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**AVALIAÇÃO DO POTENCIAL OSTEOGÊNICO DO EXTRATO DE *CRANBERRY*:
ESTUDO EM VITRO**

Florianópolis
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ESTUDO EM VITRO**

Dissertação submetida ao Programa de Pós-Graduação em Odontologia da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Mestra em Odontologia área de concentração Implantodontia.

Orientadora: Profa. Dra. Ariadne Cristiane Cabral da Cruz.

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YASMIM GUTERRES BAUER

AVALIAÇÃO DO POTENCIAL OSTEOGÊNICO DO EXTRATO DE *CRANBERRY*

O presente trabalho em nível de Mestrado foi avaliado e aprovado, em 29 de agosto de 2023, pela banca examinadora composta pelos seguintes membros:

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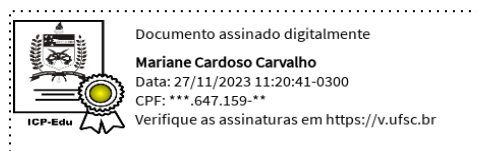
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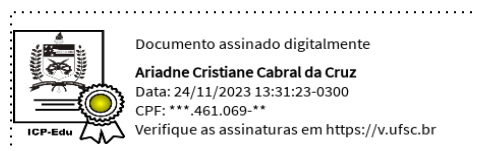
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Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de Mestra em Odontologia área de concentração Implantodontia.



Coordenação do Programa de Pós-Graduação



Profa. Dra. Ariadne Cristiane Cabral da Cruz
Orientadora

Florianópolis, 2023.

Dedico este trabalho aos meus pais,
com todo amor e gratidão.

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“I believe [declared Marie Curie] that Science has great beauty. A scientist in his laboratory is not a mere technician: he is also a child confronting natural phenomena that impress him as though they were fairy tales.” (Giroud 1986, p. 270)

RESUMO

Considerando os desafios em regeneração óssea e as variadas propriedades dos componentes do *Cranberry*, esse trabalho investigou o potencial osteogênico do extrato de *Cranberry* in vitro. Primeiramente realizamos uma revisão de literatura utilizando os descritores “*Cranberry*” e “Odontologia” nas bases de dados Pubmed, Scopus e Web of Science. Posteriormente, foi realizado um trabalho in vitro. Foi realizada a caracterização do extrato de *Cranberry*. Para tanto, foi realizada a determinação do teor de fenólicos totais usando as metodologias de Folin-Ciocalteu e Fast Blue BB. A determinação do teor de antocianinas monoméricas totais no extrato de *Cranberry* foi realizada pelo método de pH diferencial com 520 e 700 nm. Adicionalmente, a cromatografia líquida acoplada a espectrometria de massa foi utilizada para determinar os constituintes do extrato de *Cranberry*. A citotoxicidade do extrato de *Cranberry* foi avaliada pelo brometo de azul de tiazolil tetrazólio (MTT). O potencial osteogênico do extrato de *Cranberry* foi avaliado através da atividade da fosfatase alcalina (ALP), expressão da proteína óssea morfogenética-2 (BMP-2) e mineralização da matriz extracelular. Os resultados experimentais revelaram que o teor de fenólicos totais no extrato de *Cranberry* foi de $522,72 \pm 9,80$ mg GAE/ge e $364,95 \pm 12,49$ mg GAE/g pelo método Fast Blue BB e Folin-Ciocalteu, respectivamente. O extrato de *Cranberry* apresentou 140 vezes mais conteúdo de antocianina monomérica em relação ao mínimo para indicar sua autenticidade. Concentrações elevadas de extrato de *Cranberry* (62,5 mg/mL a 500 mg/mL) foram biocompatíveis para osteoblastos e células-tronco mesenquimais. Em relação à osteogênese, 20 mg/mL de extrato de *Cranberry* promoveu 2 vezes mais atividade de ALP e aumento de aproximadamente 1,5 vezes na expressão de BMP-2 do que o controle positivo. Além disso, foi observada uma correlação entre esses marcadores de osteogênese e a concentração de extrato de *Cranberry*. Por outro lado, o extrato de *Cranberry* não foi capaz de aumentar a mineralização da matriz extracelular. Concluímos que o extrato de *Cranberry* estimulou os marcadores precoces da osteogênese. No entanto, esse efeito não foi demonstrado para o marcador de osteogênese tardia. O potencial do extrato de *Cranberry* de estimular a osteogênese e a biocompatibilidade de altas concentrações do mesmo são resultados promissores para futuras aplicações do *Cranberry* incorporado em arcabouços para a regeneração óssea.

Palavras-chave: *Cranberry*; Osteogênese; Osteoblastos;

ABSTRACT

Considering the challenges in bone regeneration and the properties of Cranberry components, this study aimed to investigate the potential of Cranberry extract to stimulate the osteogenesis in vitro. First, we performed a narrative review using the descriptors "Cranberry" and "Dentistry" in Pubmed, Scopus, and Web of Science databases. Concerning the in vitro study, we determined the total phenolic and total monomeric anthocyanin contents in the Cranberry extract. Liquid chromatography coupled with mass spectrometry was used to determine the Cranberry extract constituents. The cytotoxicity of Cranberry extract was evaluated by thiazolyl blue tetrazolium bromide. Concerning the osteogenesis potential of Cranberry extract, we evaluated alkaline phosphatase (ALP) activity, bone morphogenetic protein type 2 (BMP-2) expression, and extracellular matrix mineralization. We observed a high quality of Cranberry extract based on total phenolic and anthocyanin results. Specifically, 522.72 ± 9.80 mg GAE/g and 364.95 ± 12.49 mg GAE/g of total phenolic were detected by Fast Blue BB and Folin-Ciocalteu method, respectively. Regarding the monomeric anthocyanin, the observed content was 140-fold more than the minimum to indicate Cranberry extract authenticity. Additionally, we demonstrated elevated concentrations of Cranberry extract (62.5 mg/mL to 500 mg/mL) were biocompatible for osteoblasts and mesenchymal cells. Regarding osteogenesis, 20 mg/mL Cranberry extract promoted 2-fold more ALP activity and almost 1.5-fold more BMP-2 than the positive control group. Also, a correlation was observed between the Cranberry concentration and the osteogenesis markers. Conversely, Cranberry extract was not able to increase the extracellular matrix mineralization. In summary, Cranberry extract demonstrated potential to stimulate early markers of osteogenesis. However, this effect was not demonstrated for late marker. The Cranberry extract potential to stimulate osteogenesis and the biocompatibility of its elevated concentrations are promising for future application into scaffolds for bone regeneration.

Keywords: Cranberry; Osteogenesis; Osteoblast.

LISTA DE ABREVIATURAS E SIGLAS

ALP	Fosfatase alcalina
BMP	Proteína morfogenética óssea
BMP2	Proteína morfogenética do tipo 2
DMEM	do inglês, <i>Dulbecco's Modified Eagle Medium</i>
DMSO	Dimetilsulfóxido
EAGg-1 ES	Ácido gálico por grama de extrato seco
ESI	Ionização por electrospray
FBS	Soro fetal bovino
FBBB	Fast Blue BB
FC-B	Folin-Ciocalteu
JNK	do inglês, <i>c-Jun N-terminal kinase</i>
LC/MS	Cromatografia líquida acoplada a uma espectrometria de massa
M	Molar
MAPK	Proteínas quinases ativadas por mitogênio
MC3T3-E1	Osteoblasto murino subclone 4
mg	Miligramas
mL	Mililitros
mM	Milimolar
MMP-9	Metaloproteinase 9
MTT	3- (4,5-dimetiltiazol-2-il) -2,5-brometo de difeniltetrazólio
NDM	Material liofilizado de alto peso molecular não dialisável
NFATc1	Fator nuclear de células T ativadas c1
NF-kB	Fator nuclear kappa B
Nm	Nanometros
OSX	Osterix
PDA	Fotodiodo
Runx2	Fator de transcrição 2
SHED	Células-tronco derivadas de dentes decíduos esfoliados humanos
SPARC	Proteína secretada por ácido rica em cisteína
p-NP	Para-Nitro fenol
pNPP	Para-Nitro fenol fosfato
µg	Microgramas

μL	Microlitros
μM	Micromolar

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1 INTRODUÇÃO

A atrofia moderada ou grave dos maxilares, decorrente da perda dentária, trauma ou ressecção cirúrgica, muitas vezes requer aumento ósseo vertical para permitir a colocação de implantes dentários e, posteriormente, realização da reabilitação protética por meio de próteses implantossuportadas (BEN LAGHA; HOWELL; GRENIER, 2020; ESPOSITO et al., 2010). No entanto, os procedimentos de aumento ósseo vertical apresentam desafios biológicos e técnicos. Atualmente, as técnicas cirúrgicas exibem várias limitações, incluindo morbidade associada ao sítio doador, limitada quantidade de tecido ósseo doador disponível e taxas de reabsorção inadequadas, levando ao comprometimento da estabilidade dimensional (ELGALI et al., 2017; ELNAYEF et al., 2017; RETZEPI; DONOS, 2010).

No que tange as considerações biológicas, a formação óssea envolve um processo complexo dependente da osteogênese e osteoclastogênese. Além disso, o aumento ósseo vertical na cavidade oral traz um fator adicional, tendo em vista que a cavidade oral é um ambiente contaminado. Assim, seria interessante um substituto ósseo com propriedades antimicrobianas e anti-inflamatórias, além da capacidade de promover a formação óssea (ELGALI et al., 2017; RETZEPI; DONOS, 2010). Tais propriedades poderiam vir diretamente da composição do biomaterial a ser empregado como substituto ósseo ou de substâncias incorporadas em sua composição.

O *cranberry* um fruto da espécie *Vaccinium macrocarpon* pertencente à família *Ericaceae* conhecido pelos seus inúmeros benefícios à saúde. Os componentes do *cranberry* têm atraído cada vez mais atenção na Odontologia, devido à sua capacidade antibacteriana contra patógenos cariogênicos, micóticos, endodônticos e periodontais (KRANZ et al., 2020; KUMAR et al., 2019), bem como suas propriedades anti-inflamatórias (BODET; CHANDAD; GRENIER, 2006; PELLERIN; BAZINET; GRENIER, 2021). O *cranberry* é amplamente consumido de diversas formas, como frutas frescas e secas, sucos e pós encapsulados. Ele é composto por 80% de água, 10% de carboidratos e os outros 10% são distribuídos entre flavonoides, antocianinas, catequinas, proantocianinas, triterpenóides, ácidos orgânicos e uma pequena quantidade de ácido ascórbico.

As proantocianinas são compostos polifenólicos que estão em material não dialisável (NDM) de alto peso molecular extraído do *cranberry* (NETO et al., 2017). Notavelmente, as proantocianidinas constituem os principais metabólitos ativos do *cranberry*, compostos principalmente de subunidades de epicatequina apresentando pelo menos uma ligação tipo A. Entre os atributos relevantes das proantocianidinas estão suas propriedades antiadesivas e antibacterianas, juntamente com sua capacidade de inibir a atividade da collagenase e proteinase (FELDMAN et al., 2012; LA; HOWELL; GRENIER, 2009, 2010; TIPTON; CARTER; DABBOUS, 2014). Além disso, observou-se que o NDM derivado do suco de *cranberry* inibe o fator nuclear kappa B e a metaloproteinase de matriz 3. Essa influência do NDM afeta a funcionalidade dos fibroblastos na resposta inflamatória.

As proantocianidinas do tipo A parecem interferir na maturação e fisiologia das células osteoclásticas, bem como a própria matriz óssea (TANABE et al., 2011), inibindo a formação funcional dos osteoclastos através da via NF-kB e das vias de sinalização da proteína quinase ativada por mitogênio (MAPKs) (ZHU et al., 2019). Além disso, compostos polifenólicos como os flavonoides têm sido explorados por seu potencial na preservação óssea. Esses compostos polifenólicos parecem regular as vias de sinalização celular que influenciam a diferenciação de osteoblastos e osteoclastos, promovendo a formação óssea e suprimindo a reabsorção óssea (AN et al., 2016; INCHINGOLO et al., 2022; RAMESH et al., 2021; WEAVER et al., 2012). Como faltam pesquisas sobre o impacto do *cranberry* na osteogênese, este estudo busca investigar o potencial do extrato de *cranberry* para estimular a osteogênese *in vitro*.

Outro ponto importante a ser destacado é que o *Cranberry* e seus compostos isolados não têm apresentado citotoxicidade e podem ser utilizados com segurança. Considerando os atuais desafios na regeneração óssea e as variadas propriedades dos componentes do *cranberry*, um fruto de fácil acesso e baixo custo, este trabalho se propõe a investigar o potencial osteogênico do extrato de *cranberry in vitro*. Para tanto, este trabalho se dividiu em uma revisão de literatura e experimentos *in vitro*.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o potencial osteogênico do extrato de *cranberry in vitro*.

2.2 OBJETIVOS ESPECÍFICOS

- Realizar uma revisão de literatura resumindo as aplicações e potencialidades do uso do *cranberry* nas diferentes especialidades da Odontologia.
- Caracterizar o extrato de *cranberry*, de modo a garantir a qualidade do extrato que seria empregado nos experimentos *in vitro*.
- Avaliar a biocompatibilidade de elevadas concentrações do extrato de *cranberry* sobre osteoblastos (MC3T3-E1) e células-tronco de dentes decíduos esfoliados humanos (SHED).
- Analisar o potencial do extrato de *cranberry* em estimular a diferenciação osteogênica através dos ensaios de atividade de fosfatase alcalina (ALP), expressão da proteína óssea morfogenética do tipo 2 (BMP-2) e mineralização da matriz extracelular por coloração com vermelho de alizarina.

3ARTIGO 1 – EXPLORING THE BENEFITS OF CRANBERRY IN DENTISTRY: A COMPREHENSIVE REVIEW

Exploring the benefits of Cranberry in dentistry: A comprehensive review

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ABSTRACT

Objectives: Despite the increasing scientific evidence regarding the application of Cranberries in Dentistry, there remains a need for a comprehensive elucidation of their potential benefits, constituents, and underlying mechanisms of action. Consequently, this review aims to meticulously analyze and consolidate existing scientific literature on the utilization of Cranberries for the prevention and treatment of oral diseases. **Materials and Methods:** Electronic databases (Pubmed, Scopus, and Web of Science) were searched until May 2023. This review included *in vitro*, *in vivo*, and clinical research studies. A two-phase selection process was carried out. In phase 1, two reviewers independently conducted title and abstract reading to identify potentially eligible studies. The same reviewers performed the full-text reading of eligible articles in phase 2. **Results:** Of the 72 eligible articles reviewed, most assessed the use of Cranberry in Periodontics (n=24) and Cariology (n=23). Indeed, biofilm and microorganism virulence factors (n=40) were the most studied subject. Also, Cranberry extract (n=22) and high-molecular-weight constituents of Cranberry (NDM) (n=22) were the most evaluated Cranberry fractions. Concerning cariogenesis, overall, Cranberry presented itself as a non-toxic and viable chemotherapy option against dental caries and its causative microorganisms. Regarding periodontal and peri-implantar diseases, Cranberry demonstrated host immune modulator capacity

counteracting the pathogenesis and destructive mechanisms associated with these diseases. Cranberry also demonstrated several benefits related to the inflammation concerning the periodontal disease and temporal mandibular joint lesions. Regarding dental erosion, Cranberry had the capacity to inhibited dentin erosion; however, this capacity was not demonstrated in enamel lesion. As an antioxidant agent, Cranberry showed effectiveness in preventing dental erosion. Additionally, Cranberry was able to neutralize ions immediately after dental bleaching, leading to an enhancement in bond strength. Also, Cranberry extract demonstrates the ability to counteract the oxygen ions formed on the tooth surface immediately after bleaching procedures. Concerning osteoclastogenesis, only one study was found and demonstrated that A-type proanthocyanidins can stop bone resorption. **Conclusions:** Cranberry's bioactive compounds, either in isolation or synergistically, hold substantial potential for diverse applications within Dentistry.

Clinical Relevance: This review provides insights into the plausible incorporation of Cranberry within contemporary Dentistry, offering readers an informed perspective on its potential role.

Keywords: Cranberry, dentistry, review, health benefits, bioactive compounds

INTRODUCTION

Cranberries have garnered widespread recognition for their considerable potential in enhancing human health [1]. *Vaccinium macrocarpon*, *Vaccinium oxycoccus*, and *Vaccinium microcarpum* are species of Cranberries, with *Vaccinium macrocarpon* being the most extensively studied specie in relation to its health benefits[2–4]. The therapeutic benefits of these fruits in human health have been documented since the 17th century, particularly in alleviating blood disorders, stomach ailments, scurvy, liver problems, and even cancer [1, 5]. Notably, Cranberries are predominantly acknowledged for their ability to prevent urinary tract infections [6]. The broad spectrum of positive impacts of Cranberries on human well-being can be attributed to the unique polyphenol composition found in these fruits, which has been acknowledged for their anti-bacterial, anti-adhesion, anti-tumorigenic, anti-inflammatory, and antioxidant properties [1, 7, 8].

Additionally, the phenolic compounds of Cranberries are primarily composed of phenolic acids, flavonoids (anthocyanins and flavonols), and tannins. Furthermore, flavonoids are the primary compounds identified among a repertoire of over 150 constituents classified into subgroups encompassing anthocyanins, flavonols, and proanthocyanidins[9]. As a general trend, Cranberries contains an average of 13 anthocyanins, 16 flavonols, and 26 phenolic acids and benzoates [7]. In Cranberry cultivars, forty-eight polyphenols (comprising 19 flavonols, 8 anthocyanins, 7 phenolic acids, and 14 flavan-3-ol oligomers) have been identified. The prevalence of polyphenol classes within Cranberry fruits follows the sequence: flavan-3-ols (41.5–52.2%), flavonols (18.6–30.5%), anthocyanins (8.0–24.4%), and phenolic acids (5.0–12.1%) [10, 11].

Regarding oral health, Cranberry extracts have also demonstrated several benefits, encompassing the modulating of the inflammatory response to periodontopathogens, inhibition of biofilm formation and acid production by cariogenic bacteria, and deactivation of bacteria-related proteolytic enzymes [12]. It is important to mention that dental caries, periodontal diseases, endodontic disorders, and candidal colonization represent the most prevalent oral infectious diseases[5]. Despite the increasing scientific evidence regarding the application

of Cranberries in Dentistry, there remains a need for a comprehensive elucidation of their potential benefits, constituents, and underlying mechanisms of action. Consequently, this review aims to meticulously analyze and consolidate existing scientific literature on the utilization of Cranberries for the prevention and treatment of oral diseases.

METHODS

Search Strategy

For this review, detailed research was performed in the following electronic databases: Pubmed, Scopus, and Web of Science, up to May 2023. The MeSH terms and their combinations used as fundamental concepts in the search strategy were: “cranberry” AND “dentistry”. Studies were considered eligible when Cranberry and its fractions were applied in Dentistry. Only articles published in English were deemed acceptable. This review included *in vitro*, *in vivo*, and clinical research studies. No limitations were set regarding the publication date.

Study Selection

A two-phase selection process was carried out using online software (Rayyan, Qatar Computing Research Institute, Qatar). In phase 1, two reviewers (Y.G.B. and E.B.M.) independently conducted title and abstract reading to identify potentially eligible studies. The same reviewers performed the full-text reading of eligible articles in phase 2. Any discrepancies between reviewers were resolved by discussion and consensus.

Data Extraction

The following data regarding included studies were recorded: author information, publication year, study design, Dentistry field, Cranberry properties, objective, applied tests, and outcome measures.

RESULTS

Study Selection

In phase 1, a total of 249 references were electronically retrieved from the following databases: PubMed (75), Scopus (14), and Web of Science (160). No additional references were manually identified. After removing duplicates, 188 articles remained. Subsequently, titles and abstracts were analyzed, 111 articles were excluded based on inter-reviewer agreement, leaving 77 articles for full-text analysis in phase 2. After thorough full-text reading, 5 references were excluded due to not meeting the inclusion criteria, resulting in a total of 72 articles eligible for further analysis. Refer to Figure 1 for a flowchart illustrating the process of study identification, inclusion, and exclusion.

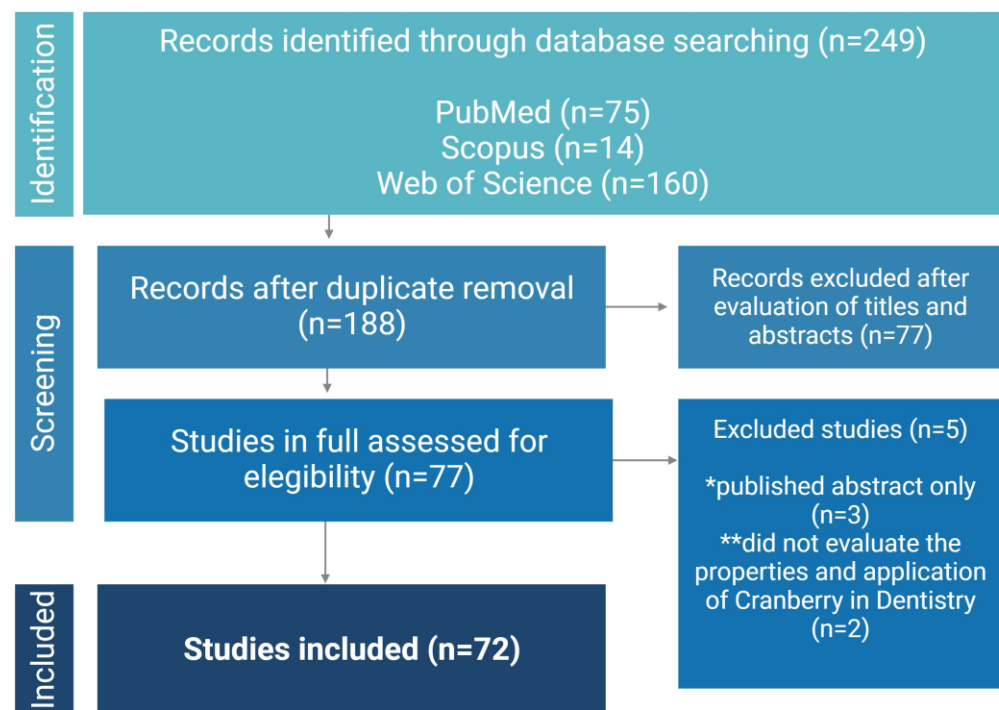


Figure 1. Flowchart of the literature search. References were selected through a two-phase process. Electronic databases (PubMed, Scopus, and Web of Science) were searched up to May 2023.

Study Characteristics

The included studies were published from 1998 up to 2022. Additionally, as despicable in Figure 2, articles assessed the use of Cranberry in Periodontics (n=24), Cariology (n=23), Direct and indirect restoration (n=9), Oral pathology and oral medicine (n=6), Cariology, Periodontics, and Endodontics (n=4),

Periodontics and Cariology (n=2), Endodontics (n=1), Periodontics and Implant dentistry (n=1), Periodontics, Implant dentistry, Direct and indirect restoration (n=1), and Implant dentistry (n=1). Moreover, most of the studies evaluated biofilm and bacterial virulence factors (n=40), followed by inflammation (n=14), alone or associated with biofilm formation (n=4). Dental erosion and antioxidant effect (n=7) were also studied, as well as inhibition of cancerous cell lines and opportunist microorganism (n=5), and osteogenesis and osteoclastogenesis (n=2), according to Figure 3.

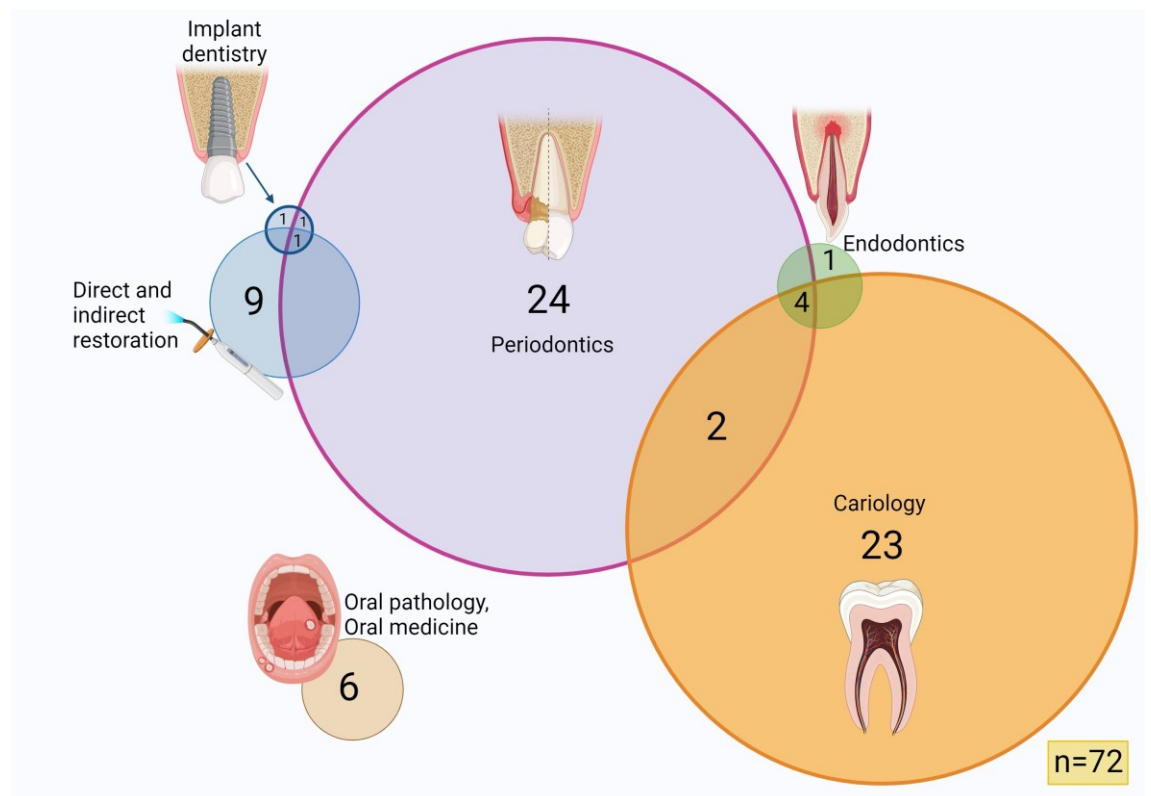


Figure 2. Venn diagram representing the included articles by area of expertise in dentistry. The white intersecting spaces represent empty sets, indicating that no articles are contained within those space.

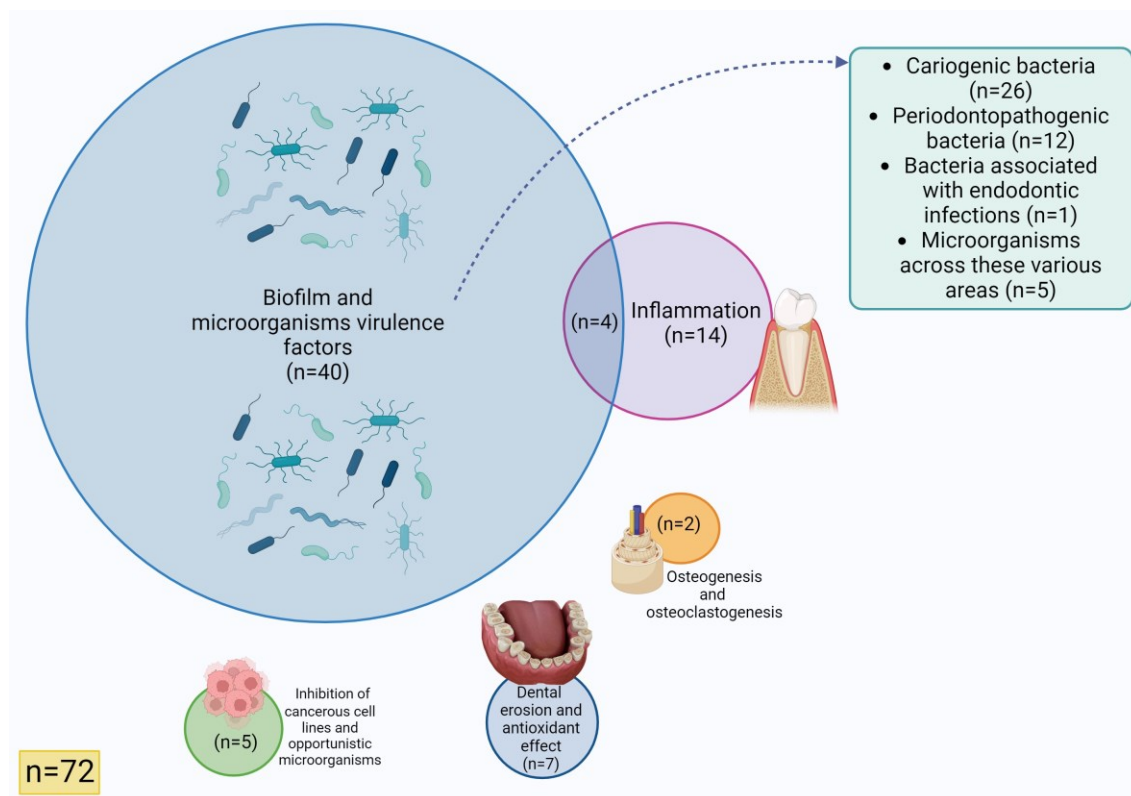


Figure 3: Venn diagram representing the included articles by research field. The white intersecting spaces represent empty sets, indicating that no articles are contained within those spaces.

As represented in Figure 4, Cranberry extract (n=22) and high-molecular-weight constituents of Cranberry (NDM) (n=22) were the most studied Cranberry fractions. Cranberry juice (n=9), A-type Cranberry proanthocyanidins (n=9), proanthocyanidin Cranberry fraction (n=3), polyphenol (n=2), Cranberry anthocyanin (n=1), Cranberry extract and cranberry juice (n=1), proanthocyanidin and flavonol (n=1), phenolic acids, flavonols, and proanthocyanidins (n=1), anthocyanins, flavonols and proanthocyanidins (n=1) were also evaluated. It is important to mention that most of the studies were performed *in vitro* (n=63), followed by clinical trials (n=7), and *in vivo* (n=2). The characteristics of the selected studies are shown in Table 1.

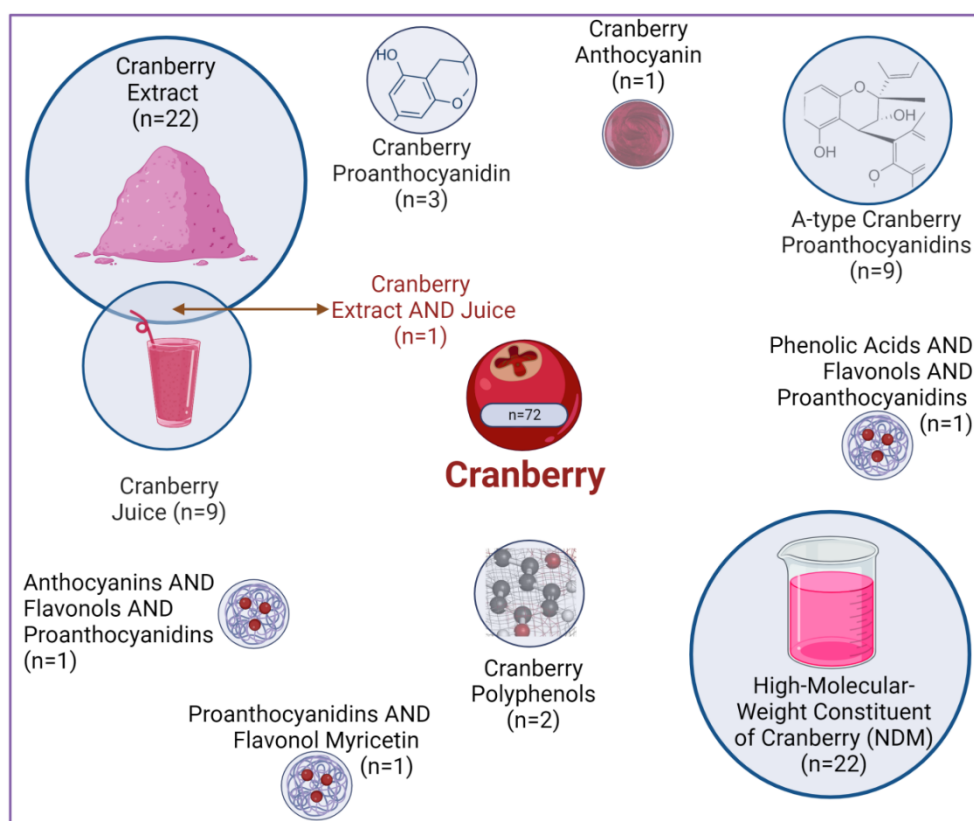


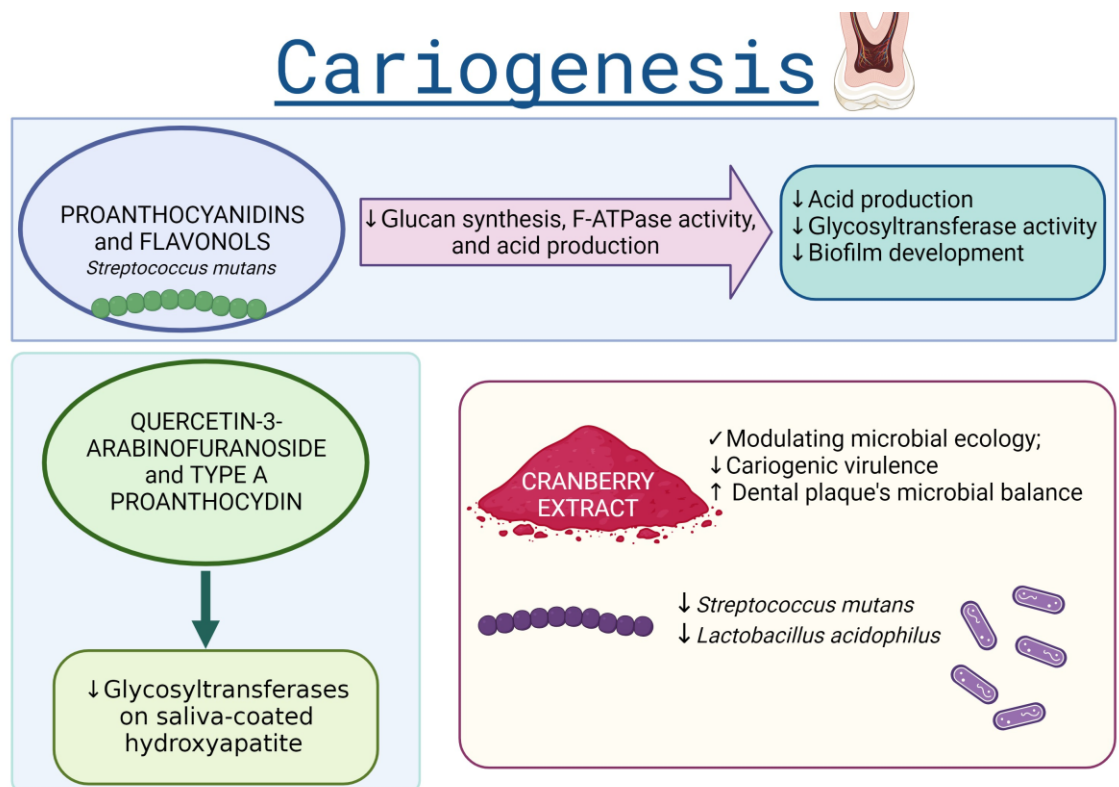
Figure 4: Different Cranberry fractions evaluated in the included articles.

Biofilm and microorganism virulence factors

Among the 72 studies included, 44 focused on biofilm and microorganism virulence factors, which of them, 12 examined periodontopathogenic bacteria [7, 11, 13–22]; 26 explored cariogenic bacteria [23–48]; 1 delved into bacteria associated with endodontic infections [49]; and 5 investigated a wide range of microorganisms across these various areas [12, 50–53]. It is worth to mention that 4 studies also focused on assessing Cranberry's anti-inflammatory properties as their primary objective, and consequently, they were revisited in the 'Gingival Inflammation' section [18, 19, 21, 48]. Concerning cariogenesis, various studies emphasize the effectiveness of proanthocyanidins and flavonols in combating the virulence factors of *Streptococcus mutans* [30, 32, 35, 40]. These compounds inhibited acid production, glycosyltransferase activity, and biofilm development on tooth surfaces. These beneficial effects seem to be related to inhibition of glucan synthesis, F-ATPase activity, and acid production [27, 30, 35, 40, 54]. Specific fractions from Cranberry extracts, such as quercetin-3-arabinofuranoside and type A proanthocyanidin, exhibited potent inhibition of

glycosyltransferases on saliva-coated hydroxyapatite [25, 52, 55]. Cranberry's capacity to hinder the adhesion of cariogenic bacteria to hydroxyapatite, reduce biofilm development, and mitigate cariogenic acid production is underlined [35, 42, 56].

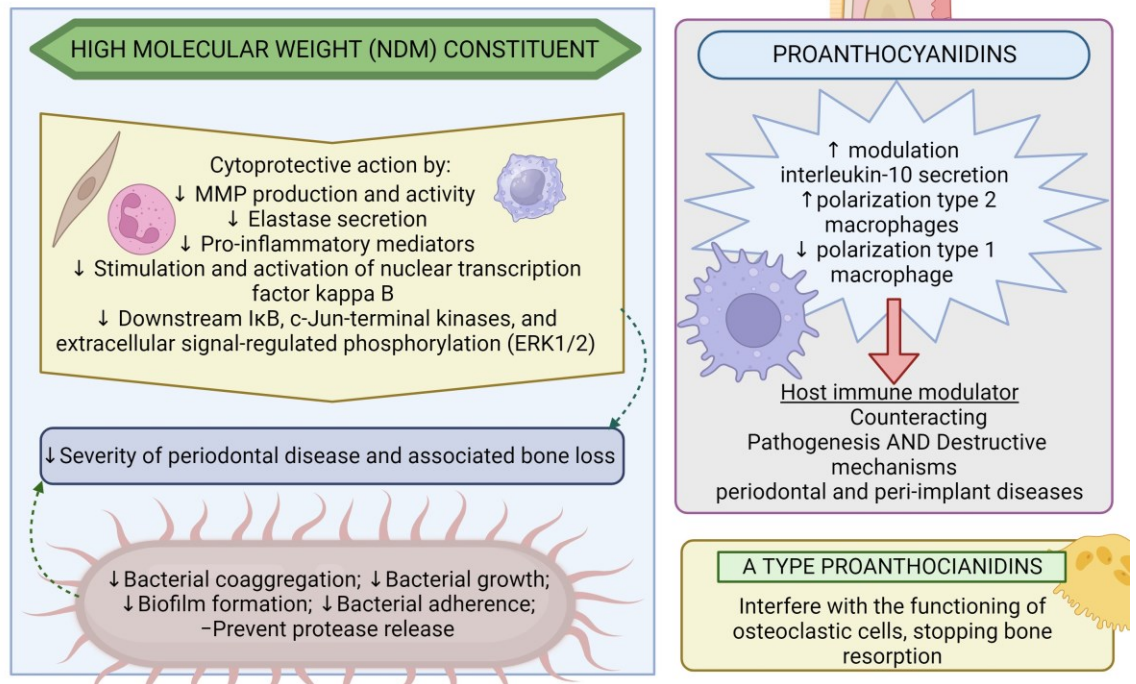
Cranberry extracts not only inhibited *Streptococcus mutans* but also *Lactobacillus acidophilus*, suggesting broader effects on the oral microbiota [43]. Also, studies highlight Cranberry's potential in mouthwash formulations, showing decreased *Streptococcus mutans* and total bacteria counts in saliva after its use; meaning comparable antibacterial efficacy to chlorhexidine [36]. In pediatric dentistry, Cranberry-based mouthwashes exhibited promising results, reducing *Streptococcus mutans* counts in plaque and saliva without adverse effects [34, 46]. Moreover, Cranberry extracts have been integrated into dentifrices, effectively modulating microbial ecology, suppressing cariogenic virulence, and improving dental plaque's microbial balance [45]. Overall, these findings endorse Cranberry's potential to offer natural and effective alternatives for oral health, presenting a non-toxic and viable chemotherapy option against dental caries and its causative organisms.



Regarding periodontitis, Cranberry's high molecular weight (NDM) constituent demonstrated effect in various periodontal pathogenic bacteria due to Cranberry's ability to inhibit bacterial coaggregation [13], adherence, growth, and biofilm formation [14], as well as to prevent protease release [15]. Additionally, clinically, Cranberry was able to mitigate the severity of periodontal disease and associated bone loss due to its capacity for diminishing bacterial adhesion and coaggregation [11]. Also, when four plant-based mouthwashes were evaluated on patients undergoing treatment with fixed orthodontic appliances, Cranberry extract mouthwash promoted a reduction in plaque index and gingivitis compared to placebo (pure distilled water)[57].

Another explored application involves the use of type A proanthocyanidins, recognized for their adhesive inhibitory characteristics, to combat *Candida albicans* [58]. Cranberry has shown the ability to effectively disrupt newly formed biofilms by *Candida albicans*, potentially impacting its biological functions without compromising the viability of the microorganism. This disruption is believed to be mediated by Cranberry's ability to inhibit the activation of nuclear factor B p65, which influences the virulence properties of *C. albicans* and attenuates inflammation. Another article focused on the incorporation of Cranberry proanthocyanidins and flavonoids into self-curing polymethylmethacrylate resin, used in the production of prostheses [2]. The results exhibited a notable reduction in the number of colony-forming units of *C. albicans* when cranberry was incorporated into the acrylic resin.

Periodontics



Inflammation

Diverse articles (n=18) have also investigated the capacity of Cranberry in inflammatory processes [18, 21, 48, 57–59]. Four out of these 18 studies are also included in the “Biofilm and Bacterial Virulence Factors” section because they explore both topics as their main objectives [18, 19, 48, 60].

Regarding NDM, it was demonstrated the protective action of this Cranberry compound on macrophages stimulated by lipopolysaccharides (LPS) of periodontopathogens [59]; the inhibition of matrix metalloproteinase (MMP) production and activity in LPS-stimulated fibroblasts and macrophages [61–63]; the ability to suppress elastase secretion by neutrophils [64]; and inhibition of the production of pro-inflammatory mediators (interleukin-6, interleukin-8, and prostaglandin E2) by gingival fibroblasts [65, 66] and epithelial cells [10]; the inhibition of stimulation and activation of nuclear transcription factor kappa B [67]; and, finally, inhibition downstream IκB, c-Jun-terminal kinases, and extracellular signal-regulated phosphorylation kinases 1 and 2 (ERK1/2) in Smulow-Glickman cells [68]. Concerning proanthocyanidins, it was observed positive modulation in interleukin-10 secretion and the polarization of type 2 macrophages, along with a reduction in type 1 macrophage polarization [69]. These findings suggest the

potential of Cranberry as a host immune modulator in counteracting the pathogenesis and destructive mechanisms associated with periodontal and peri-implant diseases.

Moreover, Regarding Temporal Mandibular Joint lesions, NDM effectively inhibited interleukin 1B. Furthermore, this inhibition displayed regulatory effects on several other pro-inflammatory mediators, such as IL-6, IL-8, vascular endothelial growth factor, kappa B nuclear transcription factor, and activator protein 1. As a result, NDM holds potential as a natural therapeutic substance that can be locally administered to prevent and treat inflammatory arthropathies of the temporomandibular joint [70].

Dental erosion and antioxidant effect

To a lesser extent, 7 studies discussed the role of Cranberry as an antioxidant agent and its effectiveness in preventing dental erosion [4, 71–76]. The main findings indicate that Cranberry had the capacity to assist in inhibiting dentin erosion [4, 71, 72]; however, this capacity was not observed in enamel erosion [73, 76]. Additionally, *in vitro* studies had revealed that Cranberry have neutralized ions immediately after dental bleaching, leading to an enhancement in bond strength [74, 75]. Also, Cranberry extract demonstrates the ability to counteract the oxygen ions formed on the tooth surface immediately after bleaching procedures [74, 75]. These ions typically hinder the polymerization and adhesive strength of composite resins and resin cements. This effect was attributed to oligomeric proanthocyanidins found in Cranberry extract, which donate electrons to the bleached surface and release free radicals through epicatechin esterification by gallic acid [74, 75]. Beyond their antioxidant properties, these proanthocyanidins influence the processes of demineralization and dentin remineralization, promoting increased remineralization and facilitating mineral deposition on the tooth surface. These minerals form protective complexes on exposed dentin, reducing collagen wear and degradation. This validates Cranberry's potential for countering dentin erosion [4, 73]. Nevertheless, attempts to prevent enamel erosion through the saliva-generated protective film formed by polyphenols in Cranberry were unsuccessful due to methodology and the extract's low pH, which counteracted the polyphenol effect [76]. The same proanthocyanidins present in Cranberry juice enhance dentin's

resistance against bacterial collagenase degradation and inhibit collagen matrix metalloproteinase, suggesting potential applications in restorative materials such as adhesives or primers [72].

Inhibition of cancerous cell lines

In the realm of oral medicine, we encountered 05 studies that examined the potential of Cranberry for controlling cancerous cell lines [3, 77, 78]. The primary objective, as highlighted in three of the articles, was to assess the chemopreventive and chemotherapeutic potential of Cranberry polyphenols in various stages of oral carcinogenesis. Notably, it was reported that proanthocyanidins effectively inhibit the induction of ornithine decarboxylase, an enzyme involved in the proliferation of epithelial tumor cells [79]. As well as *in vitro* evidence suggests that administering proanthocyanidins derived from Cranberry extract significantly reduce cell growth and proliferation in two oral tumor cell lines. Furthermore, within just 24 hours, the Cranberry extract upregulated the mRNA levels of caspase-2, an initiator of apoptosis, and caspase-8, an effector. Moreover, the extract demonstrated the ability to decrease cell adhesion in both cell lines. These findings highlight Cranberry extract's chemopreventive and chemotherapeutic capacity, selectively targeting cancer cells without affecting normal cells and tissues [77]. Another *in vitro* study reinforces these findings by demonstrating the effect of Cranberry extract in reducing tumor cell viability and proliferation while maintaining the viability of normal cells [3].

Osteogenesis and osteoclastogenesis

Two articles fall under this section [80, 81]; the first explored incorporating Cranberry extract into mesoporous bioactive glass to assess its impact on biomaterial bioactivity. This study revealed that embedded Cranberry didn't hinder hydroxyl-carbonate apatite deposition, crucial for bone regeneration [80]. The other article demonstrated that A-type proanthocyanidins can interfere with the functioning of osteoclastic cells, stopping bone resorption. This implies their potential use in treating conditions such as periodontitis [81].

Table 1: Summary of included studies descriptive characteristics in chronological order.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Weiss, E. I. et al. (1998) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Examine the ability of the high molecular weight non-dialyzable material from cranberry juice to inhibit coaggregation of selected bacterial strains inhabiting the mouth	The NDM reversed the coaggregation of 49 of the 84 bacterial pairs tested.
Weiss, E. I. et al. (2002) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i> clinical trial	Study the effect of the non-dialysable material from Cranberry juice on the coaggregation of oral bacteria.	The NDM inhibits the coaggregation of oral bacteria and their adhesion to epithelial cells. Also, NDM affects the salivary microbiota in humans.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Yamanaka, A (2004) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Evaluate the inhibitory effect of Cranberry juice on the adhesion of oral <i>streptococci</i> strains labeled with [3H]-thymidine to saliva-coated hydroxyapatite beads (s-HA).	The NDM inhibits the biofilm formation of oral <i>streptococci</i> , including cariogenic strains, suggesting that the daily use of cranberry juice could inhibit dental plaque development.
Weiss, E. I. et al. (2004) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i> Clinical trial	Study the effect of NDM-containing mouthwash on salivary bacterial counts in healthy volunteers.	The NDM inhibits the adhesion of <i>Streptococcus sobrinus</i> to saliva-coated hydroxyapatite <i>in vitro</i> . Also, the NDM reduces the total salivary bacteria. However, NDM does not influence the gingival and plaque indices.
Steinberg, D. et al, (2004) NDM	<i>In vitro</i>	Examine the effect of NDM on several constituents of the dental biofilm, glucosyltransferase (GTF), fructosyltransferase	The activity of immobilized and soluble GTF and FTF was inhibited by NDM. Also, NDM inhibited adhesion of <i>Streptococcus sobrinus</i> to hydroxyapatite.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
(<i>Vaccinium macrocarpon</i>)		(FTF), as well as on the adhesion of <i>Streptococcus sobrinus</i> .	
Koo, H. et al. (2005) Cranberry juice (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Evaluate the influence of cranberry juice on the activities of specific GTF enzymes (GTF B, C and D) adsorbed to hydroxyapatite surface. Also, investigate the influence of short-term daily exposure of cranberry on development, glucan composition and acidogenicity of <i>Streptococcus mutans</i> .	Cranberry juice inhibits glucan-mediated biofilm development and acid production, as well as holds promise as a natural product to prevent biofilm-related oral diseases. Also, Cranberry blocks the bacterial adherence mediated by surface glucans, and reduces <i>Streptococcus mutans</i> biofilm development, acidogenicity and its insoluble glucan content.
Steinberg, D. et al, (2005) NDM	<i>In vitro</i>	Examine the anti-adhesion effect of NDM on <i>Streptococcus sobrinus</i> .	NDM promoted desorption of <i>Streptococcus sobrinus</i> in the presence and absence of extracellular glucans and frutans. Precoating of the bacteria with NDM reduced their ability to form biofilm.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
(<i>Vaccinium macrocarpon</i>)			
Bodet, C. et al. (2006) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate the effect of NDM on the proinflammatory cytokine response of macrophages induced by lipopolysaccharides (LPS) from periodontopathogens.	NDM is a potent inhibitor of the pro-inflammatory cytokine and chemokine responses induced by LPS. his suggests that cranberry constituents may offer perspectives for the development of a new therapeutic approach to the prevention and treatment of periodontitis.
Labrecque, J. et al. (2006) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Study the effect of NDM on growth, biofilm formation, and adherence properties of <i>Porphyromonas gingivalis</i> .	NDM is a potent inhibitor of biofilm formation by <i>Porphyromonas gingivalis</i> . Also, NDM reduces the attachment of <i>Porphyromonas gingivalis</i> to surfaces coated with type I collagen, fibrinogen, or human serum. Cranberry may have a beneficial effect for the prevention and treatment of periodontitis.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Bodet, C. et al. (2006) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate the effect of NDM on the proteolytic activities of periodontopathogens.	NDM has the potential to reduce the proliferation of <i>Porphyromonas gingivalis</i> , <i>Treponema forsythia</i> , and <i>Treponema denticola</i> in periodontal pockets or their proteinase-mediated destructive process occurring in periodontitis.
Duarte, S. et al. (2006) Anthocyanins, flavonols, and proanthocyanidins (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Examine the influence of extracts of flavonols, anthocyanins, and proanthocyanidins on virulence factors involved in <i>Streptococcus mutans</i> biofilm development and acidogenicity.	Flavonols and proanthocyanidins, alone or in combination, inhibits the surface-adsorbed glucosyltransferases and F-ATPases activities, and the acid production by <i>Streptococcus mutans</i> .
Gregoire, S. et al. (2007)	<i>In vitro</i>	Investigate the influence of phenolic compounds on virulence properties of	Specific flavonoids exhibit biological activity against <i>Streptococcus mutans</i> . The

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Phenolic acids, flavonols and proanthocyanidins (<i>Vaccinium macrocarpon</i>)		<i>Streptococcus mutans</i> associated with glucan synthesis and acidogenicity.	biological activity of cranberry extracts may be a result from the complex mixture of flavonoids rather than a single active compound.
Bodet, C. et al. (2007) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate the effect of a NDM on MMP-3, MMP-9 and elastase activities, as well as on MMP production by human cells stimulated with lipopolysaccharide of <i>Aggregatibacter actinomycetemcomitans</i> .	NDM exhibits high capacity to inhibit MMP-3, MMP-9 and elastase activities, as well as MMP-3 and MMP-9 production by macrophages and gingival fibroblasts.
Bodet, C. et al. (2007)	<i>In vitro</i>	Determine the effect of the high- molecular-weight cranberry fraction on the production of inflammatory mediators (IL-6, IL-	NDM inhibits the IL-6, IL-8, and PGE2 response of gingival fibroblasts. This suggests that NDM, may have a beneficial effect on

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
NDM (<i>Vaccinium macrocarpon</i>)		8, and PGE2) by human gingival fibroblasts stimulated with <i>A. actinomycetemcomitans</i> LPS	slowing periodontal disease progression in combination with conventional therapy.
Yamanaka, A. et al. (2007) Polyphenol	<i>In vitro</i>	Investigate the effects of polyphenol on biofilm formation and activities of Arg-gingipain and Lys-gingipain in <i>Porphyromonas gingivalis</i> .	Cranberry polyphenol inhibits proteases of <i>Porphyromonas gingivalis</i> and its synergistic biofilm formation.
La, V.D. et al. (2009) Proanthocyanidin-rich fraction (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Evaluate the ability cranberry to protect monocyte-derived macrophages and oral epithelial cells against the cytotoxicity induced by bacterial components.	Cranberry polyphenols exert a protective effect for host cells against the toxicity induced by bacterial components.
La, V.D. et al. (2009)	<i>In vitro</i>	Investigate the effects of cranberry on the production of various MMPs by human	Cranberry inhibits both MMP production and activity, showing potential for the

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
A-type cranberry proanthocyanidins (<i>Vaccinium macrocarpon</i>)		monocyte-derived macrophages stimulated with <i>Aggregatibacter actinomycetemcomitans</i> lipopolysaccharide (LPS), and the catalytic activity of recombinant MMP-1 and MMP-9.	development of novel host-modulating strategies to inhibit MMP-mediated tissue destruction during periodontitis.
Feldman, M. et al. (2009) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate the potential anti adhesion effect of NDM via its influence on secretion, gene expression, and promoter activity of the fructosyltransferase.	NDM decreases secretion of extracellular fructosyltransferase and its down-regulated in a dose-dependent manner. NDM also markedly reduces the luciferase activity under the fructosyltransferase promoter.
Feldman, M. et al. (2010)	<i>In vitro</i>	Study the ability of NDM to bind to and inhibit the enzymatic activity of immobilized	One of the anti-biofilm modes of action of NDM is an immediate and irreversible inhibitory effect on the activity of immobilized

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
NDM (<i>Vaccinium macrocarpon</i>)		fructosyltransferase, using the real-time surface plasmon resonance (SPR) technique.	fructosyltransferase, which is due to a strong binding affinity to the immobilized enzyme.
La, V.D. et al. (2010) A-type proanthocyanidins (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Evaluate the effects of Cranberry on various virulence determinants of <i>Porphyromonas gingivalis</i> and on the inflammatory response of oral epithelial cells stimulated by this periodontopathogen.	Cranberry reduces the virulence properties of <i>Porphyromonas gingivalis</i> by inhibiting biofilm formation, adhesion, proteinase activity, and invasiveness. -Cranberry exerts anti-inflammatory activity by inhibiting the <i>Porphyromonas gingivalis</i> -induced inflammatory response in human oral epithelial cells.
Koo, H. et al. (2010)	<i>In vitro</i> <i>In vivo</i>	Determine the influence of a highly purified and chemically defined cranberry Proanthocyanidinsfraction on <i>Streptococcus mutans</i> biofilm formation on saliva-coated	Cranberry proanthocyanidins reduces the formation of biofilms by <i>Streptococcus mutans in vitro</i> and dental caries development <i>in vivo</i> , which may be attributed to the presence

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Proanthocyanidins (<i>Vaccinium macrocarpon</i>)		hydroxyapatite surface, and on dental caries development in Sprague-Dawley rats.	of specific bioactive A-type dimers and oligomers.
Tanabe, S. et al. (2011) A-type proanthocyanidins (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate the effect of A-type cranberry proanthocyanidins on osteoclast formation and bone resorption activity.	A-type proanthocyanidins interferes with osteoclastic cell maturation and physiology, as well as prevent bone resorption. These compounds may be considered as therapeutic agents for the prevention and treatment of periodontitis.
Chatelain, K. et al. (2011) Cranberry extract (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Evaluate cranberry and grape seed extracts to quantitate and compare their anti- proliferative effects on the oral squamous cell carcinoma.	All concentrations of cranberry extract (10–80 µg/ml) inhibit proliferation of CAL27 cells. Also, higher concentrations of cranberry extract inhibit proliferation of SCC25 cells in a dose-dependent inhibition.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Babu, J. et al. (2012) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Evaluate the effect of low concentrations of NDM on <i>Streptococcus gordonii</i> metabolic activity and biofilm formation on restorative dental surfaces.	NDM selectively inhibits metabolic activity of <i>Streptococcus gordonii</i> , without affecting bacterial viability, on various dental surfaces, such as titanium implant material, amalgam, and composite materials.
Feldman, M. et al. (2012) A-type proanthocyanidins (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate the potential of A-type cranberry proanthocyanidins (AC-PACs) and licochalcone A to act in synergy against <i>Porphyromonas gingivalis</i> and the host inflammatory response of a macrophage model.	A-type proanthocyanidins alone does not affect the growth of <i>Porphyromonas gingivalis</i> . However, it reduces the bacterial adherence. Also, A-type proanthocyanidins and licochalcone A possess synergistic antibacterial and anti-adherence activities and anti-inflammatory properties. This combination holds potential as a valuable therapeutic approach for the treatment and prevention of periodontitis.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Feldman, M. et al. (2012) A-type proanthocyanidins (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Study the effects of A-type cranberry proanthocyanidins on pathogenic properties of <i>Candida albicans</i> , as well as on the inflammatory response of oral epithelial cells induced by this oral pathogen.	A-type proanthocyanidins inhibits <i>Candida albicans</i> adherence to oral epithelial cells, as well decreases the secretion of IL-6 and IL-8 in a dose-dependent manner when epithelial cells were infected with <i>Candida albicans</i> .
Polak, D. et al. (2013) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i> <i>In vivo</i>	Explore the effect of high molecular weight cranberry constituent (non-dialyzable material [NDM]) on the virulence of a mixed infection with <i>Porphyromonas gingivalis</i> and <i>Fusobacterium nucleatum</i> in mice	NDM inhibits the adhesion of both species of bacteria onto epithelial cells and the coaggregation in a dose-dependent manner. Also, NDM eliminates TNF- α expression by macrophages exposed to <i>Porphyromonas gingivalis</i> and <i>Fusobacterium nucleatum</i> , without impairing their viability. additionally, NDM consumption by mice attenuated the severity of experimental periodontitis compared

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
			with a mixed infection without NDM treatment. In infected subcutaneous chambers, NDM alone reduced tumor necrosis factor.
Tipton, D. et al. (2013) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Determine the effects of cranberry on IL-6 and IL-8 production by human gingival epithelial cells and fibroblasts.	NDM inhibits IL-17, IL6, and IL-8 production by gingival fibroblasts and epithelial cells, suggesting that cranberry components could be useful as a host modulatory therapeutic agent to prevent or treat periodontitis.
Tipton, D. et al. (2013) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Use of fibroblasts to determine cytotoxicity of cranberry components or periodontopathogen (<i>Fusobacterium nucleatum</i> and <i>Porphyromonas gingivalis</i>) lipopolysaccharide associated with cranberry components, and effects of cranberry components on lipopolysaccharide-stimulated	Lack of toxicity of low NDM concentrations, and its inhibition of NF- κ B and MMP-3, suggest that cranberry components may regulate fibroblast inflammatory responses.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
		NF- κ B activation and IL-6 and MMP-3 production.	
Tipton, D. et al. (2014) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Determine the effects of cranberry on NF- κ B and MAPK/AP-1 activation of IL-1 β -stimulated IL-6 production in gingival epithelial cells.	NDM decreases nuclear levels of IL-1 β -activated NF- κ B (p65) and AP-1 (phospho-c-Jun) and strongly inhibits IL-6 production. Also, the lack of inhibition of phosphorylation of I κ B α , c-Jun or ERK1/2 suggests that NDM might affect both pathways downstream from those points in S-G cells, such as ubiquitination and proteosomal degradation of I κ B α , or inhibition of nuclear activity of c-Jun and/or ERK1/2.
Hrynash. H. et al. (2014) Anthocyanin-rich extract (<i>Vaccinium</i>	<i>In vitro</i>	Evaluate bacterial growth inhibition, mechanical properties, and compound release rate and stability of copolymers incorporated with anthocyanin.	The Anthocyanin-rich extract inhibits growth of <i>Streptococcus mutans</i> , although with far less effectiveness compared to chlorhexidine. Also, anthocyanin compounds remains stable after being released in water.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
<i>macrocarpon</i> incorporated in a resin			
Gupta, A. et al. (2015) NDM (<i>Vaccinium macrocarpon</i>)	Clinical trial	Study the effect of mouthrinse containing NDM on colonization of <i>Streptococcus mutans</i> in children.	NDM mouthrinse promotes reduction in <i>Streptococcus mutans</i> after 30 days.
Kim, D. et al. (2015) Proanthocyanidins tetramer (DP4) and monomer	<i>In vitro</i>	Investigate the effect of flavonoids to disrupt accumulation and survival of <i>Streptococcus mutans</i> , as well as on mechanical stability and the in-situ pH at the biofilm-apatite interface.	Cranberry compromises the 3D architecture of biofilms, showing a defective matrix and failure to develop microcolonies on the saliva-coated hydroxyapatite (sHA) surface. Also, topical applications of cranberry flavonoids weaken the mechanical stability of the biofilms and the pH measurements are

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
(DP9), and flavonol myricetin			higher at the biofilm-apatite interface compared to vehicle-treated biofilms.
Khairnar, M. et al. (2015) Cranberry extract (<i>Vaccinium macrocarpon</i>)	Clinical trial	Compare the efficacy of 0.2% Chlorhexidine mouthwash with 0.6% Cranberry mouthwash on <i>Streptococcus mutans</i>	Cranberry mouthwash is equally effective as Chlorhexidine mouthwash with beneficial local and systemic effects. Hence, it can be used effectively as an alternative to Chlorhexidine mouthwash
Lombardo B. et al. (2015) A-type cranberry proanthocyanidins (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Evaluate the potential of A-type cranberry proanthocyanidins (AC-PACs) and green tea epigallocatechin-3-gallate (EGCG) acting in synergy with LL-37 to reduce the secretion of inflammatory mediators by oral mucosal cells	- AC-PACs at the lowest concentration tested (25 mg/ml) significantly reduced the secretion of G-CFS (42%), GRO-a (33%), IL-8 (39%), IP-10 (72%), and MCP-1 (72%), but had no significant effect on the secretion of IL-6 and MMP - The combination of AC-PACs or EGCG and LL-37 synergistically inhibited

Author (Year)	Type of Study	Aim	Outcomes
Cranberry Fraction (Specie)			
			inflammatory cytokine secretion, suggesting a novel adjunctive therapy for treating periodontitis
Tipton, D. et al. (2016) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Determine cranberry effects on IL-6 and MMP-3 production by gingival fibroblasts, exposed to the representative advanced glycation end products (AGEs), formed during diabetic hyperglycemia, glycated human serum albumin (G-HSA), or LPS±G-HAS.	Non-toxic levels of cranberry NDM inhibits IL-6 and MMP-3 production in the presence of either G-HSA or LPS. Suggesting that cranberry phenols may be useful in regulating the host response and perhaps treating periodontitis in patients with poorly controlled diabetes.
Tipton, D. et al. (2016) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Examine effects of cranberry components on IL-1β-stimulated IL-6, IL-8, and VEGF production by human TMJ synovial fibroblast-like cells	NDM does not affect fibroblast viability, while inhibits IL-1 β and stimulates IL-6, IL-8, and VEGF production. Also, NDM reduces nuclear levels of NF-kB and AP-1, depending upon cell line and time of exposure to IL-1b+NDM. Therefore, NDM may be useful as a host

Author (Year)	Type of Study	Aim	Outcomes
Cranberry Fraction (Specie)			
			modulatory therapeutic agent to prevent or treat inflammatory arthropathies of the TMJ.
Neto, C. et al. (2017) NDM (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Characterize the polyphenol and non-polyphenol constituents of cranberry NDM	The crude NDM and the phenol-rich fraction (NDMac) are equally effective in inhibiting coaggregation of <i>Fusobacterium nucleatum</i> with <i>Streptococcus sanguinis</i> or <i>Porphyromonas gingivalis</i> . Also, NDMac reduces biofilms. The anti-adhesion activities of cranberry NDM on oral bacteria may arise from a combination of mixed polyphenol and non-polyphenol constituents.
Rajeshwari, H.R. et al. (2017)	<i>In vitro</i>	Investigate the efficacy of thermoreversible gel of cranberry juice concentrate (CJC) as local drug delivery for the treatment of periodontitis.	Cranberry demonstrates inhibitory effect against <i>Porphyromonas gingivalis</i> virulence factors. Comparison of optimized thermoreversible gel of CJC (500 mg/mL) with

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Cranberry juice concentrate (CJC) and Thermoreversible gel of CJC (<i>Vaccinium macrocarpon</i>)			commercially available chlorhexidine gluconate gel (0.2%) demonstrates similar inhibition against <i>Streptococcus mutans</i> , <i>EF</i> , <i>AA</i> , <i>PG</i> & <i>TF</i> . Therefore, the formulated thermos reversible gel of CJC could serve as an effective alternative to local drug delivery for periodontal treatment modalities.
Boteon, A. P. et al. (2017) 10% Cranberry extract gel (Shaanxi M.R Natural Product	<i>In vitro</i>	Evaluate the effect of cranberry and grape seed-enriched extract gels in inhibiting wear and degradation of demineralized organic matrix.	Cranberry extract and sodium fluoride promote similar reduction in dentin wear and degradation of demineralized organic matrix. Therefore, cranberry could be suggested as an interesting natural-based agent to prevent dentin erosion.

Author (Year)	Type of Study	Aim	Outcomes
Cranberry Fraction (Specie)			
Co., Ltd, Xi'an, China)			
Zare, A.J. et al. (2018) Cranberry juice (<i>Vaccinium macrocarpon</i>) and cranberry juice enriched with omega-3	Clinical trial	Evaluate the hypothesis that cranberry juice enriched with omega-3 will improve glycemic and lipid profiles and periodontal status in patients with diabetes with periodontal disease	Consumption of cranberry juice enriched with omega-3 can be beneficial as adjuvant therapy with nonsurgical periodontal therapy in decreasing glycated hemoglobin, increasing HDL-C, and improving periodontal status in patients with diabetes with periodontal disease.
Galarraga-Vinueza, M.E., et al. (2018)	<i>In vitro</i>	Study the potential of 58S mesoporous bioactive glass particles embedding propolis and cranberry to produce antibiofilm compounds.	Bioactive glass embedding cranberry and propolis increases hydroxyapatite crystal formation.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
A-type proanthocyanidins (<i>Vaccinium macrocarpon</i>)			
Khairnar, M. et al. (2018) Cranberry extract	<i>In vitro</i>	Assess the anti-cancer activities of Chlorhexidine and Cranberry against oral cancer cell lines.	Cranberry (80µg/mL) shows no anti-cancer property against AW13516 cell line, while demonstrates 70.6% growth inhibition against KB cell line.
Woźniewicz, M. et al. (2018) Apple juice (80 % v/v) with cranberry juice (20% v/v), and grounded cinnamon (0.25g)	<i>In vivo</i>	Determine whether the consumption of cranberry functional beverage (CFB) for a duration of eight weeks could lead to enhancements in gingival inflammation indices by inhibiting dental plaque formation. Additionally, the study aimed to examine potential changes in antioxidant status and	The cranberry functional beverage group demonstrated significantly reduced values in terms of gingival index, plaque index, and approximal plaque index when compared to the control group (water). The consumption of cranberry functional beverages resulted in enhancements of both gingival and plaque

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
		systemic inflammation among individuals diagnosed with gingivitis.	indices without introducing the risk of caries development.
Nandakumar, M.; Nasim, I. (2018) Cranberry extract	<i>In vitro</i>	Evaluate the role of grape seed extract and cranberry extract in preventing dental erosion using optical emission spectrometry.	The protective action of grape seed extract and cranberry extract was inferior to the gold standard control group of stannous fluoride role, against enamel erosion.
Abu-obaid, E. et al. (2019) Cranberry extract (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Study the antimicrobial effects of different natural and semi-natural mouthrinses on isolates of <i>Streptococcus mutans</i> obtained from the saliva of Saudi children and reference strains of <i>Streptococcus mutans</i> (ATCC 25175).	Mouthrinses containing herbal mix and cranberry have the potential to serve as effective natural alternatives to chlorhexidine mouthrinses, whether with or without alcohol, in influencing the tested parameters.
Ben Lagha, A. et al. (2019)	<i>In vitro</i>	Investigate the effects of cranberry proanthocyanidins on gene expression and cytotoxic activities of leukotoxin	Cranberry proanthocyanidins reduced the expression of the LtxA gene in <i>Aggregatibacter actinomycetemcomitans</i> and neutralized the cytolytic and pro-inflammatory

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Proanthocyanidins (<i>Vaccinium macrocarpon</i>)			responses of human macrophages treated with LtxA. This suggests that cranberry proanthocyanidins have the potential to be a promising candidate for the development of novel therapeutic agents to address localized aggressive periodontitis.
Eggula, A. et al. (2019) Cranberry extract	<i>In vitro</i>	Assess the neutralizing effect of 6% cranberry solution on the bond strength of bleached enamel compared with that of 10% sodium ascorbate solution	A 10% sodium ascorbate solution has proven superior to 6% cranberry solution in the reversal of compromised bond strength following bleaching.
Kumar, V. et al. (2019)	<i>In vitro</i>	Assess the <i>in-vitro</i> antimicrobial efficacy of hydro-alcoholic fruit extract of cranberry against Socransky complexes and predominant cariogenic, mycotic and	The extract showed satisfactory inhibitory and bactericidal effects against all test pathogens. <i>Vaccinium macrocarpon</i> can prove to be an adjunct to the existing antimicrobial

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Cranberry extract (<i>Vaccinium macrocarpon</i>)		endodontic climax communities of the oral cavity.	complexes being routinely used to combat pathogenic oral climax community.
Philip. N. et al. (2019) Extracts of Cranberry blueberry, and strawberry	<i>In vitro</i>	Evaluate if extracts of cranberry, blueberry, and strawberry, and a combination of the three berry extracts (Orophenol®) had inhibitory effects against <i>Streptococcus mutans</i> biofilms	The biofilms treated with cranberry and Orophenol® extracts exhibited the most significant reductions in metabolic activity, acid production, and bacterial/exopolysaccharide biovolumes. Among these extracts, cranberry extract proved to be the most effective in disrupting the virulence characteristics of <i>Streptococcus mutans</i> , without causing a significant impact on bacterial viability.
Philip. N. et al. (2019)	<i>In vitro</i>	Investigate the effect of cranberry extracts on saliva-derived polymicrobial biofilms with regards to biofilm biomass, acidogenicity, exopolysaccharide /microbial biovolumes,	The cranberry extract lowered biofilm biomass, acidogenicity, exopolysaccharide /microbial biovolumes, CFU counts, and

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
A-type proanthocyanidins		colony forming unit counts, and the proportion of distinct bacteria associated with caries and oral health	modulated a beneficial microbial ecological change in saliva-derived polymicrobial biofilms.
Philip. N. et al. (2019) Cranberry extract	<i>In vitro</i>	Study the effects of polyphenol-rich cranberry extracts on dual-species <i>Streptococcus mutans-Candida albicans</i> biofilms implicated in contributing to the severity of early childhood caries.	Cranberry extracts inhibited cariogenic virulence properties of <i>S. mutans-C. albicans</i> dual-species biofilms in an in vitro model
Kokubo. E. et al. (2019) Cranberry juice (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate the effects of polyphenolic compound-containing lingonberry extract on oral <i>streptococci</i> and compare them with the known anti-cariogenic activity of cranberries	Cranberry fraction I exhibited a decrease in bioactivity against <i>Streptococcus mutans</i> across all tested concentrations and against <i>Streptococcus sanguinis</i> at 0.5 or 1.0 mg/ml concentrations. There was no observable impact on <i>Streptococcus sobrinus</i> at any tested concentration. In contrast, the viability of <i>Streptococcus mutans</i> showed a minor

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
			increase, while <i>Streptococcus sobrinus</i> demonstrated a noteworthy increase when subjected to an incubation with cranberry fraction II at a concentration of 0.5 mg/ml.
Philip. N. et al. (2020) Cranberry extract	<i>In vitro</i>	Study if selected natural products could specifically target the growth of a caries-associated bacterial species (<i>Streptococcus mutans</i>) without affecting the viability of a health-associated oral commensal bacterial species (<i>Streptococcus sanguinis</i>)	None of the fruit berry extracts (cranberry, blueberry, and strawberry) demonstrated bacterial growth inhibitory effects against <i>Streptococcus mutans</i> or <i>Streptococcus sanguinis</i> . This suggests that the concentrations tested may lack bactericidal effects
Galarraga-Vinueza, M.E., et al. (2020)	<i>In vitro</i>	Evaluate cell viability, anti-inflammatory activity, and macrophage polarization properties of different cranberry concentrates	A-type proanthocyanidins did not result in a reduction in viability for human gingival fibroblasts, a human osteosarcoma cell line, or macrophages. Cranberry concentrates at concentrations of 50 and 100µg/mL exhibited a

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
A-type proanthocyanidins (<i>Vaccinium macrocarpon</i>)			downregulation of pro-inflammatory cytokine expression. Moreover, at a concentration of 100µg/mL, cranberry concentrate significantly upregulated the expression of the anti-inflammatory cytokine interleukin-10. In the case of lipopolysaccharide-stimulated macrophages exposed to cranberry concentrates, there was a notable decrease in M1 polarization and a significant increase in M2 polarization.
Singhal, R. et al. (2020) Cranberry extract (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Asses the antimicrobial and antibiofilm effect of cranberry on <i>Streptococcus mutans</i> and <i>Lactobacillus acidophilus</i>	- This study has identified bactericidal, bacteriostatic, and antibiofilm effects of cranberry extract against <i>Streptococcus mutans</i> and <i>Lactobacillus acidophilus</i> in a time-dependent and dose-dependent manner.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Abu-obaid, E. et al. (2020) Cranberry extract 0.3% mixed with chlorhexidine digluconate mouthrinse 0.06%	<i>In vitro</i>	Study the antimicrobial effects of natural and semi-natural mouthrinses on isolates of <i>Streptococcus mutans</i> , <i>Lactobacillus fermentum</i> , and <i>Lactobacillus casei</i> obtained from the saliva samples and their reference strains	- The minimum inhibitory concentration and minimum bactericidal concentration were significantly higher in the cranberry mixed with chlorhexidine than in chlorhexidine with alcohol. Herbal mix and cranberry mouthrinses could be an effective natural alternative to chlorhexidine mouthrinse with or without alcohol in improving oral health.
Philip. N. et al. (2020) Casein phosphopeptide–amorphous calcium phosphate	Clinical trial	Investigate the microbial ecological effects of 2 interventional dentifrices: a casein phosphopeptide–amorphous calcium phosphate) dentifrice and the same dentifrice supplemented with a polyphenol-rich cranberry extract	The casein phosphopeptide–amorphous calcium phosphate cranberry group showed greater mean fold decreases over time in the loads of the caries-associated bacteria and higher mean fold increases over time in the loads of the health-associated bacteria (except for <i>Corynebacterium durum</i>), compared to the

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
dentifrice supplemented with a polyphenol-rich cranberry extract			casein phosphopeptide–amorphous calcium phosphate group
Kranz, S. et al. (2020) Cranberry juice (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Study the antibacterial activity of all-fruit juices derived from blackcurrant, redcurrant, cranberry and raspberry on common oral pathogenic gram-positive and gram-negative bacteria (<i>Streptococcus mutans</i> , <i>Streptococcus gordonii</i> , <i>Streptococcus sobrinus</i> , <i>Actinomyces naeslundii</i> , <i>Fusobacterium nucleatum</i> , <i>Aggregatibacter actinomycetemcomitans</i> , <i>Porphyromonas gingivalis</i> , <i>Enterococcus faecalis</i>)	Blackcurrant juice was most efficient at suppressing bacteria; followed by the activity of redcurrant and cranberry juice. Cranberry juice induced <i>Fusobacterium nucleatum</i> and <i>A. actinomycetemcomitans</i> inhibition zones that are comparable to the inhibitory effect of chlorhexidine. Direct contact of <i>Streptococcus sobrinus</i> with cranberry juice caused a stronger antibacterial activity compared to treatment with chlorhexidine.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Ankola, A. et al. (2020) Cranberry extract (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Assess the <i>in vitro</i> anticancer efficacy of hydroalcoholic fruit extract of cranberry against oral cancer KB cell	The extract showed satisfactory antiproliferative effect on the KB cell line and a higher cell viability percentage of the normal fibroblast cell line. <i>Vaccinium macrocarpon</i> can prove to be an adjunct to the existing anticancer drug therapy against oral cancer KB cell line.
Keshaav Krishnaa, P.; Prabakar, J. (2020) Cranberry extract	<i>In vitro</i>	Determine the efficacy of cranberry distillate as a root canal irrigant.	Cranberry distillate exhibits strong antimicrobial properties, making it a potential candidate for future use as a root canal irrigant. However, additional <i>in vitro</i> and <i>in vivo</i> research is necessary to confirm its suitability for clinical application, with the aim of benefiting the broader population's oral health.
Wu, C.D. et al. (2021)	<i>In vitro</i>	Investigate if the commercially marketed plant polyphenols-containing beverages can inhibit growth and biofilm	The Cranberry juice cocktails consistently suppressed growth and biofilm formation of both <i>Streptococcus mutans</i> and

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Cranberry juice (<i>Vaccinium macrocarpon</i>)		formation of <i>Streptococcus mutans</i> and children's dental plaque	children's supragingival plaque bacteria (>90%). The plaque biofilms grown in the presence of these cranberry juice drinks were loosely attached and easily dislodged from surfaces.
Souissi, M. et al. (2021) Polyphenolic fraction of blueberry, cranberry, and strawberry (Orophenol®)	<i>In vitro</i>	Investigate a berry (wild blueberry, cranberry, and strawberry) polyphenolic fraction, commercialized as Orophenol®, for its antibacterial, anti-biofilm, and anti-adhesion properties on <i>Streptococcus mutans</i>	The berry polyphenolic preparation dose-dependently inhibited <i>Streptococcus mutans</i> biofilm formation and the adhesion to saliva-coated hydroxyapatite and saliva-coated nickel–chrome alloy. The berry fraction did not show any significant cytotoxicity in an oral epithelial cell model.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Kharche, A. et al. (2021) Cranberry extract (<i>Vaccinium macrocarpon</i>)	Clinical trial	Evaluate the efficacy and antimicrobial properties of four herbal mouthrinses on orthodontic patients with gingivitis	All the tested phytotherapeutic agents showed effective reductions in plaque and gingivitis scores in individuals with fixed orthodontic therapy. Cranberry could be used as an adjunct to non-surgical periodontal therapy.
Pellerin, G. et al. (2021) Cranberry juice (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Investigate whether the removal of organic acids (0%, 19%, 42%, 60%, and 79%) from cranberry juice by electrodialysis with bipolar membrane affects its antibacterial activity against major periodontopathogens as well as its anti-inflammatory properties in an oral epithelial cell model	Cranberry juice increased the adherence of <i>Aggregatibacter actinomycetemcomitans</i> and <i>Porphyromonas gingivalis</i> to oral epithelial cells, but reduced the adherence <i>Fusobacterium nucleatum</i> by half regardless of the deacidification rate. <i>Fusobacterium nucleatum</i> produced more hydrogen sulfide when it was exposed to deacidified cranberry juice with a deacidification rate $\geq 42\%$ compared to the raw beverage.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
			The removal of organic acids from cranberry juice lowered the cytotoxicity of the beverage for oral epithelial cells. The secretion of IL-6 by lipopolysaccharide-stimulated oral epithelial cells exposed to cranberry juice increased proportionally with the deacidification rate
Wang, Y. et al. (2021) Cranberry extract	<i>In vitro</i>	Evaluate the effects of polyphenol-rich extracts and a chemical cross-linker on the cross-linking interaction, resistance to digestion, and endogenous matrix metalloproteinase activities of dentin collagen under clinically relevant conditions	The Cranberry juice extract treated dentin collagen rapidly increased its resistance to digestion and matrix metalloproteinase inhibition. An application of cranberry juice extract as short as 30 s may be a clinically feasible approach to improving the longevity of dentin bonding in composite restorations.
Rahman, H. et al. (2021)	<i>In vitro</i>	Assess antioxidant activity of herbal antioxidants namely 6% cranberry extract, 10%	All the four antioxidants were able to reverse the compromised bond strength

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Cranberry extract		green tea, 50% aloe vera and 10% sodium ascorbate and their effect on reversal of bond strength in bleached enamel	comparatively after bleaching maximum being green tea followed by aloe vera, sodium ascorbate, and minimum in cranberry.
Niemeye, S. et al. (2021) Cranberry extract	<i>In vitro</i>	Investigate the modification of the salivary pellicle with different polyphenol-rich teas and natural extracts for the protection against dental erosion	Cranberry caused significantly more demineralization, due to its low pH, and presented the lowest surface microhardness measurement and surface reflection intensity values.
Pellerin, G. et al. (2021) Cranberry juice (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	To assess how the deacidification of cranberry juice by electrodialysis with a bipolar membrane impacts the antibacterial properties of the beverage against cariogenic (<i>Streptococcus mutans</i> , <i>Streptococcus sobrinus</i>) and commensal (<i>Streptococcus gordonii</i> , <i>Streptococcus oralis</i> , <i>Streptococcus salivarius</i>) streptococci, and how it affects oral	The removal of organic acids from cranberry juice reduced the bactericidal activity of the beverage against planktonic <i>Streptococcus mutans</i> and <i>Streptococcus gordonii</i> , whereas only the viability of <i>Streptococcus gordonii</i> was significantly impacted by cranberry juice deacidification.

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
		epithelial barrier function and inflammatory response in an in vitro model	Conditioning saliva-coated hydroxyapatite with undiluted cranberry juice samples significantly lowered the adherence of <i>Streptococcus mutans</i>, <i>Streptococcus sobrinus</i>, and <i>Streptococcus oralis</i>. Cranberry juice deacidified at a rate of $\geq 19\%$ maintained the integrity of a keratinocyte monolayer over the course of 24 h compared to raw cranberry juice. Deacidification impacted the secretion of Interleukin-6, but not of Interleukin-8, by oral epithelial cells.
Anitha, K.V. et al. (2022)	In vitro	Assess the effectiveness of <i>Vaccinium macrocarpon</i> (cranberry) extract on <i>Candida albicans</i> adhesion to cranberry added denture base biomaterial	The attachment of <i>Candida albicans</i> to a self-curing polymethyl methacrylate biomaterial enriched with cranberry was found to be reduced, regardless of the extract

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Cranberry extract (<i>Vaccinium macrocarpon</i>) incorporated into polymer of polymethyl methacrylate (PMMA)			proportions (1%, 1.5%, and 2% in PMMA). Cranberry's disinfectant efficacy was observed to result in reduced Candida growth compared to immersion in distilled water. This suggests that cranberry possesses anti-adhesive properties, serving as both a constituent material within the polymer and a disinfectant solution.
Kato, M. et al. (2022) Cranberry juice and Cranberry extract (<i>Vaccinium macrocarpon</i>)	<i>In vitro</i>	Analyze the effect of cranberry juice on the reduction of dentin erosion <i>in vitro</i>	Cranberry juice, even with the lowest pH, significantly reduced dentin wear compared to the control (water, pH 7.2), and did not significantly differ from the other acidic groups.
Vaillancourt, K. et al. (2022)	<i>In vitro</i>	Assess the effects of a berry polyphenolic fraction (Orophenol®) composed	The polyphenolic fraction from the berry demonstrated a reduction in various pathogenic

Author (Year) Cranberry Fraction (Specie)	Type of Study	Aim	Outcomes
Extracts of cranberry (<i>Vaccinium macrocarpon</i>), blueberry, and strawberry, and a combination of the three berry extracts (Orophenol®)		of extracts from cranberry, wild blueberry, and strawberry on the main pathogenic determinants of <i>Porphyromonas gingivalis</i>	characteristics associated with <i>Porphyromonas gingivalis</i> . Orophenol® hindered the growth of <i>Porphyromonas gingivalis</i> and reduced its hemolytic activity, adherence to a basement membrane matrix model, and proteinase activities. It also diminished the production of reactive oxygen species by oral keratinocytes when stimulated by <i>Porphyromonas gingivalis</i> .

Legend: NDM - high-molecular-weight constituent of cranberry.

Conclusion

In recent times, there has been a growing interest in identifying botanical or nutritional components and supplements due to their potential therapeutic benefits. A wide range of medications and active molecules are now accessible through pharmacies and health food stores. Cranberry, a fruit with extensive therapeutic potential, has garnered attention. The various elements found within it, including bioactive flavonols like anthocyanidins and proanthocyanidins, which can work on their own or together in a complementary way, present valuable potential for applications in the field of Dentistry. Given the beneficial effects promoted by Cranberry as suggested by this study, we propose that further research and product development based on Cranberry will occur, both for in-office applications and daily use by the general population, in order to fully harness the oral health benefits that this fruit can offer.

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4 ARTIGO 2 – POTENTIAL OF CRANBERRY TO STIMULATE OSTEOGENESIS: AN *IN VITRO* STUDY

Potential of cranberry to stimulate osteogenesis: An *in vitro* study

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ABSTRACT

Objective: The purpose of this study was to investigate the potential of Cranberry extract to stimulate osteogenesis in an *in vitro* setting. **Materials and Methods:** We determined the total phenolic and total monomeric anthocyanin contents in the Cranberry extract. Liquid chromatography coupled with mass spectrometry was used to identify the constituents of the Cranberry extract. To assess the cytotoxicity of the Cranberry extract, we employed the thiazolyl blue tetrazolium bromide assay. Concerning the osteogenesis potential of Cranberry extract, we determined alkaline phosphatase (ALP) activity, bone morphogenetic protein 2 (BMP-2) expression, and extracellular matrix mineralization. **Results:** The total phenolic content were 522.72 ± 9.80 mg GAE g⁻¹ ES and 364.95 ± 12.49 mg GAE/g detected by Fast Blue BB and Folin-Ciocalteu method, respectively. Regarding the monomeric anthocyanin, the observed content was 460 ± 30 mg ECG g⁻¹ ES. Moreover, we established higher concentrations of Cranberry extract (62.5 mg/mL to 500 mg/mL) were biocompatible with osteoblasts and mesenchymal stromal cells. Regarding osteogenesis, 20 mg/mL of Cranberry extract promoted 2-fold more ALP activity and almost 1.5-fold more BMP-2 than the positive control group. Additionally, concentrations of 200 mg/mL of Cranberry extract stimulated a 1.7-fold increase in extracellular matrix mineralization than the positive control group. Notably, a correlation was observed between the

Cranberry concentrations and osteogenesis markers. **Conclusions:** Cranberry extract displayed potential in stimulating early and late markers of osteogenesis. **Clinical relevance:** The ability of Cranberry extract to promote osteogenesis and its biocompatibility at higher concentrations hold promise for future application into scaffolds for bone regeneration.

Keywords: Cranberry; osteogenesis, in vitro, mesenchymal stromal cells

INTRODUCTION

Moderate or severe jaw atrophy, stemming from tooth loss, trauma, or surgical resection, often necessitates vertical bone augmentation to increase bone height, thereby facilitating the subsequent placement of dental implants followed by implant-supported prosthesis [1, 2]. Nevertheless, vertical bone augmentation procedures present biological and technical challenges. Current surgical techniques exhibit several limitations, including associated morbidity, inadequate resorption rates leading to compromised dimensional stability, suboptimal structural integrity, and challenging handling properties [2–4]. In terms of biological considerations, bone formation entails a complex process reliant on osteogenesis and osteoclastogenesis. Furthermore, vertical bone augmentation within the oral cavity introduced an additional requirement for antimicrobial and anti-inflammatory properties, owing to the presence of a contaminated environment [5, 6].

Cranberry components have garnered increasing attention within the sphere of health, including dentistry, owing to their antibacterial efficacy against cariogenic, mycotic, endodontic, and periodontal pathogens [7, 8], along with their anti-inflammatory attributes [9]. Cranberry extract, derived from *Vaccinium macrocarpon*, *Vaccinium oxycoccus*, or *Vaccinium microcarpum*, abounds in numerous bioactive compounds, with polyphenols, especially phenolic acids, flavonoids (anthocyanins and flavonols), and tannins, constituting major polyphenolic entities. Moreover, diverse fractions of cranberry have undergone investigation, encompassing cranberry extract [10–13], high-molecular-weight constituents (NDM)[14–16], cranberry juice [17–19],

total polyphenol [20–22], phenolic acids[21, 23], flavonols, proanthocyanidins, and specifically A-type cranberry proanthocyanidins [21, 24–26].

NDM extracted from *Cranberry* has demonstrated the capability to inhibit nuclear factor kappa B and matrix metalloproteinase 3, thereby impacting fibroblast functionality during inflammatory responses [27, 28]. Additionally, proanthocyanidins, present in high-molecular-weight non-dialyzable material (NDM) extracted from *Cranberry* [29] *are associated with noteworthy attributes such as* anti-adhesive and antibacterial properties, as well as the ability to hinder collagenase and proteinase activity [27, 28, 30, 31]. Furthermore, proanthocyanidins, primarily composed of epicatechin subunits featuring at least one type A bond and known as A-type Cranberry proanthocyanidins, appear to disrupt the maturation and physiology of osteoclastic cells, as well as the bone matrix itself [32]. This effect is achieved by inhibiting the functional development of osteoclasts through the NF- κ B pathway and mitogen-activated protein kinase signaling pathways (MAPKs) [33]. Polyphenolic compounds such as flavonoids sourced from materials other than cranberries, have been explored for their potential in preserving bone. These polyphenolic compounds appear to regulate cell signaling pathways that impact the differentiation of osteoblasts and osteoclasts, thereby promoting bone formation and suppressing bone resorption [34–37]. Given the absence of research evaluating the role of any cranberry fraction in osteogenesis, this study aims to investigate the potential of Cranberry extract to stimulate osteogenesis in an *in vitro* setting.

MATERIALS AND METHODS

Cranberry extract solution

For cytotoxicity analysis, we solubilized the dry Cranberry extract (*Vaccinium macrocarpon*) from 62.5 to 500 mg/mL in hydroalcoholic solutions. Additionally, for osteogenic differentiation assays, we solubilized the Cranberry extract in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Massachusetts, USA), reaching 20 mg/mL, 2 mg/mL, and 0.2 mg/mL for alkaline phosphatase activity and bone morphogenetic protein type 2 expression, and concentrations of 250 mg/mL, 200 mg/mL, 150 mg/mL, 100 mg/mL, 50 mg/mL, 20 mg/mL, 2 mg/mL, and 0.2 mg/mL for extracellular matrix mineralization.

Total phenolic content by Folin-Ciocalteu and Fast Blue BB

The total phenolic content of the Cranberry extract was ascertained employing the Folin-Ciocalteu [38] and Fast Blue BB [39] techniques. The findings were reported in mg of gallic acid equivalents per g of dry extract (mg GAE g⁻¹ ES). The analytical curve generated using gallic acid spanned concentrations from 500 to 5 µg/mL, yielding an $R^2 = 0.999$ for both analyses.

Total monomeric anthocyanin content

To quantify the total monomeric anthocyanin content in the Cranberry extract, we employed the differential pH method using wavelengths of 520 and 700 nm. An absorptivity coefficient (ϵ) of 26600 L/M/cm was applied for cyanidin-3-O-glycoside in methanol. The outcomes were reported as mg equivalent of cyanidin 3- o-glycoside per 100 g of dry extract (mg ECG 100g⁻¹ ES) [38, 39].

Liquid chromatography coupled with mass spectrometry (LC/MS)

For anthocyanin identification and separation, we employed a high-performance liquid chromatography system (Acquity-UPLC™, Waters Corp., Milford, Massachusetts, EUA) coupled with a photodiode array (PDA) and a high-resolution mass spectrometry unit (Xevo® G2 QToF model, Waters Corp.) equipped with electrospray ionization (ESI) and operating in positive mode. The Cranberry extract was solubilized in methanol and water reaching a concentration of 250 µg/mL. A Synergi™ - Phenomenex® column (Torrance, California, EUA) with 150 mm x 2 mm x 4 µm was maintained at 40°C, with and 5 µL of injection volume. The mobile phases comprised 2% formic acid (solvent A) and acetonitrile (solvent B). The gradient elution scheme consisted of: 0 min at 5% B, 10 min at 12% B, 16 min at 15% B. A flow rate of 0.4 mL/min was employed, and chromatograms were monitored at 520 nm. Mass spectrometry detection involved a full sweep from 50 to 1500 m/z, with a sweep time of 1 s. Data acquisition and processing were executed using MassLynx v4.1 software (Waters Corp). For MS/MS analyses, argon served as the collision agent with energy varying from 10, 20, and 30 eV depending on the analyzed compound. The capillary

voltage was 3.5 kV with a temperature of 90°C, while the desolvation temperature was 400°C. The gas flow (N₂) was 900 L/h, and the cone voltage was 30 V [40].

Cell lineage and culture conditions

To evaluate the cytotoxicity and osteogenic potential of the Cranberry extract, cell lineages described below were incubated at 37 °C in an atmosphere with 5% CO₂. For experiments, we seeded 2.0×10^4 cells per well in a 96-well plate. The culture media were changed every 2-3 days. To determine the sample size (n) for independent groups, we used data from a pilot study [41] and the minimum n required was 1.83.

Osteoblasts

Osteoblasts MC3T3-E1 subclone 4 (American Type Culture Collection, CRL 2593) were cultured with modified α -Minimum Eagles' Medium (Thermo Fisher Scientific, Waltham, USA), supplemented with 10% FBS, 50 μ M L-ascorbic acid (Sigma-Aldrich, St Louis, USA), and 10 mM β -glycerophosphate (Sigma-Aldrich, St Louis, USA) [41].

Mesenchymal stromal cells from human-exfoliated deciduous teeth

Mesenchymal stromal cells from human exfoliated deciduous teeth (SHED, Curityba Biotech™ Cell Processing Center, Curitiba, Brazil) were cultured in a standard medium (DMEM with 10% FBS) [42]. SHED were used in passage 4 to 6.

Cranberry extract cytotoxicity

The cytotoxicity of Cranberry extract was determined using the thiazolyl blue tetrazolium bromide (MTT, Sigma-Aldrich, USA) colorimetric test, assessing MC3T3-E1 and SHED on day 3. Upon removing the culture media, cells were exposed to the MTT solubilized in culture media (at a ratio of 1:5 v:v) and incubated for 4 h at 37 °C. Subsequently, formazan crystals were solubilized using dimethyl sulfoxide (DMSO). Absorbances were measured at 540/630 nm (Infinite M200, TECAN, Salzburg, Austria). Cellular viability percentages were calculated relative to the cellular control group (cells treated with standard medium) [43].

Osteogenic differentiation

SHED were utilized for osteogenic differentiation analysis. As previously mentioned, Cranberry extract concentrations of 0.2 mg/mL, 2 mg/mL, and 20mg/mL were evaluated for alkaline phosphatase activity and bone morphogenetic protein type 2 expression, and concentrations of 250 mg/mL, 200 mg/mL, 150 mg/mL, 100 mg/mL, 50 mg/mL, 20 mg/mL, 2 mg/mL, and 0.2 mg/mL for extracellular matrix mineralization. In the positive control group, SHED were exposed to the osteogenic medium (DMEM supplemented with FBS, 50 μ M L-ascorbic acid (Sigma-Aldrich, St. Louis, USA), and 10 mM β -glycerophosphate (Sigma-Aldrich, St. Louis, USA). The subsequent assays were carried out as follows:

Alkaline phosphatase activity

The alkaline phosphatase (ALP) activity in the cell supernatant was assessed by the liberation of p -nitrophenol (p -NP) from p -nitrophenol phosphate (p NPP) on days 3, 7, and 10. Briefly, a mixture of 0.5 mL of diethanolamine buffer (1.02 M, pH 9.8, 0.6 mM magnesium chloride, *Sigma-Aldrich, St. Louis, USA*) and 0.5 mL of 60.8 mM p NPP (*Sigma-Aldrich, St. Louis, USA*) was incubated for 2 min at 37°C. Following this, 0.1 mL of the supernatant was added. After a 30-min incubation, the reaction was stopped by the addition of 1 mL of 3 N NaOH (*Sigma-Aldrich, St. Louis, USA*). Absorbances were measured at 405 nm (Infinite M200, TECAN, Salzburg, Austria), and ALP activity was calculated based on a p NP standard curve [44].

Bone morphogenetic protein type 2 expression

To determine the bone morphogenetic protein type 2 (BMP-2) expression on day 3, we employed the Quantikine™ Human BMP-2 Immunoassay (R&D Systems, Minneapolis, USA). Briefly, we introduced 100 μ L of the RD1-19 Assay Diluent and 50 μ L of each supernatant to a well of the kit plate, sealed with adhesive tape, and subjected to 2 h incubation on a horizontal orbital microplate shaker set to 500 rpm. Subsequent to aspiration, each well received 400 μ L of wash buffer, with this step being repeated four times. Following this, 200 μ L of BMP-2 conjugate was added to each well, and the mixture was incubated for 2 h. After another aspiration/wash cycle, we introduced 200 μ L of substrate solution and incubated for 30 min. Subsequently,

50 μ L of stop solution was added, and the optical density was read at 450 nm with wavelength correction set to 540 nm or 570 nm (Infinite M200, TECAN, Salzburg, Austria) [45].

Extracellular matrix mineralization

We assessed the extracellular matrix mineralization via Alizarin Red S staining on day 15. Therefore, we fixed cells with 4% paraformaldehyde (*Sigma-Aldrich, St. Louis, USA*) for 15 min. Afterward, we treated the cells with 3% Alizarin Red S (*Sigma-Aldrich, St. Louis, USA*) for 30 min. Analysis was conducted using an stereo microscope (SZX16® Olympus Corporation, Tokyo, Japan), and images were captured (Olympus DP73®, Olympus Corporation, Tokyo, Japan) with the acquisition software (CellSens Dimension 1.12®, Olympus Corporation, Tokyo, Japan). To quantify the mineralization, Alizarin Red S staining was solubilized using 0.5 N hydrochloric acid (*Sigma-Aldrich, St. Louis, USA*) for 30 min, and the absorbances were subsequently measured (Infinite M200, TECAN, Salzburg, Austria) at 490nm [41].

Statistical analysis

The experiments were carried out in a minimum of triplicate. Dot plots display the data obtained from all distinct experiments, depicting the median. Analytical methodologies were conducted using GraphPad Prism version 8 (San Diego, California, USA). Statistical assessments encompassed the Shapiro-Wilk and one-way ANOVA tests or Anderson-Darling test and one-way ANOVA for parametric data, and alternatively, Kruskal-Wallis and Dunn's test or tests for non-parametric data. Methods of correction for multiple comparisons were implemented. A significance threshold of $p < 0.05$ was applied.

RESULTS

Cranberry with heightened total monomeric anthocyanin and phenolic content

We determined the total phenolic content of the Cranberry extract to be 522.72 ± 9.80 mg GAE/g and 364.95 ± 12.49 mg GAE/g using Fast Blue BB (FBBB) and Folin-Ciocalteu (FC-B) methods, respectively. Moreover, the FBBB/FC-B ratio was 1.43,

indicating the presence of phenolics compounds [46]. Additionally, the Cranberry extract contained 460 ± 30 mg ECG g⁻¹ ES of monomeric anthocyanin. This finding signifies that the Cranberry used in our study display a 140-fold increase in monomeric anthocyanin compared to the minimum to indicate the authenticity of the Cranberry extract [47].

Regarding the phytoconstituents, authentic Cranberry extract may encompass delphinidin-galactoside, cyanidin-3-O-glycoside, cyanidin arabinoside, peonidin-3-O-galactoside, peonidin-3-O-glycoside, and peonidin-arabinoside. Also, cyanidin-3-O-glucoside and peonidin-3-O-glucoside should be present in low concentrations. As depicted in Figure 1, our analysis identified cyanidin-3,5-glycoside, cyanidin-3-glycoside, and peonidin-3-O glycoside. In contrast, we also identified the presence of cyanidin-rutinoside in the samples, suggesting a possible contamination by *Morus nigra* (mulberry) [47].

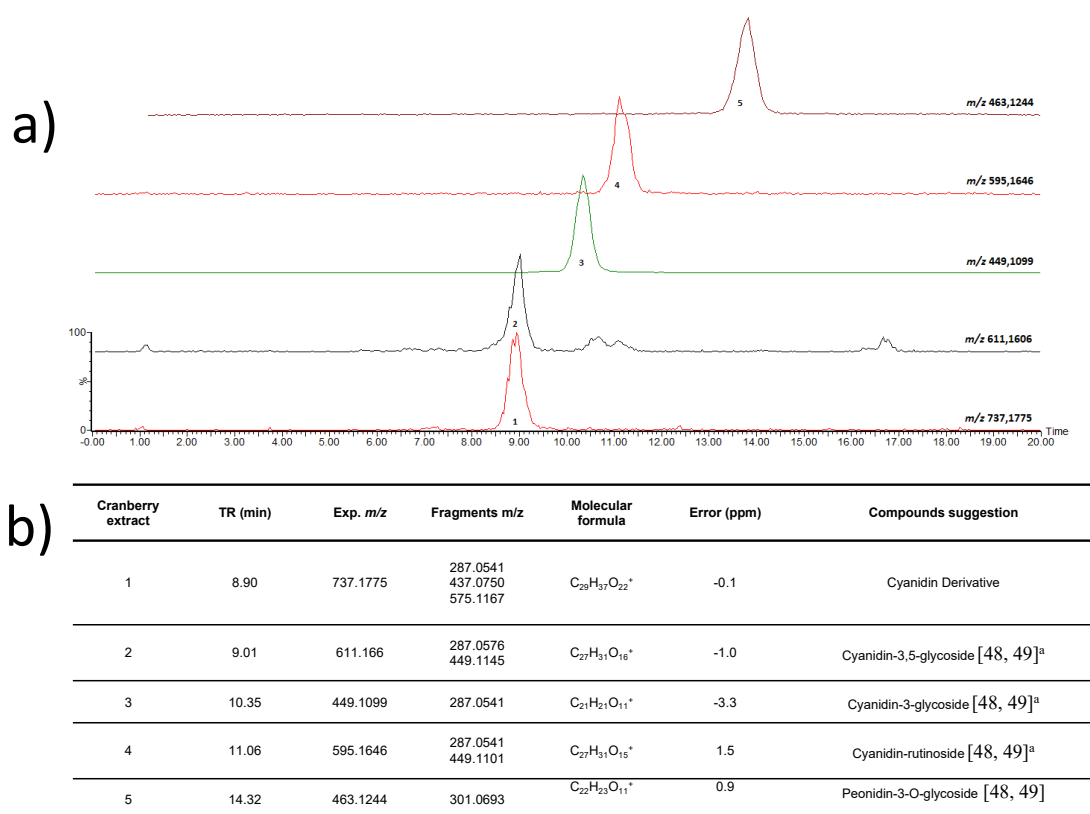


Fig1 a) Chromatographic profile of Cranberry extract depicting the ions extracted under the positive ionization mode. **b)** Phytoconstituents characterization of the of Cranberry extract. ^a injection verified with the assistance of an authentic sample.

Heightened concentrations of Cranberry extract were biocompatible

As depicted in Figure 2, concentrations of Cranberry extract ranging from 62.5 mg/mL to 500 mg/mL exhibited biocompatibility with osteoblasts and mesenchymal stromal cells since all concentrations promoted cell viability higher than 70% [50].

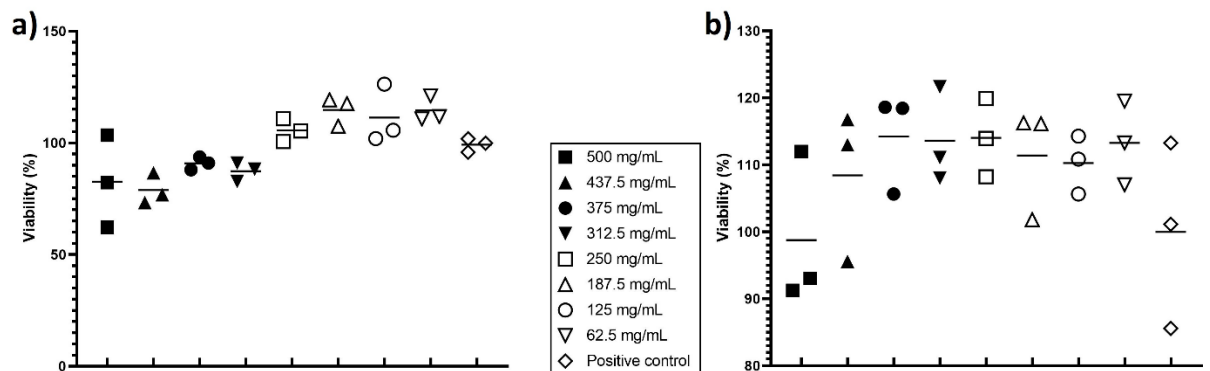


Fig2 Assessment of Cranberry extract biocompatibility in **a)** stromal cells from human exfoliated deciduous teeth (SHED) and **b)** Osteoblasts MC3T3-E1 through thiazolyl blue tetrazolium bromide (MTT) on day 3. Cell viability percentages were determined in reference to the cellular control (cell treated without Cranberry extract). Each shape format presents data from individual experiments with the median (n=3 for each experiment). Statistical analysis employed the Shapiro-Wilk and one-way ANOVA test. No significant difference was found among groups ($p > 0.05$).

Cranberry extract increased the alkaline phosphatase activity

As indicated in Figure 3, the presence of 20 mg/mL Cranberry extract resulted in a 2-fold increase in ALP activity compared to the positive control group (SHED treated with osteogenic medium, $p < 0.05$). This effect was consistent across all experimental time points. Furthermore, a correlation between the concentration of the Cranberry extract and ALP activity was identified ($r = 1$).

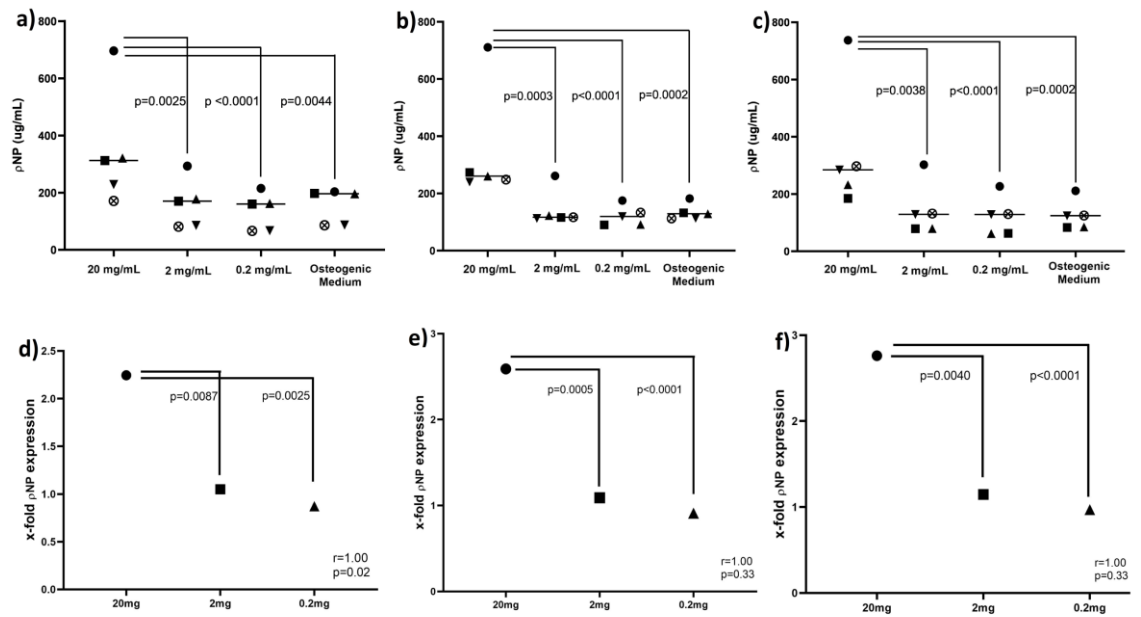


Fig3 Alkaline phosphatase (ALP) activity assessed by measuring p-nitrophenyl (pNP) produced from p-nitrophenyl phosphate (pPNPP) on days **a**) 3, **b**) 7, and **c**) 10 in stromal cells from human exfoliated deciduous teeth (SHED). Each shaped format represents data from individual experiments with the median (n=3 for each experiment). Statistical analysis was based on the kruskal-Wallis and Dunn's test. Cells were exposed to Cranberry extract (20 mg/mL, 2 mg/mL, or 0.2 mg/mL) or an osteogenic medium (Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum, 50 μ M ascorbic acid, and 10 mM β -glycerophosphate). Notably, 20 mg/mL Cranberry extract induced a 2-fold increase in ALP activity compared to SHED in osteogenic medium on days **d**) 3, **e**), 7, and **f**) 10.

Cranberry extract increased the bone morphogenetic protein-2 expression

On day 3, the presence of 20 mg/ mL Cranberry extract resulted in approximate 1.5-fold increase in BMP-2 expression compared to the positive control (SHED treated with osteogenic medium), as illustrated in Figure 5. Also, a modest correlation ($r= 0.6$, $\rho= 0.58$) between the Cranberry concentration and the BMP-2 expression was discerned.

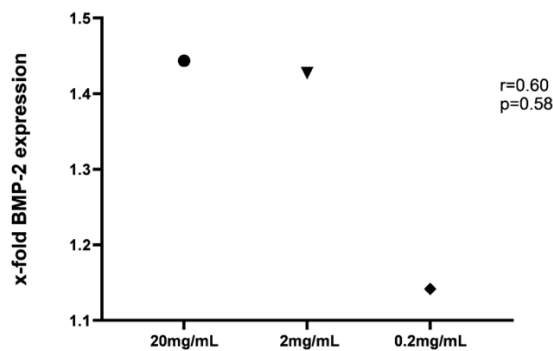


Fig4 Bone morphogenetic protein type 2 (BMP-2) expression assessed in stromal cells from human exfoliated deciduous teeth (SHED) on day 3. The normalized data presents the x-fold increase caused by the presence of Cranberry extract compared to the osteogenic medium (Dulbecco's Modified Eagles' Medium supplemented with 10% fetal bovine serum, 50 μ M ascorbic acid, and 10 mM β -glycerophosphate).

Cranberry extract increased the extracellular matrix mineralization

As depicted in Figure 6a and Figure 6b, treatment of cells with Cranberry extract resulted in elevated extracellular matrix mineralization, as evidenced by Alizarin Red S staining on day 15. Furthermore, the presence of 200 mg/mL of Cranberry extract prompted 1.7-fold increase in extracellular matrix mineralization relative to the positive control group, as evidenced in Figure 6c. Additionally, a correlation between the concentrations of the Cranberry extract and the levels of mineralization was identified ($r= 0.911$ $\rho= 0.001$).

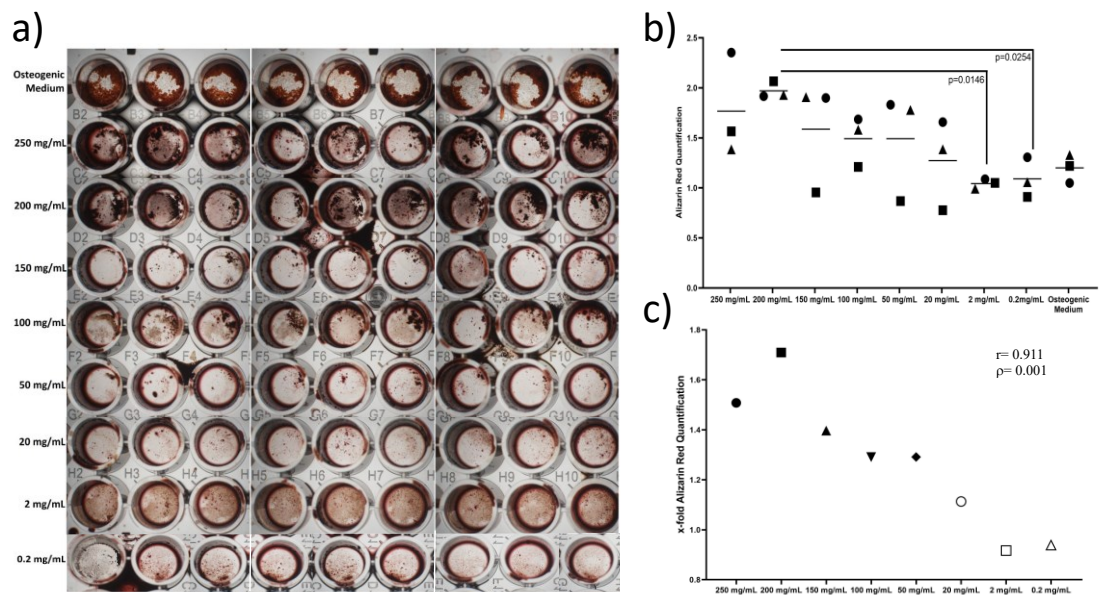


Fig5 a) This panel displays a representative image of Alizarin Red S staining conducted on day 15. Stromal cells from human exfoliated deciduous teeth (SHED) were subjected to various concentration of Cranberry extract (250 mg/mL, 200 mg/mL, 150 mg/mL, 100 mg/mL, 50 mg/mL, 20 mg/mL, 2 mg/mL, and 0.2 mg/mL) as well as an osteogenic medium (Dulbecco's Modified Eagles' Medium supplemented with 10% fetal bovine serum, 50 μ M ascorbic acid, and 10 mM β -glycerophosphate). **b)** Quantification of the Alizarin Red S. Each shaped format represents data from individual experiments with the median ($n=3$ for each experiment). The statistical analysis was performed using the Anderson-Darling test and one-way ANOVA test ($p < 0.05$). **c)** The normalized data presents the x-fold increase caused by the presence of Cranberry extract compared to the osteogenic medium.

DISCUSSION

Given the biological and technical challenges inherent to vertical bone augmentation procedures, the investigation of substances to be incorporated into scaffolds to enhance osteogenesis becomes imperative. Furthermore, considering the oral cavity's status as a contaminated environment, the incorporation of additional antimicrobial and anti-inflammatory properties would confer distinct advantages. In this context, our aim was to assess the potential of Cranberry extract to stimulate osteogenesis *in vitro*. Initially, we demonstrated that heightened concentrations of Cranberry extract (ranging from 62.5 to 500 mg/mL) exhibited biocompatibility with

osteoblasts and mesenchymal stromal cells. In terms of osteogenic potential, a concentration of 20 mg/mL of Cranberry extract prompted a 2-fold increase in ALP activity and a 1.5-fold increase in BMP-2 expression relative to the positive control group consisting of SHED treated with an osteogenic medium. Additionally, concentrations of 200 mg/mL of Cranberry extract induced approximately 1.7-fold increase in extracellular matrix mineralization. Furthermore, we established a correlation between the concentrations of the Cranberry extract and the levels of the early and late osteogenic markers.

It is imperative to underscore that, based on the parameters used to assess Cranberry extract quality in our study, the extract demonstrated high quality. Therefore, the analyzed Cranberry extract exhibited the presence of phenolic compounds devoid of interferents [46]. Additionally, this extract showcases a 140-fold surge in monomeric anthocyanin content in comparison to the minimum threshold required for indicating the authenticity of the Cranberry extract [47]. However, it is not possible to affirm that the effect of Cranberry observed was promoted by any of these compounds. Nevertheless, the specific compound within Cranberry responsible for mediating these osteogenic effects remains unidentified.

Hence, our findings revealed that the Cranberry extract led to heightened BMP-2 expression in comparison to cells treated solely with an osteogenic medium. This observation aligns with prior research that demonstrated the stimulation of ALP through anthocyanidins extracted from *Hibiscus syriacus* (Malvaceae) petals [51]. Additionally, proanthocyanidins were found to enhance ALP activity, contributing to osteogenic differentiation, in human cells derived from the periodontal ligament [52]. Likewise, grape-extracted proanthocyanidins were shown to stimulate BMP-2 gene expression [53]. These cumulative findings underscore the potential of the compounds within Cranberry extract to augment ALP and BMP-2 levels, in concordance with our results.

It is crucial to note that elevated concentrations of Cranberry extract exhibited biocompatibility with osteoblast and stromal cells. This, coupled with the observed correlation between the Cranberry extract concentrations and osteogenic markers, motivated us to explore the impact of elevated concentrations of Cranberry extract in promoting extracellular matrix mineralization. Therefore, concentrations of 200 mg/mL

of Cranberry extract induced approximately 1.7-fold increase in extracellular matrix mineralization compared to the positive control group.

This study presents the inaugural investigation into the osteogenic potential of Cranberry extract. Given that our primary objective did not encompass unraveling the precise mechanism underlying Cranberry-induced osteogenic differentiation, it remains unclear. Nonetheless, as previous alluded to, the effects of polyphenols from diverse sources have been investigated. Particularly noteworthy is their impact on osteoblasts mediated through diverse signaling pathways, including Wingless-INT/ β -catenin [54], insulin-like growth factor [55], bone morphogenetic proteins [56], Runt-related transcription factor 2 (Runx2) [57], and Osterix (OSX) [55].

Beyond its role in osteogenesis, it is pertinent to acknowledge that proanthocyanidins modulate the differentiation and physiology of osteoclastic cells, as well as bone matrix itself [32]. This influence stems from their ability to hinder the functional development of osteoclasts via the NF- κ B pathway and signaling pathways of mitogen-activated protein kinases (MAPKs), including c-Jun N-terminal kinase (JNK), which is responsible for activating the nuclear factor of activated T cells c1 (NFATc1), the primary gene regulator in osteoclast [33].

The potential of Cranberry extract to foster osteogenesis, coupled with the biocompatibility of e heightened Cranberry extract concentrations, offers promising prospects for integrating Cranberry into scaffolds intended for bone regeneration. Furthermore, the precise concentrations and amalgamations of Cranberry compounds that wield maximal efficacy, as well as the potential interactions with other factors, warrant further scrutiny. In summation, investigations into the effects of Cranberry on osteogenesis constitute an arena of ongoing scientific exploration. While the polyphenols found in Cranberries could indeed influence signaling pathways and enzyme activities related to bone formation, additional research is indispensable to comprehend and validate these effects.

CONCLUSIONS

According to the findings, Cranberry extract stimulated early and late markers of osteogenesis. Due to the biocompatibility of elevated Cranberry extract concentrations, as well as the antibacterial and anti-inflammatory properties

demonstrated in previous studies, the results observed here are promising for future application of Cranberry into scaffolds for bone regeneration.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

Yasmim Guterres Bauer declares that she has no conflict of interest. Eduarda Blasi Magini declares that she has no conflict of interest. Ingrid Vicente Farias declares that she has no conflict of interest. Flávio Henrique Reginatto declares that he has no conflict of interest. Izabella Thais Silva declares that she has no conflict of interest. Ariadne Cristiane Cabral Cruz declares that she has no conflict of interest.

ETHICAL APPROVAL

This study does not contain any studies with human participants or animals performed by any of the authors.

INFORMED CONSENT

The formal consent is not required for this type of study.

AUTHORS CONTRIBUTION

All authors contributed to the conception and design. Material preparation, data collection and analysis were performed by Y.G.B., E.B.M., I.V.F., and A.C.C.C. The first draft of the manuscript was written by Y.G.B. and A.C.C.C., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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LIST OF FIGURE LEGENDS

Fig1 **a)** Chromatographic profile of Cranberry extract depicting the ions extracted under the positive ionization mode. **b)** Phytoconstituents characterization of the of Cranberry extract. ^ainjection verified with the assistance of an authentic sample.

Fig2 Assessment of Cranberry extract biocompatibility o in **a)** stromal cells from human exfoliated deciduous teeth (SHED) and **b)** Osteoblasts MC3T3-E1 through thiazolyl blue tetrazolium bromide (MTT) on day 3. Cell viability percentages were determined in reference to the cellular control (cell treated without Cranberry extract). Each shape format presents data from individual experiments with the median (n=3 for each experiment). Statistical analysis employed the Shapiro-Wilk and one-way ANOVA test. No significant difference was found among groups ($p > 0.05$).

Fig3 Alkaline phosphatase (ALP) activity assessed by measuring p-nitrophenyl (pPNP) produced from p-nitrophenyl phosphate (pPNPP) on days **a)** 3, **b)** 7, and **c)** 10 in stromal cells from human exfoliated deciduous teeth (SHED). Each shaped format represents data from individual experiments with the median (n=3 for each experiment). Statistical analysis was based on the kruskal-Wallis and Dunn's test. Cells were exposed to Cranberry extract (20 mg/mL, 2 mg/mL, or 0.2 mg/mL) or an osteogenic medium (Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum, 50 μ M ascorbic acid, and 10 mM β -glycerophosphate). Notably, 20 mg/mL Cranberry extract induced a 2-fold increase in ALP activity compared to SHED in osteogenic medium on days **d)** 3, **e)** 7, and **f)** 10.

Fig4 Bone morphogenetic protein type 2 (BMP-2) expression assessed in stromal cells from human exfoliated deciduous teeth (SHED) on day 3. The normalized data presents the x-fold increase caused by the presence of Cranberry extract

compared to the osteogenic medium (Dulbecco's Modified Eagles' Medium supplemented with 10% fetal bovine serum, 50 μ M ascorbic acid, and 10 mM β -glycerophosphate).

Fig5 a) This panel displays a representative image of Alizarin Red S staining conducted on day 15. Stromal cells from human exfoliated deciduous teeth (SHED) were subjected to various concentration of Cranberry extract (250 mg/mL, 200 mg/mL, 150 mg/mL, 100 mg/mL, 50 mg/mL, 20 mg/mL, 2 mg/mL, and 0.2 mg/mL) as well as an osteogenic medium (Dulbecco's Modified Eagles' Medium supplemented with 10% fetal bovine serum, 50 μ M ascorbic acid, and 10 mM β -glycerophosphate). **b)** Quantification of the Alizarin Red S. Each shaped format represents data from individual experiments with the median (n=3 for each experiment). The statistical analysis was performed using the one-way ANOVA test ($p < 0.05$). **c)** The normalized data presents the x-fold increase caused by the presence of Cranberry extract compared to the osteogenic medium.

5 CONCLUSÃO

De acordo com os resultados, o extrato de *cranberry* estimulou os marcadores precoces da osteogênese. No entanto, este efeito não foi observado para o marcador de osteogênese tardia. Devido à biocompatibilidade de concentrações elevadas do extrato de *cranberry*, bem como às propriedades antibacterianas e anti-inflamatórias demonstradas em estudos anteriores, os resultados aqui observados são promissores para futuras aplicações do *cranberry* carregado em arcabouços para regeneração óssea. Além disto, é importante destacar que o extrato de *cranberry* é um produto natural, de fonte renovável e com custo acessível.

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