UNIVERSIDADE FEDERAL DE UBERLÂNDIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

DEPARTAMENTO DE ODONTOPEDIATRIA

NILSON FERREIRA DE OLIVEIRA NETO

Própolis vermelha brasileira: ação antibacteriana contra patógenos endodônticos e avaliação *in vitro* da produção de radicais livres

Brazilian red propolis: antibacterial activity against endodontic pathogens and in vitro evaluation of free radicals production

Dissertação apresentada à Faculdade de Odontologia da Universidade Federal de Uberlândia como requisito parcial para obtenção do título de mestre em Clínica Odontológica Integrada.

Uberlândia

2021

NILSON FERREIRA DE OLIVEIRA NETO

Própolis vermelha brasileira: ação antibacteriana contra patógenos endodônticos e avaliação *in vitro* da produção de radicais livres

Brazilian red propolis: antibacterial activity against endodontic pathogens and in vitro evaluation of free radicals production

Dissertação apresentada à Faculdade de Odontologia da Universidade Federal de Uberlândia como requisito parcial para obtenção do título de mestre em Clínica Odontológica Integrada.

Orientadora: Profª. Drª. Ana Paula Turrioni Hidalgo
Coorientador: Prof. Dr. Marcelo José Barbosa Silva

Banca examinadora

Profª. Drª. Ana Paula Turrioni Hidalgo
Profª. Drª. Paula Dechichi
Profª. Drª. Manoela Domingues Martins

Uberlândia
2021

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Coordenação do Programa de Pós-Graduação em Odontologia
Av. Pará, 1720, Bloco 4L, Anexo B, Sala 35 - Bairro Umuarama, Uberlândia-MG, CEP 38400-902
Telefone: (34) 3225-8115/8108 - www.ppgoufu.com - copod@umuarama.ufu.br

ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Odontologia				
Defesa de:	Dissertação de Mestrado Acadêmico, 397, PPGODONTO				
Data:	Vinte e Sete de Setembro de Dois Mil e Vinte e Um	Hora de início:	14:00	Hora de encerramento:	[15:55]
Matrícula do Discente:	11912ODO015				
Nome do Discente:	Nilson Ferreira de Oliveira Neto				
Título do Trabalho:	Própolis vermelha brasileira: ação antibacteriana contra patógenos endodônticos e avaliação da produção de radicais livres em modelo *ex vivo*				
Área de concentração:	Clínica Odontológica Integrada				
Linha de pesquisa:	Processo de Reparo				
Projeto de Pesquisa de vinculação:	Processo de Reparo				

Reuniu-se em Web Conferência pela plataforma Zoom, em conformidade com a PORTARIA Nº 36, DE 19 DE MARÇO DE 2020 da COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR - CAPES, pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Odontologia, assim composta: Professores Doutores: Paula Dechichi Barbar (UFU); Manoela Domingues Martins (UFRGS); Ana Paula Turrioni Hidalgo (UFU) orientadora do candidato.

Iniciando os trabalhos a presidente da mesa, Dra. Ana Paula Turrioni Hidalgo, apresentou a Comissão Examinadora e o candidato(a), agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

Aprovado(a).

Esta defesa faz parte dos requisitos necessários à obtenção do título de Mestre.

O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

Documento assinado eletronicamente por **Ana Paula Turrioni Hidalgo**, **Professor(a) do Magistério Superior**, em 27/09/2021, às 15:56, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Paula Dechichi Barbar**, **Professor(a) do Magistério Superior**, em 27/09/2021, às 15:57, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Manoela Domingues Martins**, **Usuário Externo**, em 27/09/2021, às 15:57, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site https://www.sei.ufu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **3045617** e o código CRC **CA736C4B**.

Referência: Processo nº 23117.060985/2021-53 SEI nº 3045617

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
com dados informados pelo(a) próprio(a) autor(a).

O48
2021

Oliveira Neto, Nilson Ferreira de, 1995-
Própolis vermelha brasileira: ação antibacteriana contra patógenos endodônticos e avaliação in vitro da produção de radicais livres [recurso eletrônico] / Nilson Ferreira de Oliveira Neto. - 2021.

Orientadora: Ana Paula Turrioni Hidalgo.
Coorientador: Marcelo José Barbosa Silva.
Dissertação (Mestrado) - Universidade Federal de Uberlândia, Pós-graduação em Odontologia.
Modo de acesso: Internet.
Disponível em: http://doi.org/10.14393/ufu.di.2021.549
Inclui bibliografia.
Inclui ilustrações.

1. Odontologia. I. Hidalgo, Ana Paula Turrioni,1985-, (Orient.). II. Silva, Marcelo José Barbosa,1976-, (Coorient.). III. Universidade Federal de Uberlândia. Pós-graduação em Odontologia. IV. Título.

CDU: 616.314

Bibliotecários responsáveis pela estrutura de acordo com o AACR2:

Gizele Cristine Nunes do Couto - CRB6/2091

Aos meus anjinhos aqui na Terra, àqueles que me apoiam e iluminam cada um dos meus dias. Mamãe, Papai e Bruninha, eu dedico cada palavra aqui escrita a vocês.

AGREDECIMENTOS

Agradeço, primeiramente, à Deus e ao universo por terem me permitido vivenciar cada segundo dessa experiência única e transformadora.

Aos meus amados: mamãe, papai e Bruninha, os quais trabalham incansavelmente por 14 longas horas diárias, e que ao final de cada dia, conseguem encontrar forças para sorrir e me abraçar. Mesmo não compreendendo o meu universo em sua totalidade ou a cultura de células pulpares, assim como as propriedades da própolis e o meu amor pela ciência, eles seguem acreditando, apoiando e motivando cada um dos meus sonhos. Esse amor me salva e me cura.

Ao meu príncipe, Akylys Barbosa de Camargos, por trazer paz, paciência e positividade ao meu coração, por segurar firme a minha mão e colocar o medo e a insegurança para darem uma volta apenas com passagem de ida. Por olhar nos meus olhos e enxergar a minha alma de uma forma tão bonita. Eu sou um homem melhor, mais feliz e abençoado por ter você comigo.

Aos grandes amores da minha vida: Sol, Lua e Flora por repousarem no meu colo nas longas noites em claro e dizerem com o seu silenciozinho que estarão ao meu lado independentemente das circunstâncias e desafios que eu decidir enfrentar. Obrigado pela leveza que vocês me proporcionam e por me deixar amá-las e cuidar de vocês.

Aos melhores amigos de toda a galáxia, em especial à Raquel Sousa, Stephany Yasmine, Camila Gontijo, Stephanie Wutke, Rafaela de Oliveira, Kamilla França, Rafael Correa, Bárbara Mattos, Carolina Nunes, Yan Kleber, João Marcos e Clara Saraiva por serem a personificação de amor em meio a tanto barulho.

Aos meus padrinhos queridos, Lucineide Vieira Coelho e Ailton Vieira Coelho, e também, aos meus vovôs Zenaide Vieira Ramos e Cosme Antônio Coelho e aos tios, primos e a toda minha família incrível, por cada oração e energias positivas enviadas a mim.

À minha incrível orientadora, Ana Paula Turrioni, pela paciência e tato comigo durante todo o tempo em que estivemos juntos. Você é força. Você é inspiração. Ter escolhido ficar em Uberlândia no mestrado foi uma das melhores decisões que tive pois escolhi ficar com você. Você deixou marcas tão bonitas no meu coração, marcas estas que estão eternizadas.

Ao professor Marcelo José Barbosa Silva, agradeço-o pela coorientação que despertou em mim extrema admiração e carinho pelo senhor. Obrigado por ter cedido, tão gentilmente, o seu espaço para que pudéssemos realizar todos os nossos experimentos. Sua ajuda viabilizou a conclusão deste estudo e de tantos outros que realizamos neste período.

Ao querido professor Carlos Henrique Gomes Martins, por ter trago imensa contribuição ao nosso trabalho. Por sempre ser tão disponível e nos receber com uma energia contagiante. Você, rapidamente, se tornou uma referência pra mim como educador, pesquisador e ser humano.

Aos meus cientistas preferidos: Jéssica Bonvicini, Gabriella Leite, Caio Candeiro e Bruna Borges por trazerem não só um jaleco para o dia a dia no laboratório e sim uma mochila recheada de boas risadas e muito amor, além de uma outra cheia de parceria e dedicação extrema a tudo que se propõem a realizar.

Às professoras Ana Paula Turrioni, Alessandra Castro, Manoela Domingues Martins, Paula Dechichi e Rainde Naiara Rezende por terem semeado valores preciosos no meu peito e terem me ajudado a construir o profissional que sou hoje.

Ao Programa de pós-graduação em Odontologia da Universidade Federal de Uberlândia, por nos proporcionar um ambiente criativo e repleto de oportunidades. Tenho muito carinho e orgulho de minha casa. Sou extremamente convicto que tive a melhor formação possível e que me despeço com o coração inundado de gratidão.

Ao Centro de Pesquisa Odontológico de Biomecânica, Biomateriais e Biologia celular da Faculdade de Odontologia da Universidade Federal de Uberlândia (CPbio/FOUFU) por acolher as nossas ideias, tirá-las do papel e colocá-las em prática.

Às agências de fomento brasileiras, que nos permitem fazer ciência dentro das universidades públicas, pela concessão de minha bolsa de mestrado (CAPES) e pelo financiamento (CNPq e FAPEMIG) deste e demais estudos realizados.

Agradeço imensamente às maravilhosas técnicas-administrativas de nosso programa: Laís e Brenda, pela eficiência e disponibilidade em sempre nos ajudar.

A todos os professores e funcionários presentes nesse período e que irão ser lembrados com muito carinho.

sou ferida aberta
sou garra
sou paixão
sou treva
sou grande
sou erro
soy libre
linda
loca
sou o que quero ser
derrapo
não paro
nessa selvageria
ninguém me devora
me salvo
me viro
sou minha
sou mulher
sou tudo
o que eu quiser.
Ryane Leão

SUMÁRIO

RESUMO

A manutenção da vitalidade pulpar é o principal objetivo das terapias pulpares vitais (TPV) e a seleção de um bom material irá contribuir para a longevidade do tratamento. A própolis vermelha brasileira é um excelente agente antimicrobiano e poderia ser utilizada como agente terapêutico em terapias pulpares. O presente estudo analisou a ação antibacteriana do extrato bruto da própolis vermelha Brasileira (PVB) e o seu efeito em contato direto com fibroblastos humanos da polpa dentária (FHPD), em comparação ao material padrão-ouro para terapias vitais pulpares: o Agregado Trióxido Mineral (MTA). A Concentração Inibitória Mínima (CIM) e a Concentração Bactericida Mínima da PVB foi determinada contra patógenos endodônticos anaeróbios. Os FHPD foram semeados em placas de 96 poços e colocados em contato, após 24h do plaqueamento, com PVB10 (10 μg/mL), PVB50 (50 μg/mL), MTA em diferentes diluições (1:1, 1:2, 1:4 e 1:8), dimetilsulfóxido a 0,5% (DMSO 0,5%) e meio de cultura (DMEM). Após 24h da exposição aos materiais, os grupos foram submetidos ao ensaio de viabilidade celular (MTT formazan) e produção de radicais livres (espécies reativas de oxigênio – EROS, sonda fluorescente 2,7-diclorodihidrofluoresceína-diacetato (DCFH-DA) e óxido nítrico - NO, reação de Griess). Os testes ANOVA One way e Tukey foram realizados considerando um nível de significância de 5%. Os valores de CIM/CBM para a PVB demonstraram ação antibacteriana para a maioria das bactérias testadas: *P. micra* (6,25/6,25 μg/mL), *F. nucleatum* (25/25 μg/mL) *P. nigrescens* (50/100 μg/mL), *P.melaninogenica* (50/100 μg/mL), *P. intermedia* (50/100 μg/mL) e *P. gingivalis* (50 μg/mL). Tanto o MTA quanto a PVB estimularam a viabilidade celular, destacando-se a PVB10 e o MTA 1:8 ($p=0.007$ e $p=0.001$, respectivamente). Em relação à produção de EROS e ON, foi observado que os grupos MTA 1:1, MTA 1:2 e PVB50 elevaram sutilmente a produção de EROS (p<,001) e ON (p=0.008, p=0.007 e p<.001, respectivamente) comparado ao grupo DMEM. Por outro lado, a PVB10 manteve os níveis de produção de EROS ($p=0,976$) e ON ($p=0,974$) semelhantes ao grupo DMEM. Portanto, a PVB se mostrou como um relevante agente antibacteriano para bactérias anaeróbias relacionadas à infecção endodôntica primária e, além disso, tanto o MTA quanto a PVB foram capazes de estimular a viabilidade dos FHPD sem aumentar consideravelmente a produção dos níveis de ON e EROS.

Palavras-chave: Própolis, Agente Antibacteriano, Radicais Livres.

ABSTRACT

Maintenance of pulp vitality is one of the purposes of vital pulp therapies (VPT) and selection of a good material strongly affects the long-term outcomes. Brazilian red propolis (BRP) is a great antimicrobial agent and may provide therapeutic solutions for pulp therapy. We hypothesized that BRP could show promising antibacterial activity and perform similar behavior with Mineral Trioxide Aggregate (MTA) in free radicals production and cell viability findings. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of BRP was determined against anaerobic endodontic pathogens. Human dental pulp fibroblasts (HDPFs) were seeded in 96 well-plates and exposed, after 24h, to BRP10 (10 µg/mL), BRP50 (50µg/mL), MTA extracts (1:1, 1:2, 1:4 e 1:8), dimethyl sulfoxide 0,5 % (DMSO) and cell culture medium (DMEM). Subsequently, 24h after the exposure to materials, the groups were tested for cell viability (MTT formazan assay), and free radicals production (reactive oxygen species - ROS, 2',7'-dichlorodihydrofluorescein diacetate fluorescent probe (DCFH-DA) and nitric oxide – NO, Griess reagent). The One-way ANOVA and Tukey's tests were employed considering significance level of 5%. MIC/MBC values of BRP performing antibacterial activity for *P. micra* (6.25/6.25 µg/mL), *F. nucleatum* (25/25 µg/mL), *P. melaninogenica* (50/100 µg/mL), *P. nigrescens* (50/100 µg/mL), *P. intermedia* (50/100 µg/mL) and *P. gingivalis* (50/200 µg/mL). In our cell viability findings, BRP and MTA were able to stimulate cell viability, emphasizing BRP10 and MTA 1:8 (*p=0.007* and *p=0.001*, respectively). Furthermore, it was observed that MTA 1:1, MTA 1:2 and BRP50 slightly increased ROS ($p<.001$) and NO production ($p=0.008$, $p=0.007$ and $p<.001$ respectively) compared to DMEM group. On the other hand, BRP10 did not raised the amount of ROS (*p=0.976*) and NO (*p=0.974*) production compared to DMEM. Therefore, BRP presented high antibacterial activity for anaerobic bacteria involved in primary endodontic infection and, both BRP and MTA promoted the viability of HDPFs without greatly increased NO and ROS production.

Keywords: Propolis, Antibacterial Agent, Cytotoxicity; Free Radicals.

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

As terapias vitais pulpares (TVPs) possuem o objetivo de manter a vitalidade de dentes decíduos e permanentes jovens em casos de exposições pulpares por lesões cariosas extensas, traumas ou iatrogenias através da utilização de um material biocompatível (Smaïl-Faugeron *et al*., 2014). O agregado trióxido mineral (MTA) é o padrão ouro para as TVPs, apresenta estímulo à diferenciação odontogênica e ausência de toxicidade (Li *etal*., 2019). Contudo, possui limitações relacionadas à alteração de cor, tempo de presa extenso, custo elevado e atividade anti-inflamatória reduzida (Shi B *et al.*, 2020).

A investigação de produtos naturais vem sendo realizada na Odontologia com o intuito de se obter materiais com melhor performance clínica, reduzida toxicidade e preços acessíveis à população (da Silva Barboza *et. al,* 2021). Neste contexto, a própolis brasileira é um conjunto de materiais resinosos coletados de diferentes fontes vegetais e elaborada por abelhas da espécie *Apis mellifera* a partir de exsudatos resinosos de cascas e botões florais. Essas substâncias são biomodificados pela adição de cera e pela ação da enzima 13-glicosidase presente nas secreções salivares das abelhas (Sousa*et al.*, 2007; Peter *et al.,* 2017; Cardoso *et al.,* 2015).

A composição química da própolis é complexa e varia de acordo com a flora da região de onde é produzida (Ítavo *et al*., 2009), assim como a época da coleta e características genéticas da abelha produtora. Esses fatores influenciam diretamente nos princípios ativos presentes na própolis, refletindo na atividade biológica e farmacêutica que os diferentes produtos apresentam (Castro *et al*., 2009).

Os principais componentes da própolis brasileira consistem em resinas e bálsamos aromáticos, ceras, óleos essenciais, grãos de pólen, compostos fenólicos (flavonóides e ácidos fenólicos), minerais e vitaminas. A própolis brasileira é classificada do tipo 1 ao 13, considerando características químicas, espécie produtora e diversidade do clima no país. A própolis vermelha, marrom e verde são alvos da maioria dos estudos relacionados às propriedades biológicas desse produto (Araújo *et al*., 2014).

A própolis verde brasileira (PVEB) é a espécie mais investigada e, também, a mais popular. Ela é encontrada no sudeste brasileiro e possui como princípio ativo a artepelina C e se origina a partir da *Baccharis dracunculifolia* (Cardoso *et al.,* 2015). Estudos mostraram que a PVEB foi capaz de inibir a progressão de câncer pulmonar (Kimoto *et al*., 2001), atividade antiviral (Gekker et al., 2005) e atividade

anticariogênica contra *Streptococus mutans*, *Streptococus sobrinus, Streptococus salivaris, Streptococus sanguinis* e *Lactobacillus casei* (Koo *et al.*, 2000; De Luca *et al*., 2014), assim como ação antifúngica em relação à *Candida albicans* (Berretta *et al.,* 2013).

A própolis marrom brasileira (PMB), apesar de comprovada antigenotoxicidade (Fernandes *et al.*, 2014; Fernandes *et al.*, 2015), atividade antimicrobiana (Bankova *et al*., 1996; Silva *et al*., 2012), esta não é tão investigada quanto à verde e vermelha. Ela é encontrada no sul do Brasil, apresenta em sua composição química principalmente benzofenonas poli-isopreniladas e têm origem botânica a partir da *Clusiarosea* pertencente à família *Gutiiferae* (Herrera, 2016).

A própolis vermelha brasileira (PVB) encontrada no litoral nordestino difere dos demais tipos devido à sua origem, sendo derivada, principalmente, do exsudato resinoso vermelho da superfície da *Dalbergia ecastophyllum* (Cardoso *et al.*, 2015). Atividade antimicrobiana, antifúngica, anti-inflamatória, antioxidante e efeito citotóxico frente a linhagens tumorais já foram descritos (Moise *et al.,* 2020).

Acredita-se que tal potencial se deva ao sinergismo existente entre seus compostos aromáticos (Machado *et al.,* 2016) como a formonetina, a pinocembrina, o ácido cafeico, a liquiritigenina, o neovestitol, os flavonóides (apigenina, flavonas, chalconas) e o vestitol (Machado *et al.,* 2017). Tais compostos não são encontrados nas demais própolis, o que confere à PVB uma composição química única (De Pontes *et al*., 2018). Além disso, os terpenos (terpineol, cânfora, ferruginol, ácido junicédrico, lanosterol e valenceno), isoflavonóides (medicarpina e isoflavona), benzofenonas preniladas e naftoquinonas já foram documentados (Trusheva *et al*., 2006; Park *et al*., 2002).

A despeito da presença de compostos anti-inflamatórios e antibacterianos, poucos estudos investigaram os efeitos da PVB em tecidos dentais, portanto, o presente estudo, pela primeira vez na literatura, pretende comparar as propriedades biológicas da PVB e do MTA em contato direto com fibroblastos humanos da polpa dentária, assim como testar a atividade antibacteriana da PVB em relação a nove bactérias envolvidas na infecção endodôntica primária.

2. **CAPÍTULO 1:** *Brazilian red propolis: antibacterial activity against endodontic pathogens and in vitro evaluation of free radicals production**

Oliveira Neto NF[a], Bonvicini JFS[a], Souza GL[b], Santiago MB[c], Veneziani RCS[d], Ambrósio SR[d], Bastos JK[e], Silva MJB[f], Martins CHG[c], Moura CCG[b], Turrioni AP[a].

[a] *Department of Pediatric Dentistry, School of Dentistry, UFU – State of Minas Gerais, Uberlândia, MG, Brazil.*

[b] *Department of Endodontics, School of Dentistry, UFU – State of Minas Gerais, Uberlândia, MG, Brazil.*

[c] *Department of Microbiology, Institute of Biomedical Sciences, UFU – State of Minas Gerais, Uberlândia, MG, Brazil.*

[d] *Nucleus of Research in Sciences and Technology, UNIFRAN – State of São Paulo, Franca, SP, Brazil*

[e] *Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, USP – State of São Paulo, Ribeirão Preto, SP, Brazil.*

[f] *Department of Immunology, Institute of Biomedical Sciences, UFU – State of Minas Gerais, Uberlândia, MG, Brazil.*

Corresponding author

Ana Paula Turrioni
Faculdade de Odontologia, Universidade Federal de Uberlândia – UFU
Departamento de Odontologia Pediátrica
Avenida Pará, 1720, Bloco 2G sala 02 - Campus Umuarama, CEP 38405-320
Tel: +55 (34) 3225-8146
e-mail: apturrioni@ufu.br

**Artigo a ser submetido no periódico: Journal of Endodontics*

ABSTRACT

Introduction: Maintenance of pulp vitality is one of the purposes of vital pulp therapies (VPT) and selection of a good material strongly affects the long-term outcomes. Brazilian red propolis (BRP) is a great antimicrobial agent and may provide therapeutic solutions for pulp therapy. We hypothesized that BRP could show promising antibacterial activity and perform similar behavior with mineral trioxide aggregate (MTA) in free radicals production and cell viability findings. **Methods:** The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of BRP was determined against anaerobic endodontic pathogens. Human dental pulp fibroblasts (HDPFs) were seeded in 96 well-plates and exposed, after 24h, to BRP10 (10 µg/mL), BRP50 (50µg/mL), mineral MTA extracts (1:1, 1:2, 1:4 e 1:8), dimethyl sulfoxide 0,5 % (DMSO) and cell culture medium (DMEM). Subsequently, 24h after the exposure to materials, the groups were tested for cell viability (MTT formazan assay), and free radicals production (reactive oxygen species - ROS, 2',7'-dichlorodihydrofluorescein diacetate fluorescent probe (DCFH-DA) and nitric oxide – NO, Griess reagent). The One-way ANOVA and Tukey's tests were employed considering significance level of 5%. **Results:** MIC/MBC values of BRP performing antibacterial activity for *P. micra* (6.25/6.25 µg/mL), *F. nucleatum* (25/25 µg/mL), *P. melaninogenica* (50/100 µg/mL), *P. nigrescens* (50/100 µg/mL), *P. intermedia* (50/100 µg/mL) and *P. gingivalis* (50/200 µg/mL). In our cell viability findings, BRP and MTA were able to stimulate cell viability, emphasizing BRP10 and MTA 1:8 (*p=0.007* and *p=0.001*, respectively). Furthermore, it was observed that MTA 1:1, MTA 1:2 and BRP50 slightly increased ROS ($p<.001$) and NO production ($p=0.008$, $p=0.007$ and $p<.001$ respectively) compared to DMEM group. On the other hand, BRP10 did not raised the amount of ROS (*p=0.976*) and NO (*p=0.974*) production compared to DMEM. **Conclusions:** Therefore, BRP presented high antibacterial activity for anaerobic bacteria involved in primary endodontic infection and, both BRP and MTA promoted the viability of HDPFs without greatly increased NO and ROS production.

Keywords: Propolis, Antibacterial Agent, Cytotoxicity; Free Radicals.

INTRODUCTION

Vital pulp therapy (VPT) is indicated to preserve the vitality of the remaining pulp tissue of primary and immature permanent teeth[1]. The selection of the material for this therapy can affect the success rate of VPTs[2]. Currently, the material of choice has been the Mineral Trioxide Aggregate (MTA), a bioactive cement that became a substitute for calcium hydroxide with better capability in disinfection, biocompatibility and lack of cytotoxicity[3]. Additionally, MTA has shown favorable properties in pulpotomies[4], perforation repairs in roots[5] and apexification procedure[6].

The search for natural products with lower toxicity, better therapeutic activity and potentially more approachable prices to the population have been performed in dentistry, especially, due to the increased popular acceptance for natural medicine[7]. In this scenario, Brazilian red propolis (BRP), found on the northeastern coast, differs from other types due to its origin which is derived mainly from the red resinous exudate on the surface of *Dalbergia ecastophyllum*[9]. Pharmacological properties of clinical interest such as anticaries effect; antibacterial, antivirus and antifungal activity; anti-inflammatory and immunomodulatory effects, antioxidant and cytotoxic activity against tumor lineages have already been described[10].

Researchers had demonstrated antibacterial activity of propolis with satisfactory results for *A. naeslundii*, *P. oralis*, *P. melaninogenica*, *P. gingivalis*, *F. nucleatum* and *V. parvula* due to biological activity of flavonoids and aromatic compounds[11]. Additionally, it was seen that Iranian propolis was able to promote anti-inflammatory effects on murine macrophage cell line (RAW 264.7) exposed to lipopolysaccharide (LPS) reducing free radicals (nitric oxide – NO and reactive oxygen species – ROS) production, also *cyclooxygenase*-2 (COX-2), interleukin-1β (IL-1β) and interleukin-6 (IL-6) expression[12]. Another study demonstrated that BRP was able to promote anti-inflammatory effects on LPS activated peritoneal macrophages by decreasing NO levels and reduced the production and expression of pro-inflammatory cytokine and genes[13].

Thus, we investigated, for the first time, the antibacterial activity of crude extract of BRP against anaerobic bacteria involved in primary endodontic infection and also cell viability and free radicals production (NO and ROS) compared to MTA in direct contact with human dental pulp fibroblasts (HDPFs). The null hypothesis was that BRP could show promising antibacterial activity and perform similar behavior with MTA regarding ROS and NO production and cell viability findings.

MATERIAL AND METHODS

Hydroalcoholic extract of BRP

BRP was obtained from the association of beekeepers of Canavieiras (Cooperativa de Apicultores de Canavieiras—COAPER, Bahia, Brazil) from March 2019 to February 2020. Concerning the extraction, the BRP samples were frozen and minced. Two hundred grams of BRP were submitted to dynamic maceration at 30 °C and 120 rpm using a shaker incubator (INNOVA 4300), with 70% hydroalcoholic ethanol solution in the ratio of 1:10 (w/v). The extracts attained were concentrated under vacuum using a rotary evaporator and then lyophilized to complete dryness. Previously, our research group isolated and identified the main compounds presented in this sample of BRP (SisGen: AF234D8), which are flavonoids and derivatives such as liquiritigenin, biochanin A, isoliquiritigenin, formononetin, calycosin, vestitol, neovestitol, 7-O-methylvestitol and medicarpin[14].

Bacterial strains

To test BRP against anaerobic endodontic pathogens, five standard strains from the American Type Culture Collection (ATCC, Manassas, VA, USA) and four clinical isolates (CI) were used, namely *Prevotella melaninogenica* (ATCC 25845*), Actinomyces viscosus* (ATCC 43146), *Prevotella nigrescens* (ATCC 33563), *Porphyromonas endodontalis* (ATCC 35406), *Veillonella parvula* (ATCC 17745), *Porphyromonas gingivalis* (CI), *Prevotella intermedia* (CI), *Parvimonas micra* (CI), *Fusobacterium nucleatum* (CI). The evaluated clinical isolates were obtained from clinical trials and kept in the Laboratory of Research on Antimicrobial Trials library under cryopreservation.

Determination of the Minimum Inhibitory Concentration (MIC) and of the Minimum Bactericidal Concentration (MBC)

MIC was determined in triplicate by using Broth microdilution method in 96-well microplates, as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. For this purpose, BRP samples were prepared at concentrations ranging from 0.195 to 400.0 µg/mL. The culture media employed for the representative strains of endodontic infections were Schadler broth or Schadler agar (Difco, Kansas City, MO, USA), both supplemented with hemin (5 µg/mL, Sigma-Aldrich, San Louis, Missouri, USA), and horse blood (5%), as recommended by Clinical Laboratory Standards Institute (CLSI, 2007)[15]. Samples were dissolved in dimethyl sulfoxide (1 mg/mL, DMSO, LGC Biotecnologia, Cotia, São Paulo, Brazil) and diluted in the desired broth. The final DMSO content was 5% (v/v) and this solution was used as negative control while metronidazole (Sigma) was used as an assay control. The inoculum was adjusted for each organism to yield a cell concentration of 5×10^6 CFU/mL. The strains were incubated in an anaerobic chamber (Don Whtiley Scientific, Bradford, UK) for 72h under an adequate atmosphere containing 5-10% H_2, 10% CO_2, and 80-85% N_2. Thereafter, resazurin (30 µL, Sigma) diluted in an aqueous solution (0.02%) was added to the microplates, to indicate microorganism viability[16]. The development of a blue and pink color indicated the absence and presence of bacterial growth, respectively. To determine MBC, an aliquot of the inoculum was removed from each well before the addition of resazurin and seeded in an appropriate culture medium. MBC was defined as the lowest concentration of the sample where no bacterial growth occurred.

MTA extracts

In this study, white MTA (Angelus, Londrina, Paraná, Brazil) was used to prepare the extracts. MTA samples were prepared in 24-well plates according to the manufacturer's recommendations and incubated at 37 ºC for 24 hours immediately after mixing. Specimens were then covered with 2.5 mL of cell culture Dulbecco modified Eagle medium (DMEM - LGC Biotecnologia, Cotia, São Paulo, Brazil) and incubated at 37 ºC in the dark for 24 hours[17]. After incubation, the original extract (1:1) was prepared according to ISO 10993-5 recommendations[18] and then, serially diluted (1:1; 1:2; 1:4; 1:8) in cell culture medium before testing[19].

HDPFs culture

This study was accomplished with a primary culture of HDPFs according to the methodology applied in previous studies[20-22]. The HDPFs were grown in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Following approval of the research project by the Ethics Committee of the Institution (protocol number 47122321.3.0000.5152), the pulp cells were obtained by the explant outgrowth method from third molars freshly extracted for orthodontic reasons.

To use non-cytotoxic BRP concentrations, a cell viability assay (MTT formazan) was performed in different concentrations (**fig. 1**). Based on these results, concentrations of 10 e 50 μg/mL were chosen and employed in the following experiments. Cells were incubated in DMEM without FBS with MTA serially diluted (1:1; 1:2; 1:4; 1:8), BRP (10 e 50 μg/mL), DMEM (assay control group), and DMSO 0.5% (negative control group).

After an incubation period of 24h, the cells were immediately tested for cell viability by MTT formazan assay, quantitative and qualitative ROS production by a fluorescent probe and release of NO by Griess method. This study was repeated three times using 4 samples for each group at every time period.

Cell viability (MTT formazan assay)

Cell viability was assessed using the methyl tetrazolium (MTT) assay (tetrazoline 3- (4,5-dimethylthiazol-2-yl) – 2,5-diphenyl bromide). Previously, this well-standardized protocol has been fully described[23,24]. In addition, antiproliferative activity was assessed by using the parameter of 50% inhibition of cell growth (IC_{50}) after 24h of treatment with different concentrations (100 – 1000 /mL) of BRP. The selective index (SI) was used to verify whether BRP has more affinity for bacteria or HDPFs. The concentrations that reduced cell viability by 50% (IC_{50}) and the MIC against the tested bacteria were calculated by using the formula: $SI=IC_{50}/MIC$.

Free radical production (NO and ROS)

NO production

NO production was evaluated using the supernatant of the cell culture. Nitrite is formed by a diazotization reaction with Griess reagent, which includes 0.1 gN (1-naphthyl) ethylenediamine dichloride (Merck KGaA), 2.5-mL orthophosphoric acid (Mallinckrodt Chemical, St. Louis, MO, USA), 1-g-sulfanilamide (Merck KGaA, Darmstadt, HE, Germanyand) and 100-mL deionized water. Three aliquots of the supernatant (50 μL) were added to 50 μL of Griess reagent in the compartments of a 96-well plate. After a 10-min incubation, the absorbance reading was assessed using a spectrophotometer (ThermoPlate, Shenzhen, China) with a 540-nm filter.

ROS production

Quantitative analysis of ROS production was performed after application of 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; InvitroGen, San Diego, CA, USA). In a 24-well plate, 300 μL of a solution containing the fluorescent probe (5 μmol/L in PBS) were added to the compartments. After a 5-min incubation, the cells were washed twice with PBS and analyzed using a GLOMAX® multimodal fluorescence microplate reader (Promega Corporation, São Paulo, Brazil) and the EVOS FL cell imaging system (Fluorescence Microscope EVOS FL Cell Imaging System, Thermo Fisher Scientific, São Paulo, Brazil) for qualitative analysis.

Statistical Analysis

First, all data were analyzed using Kolmogorov-Smirnov's test. Then, one-way ANOVA and Tukey's tests were used to compare data between the treated groups. Statistical analysis was performed using JAMOVI 1.6.23 software (New South Wales, Sydney, Australia) and considered at the pre-established significance level of 5%.

RESULTS

Determination of the Minimum Inhibitory Concentration (MIC) and of the Minimum Bactericidal Concentration (MBC)

MIC values < 100 μg/mL were identified for *P. micra CI* (6.25 μg/mL), *F. nucleatum CI* (25 μg/mL), *P. nigrescens* ATCC 33563 (50 μg/mL), *P. melaninogenica* ATCC 25845 (50 μg/mL), *P. intermedia CI* (50 μg/mL) and *P. gingivalis CI* (50 μg/mL). In addition, MIC also showed significant bactericidal activity based on MBC values for *P. micra* (6.25/6.25 μg/mL) and *F. nucleatum* (25/25 μg/mL) also bacteriostatic effect for *P. nigrescens* ATCC 33563 (50/100 μg/mL), *P. melaninogenica* ATCC 25845 (50/100 μg/mL) and *P. intermedia CI* (50/100 μg/mL).

Cell viability (MTT formazan assay)

The cytotoxicity of the BRP and MTA based on MTT formazan assay is represented in **fig. 2.** Both MTA and BRP were capable to stimulate cell viability. Higher cell viability values were found for BRP10 compared to DMEM (*p=0.007*), MTA 1:2 (*p=0.011*) and DMSO (*p=0.011*). Moreover, we performed SI based on IC_{50} results (IC_{50}=509.16). Relevant BRP levels of SI (SI ≥ 10) were detected (**Table 2**) for *P. micra* CI (81.5), *F. nucleatum* CI (20.4), *P. nigrescens* ATCC 33563 (10.2), *P. melaninogenica* ATCC 25845 (10.2), *P. intermedia CI* (10.2) and *P. gingivalis CI* (10.2).

Free radical production (NO and ROS)

BRP50, MTA 1:1 and MTA 1:2 has slightly raised NO production in HDPFs compared to DMEM (p=0.007, p<.001 and 0.008, respectively) (**fig. 3**). Similar to NO production, higher ROS values were found in BRP 50, MTA 1:1 and MTA 1:2 compared to DMEM (p<.001). On the other hand, BRP10 did not increase the amount of NO (*p=0.974*) and ROS (*p=0.976*) production compared to DMEM (**fig. 4**). According to the ROS images generated (**fig. 5**) after the fluorescent probe contact with HDPFs, it is possible to observe that quantitative analyses agreed with the images. BRP10, MTA 1:4 and MTA 1:8 had revealed lower levels of fluorescence as DMEM group, whereas BRP50 and MTA 1:1 had shown an increased ROS expression.

DISCUSSION

Maintenance of pulp vitality is one of the purposes of vital pulp therapies and the selection of a good material strongly affects the long-term outcomes[10]. BRP is a great antimicrobial agent and may provide therapeutic solutions for pulp therapy due to its many biological properties[7]. Herein, we believed that the positive effects attributed to BRP are because of the use of crude extract rather than isolated compounds, which are generally directed to test a particular activity[25]. The combined action of the various compounds allowed BRP and MTA to perform, for the first time in the literature, cell viability without increasing significantly ROS and NO production in all tested concentrations.

Antibacterial activity of natural products and medicinal plants have been widely assessed by MIC assays[26], which is a good strategy to obtain antimicrobial activity of these products instead of four other screening techniques[27]. There is different standardization for MIC results of compounds from crude plant extracts, nonetheless, in this study, we have considered that MIC values ≤ 100 μg/mL correspond to a promising antibacterial activity, as Ríos et al. (2005)[28] had established. We demonstrated that, regarding MIC and MBC values, the crude extract of BRP demonstrated positive antibacterial activity for the vast majority of anaerobic bacteria involved in primary endodontic infection which confirms the reports related to the antibacterial properties of BRP. Moreover, it is believed that flavonoids are the main component with a relevant role in the BRP antibacterial activity[29-31].

One study demonstrated that propolis may act as an inhibitor effect of glucosyltransferase enzymes, that play a crucial role in dental plaque formation, of *S. mutans* and *S. sanguis* due to apigenin (flavonoids) action. Furthermore, these authors showed that flavonoid and cinnamic compounds were found to suppress bacterial motility and viability by affecting ion permeability of the cell membrane and decreasing ATP synthesis[32].

Besides attested antimicrobial activity against *C. albicans*[33], propolis could be a promising intracanal medication or an irrigant in endodontics procedures due to its capability to eliminate *E. faecalis*[34]. In addition, one *in vitro* study established that a combination of Brazilian propolis and calcium hydroxide was effective against microorganisms collected from necrotic root canals of primary teeth[35], confirming our

findings in regards to its efficiency against bacteria related to endodontic infection. Meanwhile, the challenge in standardizing the exact components responsible for antibacterial activity present in the propolis crude extract is already known due to the variation of environmental conditions of the collection sites, the origin and type of pollen as well as the species of bee producing it[36].

An *in vitro* study[37] demonstrated great antileishmanial activity of dichlorometane fraction from Brazilian brown propolis also low cytotoxicity towards macrophages due to its highest selectivity index (SI), determined as the ratio between IC_{50} and MIC. SI is used to identify potential drugs and to evaluate their therapeutic window. Indexes ≥ 10 mean drug affinities for the pathogen and demonstrate lower toxicity towards human cells, which certify its safeness in developing new medicines[38]. Here, BRP crude extract showed marked values for *P. micra CI* (81.59) and *F. nucleatum CI* (20.4) as well as performed SI=10.2 for *P. melaninogenica* (ATCC 25845), *P. nigrescens (*ATCC 33563), *P. intermedia* (CI) and *P. gingivalis* (CI).

After the antibacterial activity, we analyzed cell viability, NO and ROS production by HDPFs on direct contact with BRP10 (10 μg/mL), BRP50 (50 μg/mL) and MTA extracts (1:1, 1:2, 1:4 e 1:8). The solvent used here (DMSO 0.5%) had no effects on the cell viability thus the cytotoxic effects were solely reported due to the presence of BRP. MTT Formazan assay demonstrated that BRP and MTA, in all tested concentrations, were able to stimulate cell viability which is a promising finding that showed similar behavior of BRP compared to a well-stablished material.

According to our results, one *in vitro* study revealed that both propolis at 10 μg/mL and MTA at 1:8 dilutions were not cytotoxic to HDPFs after 1, 5, 7 and 9 days[39]. Additionally, another study demonstrated that gray MTA and Jordanian propolis showed similar cell viability data for human periodontal ligament cells compared to the cell culture medium group (control) which confirms the favorable outcome of MTA in terms of biocompatibility to formocresol and ferric sulfate in studies analyzing primary teeth pulpotomy agents[40]. Nevertheless, propolis combined with MTA showed no inhibition of cell growth besides the promotion of differentiation and mineralization of dental pulp stem cells (DPSCs)[41]. Further animal and clinical studies are required to verify the application of isolated BRP or associated with MTA in vital pulp therapies in primary teeth and immature permanent teeth.

In moderate concentrations, NO and ROS act positively in vasodilation, cytokine signaling and expression, cell differentiation and phagocytosis of pathogens[42]. On the other hand, when their production is exacerbated, these molecules can inhibit COX and thereby induce pro-apoptotic enzymes that cause cell death with consequential tissue damage[43]. Remarkably, our results demonstrated that BRP50, MTA 1:1 and MTA 1:2 slightly increased free radicals amount compared to the other groups. However, we strongly believed that this increase would not be able to promote a pro-inflammatory environment.

It is shown that BRP attenuates multiple signaling pathways in macrophages involved in the inflammatory process when stimulated by LPS[44,45]. Considering that NO production is activated by this endotoxin, cells in the present study were not stimulated in the same manner, because we intended to analyze whether the crude propolis extract would be able to maintain the concentration of these free radicals at basal levels. Based in our results, it is possible to state that both BRP concentrations stimulated cell viability and were capable to maintain basal levels of NO and ROS. **Fig. 5** demonstrated the low fluorescent intensity of ROS to BRP10 similar to the control group (DMEM), which is in agreement with quantitative analysis.

The searching for products of natural origin in VPT increased significantly due to the growing demand for new materials presenting great therapeutic performance, good antimicrobial activity, low toxicity, affordable cost to the population, and better biocompatibility. Our promising findings suggested that BRP is a strong candidate for future animal and clinical studies due to its high antibacterial activity against anaerobic bacteria involved in primary endodontic infection. Even with limitations regarding the absence of anti-inflammatory challenge and lack of different times of cytotoxicity evaluation, both BRP and MTA promoted the viability of HDPFs without greatly increasing NO and ROS production.

ACKNOWLEDGMENTS

This study was financed in part by *Coordination for the Improvement of Higher Education Personnel* (CAPES - *88887.341526), National Council for Scientific and Technological Development* (CNPq - *434204/2018-8)* also by *The Minas Gerais Research Foundation* (FAPEMIG - APQ00315-16*)*. The authors deny any conflicts of interest related to this study.

TABLES

Table 1: In vitro antibacterial activity of the Brazilian red propolis extract against nine bacteria involved in primary endodontic infection through determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

	***Brazilian red propolis* (µg/mL)**		
Endodontic bacteria	**MIC**	**MBC**	**Metronidazole (control)**
Parvimonas micra (CI)	6.25	6.25	x
Fusobacterium nucleatum (CI)	25	25	x
Prevotella nigrescens (ATCC 33563)	50	100	x
Prevotella melaninogenica (ATC 25845)	50	100	x
Prevotella intermedia (CI)	50	100	x
Porphyromonas gingivalis (CI)	50	200	x
Veillonella parvula (ATCC 17745)	200	200	x
Porphyromonas endodontalis (ATCC 35406)	200	> 400	x
Actinomyces viscosus (ATCC 43146)	400	> 400	x
*Bacteroides fragilis (ATCC 25285)**	*x*	*x*	1.48
*Bacteroides thetaiotaomicron ATCC (29741)**	x	x	2.95

**Control strains; x: Not tested*

Table 2: Determination of the SI of Brazilian red propolis (BRP) for nine bacteria involved in primary endodontic infection.

Brazilian Red Propolis	
Bacterial strains	**Selectivity Index (IC_{50}/MIC)**
Parvimonas micra (CI)	81.5
Fusobacterium nucleatum (CI)	20.4
Prevotella nigrescens (ATCC 33563)	10.2
Prevotella melaninogenica (ATC 25845)	10.2
Prevotella intermedia (CI)	10.2
Porphyromonas gingivalis (CI)	10.2
Veillonella parvula (ATCC 17745)	2.6
Porphyromonas endodontalis (ATCC 35406)	2.6
Actinomyces viscosus (ATCC 43146)	1.3

IC50: inhibitory concentration of 50% of cell viability; MIC:, minimum inhibitory concentration.

FIGURES AND CAPTIONS

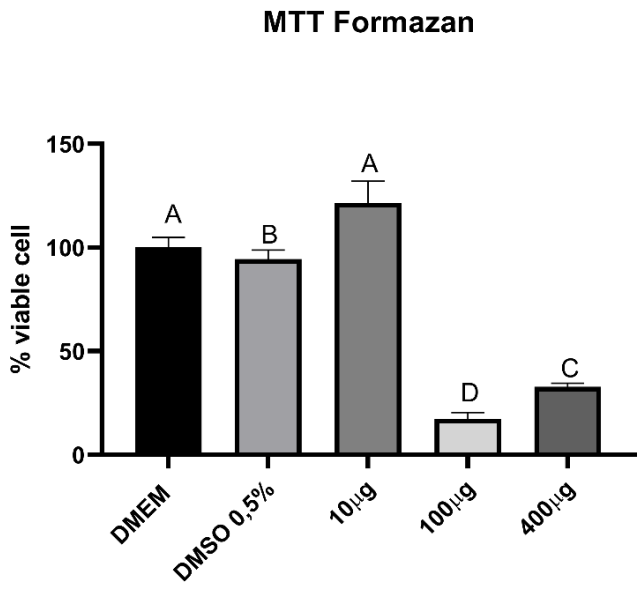

Fig 1: Cell viability assay with BRP different concentrations.

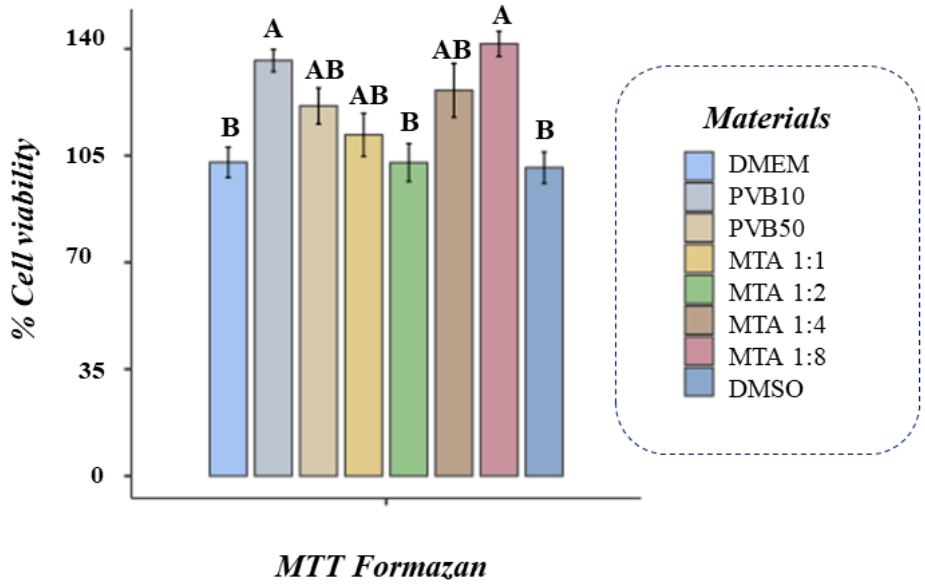

Fig. 2: Cell viability: MTT Formazan assay. Different letters represent values with statistical significance. One-way ANOVA and Tukey's tests were used to compare data between the treated groups ($p>0.05$).

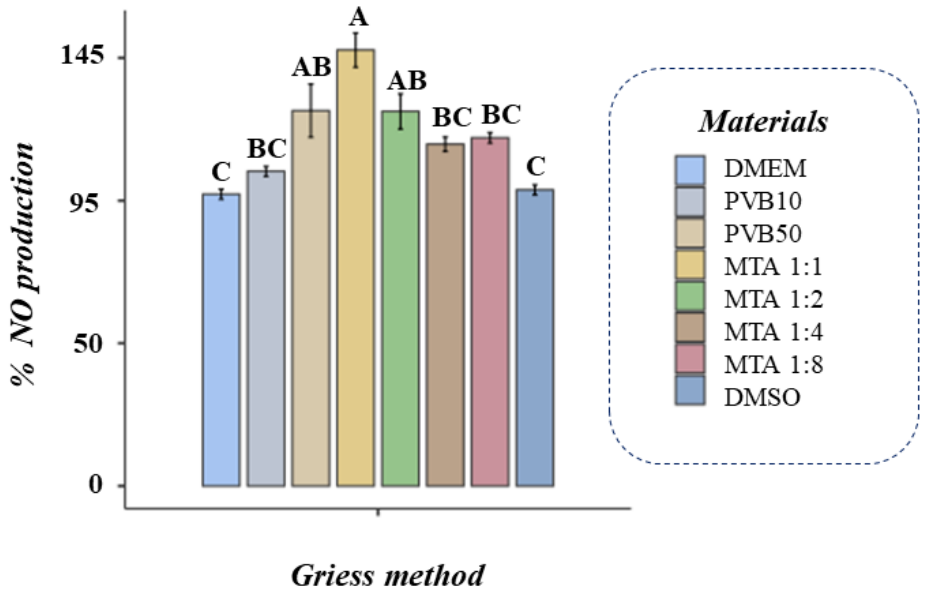

Fig. 3: NO production through Griess method. Different letters represent values with statistical significance. One-way ANOVA and Tukey's tests were used to compare data between the treated groups ($p>0.05$).

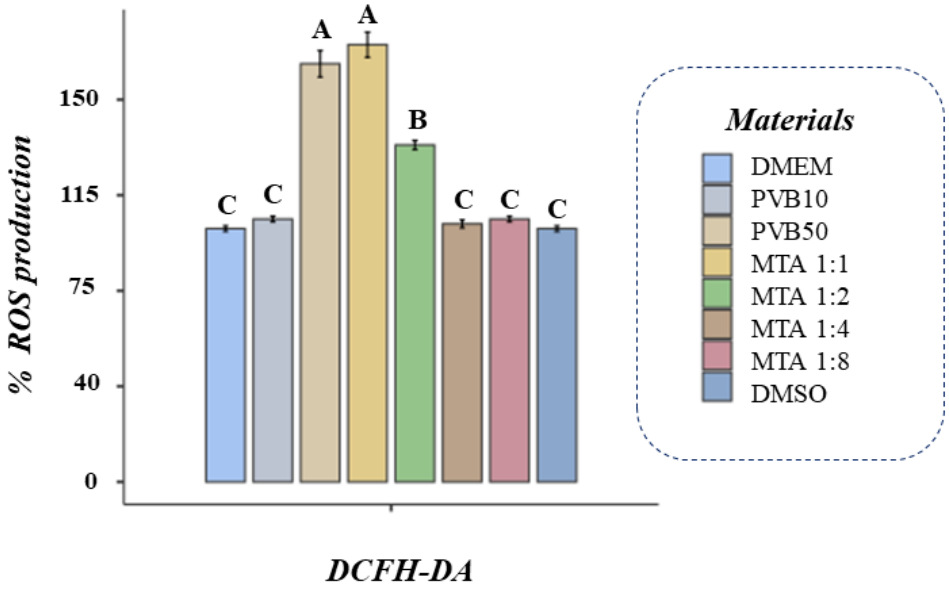

Fig. 4: ROS production through 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe. Different letters represent values with statistical significance. One-way ANOVA and Tukey's tests were used to compare data between the treated groups ($p>0.05$).

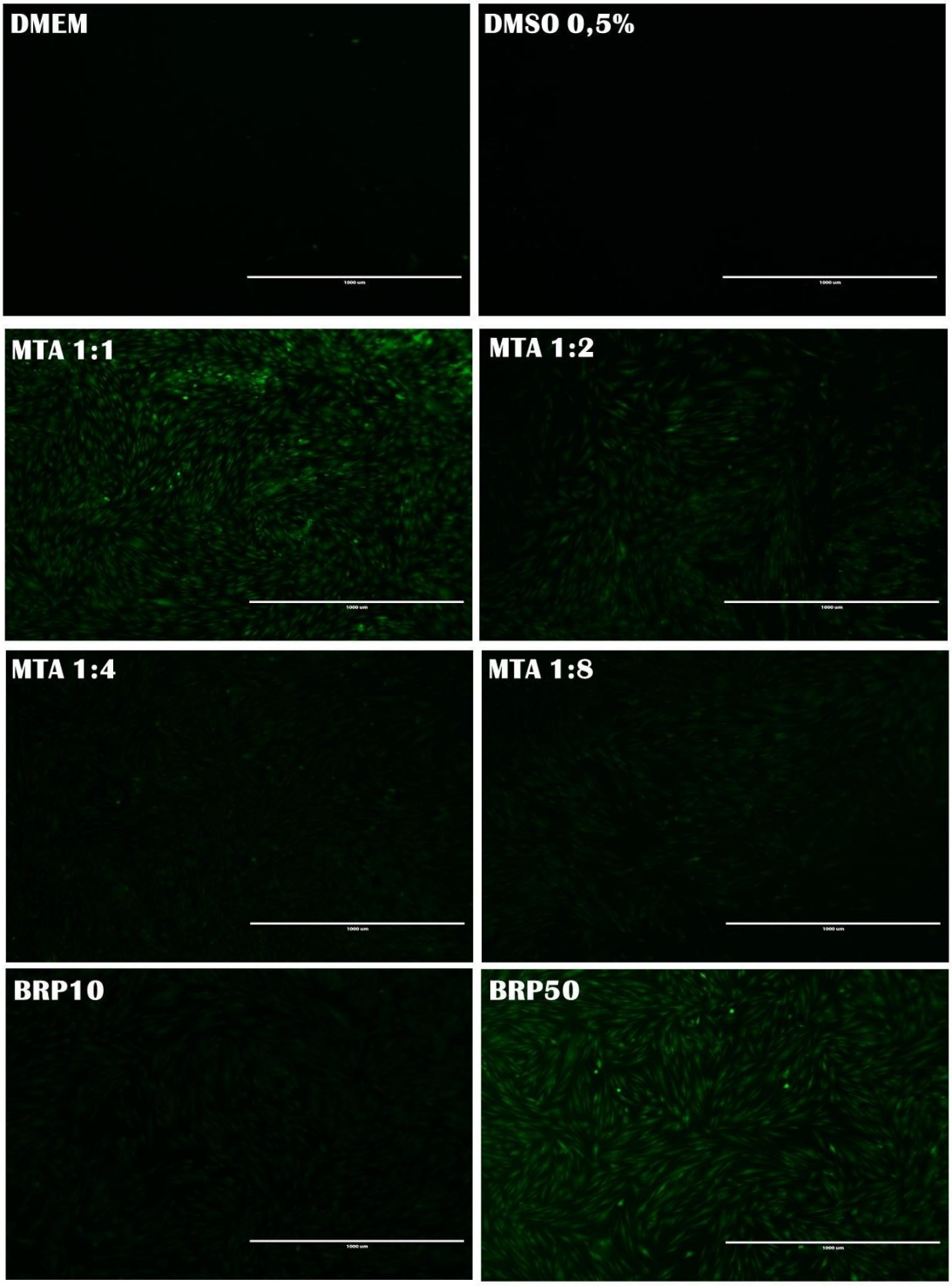

Fig.5: Images representing ROS production by HDPFs exposed to BRP, MTA and DMSO. The intensity of the DCFH-DA fluorescent probe can pervade the cell and then establish the intensity of the ROS produced by cells. Therefore, higher fluorescence indicated greater production of these oxidant agents (Fluorescence Microscope EVOS FL Cell Imaging System; scale, 400 μm).

REFERENCES

1. Hanna SN, Perez Alfayate R, Prichard J. Vital Pulp Therapy an Insight Over the Available Literature and Future Expectations. Eur Endod J 2020; 1:5(1):46-53. 3.

2. American Academy of Pediatric Dentistry. Pulp therapy for primary and immature permanent teeth. The Reference Manual of Pediatric Dentistry. Chicago, Ill.: American Academy of Pediatric Dentistry 2020:384-92.

3. Stringhini Junior E, Dos Santos MGC, Oliveira LB, Mercadé M. MTA and biodentine for primary teeth pulpotomy: a systematic review and meta-analysis of clinical trials. Clin Oral Investig. 2019 Apr;23(4):1967-1976.

4. Mc Cabe PS. The clinical applications of mineral trioxide aggregate. J. Ir. Dent. Assoc 2003; 49:123–131.

5. Baroudi K, Samir S. Sealing Ability of MTA Used in Perforation Repair of Permanent Teeth; Literature Review. Open Dent. J. 2016, 10:278–286.

6. Vijayran M, Chaudhary S, Manuja N, Kulkarni AU. Mineral trioxide aggregate (MTA) apexification: A novel approach for traumatised young immature permanent teeth. BMJ Case Rep. 2013; 10.4

7. da Silva Barboza A, Aitken-Saavedra JP, Ferreira ML, Fábio Aranha AM, Lund RG. Are propolis extracts potential pharmacological agents in human oral health? - A scoping review and technology prospecting. J Ethnopharmacol 2021; 10: 271:113846.

8. Moise AR, Bobiş O. *Baccharis dracunculifolia* and *Dalbergia ecastophyllum*, Main Plant Sources for Bioactive Properties in Green and Red Brazilian Propolis. Plants (Basel) 2020; 21;9(11): 1619.

9. Machado CS, Mokochinski JB, de Lira TO, de Oliveira FC et al. Comparative Study of Chemical Composition and Biological Activity of Yellow, Green, Brown, and Red Brazilian Propolis. Evid Based Complement Alternat Med 2016; 2016: 6057650.

10. Freires IA, de Alencar SM, Rosalen PL. A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases. Eur J Med Chem. 2016; 3; 110:267-79.

11. Boeing T, Mejía JAA, Ccana-Ccapatinta GV, Mariott M et al. The gastroprotective effect of red propolis extract from Northeastern Brazil and the role of its isolated compounds. J Ethnopharmacol. 2021. 267:113623.

12. Asgharpour F, Moghadamnia AA, Motallebnejad M, Nouri HR. Propolis attenuates lipopolysaccharide-induced inflammatory responses through intracellular ROS and NO levels along with downregulation of IL-1β and IL-6 expressions in murine RAW 264.7 macrophages. J Food Biochem 2019; 43(8):e12926.

13. Bueno-Silva B, Kawamoto D, Ando-Suguimoto ES et al. Brazilian red propolis effects on peritoneal macrophage activity: Nitric oxide, cell viability, pro-inflammatory cytokines and gene expression. J Ethnopharmacoln 2017; 31;207:100-107.

14. Ccana-Ccapatinta GV, Mejía JAA, Tanimoto MH, Groppo M, Carvalho JCASd, Bastos JK. *Dalbergia ecastaphyllum* (L.) Taub. and *Symphonia globulifera* L.f.: The Botanical Sources of Isoflavonoids and Benzophenones in Brazilian Red Propolis. *Molecules*. 2020; 25(9):2060.

15. Clinical and Laboratory Standards Institute (CLSI), Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria, Approved Standard, seventh ed., 2007. M11-A7. Wayne, PA: USA.

16. SD Sarker, L Nahar, Y Kumarasamy. Microtitre plate-based antimicrobial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antimicrobial screening of phytochemicals, Methods 2007; 42:321-324.

17. Bin CV, Valera MC, Camargo SE, Rabelo SB, Silva GO, Balducci I, Camargo CH. Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. J Endod. 2012;38(4):495-500.

18. ISO/EN10993-5. ISO 10993-5 Biological evaluation of medical devices – Part 5: Tests for cytotoxicity: in vitro methods. Int Stand ISO. 2009.

19. Bossù M, Mancini P, Bruni E, Uccelletti D, Preziosi A, Rulli M, Relucenti M, Donfrancesco O, Iaculli F, Di Giorgio G, Matassa R, Salucci A, Polimeni A. Biocompatibility and Antibiofilm Properties of Calcium Silicate-Based Cements: An In Vitro Evaluation and Report of Two Clinical Cases. *Biology*. 2021; 10(6):470.

20. Bonvicini JFS, Basso FG, de Souza Costa CA, Soares CJ, Turrioni AP. Photobiomodulation effect of red LED (630 nm) on the free radical levels produced by pulp cells under stress conditions. Lasers Med Sci. 2021.

21. Wang D, Sun S, Xue Y, Qiu J, Ye T, Zhang R, Song B, He W, Zhang Y, Jiang W. MicroRNA-223 negatively regulates LPS-induced inflammatory responses by targeting NLRP3 in human dental pulp fibroblasts. Int Endod J. 2021;54(2):241-254.

22. Wei L, Chen Y, Zhang C, Liu M, Xiong H. Leptin induces IL-6 and IL-8 expression through leptin receptor Ob-Rb in human dental pulp fibroblasts. Acta Odontol Scand. 2019;77(3):205-212.

23. Oliveira, C. F. et al. In vitro effect of low-level laser on odontoblast-like cells. Laser Physics Letters. v. 8, n. 2, p. 155-163, 2011.

24. Oliveira, C. F. et al. Increased Viability of Odontoblast-Like Cells Subjected to Low-Level Laser Irradiation. Laser Physics. v. 20, n. 7, p. 1659-1666, 2010.

25. Olmedo-Juárez A, Briones-Robles TI, Zaragoza-Bastida A, Zamilpa A et al. Antibacterial activity of compounds isolated from Caesalpinia coriaria (Jacq) Willd Against importante bactéria in public health, Microb. Pathog. 2019; 136.

26. N. Wijesundara, H Rupasinghe. Bactericidal and anti-biofilm activity of ethanol extracts derived from selected medicinal plants against *Streptococcus pyogenes*, Molecules 2019; 24:1165.

27. E.G. Alves, A.H.C Vinholis, L.A. Casemiro, N.A.J.C. Furtado, M.L.A. e Silva, W.R. Cunha, C.H.G. Martind, Comparative study of screening techniques for antibacterial activity evaluation of plant crude extracts and pure compounds, Quim. Nova 2008; 1224-1229.

28. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005; 22;100(1-2):80-4.

29. Park YK, Alencar SM, Aguiar CL. Botanical origin and chemical composition of Brazilian propolis. J. Agric. Food Chem. 2002; 50:2502–2506.

30. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol. 1998;36(4):347-63.

31. Huang, S., Zhang, C.P., Wang, K., Li, G.Q., Hu, F.L. Recent advances in the chemical composition of propolis. Molecules 2014; 19:19610–19632.

32. Koo, H., Rosalen, P.L., Cury, J.A., Park, Y.K., Bowen, W.H. Effects of compounds found in propolis on *Streptococcus mutans* growth and on glucosyltransferase activity. Antimicrob. Agents Chemother 2002; 46:1302–1309.

33. Chua, EG., Parolia A., Ahlawat P, Pau A, Amalraj FD. Antifungal effectiveness of various intracanal medicaments against *Candida albicans*: an ex-vivo study. BMC Oral Health 2014; 13:14–53.

34. Simões TMS, Silva MGB, Fernandes Neto JA, Batista ALA, Catão MHCV. Applicability of antimicrobial photodynamic therapy in the elimination of *Enterococcus faecalis.* Arch. Heal. Investig 2018; 492–496.

35. de Rezende GP, da Costa LR, Pimenta, FC, Baroni DA. *In vitro* antimicrobial activity of endodontic pastes with propolis extracts and calcium hydroxide: a preliminary study. Braz. Dent. J. 2008; 19:301–305.

36. Castro LM et al. Propolis from southeastern and northeastern of Brazil: the influence of seasonality in antibacterial activity and phenolic composition. Química Nova 2007; 30(7):1512-1516.

37. Lorena C.L.R. Santana, Sabrina Maria P. Carneiro, Laurentino B. Caland-Neto, et al. Brazilian brown propolis elicits antileishmanial effect against promastigote and amastigote forms of *Leishmaniaamazonensis*, Natural Product Research 2014, 28:5, 340-343.

38. Pavan FR., Maia PIS, Leite SRA., Deflon VM et al. F. Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: Anti - Mycobacterium tuberculosis activity and cytotoxicity. *European Journal of Medicinal Chemistry*, 2010; *45*(5).

39. Shi B, Zhao Y, Yuan X. Effects of MTA and Brazilian propolis on the biological properties of dental pulp cells. Braz Oral Res. 2020; 10;33: e117.

40. Al-Haj Ali SN. In vitro toxicity of propolis in comparison with other primary teeth pulpotomy agents on human fibroblasts. J Investig Clin Dent. 2016;7(3):308-13.

41. Kim JH, Kim SY, Woo SM, Jeong HN, Jung JY, Kim SM, Lim HS. Combination of mineral trioxide aggregate and propolis promotes odontoblastic differentiation of human dental pulp stem cells through ERK signaling pathway. Food Sci Biotechnol. 2019 May 21;28(6):1801-1809.

42. Liew FY, Cox FE. Nonspecific defense mechanism: the role of nitric oxide. Immunol Today 1991, 12(3):17–21.

43. Holder MJ, Milward MR, Palin WM, Hadis MA, Cooper PR. Effects of red light-emitting diode irradiation on dental pulp cells. J Dent Res 2012, 91(10):961–966.

44. Bueno-Silva B, Kawamoto D, Ando- Suguimoto ES, Alencar SM, Rosalen PL, Mayer MPA. Brazilian Red Propolis Attenuates Inflammatory Signaling Cascade in LPS-Activated Macrophages. PLoS ONE 2015, 10(12): e0144954.

45. Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric oxide production and signaling in inflammation. Curr Drug Targets InflammAllergy 2005; 4:471-479

3. CONCLUSÕES

A) A PVB apresentou atividade antibacteriana significativa frente às bactérias anaeróbias envolvidas na infecção endodôntica;

B) A própolis vermelha brasileira foi capaz de estimular a viabilidade de células pulpares sem causar aumento considerável da produção de óxido nítrico (ON) e espécies reativas de oxigênio (eROS).

4. REFERÊNCIAS BIBLIOGRÁFICAS*

Smaïl-Faugeron V, Courson F, Durieux P, Muller-Bolla M, Glenny AM, Fron Chabouis H. Pulp treatment for extensive decay in primary teeth. **Cochrane Database Syst Rev**. 2014. 6;(8). doi: 10.1002/14651858.

Li Y, Sui B, Dahl C, Bergeron B, Shipman P, Niu L, Chen J, Tay FR. Pulpotomy for carious pulp exposures in permanent teeth: A systematic review and meta-analysis. **J Dent.** 2019 (84):1-8. doi: 10.1016/j.jdent.2019.03.010.

Shi B, Zhao Y, Yuan X. Effects of MTA and Brazilian propolis on the biological properties of dental pulp cells. **Braz Oral Res.** 2020. 10;33: e117. doi: 10.1590/1807-3107bor-2019.vol33.0117.

da Silva Barboza A, Aitken-Saavedra JP, Ferreira ML, Fábio Aranha AM, Lund RG.Are propolis extracts potential pharmacological agents in human oral health? - A scoping review and technology prospecting. **J Ethnopharmacol** 2021. 10: 271:113846. doi: 10.1016/j.jep.2021.113846.

Sousa JPB et al. Perfis físico-químico e cromatográfico de amostras de própolis produzidas nas microrregiões de Franca (SP) e Passos (MG), Brasil. **Brazilian Journal of Pharmacognosy**. 2007 (17): 1, p. 85-93. doi: 10.1590/S0102-695X2007000100017

Peter CM et al. Atividade antiviral e virucida de extratos hidroalcoólicos de própolis marrom, verde e de abelhas Jataí (Tetragonisca angustula) frente ao herpersvírus bovino tipo 1 (BoHV-1) e ao vírus da diarreia viral bovina (BVDV). **Pesq. Vet. Bras**., v. 37, n. 7, p. 667- 675, jul., 2017. doi: 10.1590/S0100-736X2017000700003

Cardoso MN et al. Avaliação de atividade mutagênica de amostras de própolis vermelha do Estado de Sergipe (Brasil) pelo Teste de Mutação e Recombinação Somática em Drosophila melanogaster. **Scientia Plena**, v. 11, n. 1, 2015.

Ítavo CCBF et al. Características de carcaça, componentes corporais e rendimento

de cortes de cordeiros confinados recebendo dieta com própolis ou monensina sódica. **R. Bras. Zootec**., v.38, n.5, p.898-905, 2009. doi: 10.1590/S1516-382009000500017.

Castro LM et al. Própolis do sudeste e nordeste do brasil: influência da sazonalidade na atividade antibacteriana e composição fenólica. **Química Nova**, v. 30, n. 7, p. 1512-1516, 2007. doi: 10.1590/S0100-40422007000700003.

Araujo JME. Caracterização química e atividade leishmanicida dos extratos hidroetanólicos de própolis vermelha e Dalbergia ecastophyllum (Febaceae). 2014. 53p. Dissertação [Mestrado]. Universidade Federal de Sergipe. São Critóvão, 2014.

Kimoto T, Koya-Miyata S, Hino K, Micallef MJ, Hanaya T, Arai S, Ikeda M, Kurimoto M. Pulmonary carcinogenesis induced by ferric nitrilotriacetate in mice and protec- tion from it by Brazilian propolis and artepillin C. **Virchows Archiv**. 2001;438(3):259– 270. doi: 10.1007/s004280000350.

Gekker G, Hu S, Spivak M, Lokensgard JR, Peterson PK. Anti-HIV-1 activity of prop- olis in CD4(+) lymphocyte and microglial cell cultures. **Journal of Ethnopharmacol.** 2005;102(2):158–163. doi: 10.1016/j.jep.2005.05.045.

Koo H, Gomes BPFA, Rosalen PL, Ambrosano GMB, Park YK, Cury JA. In vitro anti- microbial activity of propolis and Arnica montana against oral pathogens. **Archives of Oral Biology.** 2000; 45:141–148. doi: 10.1016/s0003-9969(99)00117-x.

De Luca MP, Franca JR, Macedo FAFF, Grenho L, Cortes ME, Faraco AAG, Moreira AN, Santos VR. Propolis varnish: antimicrobial properties against cariogenic bacte- ria, cytotoxicity, and sustained-release profile. **Biomed Research International.** 2014. doi: 10.1155/2014/348647.

Berretta AA, Castro PA, Cavalheiro AH, Fortes VS, Bom VP, Nascimento AP, Mar- quele-Oliveira F, Pedrazzi V, Ramalho LNZ, Goldman GH. Evaluation of mucoadhe- sive gels with propolis (EPP-AF) in preclinical treatment of candidiasis vulvovaginal infection. **Evidence-Based Complementary and Alternative Medicine.** 2013; 2013:641480. doi: 10.1155/2013/641480.

Fernandes FH, Guterres ZR, Garcez WS, Lopes SM, Corsino J, Garcez FR. Assessment of the (anti)genotoxicity of brown propolis extracts from Brazilian Cerrado biome in a Drosophila melanogaster model. **Food Research International**. 2014; 62:20–26. doi.org/10.1016/j.foodres.2014.02.029.

Fernandes FH, Guterresb ZR, Violante IMP, Lopes TFS, Garcez WS, Garcez F R. Evaluation of mutagenic and antimicrobial properties of brown propolis essential oil from the Brazilian cerrado biome. **Toxicology Reports.** 2015; 2:1482–1488. doi: 10.1016/j.toxrep.2015.11.007.

Bankova V, Marcuccib MC, Simova S, Nikolova N, Kujumgievc A, Popov S. Antibacterial diterpenic acids from Brazilian propolis. **Zeitschrift für Naturforschung.** 1996; 51:277–280. doi: 10.1515/znc-1996-5-602.

Silva CSR, Barreto CLP, Peixoto RM, Mota RA, Ribeiro MF, Da Costa MM. Antibacterial effect of Brazilian brown propolis in different solvents against staphylococcus spp. Isolated from caprine mastitis. **Ciência Animal Brasileira.** 2012;13:247–251, 2012. doi: 10.5216/cab.v13i2.15121.

Fernandes FH, Guterresb ZR, Violante IMP, Lopes TFS, Garcez WS, Garcez F R. Evaluation of mutagenic and antimicrobial properties of brown propolis essential oil from the Brazilian cerrado biome. **Toxicology Reports**. 2015;2:1482–1488. doi: 10.1016/ j.toxrep.2015.11.007.

Fonseca YM, Oliveira FM, Vicentini FTMC, Furtado NAJC, Sousa JPB, Valim YML, Fonseca MJV. Evaluation of the potential of Brazilian propolis against UV-induced oxidative stress. **Evidence-Based Complementary and Alternative Medicine**. 2011;2012:863917. doi: 10.1155/2011/863917

Herrera YF. Atividade citotóxica de amostras de própolis brasileira e cubana contra células tumorais humanas. 2016. 101p. Tese [Doutorado]. Universidade Estadual Paulista. Botucatu, 2016.

Moise AR, Bobiş O. *Baccharis dracunculifolia* and *Dalbergia ecastophyllum*, Main Plant Sources for Bioactive Properties in Green and Red Brazilian Propolis. **Plants (Basel).** 2020 Nov 21;9(11):1619. doi: 10.3390/plants9111619.

Machado CS, Mokochinski JB, de Lira TO, de Oliveira Fde C, Cardoso MV, Ferreira RG, Sawaya AC, Ferreira AG, Pessoa C, Cuesta-Rubio O, Monteiro MC, de Campos MS, Torres YR. Comparative Study of Chemical Composition and Biological Activity of Yellow, Green, Brown, and Red Brazilian Propolis. **Evid Based Complement Alternat Med**. 2016; 6057650. doi: 10.1155/2016/6057650.

Machado NAF. Desenvolvimento e análise sensorial de bolo enriquecido com soro de leite e microencapsulado de própolis vermelha. 2017. 70p. Dissertação [Mestrado]. Universidade Federal de Alagoas. Maceió, 2017.

De Pontes LMC, Vasconceles IVA, Diniza MFFM, HLF Pêssoa. Chemical characterization and pharmacological action of Brazilian red propolis. **Acta Brasiliensis.** 2018; 1:34–39. doi: 10.22571/2526-433868.

Trusheva B, Popova M, Bankova V, Simova S et al. I. Bioactive Constituents of Brazilian Red Propolis. **Evid. Based Complementary Alternat. Med.** 2006, 3:249–254. doi: 10.1093/ecam/nel006. doi: 10.1093/ecam/nel006.

Park YK, Alencar SM, Aguiar CL. Botanical origin and chemical composition of Brazilian propolis. **J. Agric. Food Chem.** 2002; 50:2502–2506. doi: 10.1021/jf011432b.

***** *De acordo com a Norma da FOUFU, baseado nas Normas de Vancouver. Abreviaturas dos periódicos com conformidade com Medline (Pubmed) – Referências bibliográficas do item 1. Introdução e Referencial Teórico.*