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Effects of grape seed and cranberry extracts concerning the disinfection of the root canal without decreasing the strength of remnant tooth structure: a systematic review

Alexane Wladislawa Cécile FEBVEY

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

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Trabalho realizado sob a Orientação do "Professor Júlio C.M. Souza"

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Eu, acima identificado, declaro ter atuado com absoluta integridade na elaboração deste trabalho, confirmo que em todo o trabalho conducente à sua elaboração não recorri a qualquer forma de falsificação de resultados ou à prática de plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria do trabalho intelectual pertencente a outrem, na sua totalidade ou em partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores foram referenciadas ou redigidas com novas palavras, tendo neste caso colocado a citação da fonte bibliográfica.

Agradecimentos

Agradeço ao meu orientador de tese, o professor Júlio C.M Souza pela toda a sua ajuda, sua experiência e seus conselhos na redação deste trabalho de fim de grado.

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Agradeço a minha família, que sempre que apoiaram durante este curso.

Resumo

Objetivo: O objetivo deste trabalho foi realizar uma revisão integrativa sobre o efeito antimicrobiano dos extratos de sementes de uvas e de arando na desinfeção de canais radiculares sem, no entanto, prejudicar as propriedades mecânicas da estrutura dentaria remanescente.

Material e métodos: Uma pesquisa sistemática foi feita na plataforma PubMed usando as palavras-chaves seguintes: *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.* Os critérios de inclusão envolveram estudos *in vitro* publicados em língua Inglesa até 11 de fevereiro de 2021 sobre o efeito antimicrobiano das sementes de uvas (grape seed, GSE) e de arando.

Resultados. Sobre os 185 artigos identificados, 13 artigos foram selecionados para esta revisão integrativa. As sementes de uvas (GSE), compostos por proantocianidinas, apresentam uma ação antioxidante sobre a principal bactéria das infeções endodônticas secundárias, *E. faecalis*. Atuando sobre os componentes da membrana celular e sobre o metabolismo bacteriano, a eliminação de *E.faecalis* pode atingir 96,97%. O arando, igualmente constituído por proantociadinas, possui uma atividade antibacteriana comprovado sobre outras patologias da cavidade oral como a periodontite e as caries. Ainda, os compostos do GSE e arando promovem uma densificação da rede de colagénio da dentina e melhora as propriedades mecânicas da estrutura dentária remanescente. No entanto não possuem a capacidade de dissolução do *smear-layer*.

Conclusão: Os extratos de arando e sementes de uva apresentam uma significativa atividade antimicrobiana nos canais radiculares quando usados como uma solução irrigadora sem interferir nas propriedades mecânicas da estrutura remanescente. Além disso, podem ser associados a outros compostos para amplificar o efeito antimicrobiano e para assegurar a eliminação do *smear-layer*.

Palavras chaves :

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

Abstract

Objective: the main aim of this study was to perform an integrative review on the effects of grape seed and the cranberry extracts concerning the disinfection of the root canal without decreasing the strength of the remnant tooth structure.

Materials and methods: An integrative search was carried out on the PubMed electronic platform using the following key terms: *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.* The inclusion criteria involved articles published in the English language, until February 11th, 2021, reporting the antibacterial effect of grape seed and cranberry extracts.

Results: Of 185 articles identified, 13 articles were selected for this integrative review. The grape seed extract (GSE), composted of proanthocyanidins, showed an antioxidant activity against the main bacteria of the endodontic secondary infection. The bacteria removal percentage can reach around 96,97% due to the bacteria cell membrane and metabolism, and biofim formation. Also, cranberry is composed of proanthocyanidins although the antimicrobial effects were validated against bacteria related to as periodontitis and dental decay. Additionally, grape seed or cranberry allowed the dentin collagen cross-linking that maintained the 3D collagen network leading to the maintenance of the strength of the tooth root remnant structure. However, the contaminated smear layer cannot be removed by using only GSE or cranberry.

Conclusions: Grape seed extract and cranberry extracts revealed a significative antimicrobial activity in the tooth root canals when used as irrigant solutions without altering the mechanical properties of the remnant dentin tissues. Furthermore, those components can be associated with traditioanl compounds to enhance their antimicrobial effects and eliminate the smear layer.

Key words :

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

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List of abbreviations and acronyms

GSE: grape seed extract

PA: proanthocyanidin

CHX: Chlorhexidine

NaOCl: sodium hypochlorite

$CaOCl_2$: calcium hypochlorite

EDTA: Ethylenediaminetetraacetic acid

UTS: ultimate tensile strength

MOE: modulus of elasticity

ROS: reactive oxygen species

MMP: metalloproteinase

1. Introduction

The endodontic treatment consists in eliminating the bacteria in the root canal to prevent the infection, preserving the oral tissue around the teeth for further restoration (1–5). The clinical success rate oscillates between 86% and 98% and deals with the elimination of bacteria and debris from the root canal by hand-held or mechanical instrumentation although early or late failures can occur. Most part of the endodontic instruments (endodontic files) does not reach the entire area of the root canal surfaces. Previous studies have shown that around 10% to 50% surfaces remains untouched by the endodontic files leading to potential risks of persistent microbial infection (1,4–7). The major endodontic treatment failures can be related to several factors such as: (i) incompletely endodontic filling that enhance the risk of apical periodontitis, (ii) over or under endodontic filling that not reach the apex, (iii) improper coronal leakage where oral bacteria can invade, (iv) fracture of infected endodontic files, and (v) dismissed or untreated canals (i.e., in molars) (6,7).

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 (8,9). The biofilm can produce a glycoproteic barrier against aggressive substances that increase the bacterial resistance to antibiotics (9). The biofilm in primary endodontic infection is mainly composed of aerobes and facultative anaerobes: *Parviromonas* (24%), *Solobacterium moorei* (33%), *Fusobacterium nucleatum* (33%), and *Prevotella treponema*, *Eubacterium* and *Campylobacter* (8,9). 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 (1,8,10,11). It can survive as a single micro-organism in hard environmental conditions without oxygen and nutrients, as found in necrotic dentin (1,4,5,12). Also, *E. faecalis* adhere on the smear layer as well as on the dentinal surface and tubules leading to biofilm accumulation (13,14).

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 (5,11,15). Although they also can be toxic in the peri-apical tissue region. NaOCl is a proteolytic irrigant while CHX is an antiseptic and act disrupting the bacteria's membrane or altering the osmotic equilibria (2,3,9,11,12). Antimicrobial solutions should have the capability to penetrate the infected site and eradicate the remnant bacteria without causing allergic or adverse reactions to the patients (1–3,13). Even though there is a few case reports in the literature, those irrigant can generate some cytotoxic reaction on the peri-apical tissues when exposed by the apical foramen within a concentration-dependent effect. (2,4,5,12,16–18) Furthermore, remnant tooth structures are mechanically affected when using traditional synthetic irrigant solutions because of 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 (11,16,17,19,20). Grape seed (GSE) and cranberry extracts are plant derived extract rich in proanthocyanidin that have shown antioxidant properties (1,3,14,20). They also present antimicrobial activity as irrigant solution although without capability to eliminate 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. (16–19,21)

2. Objective and hypothesis

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?

3. Materials and methods

3.1. Information sources and 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 present search of studies was carried out in accordance with previous integrative or systematic review articles. 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 systematic review for additional relevant publications. The inclusion criteria encompassed articles published in the English language, until February 11th, 2021, reporting the antimicrobial effects of the grape seed and cranberry extracts on the disinfection of tooth root canal without affecting the strength of the remnant structure. The eligibility inclusion criteria used for article searches also involved: cell culture assays; *in vitro* studies; meta-analyses; randomized controlled trials; 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. Studies based on publication date were not restricted during the search process.

3.2. 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.

4. 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 27 studies were discarded because they were related to other disinfection solutions or treatment without 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 process.

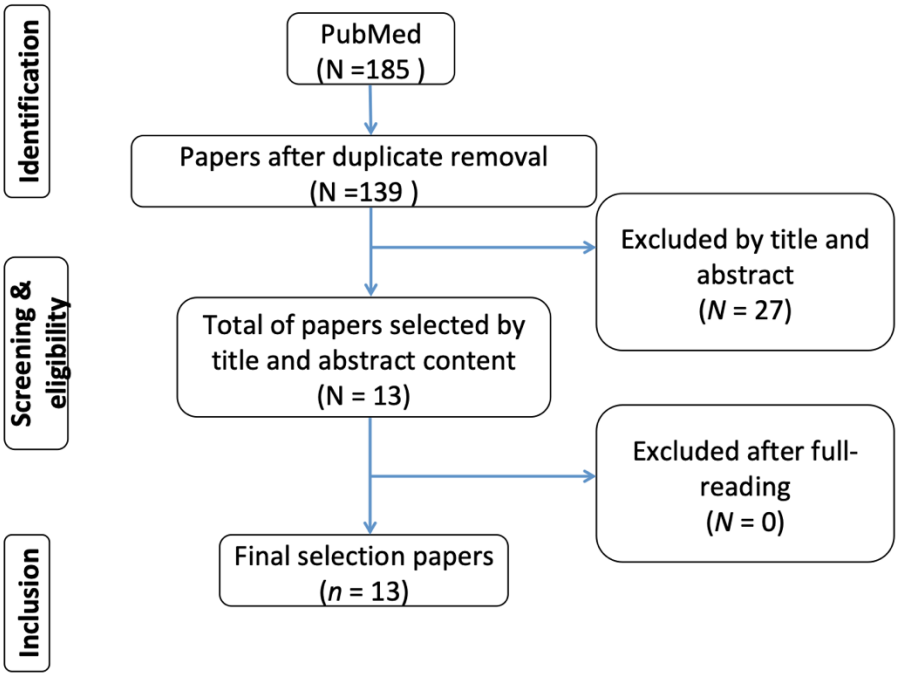

Figure 1: Flow diagram of the search strategy used in this study.

On the 13 selected studies, ten (79,92%) studies focused on grape seed extract. (1–4,10,12,16,19,21) of which six (60%) studies focused on the antimicrobial capacity of grape seed extract when compared with different disinfection solutions. (1–4,10,12) Two of those studies evaluated the zone of bacteria inhibition (2,3) and the four others counted the colony forming unit (CFU) (1,4,10,12). Two (20%) studies evaluated the effect of grape seed extract as an endodontic irrigant on the mechanical properties of the dentin (17,19). Finally, two other studies analyzed the effect of grape seed extract as a cross linker of collagen fibers (16,21). 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, predominant molecule present in the grape seed or cranberry extracts. One study focused on the bacterial reduction using confocal laser microscopy (CLSM) (11) and the other one evaluated the mechanical properties dentin after exposure the proanthocyanidin compounds (18)

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

- Grape seed extract contains proanthocyanidin that is responsible for its antimicrobial activity. ((2,3).

- Grape seed extract contains in its composition proanthocyanidin, responsible for the antimicrobial activity. On the six studies with culture of *E.faecalis* which evaluated the efficiency of grape seed extract as an antibacterial irrigant solution, two measured the diameter of the inhibition zone with GSE irrigant on the bacterial culture, and obtain respectively 7,34mm and 23mm in the second one when 5% GSE is used in conjunction with chlorhexidine and calcium hydroxide. The second one also evaluated the ROS formation and present that, at the pick value, the ROS production was decreased in the solution containing GSE (2,3).

- The four other evaluated the antimicrobial effect by measuring the colony formation units and transferring to into a percentage (1,4,10,12). One of these evaluated the efficacity with two different endodontic system: reciproc and pro-taper, and no significant differences were observed with a bacterial reduction of 96,97% with the ProTaper system and 96,72% with Reciproc 25 (1) Another study used two forms of GSE, in gel or in solution to compare the efficacity and obtain respectively 85,65% and 76,39% (12).

- Two articles assessed the mechanical properties of the dentin after irrigation with GSE (17,19) These studies tested the flexural strength, fracture resistance and ultimate tensile strength, as there are key factor to evaluate the strength of the remnant dentin after irrigation. Higher values were obtained in the groups which included GSE in the composition of the endodontic irrigant, the flexural strength is 4,50 MPa in the control group and 5,01MPa in the group with GSE. This article prove also that the fracture resistance is better when the thickness of the root is higher. The second article demonstrated no significant differences between the groups (17,19).

- Relating to the GSE, two other study focused on one of the proprieties of the GSE: it is a cross linker and can enhance the 3D network of collagen (16,21).

- Related to the cranberry extract, only one article was found (20). This one focused their experience on culture of different bacterial stain and measuring the minimum inhibitory concentration: 50µg/mL and minimal bactericidal concentration: 100µg/mL of cranberry to act on *E.faecalis.*

- Regarding the proanthocyanidin, principal antioxidant compounds found in grape seed extract and in cranberry extract, there is two articles focused on this molecule. One related the antimicrobial activity at different percentages, that can reaches 37% at 10%PA (11) and the other one measured the mechanical properties (18). Relating to the modulus of elasticity it increased from 11,35MPa at the baseline to 86,10MPa after 4hours and for the ultimate tensile strength from 8,05 MPa to 17,45MPa.

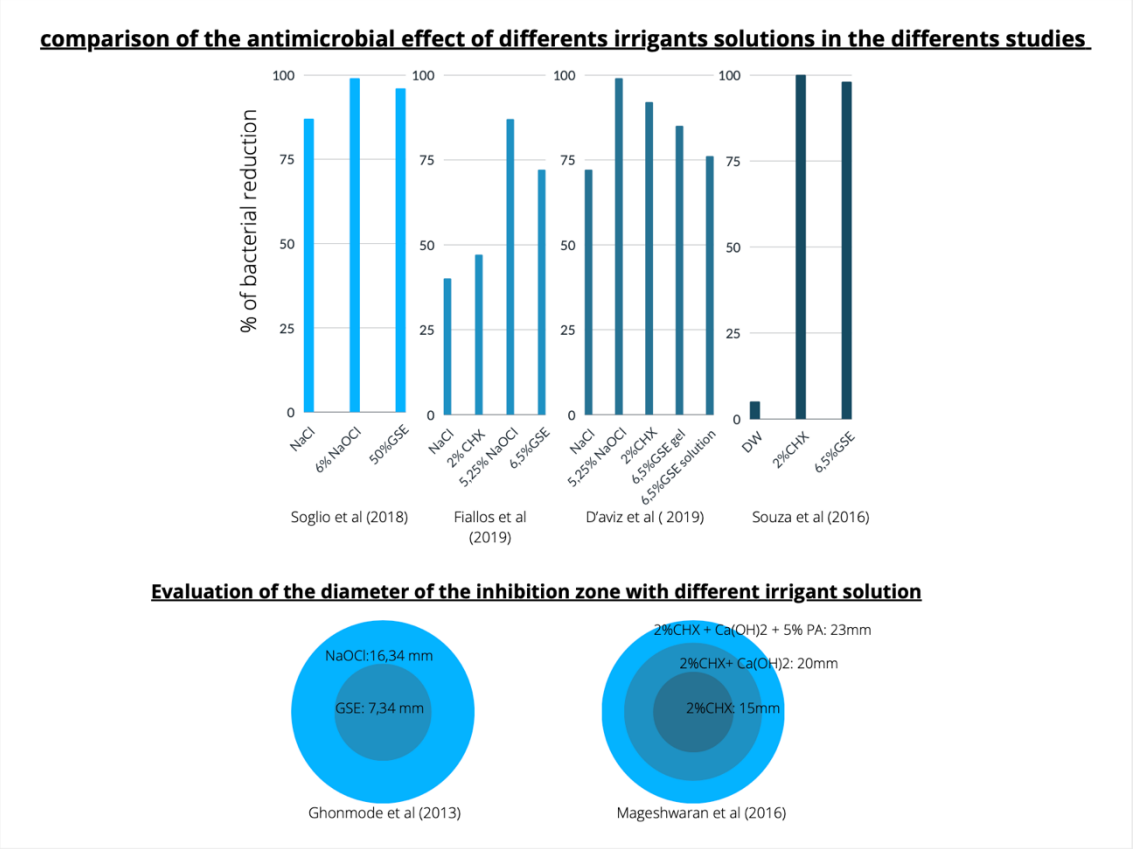

Figure 2: Main outcomes of the selected studies on the bacterial reduction by grape seed extracts (GSE) compared other solutions.

Table 1: Details of the selected studies on the antimicrobial efficacity and on the mechanical characteristics of the GSE and cranberry

Authors (year)	Purpose	Preparation of the specimen	Bacterial growth	Natural and synthetic irrigant solution	Method	Main outcomes
Soligo et al (2018)	Comparison of the efficacy of GSE, Ca(ClO)2, and NaOCl with rotary or reciprocating instruments for the disinfection of the root canal	96 mesiobucal canals of mandibular molar. Elimination of the coronal third and sterilization of the roots by autoclave and subsequently contaminate the root with *E.faecalis*	*E.faecalis* Culture on brain heart infusion (BHI) for 24H at 37°C. Inoculation 100 µL into the root canal with sterile BHI during 21 days. Culture of the sample in blood agar for 18-24H at 37°C	4 groups (n=11) CG: NaCl G1: 6% NaOCl, G2: 6% Ca(ClO)2, G3: 50% GSE	Preparation of the solution at a concentration of 6% distilled water. Injection 5mL of solution in each group. Final irrigation with 1mL EDTA 17% and 5mL NaCl. Analysis microbiological with recovery of the material at 2 stages (before and after instrumentation), culture and count of the CFU	All groups shown a reduced number of bacteria. No differences between the 2 methods of instrumentations. 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)
Ghonmode et al (2013)	Comparison of the in vitro effectiveness of neem leaf extract,	Preparation of the solutions of neem extract and grape seed extract by dilution in ethanol and filtration	*E.faecalis* Culture on brain heart infusion (BHI) broth and agar at 37°C during a night.	5 groups: G1: neem leaf extract G2: GSE G3: 3% NaOCl	Well diffusion method. 200 µL of cultures injected in the agar plates with 6mm diameter and addition	Significant differences observed in the mean values: G1: 19,57 G2: 7,34

	grape seed extracts and 3% NaOCl			G4: ethanol (control) G5: saline (control)	of 50 µ of each irritant solution. Incubation at 37°C during 24H.	G3: 16,34 G4: 0 G5: 0 All irritant solutions show an inhibition zone, but neem leaf extract has better results.
Mageshwaran et al (2016)	Analysis 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 solution and lycopene solution by dissolving into 100mL of distilled water, 5grams of each compound.	Preparation of petri dishes with each solution and inoculation of 0,5mL of *E.faecalis.* Incubation at 37°C during 24H	4 groups: 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 2% CHX gluconate + 1mL of 5% of lycopene	Evaluation of the ROS formation using a mass spectrometer Evaluation of the bacterial removal by agar diffusion method. Results analyzed by one way ANOVA and turner-Kramer test	G1: 15mm G2: 20mm G3: 23mm G4: 27mm
Fiallos et al (2019)	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 obtain 44 dentin discs decontaminated with 2,5% NaOCl and 6% citric acid. The specimens were	*E.faecalis* prepared in BHI broth and incubated in anaerobic condition during 24H at 37°C.	3 groups (n=10) G1: 5,25% NaOCl G2: 2%CHX G3: GSE vitis vinifera	Evaluation of the bacterials colonies by optical density using a spectrophotometer. Inoculation 3mL of *E.faecalis* in each disc during 21 days at 37°C Specimens were stained with	Greater proportion of dead cell in G3 and highest proportion in G1 Results : mean value of dead cells: G1: 87% G2: 47%

		subjected to UV rays during 15 min.		LIVE/DEAD BacLight fluorescence dye. Emission of different wavelength. Evaluation of the bacterial viability density. Evaluation of the results by using a Shapiro-Wilk test , one way anova test	G3: 72%
Cecchin et al (2017)	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 dentin flexural strength evaluation.	preparation of the irrigants solutions: G1: distilled water + EDTA G2: 6% NaOCl G3: 6% Ca(OCl)2 G4: 6,5% GSE	Flexural strength: Preparation of rectangular-shapes beams of dentin and incubation during 30 min. with 2mL of each solution. Beams were placing on the universal testing machine at a crosshead speed of 0,5mm/min Ultimate tennis strength : Preparation of 4 hourglass section and incubation the same way. Each specimens was tested at 0,5mm/min in a micro tensile testing grip and	Irrigation with 6% NaOCl : reduce the dentin mechanical properties. GSE don't interfere with the mechanical properties of the dentin

					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 testing in the universal machine at a rate 1mm/min until fracture occurred. Evaluation of the results by one way ANOVA test and Tukey HSD test.	
D'aviz et al (2019)	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 root of 13mm and sterilization. Injection of 100mL *E.faecalis* in the canal and incubation for 30 days, renewing BHI every 48H.	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) G3: 2%CHX gel (n=10) G4: 6,5% GSE +EDTA(n=10) G5: 6,5% GSE+ EDTA (n=10)	Evaluation microbiological at 2 steps: before and after the instrumentation. Recovery of the canal 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	No tested irrigant was able to promote complete disinfection. G1: 72% reduction bacterial growth G2: 99,69% G3: 92,05% G4: 85,65% G5: 76,39%

Souza Matheus et al (2016)	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 brain heart infusion BHI broth for 18-24H at 37°C 60 sterilized teeth divided into 6 groups (n=10) : inoculation of 100µL of *E.faecalis* and culture during 15 days replacing the BHI every 48H	6 groups: G1: distilled water G2: CHX G3: Qmix G4: 6,5% GSE G5: PDT+fiber G6: PDT+ no fiber	Evaluation microbiological at 2 steps: before and after contamination. Recovery of the canal content and culture in blood agar for 18-24H at 37° and the counting the CFU.	Bacterial reduction: G1: 0,57% G2: 100% G3: 99,97% G4: 98,02% G5: 96,67% G6: 96,04%
Duringon et al (2020)	Evaluation of the effect of different irritant solution on dentin mechanical properties and fracture resistance.	Selection of 296bovine 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 evaluated with microdurometer Microtensil test with universal testing machine Flexural strength calculated with EMIC Fracture resistance calculated with 2 thickness of root (1,5mm and 0,5mm) with EMIC test	Microhardness and flexural reduction: G3,G4, G5,G6. GSE improve the resistance to degradation and mechanical and chemical properties of the dentin
Cecchin et al (2017)	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 test Kolmogorov- smirnov test	Higher fracture with EDTA and NaOCl by degradation of the collagen fibrils G1: 2,69+- 0,84 G2: 2,50 +-0,92 G3:2,60+-0,90

						G4: 2,61+-0,78
Kumar et al (2019)	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 pathogens aerobic gram + : *S.mutans, E.faecalis, L acidophulis, C. albicans, A. Actinomycetecomitans. P. Gingivalis* in blood agar culture. All specimens were tested in BHI and incubated at 37°C	Vaccinium Macrocarpon	Determination of minimum inhibitory concentration: serial broth dilution method and minimum bactericidal concentration: agar plate subculture streaking method.	Antimicrobial effect of V.macrocarpon against *A. Actinomycetecomitans* and *P. Gingivalis*
Yang et al (2020)	Evaluation of the biofilm effect of Proanthocyanidin solution as a irritant solution against *E.faecalis* and its influence on the mechanical properties and biodegradation resistance of demineralized root dentine.	Selection of human single rooted test and molars, were cut to obtain 20 refined semi-cylindrical specimens 500µL of bacterial suspension added to the root canal and culture during 1 week	*E.faecalis* culture in BHI at 37°C overnight	5 groups: G1: sterile water G2: 2% CHX G3: 2% PA G4: 5% PA G5: 10%PA 50 µL of each solution was injected into de root canal	Evaluation with Confocal laser scanning microscopy (CLSM) examination	Better results with higher concentration of PAC G1: 15% G2: 30% G3: 32% G4: 35% G5: 50%
Epasinghe et al (2014)	Comparison of the effects of 3 flavonoids : proanthocyanidin, naringin and	30 human third molar were selected and sectioned. The specimens were demineralized with		G1: 6,5% Proanthocyanidin G2: 6.5% quercetin G3: 6.5% naringin	Evaluation with two way ANOVA test and Tukey multiple comparison	G1: highest increase after 4H : Modulus of elasticity (86.10 ▯ 22.11 MPa) and ultimate tensile

	quercetin on the modulus of elasticity (MOE) and ultimate tensile strength (UTS) of demineralized dentine.	10% phosphoric acid solution for 5H			strength (17.45 ▯ 4.42 MPa)
Srinivasulu et al (2012)	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%proanthocyanidin solution Preparation of 30 human central incisors	G1 (n=12) : control G2 (n=24) : 10% sodium ascorbate IIA: during 5 minutes IIB: during 10 min G3(n=24) 6.5% proanthocyanidin IIIA: during 5 min IIIB: during 10 min	Shear bond strength with universal testing machine 1mm/min Paired t-test	Highest mean value in G2 and G3. Results in MPa: IIA (22.12), IIB (23.05), IIIA (27.57), and IIIB (27.85)

5. Discussion

The present integrative review reported the main outcomes of relevant previous studies considering the effects of grape seed and cranberry extracts on the removal of bacteria and contaminants from tooth root canals. Also, further benefits on the maintenance of the chemical composition and properties of remnant tooth tissues were described. Thus, the findings validate the hypothesis of this study. A detailed discussion deals with the eradication of bacteria eradication, smear layer removal, and maintenance of the remnant tooth tissues' properties.

5.1. Current irrigation solutions for endodontic treatment

An irrigation solution for endodontic disinfection must meet several criteria such as having an anti-bacterial action, dissolving organic and inorganic components, and keeping a high biocompatibility with the periapical tissues (2,4,15). The main synthetic irrigant solutions used in endodontic are the NaOCl, CHX, $Ca(OCl)_2$ and $Ca(OH)_2$. NaOCl has a broad antimicrobial spectrum and it can be used in different concentration from 0.5%, the minimal concentration with antibacterial effect, up to 6% (1–4,10,12,16,17). NaOCl has a solvent action mediated by its alkaline pH (11-12,5) that can dissolve both vital and necrotic tissue (2,10,16,19). The dissociation in Na^+ and OCl^- ions can alter the microbial cytoplasmic membrane leading to the denaturation of the compounds of the bacteria (2,12). However, 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 (2,4,5,12,16–19). 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 (1,2,4,17). The proteolytic action of NaOCl can damage and break the collagen fibrils and the proteoglycans chains. The destruction of the collagen fibrils reduces the mechanical properties of the dentin and weaken the dentin structure in a demineralized dentin, as seen in Figure 3. (1,4,19)

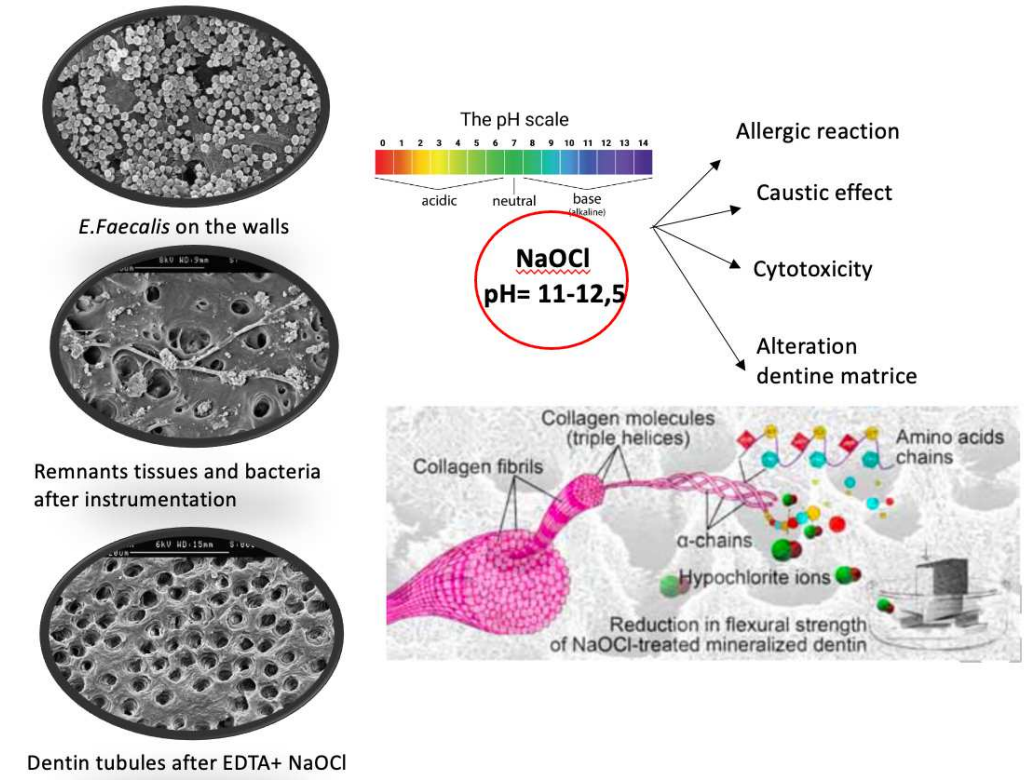

Figure 3: Presence of *E.faecalis* on the teeth canal after debridement. Negative effect of traditional irrigant solutions such as NaOCl . Adapted from Haapasalo M et al and Brito PRR and al.

Chlorhexidine (CHX) is an antiseptic solution also used in endodontic treatment as an alternative to NaOCl to ensure the elimination of pathogenic microorganisms throughout the root canal system. CHX can act against Gram negative and Gram positive bacteria (1,3,10,12,13,16,17). The antibacterial action of CHX deals with the osmotic balance in the bacteria plasmatic membrane inducing bacteria death (3,11,12). The positive charge of the CHX binds to the negative charge of the cell membrane (11,15). CHX has the advantage of maintaining mechanical properties of the root canal dentin since it is not able to dissolve the organic components of the tissues. CHX is also not capable to inhibit the metalloproteinases and cysteine cathepsin which do not weaked the tooth structure. (12,14,15,17). However, CHX also present some disadvantages, even with lower toxicity compared to NaOCl. CHX is able to induce the production of reactive oxygen species (ROS) which can lead to lethal effects on viable cells as shown in the study of Mageshwaran et al

(3). Although ROS can lead to bacterial destruction, they can also produce oxidative stress in the nucleic acid, protein and lipid of different tissue cells. (3) CHX present *in vitro* cytotoxic effect on the human osteoblastic cells, human gingival fibroblasts and periodontal ligament cells, with a dose dependent effect (15). Furthermore, CHX can only remove bacteria on the upper layers of the biofilm and has incapacity to dissolve the organic compounds and the smear layer (4,12,15). The association between NaOCl and CHX can enhance the disinfectant properties but both also precipitate that will create a cytotoxic chemical smear-layer on the dentinal tubules (15).

$Ca(OCl)_2$ is an auxiliary substance that has an antimicrobial activity against *E.faecalis* (1,19) although it can promote the soft tissue dissolution. $Ca(OCl)_2$ acts by oxidating the sulfhydryl groups of bacterial enzyme which are essential for the microbial metabolism and cell viability (1). The dissociation of $Ca(OCl)_2$ release Chlorine, which promotes the antimicrobial activity (1). $Ca(OCl)_2$ reveals acceptable outcomes regarding the decrease of bacteria migration, viability and the inflammatory response by the tissue (1,3). $Ca(OCl)_2$, shows less toxicity than NaOCl but its lower surface tension restrain its penetration into the deep dentin and therefore it has less antimicrobial activity (17,19). The dissolution of periapical tissues by $Ca(OCl)_2$ is lower than that for NaOCl (19). Finally $Ca(OH)_2$, an alkaline product which dissociate in Ca^{2+} and OH^- can inactivate bacterial enzyme and promote antibacterial activity and tissue dissolution (3).

5.2. Grape seed for tooth root canal disinfection

Grape seed extract (GSE) is a plant derived extract which has a high content of phenolic compounds rich in proanthocyanidin in oligomeric and polymeric forms (1,11,14,17). Proanthocyanidin (PA) compounds are classified as flavonoids which are found in grape seed, cranberries, berries, and nuts (21,22). GSE is composed of proanthocyanidin, oligomers of flavan-3-ol, that shows anti-microbial and potent bioactive antioxidant compounds that can act against the Gram positive and Gram negative bacteria (1,3,14). The polyphenols can eradicate bacteria by modifying the microbial cell permeability which lead to cellular death thanks to the inhibition mechanisms on: (i) nucleic acid synthesis, (ii) cell cytoplasmic membrane function, (iii) bacteria metabolism, (iv) biofilm formation, and (v) membrane

permeability. (1,4,11). Also, the PA contains in the GSE can promote the formation of reactive oxidative species (ROS) that can destroys *E.faecalis* . (3)

All the selected studies have shown significant results on GSE considering bacterial eradication when compared with the traditional irrigant 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 (12). The flowable state of GSE revealed higher bacteria elimination than that recorded in the gel form. (12). The percentage of bacterial reduction between three irrigants solutions after ten minutes of exposure time was recorded at 47% for 2%CHX; 72% for 6.5% GSE, and 87% for 5.25% NaOCl (4). Thus, GSE has a potential effect against bacteria and the effects can be enhanced depending on the concentration of GSE content (4). Lower content of GSE has revealed no effective reduction of bacteria percentage into the tooth root canals (2). Another study reported the bacterial eradication comparing different irrigant solution and two mechanical system of instrumentation (1). In 50% GSE, the elimination of the bacteria was slightly higher using protaper system (96.97%) than that for reciproc 25 system (96.72%) (1). It should be highlighted the formation of reactive oxidative species (ROS) in the presence of proanthocyanidin released from GSE (Figure 4) (3,26). ROS are subproducts of the oxygen metabolism with beneficial effect in cell's signaling and homeostasis in normal condition but with detrimental effect regarding cytotoxicity in oxidative stress conditions (3,26). The formation of ROS in equilibrium can destroy the cell wall and plasma membrane of *E.faecalis* leading to bacteria death (3). The antioxidant properties of the GSE can act as a protective agent against the ROS formation as shown in the study of Mageschawaram (3).

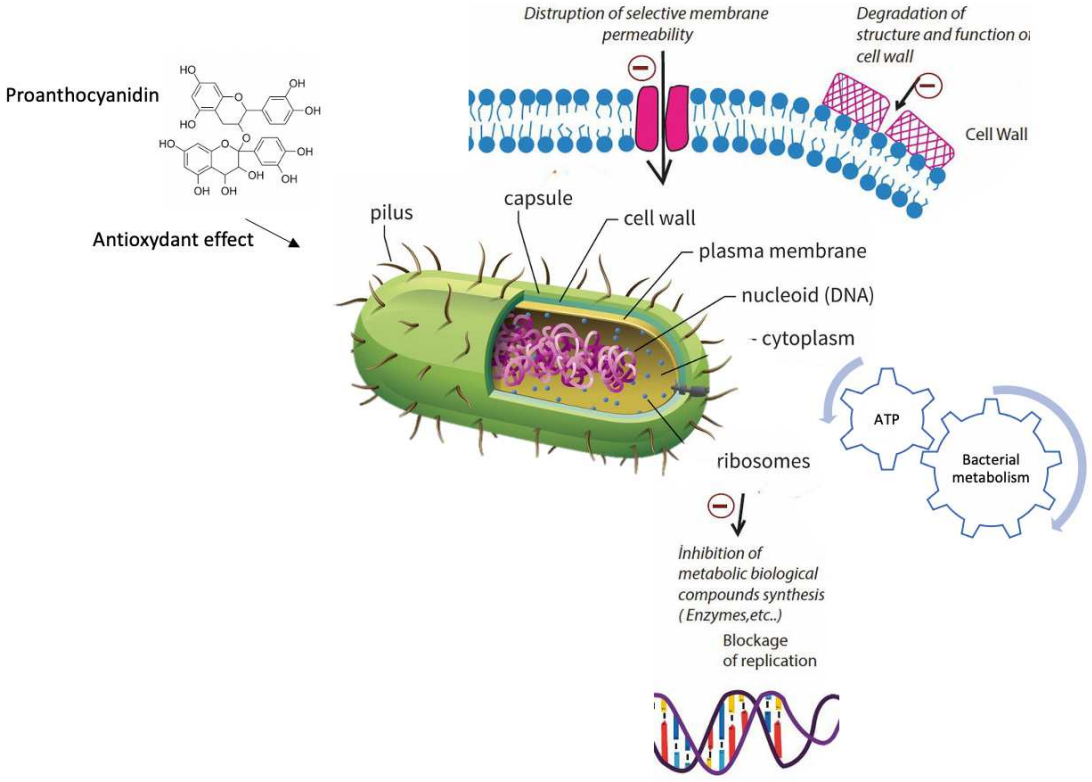

Figure 4: antimicrobial efficacity of the GSE.

Hand-held or mechanical instrumentation may cause debris that can accumulate on the tooth root canal surfaces, especially in the lateral or accessory canals and isthmus, which may prevent the availability of PA, principal molecule constituent of the GSE (5,18,24). Debris are composed by compacted inorganic particles, microbial metabolites and bacteria (5,15). Dentin demineralization with a chelating agent is important for exposing dentin tubules and thus the underlying collagen-matrix network (17). NaOCl and 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 (2,24). For this purpose, 17% EDTA is the most used solution for smear layer removal by chelating calcium (2,24). In six previous studies, 17% EDTA was used for smear layer removal (1,10,12,16,17) while one study used 6% acid citric (4), and another one used 10% phosphoric acid (18). The citric acid enlarged the dentinal tubules, and that was more effective than EDTA 17% in concentration varying between 1 to 10% (5). Considering its properties and chemical composition, GSE does not dissolve organic or inorganic

compounds and therefore it must be used with a complementary solution to dissolve smear layer (1).

On the dentin tissue, the organic matrix consists of 90% collagen proline of type I organized in fibrillary network around the peri-tubular dentin that provides it rigidity and stability (1,17,19,21). The organic matrix (~10%) is also composed of proteoglycans that connect glycosaminoglycans chains to ensure intratubular permeability (17,19). A previous study evaluated different key factors implicated in the strength of the remnant teeth structure after irrigation with GSE or other two traditional irrigant solutions. The findings revealed high values of flexural strength, tensile strength, the fracture resistance of dentin after irrigation with GSE (17,19). In fact, the fracture resistance of the remnant tooth tissues increased when 6.5% GSE was used as irrigation solution instead of NaOCl (17). However, this study showed lower results than the literature in relation to UTS, flexural strength and fracture resistance because the tests were done on mineralized dentin (17). Despite, 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 (12,16,17,19,25). GSE also inhibits metalloproteinase and cathepsin cysteine, both present in the dentin and the saliva, and implicated in the continuous degradation process of dentin in non-specific ways (10,17). Metalloproteinase and cathepsin cysteine can activate their degradation pathways in acidic environment (25). Metalloproteinases are enzymes present in the deep dentin, and have the capability to turn-over the extra-cellular matrix (15). Then, the GSE increases the resistance against collagenase degradation and thus the biostability strengthening the density of the inter- and intra-fibrillary collagen network (4,10,16,17,19).

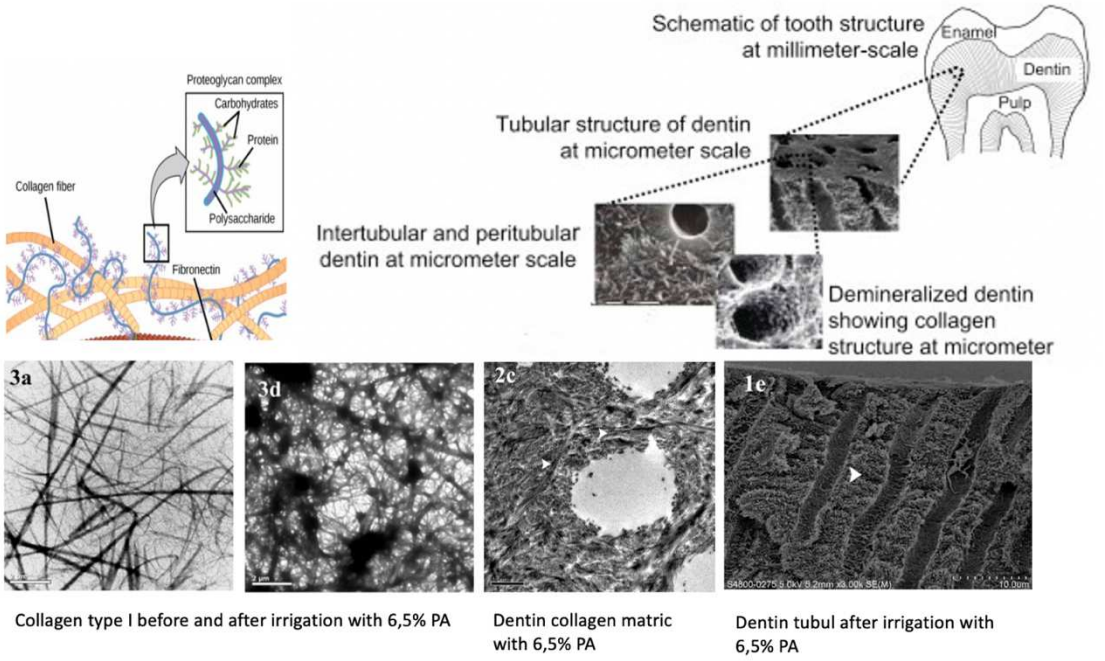

Figure 5: The dentinal tubules before and after removal of the smear-layer and the effect of GSE on the collagen matrix of the dentin. Adapted from Epasinghe et al.

Although acting on a broad bacterial spectrum, NaOCl often can damage the vital tissues and have toxic effects especially if the solution exceeds the apical foramen and reaches the periapical tissues (1,2,4,12,16,17,19). The dissociation in Na^+ and OCl^- ion causes an alteration of the bacterial cell balance as well as can cause caustic effects on human cells and allergic reactions (1,4,19). In comparison, no adverse reactions were reported with the use of PA due to its anti-allergic properties (3,4,19). However, all the selected studies were carried out on bacterial cultures or on extracted teeth. As described in the literature, PA has lower toxicity to human cells (4,12,14,20) and a potent antioxidant bioactive capability reducing the formation of ROS (3).

5.3. Beneficial effects of Cranberry

Vaccinum macrocarpon, known as cranberry, is a source of bioactive flavonoids (20). Three major classes of flavonoids, family of polyphenols, have been identified in cranberry fruit: anthocyanins, proanthocyanidins, and flavan-3-ols. (20,26). Cranberry extract contains around 65% polyphenol (25). PA contained in the cranberry already showed beneficial

effects on dental caries or periodontal diseases as an dental auxiliar product or as a natural medication in urinary tract infection (25,27–29). In case of urinary tract infection, the literature is consistent to say that the cranberry extract is a good antimicrobial auxiliary on the principal pathogen *E.coli* without developing antibio-resistance. His action is mediated by his ability to break down the bacteria accumulation and reducing the principals inflammation's mediators (29). Cranberry extract can inhibit the biofilm formation by modifying the dentin avoiding the adhesion of polysaccharide produced from the bacterial metabolism and the adhesins localized on the surface area on the bacteria (25,26). The cytokines, inflammation mediators like TNF-alpha, interleukin 1 or interleukin 17, with an important key role in the progression of periodontitis, can be inhibited. Also some invitro studies have shown the implication in the homeostasis and reducing the bone resorption (29,30). The capability of PA to disintegrate the accumulation of bacteria like *porphyromonas gengivalis* and *Aggregatibacter actinomycetemcomitans* (*A.a)* in case of periodontitis; *streptococcus mutans, lactobacillus* and *actinomyces* in case of dental caries or *C.albicans* in oral candidiasis is well known and studied (27,28,31). In case of dental decay, this biofilm modulation is benefic as it can prevent the apparition of an cariogenic ambient (32,33). The cranberry extract can disrupt the biofilm formation, alter the acid lactic formation and exercise a cariostatic effect by binding to the salivary compounds (32,33). As an endodontic irrigant, only one study evaluated the effects of PA on various microbial strains of the 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) (20).

Only two articles assessed the antimicrobial potential of the PA from cranberry for tooth root canal disinfection. A previous study reported a bacterial reduction depend on the dose and the results were higher with high concentration of PA (11). An increase in the elastic modulus of the dentin tissue was also recorded as the PA concentration increased (11). 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 PA while the elastic modulus of the dentin free of PA was recorded at 11.3 MPa (18). Also, the tensile strength was doubled between the baseline and after 4h immersion

in PA (18). The process of remineralization of the dentin can be explained by the formation of a hydro soluble complex which bind to the calcium ions (25). PA is a large molecule with hydroxyl group to establish strong hydrogen bonds. (25). The cranberry extract has the capability to inhibit the metalloproteinase (MMP), specifically MMP1 and MMP9, which reduce the dentin degradation (22).

Most studies were performed within *in vitro* models involving free-form (planktonic) bacteria or mono-species biofilms (27,30). 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 (27,31). Future *in vivo* studies should evaluate the influence of different content of cranberry extracts on several bacterial strains involved in tooth root canal infection. The main advantage of using natural sources of antimicrobial compounds is the low risks of toxicity to the oral tissues. That supports the safety of the patients and long-term success of the clinical treatment.

6. 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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