UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Odontologia
Colegiado de Pós-Graduação em Odontologia

Bruna Tavares Carneiro

POTENCIAL ANTIBACTERIANO DOS FLAVONOIDES E DESENVOLVIMENTO DE UMA RESINA COMPOSTA EXPERIMENTAL A BASE DE FLAVONOIDE

Belo Horizonte
2023

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Dissertação apresentada ao colegiado de Pós-graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Mestre em Odontologia – área de concentração em Clínica Odontológica.

Orientadora: Profa. Dra. Carolina Bosso André
Coorientadora: Profa. Dra. Ivana Márcia Alves Diniz

Versão corrigida

Belo Horizonte
2023

Ficha Catalográfica

C289p 2023 T

Carneiro, Bruna Tavares.
Potencial antibacteriano dos flavonoides e desenvolvimento de uma resina composta experimental a base de flavonoide / Bruna Tavares Carneiro. -- 2023.

88 f. : il.

Orientadora: Carolina Bosso André.
Coorientadora: Ivana Márcia Alves Diniz.

Dissertação (Mestrado) -- Universidade Federal de Minas Gerais, Faculdade de Odontologia.

1. Resinas compostas. 2. Flavonoides. 3. Materiais dentários. I. André, Carolina Bosso. II. Diniz, Ivana Márcia Alves. III. Universidade Federal de Minas Gerais. Faculdade de Odontologia. IV. Título.

BLACK - D047

Elaborada por: Mateus Henrique Silva Trindade - CRB 6/3883.

UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE ODONTOLOGIA
COLEGIADO DO CURSO DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FOLHA DE APROVAÇÃO

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BRUNA TAVARES CARNEIRO

Dissertação submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ODONTOLOGIA, como requisito para obtenção do grau de Mestre em ODONTOLOGIA, área de concentração CLÍNICA ODONTOLÓGICA.

Aprovada em 05 de julho de 2023, pela banca constituída pelos membros:

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Belo Horizonte, 5 de julho de 2023.

Documento assinado eletronicamente por **Thais Yumi Umeda Suzuki, Professora do Magistério Superior**, em 05/07/2023, às 10:52, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Carolina Bosso Andre, Professora do Magistério Superior**, em 05/07/2023, às 10:53, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Alberto Nogueira da Gama Antunes, Usuário Externo**, em 06/07/2023, às 10:44, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Dedico este trabalho aos meus pais e ao meu esposo, que me apoiaram incondicionalmente.

AGRADECIMENTOS

Agradeço a Deus por me proporcionar essa oportunidade de concluir o mestrado.

Aos meus pais que me apoiaram e incentivaram incondicionalmente a minha vida acadêmica e profissional. Ao meu esposo, parceiro de caminhada.

À minha orientadora, Profa. Carolina Bosso André por toda ajuda ao longo do curso e pelas oportunidades; à Profa. Ivana Márcia Alves Diniz pela disponibilidade em ensinar e colaborar com minha pesquisa. À aluna de iniciação científica Thayná Pinho França por colaborar com a pesquisa laboratorial. Às alunas e companheiras de pós-graduação por me ajudarem nos testes laboratoriais.

Finalmente agradeço à Fundação de Amparo à pesquisa do Estado de Minas Gerais (FAPEMIG), ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo apoio financeiro.

“Educação é uma descoberta progressiva de nossa própria ignorância.”

Voltaire

RESUMO

Os flavonoides podem ser considerados substâncias fitofarmacêuticas, ou seja, são obtidas na natureza e possuem benefícios à saúde humana. Alguns desses benefícios estão relacionados ao sistema cardiovascular, doenças crônicas como diabetes e em desordens bucais como periodontite e cárie. Assim, os objetivos do presente estudo foram: analisar efeitos antibacterianos de diferentes flavonoides contra bactérias relacionadas à doença periodontal e à cárie dentária por meio de uma revisão de escopo; avaliar as propriedades físico-químicas de uma resina composta experimental adicionada de naringina. Para a revisão de escopo, 19 artigos foram incluídos, todos estudos *in vitro*. Para bactérias relacionadas à doença periodontal, proantocianidinas, isoliquiritigenina, liquiritigenina, galangina, quercitrina, taxifolina, crisina, diosmetina, quercetina, miricetina, naringina, apigenina, catequinas, luteolina, morina e rutina foram investigadas. Para bactérias relacionadas à cárie dentária, baicaleína, naringenina, catequina, isoliquiritigenina, liquiritigenina, miricetina, quercetina e kaempferol já foram investigados. Atividade bacteriostática, bactericida e antibiofilme foram predominantemente relatadas. Para o desenvolvimento de uma resina experimental, naringina foi adicionada em concentrações de 5, 10 e 15mM. Testes laboratoriais foram conduzidos para avaliar a resistência flexural e modulo de elasticidade, alteração de cor, sorção e solubilidade e a citotoxicidade dessas resinas, comparado ao controle sem adição do flavonoide. Os dados foram analisados quanto à normalidade e homoscedasticidade. A resistência à flexão, o módulo de flexão, a sorção de água, a solubilidade em água, os parâmetros a* e C* e a citotoxicidade de 24 horas foram analisados por ANOVA de uma via seguida pelo teste de Tukey. L*, b* e WID foram analisados por Welch ANOVA seguido pelo teste T3 de Dunnett, pois os dados eram heteroscedásticos. A citotoxicidade após 48h e 72h foi analisada pelo teste de Kruskal Wallis e Dunn porque os dados não foram normalmente distribuídos e heteroscedásticos. A revisão de escopo demonstrou um potencial de ação dos flavonoides nas bactérias relacionadas à carie e doença periodontal, sendo promissor quanto ao desenvolvimento de novas terapias e novos materiais restauradores. As resinas compostas experimentais adicionadas de naringina não apresentaram citotoxicidade e não demonstraram efeitos deletérios quanto as propriedades físico-químicas, apesar de promover uma alteração na cor final da resina.

Palavras-chave: resinas compostas; flavonoides; materiais dentários.

ABSTRACT

Antibacterial potential of flavonoids and development of an experimental flavonoid-based resin composite.

Flavonoids can be considered phytopharmaceutical substances, that is, they are obtained in nature and have benefits to human health. Some of these benefits are related to the cardiovascular system, chronic diseases such as diabetes, and oral disorders as periodontitis and dental caries. Thus, the objectives of the present study were: to analyze the antibacterial effects of different flavonoids against bacteria related to periodontal disease and dental caries through a scoping review; to evaluate the physical-chemical properties of an experimental resin composite with naringin addition. For the scoping review, nineteen articles were included, all *in vitro* studies. For bacteria related to periodontal disease, proanthocyanidins isoliquiritigenin, liquiritigenin, galangin, quercetrin, taxifolin, chrysin, diosmetin, quercetin, myricetin, naringin, apigenin, catechins, luteolin, morin and rutin were investigated. For bacteria related to dental caries, baicalein, naringenin, catechin, isoliquiritigenin, liquiritigenin, myricetin, quercetin and kaempferol have been investigated. Bacteriostatic, bactericidal and antibiofilm activity were predominantly reported. For the development of an experimental composite, naringin was added in concentrations of 5, 10 and 15mM. Laboratory tests were conducted to evaluate the flexural strength and flexural modulus, color change, sorption and solubility, and the cytotoxicity of these composites, compared to the control group without flavonoids addition. Data were analyzed for normality and homoscedasticity. Flexural strength, flexural modulus, water sorption, water solubility, a* and C* parameters, and 24-hour cytotoxicity were analyzed by one-way ANOVA followed by Tukey's test. L*, b* and W_{ID} were analyzed by Welch ANOVA followed by Dunnett's T3 test, as the data were heteroscedastic. While the 48h and 72h cytotoxicity was analyzed by the Kruskal Wallis and Dunn test, because the data were not normally distributed and heteroskedastic. The scope review demonstrated a potential action of flavonoids on bacteria related to caries and periodontal disease, with potential development of new therapies and new restorative materials. The experimental composites with naringin addition did not show cytotoxicity and did not demonstrate deleterious effects in terms of physicochemical properties, despite promoting a change in the final color of the resin.

Keywords: composite resins; flavonoids; dental materials.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURA E SIGLAS

Bis-GMA	Bisfenol A Glicidil Metacrilato
BHT	Hidroxitolueno Butilado
DMEM	Dulbecco´s Modified Eagle Medium
DMSO	Dimetilsulfóxido
EDMAB	Dimetil Aminobenzoato
J/cm2	Joule/centímetro quadrado
Mg	Miligramas
MIC	Concentração Inibitória Mínima
mM	Milimolar
µm	Micrômetro
µg	Micrograma
mm	Milímetro
MTT	(3-(4,5-dimetiltiazol-2yl)-2,5-di- fenil brometo de tetrazolina)
Nar	Naringina
nm	Nanômetros
S. Mutans	*Streptoccocus mutans*
TEGDMA	Trietilenoglicol Dimetacrilato

SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

Os flavonoides são compostos largamente presentes em produtos naturais como vegetais e frutas (SINGH *et al.,* 2018), sendo responsáveis pela cor e sabor característicos desses alimentos. Além disso, promovem indiretamente a interação com o meio e demais seres vivos, atraindo, por exemplo, insetos polinizadores por meio da cor e protegendo contra agressores, como os fungos (SHEN *et al.,* 2022).

Em relação à estrutura química, possuem mais de 6.000 tipos (PANCHE; DIWAN; CHANDRA 2016). Basicamente, são constituídos por dois anéis benzenos associados a um grupo fenólico hidroxila por meio de três átomos de carbono centrais (WEN *et al.,* 2021). De acordo com as diferenças estruturais, os flavonoides podem ser divididos em 7 principais subgrupos: flavonol, flavonas, isoflavonas, antocianidinas, chalconas, flavanonas e flavan-3-ol (SHEN *et al.,* 2022).

Os flavonoides podem ser enquadrados no grupo das substâncias fitofarmacêuticas, já que possuem diversos benefícios à saúde humana, como efeito protetor em doenças cardiovasculares, atividades antioxidantes, anti-inflamatória e antitumoral (BENAVENTE-GARCIA; CASTILHO 2008; CHANET *et al.* 2012). A facilidade de obtenção, custo baixo, variedade de ações farmacológicas e longa história de uso, tornam os estudos na área da saúde com os flavonoides cada vez mais promissores. Além disso, os efeitos colaterais de seu uso são praticamente inexistentes em relação as drogas sintéticas tradicionais (SHEN *et al.,* 2022).

A ação antibacteriana possibilita sua aplicação na Odontologia como prevenção de desordens bucais como a periodontite e cárie. Esse efeito contra bactérias perpassa por diversos mecanismos já relatados, como por exemplo: rompimento da membrana bacteriana; inibição da formação do biofilme; alteração em síntese de componentes estruturais e metabólicos como ácidos nucléicos e ATP e até inibição da replicação do DNA bacteriano (GÓRNIAK; BARTOSZEWSKI; KRÓLICZEWSKI 2019; JUCÁ *et al.,* 2020).

Um estudo conduzido por Chen *et al.* (2022) investigou o efeito de flavonoides na inibição de biofilmes compostos por *Streptococcus mutans* apenas, *Candida albicans* e os dois microrganismos juntos. Os resultados demonstraram que a baicaleina, um flavonoide pertencente ao subgrupo das flavonas, reduziu significativamente a atividade metabólica dos biofilmes quando comparada ao grupo controle, tratado com

clorexidina. Outro estudo avaliou a incorporação de apigenina em compósitos resinosos na modulação da virulência do *S. mutans*, reduzindo o peso seco do biofilme e os polissacarídeos intracelulares e insolúveis formados (ANDRÉ *et al.,* 2020). Isso pode ser explicado pelo fato de os flavonoides agirem no metabolismo bacteriano. Por conseguinte, a atividade da enzima glicosiltransferase, que é usada pelo *S. mutans* para quebrar a sacarose em glucano, também é afetada, reduzindo aderência e formação do biofilme (JEFFREY *et al.,* 2020).

O *Streptococcus mutans* nem sempre é dominante no biofilme, mas é o principal microorganismo envolvido na patogênese da doença cárie. Ele pode arquitetar uma matriz polimérica insolúvel na presença de açúcares, denominada matriz de exopolissacarídeos (EPS), que age como uma estrutura de suporte e barreira à difusão de soluções tampões na estrutura do biofilme. (FALSETTA *et al.,* 2012; KIM *et al.,* 2015; KOO *et al.*, 2013). Essa matriz presente em biofilmes cariogênicos é reconhecida como um fator essencial de virulência e está associada ao início do biofilme cariogênico (KIM *et al.,* 2015).

Além da ação contra bactérias cariogênicas, parece haver um efeito também em periodonto-patógenos. Em um estudo recente, um flavonoide associado a um quitooligosacarídeo foi usado em bactérias do tipo *Streptococcus aureus* (envolvidas na periodontite) (CAO *et al.,* 2019). A mudança na morfologia dessa bactéria confirmou a atividade antibacteriana do complexo de naringina, uma flavonona, que em contato com a membrana celular, aumentou sua permeabilidade e causou seu rompimento (XING *et al.,* 2009).

Materiais dentários inovadores têm sido produzidos com propriedades antibacterianas e bioativas (CHEN *et al.,* 2018). No entanto, a adição de agentes antimicrobianos de amplo espectro aos materiais restauradores negligencia a importância dos microrganismos residentes relacionados à saúde. Isso pode resultar na seleção de bactérias resistentes aos antimicrobianos, gerando consequências indesejáveis para a saúde oral (FALSETTA *et al*., 2012; MCDONNEL; RUSSELL, 1999). Portanto, o desenvolvimento de terapias de ação alvo-específico, visando a atenuação da virulência microbiana é mais desejável e pode contribuir com a redução da cárie dental (FREIRES *et al.,* 2015; KOO; JEON 2009). Resultados prévios de diferentes flavonoides demostraram ação alvo-específico desses compostos na virulência do *S. mutans*, especialmente na redução da produção de polissacarídeos (KOO *et al.,* 2002).

2 OBJETIVOS

2.1 Objetivo geral

O objetivo desse estudo foi realizar uma revisão de escopo de estudos *in vitro* para avaliar o potencial antibacteriano dos flavonoides contra bactérias cariogênicas e relacionadas à periodontite. Além disso, foi realizada uma pesquisa *in vitro* com o objetivo de avaliar as propriedades físico-químicas e a citotoxicidade de uma resina composta experimental contendo naringina.

2.2 Objetivos específicos

a) Avaliar quais flavonoides já demonstraram ação contra bactérias relacionadas à cárie e periodontite;
b) Avaliar quais metodologias e mecanismos de ação foram descritos para os flavonoides testados contra bactérias relacionadas à cárie e periodontite;
c) Adicionar o flavonoide naringina, em concentrações distintas, na composição de uma resina composta experimental, visando avaliar os seus efeitos na resistência à flexão, módulo de elasticidade, alteração de cor, sorção e solubilidade, bem como na citotoxicidade das resinas compostas experimentais.

3 METODOLOGIA EXPANDIDA

3.1 Revisão de escopo

3.1.1 Protocolo e registro

O checklist dos itens necessários para a condução de revisões de escopo (PRISMA-scr) foi adotado na presente revisão. O protocolo de revisão de escopo foi registrado no Open Science Framework (10.17605/OSF.IO/K4XWU).

3.1.2 População e critérios de elegibilidade

Os critérios de inclusão consistiram em 1) estudos in vitro, in situ ou in vivo que analisaram o efeito antibacteriano de um ou mais flavonoides testados contra bactérias relacionadas à cárie e doenças periodontais; 2) acrescentaram-se estudos que testaram flavonoides puros ou diluídos em veículo (comercialmente disponíveis ou extraídos de plantas). Os critérios de exclusão foram 1) estudos que utilizaram flavonoides encapsulados, ou em sistema funcionalizado com nanopartículas, ou em sistemas de gel ou goma de mascar; 2) estudos nos quais o flavonoide não foi especificado; 3) revisões de literaturas e sistemáticas, para focar apenas nos estudos in vitro e suas metodologias e resultados; 4) estudos que investigaram apenas genes isolados ou fatores de virulência isolados, ou o potencial efeito antibacteriano por meio de programa computacional; 5) artigos que utilizaram flavonoides incorporados a outros materiais, o que pode comprometer sua liberação e modo de ação. Para bactérias relacionadas à doença periodontal, proantocianidinas isoliquiritigenina, liquiritigenina, galangina, quercitrina, taxifolina, crisina, diosmetina, quercetina, miricetina, naringina, apigenina, catequinas, luteolina, morina e rutina foram investigadas. A busca foi restrita a artigos publicados em inglês sem restrição de data

A pergunta a ser respondida nessa revisão foi baseada na estratégia representada pelo acrônimo PICO (População, intervenção, comparação e resultado): “Quais flavonoides possuem ação contra bactérias relacionadas à cárie e doença periodontal?” A população do estudo foi composta por bactérias relacionadas à cárie e a periodontite, que foram submetidas a ação dos flavonoides. A intervenção consistia nos diferentes testes antibacterianos com uso dos flavonoides. A comparação foi feita com soluções antibacterianas consideradas padrão ouro, como a clorexidina. Os desfechos considerados foram: o efeito dos flavonoides na inibição do biofilme bacteriano (adesão, formação e crescimento), diminuição da massa do biofilme bacteriano (peso seco) e dos polissacarídeos e a atenuação da virulência por meio da modificação da atividade das enzimas glicosiltransferases.

3.1.3 Bases de dados, estratégias de pesquisa e seleção dos estudos

Uma busca foi realizada a partir de setembro de 2022 e finalizada em dezembro do mesmo ano. As bases de dados selecionadas foram: PubMed, Embase, Scopus, Web of Science, Lilacs e a literatura cinzenta. A estratégia de busca utilizou palavras-

chave determinadas pela intervenção (flavonoides, flavonoides mais comuns e suas subclasses); testes antibacterianos mais usados e as doenças investigadas (cárie dental e periodontite). As palavras-chave foram combinadas por meio dos operadores booleanos “OR” e “AND”, e ajustadas de acordo com cada base de dados. A estratégia de busca está representada pelo Quadro 1.

Quadro 1 – Estratégia de busca.

Pubmed/Medline		
#1	#2	#3
"flavonoids"[MeSH Terms] OR "flavonoids"[All Fields] OR "flavonoid"[All Fields] OR "flavanols"[All Fields] OR "flavanols"[All Fields] OR "flavonone"[All Fields] OR "flavone"[All Fields] OR "Hesperetin"[All Fields] OR "Hesperedin"[All Fields] OR "Neohesperedin"[All Fields] OR "Naringenin"[All Fields] OR "Naringin"[All Fields] OR "Butin"[All Fields] OR "Luteolin"[MeSH Terms] OR "Luteolin"[All Fields] OR "Quercetin"[MeSH Terms] OR "Quercetin"[All Fields] OR "Apigenin"[MeSH Terms] OR "Apigenin"[All Fields] OR "Kaempferol"[All Fields] OR "Catechin"[All Fields] OR "Epigallocatechin"[All Fields] OR "Baicalein"[All Fields] OR "Galangin"[All Fields] OR "Morin"[All Fields] OR "Myricetin"[All Fields] OR "Rutin"[MeSH Terms] OR "Rutin"[All Fields] OR "EGCG"[All Fields] OR "Tangeritin"[All Fields]	"Antibacterial activity"[All Fields] OR "anti-bacterial"[All Fields] OR "biofouling"[MeSH Terms] OR "biofouling"[All Fields] OR "anti-biofouling"[All Fields] OR "biofilm*"[All Fields] OR "biofilms"[MeSH Terms] OR "biofilms"[All Fields] OR "anti-biofilm"[All Fields] OR "antibiofilm"[All Fields] OR "bacteria*"[All Fields] OR "bactericidal"[All Fields] OR "bacteriostatic"[All Fields] OR "MIC"[All Fields] OR "MBC"[All Fields] OR "MICB"[All Fields] OR "adherence"[All Fields] OR "bacteria"[MeSH Terms] OR "bacteria"[All Fields] OR "microorganisms"[All Fields] OR "virulence"[MeSH Terms] OR "virulence"[All Fields] OR "antimicrobial"[All Fields] OR "inhibitory"[All Fields] OR "disk diffusion"[All Fields] OR "halo"[All Fields] OR "kirby-bauer"[All Fields] OR "crystal violet"[All Fields]	"dental caries"[All Fields] OR "dental caries"[MeSH Terms] OR "dental decay"[All Fields] OR "carious lesion*"[All Fields] OR "periodontitis"[All Fields] OR "periodontitis"[MeSH Terms] OR "tooth demineralization"[All Fields] OR "tooth demineralization"[MeSH Terms] OR "gingivitis"[MeSH Terms] OR "gingivitis"[All Fields] OR "periodontal diseases"[MeSH Terms] OR "periodontal diseases"[All Fields]
		#1 AND #2 AND #3
Embase		
#1	#2	#3
'flavonoid'/exp OR 'flavonoids' OR 'flavonoid'/exp OR 'flavonoid' OR 'flavanols' OR 'flavonones' OR 'flavone'/exp OR 'flavone' OR 'hesperetin'/exp OR 'hesperetin' OR 'hesperedin' OR 'neohesperedin' OR 'naringenin'/exp OR 'naringenin' OR 'naringin'/exp OR 'naringin' OR 'butin'/exp OR 'butin' OR 'luteolin'/exp OR 'luteolin' OR 'quercetin'/exp OR 'quercetin' OR 'apigenin'/exp OR 'apigenin' OR 'kaempferol'/exp OR 'kaempferol' OR 'catechin'/exp OR 'catechin' OR 'epigallocatechin'/exp OR 'epigallocatechin' OR 'baicalein'/exp OR 'baicalein' OR 'galangin'/exp OR 'galangin' OR 'morin'/exp OR 'morin' OR 'myricetin'/exp OR 'myricetin' OR 'rutin'/exp OR 'rutin' OR 'egcg'/exp OR 'egcg' OR 'tangeritin'/exp OR 'tangeritin'	('antibacterial activity'/exp OR 'anti-bacterial' OR 'biofouling'/exp OR 'anti-biofouling' OR 'biofilm'/exp OR 'biofilms' OR 'anti-biofilm' OR 'antibiofilm'/exp) AND 'bactericidal' OR 'bacteriostatic' OR 'mic' OR 'mbc' OR 'micb' OR 'adherence'/exp OR 'bacteria' OR 'microorganisms' OR 'virulence'/exp OR 'antimicrobial' OR 'inhibitory' OR 'disk diffusion'/exp OR 'halo' OR 'kirby-bauer' OR 'crystal violet'/exp	'dental caries'/exp OR 'dental decay' OR 'carious lesion*' OR 'periodontitis'/exp OR 'tooth demineralization' OR 'gingivitis'/exp OR 'periodontal disease'/exp
		#1 AND #2 AND #3
SCOPUS		
#1	#2	#3
(ALL(‘flavonoids’ OR ‘flavonoid’ OR ‘flavanols’ OR ‘flavonones’ OR ‘flavone’ OR ‘Hesperetin’ OR ‘Hesperedin’ OR ‘Neohesperedin’ OR ‘Naringenin’ OR ‘Naringin’ OR ‘Butin’ OR ‘Luteolin’ OR ‘Quercetin’ OR ‘Apigenin’ OR ‘Kaempferol’ OR ‘Catechin’ OR ‘Epigallocatechin’ OR ‘Baicalein’ OR ‘Galangin’ OR ‘Morin’ OR ‘Myricetin’ OR ‘Rutin’ OR ‘EGCG’ OR ‘Tangeritin’))	(ALL(‘Antibacterial activity’ OR ‘anti-bacterial’ OR ‘biofouling’ OR ‘anti-biofouling’ OR ‘biofilm’ OR ‘biofilms’ OR ‘anti-biofilm’ OR ‘antibiofilm’ OR ‘bactericidal’ OR ‘bacteriostatic’ OR ‘MIC’ OR ‘MBC’ OR ‘MICB’ OR ‘adherence’ OR ‘bacteria’ OR ‘microorganisms’ OR ‘virulence’ OR ‘antimicrobial’ OR ‘inhibitory’ OR ‘disk diffusion’ OR ‘halo’ OR ‘kirby-bauer’ OR ‘crystal violet’))	(ALL(‘dental caries’ OR ‘dental decay’ OR ‘carious lesion’ OR ‘periodontitis’ OR ‘tooth demineralization’ OR ‘gingivitis’ OR ‘periodontal disease’))
		#1 AND #2 AND #3
Web of Science		
#1	#2	#3
((ALL=(“flavonoids” OR “flavonoid” OR “flavanols” OR “flavonones” OR “flavone” OR “Hesperetin” OR “Hesperedin” OR “Neohesperedin” OR “Naringenin” OR “Naringin” OR “Butin” OR “Luteolin” OR “Quercetin” OR “Apigenin” OR “Kaempferol” OR “Catechin” OR “Epigallocatechin” OR “Baicalein” OR “Galangin” OR “Morin” OR “Myricetin” OR “Rutin” OR “EGCG” OR “Tangeritin”))	(“Antibacterial activity” OR “anti-bacterial” OR “biofouling” OR “anti-biofouling” OR “biofilm” OR “biofilms” OR “anti-biofilm” OR “antibiofilm” OR “bactericidal” OR “bacteriostatic” OR “MIC” OR “MBC” OR “MICB” OR “adherence” OR “bacteria” OR “microorganisms” OR “virulence” OR “antimicrobial” OR “inhibitory” OR “disk diffusion” OR “halo” OR “kirby-bauer” OR “crystal violet”))	ALL=(“dental caries” OR “dental decay” OR “carious lesion” OR “periodontitis” OR “tooth demineralization” OR “gingivitis” OR “periodontal disease”)
		#1 AND #2 AND #3
LILACS		
#1	#2	#3
((flavonoids) OR (flavonoid) OR (flavanols) OR (flavonones) OR (flavone) OR (hesperetin) OR (hesperedin) OR (neohesperedin) OR (naringenin) OR (naringin) OR (butin) OR (luteolin) OR (quercetin) OR (apigenin) OR (kaempferol) OR (catechin) OR (epigallocatechin) OR (baicalein) OR (galangin) OR (morin) OR (myricetin) OR (rutin) OR (egcg) OR (tangeritin))	((antibacterial activity) OR (anti-bacterial) OR (biofouling) OR (anti-biofouling) OR (biofilm) OR (biofilms) OR (anti-biofilm) OR (antibiofilm) OR (bactericidal) OR (bacteriostatic) OR (mic) OR (mbc) OR (micb) OR (adherence) OR (bacteria) OR (microorganisms) OR (virulence) OR (antimicrobial) OR (inhibitory) OR (disk diffusion) OR (halo) OR (kirby-bauer) OR (crystal violet))	((dental caries) OR (dental decay) OR (carious lesion) OR (periodontitis) OR (tooth demineralization) OR (gingivitis) OR (periodontal disease)
		#1 AND #2 AND #3

3.1.4 Seleção dos estudos

A seleção dos artigos foi feita por dois autores (B.T.C. e F.N.A.M.C.) de forma independente, em duas etapas. Na etapa 1, os artigos disponíveis foram importados para o programa Endnote Online (New York, NY, USA) e as duplicatas foram excluídas. Os artigos que restaram foram importados para aplicativo Rayyan QCRI (Hamad Bin Khalifa University, Doha, Qatar). Os dois revisores avaliaram os estudos considerando os títulos e resumos de forma cega. Os estudos foram então classificados em incluídos, excluídos e incertos. Após essa análise inicial, o modo cego foi desativado para análise das discrepâncias. Em caso de discordância, as dúvidas eram sanadas por meio de discussão entre os dois revisores, e, em caso de necessidade, um terceiro revisor mais experiente (C.B.A.) era contatado. Na etapa 2, os textos completos de todos os artigos foram avaliados e aqueles que atenderam aos critérios estabelecidos foram incluídos. Em caso de novas discordâncias, os revisores discutiam entre si novamente e caso a dúvida persistisse, o terceiro revisor era consultado.

3.1.5 Coleta de dados

Os dois revisores (B.T.C. e F.N.A.M.C.) extraíram os dados considerados relevantes nos artigos de forma simultânea e transportaram para tabela padronizada no programa Microsoft Office Excel 2016 (Microsoft Corporation, Redmond, Washington, DC, USA). As seguintes informações foram obtidas: Autor principal, ano de publicação, periódico, dados sobre o flavonoide pesquisado (nome, concentração, solvente, método de extração, fabricante), aplicação da pesquisa (prevenção da cárie ou doença periodontal) e instituição financiadora da pesquisa. Quando outro microrganismo era investigado, como por exemplo os fungos, os resultados também foram coletados. Ao final, o terceiro revisor (C.B.A.) avaliou os dados extraídos.

3.2 Estudo laboratorial *in vitro*

3.2.1 Compósito experimental

Um flavonoide, naringina, encontrado principalmente em frutas cítricas foi incorporado à resina composta experimental. A naringina é sintetizada e comercializada

pela empresa Sigma-Aldrich (St Louis, MO, EUA) e verificada quando ao grau de pureza. Um total de 4 grupos experimentais foram obtidos, contendo as substâncias detalhadas na tabela 2 e divididos da seguinte forma: (1) Grupo controle, resina composta experimental sem adição; (2) Resina experimental adicionada de Naringina (Nar) à 5 mM; (3) Resina experimental adicionada de Naringina (Nar) à 10 mM; (4) resina experimental adicionada de Naringina à 15 mM. Todo o processo foi realizado manualmente, em sala escura com luz amarela para evitar a polimerização precoce da resina composta, que foi armazenada em frascos pretos na geladeira até a confecção dos espécimes.

Tabela 2. Composição dos materiais restauradores.

Bis-GMA	15 peso%
TEGDMA	15 peso%
Canforoquinona	0,2 peso%
EDMAB	0,8 peso%
BHT	0,1 peso%
0,7 µm vidro de bario-borosilicato	65 peso%
0,05 µm sílica	5 peso%
Adição: Naringina livre (NAR)	5, 10 ou 15 mM

Elaborada pela autora, 2023

3.2.2 Resistência flexural e modulo de elasticidade

Para cada grupo, 10 espécimes em formato de barra (25 mm X 2 mm X 2 mm) foram confeccionados usando um molde de aço inoxidável de acordo com a ISO 4049. As barras foram polimerizadas entre duas lâminas de vidro com fotoativação de 20 s em 3 pontos diferentes usando uma unidade fotopolimerizadora (Elipar – 3M, USA) com exposição de radiação de 30 J/cm2. Os espécimes foram armazenados secos por 24 h em recipientes pretos em temperatura ambiente (20ºC). A resistência flexural e o módulo de elasticidade das amostras foram testados usando o método de 3 pontos em uma máquina de ensaio universal (Ez test – Shumadzu, Japão) em uma velocidade de 0,5 mm/min. A resistência flexural (RS) em Mpa foi calculada usando a seguinte equação: RS (σ) = 3Fl/2bh2, onde F é a força aplicada no momento da fratura, I é o comprimento do apoio da barra (20 mm), e b e h são a largura e espessura dos

espécimes em mm, respectivamente. O modulo de elasticidade (E) foi determinado pelo início da curvatura da linha tensão-deformação, calculado pela equação: E= FI3/4bh3d, onde F é a força aplicada sobre um ponto da amostra; d é a deflexão causada pela força F; 1, b e h representam os mesmos conceitos já definidos anteriormente.

3.2.3 Avaliação colorimétrica

Discos de resina composta foram produzidos (n=6) através de um molde de silicone (10 mm de diâmetro X 2 mm de espessura) e polimerizados entre duas lâminas de vidro por 20 s cada lado. Quatro tomadas de cor foram feitas usando um espectrofotômetro digital (EasyShade, Vita Zahnfabrik, Bad Säckingen, Alemanha) em cada amostra, e a média foi calculada, um fundo branco de cerâmica foi usado para as tomadas de cor. Os valores de L* (eixo preto- branco), a* (eixo Vermelho-verde), b* (eixo amarelo-azul), e C* (Croma) foram obtidos para cada grupo, e o índice de branco para Odontologia foi calculado baseado na literatura.

3.2.4 Sorção e solubilidade

Discos de resina (n= 10) foram pesados diariamente em balança analítica (Shimadzu, Japão) até que uma massa constante (M1) fosse obtida (até que a perda de massa de cada espécime não fosse maior que 0,1 mg durante um período de 24h). Posteriormente, os espécimes foram armazenados individualmente em água destilada por 7 dias. Após esse período, os espécimes foram cuidadosamente secos com papel absorvente e pesados novamente (M2). Em seguida, os espécimes foram armazenados em condições secas e a massa foi registrada diariamente até atingir um valor constante (M3). Os valores de sorção e solubilidade foram calculados em μg/mm3 utilizando as seguintes equações: Sorção = (M2 - M3) / V; Solubilidade = (M1 - M3) / V, onde M1 representa a massa inicial (μg) antes da imersão em água; M2 é a massa do espécime (μg) após imersão em água por uma semana; M3 é a massa do espécime após o recondicionamento (μg); V representa o volume de cada espécime (mm3).

3.2.5 Ensaio de citotoxicidade

Uma linhagem de células imortalizadas pré-osteoblásticas (MC3T3–E1) (ATCC Subclone 4 CRL-2593) obtidas comercialmente foi descongelada e colocada em meio de cultura contendo meio essencial modificado Dulbecco´s (DMEM) (Gibco/Invitrogen, Grand Island, EUA). A suplementação do meio foi feita com 10% de soro fetal bovino, 1% de penicilina e 1% de estreptomicina (todos Gibco) e incubados em atmosfera úmida de 95% de ar, contendo 5% de CO_2 e à 37ºC. As células foram semeadas na densidade de 1 x 10^5 células/ poço em 3 diferentes microplacas de 24-poços em quadruplicada. Após 24 h, os discos de resina polimerizados medindo 5 mm de diâmetro e 1 mm de espessura foram inseridos em cada poço. Um grupo controle de células (não tratadas) cultivadas em condições ideais foi usado para comparação. A viabilidade celular foi avaliada usando sal de metiltetrazólio (MTT, Invitrogen/Vybrant MTT, Molecular Probes, Eugene, EUA), de acordo com as instruções do fabricante. Os discos de resina em contato com as monocamadas foram avaliados em 3 tempos: 24h, 48 h e 72 h. Trinta µL de MTT e 270 µL de DMEM fresco foram adicionados nas células por 3 h. Depois, os cristais de formazan foram diluídos usando 300 µL/poço de dimetilsulfóxido (Sigma-Aldrich). A densidade óptica foi medida a 540 nm usando o leitor de microplacas (Norgen™, BioTek Corporation, Ontario, Canada).

3.2.6 Análises estatísticas

Os dados foram analisados segundo normalidade e homoscedasticidade. A resistência flexural, modulo de elasticidade, sorção e solubilidade, valores de a* e C*, e a citotoxicidade após 24h foram analisados por ANOVA um fator seguido do teste de Tukey. L*, b* e o índice de branco para Odontologia foram analisados pelo teste Welch ANOVA seguido por Dunnett´s T3, porque os dados foram heterocedasticos. A citotocixidade após 48h e 72h foram analisados por Kruskal Wallis e teste de Dunn, porque os dados não tinham distribuição normal e eram heterocedasticos.

4 ARTIGO 1

O artigo: **“Flavonoids effects against periodontitis and dental caries related bacteria: a scoping review”** foi submetido ao periódico *Clinical Oral Investigations* (Qualis A1, Fator de impacto = 3.607)

Flavonoids effects against periodontal disease and dental caries related bacteria: a scoping review

Abstract

Introduction: Flavonoids are plants secondary metabolites with numerous health benefits. Many properties were already described for flavonoids, as anti-oxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic, and antibacterial activities. Natural products with antibacterial action may improve treatment of dental caries and periodontal disease. **Objectives:** In order to impulse new research involving flavonoids and the development of new products with natural products, this scoping review aimed to investigate all isolated flavonoids already tested against bacteria related to dental caries and periodontal disease. **Materials and Methods:** The search was done in PubMed/MEDLINE, Scopus, Web of Science, Embase, LILACS, and Gray Literature on March 2023. Studies that investigated a mixture of flavonoids, extract of plants (containing a mixture of compounds), flavonoids incorporated into other material than a liquid vehicle were excluded. **Results:** Nineteen articles were included in this scoping review, all *in vitro* studies. For periodontal disease related bacteria, proanthocyanidins isoliquiritigenin, liquiritigenin, galangin, quercitrin, taxifolin, chrysin, diosmetin, quercetin, myricetin, naringin, apigenin, catechins, luteolin, morin, and rutin were investigated. For dental caries related bacteria, baicalein, naringenin, catechin, isoliquiritigeni, liquiritigenin, myricetin, quercetin and kaempferol were already investigated. Bacteriostatic, bactericidal and antibiofilm activity were predominantly reported. Some authors also investigated the effect of flavonoids on bacteria virulence factors. Most studies tested myricetin and quercetin. **Conclusion:** Positive antibacterial effect was described for most flavonoids tested. **Clinical relevance:** flavonoids are a promising field for the development of therapeutical products, based on natural agents, to prevent and treat dental caries and periodontal disease.

Keywords: flavonoids, anti-bacterial agents, dental caries, periodontitis.

Introduction

Flavonoids are low molecular weight polyphenolic compounds from plants secondary metabolites [1, 2]. They are divided into different subclasses according to their chemical structure (with C6-C3-C6 basic ring structure), such as: isoflavone, flavone, isoflavanone, flavanone, flavonol, isoflavan, flavanonol, flavan-3-ol and anthocyanidin [2, 3]. Health benefits on human health are attributed to these polyphenolic compounds, including diabetes, hypertension, and obesity prevention [4]. In addition, these natural compounds can modulate inflammatory responses and exhibit great antibacterial activity [5]. Thus, research has been developed to isolate flavonoids from fruits and plant extracts to produce pharmacological substances, which can be helpful to treat human disorders [6, 7].

In Odontology, flavonoids are investigated to prevent dental caries and modulate periodontal disease. These two conditions are considered a health public problem worldwide [8]. Periodontitis is considered a multifactorial disease, which includes bacterial biofilm and host immune response [9]. This condition causes loss of supportive tissues, alveolar bone, the tooth, [10] and can increase the risk of diabetes and cardiovascular diseases [11]. The biofilm complexity formed of hundreds of bacteria, [12] promotes resistance against antimicrobial agents and to the immune response, [13-15] creating an important challenge to avoid periodontitis progression.

Regarding dental caries, it is characterized by a dysbiosis induced by modifications in the oral environment, which prompt a microbial shift favoring the proliferation of acidogenic and aciduric bacteria. The oral pH decreases as consequence of high concentration of fermentable carbohydrates, becoming acid, and bacteria such as *Streptococcus mutans* can proliferate [16]. Although, the process of biofilm formation is complex and occurs in stages, [17] the initial layer formation is composed of glyco and salivary proteins on the enamel surface. This pellicle facilitates the aggregation of early colonizer bacteria and *Streptococcus mutans*, which is the main responsible for producing an extracellular polymeric matrix in presence of sucrose [18]. The exopolysaccharide matrix is an important framework to help the adhesion of new bacteria and protect these microorganisms against changes in pH, pressure, temperature, immune attack, and even antibiotics [19]. The next step is biofilm maturation, which consists of new bacteria adhesion, co-aggregation, and encapsulation. Finally, degradation and re-colonization of the biofilm occur [17, 20].

The use of natural compounds that can benefit oral health without promoting bacterial resistance focusing on target-specific action, such as flavonoids, has gained increased interest. The knowledge of its mechanism of action and its broad spectrum can generate the development of new dental materials based on/or containing these natural substances to prevent and control the main oral disorders. Thus, the aim of this scoping review was to map all the findings related to flavonoid applications against bacteria related to periodontitis and dental caries.

Materials and methods

This scoping review followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis Extension for Scoping Reviews guideline (PRISMA - ScR) The final protocol was registered prospectively with the Open Science Framework on March 2023 (doi: 10.17605/OSF.IO/K4XWU). Two basic questions guided this review: 1) Which flavonoids were already tested against bacteria related to periodontal disease and dental caries? 2) Which methodologies were used to test its effects?

Eligibility criteria

The inclusion criteria consisted of *in vitro*, *in situ,* or *in vivo* studies that analyzed the antibacterial effect of one or more flavonoids tested against bacteria related to caries and periodontal diseases. Studies that tested flavonoids that were pure or diluted in a vehicle (commercially available or plant extracted) were added; while when encapsulated, or in nanoparticles-functionalized system, or in gel-systems or chewing gum, were not included in this review in order to investigate flavonoids natural presentation. Studies were not included when the flavonoid was not specified. The search was restricted to articles published in English without restriction on date. Literature and systematic reviews were excluded to focus only on *in vitro* studies and its methodologies and results. Studies that investigated only isolated genes or isolated virulence factors, or the potential antibacterial effect through computational program were also excluded, as well as articles that used flavonoids incorporated to other materials, which can jeopardize its release and mode of action. For periodontal disease related bacteria, proanthocyanidins isoliquiritigenin, liquiritigenin, galangin, quercitrin, taxifolin, chrysin,

diosmetin, quercetin, myricetin, naringin, apigenin, catechins, luteolin, morin, and rutin were investigated.

Information sources and search

The literature searches were performed by two independent researchers, starting on September 2022, and finished in December of the same year. The chosen databases for this review were PubMed/MEDLINE, Scopus, Web of Science, Embase, LILACS, and Gray Literature. Three groups of keywords were determined according to the intervention (flavonoid and most common flavonoids or subclasses), antibacterial testes (most common), and the related disease (dental caries and periodontitis). The research terms were adjusted according to each database. Table 1 presents all the specific terms and strategy used.

Table1 Search strategy used in each database.

Pubmed/Medline		
#1	#2	#3
"flavonoids"[MeSH Terms] OR "flavonoids"[All Fields] OR "flavonoid"[All Fields] OR "flavanols"[All Fields] OR "flavanols"[All Fields] OR "flavonone"[All Fields] OR "flavone"[All Fields] OR "Hesperetin"[All Fields] OR "Hesperedin"[All Fields] OR "Neohesperedin"[All Fields] OR "Naringenin"[All Fields] OR "Naringin"[All Fields] OR "Butin"[All Fields] OR "Luteolin"[MeSH Terms] OR "Luteolin"[All Fields] OR "Quercetin"[MeSH Terms] OR "Quercetin"[All Fields] OR "Apigenin"[MeSH Terms] OR "Apigenin"[All Fields] OR "Kaempferol"[All Fields] OR "Catechin"[All Fields] OR "Epigallocatechin"[All Fields] OR "Baicalein"[All Fields] OR "Galangin"[All Fields] OR "Morin"[All Fields] OR "Myricetin"[All Fields] OR "Rutin"[MeSH Terms] OR "Rutin"[All Fields] OR "EGCG"[All Fields] OR "Tangeritin"[All Fields]	"Antibacterial activity"[All Fields] OR "anti-bacterial"[All Fields] OR "biofouling"[MeSH Terms] OR "biofouling"[All Fields] OR "anti-biofouling"[All Fields] OR "biofilm*"[All Fields] OR "biofilms"[MeSH Terms] OR "biofilms"[All Fields] OR "anti-biofilm"[All Fields] OR "antibiofilm"[All Fields] OR "bacteria*"[All Fields] OR "bactericidal"[All Fields] OR "bacteriostatic"[All Fields] OR "MIC"[All Fields] OR "MBC"[All Fields] OR "MICB"[All Fields] OR "adherence"[All Fields] OR "bacteria"[MeSH Terms] OR "bacteria"[All Fields] OR "microorganisms"[All Fields] OR "virulence"[MeSH Terms] OR "virulence"[All Fields] OR "antimicrobial"[All Fields] OR "inhibitory"[All Fields] OR "disk diffusion"[All Fields] OR "halo"[All Fields] OR "kirby-bauer"[All Fields] OR "crystal violet"[All Fields]	"dental caries"[All Fields] OR "dental caries"[MeSH Terms] OR "dental decay"[All Fields] OR "carious lesion*"[All Fields] OR "periodontitis"[All Fields] OR "periodontitis"[MeSH Terms] OR "tooth demineralization"[All Fields] OR "tooth demineralization"[MeSH Terms] OR "gingivitis"[MeSH Terms] OR "gingivitis"[All Fields] OR "periodontal diseases"[MeSH Terms] OR "periodontal diseases"[All Fields]
		#1 AND #2 AND #3
Embase		
#1	#2	#3
'flavonoid'/exp OR 'flavonoids' OR 'flavonoid'/exp OR 'flavonoid' OR 'flavanols' OR 'flavonones' OR 'flavone'/exp OR 'flavone' OR 'hesperetin'/exp OR 'hesperetin' OR 'hesperedin' OR 'neohesperedin' OR 'naringenin'/exp OR 'naringenin' OR 'naringin'/exp OR 'naringin' OR 'butin'/exp OR 'butin' OR 'luteolin'/exp OR 'luteolin' OR 'quercetin'/exp OR 'quercetin' OR 'apigenin'/exp OR 'apigenin' OR 'kaempferol'/exp OR 'kaempferol' OR 'catechin'/exp OR 'catechin' OR 'epigallocatechin'/exp OR 'epigallocatechin' OR 'baicalein'/exp OR 'baicalein' OR 'galangin'/exp OR 'galangin' OR 'morin'/exp OR 'morin' OR 'myricetin'/exp OR 'myricetin' OR 'rutin'/exp OR 'rutin' OR 'egcg'/exp OR 'egcg' OR 'tangeritin'/exp OR 'tangeritin'	('antibacterial activity'/exp OR 'anti-bacterial' OR 'biofouling'/exp OR 'anti-biofouling' OR 'biofilm'/exp OR 'biofilms' OR 'anti-biofilm' OR 'antibiofilm'/exp) AND 'bactericidal' OR 'bacteriostatic' OR 'mic' OR 'mbc' OR 'micb' OR 'adherence'/exp OR 'bacteria' OR 'microorganisms' OR 'virulence'/exp OR 'antimicrobial' OR 'inhibitory' OR 'disk diffusion'/exp OR 'halo' OR 'kirby-bauer' OR 'crystal violet'/exp	'dental caries'/exp OR 'dental decay' OR 'carious lesion*' OR 'periodontitis'/exp OR 'tooth demineralization' OR 'gingivitis'/exp OR 'periodontal disease'/exp
		#1 AND #2 AND #3
SCOPUS		
#1	#2	#3
(ALL('flavonoids' OR 'flavonoid' OR 'flavanols' OR 'flavonones' OR 'flavone' OR 'Hesperetin' OR 'Hesperedin' OR 'Neohesperedin' OR 'Naringenin' OR 'Naringin' OR 'Butin' OR 'Luteolin' OR 'Quercetin' OR 'Apigenin' OR 'Kaempferol' OR 'Catechin' OR 'Epigallocatechin' OR 'Baicalein' OR 'Galangin' OR 'Morin' OR 'Myricetin' OR 'Rutin' OR 'EGCG' OR 'Tangeritin'))	(ALL('Antibacterial activity' OR 'anti-bacterial' OR 'biofouling' OR 'anti-biofouling' OR 'biofilm' OR 'biofilms' OR 'anti-biofilm' OR 'antibiofilm' OR 'bactericidal' OR 'bacteriostatic' OR 'MIC' OR 'MBC' OR 'MICB' OR 'adherence' OR 'bacteria' OR 'microorganisms' OR 'virulence' OR 'antimicrobial' OR 'inhibitory' OR 'disk diffusion' OR 'halo' OR 'kirby-bauer' OR 'crystal violet'))	(ALL('dental caries' OR 'dental decay' OR 'carious lesion' OR 'periodontitis' OR 'tooth demineralization' OR 'gingivitis' OR 'periodontal disease'))
		#1 AND #2 AND #3
Web of Science		
#1	#2	#3

((ALL=("flavonoids" OR "flavonoid" OR "flavanols" OR "flavonones" OR "flavone" OR "Hesperetin" OR "Hesperedin" OR "Neohesperedin" OR "Naringenin" OR "Naringin" OR "Butin" OR "Luteolin" OR "Quercetin" OR "Apigenin" OR "Kaempferol" OR "Catechin" OR "Epigallocatechin" OR "Baicalein" OR "Galangin" OR "Morin" OR "Myricetin" OR "Rutin" OR "EGCG" OR "Tangeritin"))	("Antibacterial activity" OR "anti-bacterial" OR "biofouling" OR "anti-biofouling" OR "biofilm" OR "biofilms" OR "anti-biofilm" OR "antibiofilm" OR "bactericidal" OR "bacteriostatic" OR "MIC" OR "MBC" OR "MICB" OR "adherence" OR "bacteria" OR "microorganisms" OR "virulence" OR "antimicrobial" OR "inhibitory" OR "disk diffusion" OR "halo" OR "kirby-bauer" OR "crystal violet"))	ALL=("dental caries" OR "dental decay" OR "carious lesion" OR "periodontitis" OR "tooth demineralization" OR "gingivitis" OR "periodontal disease")
		#1 AND #2 AND #3
LILACS		
#1	#2	#3
((flavonoids) OR (flavonoid) OR (flavanols) OR (flavonones) OR (flavone) OR (hesperetin) OR (hesperedin) OR (neohesperedin) OR (naringenin) OR (naringin) OR (butin) OR (luteolin) OR (quercetin) OR (apigenin) OR (kaempferol) OR (catechin) OR (epigallocatechin) OR (baicalein) OR (galangin) OR (morin) OR (myricetin) OR (rutin) OR (egcg) OR (tangeritin))	((antibacterial activity) OR (anti-bacterial) OR (biofouling) OR (anti-biofouling) OR (biofilm) OR (biofilms) OR (anti-biofilm) OR (antibiofilm) OR (bactericidal) OR (bacteriostatic) OR (mic) OR (mbc) OR (micb) OR (adherence) OR (bacteria) OR (microorganisms) OR (virulence) OR (antimicrobial) OR (inhibitory) OR (disk diffusion) OR (halo) OR (kirby-bauer) OR (crystal violet))	((dental caries) OR (dental decay) OR (carious lesion) OR (periodontitis) OR (tooth demineralization) OR (gingivitis) OR (periodontal disease)
		#1 AND #2 AND #3

Selection of Sources of Evidence

The identified articles were imported into the Online Endnote (New York, NY, USA) and the duplicates were excluded. Remaining articles were transferred to Rayyan QCRI (Hamad Bin Khalifa University, Doha, Qatar). The two independent researchers made the selection trough the title and abstract, in blind mode. They were classified in included, excluded or uncertain. After that, the blind mode was turn off to analyze the discrepancies. Conflicts were solved by discussion (after reading the full text) and, in case of doubt a third reviewer were asked. Finally, the full articles of the included papers were downloaded.

Data Charting, Data Items, and Analysis

A spreadsheet was created in Microsoft Office Excel 2016 software (Microsoft Corporation, Redmond, Washington, DC, USA) containing columns divided in: first author/ year; journal of publication; flavonoid aspects such as: name, concentration, solvent, manufacturer, obtaining method, and application in dentistry. Antibacterial data were extract from the selected published papers. When other microorganism was investigated, as fungi, the results were also collected. Two independent researchers extracted the data from the articles simultaneously.

Results

A total of 1003 articles were identified (Fig.1) and the references were imported to Online Endnote (New York, NY, USA). After the exclusion of the duplicates, 446 articles remained and transferred to Rayyan QCRI (Hamad Bin Khalifa University, Doha, Qatar). The first analyses were made through the tittle and abstracts, resulting in 73 studies that were downloaded and fully read by the reviewers, independently. Finally, 19 articles were selected following the inclusion/exclusion criteria.

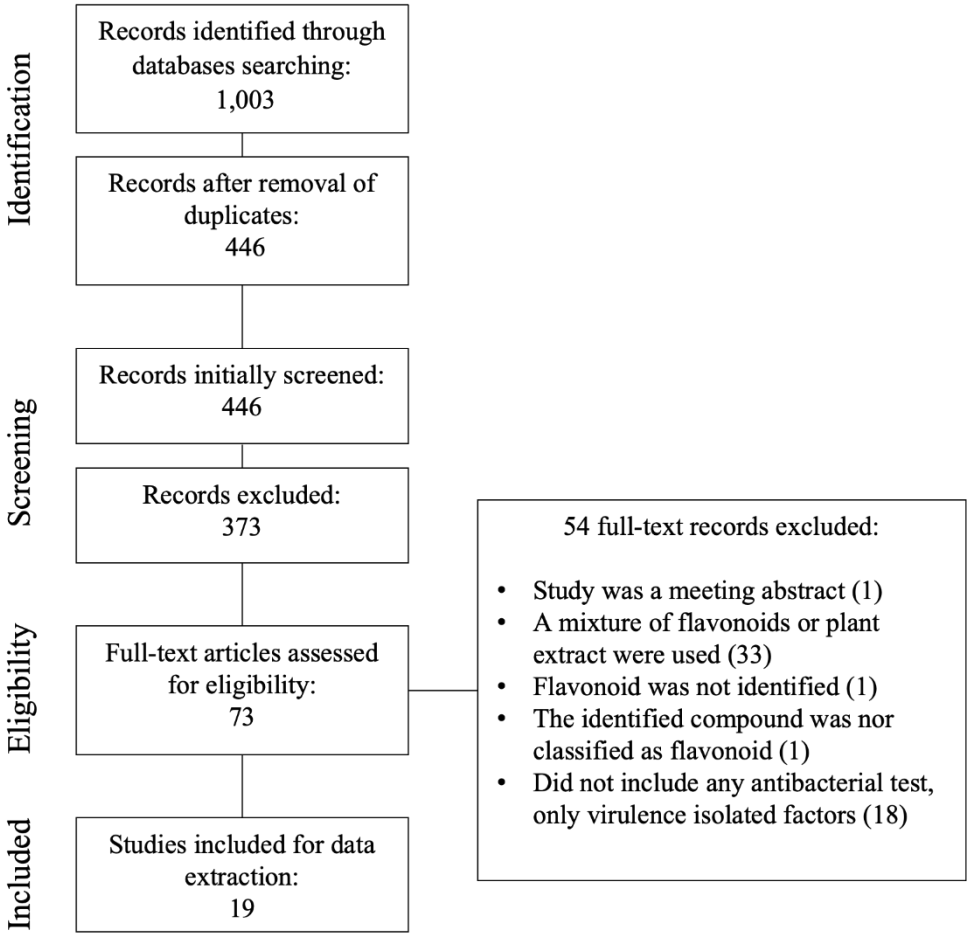

Figure 1. Flowchart of the selection process.

Characteristics and results of sources of evidence

The main characteristics of the 19 selected articles are showed in Table 2. Only *in vitro* studies fulfilled the inclusion/exclusion criteria. The publications were among 2006 to 2022 in periodicals of microbiology, periodontology, phytotherapy and phar-

macology. The most studied flavonoids in the selected articles were myricetin (7 articles) and quercetin (5 articles). Most of these flavonoids were obtained commercially (in 15 of the 19 articles, one did not inform). In one study, the extraction of blueberry was performed, and another perfomed the extraction from *Nidus vespae*. The main solvent used were dimetymethil sulfoxide (DMSO), followed by ethanol. When it comes to applications in dentistry, 8 studies investigated mainly bacteria involved in periodontitis, while 9 studies investigated bacteria related to dental caries, and 2 manuscripts investigated both bacteria related disease. A major part of the studies was financed by Chinese public institutions. Private companies did not sponsor any of the found research.

Table 2 – First author, year, flavonoid, flavonoid concentration, solvent used, flavonoid obtaining method and bacteria related disease tested against.

First author, year	Flavonoid (subgroup)	Concentration	Solvent	Obtaining method	Bacteria releted
Ben Lagha 2018	Proanthocyanidins (oligomeric flavonoid)	Stock solution: 10 mg/ml	50% ethanol	Blueberry Extraction (purity confirmed by mass spectrometry and acid catalyzed degradation with phloroglucinol)	Periodontal disease
Chen 2022	Baicalein (flavonone), naringenin (flavanone), catechin (flavonol)	0.250 mg/mL of baicalein, 1.000 mg/mL of naringenin, 0.250 mg/mL of catechin	Not informed	Commercially available (Solarbio, Beijing, China)	Dental caries
Feldman 2011	Isoliquiritigenin (chalcone) and liquiritigenin (flavanone)	1.25; 2.5; 5; 10; 20;40 µg/mL of both flavonoids	Not informed	Commercially available (Chromadex – Irvine, CA, USA)	Periodontal disease and dental caries
Gómez-Florit 2014	Galangin (flavonol), quercitrin (flavone), taxifolin (flavonol), chrysin (flavone) and diosmetin (flavone)	0.1, 1, 10, 100, 200, 500 µg	Galangin, quercitrin, and taxifolin: Absolute etanol Chrysin and diosmetin: DMSO	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Periodontal disease

Geoghegan 2010	Quercetin (flavonol)	0.1 g/mL, 0.05 g/mL, 0.025 g/mL	Distilled water	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Periodontal disease
Grenier 2015	Myricetin (flavonol)	Stock solution: 10 mg/ml	DMSO	Commercially availabble (Chromadex Inc., Irvine, CA, USA)	Periodontal disease
Guan 2012	Quercetin (flavonol) and kaempferol (flavonol)	0.125 to 16 mg/ml	DMSO	Isolated from Nidus Vespae (Chengdu, China) using Column chromatography	Dental caries
Gutiérrez-Venegas 2019	Apigenin (flavone), catechin (flavonol), luteolin (flavone), morin (flavonol), myricetin (flavonol), naringin (flavanone), quercetin (flavonol) and rutin (flavonol)	0.1 – 1.0 mg/mL (increasing dose every 0.1) and 5.0 mg/mL	DMSO	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Periodontal disease
He 2020	Quercetin (flavonol)	0 - 1600 μM	DMSO	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Periodontal disease
Hu 2021	Myricetin (flavonol)	0 - 512 μg/mL	DMSO	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Dental caries
Liu 2021	Baicalein (flavone)	512, 256, 128, 64, 32, 16, 8 and 4 μg/mL	DMSO	Not informed	Dental caries
Lobo 2021	Myricetin (flavonol)	Stock solution: 15 mg/ml 1250, 1000, 500, 250, 125, 62.5, 31.25, and 15.625 μg/mL	Ethanol and DMSO	Commercially available (AK Scientific, Inc. – Union City, CA, USA)	Dental caries
Lopes 2022	Myricetin (flavonol)	500 μg/mL and 250 μg/mL	Ethanol and DMSO	Commercially available (AK Scientific, Inc. – Union City, CA, USA)	Dental caries

Matsunaga 2010	Catechins (flavonol): (+)-catechin, (-)-epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, and (-)-epigallocatechin gallate	0.1 to 1 mM	Dissolved in bactéria media	Commercially available (Mitsui Norin, Tokyo)	Periodontal disease
Pedraza 2020	Myricetin (flavonol)	62.5 to 500 µg/ml	Ethanol and PBS	Commercially available (AK Scientific, Inc. – Union City, CA, USA)	Dental caries
Rocha 2018	Myricetin (flavonol)	2 mM	Ethanol	available (Sigma-Aldrich – St Louis, MO, USA)	Dental caries
Shu 2011	Quercetin (flavonol)	0.5 - 32 mg/ml	DMSO and PBS	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Periodontal and dental caries
Tsui 2008	Naringin (flavanone)	0.25 g/mL, 0.1 g/mL, and 0.0625 g/mL	Distilled water	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Periodontal disease
Yue 2018	Naringenin (flavanone)	3.1, 6.2, 12.5, 25, 50, 100, 200, 400, 800 µg/ml Stock solution: 20 mg/ml	DMSO	Commercially available (Sigma-Aldrich – St Louis, MO, USA)	Dental caries

Synthesis of results

The *in vitro* studies included in this scoping review showed that these natural compounds may have a potential to treat periodontitis and to prevent dental caries. The main antibacterial results are depicted in Table 3. Table 4 presents a summary of all positive antibacterial results for each flavonoid. Table 5 presents the main results described by each author at the abstract or conclusion sections.

Table 3 – Antibacterial results of flavonoids tested against periodontal and dental caries related bacteria (and other microorganisms when tested).

First author, year	Flavonoid	MIC / MBC	CFU	Other results
Ben Lagha 2018	Proanthocyanidins (PACs)			At 500 μg/ml PACs reduced *Aggregatibacter actinomycetemcomitans* growth by 62,5% recorded by optical density in microplate reader; Biofilm viability growth in presence of PACs: 31.25 μg/ml of the PACs decreased *A. actinomycetemcomitans* growth by 23.83% and promoted a biofilm inhibition by 93.98%; Treatment of a 24h-biofilm of *A. actinomycetemcomitans* for 6h: 500 μg/ml of the PACs reduced biofilm viability by 29.32%; *A. actinomycetemcomitans* caused a dose- and time-dependent release of fluorescence, suggesting that cell lysis had occurred.
Chen 2022	Baicalein, naringenin, and catechin			Cytotoxicity (CCK-8 assay) was performed to determine flavonoids concentration (similar to negative control): 0.250 mg/mL of baicalein, 1.000 mg/mL of naringenin, and 0.250 mg/mL of catechin; SEM imagens of biofilm on adhesive disks (biofilm: *Streptococcus mutans*, *Candida albicans*, and combined *S. mutans*-*C.albicans*): baicalein and naringenin treated biofilm did not fully developed even after 4h. Baicalein groups had the greatest antibacterial activity; Biofilm biomass (crystal violet staining): baicalein, naringenin, and catechin significantly reduced all biofilm biomass tested; All flavonoids decreased the polysaccharide production. Baicalein decreased more than 90% of the polysaccharide synthesis; The lactic acid production of single- and dual-species biofilms was greatly inhibited on the flavonoids; Baicalein reduced CFU of *S. mutans* biofilm by nearly 5 logs and 4 logs in *C. albicans* biofilm, respectively; Confocal images: baicalein and naringenin impaired EPS production and biofilm formation. Baicalein exhibited the lowest EPS/bacterial volume ratio, which indicated the best anti-bacterial effect of baicalein in the EPS architecture development (for the tree types of biofilms tested);

				Biofilm cultured with flavonoids, had the transcription levels of *gtfB/C/D* and *comC/D/E* genes statistically declined. The expression of *gtfB/C/D* in the baicalein group was downregulated to 0.58, 0.56 and 0.35 folds, respectively; while in the naringenin group, it was down-regulated to about 0.20 folds.
Feldman 2011	Isoliquiritigenin and liquiritigenin	MIC of isoliquiritigenin against *Prevotella intermedia* was 10 µg/mL; MBC of isoliquiritigenin against *P. intermedia* was 20 µg/mL; MIC isoliquiritigenin against *Porphyromonas. gingivalis* 40 µg/mL		Isoliquiritigenin at 40 µg/mL was able to reduce *Fusobacterium nucleatum* growth by approximately 70%; At a concentration of 40 µg/mL, liquiritigenin inhibited the growth of *P. intermedia* by 85%; Both flavonoids exhibited significant antibacterial activities against Gram-negative periodontal bacteria, while having no effect on Gram-positive streptococci.
Gómez-Florit 2014	Galangin, quercitrin, taxifolin, chrysin and diosmetin			Optical density in microplate reader: Chrysin, diosmetin, and quercitrin doses (0.1, 1, 10,100, and 200µM) inhibited *Staphylococcus epidermidis* growth rate (9%, 12% to 16%, and 33% inhibition, respectively), galangin (in all above concentrations) and 200 µM taxifolin increased it;
Geoghegan 2010	Quercetin	MIC of quercetin against: *P. gingivalis* = 0.0125 g/ml; *Actinobacillus actinomycetemcomitans* = 0.1 g/ml; *Pseudomonas cepacia*, *S. mutans,* methicillin-resistant *Staphylococcus aureus, P. gingivalis*, *A actinomycetemcomitans*, *C. albicans*, *Candida glabrata*, and *Candida. krusei*: No effect	Viability of *A. actinomycetemcomitans:* 0.25, 0.05, and 0.1 g/ml of quercetin significantly decreased the viable count compared to NaCl after 1h incubation; Viability of *Porphyromonas Gingivalis*: 0.25, 0.05, and 0.1 g/ml of quercetin significantly decreased the viable count compared to NaCl after 1h incubation. No colony forming units were observed after 24h.	
Grenier 2015	Myricetin	MIC values of myricetin against *P. gingivalis:* ATCC 33277 and ATCC 49417: 125 µg/ml; HW24D1 and W83: 62.5 µg/ml; MBC values of myricetin against *P. gingivalis:*		Myricetin at 100 µg/ml caused a decrease of 81, 93, and 64% for *fimA*, *hagA*, and *hagB*,respectively. At 50 µg/ml, only *fimA* and *hagB* significantly decreased. These are genes related to bacteria *(P. gingivalis ATCC 33277)* virulence.

		ATCC 33277 and ATCC 49417: 250 µg/ml; HW24D1 and W83: 125 µg/ml;		Myrecetin also down regulated the expression of *rgpA*, *rgpB*, and *kgp*, three cysteine protease genes related to inactivation of host defense mechanisms, tissue destruction, and nutrient acquisition.
Guan 2012	Quercetin and kaempferol	MIC - MBC (respectively) values of quercetin against: *S. mutans* = 2 mg/ml - 8 mg/ml *Streptococcus sobrinus* = 2 mg/ml - 8 mg/ml *Streptococcus sanguis* = 2 mg/ml - 16 mg/ml *Actinomyces viscosus* = 1 mg/ml - 8 mg/ml *Actinomyces naeslundii* = 1 mg/ml - 8 mg/ml *Lactobacillus rhamnosu* = 4 mg/ml - 16 mg/ml MIC - MBC (respectively) values of kaempferol against: *S. mutans* = 1 mg/ml - 8 mg/ml *S. sobrinus* = 2 mg/ml - 8 mg/ml *S. sanguis* = 2 mg/ml - 16 mg/ml *A. viscosus* = 1 mg/ml - 8 mg/ml *A. naeslundii* = 1 mg/ml - 4 mg/ml *L. rhamnosu* = 2 mg/ml - 8 mg/ml	At the MIC, quercetin and kaempferol mainly demonstrated bacteriostatic effects and the viable count of *S. mutans* slowly declined. At the MBC, bactericidal effects were observed. After 1–2 h of incubation, 8 mg/ml of quercetin and kempferol both demonstrated 100% killing capacity.	Incubation of *S. mutans* in sub-inhibitory concentrations of quercetin and kaempferol (1/8 MIC, 1/4 MIC and 1/2 MIC) exhibited an attenuation in acid production. The reduction in acid production was concentration-dependent; The acidurity of *S. mutans* was also suppressed by quercetin and kaempferol at sub-MIC levels. The survival rate of *S. mutans* at pH of 5.0 was significantly reduced in the presence of quercetin and kaempferol (0.125–1 mg/ml) compared with the nontreated control; When exposed to 1/8–1/2 MIC concentrations of quercetin and kempferol, less than 10% reduction of LDH (Lactate dehydrogenase) activity was observed. The F-ATPase activity was reduced by 47.37% at 1/2 MIC of quercetin and by 49.66% at 1/2 MIC of kempferol.
Gutiérrez-Venegas 2019	Apigenin, catechin, luteolin, morin, myricetin, naringin, quercetin and rutin			Presence of inhibition halo (significant activity): *Aggregatibacter actinomycetemcomitans:* catechin, luteolin, morin, naringin, and quercetin (concentrations not reported); *A. naeslundii:* morin (5 mg/ml), naringin (10 mg/ml), quercetin (10 mg/ml), and rutin (0.61 mg/ml); *A. viscosus:* luteolin (5 mg/ml), morin (10 mg/ml), quercetin (10 mg/ml), and rutin (0.61 mg/ml); *C. albicans:* luteolin (5 mg/ml), morin (1 mM), naringin (100 µM), quercetin (10 mg/ml), and rutin (100 µM); *Escherichia coli:* luteolin (100 µM), morin (10 mg/ml), naringin (100 µM), quercetin (10 mg/ml), and rutin (100 µM);

			Enterococcus faecalis: apigenin, luteolin, morin, naringin, and rutin (concentrations not reported); *Lactobacillus casei:* morin (1 mM), naringin (100 μM), and rutin (100 μM); *Staphylococcus aureus:* apigenin (not reported), catechin (1 mg/ml), luteolin (1 mg/ml), morin (1 mg/ml), naringin (1 mg/ml), quercetin (0.01 mg/ml), and rutin (1 mg/ml). *Streptococcus oralis:* none *Streptococcus sanguinis: n*one
He 2020	Quercetin	MIC of quercetin against *P. gingivalis* was 200 μM and MBC was 400 μM	Under Transmission Electron Microscopy, quercetin at 100, 200, and 400 μM promoted damage and discontinuity of cell membrane and the cell structures of *P. gingivalis* (planktonic culture). Gingipain activities: quercetin at sub-MIC inhibited *Rg*p and *Kgp* activities in dose-dependent manners significantly (regards to *P. gingivalis* virulence factors). Molecular docking analysis were carried out to confirm that quercetin could accommodate the binding pocket of both *Kgp* and *RgpB* by well shape matching and favorable hydrogen bond and van der Waals interactions, suggesting that quercetin is capable to inhibit the bioactivity of *Kgp* or *RgpB* by occupying the catalytic binding site. The thicknesses of quercetin-treated biofilm of *P.gingivalis* were 32.5 ± 0.1 μm at 12.5 μM, 25.6 ± 3.3 μm at 25 μM, 19.8 ± 1.3 μm at 50 μM and 13.3 ± 1.0 μm at 100 μM, respectively, which were thinner than the control group (44.2 ± 2.5 μm); Also, quercetin decreased the biofilm metabolism compared to the control group. Confocal microscopy: biofilm of *P.gingivalis* treated with quercetin were thinner. Also, as the concentration of quercetin increased, the biofilm structures were more dispersed and sparser. The hydrophobicity assay revealed that quercetin increased *P. gingivalis* surface hydrophobicity in a dose-dependent manner.

			With increasing concentrations of quercetin, the aggregation rate of *P. gingivalis* increased from 27.18 ± 1.38% at 12.5 µM to 73.49 ± 6.25% at 100 µM. Expression levels in virulence genes with quercetin were reduced ranging from 0.428- to 0.0884-fold at 50 µM and from 0.170-fold to 0.0340-fold at 100 µM (analysed under real time-PCR). The virulence factor genes were including *hagA, hagB,* hem (involved in hemagglutination); *kgp, rgpA, rgpB* (involved in gingipain); *ragA* (immunodominant surface proteins), and *vimA* (virulence modulating gene A).
Hu 2021	Myricetin	MIC of Myricetin against *S. mutans* = 512 µg/ mL (approx. 1609 µM).	Biofilm grown on polystyrene microporous plate: Myricetin (100 and 150 µM) reduced the amount of CFU in 6 h (initial adhesion) and 16 h (biofilm formation), without interfering with the bacteria growth curve; Myricetin upregulated *srtA* and *pac* expression significantly when the concentration achieved 100µM or 150µM.
Lobo 2021	Myricetin		*S. mutans* IC_{50} = 500 µg/ml; Myricetin did not demonstrate significant antimicrobial activities against *C. albicans*; Biofilm of *S. mutans* (24h): all concentrations tested demonstrated antibiofilm activity, eliminating bacterial growth; Biofilm of *S. mutans* (48h): 1 log reduction only (lower inhibitory activity); Biofilm of *C. albicans* (24h): 1 log reduction only (lower inhibitory activity); Biofilm of *C. albicans* (48h): no effect.

Lopes 2022	Myricetin		Myricetin (500 µg/ml) inhibited cell growth (*S. mutans*) over time in the mid-log setting, myricetin was less effective in the stationary setting; Myricetin (500 µg/ml) promoted gradual cell death from 4 to 24 h at the mid-log setting, but not as efficiently as in the presence of glucose.	*S. mutans* biofilm post-treatment: Myricetin (250 µg/ml) promoted a gradual decrease in cell viability, without colony recovery after three treatment cycles but with detection in the eighth cycle; *16S rRNA*, *gtfB, eno,* and *gyrA* genes (*S. mutans)* presented higher expression; Extracellular matrix components: Myricetin (250 µg/ml) promoted smaller amounts of protein, insoluble exopolysaccharides, and lower dry-weight; Confocal images (*S. mutans)*: smaller biofilm and widely spaced clusters.
Liu 2021	Baicalein	Baicalein MIC against *S. mutans* = 256 µg/mL;		Biofilm: At 8 µg/mL baicalein showed moderate inhibitory effect (24.9% reduction), while at 16 µg/mL baicalein presented 47.5% inhibition of *S. mutans* biofilm; Baicalein did not significantly affect the growth of *S. mutans* at a concentration of 16 µg/mL; Baicalein significantly reduced *LuxS* in a concentration dependent manner; Rhamnose, the hydrolyzed compound of rhamnolipids, was distinctly reduced in the baicalein treated group, from 450 µg/mL to 320 µg/mL. Baicalein repressed the pH drop of *S. mutans* culture media in a concentration dependent manner. Both testes indicate that baicalein reduced the virulence factors of *S. mutans.*
Matsunaga 2010	Catechins ((+)-catechin (C), (-)-epicatechin (EC), (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-catechin gallate (Cg), (-)-epicatechin gallate (ECg), (-)-gallocatechin gallate (GCg), and (-)-epigallocatechin gallate (EGCg))			1mM each of GC, EGC, Cg, ECg, GCg, and EGCg inhibited biofilm formation of *Eikenella corrodens* remarkably, whereas C and EC had no effect; Inhibitory effects of Cg, ECg, GCg, and EGCg on biofilm formation (*E. corrodens*) were observed even at 0.1mM, whereas effects of GC and EGC were not up to 0.5mM and 0.25mM, respectively. Bacterial growth (*E. corrodens*) fell in the presence of the catechins with the galloyl group, Cg, ECg, GCg, and EGCg, in dose-dependent manner, but we found that there is no bactericidal activity in the

				presence of the catechins without galloyl group, C, EC, GC, and EGC, because more than 80% of bacteria survived even at 1mM.
Pedraza 2020	Myricetin	MIC against *S. mutans* was 250 µg/ml; MCB not determined.	Planktonic *S. mutans*: 250 µg/ml reduced aproximatly 4 logs; 500 µg/ml reduced aproximatly 6 logs; Biofilm of *S. mutans* (polystyrene microplate): MIC and 2xMIC reduced the viable counts by 4 logs and 99% of biomass.	Biofilm of *S. mutans* (hydroxyapatite discs): myricetin reduced the soluble and insoluble exopolysaccharides; Myricetin downregulated the g*tfC* gene expression.
Rocha 2018	Myricetin			Biofilm of *S. mutans* combined with *C. albicans* treated twice daily with myrecitin: Myrecetin did not interfere with the biomass of biofilm; Myricetin did not affect the viable count of biofilm; Myricetin did not affect the water soluble and water insoluble polysaccharides.
Shu 2011	Quercetin	MIC of quercetin against: *S. sanguis* = 2 mg/ml; *P. gingivalis* = 4 mg/ml; Actinobaccilus *actinomycetemcomitans* = 2 mg/ml; *S. mutans* = 2 mg/ml; *S. sobrinus* = 1 mg/ml; *Lactobacillus acidophilus* = 2 mg/ml. MBC of quercetin against: *S. sanguis* = 16 mg/ml*;* *P. gingivalis* = 16 mg/ml; A. *actinomycetemcomitans* = 8 mg/ml *S. mutans* = 8 mg/ml; *S. sobrinus* = 8 mg/ml; *L acidophilus* = 16 mg/ml;		Inhibitory halo: 4 mg/ml of quercetin inhibited the growth of 11 main bacteria related to periodontitis and caries (*S. mutans, S. sanguis, S. sobrinus, A. viscosus, A. naeslundii, L. acidophilus, P. gingivalis, F. nucleatum, A. Actynomicetemcomitans, P. intermedia, and C. albicans*).
Tsui 2008	Naringin	MIC of naringin against: *Escherichia coli* = 0.03125 g/ml; *S. aureus* = 0.03125 g/ml; Methicillin-resistant *S. aureus* = 0.0078 g/ml; *Pseudomonas aeruginosa* = 0.125 g/ml; *Pseudomonas cepacia* = 0.0098 g/ml; C. albicans = 0.00098 g/ml; C. glabrata = 0.00098 g/ml; C. krusei = 0.0156 g/ml; Candida tropicalis = 0.0039 g/ml.	The 3 tested concentrations of naringin solutions (0.25 g/mL, 0.1 g/mL and 0.0625 g/mL) decreased significantly the viable count of *Actinobacillus actinomycetemcomitans* when compared with the controls with NaCl after 3h. After 24 h, even the lowest concentration (0.0625 g/mL) showed a significant reduction of viable cell count; For *P gingivalis,* the viable counts decreased significantly at 3h regardless of the naringin concentrations. No colony forming units could be observed	

			at 24 h for the samples treated with 0.25 g/ml and 0.1 g/ml naringin, and the count was very low for the sample treated with 0.0625 g/ml; For *S mutans*, only the highest concentration of naringin solution (0.25 g/mL) showed significant reduction of the viable cell count after 24h of incubation.	
Yue 2018	Naringenin	The MIC of naringenin was between 100 and 200 µg/ml.		At 100 µg/mL, naringenin showed 70% inhibition effect and 50 µg/mL inhibited 50% of the *S. mutans* biofilms; Field emission scanning electron microscopy: 100 and 200 µg/mL of naringenin inhibited bacterial growth. Naringenin resulted in the atypical morphology of *S. mutans* with unclear cell edges and slightly shrunk cells; Confocal: both concentrations resulted in major population of dead bacteria after 4h of growth, while 24h- biofilms increased the live bacteria (*S. mutans)*; Naringenin at 100 and 200 µg/mL increased *S. mutans* surface hydrophobicity, but naringenin at low concentration seemed more effective than in higher concentration. Both concentration (100 and 200 µg/mL) of naringenin obviously reduced bacterial aggregation; The mRNA expression of *gtfB, gtfC, comD, comE*, and *luxS* genes was downregulated by naringenin at 100 and 200 µg/ml.

Table 4 – Summary of the positive antibacterial effect of each flavonoid.

Flavonoid	**Bacteria (strain)**
proanthocyanidins	*A. actinomycetemcomitans (*ATCC 29522)

isoliquiritigenin	*F. nucleatum (*ATCC 25586) *P. intermedia (*ATCC 25611) *P.gengivalis (*ATCC 33277)
liquiritigenin	*P. intermedia* (ATCC 25611)
galangin	No positive effect
quercitrin	*S. epidermidis (*4184)
taxifolin	No positive effect
chrysin	*S. epidermidis* (4184)
diosmetin	*S. epidermidis* (4184)
quercetin	*A. actinomycetemcomitans* (ATCC 700685); *A. actynomicetemcomitans (*29523); *P. gingivalis (*ATCC 33277); *P. gingivalis (*381) *S. mutans* (ATCC 25175) *S. sobrinus* (ATCC 33478) *S. sobrinus (*6715) *S. sanguis* (ATCC 10556) *A. viscosus* (ATCC 19246); *A. viscosus (*ATCC15987); *A. naeslundii* (WVU627); *A. naeslundii (*ATCC 12104); *E. coli (*ATCC 25922) *S. aureus (*ATCC25923) *L. acidophilu (*ATCC4356) *F. nucleatum (*10953) *A. actinomycetemcomitans (*ATCC 43718) *P. intermedia (*ATCC25611)
myricetin	*P. gingivalis* (ATCC 33277) *S. mutans* (UA159)
naringin	*E. coli (*ATCC 25922) *A. naeslundii (*ATCC 12104) *A.actinomycetemcomitans (*ATCC 43718) *E. faecalis (*ATCC8043) *S. aureus* (ATCC25923) *A. actinomycetemcomitans (*ATCC 700685) *P gingivalis (*ATCC33277) *S. mutans* (ATCC35668)
apigenin	*E. faecalis* (ATCC8043) *S. aureus* (ATCC25923)
luteolin	*A. actinomycetemcomitans (*ATCC 43718) *E. coli (*ATCC 25922) *E. faecalis* (ATCC8043) *S. aureus* (ATCC25923)
morin	*A. actinomycetemcomitans* (ATCC 43718) *A. naeslundii (*ATCC 12104) *A. viscosus* (ATCC15987) *E. coli (*ATCC 25922) *S. aureus* (ATCC25923)

rutin	*A. naeslundii (*ATCC 12104) *A. viscosus* (ATCC15987) *E. coli (*ATCC 25922) *E. faecalis* (ATCC8043) *S. aureus* (ATCC25923)
baicalein	*S. mutans* (identification not informed)
naringenin	*S. mutans* (identification not informed)
catechin	*S. mutans* (identification not informed) *A. actinomycetemcomitans* (ATCC 43718); *S.aureus* (ATCC25923)
kempferol	*S.mutans* (ATCC25175)

Table 5 – Summary of the main findings according to each author*.

First author, year	
Benlagha 2018	• Blueberry Proanthocyanidins (PACs) reduced the growth of *A. actinomycetemcomitans* and prevented biofilm formation at sub-inhibitory concentrations; • The treatment of pre-formed biofilms with the PACs resulted in a loss of bacterial viability; • The antibacterial activity of the PACs appeared to involve damage to the bacterial cell membrane. • The PACs protected the oral keratinocytes barrier integrity from damage caused by *A. actinomycetem-comitans;* • The PACs also protected macrophages from the deleterious effect of leukotoxin Ltx-A and dose-dependently inhibited the secretion of pro-inflammatory cytokines (IL-1β, IL-6, CXCL8, TNF-α), matrix metalloproteinases (MMP-3, MMP-9), and sTREM-1 by A. *actinomycetemcomitans*-treated macrophages; • The PACs also inhibited the activation of the NF-κB signaling pathway.
Chen 2022	• Compared to chlorhexidine and untreated groups, the baicalein group achieved the greatest reduction in *S. mutans*, *C. albicans* and *S. mutans-C. albicans* biofilms, yielding the least metabolic activity, polysaccharide synthesis and lactic acid production ($p < 0.05$); • The biofilm CFU was decreased in baicalein group by 5 logs, 4 logs, 5 logs, for *S. mutans*, *C. albicans* and *S. mutans-C. albicans* biofilms, respectively, compared to untreated group; • When tested in a *S. mutans-C. albicans in vitro* caries model, the baicalein group substantially reduced enamel demineralization under biofilms, yielding an enamel hardness that was 2.75 times greater than that of untreated group.

Feldman 2011	• Isoliquiritigenin demonstrated significant antibacterial activity against three major periodontopathogens, *P. gingivalis*, *F. nucleatum*, and *P. intermedia*. In contrast, Liquiritigenin exerted less pronounced effects on the above bacterial species; • Neither compound was effective against cariogenic bacteria (*S. mutans* and *S. sobrinus*); • Isoliquiritigenin exhibited a stronger inhibitory activity than Liquiritigenin toward *P. gingivalis* collagenase and human matrix metalloproteinase 9; • The capacity of Isoliquiritigenin to attenuate the inflammatory response of macrophages induced by A.actinomycetemcomitans lipopolysaccharide (LPS) was much higher when compared to Liquiritigenin; • The activation of transcriptional factors nuclear factor-kB (NF-kB) p65 and activator protein-1 (AP-1) associated with the LPS-induced inflammatory response in macrophages was inhibited strongly by Isoliquiritigenin, but less affected by Liquiritigenin.
Gómez-Florit 2014	• Quercitrin showed the most promising biologic effects, in both human gingival fibroblasts (HGFs) and *S. epidermidis;* • Quercitrin was not toxic for HGFs; increased collagen IIIa1 and decorin levels; downregulated interleukin-6 messenger RNA levels; decreased the expression of profibrotic markers during wound healing; decreased reactive oxygen species levels in basal and stimulated conditions; and decreased the MMP1/TIMP1 ratio; • Quercitrin also decreased the bacterial growth rate.
Geoghegan 2010	• All quercetin solution significantly decreased the viable count of *A. actinomycetemcomitans* when compared with NaCl controls after 1 h of incubation ($p < 0.05$); • The inhibitory effects increased as the incubation period increased; • For *P. gingivalis,* viable counts decreased significantly at 1 h in all concentrations of quercetin ($p < 0.05$). No colony forming units observed at 24 h for the samples treated with the quercetin solutions; • Quercetin had an inhibitory effect against *P. gingivalis* and *A. actinomycetemcomitans*.
Grenier 2015	• Minimal inhibitory concentration values of myricetin against *P. gingivalis* were in the range of 62.5 to 125 µg/ml; • The iron chelating activity of myricetin may contribute to the antibacterial activity of this flavonol;

	• Myricetin was found to attenuate the virulence of *P. gingivalis* by reducing the expression of genes coding for important virulence factors, including proteinases (*rgpA, rgpB*, and *kgp*) and adhesins (*fimA, hagA*, and *hagB*); • Myricetin dose-dependently prevented NF-κB activation in a monocyte model. Moreover, it inhibited the secretion of IL-6, IL-8 and MMP-3 by *P.gingivalis*-stimulated gingival fibroblasts.
Guan 2012	• Quercetin and kaempferol, extracted of *Nidus Vespae*, showed remarkable antimicrobial activity against oral cariogenic bacteria; • The inhibitory actions of quercetin and kaempferol against *S. mutans* may be partly due to their impacts on some key virulence factors associated with cariogenicity, including acid production and acid tolerance.
Gutiérrez-Venegas 2019	• Results revealed that luteolin, morin, naringin, quercetin and rutin effectively inhibited bacterial (*A. actino-mycetemcomitans, A.s naeslundii, A. viscosus, E. faecalis, E. coli, L. casei, S. aureus, St. oralis and S. sanguinis)* and fungal growth (*C. albicans);* • Morin was the most effective flavonoid.
He 2020	• Quercetin exhibited antimicrobial effects and damaged the cell structure of *P. gingivalis;* • Quercetin can inhibit gingipains, hemolytic, hemagglutination activities and biofilm formation at sub-MIC concentrations; • Molecular docking analysis further indicated that quercetin could interact with gingipains; • The biofilm became sparser and thinner after quercetin treatment; • Quercetin also modulates cell surface hydrophobicity and aggregation; • Expression of the genes tested was downregulated in the presence of quercetin.
Hu 2021	• Myricetin was discovered as a potent inhibitor of *S. mutans SrtA*, with an IC_{50} of 48.66 ± 1.48 μM, which was lower than the minimum inhibitory concentration (MIC) of 512 ug/mL; • Immunoblot and biofilm assays demonstrated that myricetin at a sub-MIC level could reduce adhesion and biofilm formation of *S. mutans;* • The reduction of biofilm was possibly caused by the decreased amount of Pac (*cell surface protein antigen)* on the cells 'surface by releasing Pac into the medium via inhibiting *SrtA* activity; • Molecular dynamics simulations and mutagenesis assays suggested that *Met123, Ile191*, and *Arg213* of *SrtA* were pivotal for the interaction of *SrtA* and myricetin;

	• Myricetin can reduce biofilm formation without developing bacterial resistance in *S. mutans*, which has the benefit of dental caries prevention.
Liu 2021	• Baicalein inhibited the biofilm formation without influence on the growth of *S. mutans;* • Baicalein also distinctly reduced the production of both rhamnolipids and acids.
Lobo 2021	• Myricetin eliminated both species (*S. mutans* and *C. albicans*) on initial biofilms.
Lopes 2022	• In S. mutans biofilms formed by cells recovered from biofilms previously exposed to myricetin, cells presented higher expression of the *16S rRNA*, *gyrA* (DNA replication and transcription), *gtfB* (insoluble exopolysaccharides), and *eno* (enolase—glycolysis) genes and lower quantities of insoluble dry weight and insoluble exopolysaccharides than those derived from other agents; • Biofilms formed by *S. mutans* cells recovered after treatment with myricetin were at an earlier developmental phase than the others; • These characteristics of myricetin can reduce biofilm virulence in the absence of agents because the biofilms formed by cells preexposed to this agent presented a tridimensional structure and composition with less insoluble exopolysaccharides. This trait means that these biofilms would have a greater susceptibility to the penetration of antimicrobial agents and mechanical removal.
Matsunaga 2010	• Some catechins at sub-MIC might inhibit biofilm formation; • No inhibitory effect of catechins at sub-MIC on biofilm formation was observed in the *luxS* deletion mutant; • It is possible that some species of catechins with the galloyl group affect autoinducer 2-mediated quorum sensing and thereby inhibit biofilm formation by *E. corrodens*.
Pedroza 2020	• The author indicated major findings for the combination of myricetin with other compounds.
Rocha 2018	• The author indicated that it may be necessary to change the concentration of the tested agents so that a more robust outcome is achieved, decreasing additional virulence characteristics of the biofilm.
Shu 2011	• Quercetin had inhibitory effects on *S. mutans* with MIC of 2 mg/ml and MBC of 8 mg/ml, *S. sobrinus* with MIC of 1 mg/ml and MBC of 8 mg/ml, *L. acidophilu* with MIC of 2 mg/ml and MBC of 16 mg/ml, *S. sanguis* with MIC of 2 mg/ml and MBC of 16 mg/ml, *A. actinomycetemocomitans* with MIC of 1 mg/ml and MBC of 8 mg/ml, *P. intermedia* with MIC of 4 mg/ml and MBC of 16 mg/ml, respectively.

Tsui 2008	• *A. actinomycetemcomitans* showed a significant decrease ($p < 0.05$) in viable counts after 3 h when naringin was added at baseline; • *P. gingivalis* also showed a marked growth reduction in the presence of naringin, and no colony forming units could be observed after 24 h; • Naringin also had an inhibitory effect against all bacteria and yeasts tested; • The results suggest that naringin possesses significant antimicrobial properties on periodontal pathogens *in vitro;* • It also has an inhibitory effect on some common oral microorganisms in low concentrations
Yue 2018	• The *S. mutans* growth curves, FESEM, CLSM showed that both 100 and 200 μg/mL of naringenin inhibited *S. mutans* growth and biofilm formation, increased *S. mutans* surface hydrophobicity, reduced bacterial aggregation, and downregulated the mRNA expression of *gtfB, gtfC, comD, comE,* and *luxS*; • Naringenin at 200 μg/mL slightly decreased the growths of human dental pulp cells compared with 100 μg/mL.

*Text extract from abstract or conclusion section of each published paper.

For periodontal disease related bacteria, proanthocyanidins isoliquiritigenin, liquiritigenin, galangin, quercitrin, taxifolin, chrysin, diosmetin, quercetin, myricetin, naringin, apigenin, catechins, luteolin, morin, and rutin were investigated. Proanthocyanidins (PACs) reduced the growth of *A. actinomycetemcomitans* and prevented biofilm formation at sub-inhibitory concentrations [20]. Isoliquiritigenin demonstrated significant antibacterial activity against three major periodontopathogens, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Prevotella intermedia*; while liquiritigenin exerted less pronounced effects on the above bacterial species [21]. Quercitrin decrease the *Staphylococcus epidermidis* growth rate [22]. Quercetin had an inhibitory effect against *Porphyromonas gingivalis* and *Actinobacillus. Actinomycetemcomitans* [23]. Quercetin also exhibited antimicrobial effects and damaged the cell structure of *Porphyromonas gingivalis,* [24] Myricetin attenuated the virulence of *Porphyromonas gingivalis* by reducing the expression of genes coding for important virulence factors, including proteinases (*rgpA, rgpB, and kgp*) and adhesins (*fimA, hagA,* and *hagB*), [7] showed inhibitory and bactericidal effect on *L. acidophilu, A. actinomycetemocomitans,* and *P. intermedia* [25]. Some catechin inhibit biofilm formation of *E. corrodens* [26]. Naringin had an inhibitory effect against some bacteria and yeasts tested (*Escherichia coli, Pseudomona Aeruginosa, Pseudomonas cepaci, methicillin-resistant Staphylococcus aureus, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Candida albicans, Candida glabrata, and Candida krusei)* [27]. Apigenin showed inhibition zones against *Enterococcus faecalis* [28]. Luteolin, morin, naringin, quercetin, and rutin effectively inhibited bacterial (*Agreggatibacter actinomycetemcomitans, Actinomyces naeslundii, Actinomyces viscosus, Enterococcus faecalis, Escherichia coli, Lactobacillus casei, Staphylococcus aureus, Streptococcus oralis and Streptococcus sanguinis)* and fungal growth (*Candida albicans)* [28].

For dental caries related bacteria, baicalein, naringenin, catechin, isoliquiritigeni, liquiritigenin, myricetin, quercetin and kaempferol were already investigated. Baicalein group achieved the greatest reduction in *S. mutans*, *C. albicans* and *S. mutans-C. albicans* biofilms, yielding the least metabolic activity, polysaccharide synthesis and lactic acid production [29]. Baicalein inhibited the biofilm formation without influence on the growth of *S. mutans* [30]. Neither isoliquiritigeni or liquiritigenin was effective against cariogenic bacteria (*Streptococcus mutans* and *Streptococcus sobrinus*) [21]. Myricetin was discovered as a potent inhibitor of *S. mutans* SrtA, and at a sub-MIC level could reduce adhesion and biofilm formation of *S. mutans* [31]. Myricetin

showed antibacterial and antibiofilm activity against *S. mutans*, and some of its virulence factors [32-34]. However, when *S. mutans* were combined with *C. albicans*, no expressive results were obtained for myricetin [35]. Quercetin and kaempferol presented bacteriostatic and bactericidal effects against *S. mutans*, attenuated the acid production and suppressed S. mutans acidurity [36].

Quercetin had inhibitory and bactericidal effects on *S. mutans and S. sobrinus* [25]. The *S. mutans* growth curves showed that both 100 and 200 μg/mL of naringenin inhibited *S. mutans* growth and biofilm formation, increased *S. mutans* surface hydrophobicity, reduced bacterial aggregation, and downregulated the mRNA expression of *gtfB, gtfC, comD, comE*, and *luxS* [37].

Discussion

Flavonoids are natural compounds present in vegetables, fruits, and plants in general. It possesses a huge benefit to human health and appears to be safe according to cytotoxicity assays [38]. Regarding oral health, there is a need to control biofilm development to prevent caries, periodontitis, and other diseases. Thus, medicinal products, added with flavonoids, can be considered in the future to improve dental hygiene or to improve restorative materials [23, 39]. Another important observation is the indiscriminate use of antibiotics, that may cause a bacterial resistance, turning difficult to treat oral infections during the years. Flavonoids may act directly in the pathogen, keeping the balance of oral microflora, reducing the induction of bacterial resistance [40]. This can explain the constant increasing of studies using flavonoids to treat different disorders.

This scoping review was undertaken to identify and screen in the current literature flavonoids tested against bacteria related to dental caries and periodontal disease. Some positive effects are suggested and attributed to biofilm inhibition, bacteria growth reduction by cell lysis and disorganization of biofilm architecture by the tested flavonoids, and their effect on specific virulence factors [21, 24, 33].

The studied flavonoids were effective against the followed periodontal pathogens: *P. intermedia*, *P. gingivalis*, *A. actinomycetemcomitans*, *A. naeslundii*, *A. viscosus*, *E. faecalis* and *E. coli* [21, 23-25, 28]. Different mechanisms can explain this antibacterial property. According to Puupponen-Pimiã *et al*. (2001), some flavonoids such as myricetin possess a high degree of hydroxylation, that contributes to its strong

antimicrobial activity [41]. Grenier *et al.* (2015) concluded that myricetin also has an iron- chelating property [7]. Other molecules such as quercetin can inhibit nucleic acid synthesis, metabolism energy, and can affects cytoplasmic membrane activity [42] and others can produce hydrogen peroxide, jeopardizing membranes [29]. Quercetin can also act increasing hydrophobicity of bacteria. This property appears to help in adhesion and biofilm development [43]. Other surveyed compounds were: Galangin, taxifolin, chrysin, diosmetin, apigenin, catechin, morin, and rutin. Most of tested flavonoids exhibited positive results concerning periodontal health, preventing bacterial growth [22, 28].

In a study conducted by Feldman *et al*. (2011), Isoliquiritigenin and liquiritigenin were tested against gram positive and gram-negative bacteria, exhibiting a great result against the gram negatives, especially P. intermedia [21]. A possible explanation for these findings can be the different cell wall characteristics of these two bacterial types. The gram-positive ones appear to have a significant thickness of peptidoglycan layer, which might be helpful to resist against physical aggressors [21]. Naringin is also a promising flavonoid when it comes to prevent periodontal related bacteria. This flavonoid was able to affect the growth of two periodontopathogens: *A. actinomycetemcomitans* and *P. gingivalis* [27]. All the three concentrations utilized decreased CFUs of *A. actinomycetemcomitans* [27]. The inhibition was directly proportional of time and naringin concentration. When it comes to *P. gingivalis*, no CFU could be found after 24h of experiment [27]. In similar study, naringin was also effective against *A. actinomycetemcomitans* and other two periodontopathogens (*E. coli* and *S. aureus*). Although, it was not effective against *S. sanguinis* and *S. oralis* [28].

Regarding caries prevention, flavonoids were able to act against *S.mutans, S. sobrinus* and *S. sanguis* [21, 29, 30, 33, 36, 37]. Flavonoids mechanisms of action against *S. mutans* are related to glucosyltransferase enzymes inhibition, reducing sucrose-dependent adhesion; decrease of lactic acid production, that induces demineralization of enamel; increase of membrane permeability, causing lysis [33]; and inhibition of Quorum sensing system, decreasing the ability of cariogenic microorganisms to communicate between themselves [22]. In another study, naringenin showed a significant activity against *S. mutans*, which after 24 hours, almost the entire biofilm formation was inhibited [37]. According to the authors, naringenin appears to act in all stages of biofilm consolidation. First, it could increase bacterial hydrophobicity, avoid-

ing its aggregation to enamel. Second, inhibited the expression of two important enzymes *gtfB* and *gtfC*, which are responsible for *S. mutans* adhesion in hard surfaces of the teeth. At last, naringenin could affect biofilm maturation, reducing *S. mutans* aggregation [37].

A recent study also evaluated naringenin and baicalein action against *S. mutans*. Both were able to avoid biofilm formation even after 4 hours [29]. Baicalein presented the greatest results: at 0.250 mg/mL had the best antibacterial effect; was super effective against polysaccharide synthesis, reducing it more than 90%; had the best reduction in biofilm metabolism and colony forming units (CFUs) and the best antibacterial action in the exopolysaccharide (EPS) architecture development when EPS/ bacterial volume ratio was calculated [29]. Naringin, a similar flavonoid, did not present a great result against cariogenic bacteria. It was only effective against *S. mutans* in the highest tested concentration (0.25 g/mL) after 24 hours of experiment [27].

In terms of microbiological aspects, flavonoids were evaluated in various concentrations and dilutions. In summary, these natural compounds reduce bacterial growth and decrease bacterial colony counts [28, 30, 37]. The minimum inhibitory concentration (MIC) was also performed in most studies and showed that flavonoids solutions have low cytotoxicity and cell viability within normality [5, 30, 37], which may favor its use in medical fields. Some flavonoids were already investigated for prior application of adhesive materials or incorporated into restorative materials [39, 44, 45]. Positive results found in this scooping review may contribute to the development of new materials or techniques to improve longevity of restorative materials and adhesion to modified or affected dental substrate [46].

This scoping review restricted it screening to isolated flavonoids tested against bacteria related to periodontitis or dental caries. For this reason, the exclusion of manuscripts that investigated the effect of extracts containing a mix of flavonoids or a mix of flavonoids with other compounds, or its incorporation into other material that may affect its release, can be considered a limitation of this study. Also, many another microorganism were already investigated in literature and were not included into this review. In addition, further studies investigating the effect of flavonoids in a more complex *in vitro* oral environment are still necessary to evaluate the flavonoids effects.

Conclusions

Flavonoids has already shown its potential oral health benefits and the potential to decrease drugs resistance by targeting specific virulence factors. In dentistry, promising results against caries and periodontal pathogens has been described. These facts shed a light on how these natural compounds may improve products designed for oral hygiene or may be a source for developing new restorative materials based on the incorporation of flavonoids or flavonoids-like agents, favoring high-risk caries patients.

Acknowledgments

This study was financed in part by FAPEMIG (APQ-01185-21), CNPq (422588/2021-0), and Capes 001, for the financial support. The funding sources were not involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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5 ARTIGO 2

O artigo **“Physicochemical analysis of an experimental resin-based material containing naringin”** será submetido ao periódico *Journal of Dentistry* (Qualis A1, fator de impacto = 4,379)

Physicochemical analysis of an experimental resin-based material containing naringin

Abstract

Objectives: The aim of this *in vitro* study was to incorporate naringin in an experimental composite to analyze its effect on physicochemical properties and cytotoxicity. **Materials and methods** A flavonoid found in citric fruits (naringin) were incorporated into a resin composite. A total of 4 groups were obtained: (1) control, resin composite with no additions; (2) resin composite with Naringin (Nar) at 5mM; (3) resin composite with Naringin (Nar) at 10 mM; (4) resin composite with Naringin (Nar) at 15 mM. Flexural strength and flexural modulus, color evaluation, water sorption and solubility, and cytotoxicity (24h, 48h and 72h) on pre-osteoblastic cells (MC3T3-E1) were performed. **Results:** No decrease in flexural strength or flexural modulus was observed when the flavonoid was added into the resin composite. When it comes to color aspects, the incorporation of naringin promoted a decrease in the L*, with no significantly statistic difference between the flavonoid groups. The a* increased proportionally to naringin concentration. The b* and C* parameters increased in the naringin groups compared to the control group. The W_{ID} factor decreased in comparison of control group. Water sorption and water solubility tests did not reveal statistically significant differences between experimental composite with no addition and experimental composites with naringin addition. Regarding cytotoxicity, the results showed no statistically significant differences between the four groups of composites for all times tested, demonstrating that naringin is not cytotoxic. **Conclusions** Naringin did not affect physiochemical properties of the tested experimental composites. Despite, it changed some color parameters, resulting in a yellowish composite.

Clinical significance: Naringin incorporation into resin do not affect its physicochemical properties and may have a potential antibacterial effect.

Keywords: flavonoids, flavanones, composite resin, biomaterial.

Introduction

Flavonoids are considered secondary metabolites of plants and a source of bioactive compounds found in vegetables, fruits, and plants (1). They can be divided in several classes, for example: flavanols, flavan-3-ols, isoflavones, flavanones, anthocyanidins and flavones (2). These natural compounds have been used by industry as a food preservative, avoiding lipid oxidation, as a colorant, and as antifungal (3,4). It can also be applied to improve human health due to its anti-inflammatory, anti-carcinogenic, antioxidant and antibacterial properties (5).

Naringin is a flavonoid that belongs to flavanones group. It is usually found in citric fruits such as oranges, lemons, limes, and mandarins (6). Its chemical structure is composed of the flavonoid naringenin added to a disaccharide called neohesperidose (7). This natural compound has been receiving attention due to its low cost, ample availability, and numerous pharmacological actions. It appears to increase osteoblastic activity, leading to bone regeneration (8), reduce signaling factors expression associated with inflammatory response (9), and helps to control central nervous system diseases (10).

When it comes to Dentistry, studies have been shown promising results using naringin alone and adding in formulations of dental materials (11,12). Different aspects regarding this flavonoid potential have been evaluated. A recent study analyzed some flavonoids effects, including naringin, on modulus of elasticity and ultimate tensile strength of demineralized dentine (12). Another one evaluated dentin pre-treatment solutions containing different flavonoids on microtensile bond-strength and nanohardness (13). Others focus on its antibacterial properties against periodontopathogens (14) and cariogenic bacteria (15).

A study analyzed a naringin-chitooligosaccharide complex antibacterial potential against *Escherichia Coli* and *Sthaphyloccocus aureus.* Both bacteria presented changes in morphology, confirming the antibacterial activity of the complex that might jeopardize the surface of bacterial cells (14). Cariogenic bacteria appear to be affected by naringin as well. An *in vivo* study supplemented this flavonoid in rats diet using different concentrations. The results showed a significant reduction in dental caries when 0.72% of naringin supplementation was used. Plaque accumulation also decreased when naringin concentrations increased (15).

The fact is that several substances have been tested among the years in dental

materials formulations, in order to enhance materials properties. Silver, zinc oxide and fluorides are some examples (16). These components can be classified into antibacterial, aesthetic, reinforcing, remineralizing, radiopaque and self-healing, depending to their benefits (16). The antibacterial activity of natural compounds is one of the main properties that had gained attention in order to avoid recurrent caries, focusing on the specific pathogen and avoiding bacterial resistance (17,18). With this in mind, the objective of this *in vitro* study was to add a flavonoid, that has a potential antibacterial activity, into a resin-based material and evaluate the effect of this addition on psychochemical properties and cytotoxicity.

Materials and methods

Experimental composite formulation

A flavonoid found in citric fruit was incorporated into a resin composite. Naringin are commercially available by Sigma-Aldrich (Information). A total of 4 groups were obtained, containing the substances detailed in Table 1 and divided according to the following sequence: (1) control, resin composite with no additions; (2) resin composite with Naringin (Nar) at 5mM; (3) resin composite with Nar at 10 mM; (4) resin composite with Nar at 15 mM. The entire process was made manually, in a dark room with a yellow light to avoid early polymerization. Composites were kept in dark flasks at the fridge until the samples confection.

Table 1. Composite resin composition.

BisGMA	15 wt%
TEGDMA	15 wt%
Camphorquinone	0.2 wt%
EDMAB	0.8 wt%
BHT	0.1 wt%
0,7 µm silaneted barium borosilicate glass	65 wt%
0,05 µm silaneted fumed silica	5 wt%
Addition: Flavonoid (Naringin)	5, 10 or 15 mM

Flexural strength and flexural modulus

For each group, 10 bar-shaped specimens (25 mm X 2 mm X 2 mm) were made using a stainless-steel mold according to ISO 4049. The bars were polymerized between two glass slides with three overlapping exposures (20 s each), to cover the entire specimen, using a curing unit (Elipar-3M) with irradiance of 1378 mW/cm^2. Specimens were stored dry for 24 h in the dark at room temperature (20°C). The flexural strength and elastic modulus of the samples were tested in a 3-point bending on a universal test machine (Ez test – Shimadzu, Japan) at a crosshead speed of 0.5 mm/min. The flexural strength (FS) in MPa was calculated as FS(σ) = 3Fl/2bh2, where F is the load at fracture (N), l is the span length (20 mm), and b and h are the width and thickness of the specimens in mm, respectively. The elastic modulus (E) was determined from the slope of the initial linear part of the stress-strain curve, calculated as E = Fl3/4bh3d, where F is the load at some point on the linear region of the stress-strain curve; d is the slack compensated deflection at load F; and l, b, and h are as defined above.

Colorimetric evaluation

Disks of resin composites were obtained (n=6) within a silicon mold (10 mm diameter x 2 mm thick) and polymerized between two glass slides (20 s each side), as described above. Four color-measurements were performed using a digital spectrophotometer (EasyShade, Vita Zahnfabrik, Bad Säckingen, Germany) on each sample, and an average was obtained. A white ceramic background was used for color measurements. The L* (black-white axis), a* (red-green axis), b* (yellow-blue axis), and C* (Chroma) color parameters were obtained for each group, and the whiteness index for dentistry (W_{ID}) was calculated according to the literature (16).

Water sorption and solubility

Resin discs (n=10) measuring 5 mm diameter X 2 mm thickness were weighed daily with an analytical balance (Shimadzu, Japan) until a constant mass (M1) was recorded (until the mass loss of each specimen was not more than 0.1 mg within a period of 24 h). Afterwards, specimens were individually stored in distilled water for 1

week. Following water immersion, disk-shaped specimens were softly dried using absorbent paper, and weighed again (M2). Then, specimens were dry-stored, and the mass was daily recorded until a constant mass was obtained as described before (M3). The values of water sorption (WS) and solubility (SL) were calculated in μg/mm3 using the following equations: WS=M2−M3 /V; SL=M1−M3/V, where M1 is the conditioned mass (μg) prior to immersion in water; M2 is the mass of the specimen (μg) after immersion in water for 1 week; M3 is the mass of the reconditioned specimen (μg); V is the volume of each specimen (mm^3).

Citotoxicity assay

A commercially available pre-osteoblastic immortalized cell line (MC3T3-E1) (ATCC Subclone 4 CRL-2593) was thawed and cultured in Dulbeco's modified essential medium (DMEM) (Gibco/Invitrogen, Grand Island, EUA). It was supplemented with 10% fetal bovine serum (Gibco/Invitrogen, Grand Island, USA), 1% penicillin (Gibco/Invitrogen, Grand Island, EUA), 1% streptomycin (Gibco/Invitrogen, Grand Island, EUA), and incubated in a 95% humidified atmosphere containing 5% CO_2 at 37 °C. Cells were seeded at a density of 1×10^5 cells/well onto three different 24-well microplates in quadruplicate. After 24h, polymerized resin discs measuring 5 mm diameter and 1 mm thickness were placed in each plate. A control group of cells (untreated cells) cultured under ideal conditions was used for comparison. Cell viability was assessed using the 4,5-dimethylthiazol-2-yl) -diphenyltetrazolium bromide micro culture tetrazolium (MTT) assay (Invitrogen/Vybrant MTT, Molecular Probes, Eugene, USA), according to the manufacturer's instructions in three times: 24h, 48h and 72h after the resin discs addition into the well plate. Briefly, 30 µL of MTT and 270 µL of fresh DMEM were added to the cells for 3h. Next, formazan crystals were diluted using 300 µL/well of dimethyl sulfoxide (Sigma-Aldrich). The optical density was measured at 540 nm using a microplate reader (Norgen™, BioTek Corporation, Ontario, Canada).

Statistical analysis

Data were analyzed regarding normality and homoscedasticity. Flexural strength, flexural modulus, water sorption, water solubility, a* and C* parameters, and 24h cytotoxicity was analyzed by one-way ANOVA followed by Tukey's test. L*, b*, and W_{ID} were analyzed by Welch ANOVA followed by Dunnett's T3 test, because data were heteroscedastic. While 48h and 72h cytotoxicity were analyzed by Kruskal Wallis and Dunn's test, because data were not normally distributed and heteroscedastic.

RESULTS

Flexural strength and flexural modulus

No decrease in flexural strength or flexural modulus was observed when the flavonoid was added to the resin composite, for all concentrations tested (Table 1). The flexural strength of the experimental composite with no addition (control group) and the composites added with naringin were similar ($p>0.05$).

Table 2. Mean (SD) of flexural strength (MPa) and flexural modulus (GPa) of the experimental resin composites tested.

	Flexural strength	Flexural modulus
Control	73.2 (12.2) a	6.5 (1.0) a
Na5mM	63.1 (13.8) a	6.5 (0.8) a
Na10mM	63.1 (14.5) a	5.8 (0.9) a
Na15mM	63.8 (12.8) a	6.5 (0.5) a

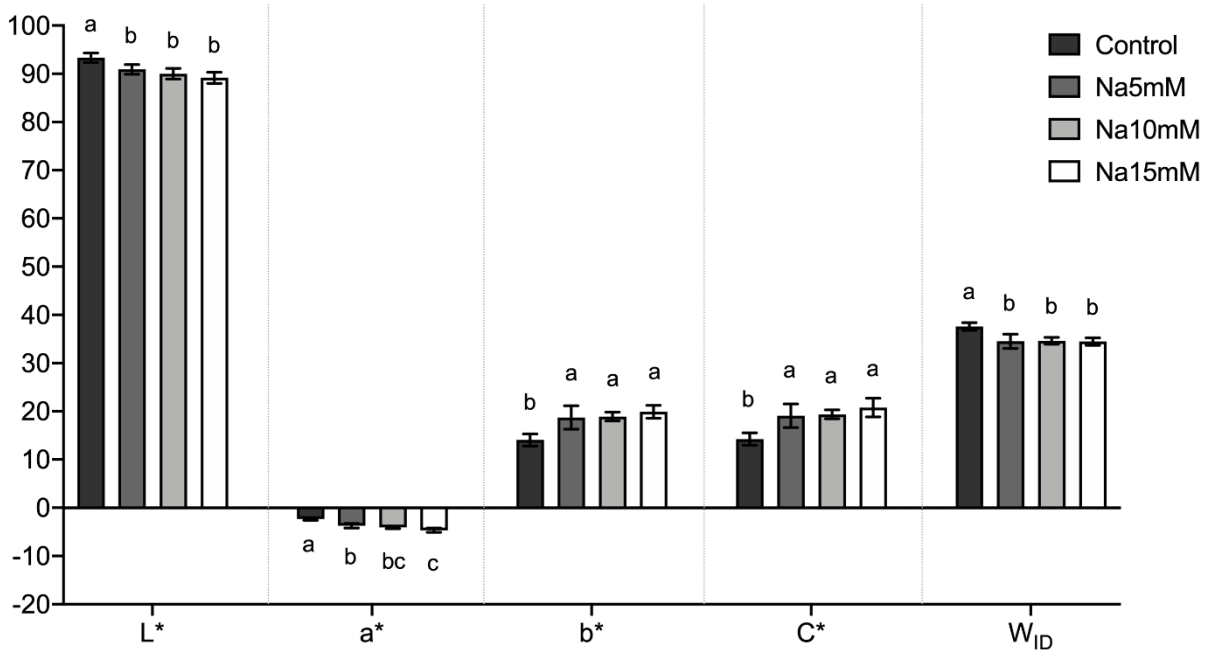
Similar letters indicate no significant difference ($p>0.05$).

Colorimetric evaluation

Colorimetric parameters were reported in Figure 1. For experimental composites tested, the incorporation of naringin promoted a decrease in the L*, with no significantly statistic difference between the flavonoid groups. The a* parameter presented significantly statistic difference between all tested groups. In general, the a* increased proportionally to naringin concentration. The b* and C* parameters increased in the naringin groups compared to the control group. The W_{ID} factor decreased in comparison of control group. Control group presented a $W_{ID} = 37.6 \pm 0.8$, while narigin containing

composites showed a W_{ID} = 34.5 ± 1.5 (Na5mM), 34.6 ± 0.7 (Na10mM) and 34.5 ± 0.8 (Na15mM) These results indicate an increase in Nar composites yellowish, which is proportional to naringin concentration.

Figure 1 – Colorimetric parameters of experimental resin composites.



Different letters indicate significant difference ($p<0.05$).

Water sorption and solubility

Water sorption and solubility results are summarized in Table 3. Water sorption and water solubility tests did not reveal statistically significant differences between experimental composite with no addition (control group) and experimental composites with naringin additions.

Table 3. Mean (SD) of water sorption and solubility (μg/mm^3) of the experimental resin composites tested.

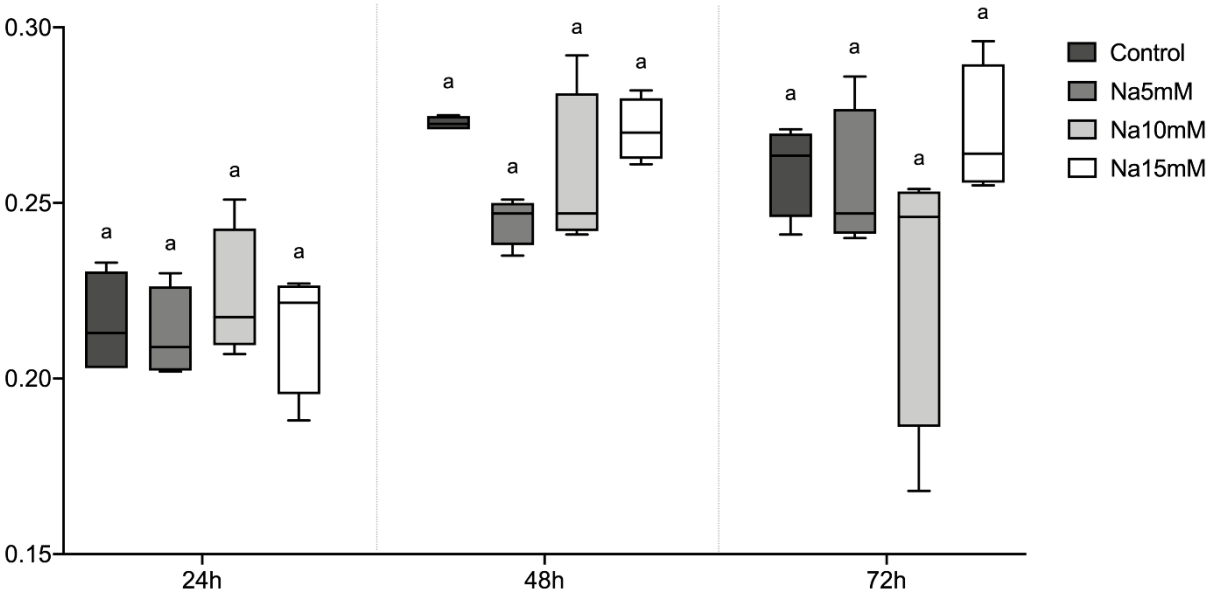
	Water sorption	Water solubility
Controle	18.6 (2.8) a	5.6 (1.7) a
Na5mM	19.5 (1.9) a	5.4 (1.8) a
Na10mM	18.5 (0.9) a	6.5 (2.1) a
Na15mM	18.1 (1.6) a	4.4 (1.4) a

Similar letters indicate no significant difference (p>0.05).

Citotoxicity assay

Figure 4 depicts the results of the MTT assay after 24h, 48h and 72h of exposure of cell cultures to the resin composite discs. The results showed no statistically significant differences between the four groups of composites tested for all times tested, demonstrating a small potential of naringin to cause cytotoxicity.

Figure 2 – Results of citotoxicity assay of experimental resin composites.



Eixo Y está sem label (Optical density (540 nm). Sugiro colocar o n. Similar letters indicate no significant difference (p>0.05).

Discussion

The use of natural substances in dental materials seems to be a trend nowadays. However, the addition of these compounds cannot change important characteristics such as the water intake and solubility, flexural strength, as well as cytotoxicity. The addition of naringin in the tested concentrations did not affect the flexural strength and flexural modulus of the experimental resin composites, which is important maintain the composite resistance to cohesive fracture. A preliminary study also presented

a similar result when another flavonoid, called apigenin, were added into a composite (19).

The addition of a new compound in dental materials is always a concern when it comes to water degradation, which can jeopardize resin-matrix and affect the restoration longevity (20). Naringin addition did not change water sorption and solubility of the tested composites. This property is related to the material composition, including the hydrophilic characteristic of monomers, type and concentration of solvents and the inorganic matrix (21-24).

Restorative materials can be bioactive, stimulate cell proliferation, migration and differentiation. In addition, must exhibit highly compatibility with pulp cells (25). The tested composites did not present cytotoxicity greater than that for the experimental composite with no addition of narigin. Another study analyzed the exposition of human cells to naringin in three concentrations for 6, 12 and 24 hours. The results showed that this flavonoid was not cytotoxic to osteoblasts and increased cell viability by 19% after 24 hours (26). Although, further studies are needed to confirm naringin non-cytotoxicity in pulp cells.

Biomodified restorative materials may also have antibacterial activities. A recent study added Brazilian red propolis into a commercial resin composite to evaluate its antibacterial activity against *S. mutans* as well as the material physical-chemical properties. The antibacterial action was confirmed using a direct contact test, the composite was not cytotoxic against 3T3 fibroblasts and physical-chemical properties were not greatly affected by the propolis addition, exhibiting a flexural resistance higher than 100 MPa (27).

Despite all the positive aspects of naringin incorporation, it showed a significant difference in all parameters related to color compared to control group. The W_{ID} showed that the perceptibility and acceptability parameters were over (around 3.0) of the threshold (0.72 and 2.70 respectively) (28,29). The yellowness increase can be mitigated using white pigments in composite resin formulation or directly to the tooth surface. Besides, if the antibacterial tests present positive results, the yellowness factor may not be so relevant comparing to the possibility of secondary caries prevention. This composite resin might be indicated for posterior restorations, where aesthetic is not mandatory; Class V, which is a critic area to retain biofilm; and also, for pediatric

and adult patients as a provisional restoration.

As any laboratorial *in vitro* study, same limitations should be described, as the absence of antibacterial tests, the absence of recurrent caries methodology, the determination of degree of conversion of the tested materials, the manual manipulation of the composites, and the basic composition of the experimental composites.

Conclusions

The addition of naringin into dental materials formulation might be promising. Naringin did not affect most physicochemical properties of the tested experimental composites, despite its concentration, and was not considered cytotoxic. However, the final shade of these composites might have a yellowish color. The yellowness increased proportionally as the naringin concentration.

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6 CONCLUSÃO

A busca por alternativas mais naturais tem sido uma preocupação das indústrias, principalmente no que diz respeito a potenciais produtos antibacterianos. Isso porque o uso indiscriminado de antibióticos convencionais de amplo espectro eleva a chance de criar uma resistência bacteriana. Nesse cenário, os flavonoides podem contribuir para modulação da doença periodontal e controle da cárie dental. Isso pode ser feito por meio da adição dessas substâncias em materiais dentários, em produtos de higiene bucal, ou ainda em produtos de uso profissional para tratamento dessas doenças citadas.

Como foi possível constatar em um dos artigos desenvolvidos, parece não haver prejuízo nas propriedades físico-químicas das resinas compostas quando a adição da naringina foi feita. A biocompatibilidade também é um importante achado, visto que o compósito não exibiu citotoxicidade, sendo aparentemente bem tolerado pelas células utilizadas neste estudo.

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APÊNDICE A – Artigo *in vitro*

Use of a flavonoid-based antioxidant after tooth whitening

Abstract

Objective To evaluate the application of a flavonoid-based antioxidant after in-office bleaching with 35% hydrogen peroxide on the shear bond strength of bovine enamel compared to 10% sodium ascorbate. **Materials and methods** Block (n=10) were obtained and randomly divided into 5 groups: without prior bleaching (CP); bleaching and without application of antioxidant (CN); bleaching and application of 10% sodium ascorbate for 1 min (ASC); bleaching and application of 5% naringin for 1 min (NA5) and bleaching and application of 10% naringin for 1 min (NA10). Samples from antioxidant groups had the color analyzed using a digital spectrophotometer (Vita EasyShade). To evaluate the failure mode, two cylinders (2 mm diameter) of flowable resin were made in each block and placed in a universal testing machine (0.5 mm/min). Shear bond strength values, ΔE_{ab} and ΔE_{00} were analyzed by one-way ANOVA followed by Tukey's test, while L*, a*, b* and C* parameters by T Test. **Results** None of tested antioxidants were able to promote bond strength like CP. However, the ASC and NA5 groups had better results than CN. NA10 obtained similar results to the CNR. Regarding color analysis, no antioxidant was able to change the L*, a*, b* and C* parameters. The predominate failure mode was adhesive between the adhesive and enamel, and mixed. **Conclusions** None of the antioxidants tested were able to promote bond strength like the control group, although ASC and NA5 obtained better results. The antioxidants did not affect color parameters. **Clinical significance** The tested flavonoid-based antioxidant might be helpful to avoid adhesion failures after bleaching.

Keywords: composite resin, tooth whitening, antioxidants, bond strength

INTRODUCTION

Aesthetic dentistry has become attractive to most of the population, who sees white smiles and aligned teeth as something fundamental to self-esteem. One of the main characteristics, reported by patients, for an aesthetically satisfactory smile is the teeth color (1). Thus, the dental surgeon should not only provide dental anatomy and function, but also involve aesthetic aspects (2). As a non-invasive and effective procedure, dental bleaching might be helpful to achieve a luminous smile. It can be performed in several ways and presents different compositions and concentrations based on hydrogen peroxide (3,4).

This substance is considered a strong oxidizing agent, with the lowest potential for deleterious effects and effective in the process of tooth whitening, so far (5). This process basically involves the decomposition of hydrogen peroxide releasing water and oxygen. This oxygen can cleave the double bonds of pigment molecules making them small enough to diffuse out of the tooth structure, or to absorb less light, and consequently obtain a lighter shade. This mechanism is known as oxide-reduction or simple oxidation (6).

However, despite being an effective procedure, tooth whitening can maintain residual oxygen in dental tissues interfering with the adhesion mechanism of resin restorations immediately after its application (7,8). Studies have shown that bleaching significantly reduced the bond strength of adhesives to enamel and dentin, suggesting incomplete infiltration of adhesives or interference with the polymerization process (7-12). Given the above, there are many studies that seek ways to reverse the reduction in bond strength after bleaching. Nevertheless, since the reduction has been shown to be temporary (13), adhesive procedures has been delaying after the last bleaching session for periods ranging from two to four weeks (14,8,15).

The use of antioxidants has been employed in in vitro studies to reverse the reduction in enamel bond strength, avoiding or reducing the waiting time required for composite resin restorations, such as in cases of closure of multiple diastems, after removal of the orthodontic appliance, or replacement of restorations in esthetic areas with compromised coloration. Some examples of these antioxidants already researched are green tea, sodium ascorbate (Vitamin C), cranberry extract, grape seed

extract (oligomeric proanthocyanidin complexes [OPCs]) and quercetin (flavonoid) (16,17,10,18,19).

According to a study (10), 10% sodium ascorbate after tooth whitening with carbamide peroxide was able to reverse the reduction in bond strength of whitened enamel caused by residual oxygen. However, when comparing 10% sodium ascorbate with 5% grape seed extract, the enamel bond strength produced by the second one, which is rich of flavonoids, was significantly higher than that resulting from the prior application of sodium ascorbate (19). Flavonoids are an extensive class of polyphenolic compounds widely distributed in photosynthesizing beings. They have thousands of varieties and can be found in fruits, vegetables, teas, and wines. According to their chemical structure, they are subdivided into subclasses, namely flavonols, flavones, flavanones, anthocyanidins, flavonoids, and isoflavones. This subdivision is determined by their molecular structural characteristic that takes into account the oxidation state of the central pyrrole ring (20,21).

Naringin, is also a flavonoid, belonging to the flavonone glycoside group, which can be found mainly in citrus fruits (22). Naringin has been shown to be effective in inhibiting demineralization, promoting remineralization of artificial root caries lesions (23), enhancing the mechanical properties of the demineralized dentin matrix (24), and more recently in improving bond strength and longevity in caries-affected dentin when applied prior to a universal adhesive (25).

Although there are already promising studies in the dental area using naringin, the evaluation for possible reversal bond strength reduction after bleaching has not been performed yet. Thus, this study aims to evaluate the effect of the application of naringin in different concentrations on the bond strength to enamel after tooth whitening using reduced time protocols. The null hypotheses were that: (1) There is no difference in naringin application compared to the CN group and (2) There is no difference in naringin application 5 and 10% compared to the ASC10 group regarding bond strength.

MATERIALS AND METHODS

Specimens preparation

Extracted bovine incisors were cleaned and stored in a 1% chloramine T 1% solution at 4°C for no more than 60 days (teeth were purchased from a cold store duly regulated by ANVISA, and it was not necessary to submit the project to the Research Ethics Committee). Teeth without cracks and without defects were selected and had their roots removed with diamond discs attached to the straight piece (2 mm below the cemento-enamel junction). The pulp chamber was cleaned and the pulp tissue was removed with endodontic files (Hedstrom files - Meillefer Dentsply) and the crowns were cleaned with Robinson brush, pumice stone and water. Then, 50 crowns (n=10) with buccal walls in similar thickness (3.5 mm) were selected. The sample calculation was performed considering α=0,05 and β=80% (result of 9 samples per group). Each crown was sectioned in a metallographic cutter (Isomet - Buehler), with a diamond cutting disk (Buehler), to obtain blocks of the central region of the tooth containing enamel and dentin with final measurements of 6 mm wide x 10 mm long x 2 mm thick. The dentin and enamel were planted with 600 grit silicon carbide sandpaper, and the final enamel thickness was standardized at 1 mm. The blocks were kept in humidity until the start of the procedures described below.

Shear bond strength (SBS)

After obtaining the blocks, 10 of them were randomly stored in humidity until the beginning of the adhesive procedure (CP). On the other blocks, a 35% hydrogen peroxide gel (Whitness HP - FGM) was applied on the enamel region for 15 min (3 applications) simulating an office bleaching session. After this period, the gel was removed with a water jet, and the blocks were randomly divided into 4 groups: application of 1 ml of distilled water for 1 min (CN), application of 1 ml of sodium ascorbate 10% for 1 min (ASC), application of 1 ml of naringin 5% for 1 min (NA5), and application of 1 ml of naringin 10% for 1 min (NA10). The antioxidant agents were applied to the entire enamel region. The naringin was diluted in distilled water and placed in the ultrasonic cleaner for 5 min to obtain a homogeneous solution. The antioxidant agent was removed with water jet, the blocks were dried with air jets and proceeded to the adhesive procedures. For the adhesive procedure, two circular regions of approximately 2.5 mm were delimited on the blocks, with the aid of adhesive tape (ground demarcation tape - 3M). In this delimited area, 35% phosphoric acid was applied for 30 seconds (Gluma Etch 35% - Kulzer), washed with a water jet for the same time, dried with an air jet and

applied two beds of a passive mode universal adhesive (Single Bond Universal - 3M) with the aid of a microbrush. The solvent was evaporated between the layers, following the adhesive manufacturer's instruction, and polymerized for 10 s (Elipar DeepCure - 3M). Then, rubber matrices with cylindrical holes of approximately 2 mm diameter were positioned in the delimited area and filled with fluid resin (Filtek Bulk Fill Flow - 3M, color A1), to build the cylinders for shearing. The blocks were then fixed in a universal testing machine (Ez-test - Shimadzu Corp.) and tested in shear until fracture, at a speed of 0.5 mm/min. The values in MPa were obtained from the average of the two cylinders in each block. The antioxidant solutions were evaluated for pH and all materials used are described in Table 1.

Table 1. Composition of the materials used, lot, shelf life and pH of the antioxidant solutions.

Material (manufacturer)	Composition	Batch - expiry	pH
Whitness HP 35% (FGM Dental Group, Joinville, BR)	Hydrogen peroxide, thickener, red colorant, glycol and inorganic filler.	300322 - 29/03/2024	-
Sodium ascorbate (ACS Científica - Orion Produtos e Serviços de Laboratório, Rio de Janeiro, BR)	Sodium L-Ascorbate PA	202111280 - 26/11/2027	10% solution = 2.670
Naringina (Merck KGaA, Darmstand, DE)	Naringina ≥ 95%	BCCG5679 - Not determined	5% solution = 7.587 10% solution = 6.872
Gluma® Etch 35 Gel (Kulzer South America Ltda, São Paulo, BR)	35% phosphoric acid, pigments, thickening agents, water	811120 - 11/2022	-
Single Bond Universal (3M, St. Paul, EUA)	2-Hydroxyethyl methacrylate, Bisphenol A diglycidyl ether dimethacrylate (Bis-GMA), propenoic 2-acid, 2-methyl-, reaction products with 1,10-decanediol and phosphorus oxide (P2O5), ethanol, water, silane treated silica, acrylic copolymer (continued)	2208000118 - 17/01/2024	-
Material (manufacturer)	**Composition**	**Batch - expiry**	**pH**
	(continued) and itaconic acid, camphorquinone, dimethylaminobenzoat (-4), 2-dimethylaminoethyl methacrylate, 2,6-Di-tert-butyl-p-cresol.		
Filtek™ Bulk Fill Flowable Restorative (3M, St. Paul, EUA)	Silanized ceramics, diurethane dimethacrylate, dimethacrylate substitute, ytterbium fluoride, BisGMA, Bisphenol A	NF00270 - 29/09/2024	-

	polyethylene glycol diether dimethacrylate, triethylene glycol dimethacrylate.		

Colorimetric evaluation

To evaluate whether the application of antioxidants interferes with the immediate bleaching result, the samples were evaluated using a digital spectrophotometer (Vita EasyShade). The samples were kept on a standardized white background for the readings. The L* (brightness), a* (green-red), b* (blue-yellow) and C* (chroma) values were noted after whitening and after antioxidant application. For the parameters L*, a*, b* and C* a numerical comparison was made after whitening and after the application of the antioxidant. In addition, the values of ΔE_{ab} and ΔE_{00} were calculated after the application of the antioxidant (KURY *et al*., 2020).

Failure pattern

The surfaces involved in the fracture of each specimen were analyzed under stereoscopic magnifier at 30x to determine fracture pattern. The blocks were evaluated by keeping the areas involved in the fractures facing upward. The classification of the fracture pattern was done according to the structures involved (70% or more): Type I: Cohesive failure in the composite resin; Type II: Adhesive failure between the composite resin and bonding agent; Type III: Adhesive failure between enamel and bonding agent; Type IV: mixed failure characterized by the presence of more than one type of failure.

Statistical analysis

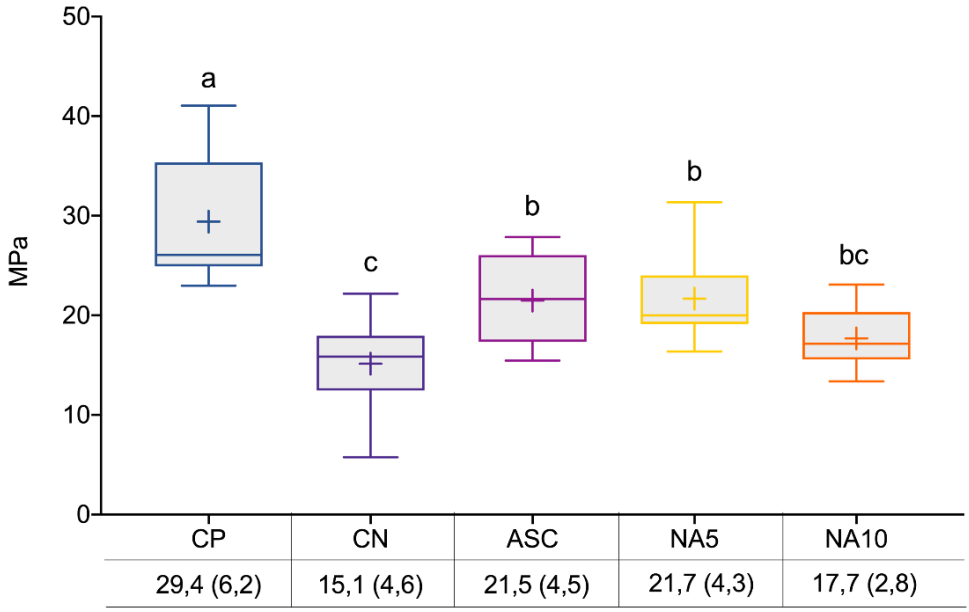
The data were evaluated for the assumptions for a parametric test using SigmaStat 3.5 software. Shear, ΔE_{ab} and ΔE_{00} data were analyzed by one-factor ANOVA and Tukey, while color parameters L*, a*, b* and C* were evaluated by T-test.

RESULTS

SBS

The results regarding the mean ± standard deviation (SD) bond strength can be observed in the graph of Figure 1. The SBS value was higher in the group without bleaching (29.4 ± 6.2) and lower in the group with bleaching and without application of antioxidant (15.1 ± 4.6). No group could reach values equal or similar to the CP group, which was statistically different from all groups. The ASC10 group was statistically different from the CP group, and statistically similar to the NA5 group, with SBS values 21.5±4.5 and 21.7±4.3, respectively. Both groups (ASC and NA5), despite being similar to each other, showed significant difference from the CN group. The NA10 group was statistically different from the CP groups, but similar to the CN group, with SBS values of 17.7±2.8.

Figure 1 - Box plot of shear strength (MPa) in each group



*Different lower case letters indicate a statistically significant difference ($p<0.05$).
Source: own authorship.

Color analysis

In the comparison between groups, at all-time intervals, no antioxidant at the tested concentrations was able to alter the parameters L*, a*, b* and C* (Figure 2). Furthermore, when comparing the color variation in the CIELAB system (ΔE_{ab}) and the

color variation in the CIEDE2000 system (ΔE_{00}) there were no statistically significant differences between the groups tested (Figure 3).

Figure 2 - Bar graph of the L*, a*, b* and C* parameters compared in each group

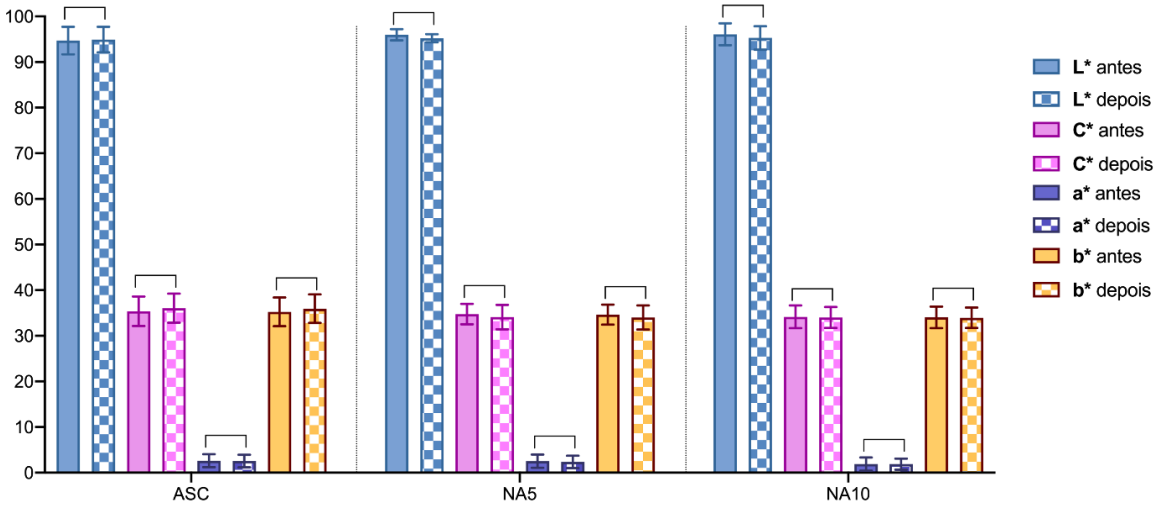
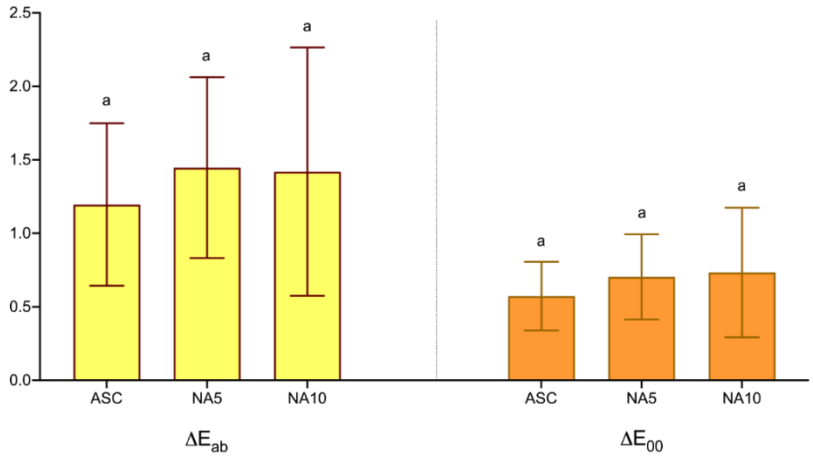


Figure 3 - Bar graph of the CIELAB system color variation comparison (ΔE_{ab}) and CIEDE2000 (ΔE_{00})



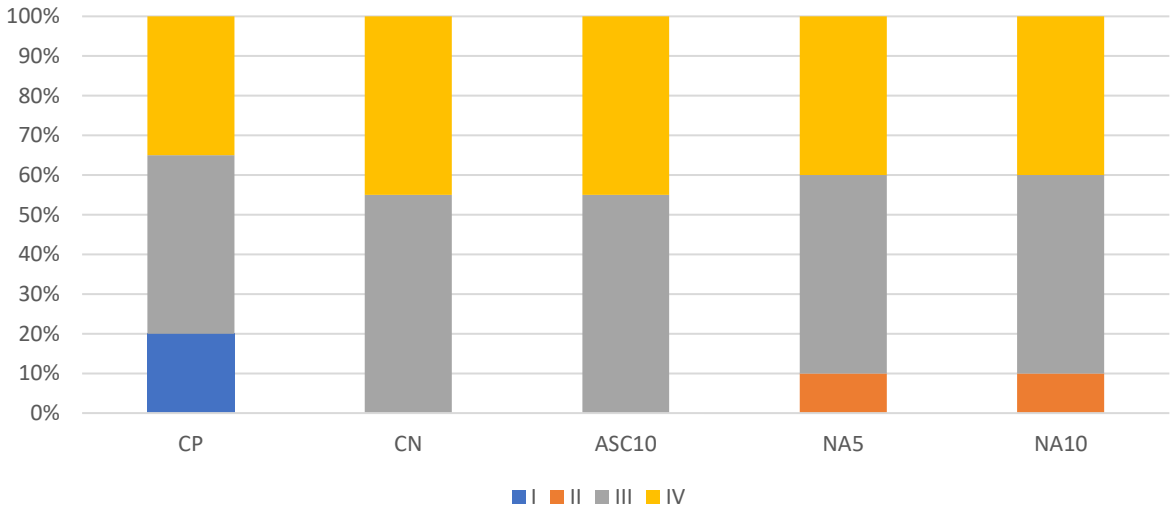
*Equal lower case letters indicate statistical similarity (p>0.05).
Source: own authorship.

Fracture pattern analysis

The percentage of each fracture pattern per group is shown in Figure 4. The CP group was the only one that showed the occurrence of type I fracture pattern. While

NA5 and NA10 groups showed the occurrence of type II fracture pattern. However, type III and type IV fracture patterns were more prevalent in all groups.

Figure 4 - Fracture pattern graph per group evaluated.



*Type I: Cohesive failure in the composite resin; Type II: Adhesive failure between the composite resin and the bonding agent; Type III: Adhesive failure between the enamel and the bonding agent; Type IV: Mixed failure characterized by the presence of more than one type of failure.
Source: own authorship.

Discussion

The first null hypothesis was partially confirmed: ASC and one concentration of naringin (NA5) showed significant difference from the CN group, despite NA10 was similar to CN group regarding bond strength. The second null hypothesis was also partially validated: NA5 was statistically different from the CP, and statistically similar to ASC10 group.

The reduction of bond strength between adhesive materials and dental enamel is one of the immediate outcomes caused by hydrogen peroxide (H2O2) (7-12). Shear bond strength (SBS) value can be useful to monitor this parameter (26). In the present study, 5% naringin obtained statistically similar results to 10% sodium ascorbate

(ASC), this is demonstrated by an increase in shear bond strength values when compared to the negative control (NC) group, in which no antioxidant was used before bonding. These findings are in agreement with the literature that highlights the antioxidant power of this bioactive compound and consequently demonstrates its potential to reverse the deleterious effects of tooth whitening by improving the micro shear bond strength in enamel due to its antioxidant and free radical scavenging properties (27,28). However, the application of naringin 5% as well as 10% ascorbate for only 1 min, was not enough to reverse the bond strength values compared to the no bleaching control (CP).

Moradian *et al*. (2022) evaluated the effects of 3 antioxidants, including the quercetin flavonoid on the SBS of whitened enamel and obtained promising results similar to this study. Quercetin, like naringin, is a flavonoid and among the groups treated with antioxidants, it obtained the highest SBS value, being significantly different from the positive control group (whitened with hydrogen peroxide at a concentration of 40% without the application of antioxidant). However, despite its ability to increase the bond strength to bleached enamel, just like sodium ascorbate, quercetin was not able to reverse it completely, not reaching SBS values similar to the positive control group (no bleaching) (29).

The present findings also showed that a higher concentration of naringin (NA10%) did not increase its efficacy, since naringin at a lower concentration (NA5%) resulted in a greater increase in SBS being statistically like the ASC10% values. This result agreed with the study by De Carvalho *et al*., (2016), who tested different concentrations of ASC and found that its antioxidant power and its ability to improve the values of bond strength to whitened enamel were inversely proportional to the increase in its concentration, with the 10% ASC applied for 60 min on whitened enamel having greater efficacy than the 20% and 30% concentrations. This compound is presented in powder form and for the viability of its application it is necessary its dilution in aqueous medium. In this research, distilled water was used. Like De Carvalho *et al*., (2016), the hypothesis for this result was that the higher the concentration of the antioxidant the higher the powder-liquid ratio. Thus, it is possible to speculate that in this increased powder-liquid ratio (NA5%-NA10%), the powder may have been deposited on the enamel surface, not being completely removed by conditioning and rinsing, interfering with the adhesion process.

Regarding sodium ascorbate 10% (ASC), it was used as a positive control because it is considered, by Moradian *et al*., (2022), as the gold standard. It is a bioavailable form of ascorbic acid (Vitamin C) and its use in food products is already well established due to its biocompatibility (31). There are numerous in vitro studies that used 10% sodium ascorbate, with different application times, as an attempt to restore the bond strength of dental enamel after bleaching with peroxides and obtained satisfactory results (30,10,32). This study employed for all groups the application time successfully used by a study (33) and the findings corroborate with those in the literature (34,30,35,33). The 10% sodium ascorbate (ASC) applied to enamel for 1 minute after 24 hours of the bleaching procedure, partially reversed the deleterious effects of 35% hydrogen peroxide and 16% carbamide peroxide, this was demonstrated by an increase in shear strength of the groups in which it was used, obtaining similar results to both the control group (no bleaching) and the group in which the restoration was performed after 14 days and no antioxidant was applied before the adhesive procedures (33).

Although, some studies have addressed some disadvantages about the use of this antioxidant, such as its low ph value (1.8), as a disadvantage for its clinical use (36). An in vitro study showed that the cumulative effect of the bleaching agent (10% carbamide peroxide) and sodium ascorbate resulted in increased porosity of the enamel surface, leading to greater adhrence of Streptococcus mutans (37). Therefore, our study evaluated naringin at concentrations of 5% and 10% as a viable alternative with a higher pH compared to sodium ascorbate.

Unlike sodium ascorbate, which when diluted has a transparent form, naringin when diluted has a beige and milky color, proportionally to its concentration. Thus, it was evaluated whether the application of the antioxidants tested could interfere with the immediate result of bleaching. Besides using the CIELAB system (□Eab) with its coordinates L*, a* and b* to detect possible color alterations and consequent alteration in the effectiveness of the tooth whitening, we also used the CIEDE2000 system (□E00), for its characteristic of greater compatibility with the perception and visual acceptance of color alterations, which its equation is adjusted by parameters such as light, hue and chroma (38). Positively, none of the antioxidants in the tested concentrations were able to interfere with the values of parameters and color variations obtained immediately after tooth whitening.

Corroborating the results described in the literature, the CP group (without tooth whitening), obtained high SBS values compared to the other groups, confirming the premise that whitening significantly reduces the bond strength of adhesive materials on enamel. This fact, which is also confirmed by the reduced SBS values in the CN group (whitened without antioxidant), which can also be attributed to incomplete infiltration of adhesives or interference in the polymerization process due to residual oxygen inherited from whitening (7-12).

The fracture pattern results demonstrate a high percentage of type III and type IV pattern for all groups. However, the CP group showed the type I pattern (in a lower percentage), which may indicate that the bond strength was higher than the cohesive strength of the resin (39). In addition, NA5 and NA10 showed type II pattern (in lower percentage), which may indicate some interference of residual naringin in the enamel on the polymerization of the adhesive. Although the results are promising to regard NA5, future studies are still needed to determine the best concentration and application time of naringin that promotes an effective antioxidative capacity, without affecting the enamel properties and without altering the polymerization reaction of resin materials.

Conclusion

Under the limitations or restrictions of this in vitro study, it could be concluded from the results obtained that:

1. None of the antioxidants tested were able to promote bond strength similar to the control group without bleaching;
2. The ASC and NA5 groups obtained better results than the whitened group without the use of antioxidants;
3. NA5 obtained similar results to ASC;
4. NA10 did not show effective values regarding the increase of the bond strength;
5. No antioxidant in the concentrations tested was able to alter the parameters L*, a*, b* and C*. The color variation after the application of antioxidants in relation to the color after bleaching was also similar for all groups tested (ΔE_{ab} e ΔE_{00}).

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