL
Xanthomonas campestris	Extracellular enzymes and polysaccharide virulence determinants	OOHL

BHL, N-butanoyl-L-homoserine lactone; HHL, N-hexanoyl-L-homoserine lactone; Od-DHL, N-3-(oxododecanoyl)-L-homoserine lactone; OOHL, N-3-(oxooctanoyl)-L-homoserine lactone.

surrounding urine attach to these crystals and form another layer of biofilm, secreting extracellular polysaccharides. Thus, these crystals are sandwiched between layers of biofilm matrix and further, cumulative crystallization takes place. This in turn results in the formation of large stones which consist of struvite and appatite crystals and the muco-substances [22]. Therefore, infection stones are generally formed as "staghorn calculi," meaning large stones which resemble the horns of a stag, branching into the infundibula and calices. Urinary tract obstructions, catheters, neurogenic bladder voiding disruptions, and medullary sponge kidney (also called as cacchi-Ricci disease) are the major risk factor of infection stones.

5 | SURVIVAL MECHANISM OF BIOFILM

The microbes present in the biofilm works as a community and communicates closely for survival advantage [2]. The Extracellular polymeric matrix of the biofilm acts as a barrier and hinders the penetration of antibiotics and immune cells. Some resistance mechanism exhibited by biofilm have been proposed [1]. It may be due to one or more of the following: (i) Penetration of antibiotic is sabotaged by the biofilm matrix; (ii) diversified growth of biofilm associated microbes; and (iii) physiological changes due to biofilm growth.

5.1 | Delay in antibiotic penetration

Antibiotic like ciprofloxacin penetrates *P. aeruginosa* within 40 s on a sterile surface. However, studies showed delayed penetration (up to 21 min) when the same organism is encased on biofilm [23]. Dispersed *Staphylococcus epidermidis* cells were more sensitive to tobramycin than cells were in intact biofilm [24]. Interaction between bacterial biofilm matrix and anti-microbial agents has been studied. Alginate isolated from *P. aeruginosa* biofilm inhibited the diffusion of gentamycin and tobramycin on 2% suspension and this effect was reversed when alginate lyase was used [25].

5.2 | Diversified growth of biofilm associated microbes

The older culture (10 days) of *P. aeruginosa* biofilm on chemostat is more resistant toward tobramycin and piperacillin than younger culture (2 days). Combined dose of 500 µg piperacillin with 5 µg tobramycin completely inactivated both planktonic cells and 2 days old biofilm. However, the viability of the cells in older (10 days) biofilm was reduced only up to 20% (approx.) with same [26,27]. This is because the biofilm associated cells grow slower than that of the planktonic cells and as a result takes up antibiotics significantly slower.

5.3 | Physiological changes in biofilm associated growth

Gram negative bacteria such as *E. coli* respond to environmental stress conditions such as nutrient limitation and oxygen deprivation by synthesizing sigma factor. Under the control of *rpo*S regulon the sigma factor regulates the transcription of genes whose product mitigates those stress condition. *E.coli* strain with and without *rpo*S gene was studied [28] and found that density and viable cell count was more in *rpoS*$^{+}$ *E. coli* biofilm. *rpo*S generally gets activated on stress condition such as nutrient limitation, toxic accumulation make the organism to grow slow and favors biofilm formation. Biofilms can display a wide range of phenotypes depending upon the nature of the microbe and the environment. *Listeria monocytogenes* predominantly develop biofilm under static condition of a homogeneous layer of cells or microcolonies that display similar morphology to that of planktonic cells [29]. Whereas *S. aureus* forms biofilm with wide matrix harboring different polymers and its maturation relies on the interplay between various regulators such as *SarA, Agr, Ica*, and *SigB* [30].

Thus, surgery is the only option for the removal of struvite stones but the chance of recurrence is very high. Postsurgical measures such as oral chemolysis and urinary acidifier are used to prevent recurrence but seem not promising as they have no impact on biofilm. Hence, an alternate strategy could be achieved by inhibiting the adherence and QS circuit of the related microbe that promotes biofilm formation.

6 | PROANTHOCYANIDINS

Proanthocyanidins (PAC) a form of condensed tannin belongs to the class of polyphenols found in many fruits especially in cranberries. They are oligomeric flavanoids of catechin, epicatechin, and their gallic acid esters [8] (Fig. 3). The structure of PAC depends on the nature of flavan-3-ol linkages substituted with hydroxyl groups along with aromatic and fused oxytane rings. Acid hydrolysis breaks the linkages and forms colored anthocyanidins and this is the basic assay for the compounds [31]. In PAC the linkage between the successive monomeric units is usually between C-4 position of the upper unit and C-5 position of the lower unit. PAC has been classified into two major types namely A-type PAC present mainly in cranberries and B-type PAC present in grapes, green tea, apple, and dark chocolates [8]. Depending on the monomeric linkages they have further sub divided into several types as shown in Fig. 4.

6.1 | B-type proanthocyanidin

They are oligomers of flavan-3-ol with interflavan bond. The linkage is commonly between C4 and C8 or C6 of upper and

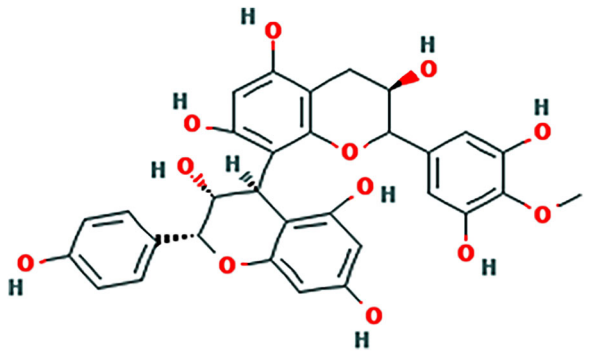

FIGURE 3 Pubchem structure of proanthocyanidins (pubchem CID 108065) (https://pubchem.ncbi.nlm.nih.gov/)

lower extension units, respectively. They have subtypes B1-B4 differs only in the spatial arrangement of (+)-catechin and (−)-epicatechin extension units. Further subtypes procyanidin B5 (epicatechin-(4β→6)-epicatechin), B6 (catechin-(4ά→6-catechin), B7 (epicatechin-(4β→6)-catechin), and B8 (catechin (4ά→6)-epicatechin) are also widely spread in plants [31]. They are dominant class of natural PAC and exhibits promising inhibitory effects against many enzymes. For example, pyocyanidin B2 from *Humulus lupus* inhibits nitric oxide synthase enzyme by scavenging reactive nitrogen species [32] and has been used for the treatment of traumatic brain injury. In rats it has been elucidated that pyocyanidin B5 (3,3′di-*O*-gallate) act as inhibitor of epoxidase enzyme on cholesterol biogenesis and through dietary many significant supplement the cholesterol level has been maintained on healthy range [33]. Likewise, findings have revealed the benefits of PAC.

6.2 | A-type proanthocyanidins

The second ether linkage between the A-ring hydroxyl group of the lower unit and C-2 of the upper unit differentiates them from the B-type. They are not as common as B-type PAC, though many A-type PAC have been isolated and they were considered as unusual structures. Majorly they are dimers of *ent*-epicatechin-(4ά→8, 2ά *O*→*7*)-ent-catechin (A1 pavetannin) and epicatechin-(4β 8, 2ά *O*→*7*)-epicatechin (A2 procyanidin). Besides these, trimers (pavetannin B1 to B8), tetramer (pavetannin C1, C2, C6, cinnamtannin B2), and pentamer (pavetannin D1) were also found on various plants [31]. Regarding nutrition and pathology the A-type PAC plays major role. This has been extensively studied through activity based fractionation. *V. macrocarpon* (cranberry) juice has been used to prevent UTI for many decade [8]. Quercetin a new moiety as terminating unit of propelargonidins (kind of anthocyanidin) was isolated from rock cherries

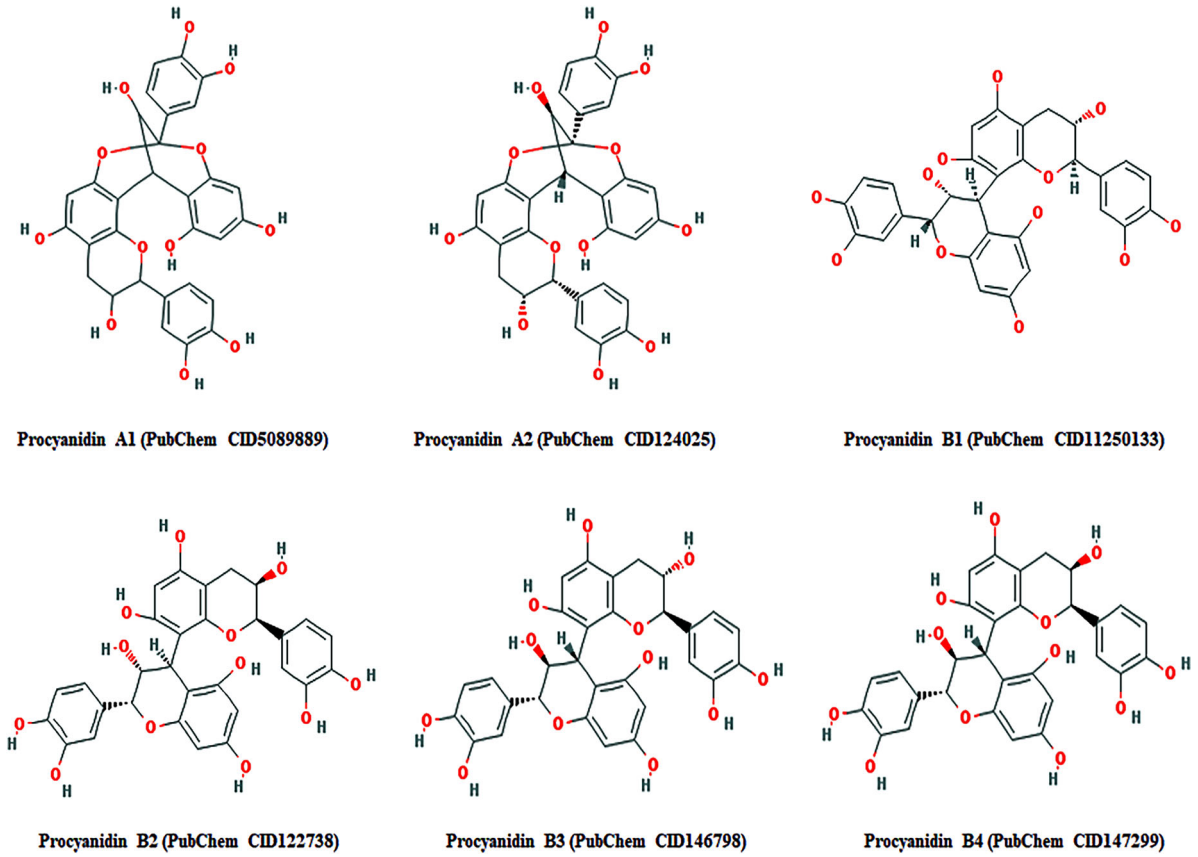

FIGURE 4 A- and B-type proanthocyanidins with flavan-3-ol monomers (https://pubchem.ncbi.nlm.nih.gov/)

(*Prunus prostrate*)which has been used as folk medicine for gastrointestinal disturbances [34]. It has been proved that A-type PAC trimers, epicatechin-(4β→6)-epicatechin-(4β→8, 2β→*O*→7)-epicatechin (4), epicatechin-(4β→8, 2β→*O*→7)-epicatechin-(4β→8)-epicatechin (5), and epicatechin-(4β→8)-epicatechin-(4β→8, 2β→*O*→7)-epicatechin (6) prevents the adherence of P-fimbriated *E.coli* to ά-Gal(1→4)β-Gal receptor surface that is similar to those present on uroepithelial cells of the urinary tract [35]. Besides the benefits on urinary tract health polyphenols and its derivatives exhibits high antioxidant activity. Cocoa products shows free radical scavenging activity, of which PAC are the major anti-oxidant components [31].

7 | EFFECT OF PROANTHOCYANIDINS ON UTI STRAINS

7.1 | Effect of proanthocyanidins against the adhesion of major UTI bacterial strains

Adhesion is the preliminary event of UTI and it begins with periurethral contamination by an uropathogen [5]. This is followed by its invasion into urethra and climb into the urinary bladder with the help of appendages such as flagella or pili. However, depends upon the host-immune interaction the uropathogen either gets eliminated or colonize the bladder successfully. In the latter case the pathogen ascend further to the kidney and releases tissue-damaging toxins and proteases to acquire nutrients from the host cell. Consequently, from kidney the pathogen can readily cross the epithelial barrier and enters into the blood thereby, initiating bacteraemia. Thus a disease condition could be prevented, if the preliminary bacterial adherence to the urinary tract is prohibited.

Several studies have shown that a positive correlation between cranberry PAC and diminution of bacterial adhesion [9,10,36–38]. The exact mechanism is still unknown however; it seems most likely that the cranberry active compound is sensitive to bacterial adhesins. The uropathogenic *E. coli* (UPEC) strain differ from other so-called harmless *E. coli* strains of gut flora, by exhibiting particular virulence factors on their cell surface namely, type-I-fimbriae and P-fimbriae. Type-I-fimbriae are mannose-sensitive (MS), which binds to glycoproteins in mannose. They mediate adherence and urinary infection in initial phase. It is supposed to be that these adhesins can able to differentiate epithelia from other structures by recognizing extra-cellular matrix proteins such as collagen, laminin, and Tamm-Horsfall protein. The second virulence factor, P-fimbriae are mannose resistant, that binds to ά-D-Gal(1→4)β–Gal of galactose on uroepethelium instead of mannose, in order to dodge the host immune response through invasivity. This also ensures the pathogen not to get eliminate with mucous in the urine. Two compounds were identified in cranberries that inhibit *E. coli* adhesins. (i) Fructose which inhibits the mannose-sensitive adhesins and (ii) PAC which inhibits the mannose-resistant adhesin of uropathogenic *E. coli*. Fructose is generally present in all kind of fruits but fruits belonging to *Vaccinium* group (especially craneberries and blueberries) predominantly contain PAC. The anti-adhesive property of cranberries probably helps to prevent UTI in two ways either it directly prevents *E. coli* adhering to uroepithelial cells or it selects for less adherent bacterial strain in the stool [39].

7.2 | Clinical and in vivo studies of the effect of proanthocyanidins on bacterial adhesion and virulence

A pilot study on the effect of sweetened dried cranberries against uropathogenic *E. coli* isolates from five women volunteers with reported UTI, showed that consumption of a single serving of dried cranberry (42.5 g) significantly reduced the adhesion of bacteria to the cell surface adhesins. Four urine samples were collected from each of the volunteer on regular time interval and the uropathogenic *E. coli* were incubated on it for specific time period and tested for anti-adhesive activity using mannose resistant hemagglutination assay. It was observed that, of the urine collected after the consumption of dried cranberry exhibit more than 50% reduction on adherence, whereas no significant reduction of adherence was observed on the urine collected after the consumption of unsweetened raisins [43]. This is further validated by clinical trial study on women volunteers aged between 18 and 65. Based on positive and negative history of recurrent cystisis they were placed into two separated group and were treated with active cranberry product and placebo. The anti-adhesive property of *E. coli* strains were tested using human bladder carcinoma cells and significant reduction of adherence was observed on the urine of the volunteers treated with active cranberry product regardless of their medical history, age, and treatment period in cross-over sequence [9]. Such kind of studies brings an insight that PAC especially from cranberry on suitable amount exhibit anti-adhesive property against uropathogen. If PAC is really effective against bacterial adhesion, then there arises a question of what would be the most effective concentration? Randomized multi-locational double blind versus placebo study [44] on human volunteers showed that PAC has its effect on dose and time dependent manner. The study has been optimized with the consumption of PAC in the form of standard cranberry powder and there was linear decrease in *E. coli* lodgment with increasing PAC dose. From the kinetic data it was evident that maximal anti-adhesion activity was observed after 6th h of PAC ingestion, since it is the critical elimination period of PAC in urine with subsequent reduction within 24 h [5]. However, only above 36 mg PAC, the residual anti-adhesion

effect was observed and was validated by mannose resistance hemagglutination assay. The effect is found to be profound with 72 mg PAC dosage per day which offers nyctohemeral protection against bacterial adhesion and it is evident in urine of all volunteers irrespective of their place. To establish bacterial virulence, an in vivo killing nematode (*Caenorhabditis elegans*) model has been used. *E. coli* grown on the urine of volunteer's regularly consuming optimized PAC dosage exhibit reduced killing of the nematode [44]. This might be due to masking or inhibition of P-fimbriae, the key virulence factor in adhesion.

7.3 | In vitro studies on effective downregulation of adhesin associated genes by proanthocyanidins

A comparative study with known UPEC strain showed that PAC is effective, also against the adhesion of *P. mirabilis* from clinical isolates. Possessing multiple virulence fimbriae on cell surface like *P. mirabilis* fimbriae (PMF), ambient-temperature fimbriae (ATF), mannose resistant proteus-like fimbriae (MR/P), and uroepithelial cell adhesin (UCA), altogether fabricate the organism as an important cause for nosocomial infections. It has been observed that 50 μg ml^{-1} PAC is most effective against adhesion of uropathogen to uroepithelium. Also % reduction seems more consistent in range 67.1–75.4% for UPEC and 61.4–75.4% for *P. mirabilis*. It is interesting to note that both uropathogens share a common virulence factor mannose resistant adhesins which is mainly composed of *Fla A* protein coded by *Fla A* gene [40]. It seems probable that PAC hinder with fimbriael expression by down-regulating protein coding gene. This subsequently reduces the bacterial adherence to the bladder [5].

Two important polyphenolic compounds identified as quercetin and tannic acid present in *A. japonica* extract is found to inhibit adhesion of another important source of nosocomial infectious pathogen *S. aureus*. Transcriptional analysis reveals that the polyphenolic compounds at 20 μg ml^{-1} have the tendency to down-regulate two intracellular adhesion genes *ica A* and *ica D* [41]. This is similar to the best understood adhesion inhibition mechanism [42]. However, it does not have any effect on house-keeping genes. Previously, it was found that quercetin moiety was the terminating unit of PAC isolated from *P. prostrate* (Rock berry) [34]. This signifies that the compounds are on target to virulence factors in due course, that plants have developed themselves with sophisticated defense mechanism to survive in their ecosystem.

Therefore, from such kind of studies, it is for certain that PAC does not exhibit any kind of antimicrobial activity. Instead it dodges only with bacterial cell surface adhesin and restrains its virulence in the urinary tract. Since adhesion is the initiating event of UTI consumption of PAC may help in preventing it.

7.4 | Effect of proanthocyanidins against the motility of major UTI bacterial strains

Earlier it has been discussed that the adhesion of pathogen to the uroepithelium is the first step in UTI [5]. Although bacterial motility is the key factor for adhesion itself and for the subsequent formation of biofilm [45]. Bacteria are able to undergo different type of motility toward surface and get attached to it through various adhesins such as type IV pili and flagella, etc. Once the bacterium gets attached, then combination of specific surface associated motilities drives them for further colonization. The interference of cranberry PAC against bacterial adhesion is reasonably well understood, although its effect on bacterial motility has not been elucidated completely. Study [46] showed that cranberry PAC (condensed tannins) could block the swarming motility of *P. aeruginosa*. Swarming is a kind of rapid, multicellular movement powered by rotating flagellum [47]. It differs from other kinds of motilities such as swimming and twitching motility by regulating through an extra-cellular signal mechanism called QS [48]. In laboratory condition the swarming nature of a bacterium is characterized by tendril formation that confers group translocation on a solid surface such as in agar plate. Study [46] showed that under control condition *P. aeruginosa* form tendrils and migrated outwards from the point of its inoculation in medium lacking PAC, whereas in medium with PAC at 60 μg ml^{-1} or above, bacterium was able to grow and form a colony but tendril formation has not been observed. This implies that the swarming behavior has been interrupted which might be due to interference of PAC in specific with QS signal that impedes cell–cell communication. Another important factor that is required for swarming motility is the production of biosurfactant called rhamnolipid. It greatly reduces the surface tension by providing hydrophilic environment and enables the bacterium to move freely on agar-associated medium [49]. Generally it is evaluated by measuring contact angle (CA) of water droplet present in the medium. CA is an angle of droplet edge generated at agar-air interface. Thus a low CA value implies that the droplet spread across the surface being hydrophilic whereas a high CA value implies that the surface is dry being hydrophobic. In the study [46] bacteria were allowed to grow on swarm medium with cranberry PAC (motility blocked) and without cranberry PAC (control) and all were ensured of exhibiting high CA (>30°) before inoculation. After inoculation and incubation of bacterium on the medium for over 16 h, the CA in control plates has been decreased (<0.5°), whereas the CA remained high (>25°) on medium that has been supplemented with cranberry PAC. This shows that the compound has some deterioration effect on biosurfactant (presumably rhamnolipid) production and correlates with the droplets distancing itself from the surface which prolongs the hydrophobicity.

7.5 | Effect of proanthocyanidins against the quorum sensing circuit of major UTI bacterial strains

QS system in bacteria relies on two components. One is the accumulation of diffusible signal molecule across the membrane on population density dependent manner and second is an activator protein in concert with the signal molecule for relevant gene expression [12]. Most of the bacteria's employ acyl-HSL as their signaling molecule. When this signal molecule reaches a threshold eventually it will bind to the respective receptor and triggers the expression of virulence factors. Thus, if the signal molecule is interrupted either by quorum quenching (signal degradation) or by signal mimicking (with structurally similar molecule to that of acyl-HSL) then the virulence gene would not get activate, that could possibly down regulate the biofilm formation. Study [50] showed that methyl gallate a phenolic compound with terminal epicatechin unit at sub-MIC level (32 μg ml^{-1}) reduces the synthesis and pre-existing activity of acyl-HSL up to 80% in *Chromobacterium violaceum.* Moreover in *P. aeruginosa* there was twofold reduction in the expression of QS genes (*lasI*, *lasR*, *RhlI*, and *Rhl*) and this is probably due to the signal degradation by methyl gallate that eventually attenuates biofilm development. Other phenolic compounds such as pholoroglucinol, pyrogallol, and resorcinol were also tested for their anti-QS property but only methyl gallate is found promising. Interestingly, like methyl gallate PAC also shares a common epicatechin moiety as the terminal unit. Presumably catechin and epicatechin plays pivotal role on quorum quenching and thus PAC may work in same way as methyl gallate against the inter-bacterial communication. Study [51] supports this, in which the bark extract of *Combretum albiflorum* predominantly contains catechin and its epimer epicathechin is found to inhibit the virulence factor pyocyanin of *P. aeruginosa* at 0.125–16 mM concentration and violacein of *C. violaceum* at 0.25–4 mM concentration. This was observed when the extract and signal molecule (HSL) were added to mutant bacterial strain (possessing QS receptor but lacks signal molecule) there was significant negative effect on virulence factor activation, and expression which implies that catechin and epicatechin might acted as quorum quencher that has eventually destructed the signal molecule (Fig. 5). Molecular docking study [52] reveals that

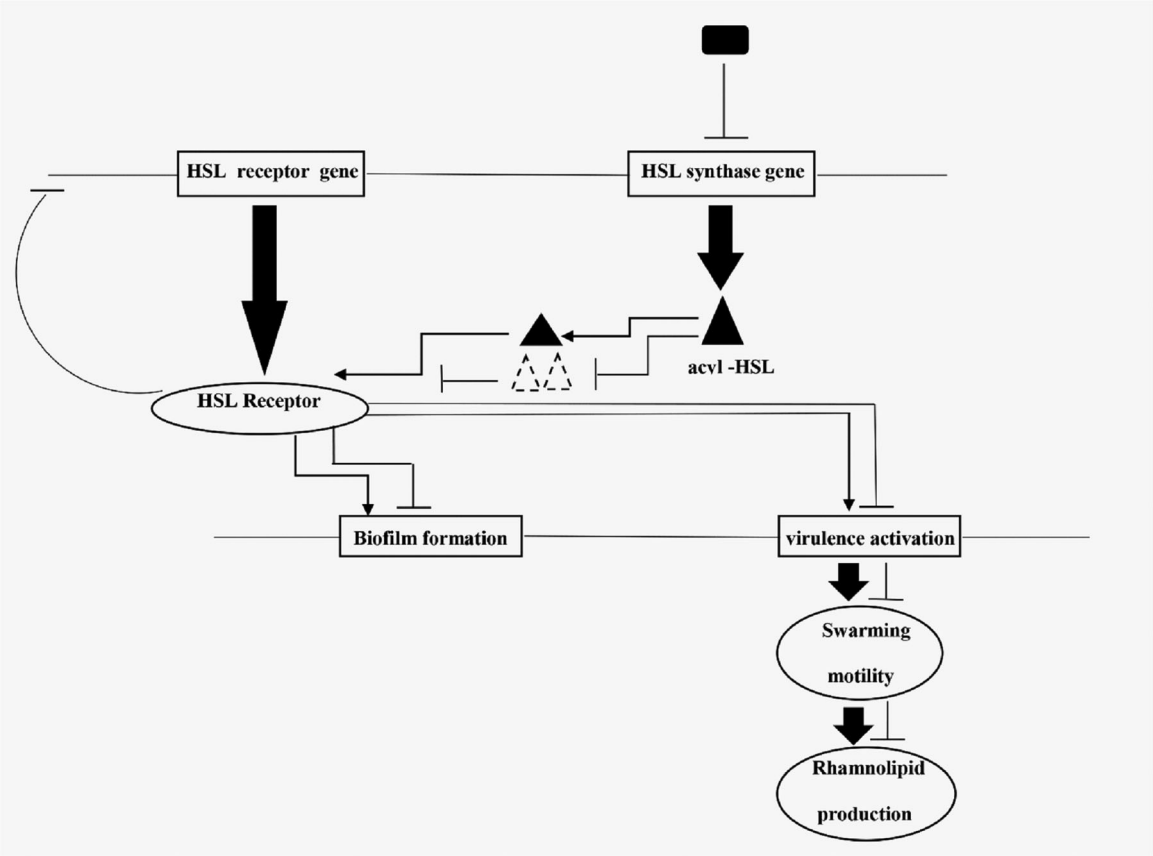

FIGURE 5 Schematic representation of plausible quorum quenching mechanism by proanthocyanidins. Quorum associated signal (acyl-HSL) synthase gene, receptor gene, and associated virulence factor production are focused on this. Positive (⇀) and negative (⊣) regulation of gene expression are indicated by arrows. HSL, homoserine lactone; acyl-HSL, N-acyl homoserine lactone. ■ -Proanthocyanidins; ▲▲ -intact signal molecule (acyl-HSL); △△ -disrupted signal molecule (acyl-HSL); ⇀ -positive regulation; ⊣ -negative regulation (quorum quenching)

PAC has better affinity in binding to LasI (synthase gene) than LasR (receptor gene). Hence the inhibition of signal molecule by PAC is through the inhibition of LasI gene. Thus from such relevant studies PAC are found to be effective in disrupting the QS-signal molecules among bacteria that plays a prime role in bringing virulence gene activation, expression (production of rhamnolipid, pyocyanin, and violacein, etc) and biofilm formation.

7.6 | Effect of proanthocyanidins against the biofilm formation of major UTI bacterial strains

Since the outer layer of biofilm is very complex and rigid, many antibiotics fails in the eradication process due to poor impermeability [24]. Thus the sensible way to shed pathogenic UTI strain's, is to disperse off the microbes being intact and make them prone to antibiotics at lower therapeutic dose (sub-MIC level). Therefore, combinatorial formulation, that is, PAC together with effective antibiotic at their sub-MIC level may work effectively. Study [8] validates this in which PAC on combination with gentamycin has offered high efficiency on the eradication of *P. aeruginosa* biofilm. The MIC of gentamycin is 1.5 $\mu g\ ml^{-1}$ [53]. However, on combination with PAC the MIC of gentamycin was observed to be 1.3 $\mu g\ ml^{-1}$. Proteomics analysis revealed that many of the down regulated proteins contain iron as cofactor (cytochrome and ferrodoxin etc.). Fe^{3+} is highly needed for the complete maturation of *P. aeruginosa* biofilm [54] but on treatment with PAC it result in the formation of flat and thin biofilm. Bacteria in such immature biofilm are more prone to antibiotics and immune cells than bacteria in fully matured biofilm. This might be due to iron-chelation effect of PAC that presumably further potentiates antibiotic activity by acting as an adjuvant. The adjuvant effect of PAC is further supported by [52].

8 | CONCLUSION

Bacterial biofilms are always a serious threat in the industrial ecosystem but their obsession on human health was first stated after discovering their relation with dental plaque [1]. They play a central role in the formation of struvite or infection stone in urinary tract, due to interaction with urine mineral components. Since alkaline nature of urine (pH 7.2–8.4) promotes mineral precipitation and brings microbial interaction, medications such as oral chemolysis and urinary acidifiers are being used but nothing seems promising as they have their own drawbacks. Thus, it is mandatory to look for an alternative that could possibly replace all such complexion. Cranberry juice and extracts have been used from ancient times for maintaining urinary tract health in women. It contains a phenolic oligomer called PAC (with repeating catechin and epicatechin monomeric units) mainly with A-type linkage in abundant. From studies it was found that they are able to provoke large amount of hippuric acid production in urine that makes the bladder acidic and prevents mineral deposition [8]. PAC does not reportedly have any potential anti-microbial activity [44]. Rather, it interrupts with the bacterial QS circuit through quorum quenching and reduces the complexity in biofilm architecture. This may really favor antibiotics to penetrate deep inside biofilm that could eradicate associated microbes. PAC with A-type linkage are only reported to be effective against adhesion and motility against common UTI pathogen. This might be due to the conformational rigidity that plays a role on bioactive urine metabolite production that effectively downregulate the virulent flagellar and fimbrieal protein expression [31]. Thus to conclude consumption of PAC either concentrated or in the form of cranberry juice on combination with antibiotics (sub-MIC level) may really offer protection against the biofilm formation of UTI bacterial strains and prevent struvite stones recurrence. While, this knowledge is available about PAC till now, further work such as pre-clinical and clinical trials on animals and human volunteers are found promising to establish it as safe and easy dietary treatment for struvite stones.

ACKNOWLEDGMENTS

Authors are also thankful to Vellore Institute of Technology for providing the resources to prepare this review.

CONFLICTS OF INTEREST

The authors declare that there was no conflict of interest.

ORCID

Pragasam Viswanathan http://orcid.org/0000-0003-3796-5589

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How to cite this article: Jagannathan V, Viswanathan P. Proanthocyanidins—Will they effectively restrain conspicuous bacterial strains devolving on urinary tract infection?. *J Basic Microbiol*. 2018;1–12. https://doi.org/10.1002/jobm.201800131