Root canal disinfection and maintenance of the remnant tooth tissues by using grape seed and cranberry extracts

Alexane Febvey[a], Filipe Silva[b,c], Bruno Henriques[b,d], Mutlu Özcan [e], Wim Teughels[f], Júlio C. M. Souza[a,b,c]*

[a]University Institute of Health Sciences (IUCS), CESPU, Gandra PRD, 4585-116, Portugal

[b]Center for MicroElectroMechanical Systems (CMEMS-UMINHO), University of Minho, Campus Azurém 4800-058, Guimarães, Portugal

[c]Associate Laboratory (LABBELS), University of Minho, 4800-058 Guimarães, Portugal

[d]Ceramic and Composite Materials Research Group (CERMAT), Federal University of Santa Catarina (UFSC), Campus Trindade, 88040-900 Florianópolis, SC, Brazil

[e]Division of Dental Biomaterials, Clinic of Reconstructive Dentistry, Center of Dental Medicine, University of Zurich, 8032 Zurich, Switzerland

[f]Department of Oral Health Sciences, University Hospitals, KU Leuven, 3000, Leuven, Belgium

*Corresponding author:

Júlio C. M. Souza, PhD, MSc, DDS

Email: jsouza@dem.uminho.pt

ORCID ID: 0001-7999-4009

Center for MicroElectroMechanical Systems (CMEMS-UMINHO), University of Minho, Campus Azurém 4800-058, Guimarães, Portugal

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Abstract

The purpose of this study was to perform an integrative review on the effects of cranberry and grape seed extracts concerning the disinfection of root canals maintaining the strength of the remnant tooth tissues' structure.

A bibliographical search was carried out on the PubMed electronic platform using the following key terms: cranberry, grape seed, vaccinium macrocarpon, proanthocyanidin, antibacterial, antimicrobial, decontamination, disinfection, bacteria removal, bacteria eradication, bacteria elimination, endodontic, root canal, faecalis, and strength. The inclusion criteria involved articles published in the English language, until March 29th, 2022, reporting the antibacterial effect of grape seed and cranberry extracts.

Of 185 studies identified, 13 studies were selected for the present review. The grape seed extract (GSE), composed of proanthocyanidins, showed an antioxidant activity against the main bacteria found in endodontic secondary infection. The percentage of bacteria removal was recorded at around 96.97% by using GSE. Studies on cranberry extracts, which are composed of proanthocyanidins, revealed antimicrobial effects against bacteria related to as periodontitis and dental caries. Additionally, GSE or cranberry allowed the dentin collagen cross-linking that preserved the 3D collagen network leading to the maintenance of the strength of the remnant tooth structure. However, the contaminated smear layer could not be removed by using only GSE or cranberry.

Cranberry extracts and GSE revealed a significant antimicrobial activity in endodontic disinfection without changing the mechanical properties of the remnant dentin tissues. Furthermore, those components can be associated with traditional compounds to enhance their antimicrobial effects and eliminate the smear layer.

Keywords: Grape seed extract, Cranberry, Antimicrobial, Endodontic, *E. faecalis,* strength

Introduction

The endodontic treatment consists in eradicating the bacteria persisting in the tooth root canal to prevent the infection [1–3]. The tooth root canal disinfection provides the preservation of the remnant tooth structures and surrounding tissues for further dental restoration [1,4–6]. The clinical success rate of endodontic treatment has been recorded between 86% and 98% and deals with the elimination of bacteria and debris from the root canal by hand-held or mechanical instrumentation [7–9]. However, early or late failures can occur due to several factors such as: : (i) incompletely endodontic filling that enhance the risk of apical periodontitis, (ii) improper coronal leakage with bacteria infiltration, (iii) fracture of tooth structures on occlusal loading or trauma, (iv) fracture of infected endodontic files, and (v) dismissed or untreated canals (i.e., molars) [7,10–13]. Most part of the endodontic instruments (endodontic files) does not reach the entire area of the root canal surfaces [7,14]. Previous studies have shown that around 10-50% intracanal surfaces remains untouched by the endodontic files leading to potential risks of persistent microbial infection [9,15,16].

The biofilm is a highly organized structure composed of colonies of bacteria and their metabolism products that are attached on surfaces and englobed in an extracellular matrix composed of glycoproteins, nucleic acids, minerals, and water [17–20]. The biofilm in primary endodontic infection is mainly composed of aerobic and facultative anaerobic species such as: *Parviromonas* (24%), *Solobacterium moorei* (33%), *Fusobacterium nucleatum* (33%), and *Prevotella treponema*, *Eubacterium* and *Campylobacter* [2,21]. On the endodontic disease progression, further micro-organisms can appear even in hard condition (necrotic tissue) such as *Enterococci, Streptococci, Lactobacilli, Actinomyces and E. faecalis*. *Enterococcus faecalis* is a facultative anaerobic gram positive that can invade the tooth root canals in 18% primary endodontic infection and in 67% to 89.6% of endodontic failures [14,21–23]. It can survive as a single micro-organism in hard environmental conditions without oxygen and nutrients, as found in necrotic dentin [21,23,24]. Also, *E. faecalis* adhere on the smear layer as well as on the dentinal surface and tubules leading to biofilm accumulation [14,21].

The most used synthetic antimicrobial solutions are the sodium hypochlorite (NaOCl), calcium hypochlorite ($CaOCl_2$), and chlorhexidine (CHX). Such substances play a key role on the disinfection of the root canals including accessory canals, isthmus, or apical delta although they also can be toxic in the peri-apical tissue region [1,22,25]. The narrow anatomic structure of the endodontic canals may lead to the exposure of the endodontic biofilm to sub-inhibitory concentration of antimicrobial solutions, leading to the selection of particular antibiotically-resistant microorganism [26,27]. A previous study reported a significant increase in biofilm formation of up to more than 50% was found in the isolates exposed to subinhibitory concentrations of several antibiotics [26].

Also, antimicrobial synthetic solutions can generate some cytotoxic reactions on the peri-apical tissues when exposed by the apical foramen within a concentration-dependent effect [1,5,24,28–31]. Then, remnant tooth structures are mechanically affected when using traditional synthetic disinfection solutions due to their actuation on the collagen matrix of the dentin. That can breaks the main component of the dentin and reduce the elasticity, ultimate tensile strength, flexural strength, and the fracture resistance of the remnant tooth root tissues [5,22,28,32,33]. Grape seed (GSE) and cranberry extracts are plant derived extract rich in proanthocyanidin (PA) that have shown antioxidant properties [4,33–37]. They also reveal antimicrobial activity as disinfection solution although without capability to remove the contaminated smear layer. At last, grape seed and cranberry extracts have shown no detrimental effects to the dentin collagen network leading to the maintenance of the mechanical properties of the dentin [5,28,29,32,38].

The purpose of this study was to perform an integrative review on the antimicrobial effects of grape seed and the cranberry extracts without decreasing the strength of the remnant tooth structure. In this study, three research questions were assessed: (i) do grape seed or cranberry extracts have antimicrobial activity on the main bacteria of endodontic infections? (ii) can grape seed or cranberry extracts change the structure of dentin and collagen of tooth root canal structures? (iii) Do grape seed or cranberry extracts affect mechanical properties of tooth root canal structures?

Materials and Methods

Search strategy

A bibliographic review was performed on PubMed (via National Library of Medicine) considering such database includes the major journals in the field of dentistry and biomaterials. The following search terms were applied: grape seed OR cranberry OR vaccinium macrocarpon OR proanthocyanidin AND antibacterial OR antimicrobial OR disinfection OR decontamination OR bacteria removal OR bacteria eradication OR bacteria elimination AND endodontic OR root canal AND *faecalis* AND strength. Also, a hand-search was performed on the reference lists of all primary sources and eligible studies of this integrative review for additional relevant publications. The present search of studies was carried out in accordance with the method used in previous integrative review articles [39–43].

The inclusion criteria encompassed articles published in the English language, until March 29th, 2022. Studies based on publication date were not restricted during the search process. The studies reported the antimicrobial effects of the grape seed and cranberry extracts on the disinfection of tooth root canal maintaining the mechanical properties of the remnant structure. Also, the eligibility inclusion criteria used for article searches involved studies on cell culture, microscopic analyses, animal assays, and prospective cohort studies. The exclusion criteria were the following: papers without

abstract; case report with short follow-up period; articles assessing only the effects of other natural compounds or synthetic chemical solutions.

Study selection and data collection process

The study selection and data collection were performed into three steps. At first, studies were primarily scanned for relevance by title, and the abstracts of those that were not excluded at this stage were assessed. Two of the authors (JCMS, AF) independently analyzed the titles and abstracts of the retrieved, potentially relevant articles meeting the inclusion criteria. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager (Elsevier). The second step comprised the evaluation of the abstracts and non-excluded articles, according to the eligibility criteria on the abstract review. Selected articles were individually read and analyzed concerning the purpose of this study. At last, the eligible articles received a study nomenclature label, combining first author names and year of publication. The following variables were collected for this review: authors' names, journal, publication year, aims, details of the natural compounds (scientific term, concentration, purity degree, etc), antimicrobial effects, assessed bacteria, methods, and main outcomes. PICO question was adjusted to the issue where "P" was related to the patients, animals, or specimens while "I" referred to the methods of analyses. Data of the reports were harvested directly into a specific data-collection form to avoid multiple data recording regarding multiple reports within the same study (e.g., reports with different set-ups). This evaluation was individually

carried out by two researchers, followed by a joint discussion to select the relevant studies.

Results

The initial search in the available database yielded a total of 185 articles of which 139 duplicate studies were eliminated. Of the remaining 46 studies, the titles and abstracts were read seeking concordance with the inclusion criteria of the present study and then 33 studies were discarded because they were related to other disinfection solutions or treatment without tooth root canal treatment. The evaluation of titles and abstracts resulted in the selection of 13 potentially studies which were maintained for this review. The results of the selection of articles are shown in Figure 1 that summarizes the search strategy and selection of studies.

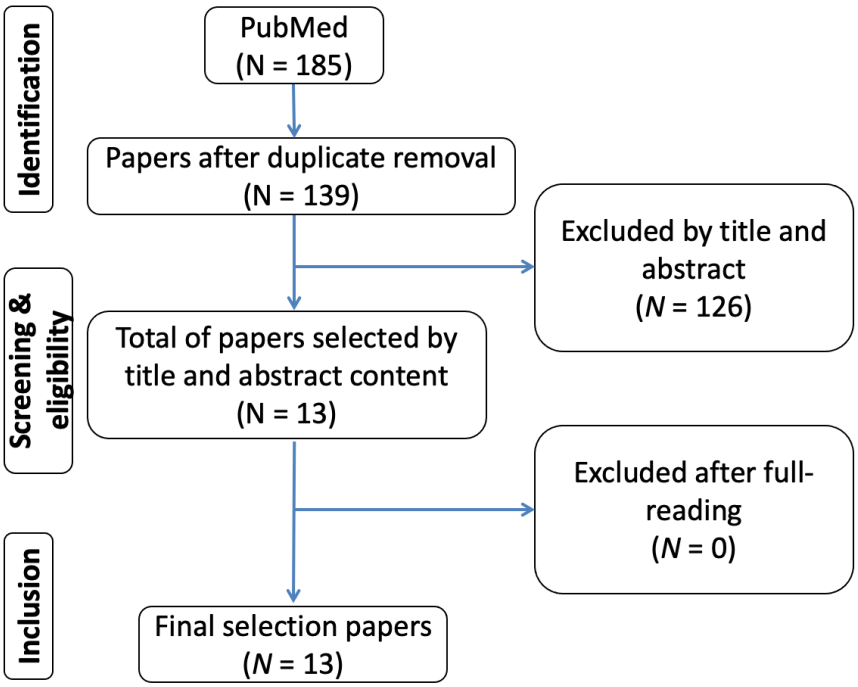

Figure 1. Flow diagram of the search strategy used in this study.

On the 13 selected studies, ten (79 %) studies focused on grape seed extract (GSE). [14,23,24,28,30–32,34,38] of which six (60%) studies focused on the antimicrobial capability of GSE when compared with different disinfection solutions. [14,23,24,30,31,34] Two of those studies evaluated the zone of bacteria inhibition [30,34] and the four others counted the colony forming unit (CFU) in agar plates [14,23,24,31]. Two (20%) studies evaluated the GSE effects as endodontic disinfection solution maintaining the mechanical properties of the remnant dentin [5,32]. Finally, two other studies analyzed the effect of grape seed extract as a cross linker of collagen fibers [28,38]. Regarding the cranberry extract, only one article evaluated the minimal inhibitory content of cranberry against *E.faecalis*. The ultimate two articles reported the benefits effects of the proanthocyanidins (PA), predominant molecule present in the GSE or cranberry extracts. One study recorded the bacterial eradication using confocal laser microscopy (CLSM) [22] and the other one evaluated the mechanical properties of dentin after exposure the PA-based compounds [29].

The main results shown in Table 1 and Figure 2 are described as follow:

- Grape seed and cranberry extracts contains PA in its chemical composition which is responsible for the antimicrobial activity [30,34] [33]. GSE solution showed a bacteria eradication of around 85 % while the GSE gel revealed a bacteria eradication of around 76 % [31];

- On agar plates, the colony forming unit for *E.faecalis* growth decreased in the presence of 5% GSE with a higher the bacteria inhibition when GSE was associated with

chlorhexidine and calcium hydroxide. The formation of ROS decreased in the groups tested with GSE [30,34];

- A high percentage of bacteria eradtcation of around 97% was recorded when the tooth root canals were disinfected with GSE solutions after using two different endodontic rotatory files, namely Reciproc™ and ProTaper™ [23]. However, there no significant differences in the bacteria eradication [23];

- After irrigation with GSE, two studies assessed the mechanical properties of the dentin such as flexural strength, fracture resistance, and ultimate tensile strength [5,32]. Higher mean values were recorded in the groups which included GSE in the chemical composition of the endodontic disinfection solution. The flexural strength was recorded at 4.5 MPa for dentin free of GSE while the dentin after exposure with GSE revealed a flexural strength at 5 MPa [5,32];

-The minimum inhibitory concentration of cranberry extracts was assessed for 50 and 100µg/mL against *E.faecalis.* However, studies focused on cranberry-based PA and they reported antimicrobial activity for PA concentration from 37% down to 10% PA [22]. After contact with cranberry extracts, the elastic modulus of dentin increased from 11.3 to 86 GPa the ultimate tensile strength increased from 8 up to 17.4 MPa [29].

Discussion

The present integrative review reported the main outcomes of relevant previous studies considering the effects of grape seed (GSE) or cranberry extracts on the bacteria eradication and smear layer removal from tooth root canals. Further benefits of GSE or

cranberry extracts on the maintenance of the chemical composition and properties of remnant tooth tissues were discussed in the present review. Previous selected studies compared the effects of GSE and cranberry extracts against traditional synthetic disinfection solutions such as NaOCl, CHX, $Ca(OCl)_2$ and $Ca(OH)_2$ .Thus, the findings validate the hypothesis of this review study and therefore a detailed discussion on the bacteria eradication, smear layer removal, and the remnant tooth tissues' maintenance has been accomplished as follow.

The selected studies have shown significant outcomes on the use of GSE considering bacterial eradication when compared with the traditional disinfection solutions. The effects of GSE depend on the concentration, physical state, and exposure time. In a previous study, two different types of GSE were also tested: solution and gel [31]. The flowable state of GSE revealed higher bacteria elimination than that recorded in the gel form [31]. A previous study recorded the percentage of bacteria eradication at 47% for 2%CHX, 72% for 6.5% GSE, and 87% for 5.25% NaOCl after 10 minutes of exposure time [24]. Thus, GSE has a potential effect against bacteria that depends on its concentration [24]. Lower content of GSE has revealed no effective bacteria eradication percentage into the tooth root canals [30]. Another study reported the bacteria eradication comparing different disinfection solutions and two mechanical system of root canal instrumentation [23]. In 50% GSE solution, the elimination of the bacteria was slightly higher using ProTaper™ system (96.97%) than that for Reciproc™ 25 system (96.72%) [23].

Thus, grape seed extract is a plant derived extract which has a high content of phenolic compounds rich in proanthocyanidin in oligomeric and polymeric forms

[4,5,22,23]. Proanthocyanidin (PA) compounds are classified as flavonoids which are found in grape seed, cranberries, berries, and nuts [35,38]. For instance, GSE is composed of PA, oligomers of flavan-3-ol, that shows anti-microbial and potent bioactive antioxidant compounds. Such molecules can act against the Gram positive and Gram negative bacteria [4,23,34]. The polyphenols can eradicate bacteria by modifying the microbial cell permeability which lead to cellular death due to the following inhibition pathways: (i) nucleic acid synthesis, (ii) cell cytoplasmic membrane function, (iii) bacteria metabolism, (iv) biofilm formation, and (v) membrane permeability. [22–24]. Additionally, GSE-derived PA can promote the formation of reactive oxidative species (ROS) that can destroys *E.faecalis* [34]. ROS are subproducts of the oxygen metabolism with beneficial effect in cell's signaling and homeostasis in normal condition although revealing detrimental effect regarding cytotoxicity in oxidative stress conditions (3,26). It should be highlighted the equalized formation of reactive oxidative species (ROS) takes place in the presence of PA released from GSE (Figure 4) (3,26). The balanced formation of ROS can destroy the cell wall and plasma membrane of *E.faecalis* leading to bacteria death [34]. Thus, the antioxidant properties of the GSE can act as a protective agent against the ROS formation [34].

On tooth root canal treatment, hand-held or mechanical instrumentation can cause debris that can accumulate on the tooth root canal surfaces, mainly in the lateral or accessory canals and isthmus [1,29,45]. The narrow dimensions of some regions may inhibit the availability of GSE-based PA [1,29,45]. Also, instrumentation debris are composed of compacted inorganic particles, microbial metabolites, and bacteria [1,25]. Considering its properties and chemical composition, GSE does not dissolve organic or

inorganic dentin compounds and therefore it must be used with a complementary solution to dissolve smear layer [23]. The dentin organic matrix consists of 90% type I collagen which is organized in a fibrillary network and crosslinking with proteins and proteoglycans resulting in a intratubular permeability [5,23,32,38] [46,47]. Dentin demineralization using a chelating agent is important for exposing dentin tubules and thus underlying the collagen-matrix network [5].

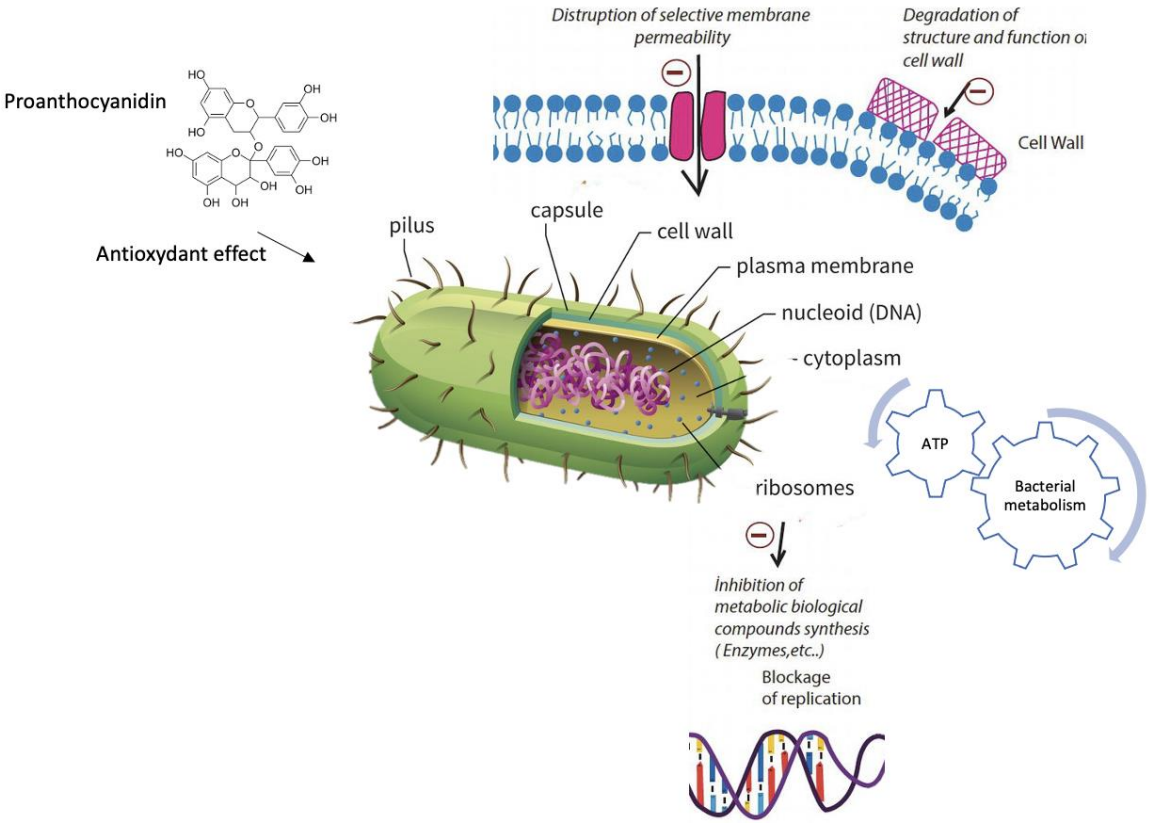

Figure 2. Antimicrobial efficacity of the GSE.

A previous study evaluated different key factors implicated in the strength of the remnant teeth structure after irrigation with GSE or other two traditional disinfection solutions. The findings revealed high values of flexural strength and tensile strength of

dentin after irrigation with GSE [5,32]. In fact, the fracture resistance of the remnant tooth tissues increased when 6.5% GSE was used as irrigation solution instead of NaOCl [5]. It should be emphasized that a decreased thickness of dentin negatively affects the strength of the remnant tooth tissues. The enhancement of the mechanical properties of the remnant tooth tissues after irrigation with GSE occurs due to its ability to establish strong bonds such as covalent, hydrophobic and ionic bonds between the amide carbonyl protein and the hydroxyl phenol group of PA in the endogenous and exogenous collagen network [5,6,28,31,32]. That increase the inter- and intra-fibrillary collagen network of the dentin [5,14,24,28,32]. GSE also inhibits metalloproteinase and cathepsin cysteine, both present in the dentin and the saliva [5,14]. Metalloproteinases are enzymes present in the dentin, and have the capability to turn-over the extra-cellular matrix [25]. Metalloproteinase and cathepsin cysteine can activate their degradation pathways in acidic environment leading to the continuous degradation process of dentin in non-specific ways [5,14] [6].

The proteolytic action of traditional NaOCl solutions can damage and break the collagen fibrils and the proteoglycans chains [5,48]. The dissociation of NaOCl in Na^+ and OCl^- ions can alter the microbial cytoplasmic membrane leading to the denaturation of the compounds of the bacteria [30,31]. However, NaOCl or chlorhexidine (CHX) do not have the capability to dissolve the smear layer. Thus, NaOCl can only break down the organic compounds of the smear-layer and therefore that needs to be associated with a chelating agent such as 17% EDTA [30,45]. The alkaline pH of NaOCl (11-12,5) induces changes in the dentinal structures that affects the dentin elasticity, its flexural strength

,and augment the risk of vertical fracture in the remnant tooth structures [5,23,24,30]. The destruction of the collagen fibrils reduces the mechanical properties of the dentin and weaken the dentin structure in a demineralized dentin [23,24,32]. In six previous studies, 17% EDTA was used for smear layer removal [5,14,23,28,31] while one study used 6% citric acid [24], and another one used 10% phosphoric acid [29]. The citric acid enlarged the dentinal tubules, and that was more effective than EDTA 17% in concentration varying between 1 to 10% [1]. Also, the solvent action of NaOCl on both vital and necrotic tissue can lead to caustic and toxic effects, and the complication appears on peri-apical tissues when exposed by the apical foramen during instrumentation. The adverse effects of NaOCl are dose dependent and studies revealed that NaOCl can also induce genotoxic effect [1,5,24,28–32]. That is the major issues of using NaOCl and therefore GSE or cranberry extracts become alternative solutions to avoid such adverse effects.

Vaccinum macrocarpon, known as cranberry, is a source of bioactive flavonoids at around 65% [33] [6]. Three major classes of flavonoids, family of polyphenols, have been identified in cranberry fruit: anthocyanins, proanthocyanidins (PA), and flavan-3-ols. [33,36]. As mentioned, proanthocyanidins can also disrupt the biofilm formation, alter the acid lactic release, and perform a cariostatic effect by binding to the salivary compounds [49,50]. In case of urinary tract infection, the literature is consistent to report that the cranberry extract is an effective antimicrobial auxiliary on the main pathogen, *E.coli,* without developing bacteria resistance. Cranberry-based PA action is mediated by its capability to break down the bacteria accumulation and reducing the main inflammation's mediators [51]. Cytokines such as TNF-alpha, interleukin 1 or

interleukin 17 can be inhibited avoiding the progression of inflammatory reactions at soft and hard tissues [37,51].

In case of dental decay, the biofilm modulation can prevent the accumulation of pathogenic bacteria species such as *Streptococcus mutans, Lactobacillus* and *Actinomyces* [49,50]. Cranberry extract can inhibit the biofilm formation by modifying the dentin surface and avoiding the adhesion of bacteria via two pathways: polysaccharide produced from the bacteria metabolism and the bacterial adhesins [6,36]. Thus, cranberry-based PA can inhibit the accumulation of pahotgenic bacteria such as *Porphyromonas gengivalis* which is responsible for periodontitis [52–54] [6,51,53,54]. Only two articles assessed the antimicrobial potential of the cranberry-based PA on tooth root canal disinfection. A previous study reported a bacterial eradication dependent on the dose and the results were higher with high PA concentration [22]. As an endodontic disinfection solution, only one study evaluated the effects of PA on various microbial strains on oral cavity culture. The minimum inhibitory concentration (MIC) against *E.faecalis* was at 50µg/mL while the minimum bactericidal concentration (MBC) against *E.faecalis* was at 100µg/mL. Those results shown that PA contained in cranberry extracts has an antimicrobial efficiency but not as effective against *Aggregatibacter actinomycetemcomitans* (*A.a*) [33].

An increase in the elastic modulus of the dentin tissue was also recorded as the cranberry-derived PA concentration increased [22]. Thus, the enhancement of the mechanical properties of the dentin is dependent on the PA content. A previous study reported the elastic modulus of the dentin at 86 MPa after contact with cranberry-derived PA while the elastic modulus of the dentin free of PA was recorded at 11.3 MPa

[29]. Also, the tensile strength was doubled between the baseline and after 4h immersion in PA [29]. The process of remineralization of the dentin can be explained by the formation of a hydro soluble complex which bind to the calcium ions [6]. PA is a large molecule with hydroxyl group to establish strong hydrogen bonds [6]. The cranberry extract has the capability to inhibit the metalloproteinase (MMP), specifically MMP1 and MMP9, which reduce the degradation mechanism of dentin [35].

The present review reveals beneficial effects of two natural compounds to inhibiting bacteria without damage of tooth tissues. Indeed, the main advantage of using natural sources of antimicrobial compounds is the low risks of toxicity to the oral tissues [22,34,37,49,55]. That supports the safety of the patients and long-term success of the clinical treatment. Thus, grape seed and cranberry extracts are alternative compounds that have shown no detrimental effects to the dentin collagen network leading to the maintenance of the mechanical properties of the dentin [5,6,22,32]. On the other hand, remnant tooth structures are mechanically affected after using traditional synthetic disinfection solutions for endodontic disinfection such as NaOCl. The synthetic disinfection solutions can generate some cytotoxic reaction on the peri-apical tissues when exposed by the apical foramen. Nevertheless, the previous studies showed limitations related to the in vitro conditions of assessment. A lack of tested variables is noticeable regarding extract concentration and bacteria species. On GSE, different concentrations should be assessed against several pathogenic species in both planktonic and biofilm forms. Also, the models for biofilm assays should consider the morphological aspects of the surfaces such as roughness, pores, smear layer over dentin. On the other hand, only a few studies reported the antimicrobial activity of cranberry-based extracts

for dentin disinfection [33,36,52]. Also, most of studies on cranberry extracts were performed within *in vitro* models involving free-form (planktonic) bacteria or mono-species biofilms [37,53]. The *in-vivo* test are commonly related to the daily ingestion of cranberry or used as a mouth-rinse in association or not with other product such as fluor in dental decay [52,53]. Most of studies focused on the antimicrobial and anti-inflammatory effects of cranberry extracts concerning urinary or periodontal diseases [35,37,51]. Future *in vivo* studies should evaluate the influence of different content of cranberry extracts on several bacterial strains involved in tooth root canal infection.

Conclusions

The present review reported significant findings on the antimicrobial activity of grape seed and cranberry extracts for tooth root canal disinfection maintaining the mechanical properties of the remnant tissues. Within limitations of this review study, the following concluding remarks can be drawn as follow:

- The highest percentage of major bacterial reduction was recorded at 96.97% after exposure to 50% grape seed extract. The effect of the grape seed extract is time and concentration dependent.
- Proanthocyanidin from cranberry also showed a decrease in the bacteria percentage although further studies are required to validate the findings considering applications in tooth root canal disinfection.
- The mechanical properties of the tooth root tissues were not negatively affected after contact with grape seed or cranberry extracts. Proanthocyanidin from

grape seed or cranberry extract acts as cross-a linker on the collagen network that enhance the mechanical properties of the dentin.

- Future studies should consider different shape of tooth root canal after preparation. Also, the presence of smear layer including bacteria and their metabolites should be analyzed as a function of the content of proanthocyanidin. The combination of chelating agents and proanthocyanidin could be a potential approach although that might alter the interaction between proanthocyanidin and the collagen fiber network.

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Table 1: Details of the selected studies on GSE and cranberry extracts.

Authors (year)	Purpose	Preparation of the specimen	Bacterial growth	Natural and synthetic irrigant solution	Method	Main outcomes
Durigon et al (2020) [5]	Evaluation of the effect of different irritant solution on dentin mechanical properties and fracture resistance.	Selection of 296 bovine incisors		G1: distilled water + EDTA 17% G2: GSE+EDTA G3: NaOCl+EDTA G4: NaOCl+ GSE+ EDTA G5: (Ca(ClO)2)+EDTA G6: (Ca(ClO)2)+EDTA +GSE G7: CHX+EDTA G8: CHX+EDTA+GSE	Microhardness using a micro-indeter tester . Micro-scale tensil tests using a universal testing machine Flexural strength recorded by EMIC. Fracture resistance evaluated regarding 2 thickness of root dentin(1.5 and 0.5mm) using EMIC test	Microhardness and flexural strength decreased for G3, G4, G5,G6. GSE improved the degradation resistance and mechanical properties of the dentin
Yang et al (2020) [22]	Evaluation of the biofilm effect of Proanthocyanidin solution as a irritant	Selection of human single teeth. Molars, were cut to obtain 20	*E.faecalis* culture in BHI at 37°C overnight	G1: sterile water G2: 2% CHX	Morphological evaluation by Confocal laser	Proper results for higher concentration of PAC.

	solution against *E.faecalis* and its influence on the mechanical properties and biodegradation resistance of demineralized root dentine.	refined semi-cylindrical specimens 500µL of bacterial suspension added to the root canal and culture over a period of 1 week		G3: 2% PA G4: 5% PA G5: 10%PA 50 µL of each solution was injected into de root canal	scanning microscopy (CLSM)	Bacteria eradication (%): G1: 15 G2: 30 G3: 32 G4: 35 G5: 50
Kumar et al (2019) [33]	Elaboration of a standardized hydro ethanolic extract of vaccinium macrocarpon and assessing its antimicrobial activity	Preparation of samples of *V. Macrocarpon* with maceration method.	6 oral aerobic pathogens and Gram positive in blood agar culture : *S.mutans, E.faecalis, L acidophulis, C. albicans, A. Actinomycetecomitans. P. Gingivalis* All specimens were tested in BHI and incubated at 37°C	*Vaccinium macrocarpon*	Determination of minimum inhibitory concentration (MIC): serial broth dilution method and minimum bactericidal concentration (MBC): agar plate subculture streaking method.	Effective antimicrobial effect of *V.macrocarpon* against *A. Actinomycetecomitans* and *P. Gingivalis*
D'aviz et al (2019) [31]	Comparison of the effectiveness of 5.25% NaOCl, 2% CHX, 6.5% GSE.	Collection of 45 mesio-buccal roots of maxillary molars, preparation to obtain	Culture of *E.faecalis* in aerobiosis brain heart infusion BHI for 24H at 37°C.	G1: NaCl (n:5) G2: 5.25% NaOCl (n=10)	Evaluation microbiological at 2 steps: before and after the instrumentation. Recovery of the canal	No tested irrigant was able to promote complete disinfection.

		root of 13mm and sterilization. Injection of 100mL *E.faecalis* in the canal and incubation for 30 days, renewing BHI every 48H.		G3: 2%CHX gel (n= 10) G4: 6.5% GSE +EDTA(n=10) G5: 6.5% GSE+ EDTA (n=10)	content and incubation in Plate count agar for 48h and then counting the CFU. Analysis of the results by two-way ANOVA test and Tukey	Bacteria eradication (%): G1: 72 G2: 99.69 G3: 92.05 G4: 85.65 G5: 76.39
Fiallos et al (2020) [24]	Evaluation of the antibacterial effectiveness of GSE against *E.faecalis* biofilm through the confocal laser scanning microscopy	Human single root teeth were selected and sectioned to produce 44 dentin discs decontaminated with 2.5% NaOCl and 6% citric acid. The specimens were subjected to UV rays for 15 min.	*E.faecalis* prepared in BHI broth and incubated in anaerobic condition during at 37°C over a priod of 48h.	G1: 5.25% NaOCl G2: 2%CHX G3: GSE vitis vinifera	Evaluation of the optical density using a spectrophotometer. Inoculation 3mL of *E.faecalis* in each disc at 37°C for 21 days Specimens were stained with LIVE/DEAD BacLight fluorescence dye.	Bacteria eradication (%): G1: 87 G2: 47 G3: 72
Soligo et al (2018) [23]	Comparison of the efficacy of GSE, Ca(ClO)2, and NaOCl with rotary or reciprocating	96 mesiobucal canals of mandibular molar. Elimination of the coronal third and sterilization of the	*E.faecalis* Culture in BHI at 37°C for 24h.	CG: NaCl G1: 6% NaOCl, G2: 6% $Ca(ClO)_2$	CFU counting	All groups shown a reduced number of bacteria. No differences between

	instruments for the disinfection of the root canal	roots by autoclave and subsequently contamination of the root with *E.faecalis*	Inoculation of 100 µL into the root canal with sterile BHI for 21 days. Culture of the sample in blood agar at 37°C for 18-24h.	G3: 50% GSE Injection 5mL of solution in each group. Final irrigation with 1mL EDTA 17% and 5mL NaCl.		the 2 methods of instrumentations. Bacteria eradication (%): GG: 87.96 (PTN) and 89.23 (R25) G1: 99.70 (PTN) and 99.56 (R25) G2: 98.02 (PTN) and 99.65 (R25) G3: 96.97 (PTN) and 96.72 (R25)
Cecchin et al (2017) [32]	Examination of the effects of different roots canals irrigants on dentin mechanical properties.	50 teeth with a single canal were selected for the flexural strength evaluation and UTS and 10 molars for flexural strength evaluation of dentin. Preparation of rectangular-shapes beams of dentin and incubation during 30 min.		G1: distilled water + EDTA G2: 6% NaOCl G3: 6% Ca(OCl)2 G4: 6.5% GSE	Flexural strength tests using a universal testing machine at a crosshead speed of 0.5mm/min Ultimate tensile strength : Preparation of 4 hourglass section and incubation the same way.	Irrigation with 6% NaOCl decreased the dentin mechanical properties. GSE did not interfere with the mechanical properties of the dentin

				Each specimen was tested at 0.5mm/min in a micro tensile testing grip and measuring results with digital caliper. Fracture resistance: Selection of root of 12mm and placing the specimens into acrylic resin exposing 6mm of the root. Root were tested in the universal machine at a rate 1mm/min until fracture occurred.	
Cecchin et al (2017) [32]	Evaluation of the effects of NaOCl, CHX and 2 naturally substances	60 single roots selected	G1: 5% NaOCl G2: 2% CHX Subdivided into 3 groups: Control group, GSE group and GT group.	Push out testing Kolmogorov- smirnov test	Higher fracture loading (N) after immersion in EDTA and NaOCl due to the degradation of the collagen fibrils G1: 2.69 G2: 2.50 G3: 2.60

						G4: 2.61
Albino Souza et al (2017) [14]	Evaluation of the effectiveness of final decontamination protocols against *E.faecalis* and their bond strength of filling material to root canal dentin	90 single root human teeth selected and preparation of the root canal with ProTaper™ system.	*E.faecalis* cultivated in BHI broth for 18-24H at 37°C 60 sterilized teeth divided into 6 groups (n=10): inoculation of 100µL *E.faecalis* and culture over a period of 15 days. Renewing the BHI every 48h	G1: distilled water G2: CHX G3: Qmix G4: 6.5% GSE G5: PDT+fiber G6: PDT+ no fiber	CFU counting in agar plates before and after contamination.	Bacteria eradication (%): G1: 0.57 G2: 100 G3: 99.97 G4: 98.02 G5: 96.67 G6: 96.04
Mageshwaran et al (2016) [34]	Analysis of the role of grape seed extract and tomato extract reducing the ROS formation. Evaluation of their antibacterial effect against *E.faecalis*	Preparation of 5mL Proanthocyanidin (PA) solution and lycopene solution by dissolving 5g of each compound into 100mL distilled water.	Preparation of petri dishes with each solution and inoculation of 0.5mL of *E.faecalis*. Incubation at 37°C for 24h	G1: 2% CHX gluconate G2: 125mg $Ca(OH)_2$ + 1mL of 2% CHX gluconate G3: 125mg $Ca(OH)_2$ + 1mL of 2% CHX gluconate + 1mL of 5% PA G4: 125mg $Ca(OH)_2$ + 1mL of	Evaluation of the ROS formation using a mass spectrometer Evaluation of the bacterial removal by agar diffusion method.	G1: 15 mm G2: 20 mm G3: 23 mm G4: 27 mm

				2% CHX gluconate + 1mL of 5% lycopene		
Epasinghe et al (2014) [29]	Comparison of the effects of 3 flavonoids : proanthocyanidin, naringin and quercetin on the modulus of elasticity (MOE) and ultimate tensile strength (UTS) of demineralized dentine.	30 human third molar were selected and sectioned. The specimens were demineralized with 10% phosphoric acid solution for 5H		G1: 6.5% PA G2: 6.5% quercetin G3: 6.5% naringin	Ultimate tensile strength testing	G1: highest increase after 4H : Elastic modulus (86.10 and 22.11 GPa) and ultimate tensile strength (17.45 and 4.42 MPa)
Ghonmode et al (2013) [30]	Comparison of the *in vitro* effectiveness of neem leaf extract, grape seed extracts and 3% NaOCl	Preparation of the solutions of neem extract and grape seed extract by dilution in ethanol and filtration	*E.faecalis* Culture in BHI broth and agar at 37°C for 18h.	G1: neem leaf extract G2: GSE G3: 3% NaOCl G4: ethanol (control) G5: saline (control)	Well diffusion method. 200 µL of cultures injected in the agar plates with 6mm diameter and addition of 50 µl each irrigant solution. Incubation at 37°C for 24h.	Significant differences in the mean inhibition zone values: G1: 19.57 G2: 7.34 G3: 16.34 G4: 0 G5: 0

Srinivasulu et al (2012) [38]	Determination of the shear bond strength of composite resin to deep dentin using a total etch adhesive after treatment with different collagen cross-linking agents at varying time intervals.	2 solutions: 1- sodium ascorbate powder 2- 6.5% PA solution Preparation of 30 human central incisors	G1 (n=12) : control G2 (n=24) : 10% sodium ascorbate IIA: for 5 min IIB: for 10 min G3 (n=24) 6.5% PA IIIA: for 5 min IIIB: for 10 min	Shear bond strength at 1mm/min using a universal testing machine	Highest mean value in G2 and G3. Results in MPa: IIA (22.12), IIB (23.05), IIIA (27.57), and IIIB (27.85)