



orophenol® 

A blend of polyphenols, from Canadian wild blueberries, cranberries, and strawberries, developed for the support of oral health.

Orophenol[®]: promoter of your health

What is Orophenol[®] extract?

Orophenol[®] is a standardized water soluble functional extract of polyphenols obtained from Canadian wild blueberries, cranberries, and strawberries. Polyphenols are a family of plant metabolites involved in plant protection from oxidative stress and aggression by pathogens. The polyphenol profile varies among plants. Blueberries, cranberries, and strawberries are rich sources of polyphenols including phenolic acids, flavonoids, and procyanidins, with overlapping and complementary activities. For each of the three types of berries, **Diana Food** has identified polyphenolic fractions capable of interfering with the growth and biofilm formation of bacteria associated with dental plaque formation and periodontal disease, such as *F. nucleatum*. The most efficient inhibitory fractions of each fruit were combined in **Orophenol[®]**.



EFFECT OF OROPHENOL [®] COMPONENTS ON <i>F. NUCLEATUM</i> GROWTH AND BIOFILM FORMATION					
Extract	MIC (µg/mL)	MBC (µg/mL)	MBFC (µg/mL)	MBFC ₅₀ (µg/mL)	
 Blueberry	500	1000	125	62.5	
 Cranberry	500	1000	250	125	
 Strawberry	2000	4000	125	62.5	

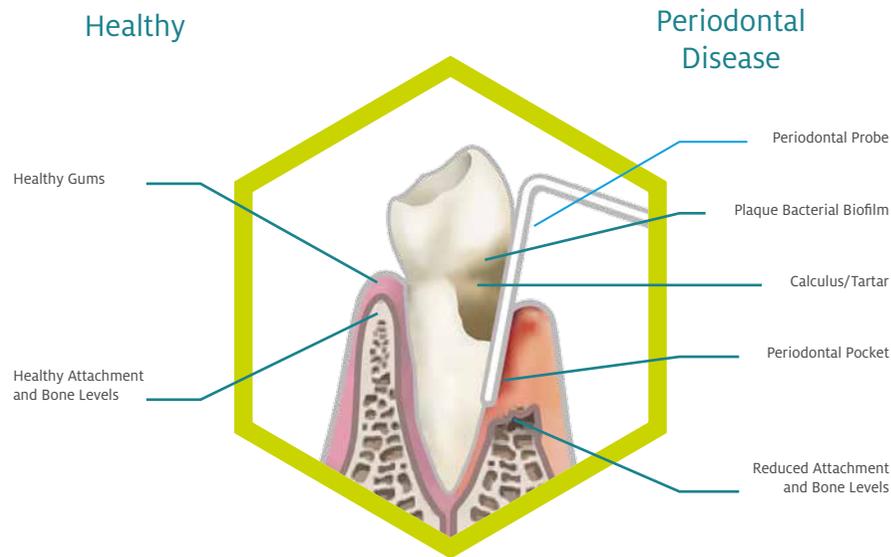
MIC = minimum inhibitory concentration
MBC = minimum bactericidal concentration
MBFC = minimum biofilm inhibitory concentration
MBFC₅₀ = minimum concentration of 50% inhibition of biofilm formation

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D. Grenier, 2015, Université Laval

Orophenol®: what for?

Orophenol® is indicated in the support of oral health. In the mouth, healthy gums and bone are essential to hold teeth firmly in place. Unfortunately, the attachment zone between gums and teeth is vulnerable to the formation of dental plaque. The latter is a biofilm consisting in a mixture of sticky mucus, bacteria and food that prompts tooth decay and periodontal disease. Biofilm bacteria attack gums, first causing a state of inflammation called gingivitis with symptoms such as red, puffy and bleeding gums. If the condition is left untreated, gingivitis may progress to periodontitis, a serious chronic disease, where the gum and bone pull away from the teeth, forming pockets that deepen below the gumline. Sustained inflammation in periodontitis leads to the destruction of connective and bone tissues that support teeth causing them to eventually fall off. Importantly, the consequences of periodontal disease extend beyond the mouth, increasing the risk for heart disease, diabetes and complications during pregnancy (Shangase et al, 2013). The condition is widespread in the population. Recent reports from the Centers for Disease Control and Prevention (CDC) revealed that nearly 50% of American adults aged 30 years and older and 64% of those aged 65 and older have some form of periodontitis.

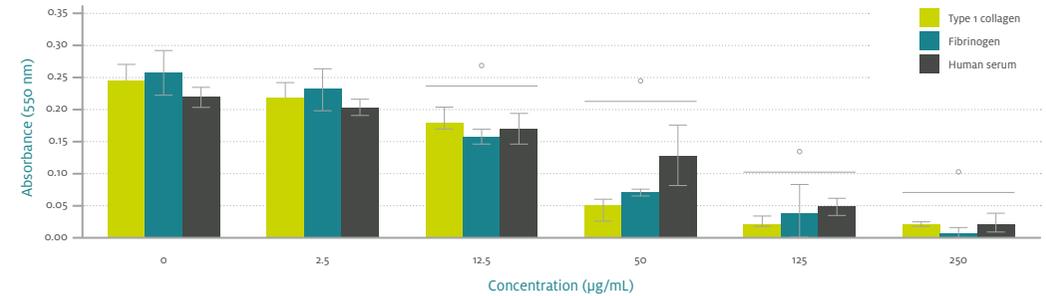


Healthy versus diseased gum

Results from *in vitro* studies Inhibition of pathogen colonization

INHIBITION OF *P. GINGIVALIS* ADHERENCE BY CRANBERRY EXTRACT

Adherence studies were performed using a microtitre plate assay in which proteins, as those found in gingival crevice, were immobilized onto the bottom of wells. *P. gingivalis* adherence to these proteins was revealed with Crystal Violet staining. This strain of bacteria was chosen for its frequent association with the development of periodontal disease. The bacteria were found to attach to a similar extent to type 1 collagen, fibrinogen and human serum. When cranberry extract ($\geq 50 \mu\text{g/mL}$) was present in the mixture, *P. gingivalis* attachment to all three proteins was significantly reduced.

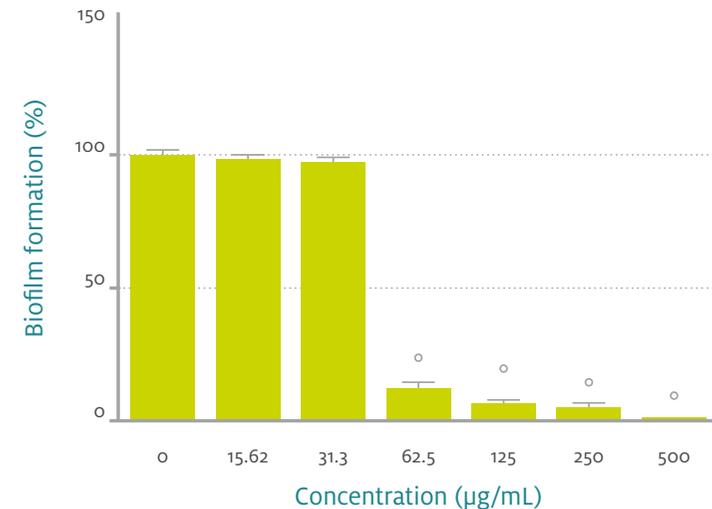


Source: Labrecque et al, 2006

INHIBITION OF *F. NUCLEATUM* BIOFILM FORMATION BY BLUEBERRY EXTRACT

Using a similar microtitre plate assay as above, *F. nucleatum* (also associated with periodontal disease) was grown anaerobically for 48 h in the presence or absence of blueberry extract. At concentration ranging from 62.5 to 250 $\mu\text{g/mL}$, the extract significantly inhibited biofilm formation.

Source: Latha et al, 2015



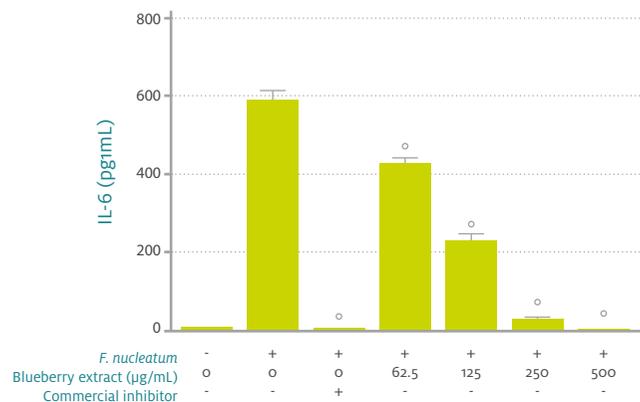
Results from *in vitro* studies

Inhibition of inflammatory response

INHIBITION OF MACROPHAGE IL-6 SECRETION BY BLUEBERRY EXTRACT

PMA-stimulated U937 cells (a macrophage-like model) were pre-treated or not with blueberry extract and next challenged with *F. nucleatum*. The release of various cytokines (IL-6, IL-1b, TNF-a) was then quantified using specific ELISA kit assays. Blueberry extract significantly and dose-dependently inhibited the bacterial-induced cytokine release, without affecting bacterial growth. Results are shown for IL-6 but inhibition was also documented for IL-1b and TNF-a.

Source: Lagha et al, 2015

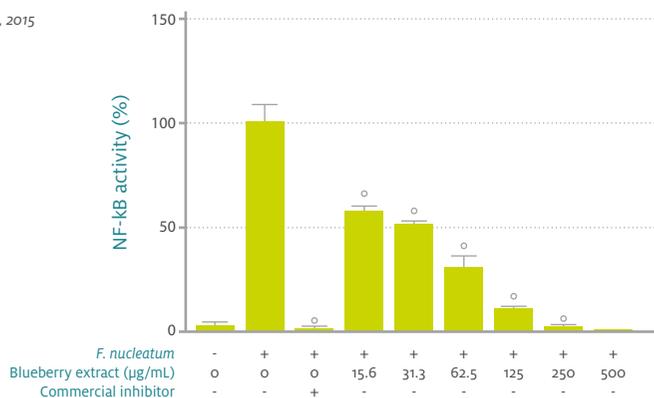


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INHIBITION OF NF-KB ACTIVITY IN MONOCYTES BY BLUEBERRY EXTRACT

The activity of NF-kB (a nuclear factor activated by inflammatory stimuli) was monitored in U937 monocytes stably transfected with a Luciferase gene coupled to three NF-kB binding sites. Pre-treatment of cells with blueberry extract dose-dependently inhibited *F. nucleatum*-induced NF-kB activation.

Source: Lagha et al, 2015



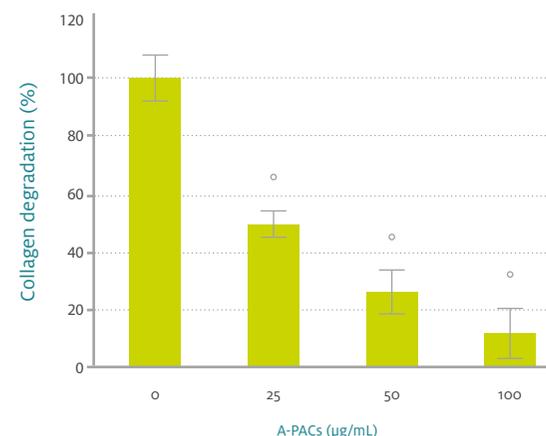
Results from *in vitro* studies

Inhibition of connective tissue and bone destruction

INHIBITION OF P. GINGIVALIS COLLAGENASE ACTIVITY BY CRANBERRY EXTRACT

Matrix metalloproteinase (MMP) are enzymes that degrade collagen. Within gum tissues, MMPs are produced by both *P. gingivalis* and host cells causing connective tissue loss in the course of periodontal disease. To investigate the effect of cranberry extract on collagenase activity, aliquots of cell-free culture supernatant of *P. gingivalis* were mixed with increasing concentrations of cranberry extract. Next, fluorescein-conjugated collagen was added as a substrate for MMP. The fluorescence generated upon collagen cleavage was measured every 4 hours using a fluorimeter (Ex/Em: 490 nm / 520 nm). *P. gingivalis* MMP activity was dose dependently inhibited in the presence of cranberry extract. A similar effect was also recorded for bacteria-induced host cells' MMP activity.

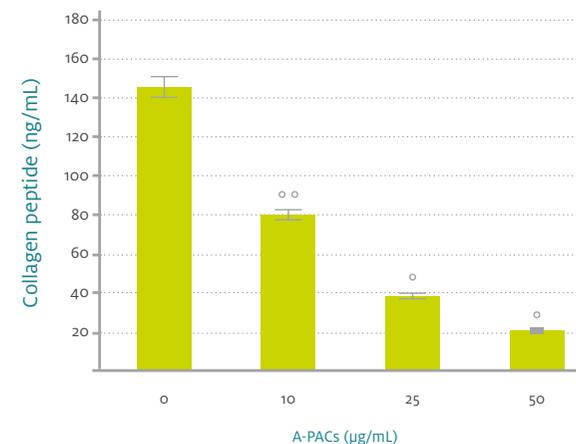
Source: La et al, 2010 Q 2009

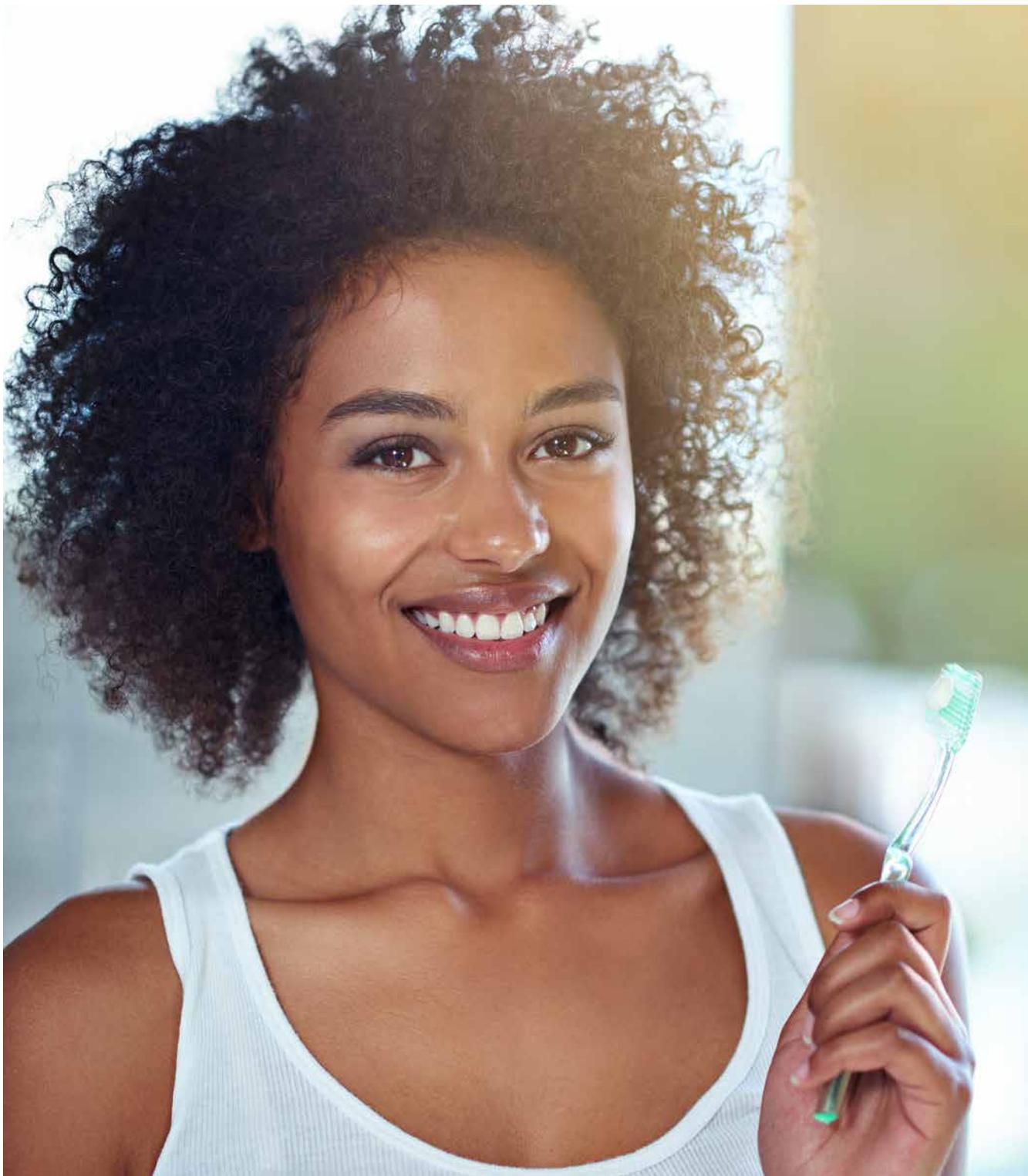


INHIBITION OF OSTEOCLAST ACTIVITY BY CRANBERRY EXTRACT

Osteoclasts were cultured on OsteoAssay™ human bone plates in the presence of cranberry extract for four days. Supernatants were then collected and analyzed by enzyme immunoassay, for the presence of a specific peptide released from collagen through the bone resorption activity of osteoclasts. Cranberry extract dose-dependently inhibited the release of the peptide demonstrating its potential to halt osteoclastic activity.

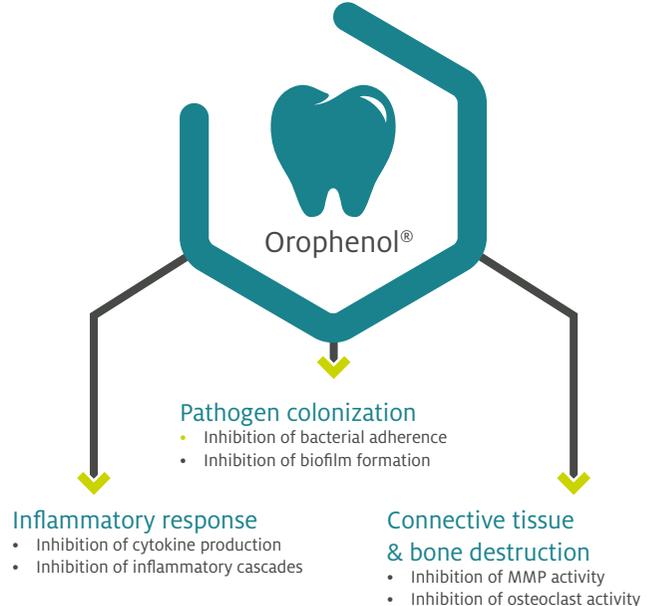
Source: Tanabe et al, 2011





How Orophenol® works

In vitro studies have shown that **Orophenol®** blocks pathogen colonization by inhibiting the adhesion of bacteria to gingival sites and by interfering with their capacity to form a biofilm. Such activities has been demonstrated for *P. gingivalis* and *F. nucleatum*, two species associated with periodontitis development. **Orophenol®** also modulates the host immune response, preventing the release of inflammatory cytokines, including IL-6, TNF-a, and IL-1b, by macrophages stimulated with *F. nucleatum*. The anti-inflammatory action of **Orophenol®** extends to the NF-kB pathway, which is shut down in monocytes challenged with *F. nucleatum* in the presence of the extract. The host inflammatory response is a significant factor in the progression of periodontal disease. Under pathogen stimulation, the host immune cells release proteases (MMPs) that destroy the connecting tissues supporting teeth, thus creating opportunities for deep bacterial infiltration and bone destruction. MMPs can also be directly released by bacteria such as *P. gingivalis*. In line with its anti-inflammatory activity, **Orophenol®** prevents MMP activation and collagen destruction. Lastly, **Orophenol®** modulates the osteoclast osteoblast activities in favor of bone maintenance. **Orophenol®** is thus a multivalent extract capable of maintaining optimal oral health.







Who may benefit from Orophenol® supplementation?

Anyone who cares about **maintaining healthy teeth and gums**.

Maintaining a good gum health is a primary importance especially in the aging population, considering the recent evidences showing links between increase of cardiovascular disease risk and periodontal disease. Healthy gums (by normal brushing and washing), healthy diet rich in fruits and vegetables, and a regular consumption of **Orophenol®** are parts of a good and natural plan to help prevent development of periodontal disease and tooth decay. Everybody who care about her or his good oral health should regularly use **Orophenol®**.

Suggested applications

- Healthy beverages
- Chewable tablets
- Mouthwash
- Toothpaste
- Tooth health strips
- Chewing gums
- Soft chews and gummies

Suggested concentration

A total of 62.5 mg of **Orophenol®**, providing 14.4 mg of polyphenols from blueberries, cranberries, and strawberries, is suggested on a daily basis.

Stability data

The polyphenol content will remain stable for 24 months, when kept at room temperature and away from light.

REFERENCES

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