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Review

Proanthocyanidins and Flavan-3-ols in the prevention and treatment of Periodontitis - immunomodulatory effects, animal and clinical studies.

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Abstract: This paper continues the review study on antimicrobial properties relevant to the periodontal diseases. Inflammation as a major response of the periodontal tissues attacked by pathogenic microbes can significantly exacerbate the condition. However, the bidirectional activity of phytochemicals that simultaneously inhibit bacterial proliferation and proinflammatory signaling can provide a substantial alleviation of both cause and symptoms. The modulatory effects on various aspects of inflammatory and overall immune response has been covered, including confirmed and postulated mechanisms of action, structure activity relationships and molecular targets. Further, the clinical relevance of flavan-3-ols and available outcomes from clinical studies has been analyzed and discussed.

Among the numerous natural sources of flavan-3-ols and proanthocyanidins the most promising are, similarly to antibacterial properties, constituents of various foods, such as fruits of *Vaccinium* species, tea leaves, grape seeds, and tannin-rich medicinal herbs. Despite a vast amount of *in vitro* and cell-based evidence of immunomodulatory there is still much less studies using animal models and only a few clinical studies. Most of the studies, regardless of the used model indicated efficiency of these phytochemicals from cranberries and other *Vaccinium* species and tea extracts (green or black). Other sources such as grape seeds and traditional medicinal plants, were seldom. In conclusion, the potential of flavan-3-ols and their derivatives in prevention and alleviation of periodontitis is remarkable but clinical evidence is urgently needed for issuing credible dietary recommendation and complementary treatments.

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

1. Introduction

In the previous paper, we have demonstrated that both free flavan-3-ols and oligomeric proanthocyanidins are very promising constituents for combating various bacteria involved in periodontitis pathogenesis [1]. Here, using the same systematic approach, we have selected and discussed the recent data on anti-inflammatory and immunomodulating activities of these compounds, including *in vivo* models and clinical studies. Search strategy as well as inclusion, exclusion criteria and data organization are described in first part of review, in which the antimicrobial activity is discussed. According to the latest concept of periodontitis etiopathology, the development of the disease requires the co-existence of dental plaque and the host's immune-inflammatory response [2]. Periodontal bacteria cause the mobilization of innate immune response (e.g. macrophages, dendritic cells, natural killer cells, neutrophils) as well adaptive immunity mechanisms (B and T lymphocytes) leading to release of pro-inflammatory molecules (e.g., interferon-gamma, interleukin-17, tumor necrosis factor, interleukin-1, interleukin-6) and enzymes

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(e.g., collagenases such as matrix metalloproteinases) [3](Figure 1). By the inflammatory response body protects itself against the bacteria and their invasion inside the deeper tissues (such as bone). However, if the inflammation persists and is poorly regulated, it causes the most troublesome detrimental changes in periodontium tissue form and function such as periodontal pockets, attachment loss, gingival recessions, tooth mobility, tooth migration and tooth loss [4]. Literature provides the data about anti-inflammatory and antioxidant effects of polyphenols which include falvan-3-ols and proanthocyanidins, drawing conclusions that they can play a beneficial role in the prevention and the progress of chronic diseases related to inflammation such as diabetes, obesity, neurodegeneration, cancers, and cardiovascular diseases, among other conditions [5]. Our review focused on the activity a high potent compounds among polyphenols in relation to periodontitis-a disease closely related to the inflammatory diseases mentioned above [2].

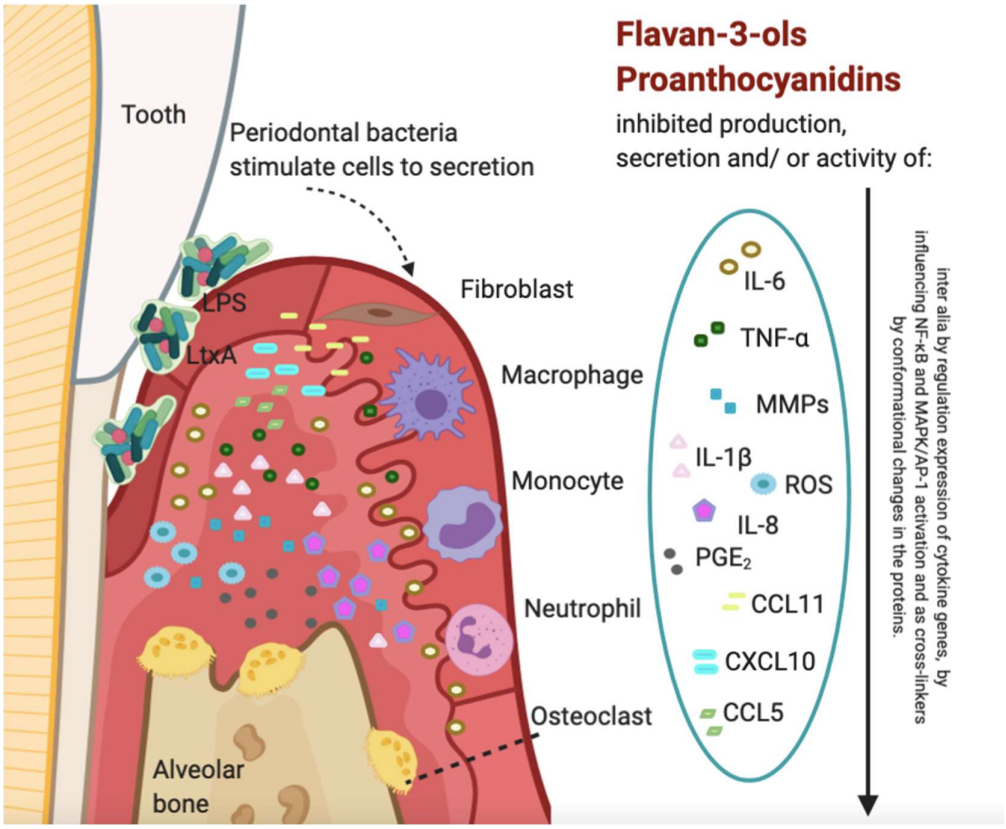

Figure 1. Schematic picture illustrating immunomodulatory activities of flavan-3-ols and proanthocyanidins in periodontitis. Some of the more important cytokines are shown in the figure. The figure was created by using BioRender.com.

2. Immunomodulatory effects of proanthocyanidins or flavan-3-ols on host cells and tissues, *in vitro* studies

2.1. Influence on matrix metalloproteinases (MMPs)

It was already proved that matrix metalloproteinases (MMPs) play important roles in the connective tissue destruction of the periodontal complex. Matrix metalloproteinases (MMPs) are a calcium-dependent zinc-containing endopeptidases, responsible for the tissue remodeling and degradation of the extracellular matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan [6]. Major cell types found in the periodontium, like fibroblasts, neutrophils, and macrophages release these proteolytic enzymes [7], which secreted as latent proenzymes (except membrane type (MT)-MMPs)) must be activated extracellularly or at the cell surface by tissue, plasma or bacterial proteinases. Under normal condition, MMPs play an important

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role in wound healing, angiogenesis, and gingival tissue remodeling [8]. However, in periodontitis condition, when host cells are threatened by periopathogens and their products such as lipopolysaccharides (LPS), production of MMPs increases significantly leading to the destruction of periodontal tissues by the degradation of periodontal ligaments, the loss of gingival collagen, and the resorption of alveolar bone [9]. Increased activities of collagenases (MMP-1 and MMP-8) and gelatinases (MMP-2 and MMP-9) have been detected in inflamed gingival tissues and in the gingival crevicular fluid of periodontitis patients [10]. MMPs can activate but also inactivate or antagonize the biological functions of cytokines and chemokines by proteolytic processing and this way either promote or suppress inflammation. On the other hand, stimulation of inflammatory cells by cytokines and chemokines may induce production of MMPs [11]. Many studies proved that proanthocyanidins could inhibit activity of matrix metalloproteinases (Table 1).

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Tabel 1 Immunomodulatory effects of proanthocyanidins or flavan-3-ols on host cells and tissues - *in vitro* studies.

Active compound/ extract/fraction	**Cells/tissues**	**Methods**	**Results**	**Author, Years**	**Ref.**
Cranberry *(Vaccinium macrocarpon)* concentrate from capsules (Uriach-Aquilea OTC) containing 130 mg A-Type PACs	Human gingival fibroblasts (HGF), human osteosarcoma-derived osteoblasts (SAOS-2 cell line), PMA- induced macrophages (from THP-1 cells, a monocytic leukemia cell line).	All tested cells were 24 h exposed to different cranberry concentrates (25, 50, and 100 µg/mL). After 0, 3 and 7 days cell viability assay was performed. Interleukin (IL)-8, IL-1*ß*, IL-6, and IL-10 expression of lipopolysaccharides (LPS from *E.coli*) -stimulated macrophages, and macrophage polarization markers were evaluated through determination of live-cell protease activity, enzyme-linked immunosorbent assay, and immunofluorescence staining semi-quantification.	Cranberry concentrates did not reduce HGF, SAOS-2, and macrophages viability after 24 hours of exposure. Expression of proinflammatory IL-8 and IL-6 was downregulated by PACs concentrates at 50 and 100 µg/mL but expression of anti-infammatory IL-10 was upregulated at 100 µg/mL. No influence on expression of IL-1 ß was seen. Exposed LPS-stimulated macrophages to PACs significantly decreased M1 polarization and increased M2 polarization.	(Galarraga-Vinueza et al. 2020)	[12]
Unverified commercial proanthocyanidin (PA) purchased at ChemFace, with molecular weight =594.52 and untypical structure.	Human periodontal ligament fibroblasts (HPDLFs)	HPDLFs were stimulated by tumor necrosis factor-α (TNF-α), PA, or their combination, and osteogenic differentiation- and mineralization-associated markers were detected by quantitative real-time polymerase chain reaction (qRT-PCR), alizarin red S staining, and alkaline phosphatase (ALP) activity assay.	PA (0.1, 1, 10 µg/ml) significantly upregulated expression of osteogenesis-related genes and proteins and ALP activity in HPDLFs compared with the control in non-inflammatory environment. PA (1 µg/ml) significantly reversed inhibition of osteogenesis-related gene and protein expression, ALP activity, and mineralization caused by TNF-α. The underlying mechanism was that PA could regulate osteogenesis of HPDLFs via suppressing nuclear factor-kappa beta (NF-κB) signal pathway.	(Huang et al. 2020)	[13]

Catechin	THP-1-derived macrophages	THP-1 cells were pre-treated with catechin (40 μM) and then infected with *P.gingivalis*. The cytokine levels (IL-1β and TNF-α) and relevant protein expression in THP-1 cells (e.g. pro-IL-1β, TLR2, TLR4, NF-κB, MAPK and others) were measured using an ELISA kits and Western blot analysis, respectively. An apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) pyroptosome formation was measured by confocal laser scanning microscopy.	Catechin inhibited *P. gingivalis*-induced IL-1β and TNF-α secretion in THP-1 macrophages. The decreased production of IL-1β caused by catechin was due to its inhibition of pro-IL-1β expression via the downregulation of NF-κB, p38 mitogen-activated protein kinase, and Toll-like receptor signaling. In addition, catechin inhibited the activation of inflammasomes induced by *P. gingivalis*, but did not affect the growth of *P. gingivalis*.	(Lee et al. 2020)	[14]
Pelargonium sidoides DC root extract (PSRE) and proanthocyanidin (prodelphinidins) fraction from PSRE (PACN)	Rat Gingival Fibroblast Cell Culture; Bone marrow-derived macrophages (BMDM); Human peripheral blood mononuclear cells (PBMCs).	Cells (rat gingival fibroblast, BMDM or PBMCs) were stimulated with PSRE and PACN and LPS and IFNγ (for BMDM). Medium collected after treatments was assayed for TNF-α, IL-6, IL-8 and PGE2 production using TNF-α mouse, IL-6 human IL-8 rat and PGE2 rat ; Examine the capacity of PSRE and PACN to modulate expression of inflammatory genes IL-1β, TNF-α and iNOS in BMDM and PBMCs. Expression of proinflammatory cell surface markers CD80 and CD86 analyzed by flow cytometry 24 h after treating LPS + IFNγ-activated BMDMs with PSRE or PACN.	PSRE and PACN (50 - 100 μg/mL) efficiently suppress LPS-induced IL-8 and PGE2 release from gingival fibroblasts and IL-6 release from mononuclear leukocytes. PACN had slightly stronger IL-8 and IL-6 release suppressing activity, and significantly stronger PGE2 release suppressing activity than PSRE. PSRE and PACN at a dose of 100 μg/mL significantly suppressed the mRNA transcription of IL-1β, iNOS and COX-2 but not TNF-α. PSRE and PACN (100 μg/mL) reduced the level of CD80 and CD86-positive cells. The population of cells with the exposed markers after treatment with PSRE was by 58% lower, and after treatment with PACN by 71% lower than after LPS and IFN-γ stimulation without the treatment	(Jekabsone et al. 2019)	[15]
Commercial green and black tea extracts,	U937 human monocytes differentiated to macrophage-like cells,	The macrophage-like cells were treated with green tea extract, black tea extract, EGCG, or theaflavins prior to being stimulated with 10 or	The green and black tea extracts, EGCG and theaflavins in range 7.9-62.5 μg/mL significantly and dose-dependently reduced	(Amel Ben Lagha	[16]

epigallocatechin-3-gallate (EGCG), theaflavin fraction (mixture of theaflavin, theaflavin-3- gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate) (80% purity).	gingival keratinocyte cell line B11, human oral epithelial cell line GMSM-K	100 ng/mL of recombinant human TNF-α. An ELISA kit was used to quantify the levels of intracellular and released IL-1β. Caspase-1 activation and quantification as well activation of the NF-κB signaling pathway were determined by commercial assay. Gingival keratinocytes were used to evaluate the effect of tea polyphenols on the TNF-α-induced disruption of tight junction integrity which was determined by measuring transepithelial electrical resistance (TER). ELISA kit was used to quantify IL-8 secretion by oral epithelial cells.	IL-1β secretion by TNF-α-treated macrophages (at 62.5 μg/mL all substances inhibited the secretion of IL-1β by more than 94%, except black tea-64.5%), as well as reduced the activation of caspase-1 and NF-κB activation. Green tea extract, EGCG, theaflavins, and to a lesser extent, black tea extract protected keratinocytes against the TNF-α-mediated breakdown of barrier integrity. The treatment of keratinocytes with tea polyphenols markedly mitigated the morphological changes of tight junction proteins such as zonula occludens-1 and occludin compared to cells exposed only to TNF-α. At a concentration of 62.5 μg/mL, the green tea extract, EGCG, and theaflavins reduced the secretion of IL- 8 by 93.1%, 98.8% and 70.8%, respectively. A much higher concentration of black tea extract (250 μg/mL) was required to reduce the secretion of IL-8 (78%).	and Grenier 2019)	
Cranberry proanthocyanidins (PACs) isolated from cranberries (*Vaccinium macrocarpon*)	U937 human monocytes differentiated to macrophage-like cells	Adherent macrophages were exposed to leukotoxin LtxA (1 μg/mL) in the absence or presence of cranberry PACs. RealTime-Glo™MT Cell Viability Assay was performed. The amounts of IL-1β, IL-18, and caspase-1 secreted into the culture medium or contained in the macrophages were quantified by ELISA. Quantitative real-time PCR analysis to determine the levels of P2X7 and CIAS mRNA expression was determined.	Cranberry PACs markedly reduced the cytotoxicity of LtxA on macrophages. In the presence of 125 μg/mL of cranberry PACs, the release of caspase-1, IL-1β, and IL-18 was reduced 100%, 99.3%, and 98.7%, respectively, relative to cells treated with LtxA alone. However, this strong reduction was already seen with lower concentration-15.625 μg/mL of PACs (about 80-90% reduction -as can be read from the graph). In	(Amel Ben Lagha, Howell, and Grenier 2019)	[17]

		Intracellular ROS generation was measured using a total ROS/superoxide detection kit Influence of cranberry PACs on binding of FITC–LtxA to macrophages was examined by flow cytometry.	contrast, intracellular pro-caspase-1, pro-IL-1β, and pro-IL-18 levels were comparable to those of control cells. In the presence of 125 μg/mL of cranberry PACs, the increase in the expression of CIAS and P2X7 was reduced by 30.2% and 45.8%, respectively, Compared to the control with LtxA alone. ROS and superoxide production was reduced by 92.2% and 72.7%, respectively, in the presence of 125 μg/mL of cranberry PACs on LtxA-induced macrophages. PACs at a concentration of 125 μg/mL and 62.5 μg/mL blocked the binding of FITC–LtxA to macrophages by 46.6% and 55.7%, respectively.		
The buds of *Castanopsis lamontii* water extract (CLE) rich in epicatechin and procyanidin B2; epicatechin (EC); procyanidin B2 (PB2).	Mouse macrophage RAW264.7 cells	RAW264.7 cells were co-treated or pretreated with LPS (not defined) and CLE/EC/PB2 and then the expression of TLR-4 pathway-related proteins (TLR-4, p-NF-κB (p65), iNOS, and COX-2) and the release of NO, PGE2, TNF-α were determined. The NO, PGE2, TNF-α concentrations were measured using the NO assay kit, PGE2 ELISA kit, and TNF-α kit, respectively. The Western blot assay was conducted to determined expression of TLR-4, p-NF-κB (p65), iNOS, and COX-2.	CLE (400 μg/mL) and two compounds-PB2 (34.4 μg/mL) and EC (120 μg/mL) (equivalent to the concentration of PB2 and EC in 400 μg/mL CLE) significantly decreased the release of NO, PGE2, and TNF-α from LPS-stimulated macrophages in LPS- cotreated and pretreated group as well as decreased LPS-stimulated up-regulation of TLR4, p-NF-κB (p65), COX-2, iNOS in RAW264.7 cells. Compared with EC, PB2 showed much more potency in suppressing the LPS-stimulated inflammatory response.	(Gao et al. 2019)	[18]

Highbush blueberry (*Vaccinium corymbosum*) proanthocyanidins (PACs)	U937 human monocytes differentiated to macrophage-like cells.	Adherent macrophage-like cells were pre-treated with the PACs and then were stimulated LPS-*A. actinomycetemcomitans*. Secreted pro-inflammatory cytokines (IL-1β, TNF-α, IL-6 and CXCL8) and MMPs (MMP-3 and MMP-9) were then quantified by ELISA. The U937-3xκB-LUC monocyte cell line transfected with a luciferase reporter gene was used to monitor NF-κB activation. MTT assay was used to determine viability of cells treatment with PACs.	The secretion of pro-inflammatory cytokines by macrophages stimulated with LPS was significantly and dose-dependently attenuated by the PACs compared to the controls. PACs at 125 µg/mL reduced the secretion of IL-1β, TNF-α, IL-6, and CXCL8 by 75.34%, 81.64%, 48.27% and 90.19%, respectively, whereas MMP-3 and MMP-9 secretion was attenuated by 93.04% and 68.78% respectively. The PACs also inhibited the activation of the NF-κB signaling pathway.	(Amel Ben Lagha, LeBel, and Grenier 2018)	[19]
Mixture of theaflavins (TFs) from black tea (theaflavin- 3-gallate, theaflavin-3′-gallate and theaflavin-3-3′-digallate, with more than 80% purity)	The gingival keratinocyte cell line, B11; U937 human monocytes differentiated to macrophage-like cells.	Adherent macrophage-like cells were pre-treated with the TFs and then were stimulated with *P. gingivalis*. Secreted pro-inflammatory cytokines (IL-1β, TNF-α, IL-6 and CXCL8) and MMPs (MMP-3, MMP-8, MMP-9) were then quantified by ELISA. Effect of theaflavins (TFs) on the activity of MMP-9 was monitored using a fluorogenic assay. The U937-3xκB-LUC monocyte cell line transfected with a luciferase reporter gene was used to monitor NF-κB activation. MTT assay was used to determine viability of cells treatment with TFs.	At a concentration of 125 µg/mL, the TFs reduced the secretion of IL-1β by 98.4% , TNF-α by 98.8%, IL-6 by 97.7% and of CXCL8 by 84% compared to the controls as well reduced the secretion of MMP-3 by 97.3%, MMP-8 by 99.9%, MMP-9 by 95.7%. The TFs mixture at 125 µg/mL reduced MMP-9 activity by 100% . The TFs inhibited the activation of the NF-κB signaling pathway.	(A Ben Lagha and Grenier 2017)	[20]
80% methanol extract of green tea (*Camellia sinensis*) and commercially purchased epigallocatechin-gallate (EGCG)	human neutrophils	Methanol extract and EGCG were tested *in vitro* for their ability to inhibit MMP-9 activity and/or its release from neutrophils using a b-casein cleavage assay and gelatin zymography, respectively.	Methanol extract and EGCG at 0.1% (w/v) completely inhibited the activity of MMP-9, as well significantly inhibited the release of MMP-9 from (FMLP)- stimulated human neutrophils by 62.01% and 79.63%, respectively and from unstimulated neutrophils (52.42% and 62.33%, respectively).	(Kim-Park et al. 2016)	[21]

70% ethanolic blueberry extract (*Vaccinium angustifolium)* - phenolic acids, flavonoids and procyanidins made up 16.6, 12.9, and 2.7% of the blueberry extract, respectively.	U937 human monocytes differentiated to macrophage-like cells.	The macrophage-like cells were pre-treated with the blueberry extract and then stimulated with *F. nucleatum.* ELISA kits were used to quantify IL-1β, IL-6, CXCL8, TNF-α, MMP-8, and MMP-9 concentrations. Activity of MMP-9 was monitored using a fluorogenic assay. The ability of the blueberry extract to inhibit the NF-κB signaling pathway in U937-3xκB cells was investigated	The blueberry extract dose-dependently inhibited the activation of NF-κB induced by *F. nucleatum*. A pretreatment of macrophages with the blueberry extract (62.5μg/mL) inhibited the secretion of IL-1β, TNF-α, and IL-6 by 87.3, 80.7, and 28.2%, respectively. The secretion of the chemokine CXCL8 was not affected by 62.5 μg/mL of the blueberry extract, but 500, 250, or 125μg/mL of extract decreased CXCL8 secretion by 79, 57.9, and 11.2 % respectively. The secretion of MMP-8 and MMP-9 was also dose-dependently inhibited as well MMP-9 activity.	(Amel Ben Lagha et al. 2015)	[22]
A-type cranberry proanthocyanidins (AC-PACs) and epigallocatechin-3-gallate (EGCG)	A 3D co-culture model composed of gingival fibroblasts embedded in a collagen matrix and overlaid with gingival epithelial cells.	The 3D co-culture model treated with non-cytotoxic concentrations of AC-PACs (25 or 50 μg/mL), EGCG (1 or 5 μg/mL) and LL-37 (peptide cathelicidin) individually and in combination (AC-PACs + LL-37 and EGCG + LL-37) were stimulated with *Aggregatibacter actinomycetemcomitans* LPS. Multiplex ELISA assays were used to quantify the secretion of 54 host factors, including chemokines, cytokines, growth factors, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs).	Of the 41 different cytokines, chemokines, and growth factors analyzed, *A. actinomycetemcomitans* LPS significantly increased the secretion only G-CFS, GRO-a, IL-6, IL-8, IP-10, and MCP-1 by the 3D co-culture model compared to the unstimulated control. When used individually, AC-PACs at the lowest concentration tested (25 μg/mL) significantly reduced the secretion of G-CFS (42%), GRO-a (33%), IL-8 (39%), IP-10 (72%), and MCP-1 (72%), but had no significant effect on the secretion of IL-6, while EGCG at 5 μg/mL significantly reduced the secretion of GRO-a (13%), IL-8 (34%), IP-10 (22%), and MCP-1 (70%), but had no significant effect on the secretion of G-CSF and IL-6. AC- PACs and LL-37 acted in synergy to reduce the GRO-a, G-CSF, and IL-6, and had	(Telma Blanca Lombardo Bedran, Palomari Spolidorio, and Grenier 2015)	[23]

			an additive effect on reducing the secretion of IL-8, IP-10, MCP-1. EGCG and LL-37 acted in synergy to reduce the secretion of GRO-a, G-CSF, IL-6, IL-8, and IP-10, and had an additive effect on MCP-1 secretion. *A. actinomycetemcomitans* LPS also induced the secretion of MMPs and TIMPs by the 3D co-culture model, although none of the concentrations of AC-PACs and EGCG tested, individually or in combination decreased the secretion of MMPs (-1, -2, -3, -7, -8, -9, 10, -12 and -13) or TIMPs (-1, -2, -3 and -4).		
The commercial black tea extract (with theaflavin content of 40.23%); theaflavin, theaflavin-3,3`-digallate, epigallocatechin-3-gallate EGCg	The oral epithelial cells (OBA-9)	The epithelial cells were pre-treated with the black tea extract, theaflavin, theaflavin-3,3′-digallate or EGCg prior to being stimulated with *A. actinomycetemcomitans* LPS. ELISA assays were used to quantify the secretion of IL-8, hBD-1, hBD-2 and hBD-4 by oral epithelial cells.	The black tea extract (200 μg/ml), as well as theaflavin and theaflavin-3,3′-digallate (50 μg/ml) reduced IL-8 secretion by 85%, 79%, and 86%, respectively. EGCg used as a positive control reduced IL-8 little stronger. The secretion of all three antimicrobial peptides hBDs was dose-dependently up-regulated. Only theaflavin did not induce the secretion of significant amounts of hBDs by oral epithelial cells.	(Telma Blanca Lombardo Bedran et al. 2015)	[24]
The commercial green tea extract with polyphenol content ≥ 98%, including 45% epigallocatechin-3-gallate EGCg	The immortalized human gingival epithelial cell line, B11.	Gingival epithelial cells were treated with various amounts (25–200 μg/mL) of green tea extract or EGCg. The secretion of hBD1 and hBD2 was measured using ELISAs, and gene expression was quantified by real-time PCR. The ability of green tea extract and EGCG to prevent hBD degradation by proteases of *P. gingivalis* present in a bacterial culture supernatant was evaluated by ELISA	Green tea extract and EGCg dose-dependently induced the secretion of hBD1 and hBD2 from gingival epithelial cells. They increased expression of the *hBD* gene in gingival epithelial cells. Green tea or EGCg-induced secretion of hBD1 and hBD2 appeared to involve ERK1/2 and p38 MAPK. Green tea extract and EGCg prevented the	(T B Lombardo Bedran et al. 2014)	[25]

			degradation of recombinant hBD1 and hBD2 by a culture supernatant of *P. gingivalis*.		
Non-dialyzable material (NDM) prepared from concentrated cranberry (*Vaccinium macrocarpon*) juice , containing 65.1% proanthocyanidins.	The Smulow–Glickman (S-G) human gingival epithelial cell line	S-G cells were incubated with IL-1β in the presence or absence of NDM or inhibitors of NF-κB, (NBD) or AP-1 (SP600125), and IL-6 levels were measured by ELISA kit. Effects of NDM on IL-1β -activated NF-κB and AP-1 and phosphorylated intermediates in both pathways were measured in cell extracts via binding to specific oligonucleotides and specific sandwich ELISAs	IL-1β caused a time- and dose-dependent stimulation of S-G epithelial cell IL-6 production. NDM appeared to be a stronger inhibitor of IL-1β-stimulated IL-6 production than either NBD peptide or SP600125 alone and was similar to inhibition caused by the combination of the NBD peptide and SP600125. IL-1β stimulated NF-κB and AP-1 activation, which was inhibited by NDM. However, NDM did not significantly affect IL-1β-stimulated levels of phosphorylated intermediates in the NF-κB pathway (IκBα) or the AP-1 pathway (c-Jun, ERK1/2).	(Tipton, Carter, and Dabbous 2014)	[26]
same as above	The Smulow–Glickman (S-G) human gingival epithelial cell line; normal human gingival fibroblasts	S-G and normal human gingival fibroblasts were incubated with NDM, IL-17, or NDM + IL-17. IL-6 and IL-8 in culture supernatants were measured by ELISA.	In both cell lines, IL-17 significantly stimulated production of IL-6 and IL-8. Non-toxic levels of NDM (5–50 μg/ml) inhibited constitutive IL-6 and IL-8 production as well their IL-17-stimulated cytokine production by epithelial cells and fibroblasts.	(Tipton et al. 2013)	[27]
same as above	Human gingival fibroblast cell line derived from a patient with Aggressive periodontitis (AgP);	AgP or normal fibroblasts were incubated with NDM or LPS (from *Fusobacterium nucleatum* or *Porphyromonas gingivalis*) ± NDM. Membrane damage and viability were assessed by enzyme activity released into cell supernatants and MTT assay, respectively. Secreted IL-6 and MMP-3 were measured by ELISA. NF-κB p65 was	NDM ≤ 100 μg/ml had no significant effect on AgP gingival fibroblast viability, but higher concectration decreased their viability. Short-term exposure to NDM, or LPS ± NDM caused no membrane damage. NDM (50 μg/ml) inhibited LPS-stimulated p65 and constitutive or LPS- stimulated	(Tipton, Babu, and Dabbous 2013)	[28]

	Normal human gingival fibroblast cell lines (GN23, GN56, GN60)	measured via binding to an oligonucleotide containing the NF-κB consensus site.	MMP-3 in AgP fibroblasts. NDM increased IL-6 in LPS-stimulated AgP fibroblast, but decreased in normal human gingival fibroblast.		
Non-dialyzable material (NDM) prepared from concentrated cranberry (*Vaccinium macrocarpon*) juice , rich in proanthocyanidins.	RAW 264.7 mouse macrophages,	RAW 264.7 mouse macrophages infected with *P. gingivalis* and *F. nucleatum* were exposed to culture media with or without NDM. The secreted form of mouse TNF-α was quantified using two-site ELISA. Macrophage functionality was investigated using a phagocytosis assay.	NDM eliminated TNF-α expression by macrophages that were exposed to *P. gingivalis* and *F. nucleatum*, without impairing their viability. Furthermore, NDM increased the phagocytosis of *P. gingivalis*, but mild attenuation of the phagocytosis of *F. nucleatum.*	(Polak et al. 2013)	[29]
(-)-Epigallocatechin gallate (EGCG)	Human gingival fibroblasts (HGFs)	HGFs were cultured in the presence or absence of EGCG (3.125, 6.25, 12.5, 25, or 50 μg/ml) prior to their incubation with IL-1β+IL-4 or or TNF-α+IL-4- stimulations, then the CCL11 concentrations were measured with ELISA. To confirm the effects of EGCG on IL-1β+IL-4 or TNF-α+IL-4-induced phosphorylation of signal transduction molecules, Western blot analysis was performed.	IL-4 synergistically enhanced CCL11 production in IL-1β or TNF-α-stimulated HGFs. EGCG prevented IL-1β/ IL-4 or TNF-α/IL-4-mediated CCL11 production in a concentration dependent manner. CCL11 production in HGFs was positively regulated by p38 MAPK, ERK, and JNK. EGCG treatment prevented IL-1β/IL-4 or TNF-α/IL-4-induced ERK and JNK activation in HGFs but not p38 MAPK.	(Hosokawa et al. 2013)	[30]
A-type cranberry proanthocyanidins (APAC) and Licochalcone A (LA)-chalcone, not proanthocyanidin	U937 human monocytes differentiated to macrophages.	IL-1 β, TNF-α, IL-6 and IL-8 production by macrophages treated with the APAC (or/and LA) and stimulated by *A. actinomycetemcomitans* LPS was evaluated by ELISA kits. Influence of APAC (or/and LA) on MMP-9 and *P. gingivalis* collagenase activities was measured by fluorometric assays.	ACPAC in 25 or 50 μg/ml concentration reduced the LPS-induced secretion of TNF-α, IL-6 and IL-8 in a macrophage model, but not IL-1 β. A significant reduction in IL-1 β secretion was seen when ACPAC was used together with LA. ACPAC (25 μg/ml) inhibited MMP-9 activity by 32 % and *P. gingivalis* collagenase by 66 %.	(Feldman and Grenier 2012)	[31]

		Fmacrophages viability was evaluated by an MTT assay			
AC-PACs fraction from cranberries *(Vaccinium macrocarpon)*	Human osteoclast precursor cells	This study investigated the effect of AC-PACs on osteoclast formation and bone resorption activity. The degree of osteoclast formation was evaluated by quantification of TRAP-positive stained multinucleated cells, while the secretion of IL-8 and MMP-2, MMP-9 was measured by ELISA. Bone resorption activity was investigated by using a human bone plate coupled with an immunoassay that detected the release of collagen helical peptides. Cytotoxic effect of AC-PACs on osteoclastic cells was measured by the MTT assay.	AC-PACs at 10, 25 and 50 µg/mL caused a 38%, 84% and 95 % inhibition of RANKL-dependent osteoclast differentiation, respectively. AC-PACs increased the secretion of IL-8 and inhibited the secretion of both MMP-2 and MMP-9 in a dose-dependent manner. AC-PACs significantly decreasing the release of collagen helical peptides suggested that can prevent bone resorption. AC-PACs did not exhibit any toxic effect on osteoclastic cells ranging from 10 to 100 µg/mL.	(Tanabe et al. 2011)	[32]
50% EtOH extract from *Myrothamnus flabellifolia* (MF)	KB cells (ATCC CCL-17, HeLa)	KB cells were pre-treated with MF (10 and 100 µg/ml) and infected with *P. gingivalis*. The influence of MF on *P. gingivalis*-induced cytokine gene expression was monitored by RT-PCR and IL-6 titres by ELISA.	10 and 100 µg/ml of MF significantly decreased (upregulated by *P. gingivalis)* gene expression for IL-1β, IL-8 and TNF-α, but not IL-6 compare to control cells (not exposed to MF). However, pre-incubation of the KB cells with MF before exposure to *P.gingivalis* resulted in significant lower concentration of IL-6 in the cells than in MF-untreated control group.	(Löhr et al. 2011)	[33]
Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG)	Human gingival fibroblasts (HGFs) isolated from healthy gingiva	HGFs were cultured in the presence or absence of EGCG or ECG prior to their incubation with Oncostatin M (OSM), then the CXC chemokine ligand 10 (CXCL10) concentrations of the culture supernatants were measured with ELISA. The effects of EGCG and ECG on the p38 MAPK, JNK, Akt and STAT3 phosphorylation induced by OSM in HGFs was measured using Western blotting analysis with antibodies.	EGCG or ECG (50 µg/ml) significantly inhibited (about 60%) the CXCL10 production induced by OSM treatment. EGCG (50 µg/ml) significantly prevented OSM-induced phosphorylation of JNK, Akt (Ser473) and STAT3 (Tyr705 and Ser727), whereas ECG (50 µg/ml) prevented phosphorylation of JNK and Akt (Ser473).	(Hosokawa et al. 2010a)	[34]

		The effects of EGCG or ECG on OSMRβ expression on HGFs were measured using flow cytometry	EGCG and ECG attenuated OSMRβ expression on HGFs.		
Epigallocatechin gallate (EGCG), epicatechin gallate (ECG), theaflavin-3,3′-digallate (TFDG)	Human gingival fibroblasts (HGFs) isolated from healthy gingiva	HGFs were cultured in the presence or absence of EGCG, ECG, TFDG (5 or 50 μg/ml) prior to their incubation with TNFSF14, then the IL-6 concentrations of the culture supernatants were measured with ELISA. The effects of EGCG, ECG, TFDG on MAPKs and NF-κB pathways in TNFSF14-stimulated HGFs were measured using Western blotting analysis with antibodies. The effects of EGCG, ECG, TFDG on TNFSF14 receptor expression (HVEM and LTbR) on HGFs were measured using flow cytometry	Treatment with 50 μg/ml of EGCG, ECG, or TFDG significantly inhibited IL-6 production in TNFSF14-stimulated HGFs, without harming cells. EGCG, ECG and TFDG inhibited TNFSF14-induced ERK, JNK, and NF-κB activation and suppressed TNFSF14 receptor expression in HGFs. It is supposed that EGCG, ECG, and TFDG suppressed IL-6 production from TNFSF14-stimulated HGFs through the inhibition of ERK, JNK, or NF-κB activation.	(Hosokawa et al. 2010b)	[35]
A-Type Cranberry Proanthocyanidins (AC-PACs) were isolated from cranberries (*Vaccinium macrocarpon*)	Oral epithelial cells (GMSM-K)	The epithelial cells were pretreated with increasing concentrations of AC-PACs (25 - 100 μg/mL) before the stimulation with *P. gingivalis*. ELISA kits were used to quantify IL-6, IL-8, and chemokine (C-C motif) ligand 5 (CCL5) concentrations in the free-cell supernatants. To understand the mechanism of action of AC-PACs, their effect NF-κB p65 activation was investigated.	AC-PACs significantly decreased the secretion of IL-8 and CCL5 at all concentrations tested in a dose-dependent manner, where 100 μg/mL reduced secretion of IL-8 and CCL5 by more than 80%. Decreased secretion of IL-8 and CCL5 was not related to loss of cell viability. AC-PACs did not affect the secretion of IL-6. A pretreatment of epithelial cells with 50 μg/mL of AC-PACs prior to the stimulation with *P. gingivalis* significantly decreased the *P. gingivalis*-induced activity of NF-κB p65 to 91%	(Vu Dang La, Howell, and Grenier 2010)	[36]
same as above	Human monocyte-derived macrophages	Investigate the effects of AC-PACs on: (1) the production of various MMPs by human monocyte-derived macrophages stimulated with *A. actinomycetemcomitans* LPS- using ELISA combined with piezoelectric printing technology,	No toxic effects toward macrophages were detected following a 24-hour treatment with100 μg/ mL of AC-PACs. AC-PACs significantly reduced the production of MMP-7, MMP-8, and MMP-13	(V D La, Howell, and Grenier 2009)	[37]

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		(2) the catalytic activity of recombinant MMP-1 and MMP-9, (3) the expression of 5 protein kinases and the activity of nuclear factor-kappa B (NF-κB) p65 in macrophages stimulated with LPS -using commercial kits. Determination of cytotoxicity was using MTT assay.	from stimulated macrophages at all concentrations tested (25, 50, and 100 µg/mL), whereas production of MMP-3 reduced significantly only at the highest 100 µg/mL and MMP-1, MMP-9 at 50, and 100 µg/mL of AC-PACs. The catalytic activity of MMP-1 and MMP-9 was also inhibited. The inhibition of MMP production was associated with reduced phosphorylation of key intracellular kinases and the inhibition of NF-κB p65 activity.		
Epigallocatechin-3-gallate (EGCG)	MG-63, a human osteosarcoma cell line.	MG-63 cells were incubated with OSM (oncostatin M) alone or in combination with 10 µg/mL EGCG (before the addition of OSM). The levels of Cyr61 was measured using Western blot analysis. Moreover, MG-63 cells were treatment with Cyr61 and level of CCL2 was measuered ELISA kit.	OSM stimulated Cyr61 synthesis in MG-63 cells in a time- dependent manner, whereas EGCG readily attenuated this effect. Cyr61 treatment of MG-63 cells induced the release of CCL2.	(Y.-L. Lee et al. 2009)	[38]
fraction from Cranberries (*Vaccinium macrocarpon*) , obtained after dialysis; Non-dialysable material (NDM) contains 65.1% proanthocyanidins.	Human gingival fibroblasts (HGF-1), U937 human monocytes differentiated to macrophages.	MMP-3 and MMP-9 production by HGF-1 and macrophages treated with the cranberry fraction and then stimulated with LPS from *A. actinomycetemcomitans* was measured by ELISA kits. MMP- 3, MMP-9 and elastase activities in the presence of the cranberry fraction were evaluated using colorimetric or fluorogenic substrates. The changes in expression and phosphorylation state of fibroblast intracellular signaling proteins induced by LPS and the cranberry fraction were characterized by antibody microarrays. MTT assay for cells.	MMP-3 and MMP-9 production by macrophages after treated with the cranberry fraction and stimulated with LPS were inhibited significnatly ,in a dose-dependent manner, similary MMP-3 production by fibroblast. However, MMP-9 response after stimulated LPS wasn't observed. Cranbery fraction wasn't toxic towards fibroblast and macrophages. Cranberry fraction inhibited fibroblast intracellular signaling proteins, a phenomenon that may lead to a down-regulation of activating protein-1 activity.	(C Bodet, Chandad, and Grenier 2007)	[39]

			MMP-3, MMP-9 and elastase activities were significantly inhibited by cranberry fraction even at low concentration -10 μg/mL (about 50%).		
same as above	Human gingival fibroblasts HGF-1	Interleukin (IL)-6, IL-8, and PGE2 production by fibroblasts treated with the cranberry fraction (10, 25 or 50 μg/mL) and stimulated by *A. actinomycetemcomitans* LPS was evaluated by ELISA. Changes in the expression and phosphorylation state of fibroblast intracellular signaling proteins were characterized by antibody microarrays. Fibroblast viability was evaluated by an MTT assay.	The LPS-induced IL-6, IL-8, and PGE2 responses of gingival fibroblasts were inhibited by cranberry fraction at concentrations 10-50 μg/mL. At a final concentration of 50 μg/mL cranberry fraction completely inhibited the IL-8 production, whereas a 72% inhibition was noted at a concentration of 10 μg/mL. The PGE2 and IL-6 response of fibroblasts induced by LPS was also significantly reduced by cranberry treatments in range 25 and 50 μg/mL. The results suggest that the cranberry fraction can act by reducing the activator protein-1 (AP-1) activity. Cranberry components also reduced cyclooxygenase 2 expression. Cranberry fraction (10-50 μg/mL) had not cytotoxic effect towards fibroblast.	(Charles Bodet, Chandad, and Grenier 2007)	[40]
Water-alcohol Grape Seed Extract (GSE) from red grape seeds containing 95% oligomeric proanthocyanidins (PAs), gallic acid (GA) , epigallocatechin gallate (EGCG)	The murine macrophages cell line RAW 264.7	Cells were preincubated with non-toxic concentrations of GA (4 μg/ml), EGCG (0.5 μg/ml), or GSE (4 μg/ml) and stimulated with LPS of *A. actinomycetemcomitans*, *F. nucleatum*. iNOS expression was evaluated by immunoblotting, NO production was quantified using the colorimetric Griess assay, whereas ROS production was measured with the fluorescent 123-dihydrorhodamine dye.	GSE as well EGCG strongly decreased NO and ROS production and iNOS expression by LPS-stimulated macrophages. GA also revealed a strong inhibitory effect on NO production without affecting iNOS expression but slightly increasing ROS production.	(Houde, Grenier, and Chandad 2006)	[41]

EGCG - (-)-epigallocatechin gallate	Mouse calvarial primary osteoblastic cells	The effect of EGCG on the gene expression of MMPs was examined by treating mouse calvarial primary osteoblastic cells with EGCG (20 μM) in the presence of sonicated *P. gingivalis* extracts. The transcription levels of MMP-2, -9 and -13 were assessed by (RT- PCR). The effect of EGCG on osteoclast formation was confirmed by (TRAP) staining in a co-culture system of mouse bone marrow cells and calvarial primary osteoblastic cells.	Treatment with the sonicated *P. gingivalis* extracts stimulated only the expression of MMP-9 mRNA (215% increase) and this effect was significantly reduced by EGCG, reaching the same level of expression of MMP-9 mRNA as that observed in the untreated cells. The transcription levels of MMP-2 and MMP-13 were not affected by either the sonicated *P. gingivalis* extracts or EGCG. EGCG significantly inhibited osteoclast formation in the co-culture system.	(JH et al. 2004)	[42]
fraction of green tea polyphenols (GTP), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (+)-catechin (C)	MMP-12 was partially puried from the conditioned medium of the mouse macrophage cell line NCTC 3749, Human proMMP-2 and mouse proMMP-9 were activated with APMA (aminophenyl-mercurin acetate)	GTP and 5 catechins were tested for their ability to inhibit matrix metalloproteinase (MMP)-2, MMP-9 and MMP-12 activities, measured by fluorescence and by gelatin or casein zymography. In addition, the activation of proMMP-2 by the lectin Con A was determined following exposure to GTP.	IC_{50} values for the inhibition of MMP-2 and MMP-9 activities was 10 μg/ml and 0.6 μg/ml for GTP, 95 μM and 28 μM for ECG and 6 μM and 0.3 μM for EGCG, respectively. MMP-12 was inhibited more than 60% by 1 μM of ECG or EGCG. MMP-2, MMP-9 and MMP-12 activities were unaffected by C, EC and EGC. The activation of MMP-2 by Con A was reduced by 50% at 17.5 μg/ml of GTP and was almost completely inhibited at 35 μg/ml. Among catechins (at 100 μM), only EGCG inhibited the activation of MMP-2 by Con A. The activation of proMMP-2 by EGCG was inhibited in a dose-dependent manner. EGCG at 25 μM completely abolished the activation of proMMP-2 by Con A.	(Demeule et al. 2000)	[43]

Elm extract (EE) (n-butanol fraction from extract of *Ulmi macrocarpi cortex*) containing 20% of procyanidins) and the mixture of procyanidin oligomers (PO)	Gingival crevicular fluid (GCF) collected from periodontitis patients; Cultures of periodontal ligament (PDL) cells treated with *Treponema lecithinolyticum.*	The inhibitory effect of EE and PO on the MMPs in GCF (mostly MMP-8 and MMP-9) were assessed by gelatin zymography. The MMP-2 was verified by immunoblotting. Effects of EE and PO on cell proliferation testing by MTT assay.	EE and PO inhibited activity of MMPs in GCF (most abundant in MMP-8 and MMP-9), as well pro and active forms of MMP-2. PO was more effective than the EE. The IC50 values of the EE were 29 and 45 μg/ml for GCF collagenases (mostly MMP-8 and MMP-9 detected in GCF) and MMP-2, respectively. The corresponding IC50 values of the PO were 25 and 33 μg/ml, respectively. Contrary to PO, the elm extract at concentrations of 0.05–0.1% had cytotoxic effect towards PDL cells.	(Song et al. 2003b)	[10]
Ethyl acetate fraction from the tea leaf (*Camellia sinensis.*) (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECg), (-)-epigallocatechin gallate (EGCg).	Gingival crevicular fluid (GCF) collected from periodontitis patients; purified collagenase;*in vitro* study	Ethyl acetate fraction and 6 isolated catechines were tested for their ability to inhibit purified collagenase activities using collagenase of *Clostridium histolyticum* and supernatant of *Porphyromonas gingivalis* as well collagenase activity in GCF. Collagenase activity was determined using a commercially available kit.	Total inhibition of the collagenase activity was achieved by 100 μg/ml of ethyl acetate fraction from tea and 50 μg/ml (-)-epicatechin gallate (ECg), (-)-epigallocatechin gallate (EGCg). Other catechins, without the gallate residue had no effect on collagenase.	(Makimura et al. 1993)	[44]

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Particularly, the *n*-butanol fraction from the *Ulmus macrocarpa* Hance bark, defined as elm extract (contain 20% of procyanidins) and the mixture of procyanidin oligomers (composed of 3 to 12 flavan-3-ol monomers, an average molecular weight of 1518) isolated from elm extract in range 100-1,000 μg/ml exhibited inhibitory effects on the MMPs, present in gingival crevicular fluid (GCF) of adult periodontitis patients (mainly, MMP-8 and MMP-9) and on the pro and active forms of MMP-2 (from the conditioned media of cultured periodontal ligament (PDL) cells treated with a periodontopathogen, *Treponema lecithinolyticum*) [10]. The inhibition of enzyme activity by procyanidin oligomers was more effective than by the elm extract, with IC50 values 25 and 33 μg/ml for GCF collagenases (mostly MMP-8 and MMP-9) and MMP-2, respectively. Moreover, elm extract and procyanidin oligomers inhibited proteolytic enzymes of two periopathogens, *T. denticola* and *P. gingivalis* responsible for degradation of the interstitial and basement membrane collagens as well as activating of matrix metalloproteinases e.g. MMP-8, MMP-9 or MMP-1, MMP-3 and MMP-9 [10].

Free (non-polymerized) galloylated flavan-3-ols such as (-)-epicatechin gallate and (-)-epigallocatechin gallate (EGCG) from green tea also inhibited collagenase activity, achieved total inhibition at 50 μg/ml [44]. Other tested compounds, without the gallate residue such as catechin, epicatechin, gallocatechin, and epigallocatechin had no effect on collagenase. Makimura reported also that ethyl acetate fraction from tea leves (*Camellia sinensis*), which contained six above catechins inhibited collagenolytic proteases in gingival crevicular fluids (GCF) and in culture supernatants of *P. gingivalis* [44]. Similar results were observed by Demeule et al. [43]. Green tea polyphenols, especially (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG), inhibited matrix metalloproteinase (MMP)-2, MMP-9, MMP-12 and proMMP-2 activities in the range of micromolar concentrations. In the following years, more studies confirmed the inhibitory effects of proanthocyanidins and its galloylated monomers on production and/or activity of matrix metalloproteinases MMPs (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13) involved in periodontitis (Table 1). Among them were proanthocyanidins and gallate catechins from green tea [21], [42], theaflavins from black tea [20], A-Type cranberry proanthocyanidins (AC-PACs) isolated from cranberries(*Vaccinium macrocarpon* fruits) [37], [39], [31], [32], [28], proanthocyanidins from blueberries of two North American species - *Vaccinium corymbosum* [19], and (*V. angustifolium* [22] .

In opposition to the above results are results obtained by Lombarto et al. who showed [23] that A-type cranberry proanthocyanidins (AC-PACs) and epigallocatechin-3-gallate (EGCG), individually or in combination, had no effect on the regulation of MMP (-1, -2, -3, -7, -8, -9, 10, -12 and -13) and tissue inhibitors of metalloproteinases -TIMP (-1, -2, -3 and -4) secretion but inhibited the secretion of several cytokines in the (3D) co-culture model of gingival epithelial cells and fibroblasts stimulated with *A. actinomycetemcomitans* LPS (Table 1).

2.2. Influence on bone tissue resorption

Yun et al.[42] reported an inhibitory effect of (-)- epigallocatechin gallate (EGCG) (20 μM) on the gene expression of MMP- 9 in osteoblasts and on the formation of osteoclasts, what suggested that EGCG may prevent the alveolar bone resorption that occurs in periodontal diseases leading to teeth loss. Importantly, in the periodontal disease an enhanced osetoclastogenesis can occur due to the presence of the of inflammatory cytokines that stimulates osteoclast proliferation or promotes the differentiation of progenitor cells. Mature osteoclasts that derive from hematopoietic monocyte/macrophage precursors under the action of RANKL (receptor activator of nuclear factor kappa-B ligand) and M-CSF (macrophage colony-stimulating factor) mediate the destruction of the alveolar bone by attaching to the bone surface and promoting mineral dissolution. The demineralized bone matrix is later degraded by proteases such as cathepsin K and metalloproteinases (MMPs) [32]. Tanabe et al. [32] showed that A-type cranberry proanthocyanidins (AC-PACs) have influence the osteoclast formation and bone resorption activity. In a range of 10-100 μg/ml, AC-PACs inhibited RANKL-dependent osteoclast differentiation, as well as secretion of both MMP-2 and MMP-9 but secretion of IL-8 was increased. IL-8 from normal human bone marrow stromal cells inhibits the bone resorbing activity of osteoclasts. [45]

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Huang et al. [13] reported that proanthocyanidins (PA) may contribute to bone generation in inflammatory microenvironment via suppressing NF-κB signaling pathway and therefore may be a potential inducer of periodontal bone regeneration. In this study an effect of PA on osteogenic differentiation of human periodontal ligament fibroblasts (PDLFs) with or without TNF-α stimulation was tested and the underlying mechanism was explored. The assumption was that PDLFs are capable of differentiating into osteoblasts, but pro-inflammatory cytokines like TNF-α inhibit this process. Osteogenic differentiation- and mineralization-associated markers were detected by qRT-PCR, alizarin red S staining, and alkaline phosphatase (ALP) activity assay. In result, PA in low concentration (0.1, 1, 10 μg/ml) significantly upregulated expression of osteogenesis-related genes and proteins and ALP activity in PDLFs compared with the control. However, PA at higher concentrations of 30 and 50 μg/ml significantly suppressed the ALP activity of PDLFs. For the rest assay, authors used only lower concentration of PA -(0.1, 1, 10 μg/ml). PA in concentration of 1μg/ml significantly reversed inhibition of osteogenesis-related gene and protein expression, ALP activity, and mineralization caused by TNF-α. The authors also suggesteded that PA may reverse TNF-α inhibited osteogenic differentiation via NF-κB signaling pathway.

These authors used commercial proanthocyanidins claimed to possess an untypical for proanthocyanidins structure (figure 1) and with molecular weight =594.52. The supplier's website states that proanthocyanidins have been isolated from grapes (the fruits of *Vitis vinifera* L.)

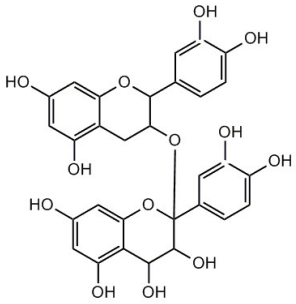

Figure 1. Structure of untypical proanthocyanidins isolated from fruits of *Vitis vinifera* used in the study of Huang et al [2].

2.3. Influence on cytokines

The overproduction and secretion of inflammatory cytokines by resident and immune cells modulate the progression and severity of periodontitis. Increase of such proinflammatory cytokines as: IL-1α, IL-1β, TNF-α, IL-6, and IL-17 were shown in patients with acute or chronic periodontitis [46]. More specifically, TNF-α is present at high levels in gingival crevicular fluid (GCF) and in diseased periodontal tissues, where it is positively correlated with MMPs and RANKL expression. Human and animal studies confirmed that TNF-α plays a central role in inflammatory reaction, alveolar bone resorption, and the loss of connective tissue attachment. Moreover, TNF-α up-regulates the production of other pro-inflammatory innate immunity cytokines, such as IL-1β and IL-6 associated with inflammatory cell migration and osteoclastogenesis [46]. IL-1β plays an important role in the pathogenesis of periodontitis also by regulation of the IL-6 production in a variety of cell types, including fibroblasts and epithelial cells [26]. Similarly to bacterial LPS, cellular response to cytokines or chemokines (e.g. IL-1β) can be mediated via signaling cascades, including NF- κB and MAPK/AP-1 pathways, which lead to gene expression of certain proteins (for example IL-6). There are more and more studies proving that proanthocyanidins and flavan-3-ols inhibit the secretion of cytokines by influencing NF-κB and MAPK/AP-1 activation (table 1), [14], [13], [18], [19], [20], [22], [37], [26], [28].

Many studies have shown inhibition of production and / or secretion of inflammatory cytokines by proanthocyanidins. Bodet et al. [40] demonstrated that proanthocyanidin-enriched cranberry fraction at concentrations 25-50 μg/ml, significantly inhibited the IL-6, IL-8, and PGE_2 production by gingival fibroblasts stimulated with the *Aggregatibacter actinomycetemcomitans* lipopolysaccharide (LPS) . The

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most spectacular inhibitory effect was seen towards IL-8 that belongs to chemokines (CXCL8) also known as neutrophil chemotactic factor. It directs the migration of polymorphonuclear leukocytes, monocytes, and macrophages to the site of infection. Increased level of IL-8 was observed in the gingival crevicular fluid of inflamed periodontal sites [40]. PGE_2 is another proinflammatory molecule involved in destructive process in periodontal disease. It is secreted in response to pro-inflammatory cytokines, periodontopathogens and LPS. The cranberry fraction significantly inhibited PGE2-response even at low tested concentration-25 μg/ml, and reduced COX 2 protein expression- the enzyme involved in PGE2 production. Moreover, cranberry fraction influence on the phosphorylation and expression of various intracellular proteins (Jun, Fos, MKK3, MKK6, Rac1, Mnk1) which are implicated in cytokine production. Bodet et al. concluded that cranberry fraction may act especially via a downregulation of AP-1 activity [40]. Feldman and Grenier [31] showed an inhibitory effect of 25 or 50 μg/ml of A-type cranberry proanthocyanidins (APAC) on TNF-α, IL-6, and IL-8 secretion in a macrophage model. The 50 μg/ml concentration of APAC reduced the LPS-induced secretion of TNF-α, IL-6 and IL-8, by about 50%, but had not influence on IL-1 β. A significant reduction in IL-1 β secretion was seen when ACPAC was used together with Licochalcone A (chalcone, not proanthocyanidin). Further studies on proanthocyanidins, in the predominant amount on A-type cranberry proanthocyanidins, prove their influence on the secretion and production of interleukins, as well as provided explanation of molecular mechanisms responsible for this activity [12], [17], [23], [26], [27], [28] [29], [36] (Table 1).

Galarraga-Vinueza et al. [12], revealed that cranberry concentrate from capsules (Uriach-Aquilea OTC) containing 130 mg A-Type PACs significantly decreased M1 polarization and increased M2 polarization in LPS-stimulated macrophages. M1 phenotype of macrophage are activated by bacteria sub-products like lipopolysaccharides (LPS) and are associated with the secretion of pro-inflammatory cytokines (e.g. IL-1ß, IL-6, IL-8), whereas a M2 phenotype of macrophages are activated by alternative ways and are associated with the secretion of anti-inflammatory cytokines (e.g. IL-10) and growth factors which enhance tissue repair. Galarraga-Vinueza et al. [12] confirmed the effect of A-Type PACs (50 and 100 μg/mL) on cytokine expression - proinflammatory cytokines: IL-8 and IL-6 were significantly downregulated in LPS-stimulated macrophages and A-Type PACs, whereas an anti-inflammatory IL-10 was upregulated. No influence on expression of IL-1 ß was seen. Lagha et all. [17] showed that fraction of proanthocyanidins (PACs) from cranberries at a concentration of 15.625-125 μg/mL markedly reduced cytotoxicity of leukotoxin on macrophages and significantly reduced (by about 80-90% at 15.625 and more than 98% at 125 μg/mL) release of caspase-1, IL-1β, and IL-18 from LtxA-induced macrophages. Leukotoxin (LtxA), released by *A. actinomycetemcomitans* is one of the important virulence factor playing a critical role in the pathogenic process of localized aggressive periodontitis (LAP). LtxA affects immune cells by activates pyroptosis of monocytes and macrophages and inducing the release of pro-inflammatory cytokines. Pyroptosis in turn, is inflammatory form of programmed cell death, involves the activation of caspase-1, which in turn covert of pro-IL-1β and pro-IL-18 to the biologically active forms. Pyroptosis in macrophages leads to the formation of pores in the plasma membrane which allow secretion of IL-1β and IL-18, cytokines known as damage-associated molecular patterns (DAMPs) and contribute to the progression of periodontitis by increasing cell migration and osteoclastogenesis [47], [48]. Moreover, PACs reduced the expression of CIAS and P2X7 genes (increase by LtxA, in macrophages) by about 30-45%, similarly for a range 15.625-125 μg/mL [17]. This is important because the activation of P2X7 receptors and CIAS leads to the rapid formation of membrane pores and to the release of IL-1β and IL-18. Lastly, cranberry PACs blocked the binding of leukotoxin (LtxA) to macrophages as well reduced ROS and superoxide production in LtxA-induced macrophages.

Cranberry proanthocyanidins (PACs) can have differently affect interleukines secretion/production, depending on a cell type. In LPS-stimulated normal human gingival fibroblast, cranberry non-dialyzable material (NDM) rich in proanthocyanidins decreased level of IL-6, what is consistent with other studies, but NDM significantly increased IL-6 in LPS-stimulated human gingival fibroblast cell line derived from a patient with aggressive periodontitis (AgP fibroblasts) [28]. This increasing level of IL-6 occurred only in the presence of LPS; NDM alone did not

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significantly increase constitutive IL-6 production. Simultaneously, NDM inhibited NF-κB activity (increased by LPS treatment) in AgP fibroblasts what suggested involvement of other mechanisms of IL-6 regulation in these cells.

Influence of proanthocyanidins and flavan-3-ols from other source than cranberries on the secretion and production of interleukins was also demonstrated in several studies (table 1). Jekabsone et al. [15] reported that *Pelargonium sidoides* root extract (PSRE) and especially proanthocyanidin fraction from PSRE (PACN) possess strong antibacterial (against *Aggregatibacter actinomycetemcomitans)*, anti-inflammatory and gingival tissue protecting properties under periodontitis-mimicking conditions. The cells (gingival fibroblast, bone marrow-derived macrophages (BMDM) or human peripheral blood mononuclear cells (PBMCs) were stimulated using LPS (and IFNγ for BMDM) and treated with 50 and 100 ug/mL of PSRE or PACN. The extracts protected human gingival fibroblast from *A. actinomycetemcomitans* infection, decreased LPS-induced release of IL-8 and prostaglandin E2 from gingival fibroblasts and IL-6 from leukocytes, blocked expression IL-1β, iNOS and COX-2 but not TNF-α. Stronger anti-inflammatory activity of proanthocyanidin fraction (PACN) than root extract (PSRE) was associated with higher amounts of prodelphinidins. The study also reported that PSRE and PACN (100 μg/mL) blocked the surface presentation of CD80 and CD86 (surface markers of proinflammatory M1 phenotype) in LPS+IFNγ-treated macrophages, whereas PACN was characterized by stronger activity. These results indicate that both PACN and PSRE are potent in preventing macrophage conversion to proinflamatory M1 phenotype under exposure to LPS.

Low concentrations (7.9-62.5 μg/mL) of green and black tea extracts as well as their flavan-3-ols (epigallocatechin-3-gallate, theaflavins) have influence on production and secretion proinflammatory cytokines. They attenuate the gingival epithelial barrier dysfunction caused by TNF-α and modulate the inflammatory host response. They inhibited the activation of NF- κB and caspase-1 as well as reduced IL-1β secretion by macrophages (at 62.5 μg/mL by more than 94%, except black tea-64.5%), and secretion IL- 8 (only black tea required higher concentration than 62.5 μg/mL for more than 70% inhibition) by oral epithelial cells stimulated with recombinant TNF-α [16]. The green tea extract showed higher activity than black tea extract. Other studies have confirmed the inhibitory effect of flavan-3-ols obtained from tea on the secretion of pro-inflammatory cytokines from LPS stimulated macrophages as well from cytokines-stimulated gingival cells (table 1),[14], [23], [24], [35]. Ben Lagha et al. [20] presented consistent results in which they proved inhibitory effect theaflavins (TFs) from black tea on the secretion of pro-inflammatory cytokines from *P. gingivalis*-treated macrophages and on the activation of the NF-κB signaling pathway (table 1). Lombardo Bedran et al. [25], [24] in studies on green and black tea and their main galloylated flavan-3-ols revealed the ability of these compounds to induce human beta-defensin (hBD) secretion in gingival epithelial cells. Human beta-defensins (hBDs) are antimicrobial peptides secreted by gingival epithelium in response to periopathogens. They interact with the bacterial cell membrane, leading to pore formation and finally to the lysis of major periopathogens. Evidence indicated that level of hBDs are higher in healthy gingival tissues than in diseased gingival tissues and that some periodontopathogenic bacteria, such as *P. gingivalis*, are capable to down-regulate hBD expression by epithelial cells and/or to inactivate hBDs by proteolytic cleavage [25]. Both green and black teas and their galloylated flavan-3-ols stimulated secretion of hBDs and increased expression of the *hBD* gene in gingival epithelial cells as well as prevented the degradation of hBD1 and hBD2 by *P. gingivalis.* Again, the tested non – galloylated flavan-3-ols - theaflavins did not induce secretion of significant amounts of hBDs by oral epithelial cells.

In addition to the inhibitory effect of (-)-epigallocatechin gallate (EGCG) on innate immune response (e.g. IL-1, IL-6, TNF-α), an influence on adaptive immunity mechanisms (Th1, Th2, Th17, and Tregs) was demonstrated. Hosokawa et al. [30] showed influence of EGCG on Th2-type chemokines, such as CCL11 production. The EGCG in range 3.125- 50 μg/ml decreased CCL11 production in IL-1β/IL-4 or TNF-α/IL-4-stimulated human gingival fibroblasts (HGFs) in a concentration dependent manner (almost total reduction at 50 μg/ml) . Moreover, they demonstrated that ERK and JNK activations, related to CCL11 production in HGFs, are inhibited by EGCG

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treatment. The same group demonstrated an inhibitory effect of epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) on CXC chemokine ligand 10 (CXCL10) production (about 60% inhibition by 50 μg/ml) in human gingival fibroblasts (HGFs) stimulated oncostatin M (OSM) – cytokine belonging to the interleukin IL-6 family [34]. CXC chemokine ligand 10 (CXCL10) is a Th1-type chemokine which plays a key role in the recruitment of Th1 cells, and thus in the development of periodontal disease. It is supposed that EGCG and ECG suppressed production of CXCL10 through the inhibition of phosphorylation of signal transduction molecules like JNK, Akt and STAT3 phosphorylation as well as by suppressed OSMRβ expression in stimulated HGFs [34]. Influence on adaptive immunity mechanisms was also proved for cranberry AC-PACs [36]. AC-PACs significantly decreased the secretion of CCL5 from *P. gingivalis*-stimulated oral epithelial cells (100 μg/mL of AC-PACs reduced secretion of CCL5 and also IL-8 by more than 80%). The chemokine (C-C motif) ligand 5 (CCL5) has significant chemotactic activity for Th1 cells as well basophiles, eosinophiles, monocytes [36].

In addition to the above mentioned, there is a couple of other well studied sources of proanthocyanidins with proven anti-inflammatory activities linked with periodontitis.

Castanopsis lamontii water extract (CLE), (400 μg/mL) rich in epicatechin (EC) and procyanidin B2 (PB2) as well as EC (120 μg/mL) and PB2 (34.4 μg/mL) alone, significantly suppressed LPS-stimulated inflammation by inactivating the TLR-4/NF-κB/iNOS and TLR-4/NF-κB/COX-2 pathways [18]. All tested samples (CLE, EC, PB2) decreased the release of NO, PGE2, and TNF-α from stimulated-LPS mouse macrophage RAW264.7. PB2 showed much more potency in suppressing the LPS-stimulated inflammatory response than EC.

Ben Lagha et al. [19] proved inhibitory effect of proanthocyanidins isolated from highbush blueberry (*Vaccinium corymbosum*) on the secretion of pro-inflammatory cytokines from LPS-*A. actinomycetemcomitans*-treated macrophages and on the activation of the NF-κB signaling pathway. PACs at 125 μg/mL reduced the secretion of IL-1β, TNF-α, IL-6, and CXCL8 by 75.34%, 81.64%, 48.27% and 90.19%, respectively [19]. Similarly promising results were reported in the study on ethanolic lowbush blueberry extract (*Vaccinium angustifolium)* [22]. A pretreatment of macrophages with the blueberry extract (62.5μg/mL) and then stimulated with *F. nucleatum* inhibited the secretion of IL-1β, TNF-α, and IL-6 by 87.3, 80.7, and 28.2%, respectively. The secretion of the chemokine CXCL8 was affected by 500, 250, or 125 μg/mL of extract, decreased CXCL8 secretion by 79, 57.9, and 11.2 % respectively [22].

Proanthocanydin- enriched extract from *Myrothamnus flabellifolia*, plants traditionally used for treatment of gingivitis and periodontitis in South Africa, decreased gene expression of IL-1β, IL-8 and TNF-α, and level of IL-6 in KB cells, pre-incubated with MF (10 and 100 μg/ml) and infected with *P. gingivalis* [33].

2.4. Influence on reactive oxygen species (ROS) production

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) production by immune cells stimulated by periopathogens is an imortant factor in pathogenesis of periodontitis [41]. Their overproduction can lead to oxidative damage to gingival tissue, periodontal ligament, and alveolar bone. Study of Houde et al. [41] showed that the stimulation of macrophages with LPS of *A. actinomycetemcomitans* and *F. nucleatum* induces increased NO and ROS release. However, macrophages pretreated with non-toxic concentrations of grape seed extract (GSE) containing 95% oligomeric proanthocyanidins significantly inhibited free radical generation by inhibiting the production of the proinflammatory mediators NO and ROS and by modulating iNOS protein expression.

3. In vivo studies reporting influence proanthocyanidins or flavan-3-ols on periodontitis in animal models

Toker et al. [49] presented results which indicated that grape seed proanthocyanidin extract (GSPE) can decrease periodontal inflammation and alveolar bone loss via decreasing MMP- 8 and HIF-1α levels and increase osteoblastic activity in diabetic rats with experimental periodontitis (Table

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2). 100 and 200 mg/kg doses of grape seed proanthocyanidin extract (GSPE) administered by oral gavage to rats with induced diabetes (D) and periodontitis (P) significantly decreased alveolar bone loss, inflammatory cell numbers, MMP-8 and HIF-1α levels compared to rats with D+P but without GSPE. Moreover, the osteoblast number increased significantly in the GSPE groups compared to the P and D+P groups.

Cai et al. [50] study indicated that epigallocatechin-3-gallate (EGCG) alleviates *Porphyromonas gingivalis*-induced periodontitis in mice. The mice orally inoculated with *P. gingivalis* in PBS, received sterile food and drinking water with 0.02% solution of EGCG from 8 weeks to 15 weeks. EGCG significantly reduced alveolar bone resorption as well as decreased the high expressions (caused by *P. gingivalis* infection) of inflammatory cytokines and other mediators both in serum and in gingival tissue (details in the table 2) what is consist with previous study of Lee y et al. [38], in which EGCG suppressed the progression of apical periodontitis, by diminishing Cyr61 expression (a potential osteolytic mediator) in osteoblasts and, subsequently, macrophage chemotaxis into the lesions.

Cho et al. [51] observed decreased interleukin (IL)-6 and tumor necrosis factor (TNF) expression in rats tissue orally fed EGCG compare to group without EGCG. Downregulation of TNF and IL-6 expression by EGCG led to a decrease in osteoclast number and activity, which resulted in reduced bone loss. They also noticed reduced collagen destruction in EGCG group. Similar results achieved Lee at al. [14] studying catechin, another of major flavan-3-ols in green tea. They showed that catechin reduced the level of alveolar bone loss in a *P. gingivalis*-induced periodontitis mouse model. In turn, Polak et al. [29] showed that cranberry non-dialyzable material (NDM) consumption by mice infected by *P. gingivalis* and *F. nucleatum* attenuated the alveolar bone loss compared to the mice with infection but without NDM treatment. Moreover, in subcutaneous chamber model of inflammation, NDM alone reduced tumor necrosis factor-α (TNF-α) levels induced by the mixed infection. *In vivo* studies were supported by *in vitro* study (Table 1). Oral administration of commercial grape seed proanthocyanidins (PC) [52] to rats with experimentally induced periodontitis (EP) revealed that PC enhanced the host resistance and inhibited the oxidative stress. In serum, proanthocyanidins (PC) significantly decreased reactive oxygen species, lipid peroxides, lysosomal enzymes, acute phase proteins and they increased antioxidant levels. Histopathological evidence of experimental periodontitis without PC showed cellular infiltration of inflammatory cells whereas the proanthocyanidin treated groups demonstrated only scattered inflammatory cells.

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Tabel 2 *In vivo* studies reporting influence proanthocyanidins or flavan-3-ols on periodontitis in animal model.

Active compound/ extract/fraction	Animal model	Methods	Results	Author, Years	Ref.
Catechin	Six-week-old C57BL laboratory mice were divided into four groups: untreated normal control group, *P. gingivalis*-infected group *P. gingivalis*-infected + catechin group, catechin only-treated group.	In the *P. gingivalis*+catechin and catechin group, catechin (40 mg/kg body weight) was administrated orally to the mice 30 minutes before the *P. gingivalis* injection for 2 weeks, and subsequently every 2 days for an additional 2 weeks. The mice were euthanized and evaluated on day 49. For quantitative analysis of alveolar bone loss, themaxilla was examined using a microcomputed tomography (micro-CT) system.	In the *P. gingivalis*+catechin group (3), the bone loss area was reduced significantly compared with the periodontitis group (2).	(H. A. Lee et al. 2020)	[14]
A commercial Grape seed proanthocyanidine extract (GSPE) containing 50 mg polyphenols and 30 mg flavonoids in 100 mg	Animal study: 40 Wistar male rats: Control group (C group, 6 rats) Periodontitis group (P group, 6 rats) Diabetes group (D group, 6 rats) Diabetes and periodontitis group (D+P group, 6 rats) Diabetes, periodontitis and 100 mg/kg/day GSPE group (GSPE-100 group, 8 rats) Diabetes, periodontitis and 200 mg/kg/day GSPE group (GSPE-200 group, 8 rats)	100 and 200 mg/kg doses of GSPE were administered by oral gavage. After 30 days, all rats were killed. Alveolar bone loss was measured morphometrically via a stereomicroscope. For histopathological analyses, alizarin red staining, and matrix metalloproteinase (MMP)-8, VEGF and HIF-1α immunohistochemistry were performed. Tartrate- resistant acid phosphatase- positive osteoclast cells were also determined. For the evaluation of inflammation, total inflammatory cells in an area of 10 000 μm2 (neutrophil, lymphocyte, eosinophil and macro-phage cells) were counted in the periodontal ligament area.	The highest alveolar bone loss was observed in the D+P group ($P < .05$). GSP-200 group decreased alveolar bone loss ($P < .05$). The D+P group had the highest osteoclast counts, but the difference was not significant compared to the P, GSPE-100 and GSPE-200 groups. GSPE-100 and GSPE-200 groups significantly decreased inflammatory cell numbers compare to D+P group. The osteoblast numbers increased in the GSPE-100 and GSPE-200 groups compared to the P and D+P groups ($P < .05$). MMP-8 and HIF-1α levels were highest in the D+P group and GSPE significantly decreased these levels ($P < .05$)	(Toker et al. 2018)	[49]

Epigallocatechin-3-gallate (EGCG)	Animal study: 24 female mice divided into three groups (n= 8 per group-1,2,3). The mice received sterile food and drinking water ad libitum, with (1) and (2) distilled water or (3) 0.02% solution of EGCG from 8 weeks to 15weeks.	Mice were orally inoculated with (1) PBS or (2) and (3) *P. gingivalis* in PBS. At the age of 15 weeks, the mice were sacrificed to collect blood, gingival tissue and maxillae samples. Mouse inflammation antibody array C1 was used to detect the intensities of 40 mouse inflammatory mediators in serum. Cytokine levels were detected using ELISA kits for IL-17 and IL-1β in serum. The sections of gingival tissue were stained by using IL-17 and IL-1β Abs. The level of gene expression in the gingival tissue was determined by real-time PCR. Alveolar bone resorption was analyzed by forming three dimensional structures using aMicro-CT scanner.	EGCG significantly reduced *P. gingivalis*-increased alveolar bone resorption. In serum sample, EGCG significantly decreased the high expressions (caused by *P. gingivalis* infection) of proteins such as: IL-1β, IL-6, IL-9 and IL-12p70, exotain-1, exotain-2, fas ligand, MCP-1, MIG, MIP-1α , whereas IL-17 and TNF-α were slightly decreased without being statistically significant. ELISA assay showed that EGCG reduced level of IL-17 and IL-1β in serum, however IL-17 level was not statistically significance. In the gingival tissue, EGCG reduced, increased by *P. gingivalis* infection, expression of IL-17 and IL-1β as well as significantly down-regulated the level of gene expression: IL-1β, IL-6, TNF-α, RANKL, CCL2 and MMP-9, but not IL-23. The expression of IL-17 and MMP-2 were slightly down-regulated but without statistically significant.	(Cai et al. 2015)	[50]
Non-dialyzable material (NDM) prepared from concentrated cranberry (*Vaccinium macrocarpon*) juice, rich in proanthocyanidins.	Female BALB/c mice	Mice (16) were oral infected with *P. gingivalis* and *F. nucleatum* mixture (1:1). NDM (4 mg/mL) was added to the bacteria (in PBS) and the drinking water, whereas the control group received the infection in PBS alone. The maxillary jaws were harvested and alveolar bone loss was evaluated by microtomography. Mice (12) were challenged by an injection of PBS containing a mixture of *P. gingivalis* and *F. nucleatum* into the chambers of dorsolumbar area. In the experimental groups, NDM was added to the	The NDM addition to the mixed infection attenuated the alveolar bone loss induced by the mixed infection by approximately 20%. In subcutaneous chamber model of inflammation, the addition of NDM resulted in attenuation of TNF-α levels, compared with group without NDM, at all tested times, however results were statistically significant only at 24 hours post-infection, not at 2 hours.	(Polak et al. 2013)	[29]

		bacteria (in PBS) or to the PBS at a final concentration of 4 mg/mL, whereas the control group received the infection in PBS alone. Chamber exudates were harvested for analysis-TNF-α, quantified using two-site ELISA			
Epigallocatechin-3-gallate (EGCG)	Male Sprague–Dawley rats were randomly divided into two groups: (1) control group (n = 24; fed PBS vehicle after inducing experimental periodontitis), (2) EGCG group (n = 24; fed EGCG after inducing experimental periodontitis). All administration was conducted via oral gavage on a daily basis. Rats were killed 1, 2 and 4 week after EGCG or PBS administration.	Histologic and histomorphometric analyses, tartrate resistant acid phosphatase staining and immunohistochemistry were carried out.	Decreased interleukin (IL)-6 expression was shown from the early stage of EGCG administration, followed by reduced tumor necrosis factor (TNF) expression at week 4 EGCG group. Downregulation of TNF and IL-6 expression by EGCG led to a decrease in osteoclast number and activity, which resulted in reduced bone loss. EGCG reduced collagen destruction.	(Cho et al. 2013)	[51]
Commercial grape seed extract containing 95% Proanthocyanidins (PC)	Male Wistar rats with experimentaly induced periodontitis (EP) by injecting *E.coli* endotoxin into the labial and palatal aspects of maxillary anterior gingivae, buccal and palatal aspects of maxillary molars. The animals were divided into 2 groups: 1): control; 2): experimental periodontitis (EP), in which animals	After the experimental period, the animals were sacrificed and blood was collected. Bone and teeth of the right maxillary halves were dissected out and the histopathological evaluation was performed on right maxillary halves.	Proanthocyanidins at an effective dose of 30mg / kg body weight, sc, for 30 days effected a decrease in serum reactive oxygen species, lipid peroxides, lysosomal enzymes, acute phase proteins and an increase in antioxidant levels. Histopathological evidence of experimental periodontitis showed cellular infiltration of inflammatory cells while proanthocyanidin treated groups demonstrated only scattered inflammatory cells.	(Govindaraj et al. 2010)	[52]

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	received orally different amount of PC and one group received metronidazole as control positive for 30 days.				
Epigallocatechin-3-gallate (EGCG)	20 four-week-old Wistar rats divided on 2 groups: the rats (10 per each group) were given intraperitoneal injections of EGCG (80 mg/kg) or the rats (10 per each group) were given intraperitoneal injections of normal saline (NS, as control) on a daily basis until death. The animals were killed 20 days after induced apical periodontitis.	The jaws were dissected and radiographs were taken. Cyr61 and CCL2 were measured using immunohistochemistry assays.	Radiographs showed that administration of EGCG significantly attenuated periapical bone resorption compared with the control. Image analysis revealed that EGCG suppressed periapical osteolysis by an average of 57.2% in experimental model. In EGCG group, the numbers of Cyr61-synthesizing osteoblasts and infiltrating macrophages were also decreased. EGCG also markedly diminished the numbers of CCL2-producing osteoblasts. Statistical analysis revealed a lower percentage of Cyr61-positive osteoblasts in the EGCG-treated group (21.3%), compared with that in the control group (62.1%).	(Lee et al. 2009)	[38]

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4. Clinical studies

Until this moment, only three studies have been published in the field of the use of proanthocyanidins or flavan-3-ols in periodontal disease in humans [53], [54], [55]. Two of them relate to the use of local drug therapy with green tea extracts. Specifically, a thermo-reversible sustained-release system with incorporated green tea extract and hydroxypropylcellulose strips containing green tea catechin were used. Local therapeutic systems turned out to be effective in reducing periodontal pockets and inflammation [53], [55] (details in table 3). However, the weakness of Hirsawa et al. study [53] was the limited number of subjects in experimental group, as only 6 patients were treated. Díaz Sánchez. et al. [54] were the only one to design study using pill rich in oligomeric proanthocyanidins. In this clinical study, 10 of 20 healthy volunteers with an induced gingivitis took the experimental treatment with oligomeric proanthocyanidins supplement administered orally as a dissoluble pill . According to Diaz Sánchez et al. study the supplement induced improvement in the periodontal tissues condition during the period of treatment. Although this study does not refer to periodontics but to reversible gingivitis, the positive effect of the use oligomeric proanthocyanidins draws attention and encourages further clinical research.

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Table 3. Clinical study

Treatment	Study design and population	Methods	Results	Author, Years	Ref.
The experimental treatment consisted of 90 mg supplement based on blueberry and red fruit rich in oligomeric proanthocyanidins (OPCs) (equivalent to 36 mg OPCs-oligomeric proanthocyanidins) and 120mg of vitamin C.	A prospective, double-blind, randomized, controlled clinical trial in the gingivitis prevention. 20 healthy volunteers took the experimental or placebo treatment during 21 days. The pill was maintained in the mouth until complete dissolution.	Two evaluation visits were performed on days 14 and 21 of the study for an oral clinical examination and to register the Silness and Löe index, the gingival bleeding index, the Turesky plaque index, the inflammatory crevicular fluid study (IL6), and changes in the brightness of the gingiva.	The Silness and Löe gingival index was higher in the control group than in the experimental group. The bleeding was lower in the experimental group versus the control group. In contrast to the above results, the amount of dental plaque was slightly higher (33%) in the experimental group versus in the control group. No significant differences between the study group and the control group was seen in brightness of the gingiva. Statistically significant differences in level of IL-6 were found at the baseline between the experimental group and the control group and in the subsequent visits. However, in experimental group level of IL-6 was lower.	(Díaz Sánchez et al. 2017)	[54]
Thermo-reversible sustained-release system incorporated with green tea extract .	A controlled, split-mouth single-evaluator masked study was conducted to evaluate the effect of green tea extract as a sustained-release system in patients with chronic periodontitis (CP). 30 patients, each with 2 sites (test and control) having probing depths (PDs) of ≥4 mm were selected. Green tea and placebo gels were placed at test and control sites as	Assessment of gingival index (GI), pocket depth (PD), and relative clinical attachment levels (rCALs) was done at baseline and at 4 weeks.	When the comparison of means of GI, PD, and rCAL was done between baseline values and at the end of 4 weeks within the test group, and control group all the parameters were lowered and statistically highly significant. The test group showed significantly better results when compared with controls. Adjunctive local drug therapy with thermo-reversible green tea gel has revealed to reduce pockets and inflammation during the 4 weeks of the clinical trial in patients with CP.	(Chava and Vedula 2013)	[55]

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	an adjunct to Phase 1 periodontal therapy.				
Hydroxypropylcellulose strips containing green tea catechin (Taiyo Kagaku, Yokkaichi, Mie, Japan)	6 volunteers with advanced periodontitis, but with no systemic disorders. From each volunteer two pockets were selected; one for administration of the test agent and the other for placebo. Strips were applied in pockets in patients once a week for 8 weeks. The subjects were divided randomly into the scaled group (3 subjects) non-scaled group (3 subjects) were applied in pockets in patients	The clinical (pocket depth (PD)), enzymatic (peptidase activities) and microbiological effects (the proportion of black-pigmented, Gram-negative anaerobic rods (BPR) of the catechin)were determined.	The PD and the BPR were markedly decreased in the catechin group with mechanical treatment at week 8 compared to baseline. The peptidase activities in the gingival fluid were maintained at lower levels during the experimental period in the test sites with catechin, while it reached 70% of that at baseline in the placebo sites.	(H. M et al. 2002)	[53]

5. Conclusions

Among the numerous *in vitro* studies (34) on the immunomodulatory effect of proanthocyanidins or flavan-3-ols on the host cells, most concern the tea leaves extract and its compounds- catechines with presence of the galloyl moiety as the most active, as well as of A-type proanthocyanidins from fruits of *Vaccinium* species. Other sources of proanthocyanidins such as grape seeds and traditional medicinal plants, were seldom. The *in vitro* studies proved their immunomodulatory activity, among others by influencing on immune cell regulation, proinflammatory cytokines' synthesis and gene expression as well as by radical scavenging and inhibition of certain enzymes. They modulate NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and mitogen-activated protein Kinase (MAPk) pathways. Despite these promising results there is still much less studies using animal models (7) and only a few clinical studies (3). In conclusion, the potential of flavan-3-ols and their derivatives in prevention and alleviation of periodontitis is remarkable but clinical evidence is urgently needed for issuing credible dietary recommendation and complementary treatments

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