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Clinical Assessment of a Pharmaceutical Polyphenol Fruit Extract Gel to Inhibit Dental Plaque

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Abstract

Oral bacteria (e.g. *Porphyromonas gingivalis*) are associated and potentially co-causative in several medical conditions including Alzheimer's disease and increased cardiovascular risk. Naturally occurring polyphenols are a developing class of antimicrobial pharmaceutical agents. The use of plant-based polyphenol compounds is an attractive area of medical and dental research due to their high safety factor from their presence in vegetables and fruits. This preliminary study evaluated polyphenols found in berries to reduce bacterial dental plaque accumulation. The study was an *in vivo* single blind randomized controlled trial of seven subjects with a novel tooth gel using validated measurements. Results showed a significant reduction in plaque accumulation ($p = 0.005$) and a non-significant trend to reduce gingivitis ($p = 0.08$). The bioactive gel showed no local or systemic adverse effects and was well tolerated by subjects.

Keywords

Dental Plaque, Gingivitis, Polyphenol, Quercetin, Randomized Controlled Trial

1. Introduction

The oral cavity contains an impressive and diverse array of microorganisms of bacteria, viruses, and fungal elements. In addition, the human dietary intake may contain multiple environmental toxins from their incorporation into the food chain. Direct breathing of polluted air from smog and industrial chemical exposures adds to an altered oral microbiome. While most oral bacteria are

commensal, one anaerobic type is *Porphyromonas gingivalis* (*P. gingivalis*) and forms part of the "red complex" that is responsible for destructive periodontal disease. There is an accumulating body of evidence from animal models and human cadaver studies that *P. gingivalis* is a pathogen of interest in the causation of Alzheimer's disease. This bacterium secretes proteases that cleave amyloid and tau causing the formation of β-amyloid proteins and neurofibrillary tangles [1]. *P. gingivalis* is an opportunistic bacteria that inhabit periodontal pockets. The pockets are initially established by local inflammation and calcification of supragingival dental plaque. The reduction of dental plaque would be a logical and feasible first step approach to prevent habitation of *P. gingivalis*.

From a dental perspective, there have been extensive epidemiological, clinical and bacteriological studies that have long demonstrated the direct link between the accumulation of dental plaque, gingivitis, periodontitis and caries [2] [3] [4] [5]. Current global oral health techniques of plaque removal employ tooth brushing with a dentifrice and the use of interdental devices including dental floss, toothpicks and interdental brushes [6]. A key research goal for clinical translation has been to assess the nature of dental plaque for disruption of the bacterial biofilm via chemical means [2]. To achieve this disruptive mechanism many dentifrices now contain synthetic pharmaceutical agents such as chlorhexidine, metal fluoride complexes (stannous fluoride) and triclosan. These common chemical additives have been shown to reduce plaque accumulation with chlorhexidine being the most effective [6] [7].

Current estimates suggest that less than one-third of the population of developed countries can be expected to practice adequate mechanical plaque removal [8]. In developed countries, there is an increased awareness for the aging population to have a decline in oral health and increased caries due to the physical limitations of personal oral cleansing habits. Therefore, it can be argued that supplementation of oral hygiene practices with a chemotherapeutic agent would be beneficial in limiting the oral disease burden to hard and soft dental tissues [7]. Chlorhexidine whilst effective at plaque control has unwanted side effects of the staining of the teeth, propensity to calculus formation and altered taste [9] [10] [11].

Increasingly, herbal compounds have been studied for their efficacy in inhibiting the formation of dental plaque by their antibacterial effect on periopathogenic and cariogenic bacteria [12]. Herbal compounds have been shown to have antimicrobial properties, increase salivary pH buffering capacity, reduce inflammation and assist in wound healing [13]. Berries are a particular area of interest within the herbal research domain as they have numerous bioactives termed polyphenols that have demonstrated anti-inflammatory activities. Studies of these naturally occurring polyphenols have found these chemicals exert an inhibitory effect on microorganisms commonly found in dental plaque that is a precursor to periodontitis, periodontal pocket formation and dentinal caries [14] [15]. Quercetin is one such polyphenol that has an inhibitory effect on periodontal

pathogens *in vitro* [16]. Recently raspberry leaf, containing quercetin and kaempferol polyphenols, has been shown to significantly reduce pain and reverse disease progression in the mucosal autoimmune disease of oral lichen planus *in vivo* [17].

The current aim of this pilot study was to assess the efficacy of a novel herbal topical gel formulation comprising of a concentrated extract obtained from several professional herbal grade berry sources that contain quercetin. Herbal extracts containing quercetin include raspberry leaf, juniper berries and blueberry fruit extract. Specifically, the study was to quantify the accumulation of dental plaque and its corresponding gingival health *in vivo* using the herbal extract in a tooth gel. An additional aim was to evaluate if the test polyphenol formulation caused any unwanted oral esthetic changes similar to the detrimental cosmetic consequences of chlorhexidine that have significant tooth enamel staining characteristics. A final aim was to evaluate any adverse local or systemic effects.

2. Materials and Methods

2.1. Materials

The concentrated polyphenol berry extract was prepared in a suitable, easy to dispense gel formulation to replicate commercial toothpaste gels used by the public. The active treatment gel contained 0.65% polyphenol concentration adjusted to pH 7.0. The treatment gel was yellow due to the polyphenols as pure quercetin has a strong yellow base color. A corresponding yellow placebo gel was formulated with a neutral commercial supermarket food coloring additive (https://queen.com.au/). The constituents of the placebo gel were water, food colors (102, 122) with 2.3% total dyes, food acid (330) and preservatives (211, 202). No preservatives were included in the treatment gels, such as phenoxyethanol, to avoid potential confounding results as preservatives have intrinsic antimicrobial activity. The active and placebo gels were comparable for taste, viscosity and color.

Preliminary *in vitro* studies by author ERV (unpublished data) were conducted to identify a polyphenol concentration that would inhibit plaque accumulation. This was performed on extracted 3rd molar teeth that were bathed in a glucose enriched saliva at 37˚C for seven days. A concentration of 0.65% quercetin polyphenol in the gel was found to visibly inhibit plaque. A volume of 1 - 2 mL of active/placebo gel was necessary to coat the buccal surfaces of eight teeth *i.e.* four premolars and four molars that would be tested on the ipsilateral quadrants. The maximum polyphenol concentration applied to these quadrants was estimated to be 1.5 mg. This provided reassurance on avoiding any potential systemic or local toxic effects from quercetin as the generally recommended oral supplemental daily dose is 500 mg of quercetin. The gel was adjusted to pH 7 to maintain homeostasis of resting saliva and not encourage spontaneous salivary output from an acidic gel formulation. Laboratory testing of the gel showed continued efficacy over seven days indicating stability of the formulation however

long term stability studies with chromatography are still required.

2.2. Data Measurements

The study was conducted as a single blinded, placebo controlled, randomized trial. Prior to the commencement of the study each subject had all teeth undergo cleaning and prophylaxis and confirmed by erythrosine plaque disclosing tablet (CareDent, Sydney, Australia). Clinically validated measurements and photographic images were recorded at day 0 (baseline) and at day 5 (termination of the study). Photographic images were used to visually confirm the plaque accumulation in subjects using the erythrosine plaque disclosing method at baseline and day 5. Images were captured with a high resolution digital Canon SLR 550D camera with flash using intraoral mirrors.

For each gel application the subjects were to rinse with water and then apply the gel formulation to the buccal, mesial and distal surfaces of the posterior teeth in each quadrant (tooth numbers 14 to 17, 24 to 27, 34 to 37 and 44 to 47 International Dental Notation) using a separate micro-brush for each side. Prior testing showed the micro-brush delivered the gel carefully without disturbing the accumulated plaque. Subjects applied the gel formulation three times each day after each main meal. Subjects were instructed not to rinse for 30 minutes after the application of the gels. Tooth brushing was only permitted on the buccal, lingual or palatal surfaces of the anterior teeth (13 to 23, 33 to 43). Subjects were permitted to use their current toothpaste on the anterior teeth. However, subjects avoided flossing and the use of any other forms of mouth wash or chewing gum.

Clinical scores of plaque accumulation on the selected teeth used the Silness and Löe Plaque Index (PI) [18]. Gingival inflammation was scored with the Gingival Bleeding Index (GBI) validated by Ainamo and Bay [19] and the widely accepted clinical three point per surface "bleeding on probing" measurement of the buccal surface of the teeth, namely the mesial, buccal and distal gingival crevice points per tooth. Collated scores from the three point per tooth yield a two point per tooth per quadrant value in the analysis when applied to multiple adjacent teeth measurements as used in this study (*i.e.* distal of 25 and mesial of 26 are counted as a single score). The periodontal measuring instrument was a Hu-Friedy WHO periodontal probe (Henry Schein Pty Ltd, Sydney, Australia).

2.3. Subjects

The study recruited seven volunteers (three female and four males) ranging from 26 to 62 years (mean 42.9, SD 16.6) from a private dental clinic setting. Subjects were in good oral health with no evidence of dental pathology such as enamel decalcification, active dental caries, gingival inflammation or periodontal disease. The inclusion criteria specified eligible participants having no more than one missing tooth per side. Participants with crowned teeth were included in the study because the Silness and Löe PI measures plaque at the gingival one-third

and it is well reported that crown margins, particularly subgingival margins, are locations particularly susceptible to plaque accumulation and subsequent gingival inflammation [20] [21] [22] [23]. Furthermore, subjects were in good health and their medical history showed no systemic conditions that have known oral mucosal manifestations of disease. The study was conducted in accordance to the guidelines of the Declaration of Helsinki. All subjects gave written informed consent.

2.4. Statistics

Statistical methods employed student's paired t-test with a level of significance $p < 0.05$.

3. Results

No adverse local or systemic effects were reported by the subjects. Thorough intraoral examination showed no tooth, mucosal, deep periodontal inflammatory or pathological changes due to the active or placebo gels. Subject 5 was missing one tooth (36) that reduced the number of available tested teeth from 112 teeth to 110 teeth (subject 5 had missing tooth 36 and thus the contralateral 46 excluded in the statistical analyses where appropriate). In addition, there were seven teeth that were crowned with porcelain or gold.

PI scores showed all seven subjects had reduced plaque accumulation on the treatment side compared with the control side ($p = 0.005$) *i.e.* ipsilateral quadrants compared with contralateral quadrants. When comparing tooth location there was a significant difference on PI scores for premolar teeth ($p = 0.004$) but not molar teeth ($p = 0.09$). GBI two bleeding point analysis per tooth showed no significant difference at the commencement of the study between treatment and control sides ($p = 0.6$) but a non significant trend at the end of the study ($p = 0.078$). The active gel showed an impressive reduction of plaque accumulation (raw data) of 46.3% and was slightly improved in the premolar teeth compared with the molar teeth of 48.5% and 44.5% respectively. The reduction in plaque (raw data) between from the active gel varied considerably in the test subjects ranging from subject 4 (16.7%) to subject 6 (73.3%). On the Plaque Index adjusted scores the group showed a reduction in total plaque buildup of 39.5% with premolars having less plaque than the molar teeth of 48.8% and 40.7% respectively. The adjusted Gingival Bleeding Index showed a rapid increase of plaque for the placebo (+475%) and a slight reduction (−16.7%) for the active polyphenol gel (**Tables 1-3**; **Figures 1-6**).

4. Discussion

This study showed very promising results by day 5 to reduce plaque from the polyphenol gel. Plaque is the major etiological factor of dental caries, gingivitis and periodontitis [2] [3] [4]. These conditions manifest as the most frequently encountered global disease affecting hundreds of millions of people. Despite the

Table 1. Plaque accumulation raw data scores (Silness and Löe).

Subject	Age	Gender	Control gel total	Active gel total	Premolars (control)	Premolars (active)	Molars (control)	Molars (active)
1	57	Female	53	36	26	15	27	21
2	62	Male	40	15	18	10	22	5
3	33	Male	33	22	14	11	19	11
4	29	Male	30	25	13	11	17	14
5*	62	Male	32	25	24	10	9	15
6	26	Female	45	8	21	4	24	4
7	30	Female	42	17	20	9	22	8
Mean	42.9		39.3	21.1	19.4	10.0	20.0	11.1
SD	16.6		7.6	8.3	4.5	3.0	5.4	5.6
% plaque reduction				46.3%		48.5%		44.5%

*Subject 5 missing tooth 36, scores adjusted for teeth 36 and 46.

Table 2. Plaque Index adjusted scores (PI) (Silness and Löe).

Subject	PI control total (day 5)	PI active total (day 5)**	PI control premolars (day 5)	PI active premolars (day 5)***	PI control molars (day 5)	PI active molars (day 5)
1	2.21	1.50	2.17	1.25	2.25	1.75
2	1.67	0.63	1.50	0.83	1.83	0.42
3	1.38	0.92	1.17	0.92	1.58	0.92
4	1.25	1.04	1.08	0.92	1.42	1.17
5*	1.52	1.19	2	0.83	0.75	1.67
6	1.88	0.57	1.75	0.33	2	0.33
7	1.75	1.21	1.67	0.75	1.83	0.67
Mean	1.67	1.01	1.62	0.83	1.67	0.99
SD	0.30	0.31	0.37	0.25	0.45	0.53
% plaque reduction		39.5%		48.8%		40.7%

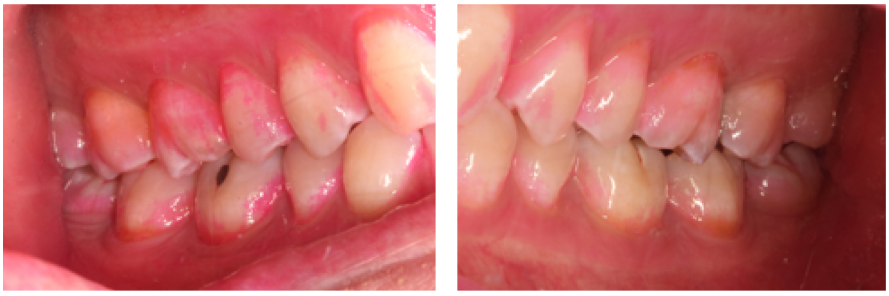
*Subject 5 missing tooth 36, scores adjusted for teeth 36 and 46. **Reduced total plaque accumulation on the treatment side compared with the control side ($p = 0.005$). ***Reduced premolar plaque accumulation on the treatment side compared with the control side ($p = 0.004$).

advances in technology and education, dental disease is still the cause of widespread daily oral pain with a high socioeconomic burden. Loss of teeth results in decreased masticatory efficiency and potential malnutrition. In addition, teeth have a high priority in establishing and maintaining an attractive appearance, and for clarity in speaking. Many forms of employment and social interaction rely on the appearance of teeth. The gel was a formulation specifically tailored for the research trial and did not include any cosmetic or taste considerations. After unmasking the gels several subjects reported the active gel to have a slightly

Table 3. Total bleeding points and Gingival Bleeding Index (GBI).

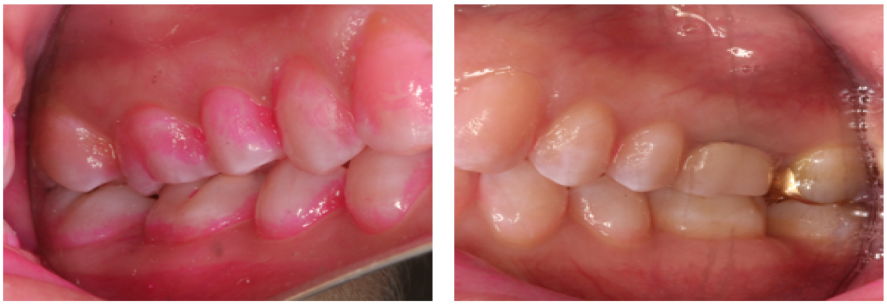
Subject	Total bleeding points (raw data)				Gingival Bleeding Index (adjusted)			
	Control (day 0)	Control (day 5)	Active (day 0)	Active (day 5)	Control (day 0)	Control (day 5)	Active (day 0)	Active (day 5)
1	0	3	0	0	0	0.19	0	0
2	1	1	2	1	0.06	0.06	0.13	0.06
3	0	2	0	0	0	0.13	0	0
4	0	0	0	0	0	0	0	0
5*	2	10	1	3	0.14	0.71	0.07	0.21
6	1	4	0	1	0.06	0.25	0	0.06
7	0	0	3	0	0	0	0.19	0
Total**	4	20	6	5				
Mean	0.57	2.86	0.86	0.71	0.04	0.19	0.06	0.05
SD	0.73	3.23	1.12	1.03	0.05	0.23	0.07	0.07
% change		(+) 502%		(−) 17.4%		(+) 475%		(−) 16.7%

*Subject 5 missing tooth 36, scores adjusted for teeth 36 and 46. Adjusted GBI not significant between control and active groups ($p = 0.078$). **Chi-square test $p = 0.02$ for total number of bleeding points between placebo control gel and active treatment gel.



(a) (b)

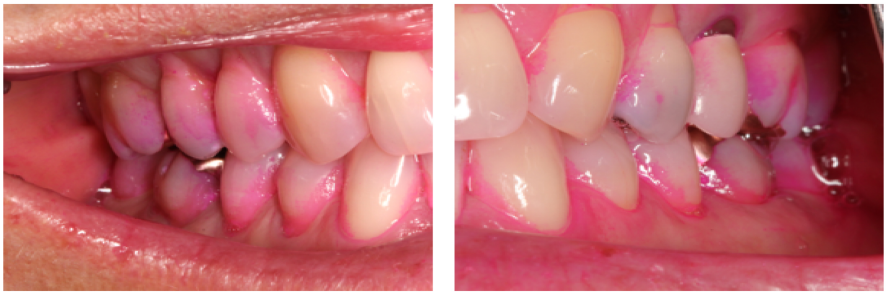
Figure 1. Subject #3 at day 5. (a) Control side; (b) active side.



(a) (b)

Figure 2. Subject #6 at day 5. (a) Control side; (b) active side.

bitter taste but was quite tolerable. Similarly, the yellow color was commented on by several subjects as they preferred to usually purchase "bleached white" color supermarket toothpaste. Cosmetic improvements in the taste and color of the active gel would be a logical development for commercial purposes.



(a) (b)

Figure 3. Subject #1 at day 5. (a) Control side; (b) active side.

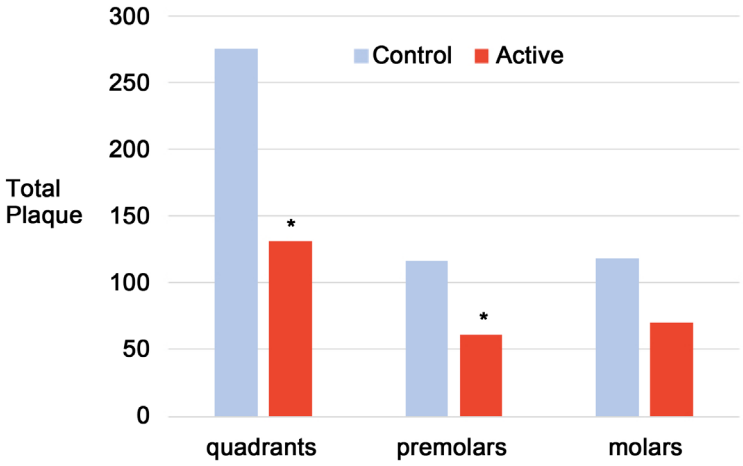


Figure 4. Total plaque accumulation comparison between control and active gel treatments from baseline (day 0) to day 5. Each column represents total score of the subjects (*$p < 0.01$).

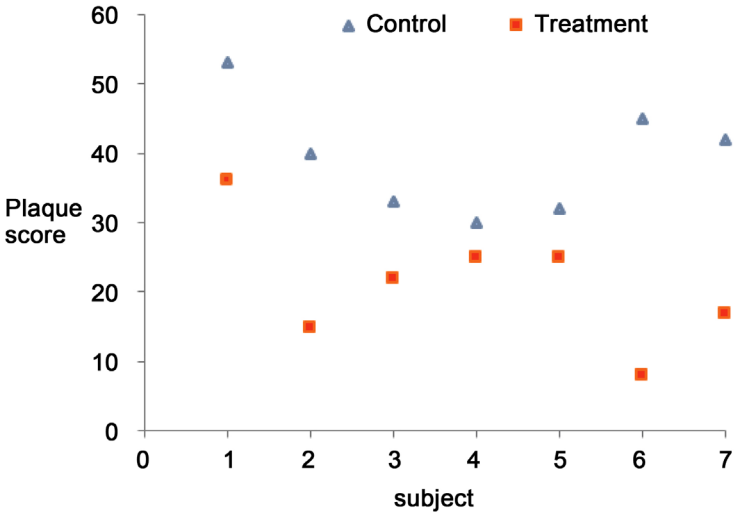


Figure 5. Plaque accumulation comparison (total number for each subject) of control versus active gels treatments from baseline (day 0) to day 5.

The results in this study showed a highly significant improvement by reducing plaque, and remarkably demonstrated a positive outcome in oral health measures

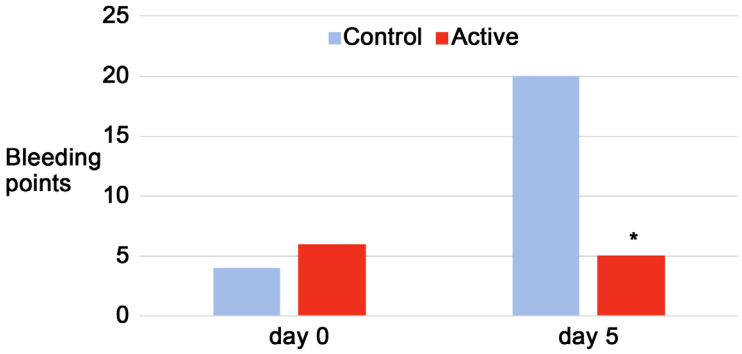


Figure 6. Total number of bleeding points comparison between control and active gel treatments from baseline (day 0) to day 5. Each column represents total score of the subjects (*$p < 0.05$).

in all seven (100%) subjects. From a clinical perspective, this has an important therapeutic value in a new treatment modality by providing assurance when advising patients of the likelihood of success. In contrast, typical study outcomes from a novel drug therapy invariably have subjects who fail treatment. However, in this study despite limited numbers, every subject was a positive outcome. The randomized single blind trial design confirmed the potential utility of the polyphenol approach. The PI and GBI are well-validated instruments [24] [25] [26] [27] [28]. However, one limitation of the PI and GBI in its current form as used in this study was that it does not correct for missing teeth that resulted in a reduction of three reading points for the PI and two reading points for the GBI for subject 5. However, this did not affect the level of significance. Another limitation is that the GBI measures absence or presence of gingival inflammation using only one clinical sign of bleeding (dichotomous index), whereas other measurable signs such as redness and swelling that indicate gingival inflammation are not taken into account. The analysis of the GBI showed there was a trend to reduce gingival inflammation from the active treatment gel ($p = 0.078$). Results showed a significant reduction in plaque and gingival inflammation by the end of the study (day 5). Plaque is the initial step in the development of periodontal pockets. The presence of plaque allows calcification and the formation of supragingival plaque. This in turn protects bacteria in the gingival crevice to commence the destructive chronic inflammatory process of pocket formation and bone loss. Loss of alveolar bone requires surgical intervention and meticulous oral hygiene to prevent tooth loss. Clinical outcomes of uncontrolled periodontal disease manifest with localized tooth mobility and tooth loss. More insidious is the systemic infection of bacteria such as *P. gingivalis* with its potential for brain infection and Alzheimer's disease. Clearly, a simple strategy of plaque prevention would be very helpful for improved oral hygiene and general medical health. The gel showed an approximate 50% decrease in plaque. Conversely, for the control gel, the effect of plaque accumulation caused a dramatic rapid increase of (+) 475% in gingival inflammation as measured on the adjusted GBI. The gel showed no side effects and has inherent safety for oral and gastrointes-

tinal absorption of active compounds. The variation in plaque reduction shows research is needed into the pharmaceutical mechanisms of plaque inhibition and optimal concentrations of the berry polyphenols.

Herbal medicine including dental use of plant extracts has cultural and economic significance in many third world countries. First-world countries are now recognizing the benefits of researching natural compounds containing polyphenol bioactive compounds. Several of these compounds such as curcumin derived from turmeric have anti-cancer properties and are undergoing intense scrutiny in hospital cancer research trials. Quercetin is also receiving considerable interest as it has demonstrated multimodal inhibitory effects in several key pathways in cancer progression [29]. This was tested in the oral environment where the quercetin-containing herb raspberry leaf reversed disease progression in the precancerous lesions of oral lichen planus [16]. It is probable that further research will identify other dental applications of herbal and natural compounds.

From a dental health management perspective, increased plaque particularly on exposed root surfaces can trigger rapid and widespread tooth decay. This area is specifically vulnerable as it leads to problematic restorative dentistry where root caries mechanically undermines the functional load-bearing capacity of the tooth with a resulting crown fracture. In addition, pulpal tissue is quickly involved from root caries leading to non-vitality and periapical infection. Several "at-risk" groups of the population are vulnerable to rampant caries and its devastating outcomes, for example, aged care residents and the elderly have declining dexterity and mental aptitude to perform adequate oral health care. This is exacerbated by the multiple medications used in aged care where the side effect is a reduction in salivary flow rate and its anti-cariogenic pH buffering capacity. Other patients who have received head and neck radiotherapy for cancer management suffer a similar dental crisis [30]. Maintaining optimal oral health with polyphenols would be helpful for bis-phosphonate patients where dental extractions can cause major medical complications of infection, osteomyelitis, osteonecrosis and life-threatening cellulitis.

The inhibition of dental plaque can delay or prevent periodontal pocket formation and potentially the subsequent presence and pathogenic disease states attributed to *P. gingivalis.* This bacterium is a strong risk factor for Alzheimer's disease (AD) that now affects over 24 million people in the world. Over the last 20 years there have 160 drugs trialed to treat AD but all have failed USA Food and Drug Administration clearance due to safety issues or lack of efficacy. Prevention of AD by *P. gingivalis* inhibition is a logical step forward, particularly if safe and efficacious naturally sourced antimicrobial compounds are available. This preliminary investigation used limited subject numbers but was conducted in a randomized, placebo-controlled manner. The early results are highly encouraging to perform a follow-up trial of a larger number of subjects focusing on a group with high plaque accumulation and caries incidence. An advantage of conducting research on the oral cavity is the ability to use blinded place-

bo-controlled trials comparing active to placebo tooth gels and documenting the changes with high-resolution photography. Use of *in vitro* techniques of assessing plaque accumulation on extracted redundant 3rd molar wisdom teeth is another area of research. This approach could investigate parameters of low pH and high carbohydrate loading to identify optimal concentrations of the polyphenols. Moreover, and importantly, the polyphenols are found abundantly in natural food sources and can provide high intrinsic safety margins for rapid clinical translation to improve oral and systemic health.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Author Contributions

SPG and ERV conceived and designed the experiments.
ERV formulated the control and active test agents.
SPG and DMG conducted the experiments.
SPG and ERV analyzed the data.
SPG and ERV wrote the paper.

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