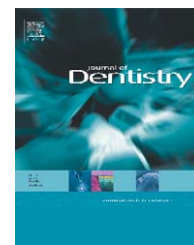


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## Review

# Polyphenols, oral health and disease: A review

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### ABSTRACT

**Objective:** Polyphenols (PPs) are reactive metabolites abundant in plant-derived foods, particularly fruits, seeds and leaves. In the tissues of the digestive tract, particularly the oral mucosa, active PPs reach the highest concentration. Although excessively high and monotonic intake may be toxic (such an effect is countered by salivary proline-rich proteins), PPs exert preventive activity against infectious and degenerative diseases and may also help prevent oral diseases, via mechanisms like antioxidant activity and neutralisation/modulation of human/bacterial/viral proteins/enzymes. This review seeks to investigate the preventive PP activity against oral diseases.

**Methods:** Studies published during the last 15 years were searched using MEDLINE, PUBMED, EMBASE. The quality of evidence was rated using the three-point scale used by the US Preventive Services Task Force to assess the merits of preventive measures.

**Results:** Consistent, well-designed, epidemiological, animal and *in vitro* studies directly and indirectly support the preventive PP effect against oral cancer with good evidence. Consistent studies showing that PPs inactivate periodontal pathogens and increase antioxidant capacity of oral fluids, suggest a preventive effect against periodontal disease with fair evidence. Animal and *in vitro* studies showing that PPs decrease mutans streptococci virulence and dental plaque accumulation, suggest a preventive effect against dental caries with fair evidence. Meta-analysing clinical trials on subjects refraining from oral hygiene, PP-containing mouthrinses decrease mutans streptococci level by almost 50% and Plaque Index score.

**Conclusions:** Regular and frequent PP intake may help protect against oral cancer, while human studies are needed to confirm the promising preventive activity against periodontal disease and caries.

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## 1. Introduction

### 1.1. Polyphenols in the plant kingdom

Polyphenols (PPs) are plant metabolites characterized by the presence of several phenol groups (i.e., aromatic rings with

hydroxyls), which derive from L-phenylalanine.<sup>1–3</sup> The most important PP classes are phenolic acids, which include polymeric structures, such as hydrolyzable tannins, lignans, stilbenes, and flavonoids. Flavonoids include flavonols (e.g., quercetin and kaempferol, the most ubiquitous flavonoids in foods), flavones, isoflavones, flavanones, anthocyanidins

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(pigments responsible for the colour of most fruits), flavanols (catechins—monomers and proanthocyanidins—polymers, known as condensed tannins).<sup>4,5</sup>

Some PP hydroxyls are very reactive in (i) neutralizing free radicals ( $-R^{\bullet}$ ) by donating a hydrogen atom ( $-RH$ ) or an electron ( $-R^{-}$ ),<sup>6</sup> (ii) chelating metal ions in aqueous solutions,<sup>6-8</sup> and (iii) binding and precipitation of proteins, due to extensively coating of hydrophobic surfaces of peptides and then to co-operative bridge formation.<sup>9,10</sup>

PPs are essential in a wide range of plant functions. For example, they provide flower, fruit and seed pigmentation, thus attracting pollinators and seed dispersers; promote plant fertility and germination of pollen; act as signal molecules in plant-microbe interactions; protect against ultraviolet light; and have other defensive functions.<sup>11,12</sup> Two defensive functions have important consequences in the study of the effect of PPs on dental diseases, namely, the defences against pathogenic micro-organisms and plant predators.

(I) Plant defence against pathogenic micro-organisms. From the point of view of their antimicrobial activity PPs may be classified into simple phenols/phenolic acids (e.g., p-cresol, 3-ethylphenol, vanillic, gallic, ellagic acids, hydroquinone), hydroxycinnamic acid derivatives (e.g., p-coumaric, caffeic, ferulic, sinapic acids), flavonoids, and tannins.<sup>13</sup> Mechanisms such as hydrogen peroxide production, bacterial protein/enzyme inhibition and disinfectant activity of phenolic acids are all well documented.<sup>14</sup> Phenolic acids are antimicrobials and are directly involved in the response to micro-organisms. Indeed, their concentration raises after infection,<sup>15</sup> and the phenolic acid content of vegetables produced by organic or sustainable agriculture is higher than that of vegetables grown without stress, such as those grown in conventional or hydroponic conditions.<sup>5</sup> Hydrogen peroxide is formed in aqueous solution through proton dissociation from reactive hydroxyl groups. The mobilized electrons in the phenol rings reduce the oxygen molecules, forming  $O_2^{2-}$ , while the free protons combining with  $O_2^{2-}$ , generate hydrogen peroxide.<sup>16</sup> The activity of PPs against microbial enzymes and proteins is concentration-dependent: at low concentration, PPs interfere with specific sites, whereas at high concentrations they cause denaturation.<sup>17</sup> PPs interact with microbial membrane proteins, enzymes and lipids, thereby altering cell permeability and permitting the loss of protons, ions and macromolecules.<sup>17-19</sup> PPs that have crossed the bacterial cellular membrane are active against enzymes and proteins.<sup>13,20</sup> In addition, micro-organisms stressed by exposure to PPs, up-regulate proteins related to defensive mechanisms, which protect cells or help cells survive, while simultaneously they down-regulate various metabolic and biosynthetic proteins involved, for example, in amino-acid synthesis and protein formation, phospholipid, carbon and energy metabolism.<sup>21</sup>

(II) Plant defence against predators. PPs protect plant leaves, fruits (until they are ripened) and seeds (until they are ready to disperse) from mammalian herbivores/omnivores and other predators. For example, tannins increase the resistance of sorghum to bird predation<sup>22</sup> and cause

weight loss in rats and chicks.<sup>23-25</sup> Tannins ingested by herbivores are toxic, causing gastrointestinal mucosal necrosis, and renal and hepatic failure, probably due to intestinal tannin hydrolysis by esterases with release and absorption of toxic phenolic acid.<sup>26-29</sup> PPs disturb animal digestion and metabolism via several mechanisms. First, tannins inhibit digestive enzymes (e.g., trypsin, chymotrypsin,  $\alpha$ -amylase,  $\alpha$ -glucosidase, and lipase), by the same mechanisms used to inhibit bacterial proteins and enzymes. Neutralization of such enzymes interferes with digestion and absorption of amino-acids, first, but also starch and lipids.<sup>30-35</sup> Second, PPs insolubly complex with ferrous iron, thus decreasing the bioavailability of inorganic, non-haem iron. In resource-poor countries, where plant foods are the major staples of diet and the dietary iron source is mostly non-haem iron, this is a major nutritional problem. In developing countries, with an absorption to consumption ratio close to 10%, almost 40% of children under the age of five and of women are anaemic, one half having iron deficiency anaemia.<sup>36-38</sup> Third, PPs can inactivate thiamine and lead to enhanced excretion of endogenous proteins.<sup>37</sup>

Salivary tannin-binding proteins, a heterogeneous group of proteins which include proline-rich proteins (PRPs) and histatins, are the main animal counter-measure to tannins.<sup>29,39</sup> PRPs account for two thirds of all salivary proteins among mammalian herbivores and omnivores, and have a high affinity for tannins.<sup>29,40</sup> Indeed, PPs and PRPs form stable complexes in the oral cavity, which remain stable throughout the whole gastrointestinal tract,<sup>41,42</sup> thus preventing tannins from interacting with digestive enzymes.<sup>43,44</sup> PRPs are generally classified into basic or acidic. According to the evolutionary hypothesis, acidic PRPs are involved in maintaining oral homeostasis, and basic PRPs, with their affinity for tannins, evolved from acidic PRPs when mammals began to consume diets with high tannin content. Interestingly, PRP production is often induced by tannin ingestion, and animals with high tannin contents in their natural diets have higher salivary basic PRP levels than do animals with low tannin contents.<sup>29,45</sup> Like PRPs, histatins, which account for only 3% of all human salivary proteins,<sup>46</sup> readily bind tannins<sup>47</sup> forming stable complexes throughout the gastrointestinal tract. Histatins must play a secondary role in PP inactivation.

## 1.2. Polyphenols in human diet and bioavailability

PPs are common in all human diets wherever fruit and vegetables are consumed. However, available information on quantitative PP intake is incomplete and comprehensive reference food composition tables are not available, because of the wide range of PPs and the considerable number of factors that modify their concentration in foods. For example, flavonoids and phenolic acids in apples vary by a factor of 1:4; flavonols, which are found in the peel of apples, are not found in pulp; and PP composition and level vary in time and space within the same fruit according to sun exposure and maturation degree.<sup>4,48,49</sup>

PP intake and bioavailability are generally assessed using the total antioxidant capacity of plasma, which is based on the

**Table 1 – Total antioxidant capacity of various plant foods and beverages, assessed as Trolox – a water soluble vitamin E analogue – equivalent antioxidant capacity (mmol Trolox per kg of food or l of beverage).<sup>51</sup>**

Food/beverage	mmol Trolox/kg or l	Food/beverage	mmol Trolox/kg or l
Spinach	8.5	Cola	0.1
Pepper	7.6–8.4	Black tea	3.6
Broccoli	3.0	Green tea	6.0
Carrot	0.4	Coffee (espresso)	36.5
Potato	0.8	Coffee (soluble)	32.5
Tomato	1.7	Beer (lager)	1.0
Blackberry	20.2	Cognac	1.3
Grape	2.5–3.9	Whiskey	1.7
Olive	10.4–14.7	Rum	<0.1
Pineapple	9.9	White wine	1.6–1.9
Strawberry	10.9–11.3	Rosè wine	1.5–2.4
Orange juice	3.0	Red wine	9.0–12.1

characteristic antioxidant activity of PPs. However, this method is not free from flaws, because there are many other micronutrients, such as vitamins E and C, with greater antioxidant activity and because antioxidant capacity is not directly correlated to the *in vivo* mechanisms of defence, which are mainly enzymatic.<sup>50</sup> A list of total antioxidant capacity of plant foods and beverages is displayed in Table 1.<sup>51</sup>

More recently, PP intake has been assessed using dietary diaries. The mean daily PP intake among Finnish adults is 863 mg, with phenolic acids representing 75% of total intake, followed by proanthocyanidins (14%), anthocyanidins and other flavonoids (10%). The main PP sources in this sample were coffee, cereals, fruits, mostly berries and berry products.<sup>52</sup> The mean daily PP intake from fruits and vegetables among French adults is 219 mg (males), 193 mg (females) from fruit and 78 mg (males), 67 mg (females) from vegetables.<sup>53</sup>

Little is known about PP absorption, bioavailability, biodistribution and metabolism, although there is probably a common pathway. The aglycones, that is, the non-conjugated forms, are generally absorbed intact from the digestive tract, while esters, glycosides, or polymers must be hydrolyzed before being absorbed. Oral and intestinal microorganisms also are responsible for PP degradation into aglycones and, occasionally, production of various simple aromatic acids. Absorbed PPs are conjugated into methylated, glucuronidated or sulphated derivatives, a metabolic detoxication process common to many xenobiotics. Such mechanisms are so efficient, that aglycones are generally absent or present at low concentrations in blood after consumption of nutritional doses. PPs are detected in many tissues, but mainly in the mucosae of the digestive tract and, principally, the oral mucosa. The best absorbed PPs are isoflavones and gallic acid, followed by flavanones, catechins and quercetin glycosides. The least well absorbed PPs are proanthocyanidins, anthocyanins and galloylated catechins. All PPs are excreted chiefly in the urine and bile.<sup>5,54–58</sup>

### 1.3. Polyphenols and human health

PPs have powerful antioxidant activity *in vitro* being capable of scavenging a wide range of reactive oxygen, nitrogen, and chlorine species, such as superoxide anion, hydroxyl radical, peroxy radicals, hypochlorous acid and peroxynitrous acid. They also chelate metal ions, thus decreasing their pro-

oxidant activity. Since considerable evidence indicates that increased oxidative damage is associated with the development of most major age-related degenerative diseases, it has been speculated that PPs may have protective effects against such conditions. High PP intake has allegedly been associated with decreased risks for cancers, cardiovascular diseases and neurodegenerative disorders. Such biological activity has often been evaluated *in vitro* on pure enzymes, cultured cells, or isolated tissues using food aglycones or glycosides.<sup>5,59</sup> Although there are myriads of epidemiological studies regarding the effects of PPs on human health, comprehensive data are available only for flavonoids. Epidemiological studies suggest with convincing evidence that high flavonoid intake decreases mortality from coronary artery disease by up to 65% and generally decreases the risk for stroke, as well as for lung and rectal cancers, asthma and chronic obstructive pulmonary disease. In contrast, the effect on colon, epithelial, ovarian, testicular cancers and cancers of the stomach, urinary tract, prostate and breast are not clear.<sup>11,60,61</sup>

The antimicrobial activity of PPs<sup>14</sup> is useful against infectious diseases. For example, the alleged anti-HIV activity would be due to inhibition of enzymes, such as reverse transcriptase,<sup>62</sup> proteinase and integrase, and of CD4 receptors.<sup>63</sup> PP activity against human and avian influenza viruses appears to be mainly due to the inhibition of viral haemagglutinin,<sup>64</sup> while the activity against cytomegalovirus is attributed to inhibition of epidermal growth factor receptors and immediate early protein function.<sup>65</sup> Animal and *in vitro* models have demonstrated other important effects of PPs, such as decreased leukocyte immobilization, apoptosis induction, cell proliferation and angiogenesis inhibition, and phytoestrogenic activity.<sup>66–69</sup>

Interestingly, the beneficial PP activity against human diseases in animal and *in vitro* studies has generally not been confirmed by human studies, thus raising the question as to whether experimental studies are relevant for human disease outcomes. Such discrepancies between epidemiological and experimental studies may partly be explained by the many methodological problems in human studies. Indeed, the aforementioned methods employed to assess PP intake do not provide useful information concerning PP bioavailability in active forms and at tissue level.<sup>50</sup> In addition, the various PPs have different biological properties.<sup>4</sup> Another explanation for the discrepancy between human and experimental studies

is that the mechanism of action of PPs *in vivo* could be different from the *in vitro* mechanism. In fact, the classical antioxidant activity of PPs is unlikely to be the principal explanation for cellular effects in humans, where non-specific protein/enzyme modulating mechanisms are primarily involved. This hypothesis is based on two lines of reasoning. First, PP metabolism significantly alters their redox potentials. In fact, the antioxidant capacity of PP conjugates and metabolites is clearly lower than that of their parent aglycones. Second, despite PP concentration in plasma and organs (between high nanomolar and low micromolar) is lower than concentration of other antioxidant micronutrients, such as ascorbic acid and  $\alpha$ -tocopherol (between high micromolar and low millimolar), PPs are more efficient than ascorbate against oxidative stress at tissue level.<sup>70</sup> According to this hypothesis, PP activity against several forms of cancer, proliferative diseases, inflammation, and neurodegeneration is mainly exerted through the inhibiting and modulating activities against a wide range of receptors, enzymes and transcription molecules.<sup>71</sup> For example, the anti-cancer activity of several PPs is due to their ability to inhibit enzymes involved with carcinogenesis and tumour development.<sup>71,72</sup> Similar modulating activities are primarily involved in explaining the protective PP effect against cardiovascular diseases.<sup>60</sup> Interestingly, some oxidized PP metabolites act as pro-oxidants, but induce apoptosis by production of toxic reactive oxygen species against cancer cell mitochondria.<sup>73</sup> On the other hand, such pro-oxidant activity suggests that high dietary PP intake could be potentially more of an oxidative risk than a benefit—a concern corroborated by one epidemiological study reporting a direct association between flavonoid intake and colon cancer.<sup>74</sup> This suggests that dietary supplementation with large amounts of single PPs may actually be deleterious to human health.<sup>3,72</sup>

## 2. Polyphenols and oral diseases

### 2.1. Methods

A literature search was conducted to identify studies published between the years 1993 and 2008, investigating the association between PPs and dental diseases. The databases used were MEDLINE, PUBMED, EMBASE. The following groups of search terms were linked: (i) PP related terms, such as PPs, PP classes and subclasses (e.g., flavonoids, catechins, tannins), specific PPs (e.g., quercetin, gallic acid, galloylated catechins) and (ii) oral disease related terms (e.g., dentistry, oral cancer, leukoplakia, periodontitis, periodontal disease, dental caries) and their etiological and risk/protective factors (e.g., periodontal pathogens, mutans streptococci).

The quality of the evidence of the association between PPs and oral diseases was rated using the three-point scale used by the US Preventive Services Task Force to assess the merits of preventive measures. Such method was preferred to other methods because of its simplicity and because the quality of evidence can be assessed merging animal, *in vitro* and human studies. According to this method, the evidence in systematic reviews is classifiable as “good”, “fair”, “poor”. When evidence is good the preventive measure is proven to exert

a direct effect on the health outcome; when it is fair, the measure could exert an effect on the health outcome; when it is poor the measure is not proven to exert an effect on the health outcome. Good evidence is achieved by consistent results from well-designed, well-conducted studies, not necessarily clinical trials, in representative samples that directly assess effects on health outcomes. Fair evidence is achieved by limited number, quality, or consistency of individual studies, reduced generalizability to routine practice, or indirect nature of the evidence on health outcomes. Poor evidence is achieved by limited number of studies or studies with low power, with important flaws in their design or conduct, gaps in the chain of evidence, or lack of information on important health outcomes.<sup>75</sup>

The results concerning every oral disease were exposed organically and descriptively, while the quality of the evidence of the association between diseases and PPs was displayed in a distinct section.

### 2.2. Polyphenols in the oral cavity

The earlier hypothesis of the direct antioxidant activity of PPs is potentially valid in explaining their preventive effect against diseases of the oral cavity, where PPs come into direct contact with tissues before being absorbed and metabolised<sup>76</sup> and are activated into aglycones by human and bacterial enzymes.<sup>56</sup> Indeed, oral mucosa, where PPs reach the highest concentration with respect to all other tissues, is constantly exposed to oxidative stress from environment and diet.<sup>59,77</sup>

### 2.3. Polyphenols and oral cancer

The potential preventive activity of several PPs against oral squamous cell carcinoma, the most common form of oral cancer, is reported in a multitude of animal and *in vitro* studies. Namely, catechins from tea inhibit the production of important metalloproteases, thus potentially reducing invasion and migration,<sup>78</sup> inducing apoptosis<sup>79,80</sup> and growth arrest in both oral cancer<sup>80</sup> and oral leukoplakia cell lines.<sup>81</sup> Methoxylated flavonoids, present in citrus fruits, pepper and betel, inhibit DNA adduct formation promoted by known carcinogens, such as tobacco nitrosamines.<sup>82</sup> Proanthocyanidins, highly concentrated in red wine, pigmented fruits, nuts and chocolate, reduce cell proliferation in human oral cancer cells infected by human papillomavirus, implicated in the development of some oral cancers, inhibit proliferation in non-infected cells,<sup>83</sup> show cytotoxic activity<sup>84</sup> and induce apoptosis and cell differentiation.<sup>85</sup> In addition, several cancer preventive PPs selectively accumulate in the epithelial cells of the upper digestive tract of rats, with an ATP- and Na<sup>+</sup>-dependent organic anion transport mechanism.<sup>86</sup>

Epidemiological study results are consistent with experimental studies. Specifically, a recent South African retrospective study reported that diets poor in fruit and vegetables, with consequent low PP content, are responsible for 7% and 10% of oesophageal cancers among females and males, respectively.<sup>87</sup> According to a large Danish cohort study, the oral cancer risk of alcohol drinkers, who consume wine alone or in combination with other alcoholic drinks, is the same as in the reference group of non-drinkers. The authors attribute this

result to the high PP concentration in wine, which might protect against the carcinogenic effects of alcohol.<sup>88,89</sup> An Italian case-control study reports that high dietary flavonoid intake decreases the probability to develop oral and pharyngeal cancers by 50%.<sup>90</sup>

#### 2.4. Polyphenols and periodontal disease

Inflammatory stimulation by periodontal pathogens increases the production of crevicular fluid and induces the chemotaxis of polymorphonuclear leukocytes, which, in order to inactivate periodontal pathogens, release singlet oxygen and hypochlorous acid into the crevicular fluid. The consequent oxidative stress is countered by the antioxidant activity of ascorbate, albumin and urate present in the crevicular fluid and derived from plasma. However, this local oxidative stress may be increased by external factors or systemic conditions, such as smoking, diabetes, obesity and metabolic syndrome. When there is a disequilibrium between oxidative stress and antioxidant activity, periodontal tissue destruction may appear. These observations suggest that antioxidant rich diets might inhibit periodontal disease development and progression, particularly in subjects exposed to environmental and dietary sources of oxidative stress.<sup>91–94</sup> Several studies also report that decreased antioxidant activities of crevicular fluid and saliva are associated with the development of periodontitis.<sup>95–97</sup> PPs may contribute to increase the antioxidant activity of oral fluids. Indeed, delivery of tea PPs by holding green or black tea in the mouth for 2–5 min increases the antioxidant capacity of saliva,<sup>98</sup> and daily consumption of two fresh grapefruits for 2 weeks increases the phagocytic capacity of the polymorphonuclear leukocytes in the gingival crevicular fluid.<sup>99</sup>

PPs have an *in vitro* antibacterial activity against periodontal pathogens. Cranberry PP fraction prevents biofilm formation by *Porphyromonas gingivalis* and *Fusobacterium nucleatum* and inhibits some *Porphyromonas gingivalis* proteases.<sup>100</sup> Wine catechins have strong antimicrobial activity against *Porphyromonas gingivalis* and *Prevotella intermedia*.<sup>101</sup> Green tea catechins, used in a slow-release local delivery strip system applied in the periodontal pockets, decrease the pocket depth and the proportion of Gram negative anaerobic rods, while the same catechins show an *in vitro* bactericidal effect against *Porphyromonas gingivalis* and *Prevotella* spp.<sup>102</sup> Several PPs inhibit the proteolytic activity of *Porphyromonas gingivalis*.<sup>103</sup> In addition, hop-derived PPs counteract the production of prostaglandin E2 induced by *Porphyromonas gingivalis*.<sup>104</sup>

#### 2.5. Polyphenols and dental caries

The effect of PPs against dental caries has been generally investigated indirectly. The studies can be divided into *in vitro* studies investigating the effect of plant extracts against mutans streptococci, *in vitro* studies investigating the effect of specific PPs against mutans streptococci, studies on animals, and studies on humans.

(I) *In vitro* studies investigating the effect of plant extracts against mutans streptococci. Eight studies consistently report – one of them non significantly – that plant extracts

inhibit glucosyltransferases (GTFs) activity and insoluble glucan synthesis<sup>105–112</sup>; 10 studies, with heterogeneous designs, report adherence inhibition on hard surfaces<sup>106,108–116</sup>; three of the four studies investigating the inhibition of acid production from sucrose or glucose report this effect,<sup>106,112,117</sup> while one does not.<sup>113</sup> Eight studies report bacteriostatic activity against mutans streptococci<sup>105–107,110–112,114,118</sup> and one does not,<sup>113</sup> while one study reports bactericidal activity<sup>119</sup> and another does not.<sup>109</sup> In addition, plant extracts induce the down-regulation of a series of enzymes essential for *Streptococcus mutans* metabolism, such as those responsible for amino-acid, carbohydrate, lipid and nucleotide syntheses and for translation.<sup>117</sup>

Thus, studies on plant extracts consistently suggest an activity against several metabolic activities of mutans streptococci, resulting in a decrease in growth and virulence but probably not in viability, *in vitro*. These effects cannot be attributed solely to PPs, because plant extracts include several other components.

(II) *In vitro* studies investigating the effect of specific PPs against mutans streptococci. Some of them report the inhibition of GTF-dependent insoluble glucan synthesis.<sup>105,120–125</sup> However, only a fraction of all PPs exhibits such activity, like the polymeric fraction of tea PPs<sup>120,122</sup> and the mono-, di-, tri-meric fractions of apple PPs.<sup>121</sup> In contrast, PPs in cranberry and grape are more active taken as a whole than independently.<sup>123–125</sup> Four studies report the inhibition of acid production by mutans streptococci<sup>123–126</sup> and partly ascribe it to the inhibition of the bacterial enzyme proton translocating F-ATPase,<sup>123–125</sup> which transports protons out of cells and alleviates the negative influence of acidification on metabolic processes, thus decreasing the extracellular environmental pH.<sup>127</sup> One study reports the inhibition of mutans adherence to hydroxyapatite,<sup>128</sup> while another reports no effect on enamel remineralisation.<sup>129</sup> Two studies report bacteriostatic effect with growth inhibition,<sup>123,126</sup> while another does not,<sup>121</sup> and three studies report no bactericidal effect.<sup>124–126</sup>

According to two studies, tannins inhibit human salivary alpha-amylase,<sup>33,121</sup> which catalyzes the hydrolysis of starch to oligosaccharides and binds to viridans streptococci and enamel, thus providing an acidogenic food source for cariogenic micro-organisms on the tooth surface.<sup>130</sup> In addition, cocoa flavanols stimulate peripheral blood mononuclear cells to secrete interleukin-5, which, in turn, stimulates IgA production, thus possibly protecting against mutans streptococci.<sup>131</sup>

Everything considered, although some PPs are able to inhibit bacterial and human enzymes involved in caries aetiology, there is no evidence for direct antibacterial activity.

(III) Studies on animals. The studies on rats show generally similar design, with animals initially infected with mutans streptococci, successively fed highly cariogenic diets supplemented (test group) or not (control group) with plant extracts or PPs. Caries scores and dental plaque

**Table 2 – Pooled estimates of the treatment effect of polyphenol-containing mouthrinses on plaque index (values ranging between 0 and 5) using the fixed and the random effect methods (the best fitting method is in bold character).**

Study	Mean difference (test-control)	Variance	95% confidence interval for difference
Ooshima et al. <sup>134</sup>	-0.64	0.024	-0.94 to -0.34
Matsumoto et al. <sup>114</sup>	-1.17	0.009	-1.35 to -0.99
Shinada et al. <sup>135</sup>	-0.80	0.034	-1.16 to -0.44
Fixed effect method	-0.87	0.005	-0.88 to -0.86
Random effect method	<b>-0.88</b>	<b>0.093</b>	<b>-1.06 to -0.70</b>

Q statistic to assess between study heterogeneity  $\chi^2_{2df} = 12.35; p < 0.01$ .

levels are used as outcome measures. Cacao extracts, containing 10–13% w/w PPs,<sup>106,113</sup> and oolong tea leaves, containing 16% w/w PPs,<sup>105</sup> reduce caries increments and dental plaque levels. Naringenin exhibits dose-dependent anti-caries/anti-plaque effects, and while 0.57% w/w quercetin and naringin decrease caries increment, rutin does not.<sup>132</sup>

Thus, studies on rats are suggestive of anti-caries and anti-plaque activities of PPs among rats fed highly cariogenic diet.

(IV) Studies on humans. According to one observational study, high consumers of coffee, barley coffee, tea and wine show lower lactobacilli and mutans streptococci levels in plaque and saliva and lower dental plaque scores, than low/none consumers.<sup>133</sup> However, the quality of this study is low, since the authors did not take into account the effect of important confounders, such as oral hygiene and other dietary variables.

Three double blind clinical trials investigating the effect of PP-containing mouthrinses on plaque accumulation and mutans streptococci have similar design. Namely, levels of plaque accumulation and of mutans streptococci in plaque or saliva were assessed among adult volunteers who received thorough prophylaxis to remove dental plaque at baseline and refrained from oral hygiene except for rinsing with the test or the placebo mouthrinses throughout the following 72 h. In the first study, with a mouthrinse produced from oolong tea leaves (0.2 mg/ml PPs), volunteers rinsed with the test mouthrinse nine times daily and refrained from drinking tea or coffee. After 1 week, subjects repeated the same protocol using the placebo mouthrinse. Significantly lower mean Plaque Index and non-significantly higher mutans streptococci level were observed during the test period.<sup>134</sup> In the second study, with a mouthrinse produced from cacao bean husk (approximately

0.13 mg/ml PPs), the same study protocol used in the previous trial was adopted, with the difference that volunteers were asked to refrain from drinking tea, coffee, cocoa and from eating chocolate. Significantly lower mean Plaque Index and mutans streptococci levels were detected during the test period.<sup>114</sup> In the third study, with a mouthrinse produced from the bract part of hops (approximately 0.35 mg/ml PPs), volunteers were randomly located to the test and placebo groups and, after 2 weeks, the protocol was repeated with a crossover design. Volunteers rinsed five times daily. Significantly lower mean Plaque Index and mutans streptococci level were reported in the test subjects.<sup>135</sup>

The data from these trials can be pooled and meta-analysed. The outcome measures used were mean Plaque Index (expressed through a scale ranging between 0, that is, absence of visible plaque and 5, that is, plaque covering the entire surface of the teeth) and mean mutans streptococci level (expressed as logarithm of colony-forming units per ml, log cfu/ml). In each trial the effect of the treatment, that is, the use of PP-containing mouthrinses, was calculated as the difference between means during the test and the control periods. The heterogeneity between studies was assessed using the Q statistic. The pooled estimates of the treatment effect were calculated with the inverse variance weighted method, using the general fixed effect method, applicable in case of low between-study heterogeneity, and the random effect method, preferable in case of high heterogeneity. More details on the method used in this meta-analysis are described by Sutton and colleagues.<sup>136</sup> The results are shown in Tables 2 and 3. Using Plaque Index as outcome measure, the studies resulted significantly heterogeneous ( $p < 0.01$ ). Therefore, according to the random effect method, among subjects with no other oral hygiene measure than mouthrinsing, the daily use of PP-containing mouthrinses from 5 to 9 times may reduce the Plaque Index score by 0.7 and 1.1 with 95% probability (Table 2). Using the level of mutans streptococci as

**Table 3 – Pooled estimates of the treatment effect of polyphenol-containing mouthrinses on the mean level of mutans streptococci (expressed in log colony forming units (cfu)/ml) using the fixed and the random effect methods (the best fitting method is in bold character).**

Study	Mean difference (test-control)	Variance	95% confidence interval for difference
Ooshima et al. <sup>134</sup>	+0.12	0.087	-0.05 to +0.29
Matsumoto et al. <sup>114</sup>	-0.34	0.010	-0.36 to -0.32
Shinada et al. <sup>135</sup>	-0.40	0.066	-0.53 to -0.27
Fixed effect method	<b>-0.31</b>	<b>0.008</b>	<b>-0.33 to -0.29</b>
Random effect method	-0.29	0.013	-0.31 to -0.27

Q statistic to assess between study heterogeneity  $\chi^2_{2df} = 2.34; p = 0.3$ .

**Table 4 – Quality of the evidence of the protective effect of polyphenols against oral diseases rated using the three-point scale used by the US Preventive Services Task Force.<sup>75</sup>**

Oral disease	Quality of evidence	Comments
Oral cancer	Good	Sufficient, direct evidence. Consistent, well-designed, well-conducted studies
Periodontal disease	Fair	Sufficient, indirect evidence. Consistent, well-designed, well-conducted studies
Dental caries	Fair	Sufficient indirect evidence. Consistent studies
Dental caries (mouthrinses)	Fair	Sufficient indirect evidence. Consistent, well-designed, well-conducted studies

outcome measure the trials resulted homogeneous. Therefore, according to the fixed effect method, PP-containing mouthrinses may decrease mutans streptococci levels by 0.29 and 0.33 log cfu/ml with 95% probability (Table 3). Converting the log values into cfu/ml, PP-containing mouthrinses decrease the mutans streptococci level approximately by one half.

## 2.6. Quality of the evidence

The quality of evidence regarding the effect of high/very high PP intake on oral diseases is displayed in Table 4. There is good evidence that high PP intake has a preventive effect against oral, oesophageal and pharyngeal cancers, as such effect is consistently directly and indirectly reported by both experimental and epidemiological studies. There is fair evidence that high PP intake has a preventive effect against periodontal disease. In fact, such an effect is not directly reported, but it is biologically plausible and indirectly deducible from studies consistently showing that PPs are active against periodontal pathogens and strengthen the antioxidant activity of saliva and crevicular fluid. There is fair evidence that PPs decrease the risk for caries, because biological plausibility and indirect evidence are sufficiently sustained by several consistent studies. Despite the three clinical trials using PP-containing mouthwashes showing a consistent preventive effect, the evidence that these products are an active anti-caries measure is only classified as fair because they demonstrated effective in decreasing mutans streptococci and Plaque Index levels and not caries directly.

In summary, regular and frequent consumption of foods/beverages containing PPs may help prevent oral cancer, effectively. With regard to periodontal disease and dental caries, studies on humans are needed to confirm the promising results provided by the many experimental studies.

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