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Review.

Proanthocyanidins and Flavan-3-ols in the prevention and treatment of Periodontitis - Antibacterial effects.

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Abstract:

Flavan-3-ols and their oligomeric forms called proanthocyanidins are polyphenolic compounds occurring in several foodstuffs and in many medicinal herbs. Their consumption is associated with numerous health benefits. Their bioactivities include antioxidant, anti-inflammatory, cytoprotective, as well as antimicrobial. The latter property is important in prevention and treatment of periodontal diseases. Periodontitis is a multifactorial polymicrobial infection characterized by a destructive inflammatory process affecting the periodontium. Using non-toxic and efficient natural products such as flavanol derivatives can significantly contribute to alleviating of periodontitis symptoms and prevent the disease progress. In this paper, we systematically review the state-of-the art in antibacterial effects of these compounds from the viewpoint of gum health. There is a significant evidence supporting an importance of antibacterial action exerted by proanthocyanidins from edible fruits, tea and medicinal herbs in inhibition of periodontitis-causing pathogens.

Keywords: condensed tannins; proanthocyanidins; flavan-3-ols; periodontitis; gingivitis; gum disease; cranberry; *Camellia sinensis*; polyphenols.

1. Introduction

Periodontitis is a multifactorial polymicrobial infection characterized by a destructive inflammatory process affecting the periodontium which comprises a set of teeth supporting structures: gingiva, cementum, periodontal ligament, and alveolar bone. Approximately 5 to 15% of the world population is affected by severe forms of the disease which, if left untreated, may result in tooth loss and systemic complications [1], [2],[3]. In the last 30 years, the classification of periodontitis has been modified in an attempt to align it with emerging scientific evidence. On the World Workshop for the Periodontology in 2017 it was agreed that, consistent with current knowledge on pathophysiology, three forms of periodontitis can be identified: necrotizing periodontitis, periodontitis as a manifestation of systemic disease, and the forms of the disease previously recognized as "chronic" or "aggressive", now grouped under a single category, "periodontitis" [4]. The most current concept of the etiopathology of the periodontitis involves the co-existence of the dental plaque and host immune-inflammatory response. Socransky divided the periopathogens involved in periodontitis into six clusters -red, orange, yellow, green, blue and purple. First to colonize of the surface of the teeth are purple and yellow complexes comprised mostly by *Actinomyces* species and *Streptococci* including *S. sanguinis* and *S. oralis*. The next complex, involved in periodontits progression includes *Capnocytophaga spp.*, *Campylobacter concisus*, *Eikenella corrodens*, and *Actinobacillus actinomycetemcomitans*, the bacteria contributing to the primary changes

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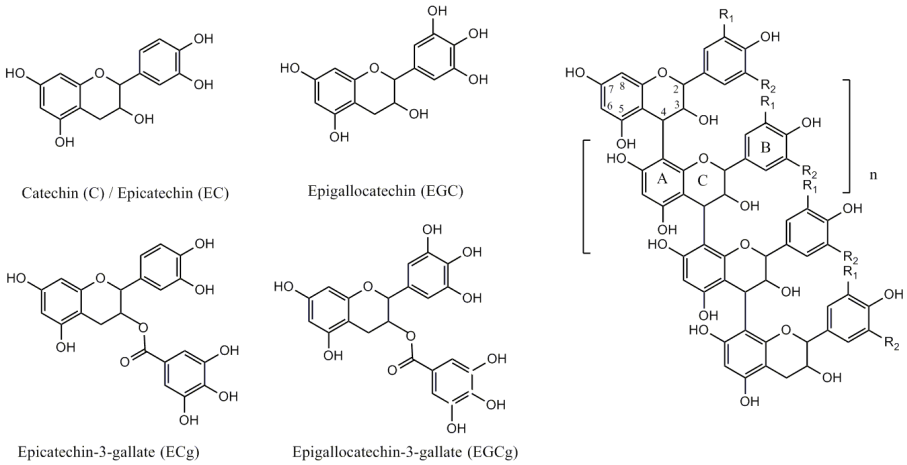
in the host. The "bridging species" formed the orange cluster are as follows: *Prevotella spp*., *Micromonas micros*, *Fusobacterium spp*., *Eubacterium spp*. and *Streptococcus constellatus*. That cluster included the species capable of using and secreting nutrients in the biofilm, in addition to expressing cell surface molecules facilitating binding to early colonizers and the individual of the red complex. Finally, *Porphyromonas gingivalis* and *Treponema denticola* in addition to *Tannerella forsythia* refer to the red cluster are responsible for further progression of the periodontitis [5].

The presence of bacteria is necessary, but insufficient to cause the periodontal disease. The exposure to bacteria must be connected with the individual susceptibility. However, the individual susceptibility to bacteria is dependent on genetic factors. The structure of the periodontium, which itself is a barrier to periodontal pathogens is also genetically predetermined. Moreover, the individual response for the inflammation of periodontal tissue, and its medical course is under the influence of a number of the environmental factors [6]. Periodontal bacteria lead to the mobilization of innate immune response (e.g. IL-1, IL-6, TNF-α) as well adaptive immunity mechanisms (Th1, Th2, Th17, and Tregs). The host response to periopathogens, especially the overproduction by resident and immune cells of inflammatory mediators such as pro-inflammatory cytokines and prostanoids as well matrix metalloproteinases MMPs, which can modulate the progression and severity of periodontitis plays a leading role in the pathogenesis of periodontitis next to bacteria.

High antimicrobial and immunomodulatory activities of proanthocyanidins make them an interesting object for prevention and treatment of periodontal diseases [7], [8]. An additional benefit is their natural dentin cross-linker activity and inhibition of matrix metalloproteinases (MMP) that may be helpful in adhesive dentistry [9]. Bioactivity of proanthocyanidins arises from their unique chemical structure [10]. Proanthocyanidins, also known as condensed tannins, are highly hydroxylated structures capable of creating an insoluble complex with carbohydrates and proteins [9]. They are built from flavan-3-ol blocks, forming oligomeric structures of various numbers of units (from 2 to many). Mostly, the flavan-3-ol units are catechin (C), epicatechin (EC) or their substituted derivatives connected through C4-C8 or C6 bonds (B-type). Due to the number of hydroxyl substitutions on the B ring, proanthocyanidins can be categorized as propelargonidin (one hydroxyl substitution), procyanidin (two hydroxyl substitution) and prodelphinidin (three hydroxyl substitutions) (figure 1).



R1	R2	Proanthocyanidins
H	H	Propelargonidin
OH	H	Procyanidin
OH	OH	Prodelphinidin

Figure 1. Structure of flavan-3-ols and proanthocyanidins

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B-type proanthocyanidins are found in common food sources such as grapes, red wine, chocolate, black chokeberry as well in many plants used in traditional medicine like rhizome of *Reynoutria japonica* Houtt. (synonym *Polygonum cuspidatum*) [11],[12] or *Sanguisorba officinalis* L [13] and many others [14]. A-type polymers isolated from cranberry are less common. They possess at least one intermolecular bond between O7 and C2 atoms in addition to the carbon-carbon bond [15] (figure 2).

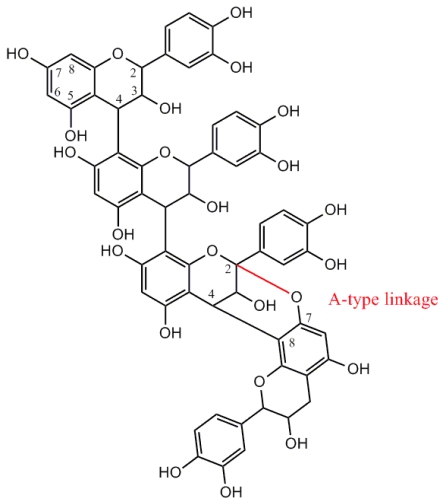

Figure 2. Structure of cranberry proanthocyanidins with A-linkage.

The unique composition of a particular proanthocyanidin structure can influence its biological activity in periodontitis. Some simple (not condensed) structures such as catechin, epicatechin and their derivatives from tea are also helpful in periodontal diseases [16]. The aim of this review was to verify and discuss evidence that proanthocyanidins and flavan-3-ols are beneficial in the prevention and treatment of periodontitis.

2. Materials and Methods

2.1. Search strategy

This systematic review adhered to the Preferred Reporting Items for Systematic Reviews and MetaAnalysis (PRISMA) guidelines [17]. An electronic database search was conducted using PubMed (access date to 6th August 2020). The search terms included all combinations of the following key words: periodontitis OR periodontal diseases OR gingivitis AND proanthocyanidins OR condensed tannins OR flavan-3-ols OR catechin OR epicatechin AND anti-bacterial OR antiadhesive OR anti-inflammatory, respectively. All titles with abstracts were imported into a citation manager program "Mendeley" (Elsevier, UK), and all duplicates were removed. Bibliographies of imported studies were also screened for relevant articles. Two investigators (N-H I and K-R P) independently reviewed the titles and abstracts of the imported studies to determine whether they met the inclusion and exclusion criteria. Disagreements were resolved via consensus and by a third investigator (H J).

2.2. Inclusion criteria

The inclusion criteria were as follow: a) all relevant studies reporting the influence of proanthocyanidins or flavan-3-ols on growth, colony formation and metabolic activity of periopathogens and studies reporting the possible inhibition of periopathogens adhesion to potential oral mucosa cells b) all relevant *in vitro* studies reporting immunomodulatory effects of proanthocyanidins or flavan-3-ols on host cells or periodontal tissue treated with exotoxins from periopathogenes c) all relevant *in vivo* studies reporting influence proanthocyanidins or flavan-3-ols on periodontitis in animal model, d) clinical trials studying influenced of proanthocyanidins or

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flavan-3-ols on periodontitis. Only studies published in English language were taken into consideration.

2.3. Exclusion criteria

The review studies and prospective or only *in-silico* studies were excluded from the present study. Poorly characterized plant extracts or extracts without proanthocyanidins or flavan-3-ols were also excluded from the present study. Moreover, studies with oral pathogens but without specific periodontal pathogens were excluded. Also studies reporting application of proanthocyanidins or flavan-3-ols in combination with others pharmaceuticals, e.g. chlorhexidine or antibiotics were excluded.

2.4. Data organisation

Authors, year of publication, type of study, type of compounds, plant source of compounds, compound concentration, type of bacteria, type of cells and tissues, methods and principle findings of each study were noted in a standard document. The studies were divided into four groups follows inclusion criteria: 1) studies reporting the antibacterial effects on periopathogens and inhibiting bacterial proteolytic enzymes by proanthocyanidins or flavan-3-ols 2) *in vitro* studies reporting immunomodulatory effects of proanthocyanidins or flavan-3-ols on host cells and tissues, 3) *in vivo* studies reporting influence proanthocyanidins or flavan-3-ols on periodontitis in animal model, 4) clinical studies.

3. Results and Discussion

After duplicate removal, 99 articles were further screened by the title and abstracts (Figure 3).

Finally 58 studies met the inclusion criteria. 30 of these *in-vitro* studies reporting the influence of proanthocyanidins or flavan-3-ols on growth, colony formation, metabolic activity of potential periopathogens and inhibition of periopathogens adhesion to oral mucosa cells; 34 *in-vitro* studies reporting the action of proanthocyanidins or flavan-3-ols in immunological response of the periodontal tissues; 7 *in vivo* studies reporting influence proanthocyanidins or flavan-3-ols on periodontitis in animal model and (3) controlled clinical trials reporting application of proanthocyanidins or flavan-3-ols in periodontitis.

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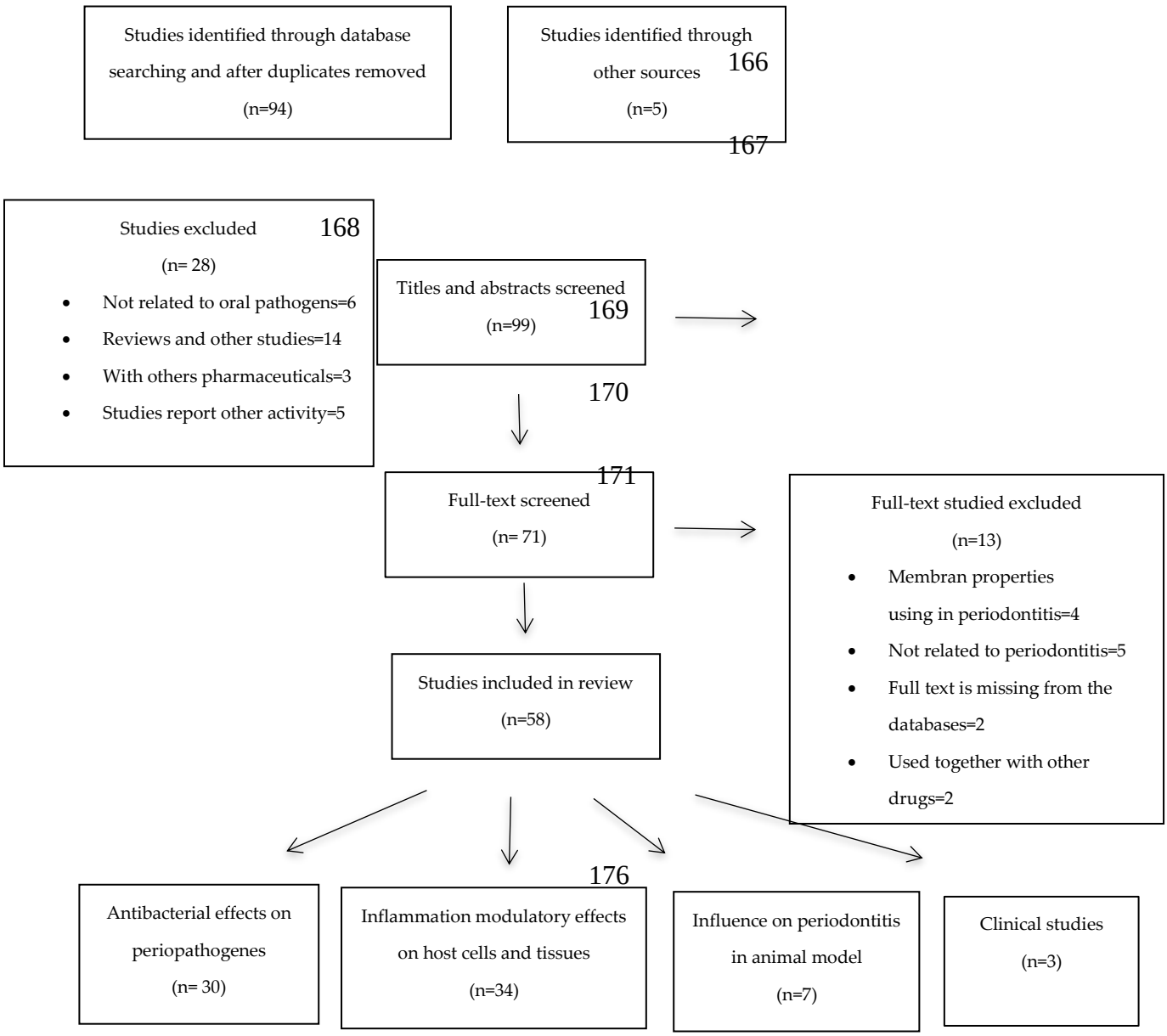

Figure 3. Flowchart of the article search strategy, exclusion criteria, study selection, and data management process

3.1.Antibacterial effects of proanthocyanidins or flavan-3-ols on periopathogens.

An overview of the antibacterial effects of the proathocyanidins or flavan-3-ols is presented in Table 1.

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184 Table 1. Influence of proanthocyanidins or flavan-3-ols on periopathogens and their proteolytic enzymes

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Active compound/extract/fraction	Periopathogen and its proteinase/toxine	Results	Author, Year	Ref.
Catechin	*P. gingivalis*	Catechin did not influence the growth of *P. gingivalis* at the concentration tested (20, 40, or 60 μM)	(Lee et al. 2020)	[18]
Pelargonium sidoides DC. root extract (PSRE) and proanthocyanidin fraction from PSRE (PACN)	*A. actinomycetemcomitans*	1) PSRE and PACN significantly reduced bacterial metabolic activity in comparison to the untreated control 80 μg/mL PSRE- decreased by 57% 80 μg/mL PACN - decreased by 99%; 2) PSRE and PACN at 100 μg/mL were effective in protecting human gingival fibroblast from *A. actinomycetemcomitans* infection. 3) PSRE and PACN protected rat gingival fibroblasts from bacterial LPS-induced necrosis.	(Jekabsone et al. 2019)	[19]
The buds of *Castanopsis lamontii* Hance water extract (CLE) rich in epicatechin and procyanidin B2; epicatechin (EC); procyanidin B2 (PB2).	*P. gingivalis*	MICs of CLE, EC and PB2 against *P. gingivalis* were 0.625, 1.25 and > 1.25 mg/mL, respectively	(Gao et al. 2019)	[20]
Cranberry proanthocyanidins (PACs) isolated from the cranberry fruit (*Vaccinium macrocarpon* Aiton)	*A. actinomycetemcomitans*, leukotoxin	PACs dose-dependently reduced leukotoxin gene expression (ltxB and ltxC but not ltxA and ltxD) in the two strains of *A. actinomycetemcomitans* tested.	(Amel Ben Lagha et al. 2019)	[21]
Highbush blueberry (*Vaccinium corymbosum* L.) proanthocyanidins (PACs)	*A. actinomycetemcomitans*	At a concentration of 500 μg/ml, the PACs reduced the growth of *A. actinomycetemcomitans* by 62.5% The PACs at concentrations ranging from 500 to 3.9 μg/ml significantly and dose-dependently reduced biofilm formation. More specifically, 31.25 μg/ml of the PACs reduced the growth of bacteria by 23.83% and inhibited biofilm formation by 93.98%.	(Amel Ben Lagha et al. 2018)	[22]

		PACs revealed capacity to reduce biofilm viability but not biofilm desorption at 500 µg/ml. PACs reduced LtxA cytotoxic towards macrophage- like cells by 100%, 95.4%, and 69.70%, at 125, 62.5, and 31.25 µg/ml, respectively. The PACs protected the oral keratinocytes barrier integrity from damage caused by *A. actinomycetemcomitans*.		
The commercial green tea extract with polyphenol content of 98.42%, including 47.92% of EGCg. (-)-Epigallocatechin gallate (EGCg).	*P. gingivalis*, Arg-gingipain, Lys-gingipain	62,5 µg/mL of both the green tea extract and EGCg inhibited the degradation of type I collagen by a *P. gingivalis* culture supernatant by 91.1% and 94.5%, respectively. The green tea extract caused more significant inhibitions of both Arg-gingipain, Lys-gingipain activities than EGCG. More specifically, 125 µg/mL of the green tea extract and EGCg reduced Arg-gingipain activity by 61.82% and 16.46%, while inhibiting Lys-gingipain activity by 51.28% and 7.97%, respectively. Green tea extract and EGCg enhanced the barrier function of a gingival keratinocyte model and exert a protective effect against invasion by *P. gingivalis*.	(Amel Ben Lagha et al. 2018)	[23]
Pelargonium sidoides root DC. extract (PSRE) and proanthocyanidin fraction from PSRE (PACN)	*P. gingivalis*	PSRE extract was effective in reducing the viability of *P. gingivalis* in a significant manner in comparison to the untreated control starting from the lowest 0.02 g/mL. PACN reduced tha viability at lower concentration, it reduced *P. gingivalis* viability from ≈90% (0.01–0.03 mg/mL) to ≈10% (0.05–0.09 mg/mL).	(Savickiene et al. 2018)	[24]
Mixture of theaflavins (TFs) from black tea (theaflavin- 3-gallate, theaflavin-3′-gallate and theaflavin-3-3′-digallate, with more than 80% purity)	*P. gingivalis*	TFs dose-dependently inhibited the expression of genes *(fimA, hagA, rgpA and kgp)* encoding the major virulence factors of *P. gingivalis* and attenuated its adherence to gingival keratinocytes. A treatment of gingival keratinocytes with TFs significantly enhanced tight junction integrity and prevented *P. gingivalis*-mediated tight junction damage as well as bacterial invasion.	(A Ben Lagha and Grenier 2017)	[25]
70% aceton extract from rhizomes of *Limonium brasiliense* (Boiss.) Kuntze (LBE), rich in proanthocyanidins and gallic acid, ,epigallocatechin-3-O-gallate. LBE contain high amount untypical double linked proanthocyanidins named-samarangenins A and B	*P. gingivalis*, Arg-gingipain	LBE at 100 µg/mL reduced the adhesion of *P. gingivalis* to the human epithelial KB cells by about 80% and at 20 µg/mL reduced the proteolytic activity of the arginin-specific Rgp gingipain by about 75%.	(de Oliveira Caleare et al. 2017)	[26]

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The commercial green tea extract with polyphenol content of 98.42%, including 47.92% of EGCg. (-)-Epigallocatechin gallate (EGCg).	*P. gingivalis;* expression of several *P. gingivalis* genes involved in host colonization (*fimA, hagA, hagB*), tissue destruction (*rgpA, kgp*), heme acquisition (*hem*), and stress response (*htrA*) was investigated.	The MIC values of the green tea extract ranged from 250 to 1000 µg/mL, while those of EGCg ranged from 125 to 500 µg/mL. Synergistic antibacterial effects were observed for the green tea extract or EGCG in combination with metronidazole. The combination of the green tea extract or EGCg and tetracycline resulted mostly in an additive effect. Both substances caused a dose-dependent inhibition of bacterial adherence to oral epithelial cells. Green tea extract and EGCg dose-dependently inhibited the expression of *fimA, hagA, hag, rgpA, kgp, hem*. However, both compounds increased the expression of the stress protein *htrA* gene. Green tea extract and EGCg revealed inhibit quorum sensing.	(Fournier-Larente, Morin, and Grenier 2016)	[27]
Persimmon fruit (*Diospyros kaki* Thunb.) extract (PS-M) contained 21.5 wt % of condensed tannin (proanthocyanidins).	Oral polymicrobial biofilms	The colony forming units (CFUs) were lower in all PS-M and CHX (chlorhexidine) groups compared to the control group. PS-M exerted a dose-dependent effect. PS-M at a dose of 4.0 wt% had the same effect as 0.2 wt% CHX. SEM revealed the biofilm structures were considerably destroyed in the 4.0 wt% PS-M and 0.2 wt% CHX.	(Tomiyama et al. 2016)	[28]
70% aceton extract from aerial parts of *Rumex acetosa* L., after removal of lipohilic compounds (RA1); According structural features of Fig. 1: 1) Epicatechin, 2) Catechin, 3) Epigallocatechin 4) Gallocatechin 5) Epicatechin-3-O-gallate 6) Epigallocatechin-3-O-gallate 7) Procyanidin B2 ,	*P. gingivalis,* Arg-gingipain, Lys-gingipain	RA1 (5 to 15 µg/mL) reduced *P. gingivalis* adhesion to KB cells in a dose-dependent manner to about 90%. Galloylated flawan-3-ols and proanthocyanidins (5, 6, 8, 12) were confirmed to be responsible for this antiadhesive effect with (8) procyanidin B2-di-gallate being the lead compound. Ungalloylated flavan-3-ols and oligomeric proanthocyanidins (1,2,3,4,7,11) were inactive. RA1 and the galloylated proanthocyanidins (5,6,8,9,10,12,13) strongly interact with the bacterial virulence factor Arg-gingipain, while the corresponding Lys-gingipain was hardly influenced. RA1 does not influence gene expression of rgpA, kgp and fimA. RA1 inhibited also hemagglutination. *In silico* docking studies indicated that (8) procyanidin B2-di-gallate interacts with the active side of Arg-gingipain and hemaglutinin from *P. gingivalis* and the	(Schmuch et al. 2015)	[29]

8) Procyanidin B2-di-gallate, 9) Epicatechin-(4β→6)-epicatechin-3-O- -gallte 10) Epicatechin-3-O-gallate-(4β→6)- epicatechin-3-O-gallate 11) Epicatechin-(4β→8)-epicatechin-(4β→8)- -catechin 12) Epicatechin-3-O-gallate-(4β→8)- -epicatechin-3-O-gallate-(4β→8)- epicatechin-3-O-gallate 13) Epiafzelechin-3-O-gallate-(4β→8)- -epicatechin-3-O-gallate 14) Cinnamtannin B1 15) Quercetin-3-O-glucuronide		galloylation of the molecule seems to be responsible for fixation of the ligand to the protein.		
The commercial black tea extract (with theaflavin content of 40.23%); theaflavin (TF), theaflavin-3,3`-digallate (TFg)	*P. gingivalis, Prevotella intermedia, Fusobacterium nucleatum, A. actinomycetemcomitans*	MIC/MBC values (μg/ml) of black tea, TF and TFg for *P. gingivalis* and *P. intermedia* was very similar 500/1000, 125/500, 250/500, respectively, and significant higher for *F. nucleatum* 2000/4000, 250/>1000, 250/>1000 and *A. Actinomycetemcomitans* 2000/8000, 250/>1000, 500/1000. The black tea extract, theaflavin and theaflavin-3,3'-digallate can potentiate the antibacterial effect of metronidazole and tetracycline against *P. gingivalis*.	(Telma Blanca Lombardo Bedran et al. 2015)	[30]
70% ethanolic blueberry extract (*Vaccinium angustifolium* Ait.) - phenolic acids, flavonoids and procyanidins made up 16.6, 12.9, and 2.7% of the blueberry extract, respectively.	*Fusobacterium nucleatum*	The MIC of the blueberry extract against *F. nucleatum* was 1 mg/mL. This concentration also corresponded to the MBC. It was sugested that this property may result from the ability of blueberry polyphenols to chelate iron. Moreover, the blueberry extract at 62.5 μg/mL inhibited *F. nucleatum* biofilm formation by 87.5 %.	(Amel Ben Lagha et al. 2015)	[31]

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Epigallocatechin gallate (EGCg)	*P. gingivalis*	EGCg demonstrated a dose- dependent inhibitory effect of on *P. gingivalis* growth. EGCg at 500 μg/mL exhibited 99,9% decrease and at 1 mg/mL 100% decrease of growth. EGCg (500 μg/mL or 5 mg/mL) higher than its MIC disrupted established *P. gingivalis* biofilms, what is caused by the destruction of the bacterial cell membrane of *P. gingivalis.* Moreover, EGCg at sub-MIC levels inhibited *P. gingivalis* biofilm formation. EGCg at 10 μg/mL efficiently inhibited biofilm formation without affecting the growth rate. At sub-MIC EGCg did not damage the cytoplasmic membrane of *P. gingivalis.*	(Asahi et al. 2014)	[32]
Cranberry non-dialyzable material (NDM) prepared from concentrated juice of *Vaccinium macrocarpon* Ait., rich in proanthocyanidins.	*P. gingivalis* and *F. nucleatum* mixed infection	NDM inhibited coaggregation between *P. gingivalis* and *F. nucleatum* in a dose-dependent manner (starting from 1 mg/ml). NDM inhibited *P. gingivalis* and *F. nucleatum* adhesion to human epithelial cells. The addition of 4 mg/ml NDM fully inhibited the adhesion of *F. nucleatum* and *P. gingivalis* onto the epithelial cells, leaving the cells entirely free of bacteria.	(Polak et al. 2013)	[33]
Commercial proanthocyanidins from grapeseed extract (Leucoselect ®, Indena, Italy) were combined withH2O2 and photo-irradiation	*P. gingivalis, S. mutans*	The photolysis of H2O2 in combination with proanthocyanidin synergistically induced damage to *P. gingivalis* and *S. mutans*	(Ikai et al. 2013)	[34]

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Epigallocatechin gallate (EGCg)	*A. actinomycetemcomitans*	Antimicrobial activity was observed at >0.5 mg/ml of EGCg. Alpha-amylase reduced the antimicrobial activity of EGCG and the other way EGCG inhibited the activity of alpha-amylase. The reason was precipitated alpha-amylase by EGCg after adding to saliva.	(Hara et al. 2012)	[16]
A-type cranberry proanthocyanidins (APAC) and Licochalcone A (LA)-chalcone, not proanthocyanidin	*P. gingivalis*	APAC at the highest concentration tested did not affect the growth of *P. gingivalis*, whereas licochalcone A completely prevented growth at 10 μg/ml. When the two compounds were used in combination, *P. gingivalis* growth was inhibited in a synergistic manner. Contrary, licochalcone A had no effect on the adherence of *P. gingivalis* to epithelial cells, but 50 μg/ml of APACs reduced bacterial adherence by approximately 25%. When used in combination, they acted in synergy to inhibit the adherence of *P. gingivalis* to oral epithelial cells. APACs 25 μg/ml inhibited *P. gingivalis* collagenase by 66 %.	(Feldman and Grenier 2012)	[35]
50% EtOH extract from *Myrothamnus flabellifolia* Welw. (MF), rich in flavan-3-ols and oligomeric proanthocyanidins	*P. gingivalis*, Arg-gingipain, Lys-gingipain	100 μg/ml of MF reduced *P. gingivalis* adhesion/invasion about 50%. Fimbrillin and Arg-gingipain encoding genes were up-regulated by MF. On the protein level, inhibition (70-80% at 50 μg/ml) of Arg-gingipain activity was observed, while the corresponding Lys-gingipain was hardly influenced. MF also inhibited haemagglutination.	(Löhr et al. 2011)	[36]
A-Type Cranberry Proanthocyanidins (AC-PACs) were isolated from cranberry fruit (*Vaccinium macrocarpon* Ait.)	*P. gingivalis*	AC-PACs inhibited biofilm formation by 45% and 60% at concentrations of 50 and 100 μg/ml, and inhibited *P. gingivalis* adherence to epithelial cells by 37.5% % and 54.1%, respectively. AC-PACs also inhibited the adherence of *P. gingivalis* to Matrigel-coated polystyrene surfaces. AC-PACs inhibited type I collagen degradation by extracellular proteinases produced by *P. gingivalis* in dose dependent	(Vu Dang La, Howell, and Grenier 2010)	[37]

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		manner. At all the concentrations tested AC-PACs did not significantly affect the growth of *P. gingivalis*.		
Cranberry non-dialyzable material (NDM) prepared from concentrated juice of *Vaccinium macrocarpon* Ait., contain 65.1% proanthocyanidins.	*Peptostreptotoccus micros*	Treatment of monocyte-derived macrophages as well oral epithelial cells with cell wall of *P. micros* decreased their cell viability, however adding the cranberry fraction (25-50 μg/ml) prior to treating cells with *P. micros* cell wall dose-dependently protected these cell lines from the toxic effect.	(Vu Dang La, Labrecque, and Grenier 2009)	[38]
Cranberry non-dialyzable material (NDM) prepared from concentrated juice of *Vaccinium macrocarpon* Ait., contain 65.1% proanthocyanidins.	*P. gingivalis*	NDM prevented significantly the attachment of *P. gingivalis* to surfaces coated with type I collagen, fibrinogen or human serum. NDM inhibited the biofilm formation of *P. gingivalis*, however, it has no effect on growth and viability of bacteria.	(Labrecque et al. 2006)	[39]
Cranberry fraction from *Vaccinium macrocarpon* Ait. friuts, obtained after dialysed; Non-dialysable material (NDM) contains 65.1% proanthocyanidins.	Arg-gingipain, Lys-gingipain, dipeptidyl peptidase IV of *P. gingivalis*; Trypsin-like protease of *T. forsythia* Chymotrypsin- like protease of *T. denticola*	NDM dose-dependently inhibited the proteinases of *P. gingivalis*, *T. forsythia* and *T. denticola* (10-150 μg/ml), however the trypsin-like activity of *T. forsythia* was the slightest sensitive to NDM. 50 μg/ml of NDM significantly reduced the collagenase activity of *P. gingivalis* (by 30%) and capability of *P. gingivalis* to degrade transferrin (by abaout 20%). Degradation of type I collagen and transferrin by *P. gingivalis* was completely or almost completely inhibited by 100 μg/ml and 150 μg/ml of NDM, respectively.	(Charles Bodet et al. 2006)	[40]
Apple fraction (AP) rich in proantocyanidins. Apple condensed tannin (ACT) isolated from AP. Hop bract polyphenols (HBP) fraction rich in proanthocyandins. HMW-HBP (high molecular weight fraction) and LMW-HBP (low molecular weight fraction) separated from HBP. HMW-HBP	*P.gingivalis,* Arg- and Lys-gingipains	None of the fractions revealed bactericidal activity or suppression of bacterial growth at concentrations of 1 and 10 μg/ml. Studied fractions at 10 μg/ml significantly protected PDL cells viability from the effect of *P. gingivalis* infection, although EGCg and LMW-HBT showed slightly lower effects than the others. Even at 1 μg/ml, AP, ACT, HBP, and HMW- HBP demonstrated protective effects. All of the fractions revealed significant inhibitory effects toward the proteolytic activities of Rgp and Kgp in a dose depen- dent manner, with the ratios ranging from 70% to 95% at 10 and100 μg/ml. At lower doses (0.1 and 1 μg/ml), EGCg showed the greatest effect, followed by ACT and AP.	(Inaba et al. 2005)	[41]

mainly contains 8 to 22 mer proanthocyanidins. EGCg:(-)-Epigallocatechin gallate				
(-)-Epigallocatechin gallate (EGCg), Epicatechin gallate (ECg), Epigallocatechin (EGC), Epicatechin (EC), (-)-Gallocatechin gallate (GCg), Catechin gallate (Cg), Gallocatechin (GC), (-)-Catechin (C), Gallic acid (G)	*P.gingivalis,* Arg- and Lys-gingipains	Catechin derivatives, containing the galloyl moiety which included EGCg, ECg, GCg Cg signigcantly inhibited the Arg-gingipains. The 50% inhibitory concentrations (IC50s) of these catechin derivatives for Arg-gingipains ranged from 3 to 5 mM. While ungalloyleted catechins: EGC and GC moderately inhibited Arg-gingipains activity (IC50s, 20mM), EC, C and G were not effective, with IC50s greater than 300mM. Further, some of the catechin derivatives (galloylated) also inhibited the Lys-gingipains activity, though to a lesser extent than inhibition of the Arg-gingipains activity.	(Okamoto et al. 2004)	[42]
Tea polyphenol mixture (TP) (+) Catechin (C), (-) Epicatechin (EC), (+) Gallocatechin (GC), (-) Epigallocatechin (EGC), (-) Epicatechin gallate (ECg), (-) Epigallocatechin gallate (EGCg), (-) Gallocatechin gallate (GCg)	short-chain fatty acid (n-butyric and propionic acid) as well as phenylacetic acid production by *P. gingivalis*.	The production of n-butyric and propionic acid in general anaerobic medium (GAM) was inhibited by TP in dosage dependent manner; completely inhibition was seen at a concentration of 1.0-2.0 mg/mL. EGCg-a major component of tea polyphenols inhibited the production of phenylacetic acid at 0.5 mg/mL. EGCg and other galloylated catechins: Ecg, GCg inhibited reaction leading to the production of phenylacetic acid from L-phenylalanine and phenylpyruvic acid. However, C, GC, EC, EGC did not inhibit those reactions. Moreover, growth of *P. gingivalis* was inhibited by EGCg (strong at 0,5 mg/ml)	(Senji Sakanaka and Okada 2004)	[43]
Elm extract (EE) (n-butanol fraction from extract of *Ulmi cortex (Ulmus macrocarpa* Hance*)*) containing 20% of procyanidins) and the mixture of procyanidin oligomers (PO)	trypsin-like enzymes from *T. denticola* and *P. gingivalis*.	Both inhibitors (EE and PO) effectively inhibited the *T. denticola* proteases, whereas the elm extract was less effective on *P. gingivalis* proteases than that of the procyanidin oligomer (PO). PO (0.1–0.05%) reduced the enzyme activity to 34–58% in *T. denticola* and 39–73% in *P. gingivalis* in a dose-dependent manner, whereas the elm extract reduced enzyme activity to 40–89% in *T. denticola* and 49–91% in *P. gingivalis*	(Song et al. 2003)	[44]
The green tea catechin was well-purified Sunphenon ® (Taiyo Kagaku, Yokkaichi, Mie, Japan) prepared from Japanese green tea Details about composition of extract are not provided.	*P.gingivalis, Prevotella species*	The MICs for *P. gingivalis*, *P. intermedia* and *P. nigrescens* were 1.0 mg/mL. Green tea catechin showed bactericidal effects against all three bacteria. However, high concentration of catechin was used (4 mg/ml).	(Hirasawa et al. 2002)	[45]

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Tea polyphenol mixture (TP) (+) Catechin (C), (-) Epicatechin (EC), (+) Gallocatechin (GC), (-) Epigallocatechin (EGC), (-) Epicatechin gallate (ECg), (-) Epigallocatechin gallate (EGCg), (-) Gallocatechin gallate (GCg)	*P.gingivalis*	EGCg completely inhibited the growth of three strains of *P. gingivalis* at concentrations of 250 or 500 μg/ml. MICs for others polyphenols were 1000 μg/ml. At the concentration of 100 μg/ml of TP, the adhered bacterial cells onto Human Buccal Epithelial Cells were reduced by about 70%. All of the compounds inhibited the adherence of *P. gingivalis* onto epithelial cells. However, the inhibitory effect was pronounced with catechin derivatives having a galloyl moiety: EGCg, GCg and Ecg (at 250 μg/ml almost completely inhibited adherence). EGCg or ECg, at 7.8 μg/ml reduced the adhered bacterial cells about 30% of the control. Inhibition of the adherence of *P. gingivalis* onto epithelial cells was much more effective when EGCg was preincubated with bacteria than with epithelial cells.	(Sakanaka et al. 1996)	[46]

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22 studies reported their effects against *Porphyromonas gingivalis,* 5 studies, against *Actinobacillus actinomycetemcomitans*, 3 studies against *Fusobacterium nucleatum*, 2 studies against *Prevotella intermedia, 2* studies against *Treponema denticola*, 1 study against *Tannerella forsythia,* 1 study against *Peptostreptotoccus micros* cell and one against oral polymicrobial biofilms. Influence proathocyanidins or flavan-3-ols on bacterial enzymatic activity was also reported by most of these studies.

The Gram-negative anaerobic rod *P. gingivalis* is the most studied bacterium, able to adhere epithelial cells of the gingival mucosa and endothelial cells using fimbriae OMPs (outer membrane proteins). It can produce a series of high virulence factors like proteases (e.g. collagenase), hemolysins, endotoxins, fatty acids, ammonia, hydrogen sulfide, indole and others that affect the host response and are important for adherence, colonization, as well as for nutrients acquisition and targeting the host immune response [14,15]. Some of them, like lipopolysaccharide (LPS) binds the toll-like receptors-TLRs (expressed in various immune cells, such as neutrophils, macrophages, and dendritic cells) and activates inflammatory signaling pathways, promotes the secretion of pro-inflammatory cytokines, nitric oxide (NO) and eicosanoids and finally causes symptoms of inflammation [20]. However, it is supposed that the most important virulence factors are cysteine proteases- the arginine-specific (RgpA and RgpB) and lysine-specific (Kgp) gingipains, which are attributed to 85% of the total proteolytic activity of *P. gingivalis* [47]. Moreover, they are the most potent adhesins of *P. gingivalis.* They are located on the surface of *P. gingivalis* cells from where subfractions are secreted into the extracellular fluid [29]. Gingipains execute pathological actions due to their reactivity against a broad-range of proteins, e.g cytokines. They are essential for tissue degradation and may contribute to the penetration of this bacterium into the periodontium [48].

Fractions reach in proanthocyanidins (PACs, often named APACs because of A-type bond) from cranberry fruits (*Vaccinium macrocarpon*) count to the best studied natural substances against periopathogenes. Proanthocyanidins isolated from cranberry are mainly composed of epicatechin subunits with at least one A-type bond (intermolecular bond between O7 and C2 in addition to the carbon-carbon bond). Taking into account the collected literature data (Table 1), it can be concluded that, cranberry PACs can inhibit *P. gingivalis* attachment to the periodontal tissue, reduce bacterial biofilm formation, collagenase activity, and invasion by neutralizing periodontopathogen proteinases and cytotoxicity, however they do not interfere with the growth of *P. gingivalis* [19,20], [33], [35], [37], [40]. La et al. [37] in addition to the above activities, showed that A-type cranberry proanthocyanidins (PACs) inhibited the adherence of *P. gingivalis* to Matrigel-coated polystyrene surfaces and inhibited type I collagen degradation by extracellular proteinases produced by *P. gingivalis* in dose dependent manner. Despite that, PACs not influence on growth of *P. gingivalis* by themselves, Ikai H et al. [34] showed that bactericidal activity against *P. gingivalis* and *S. mutans* of hydrogen peroxide photolysis system was augmented in the presence of 2-8 mg/mL commercial grapeseed proanthocyanidins. A putative mechanism of action could involve an additional H_2O_2 generation up to 1 mM by irradiated PACs dissolved in an aqueous buffer (PBS) as was demonstrated using an EPR detection.

Feldman and Grenier [35] also proved that bactericidal effect of proanthocyanidins, more specifically proanthocyanidins from cranberry, could be improved in a presence of another polyphenol. They observed that when PAC and licochalcone A were used in combination, *P. gingivalis* growth was inhibited in a synergistic manner. Bodet at al. [40] presented the effect of PACs fraction from cranberry juice on the proteolytic activities of *P. gingivalis*, and two other periopathogenes - *Tannerella forsythia* and *Treponema denticola*, belonging to the "red cluster", the most responsible for progression of the periodontitis. Both *Tannerella forsythia* and *Treponema denticola* produced proteases which contribute to bacterial virulence in multiple ways; such as by degrading host periodontal tissues, activating host degradative enzymes, modifying host cell proteins, cleaving components involved in innate (cytokines/chemokines, complement factors) and adaptive immunity (immunoglobulins) [49]. Bodet at al. [40] noticed that PACs fraction dose-dependently inhibited proteinases of *P. gingivalis* (Arg-gingipain, Lys-gingipain, dipeptidyl peptidase IV (DPP IV)) *T. forsythia* (trypsin-like proteinase) and *T. denticola* (chymotrypsin- like proteinase) (10-150µg/ml), however the trypsin-like activity of *T. forsythia* was little sensitive to PACs. Moreover,

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proanthocyanidins fraction significantly reduced the collagenase activity of *P. gingivalis* and capability of *P. gingivalis* to degrade transferrin. Degradation of type I collagen and transferrin by *P. gingivalis* was completely or almost completely inhibited by 100 μg/ml and 150 μg/ml of NDM, respectively [40].

Proanthocyanidins from other sources than cranberry, and with different structures were also studied. De Oliveira Caleare et al. [26] studied 70% acetone extract from *Limonium brasiliense* rhizomes (LBE), rich in proanthocyanidins, EGCG and gallic acid. LBE contained high amount of untypical double linked proanthocyanidins - samarangenins A and B (figure 4).

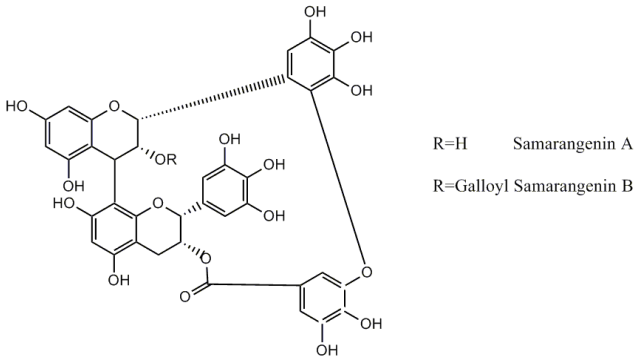

Figure 4. Structure of samarangenins A and B

LBE at 100 μg/mL reduced the adhesion of *P. gingivalis* to the human epithelial KB cells by about 80% and at 20 μg/mL reduced the proteolytic activity of the arginin-specific Rgp gingipain by about 75%. LBE at ≤ 100 μg/mL had no cytotoxicity against the bacteria and did not influence the cell physiology of human epithelial KB cells. Findings from study of Lohr et al. [36] showed that 50% EtOH extract from *Myrothamnus flabellifolia* (MF**),** rich in flavan-3-ols and oligomeric B-type proanthocyanidins, dose-dependently inhibited *P. gingivalis* epithelial cell attachment or invasion to KB cells, however bacterial growth was not influenced. Moreover, MF extract at 50 μg/mL reduced Arg-gingipain by 70–80% and also inhibited Lys-gingipain, but to a lesser extent. Schmuch at al. [29] carried out extensive study about the influence of a proanthocyanidin-enriched extract from aerial parts of *Rumex acetosa* (sorrel) (RA1) and isolated compounds (details in table 1) against the adhesion of *P. gingivalis.* It was revealed that RA1 (5 to 15 μg/mL) reduced *P. gingivalis* adhesion to KB cells in a dose-dependent manner to about 90% at 15 μg/mL. Galloylated proanthocyanidins were confirmed to be responsible for this antiadhesive effect with procyanidin B2-di-gallate being the lead compound. A trigalloylated trimeric procyanidin (epicatechin-3-O-gallate-(4β→8)-epicatechin-3-O-gallate-(4β→8)-epicatechin-3-O-gallate) was even more active than procyanidin B2-di-gallate, but it was a minor compound in the RA1 fraction. The compounds not esterified with gallic acid - flavan-3-ols (epicatechin, catechin, epigallocatechin and gallocatechin) and oligomeric proanthocyanidins (procyanidin B2 and epicatechin-(4β→8)-epicatechin-(4β→8)-catechin) were inactive similar like quercetin-3-O-glucuronide present in large amounts in the RA1. Interestingly, a non-galloylated, mixed A/B-type proanthocyanidin alos present in the RA1 fraction - cinnamtannin, also reduced *P. gingivalis* adhesion to KB cells but only moderately. Moreover, RA1 and the galloylated proanthocyanidins strongly inhibited the Arg-gingipain. The inhibition force increased with the degree of polymerization –the galloylated trimer had the higher activity than dimers and monomers were barely active. No differences were observed between the dimeric galloylated 4→8-linked and the 4→6-linked proanthocyanidins. Moreover, monogalloylation in the lower building block seemed to be sufficient for activity, while di-galloylation did not seem to be necessary. Again, a mixed A/B-type proanthocyanidin – cinnamtannin was less active. Contrary to Arg-gingipain, Lys-gingipain was hardly influenced by RA1 and its constituents. Lys-gingipain activity was only influenced to a minor extent by the di- and trimeric galloylated procyanidins. RA1 also inhibited *P. gingivalis* induced hemagglutination but did not influence gene expression of *rgpA* (for Arg-gingipain*), kgp* (for Lys-

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gingipain) and *fimA* (for fimbrillin). *In silico* docking studies indicated that procyanidin B2-di-gallate interacts with the active side of Arg-gingipain and hemagglutinin from *P. gingivalis* and that the galloylation of the molecule seems to be responsible for fixation of the ligand to the protein. Expectedly, the total amount of H-bond-donors was an important factor, indicating a tannin-like effect and therefore suggesting an unspecific interaction with the hemagglutination domain [29].

Results of Okamoto et al. [42] are consistent with the above report. Only tea catechin derivatives containing the galloyl moiety inhibited Arg-gingipain and to lesser extent Lys-gingipain of *P. gingivalis*..

Amel Ben Lagha et al. [23] have shown that the green tea extract and epigallocatechin gallate (EGCG) inhibited both Arg-gingipain and Lys-gingipain activities, however the green tea extract was stronger inhibitor. Both similarly inhibited the degradation of type I collagen by a *P. gingivalis*. Collagen degradation by *P. gingivalis* is mainly related to its Arg-gingipain activity [50]. In the same study, the green tea extract and EGCG protected the epithelial barrier against the *P. gingivalis*-mediated damage and prevented the penetration of bacteria through a keratinocyte monolayer. It was linked with the ability of these substances to enhance gingival epithelium barrier function and with their influence on gingipains [23]. Similar effect towards the epithelial barrier against the *P. gingivalis*-mediated damage were earlier reported for black tea theaflavins (figure 5)[25].

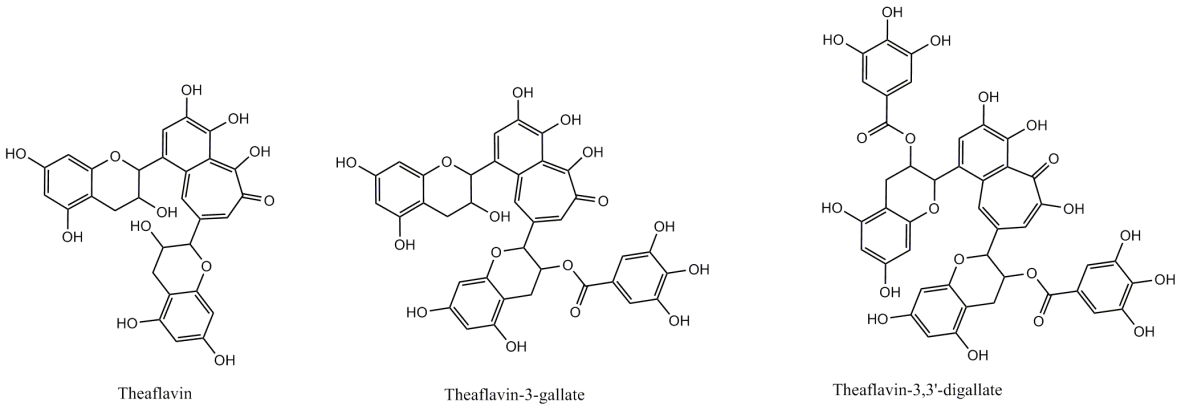

Figure 5. Structure of theaflavins.

Moreover, theaflavins dose-dependently inhibited gene expression of major virulence factors of *P. gingivalis*, such as : *fimA, hagA,* involved in colonization and *rgpA and kgp* responsible for host tissue destruction. Fournier-Larente et al. [27] showed the same activity for green tea extract and EGCG, extending the studied genes by *hagB* and *htrA* (involved in stress response) as well as *hem* (involved in heme acquisition). Sakanaka and Okada have shown that green tea polyphenols reduce the ability of *P. gingivalis* to produce toxic end metabolites (n-butyric, propionic acid and phenylacetic acid) which injure periodontal cells and disturb host cell activity. The inhibitory effect on the production of toxic end metabolites can be attributed to the presence of the galloyl moiety in the catechins. The growth of *P. gingivalis* was inhibited by EGCG but at high concentration- 0.125-0.5 mg/ml, only [43]. In the earlier study, Sakanaka et al. [46] the same extract and galloylated flavan-3-ols ((-) epicatechin gallate, EGCG, (-) gallocatechin gallate) significantly inhibited adherence of *P. gingivalis* onto the buccal epithelial cells and this activity was attributed to the presence of the galloyl moiety. EGCG also completely inhibited the growth of three strains of *P. gingivalis* at concentrations of 250 and 500 µg/ml, whereas MIC for others polyphenols was 1000 µg/ml. Similarly, in the study by Hirasawa et al. [45] the MICs of green catechins from Japanese green tea (not specified catechins) for *P. gingivalis*, *P. intermedia* and *P. nigrescens* were 1.0 mg/mL. Asahi et al. [32] demonstrated that EGCG (500 µg/mL or 5 mg/mL) disrupted established *P. gingivalis* biofilms, by the destruction of the bacterial cell membrane. Moreover, EGCG at sub-MIC levels inhibited *P. gingivalis* biofilm formation. EGCG at 10 µg/mL efficiently inhibited biofilm formation without affecting the growth rate. At sub-MIC EGCG, did not damage the cell membrane of *P. gingivalis* Hence, inhibition of *P. gingivalis* biofilm formation

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is liklely based on a mechanism distinct from that responsible for its bactericidal activity at high concentrations. Gao et al [20] studied the buds of *Castanopsis lamontii* Hance water extract (CLE) rich in epicatechin and procyanidin B2, confirming inhibition of *P. gingivalis* growth by flavan-3-ols or proanthocyanidins only at high concentrations.

Other plant materials rich in procyanidins, studied against *P. gingivalis* were *Pelargonium sidoides* roots [24], *Ulmus macrocarpa* bark [44], apples and hop bracts. [41]. Savickiene et al. [24] demonstrated that *Pelargonium sidoides* root extract (PSRE) rich in monomeric flavan-3-ols (epigallocatechin, catechin, EGCG) with a minor contribution of proanthocyanidins and a proanthocyanidin-enriched fraction from PSRE (PACN) reduced viability of *P. gingivalis* and the non-pathogenic comensal *Streptococcus salivarius.* However, PACN fraction that impaired the bacteria viability only at much lower concentrations than the PSRE (50-90 μg/mL vs. 10-90 mg/mL, respectively) was partially selective against *P.gingivalis*. Song et al. [44] studied partially purified extract from the bark of *Ulmus macrocarpa*, defined as elm extract (contained 20% of procyanidins) and its active ingredient, a mix of proanthocyanidin oligomers (composed of 3 to 12 monomers, an average molecular weight of 1518) for a possible inhibitory effect against proteases - trypsin-like enzymes from *P. gingivalis* and *Treponema denticola.* Both fractions inhibited proteases of these pathogens, but proanthocyanidin oligomer mixture inhibited them more effectively than the elm extract. The trypsin-like activity of *T. denticola* was slightly more susceptible to these inhibitory effects than *P. gingivalis*. Inaba et al. [41] studied fractions rich in proanthocyanidins from immature apples (*Malus sp*.) and hop bracts (*Humulus sp.* from Japan). Apple fraction (AP) and more purified fraction called apple condensed tannins (ACT) are oligomeric, whereas hop bract polyphenols fraction (HBP) and its high molecular weight fraction (HMW-HBP) are polymeric proanthocyanidins. Studied fractions at very low concentrations: 1-10 μg/ml significantly protected periodontal ligament (PDL) cells viability from the effect of *P. gingivalis* infection, although EGCG and LMW-HBT (low molecular weight fraction of HBT) showed lower effects than the others (AP, ACT, HBP, HMW-HBP). All fractions inhibited the proteolytic activities of Rgp and Kgp in a dose dependent manner, with AP, ACT, and HBP more effective toward Kgp. Moreover, AP, ACT, HBP, and HMW-HBP significantly protected Enamel matrix derivative (EMD)-stimulated PDL cells from *P. gingivalis*, suggesting a potential benefit of using proanthocyanidins to enhance periodontal tissue regeneration in response to EMD. In contrast, EGCG and LMW-HBP were inactive, suggesting that higher polymerized procyanidins are responsible for above effect.

Ben Lagha et al. [22] reported that proanthocyanidins isolated from highbush blueberry (*Vaccinium corymbosum*) reduced the growth of *Aggregatibacter actinomycetemcomitans* and prevented biofilm formation at sub-inhibitory concentrations. This effect was linked to an ability of PACs to damage the bacterial cell membrane. The application of PCAs on pre-formed biofilms resulted in a loss of bacterial viability. Moreover, PACs significantly reduced LtxA cytotoxicity towards macrophage-like cells and protected the oral keratinocytes barrier integrity from damage caused by *A. actinomycetemcomitans*. A next study of the same group [21] tested the influence of cranberry PACs from cranberries on mRNA expression of *A. actinomycetemcomitans* leukotoxin encoding genes. PACs (60 μg/mL) treatment down regulated mRNA level of ltxB by 65.3% and 88.7% and of ltxC by 94.4% and 86.1% in the Y4 and JP2 strains, respectively. LtxB encodes components required for the transport of LtxA to the *A. actinomycetemcomitans* outer membrane and ltxC encodes components involved in posttranslational acylation.

The mentioned above *Pelargonium sidoides* root extract (PSRE) and proanthocyanidin fraction (PACN) were also active against *A. actinomycetemcomitans* [19]. PSRE and PACN at 80 μg/mL significantly reduced bacterial metabolic activity in comparison to the untreated control, whereas PACN was more effective than PSRE. Moreover, PSRE and PACN protected human gingival fibroblast from *A. actinomycetemcomitans* infection through lowering bacteria proliferation and prevented LPS-induced necrosis.

Hata et al. [16] reported antimicrobial activity of epigallocatechin gallate EGCG against *A. actinomycetemcomitans* at >0.5 mg/ml. However, EGCG also precipitated several salivary proteins including α-amylase thus inhibiting the enzymatic activity. On the other hand, α-amylase reduced the antimicrobial activity of EGCG. It was suggested that EGCG–salivary protein interactions may

have both protective and detrimental effects to oral health. This should certainly be considered when assessing the effects of EGCG on the oral cavity, and probably also applies to many proanthocyanidins with protein-binding activity.

4. Conclusions

Among the reviewed *in-vitro* studies, thirtyreported on the influence of proanthocyanidins or flavan-3-ols on periopathogens, mainly *Porphyromonas gingivalis* (22 studies). Much fewer studies concerned other oral pathogens: *Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Prevotella intermedia, Treponema denticola, Tannerella forsythia, Peptostreptotoccus micros.* Both proanthocyanidins and simple flavan-3-ols affected attachment of periopathogens, mostly *P.gingivalis,* to the periodontal tissue, depending on their chemical structure. The antiadhesive effect was attributed to the presence of the galloyl moiety in the B-type proanthocyanidins or flavan-3-ols (e.g. from tea) and an A-type linkage in the case of A-type proanthocyanidins from cranberry. Similarly, these structural pattern were also important for other activities, such as reduction of bacterial biofilm formation, collagenase activity, as well as in neutralizing periodontopathogen proteinases activity and cytotoxicity. The above-mentioned activities were manifested at low, micromolar concentrations, at which they only slightly interfered with periopathogen growth. However, the inhibition often occurred at higher concentrations.

Using flavanol derivatives at their non-toxic but active concentrations can significantly contribute to alleviating of periodontitis symptoms and prevent the disease progress. However, considering the usage of this compounds in the prevention and treatment of periodontitis, their interaction with saliva proteins should be taken into account, because it may alter their level of antimicrobial activity, what needs to be looked at in the future.

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