ANTIMICROBIAL ACTION OF *PSIDIUM GUAJAVA* L. EXTRACT AGAINST *ENTEROCOCCUS FAECALIS* AND *CANDIDA ALBICANS* STRAINS: A PILOT STUDY

Recebido em: 19/06/2023
Aceito em: 20/07/2023
DOI: 10.25110/arqsaude.v27i7.2023-015

Giovana de Abreu Baldoni [1]
Vanessa Marques Meccatti [2]
Lara Steffany de Carvalho [3]
Cláudio Antonio Talge Carvalho [4]
Luciane Dias de Oliveira [5]
Amjad Abu Hasna [6]

ABSTRACT: Endodontic treatment consists of decontamination of the root canal using irrigants like Sodium Hypochlorite (NaOCl). However, some microorganisms resist conventional therapies, causing relapse and secondary infections. Substances with microbicidal power and potential to become new irrigants need to be studied. Thus, the objective of the present study was to evaluate the antimicrobial potential of *Psidium guajava L.* (guava tree) extract against clinical and standard (ATCC) strains of *Enterococcus faecalis* and *Candida albicans.* For this purpose, the extract was produced from the raw material (leaves and shoots) diluted in the hydroethanolic vehicle (EtOH: H2O / 50:50) for subsequent microbiological analysis. The Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) of the plant extract were tested, according to the Clinical and laboratory standards institute (CLSI) guidelines, on four strains: *E. faecalis* (ATCC and clinic) and *C. albicans* (ATCC and clinic). The extract of *P. guajava* did not produce antifungal action on C. albicans, however, it showed microbicidal potential against strains of *E. faecalis*, showing MIC of 0.20%. This concentration was lower than the MIC of NaOCl which was 0.31%, a solution that is commonly used in dental clinics. In conclusion, the hydroethanolic extract of *P. guajava* presents bactericidal action against *E. faecalis*, being a natural product with potential for future studies regarding the development of new endodontic irrigants.
KEYWORDS: *Enterococcus Faecalis*; *Candida Albicans*; Phytotherapy; Endodontics.

[1] Under-Graduate Student in Dentistry. Instituto Nacional de Ensino Superior e Pós-Graduação Padre Gervásio. E-mail: giovana_abreubaldoni@hotmail.com
[2] PhD in Sciences Applied to Oral Health. Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University (ICT-UNESP). E-mail: vanessa.meccatti@unesp.br
[3] Master's Student in Sciences Applied to Oral Health. Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University (ICT-UNESP).
E-mail: lara.s.carvalho@unesp.br
[4] PhD in Restorative Dentistry. Department of Restorative Dentistry, Endodontics Division, Institute of Science and Technology, São Paulo State University (ICT-UNESP). E-mail: claudio.talge@unesp.br
[5] PhD in Oral Biopathology. Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University (ICT-UNESP). E-mail: luciane.oliveira@unesp.br
[6] PhD in Restorative Dentistry. Instituto Nacional de Ensino Superior e Pós-Graduação Padre Gervásio. Department of Restorative Dentistry, Endodontics Division, Institute of Science and Technology, Universidade Estadual Paulista (ICT-UNESP). E-mail: d.d.s.amjad@gmail.com

AÇÃO ANTIMICROBIANA DO EXTRATO DE *PSIDIUM GUAJAVA* L. CONTRA AS CEPAS DE *ENTEROCOCCUS FAECALIS* E *CANDIDA ALBICANS*: UM ESTUDO PILOTO

RESUMO: O tratamento endodôntico consiste na descontaminação do canal radicular com emprego de soluções irrigadoras, como por exemplo, o Hipoclorito de Sódio (NaOCl). Contudo, alguns microrganismos resistem as terapias convencionais, causando recidiva e infecções secundárias. Substâncias com poder microbicida e potencial para se tornarem novas soluções irrigadoras precisam ser estudadas. Assim, o objetivo do presente estudo foi avaliar o potencial antimicrobiano do extrato de *Psidium guajava* L. (goiabeira) contra cepas clínicas e padrão (ATCC) de *Enterococcus faecalis* e *Candida albicans.* Para tanto, o extrato foi produzido a partir da matéria bruta (folhas e brotos) diluído no veículo hidroetanólico (EtOH: H2O / 50:50) para posterior análise microbiológica. Foram testadas a Concentração Inibitória Mínima (CIM) e Concentração Microbicida Miníma (CMM) do extrato vegetal, de acordo com as normas da *Clinical and laboratory standards institute* (CLSI), sobre quatro cepas: *E. faecalis* (ATCC e clínica) e *C. albicans* (ATCC e clínica). O extrato de *P. guajava* não produziu ação antifúngica sobre *C. albicans*, contudo, apresentou potencial microbicida contra cepas de *E. faecalis*, exibindo CIM de 0,20%. Esta concentração foi menor que a CIM do NaOCl que foi de 0,31%, uma solução que é comumente utilizada nas clínicas odontológicas. Em conclusão, o extrato hidroetanólico de *P. guajava* apresenta ação bactericida contra *E. faecalis*, sendo um produto natural com potencial para futuros estudos quanto ao desenvolvimento de novos irrigantes endodônticos.
PALAVRAS-CHAVE: *Enterococcus Faecalis; Candida Albicans;* Fitoterapia; Endodontia.

ACCIÓN ANTIMICROBIANA DE LA EXTRACCIÓN DE *PSIDIUM GUAJAVA* L. CONTRA LOS CEPES DE LOS *ENTEROCOCCUS FAECALIS* Y LOS *CANDIDA ALBICANS*: UN ESTUDIO PILOTO

RESUMEN: El tratamiento endotónico consiste en la descontaminación del canal raíz con el uso de soluciones de irrigación, como el Hipoclorito de Sodio (NaOCl). Sin embargo, algunos microorganismos se resisten a las terapias convencionales, causando recaídas e infecciones secundarias. Es necesario estudiar las sustancias con poder de microbicida y el potencial para convertirse en nuevas soluciones de riego. Por lo tanto, el objetivo de este estudio fue evaluar el potencial antimicrobiano del extracto de *Psidium guajava* L. (guava) frente a cepas clínicas y estándar (ATCC) de *Enterococcus faecalis* y *Candida albicans*. Con este fin, el extracto se produjo a partir de la materia prima (hojas y brotes) diluida en el vehículo hidroetanólico (EtOH: H2O / 50:50) para análisis microbiológicos posteriores. La Concentración Mínima Inhibidora (CMI) y la Concentración de Microbicidas Minima (CCM) del extracto vegetal se probaron de acuerdo con el *Clinical and laboratory standards institute* (CLSI) en cuatro cepas: E. *faecalis* (ATCC y clínico) y C. *albicans* (ATCC y clínico). El extracto de *P. guajava* no produjo acción antifúngica sobre C. *albicans*, sin embargo mostró potencial microbicida frente a cepas de E. *faecalis*, presentando CIM 0,20%. Esta concentración fue menor que la CMI de NaOCl, que fue del 0,31%, una solución comúnmente utilizada en clínicas dentales. En conclusión, el extracto hidroetanólico de *P. guajava* muestra una acción bactericida frente a *E. faecalis*, siendo un producto natural con potencial para futuros estudios sobre el desarrollo de nuevos irrigantes endotónicos.

PALABRAS CLAVE: *Enterococcus Faecalis*; *Candida Albicans*; Fitoterapia; Endodontia.

1. INTRODUCTION

Root canal treatment seeks maintaining the teeth functionally, by eliminating inflammation or infection of the pulp tissues and periapical region. The treatment is performed using mechanical instrumentation of the canals, associated with the correct use of endodontic irrigants (ABU HASNA et al., 2020, 2021; DE; LEONARDO; LEONARDO, 2012; HASNA et al., 2019), which are used for different finalities, but principally because of its antimicrobial action against the microorganisms in the root canal system. These microorganisms are often difficult to eliminate because of the complex anatomy of the root canal system, that limits the mechanical action of endodontic instruments, therefore, the use of chemical irrigants with antimicrobial activity is essential (ABU HASNA et al., 2020, 2021; CARVALHO et al., 2020).

Sodium hypochlorite (NaOCl) has been the most used substance during root canal preparation due to its excellent properties and effects, such as solvent action of organic matter, lubricant, whitening, deodorizing, antimicrobial activity and removal of necrotic tissues (Spencer et al., 2007) However, this irrigant principal disadvantage is its toxicity (MOHAMMADI et al., 2017).

Studies of plants with therapeutic properties, including those with antimicrobial activity, have grown a lot (AL-ABDULLAH et al., 2022; DE SÁ ASSIS et al., 2022; DOMINGUES et al., 2022; DOS SANTOS LIBERATO et al., 2022; FERREIRA et al., 2021; MARQUES MECCATTI et al., 2022; MECCATTI et al., 2022, 2023;CESTARI et al., 2013). *Psidium guajava*, commonly known as guava, cultivated in countries of Central and South America, which are used by the population as antimicrobial agents was tested against *E. faecalis* and can be used to reduce root canal microflora and root canal failures (DUBEY, 2016).

Therefore, the aim of this pilot study was to evaluate the antimicrobial action of 2.5% NaOCl and *P. guajava* extract against *E. faecalis* and *C. albicans* for possible future use as endodontic irrigant. The null hypothesis was that *P. guajava* extract has no antimicrobial action against *E. faecalis* and *C. albicans*.

2. MATERIAL AND METHODS

2.1 Plant Extract Preparation

The hydroethanolic extract of *Psidium guajava* was prepared from the buds of leaves that, after being harvested, were dried in the dark between 20 - 27 °C for 5 days. The dried plant material was ground in a blender. The vehicle chosen for extraction was absolute ethanol (ethyl alcohol 99.5% - Merck Darmstadt, Germany) and ultrapure water obtained in the Milli-Q® system (EtOH: H2O / 50:50), following the proportion 30 g of raw material for each 200 mL of the vehicle, with an extraction time of 48 h. The extract was filtered in two stages: filtration to remove solid residues (paper filter with micro holes) and filtration for sterilization (0.22 µm membrane filter).

2.2 Determination of the Soluble Solids Content

Six empty 25 mL beakers were weighed and the weights recorded. Then, 5 mL of the extract were pipetted into each of them (triplicate) and then they were dried in at 80º C between 24 - 48 hours. After drying, the beakers were placed in a desiccator until completely cooled and then weighed. The quantity of soluble solids in the extract was quantified using the formula below:

$$\% \text{ soluble solids (m/v)} = \frac{(m-b)x\,100}{V_a}$$

% soluble solids (m/m) = % soluble solids (m/v) / density

Where:

b = beaker mass
m = final mass of the extract, after drying
V = pipetted volume in the beaker

2.3 Microorganisms and Culture Conditions

The microorganisms obtained from American Type Culture Collection (ATCC) *Candida albicans* (ATCC 18804), *Enterococcus faecalis* (ATCC 4083) in addition to a clinical strain of *Candida albicans* and *Enterococcus faecalis* were grown in specific culture medium at 37°C for 24 h, the Agar Sabouraud Dextrose (Kasvi, Italy)

supplemented with chloramphenicol 50 mg/L (Union Química, São Paulo, SP, Brazil) for *C. albicans,* and the *Enterococcus* Agar (Difco, Detroit, USA) for *E. faecalis*.

2.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicide Concentration (MMC)

To determine MIC value, broth microdilution method based on the Clinical and Laboratory Standards Institute (CLSI), standard M7-A10 (2015) was used. A standardized bacterial and fungal inoculum of each strain was prepared of culture seeded on BHI agar (Himedia, Mumbai, India) or Sabouraud Dextrose (SD) Agar (Kasvi, São José dos Pinhais, PR, Brazil) after 24 h of incubation at 37 °C and adjusted at 760 and 530 nm, respectively, in a concentration of 10^6 cells/mL by the spectrophotometer (V-5000, Shanghai Metash Instruments Co., Ltd, China). The test was performed in 96-well microplates (TPP, Trasadingen, Switzerland), performing the serial dilution of pomegranate extract in Mueller Hinton broth (Himedia, Mumbai, India) or RPMI 1640 (INLAB, São Paulo, Brazil), with glutamine and phenol red indicator, but without bicarbonate, testing 10 different concentrations, ranging from 0,83% to 0,001% (n=10 for each group). All the experimental groups were tested in duplicate for each strain.

The plates were incubated for 24h at 37 °C after inoculation of 100 µL of the bacterial suspension. Then, the MIC value was determined where the well with the lowest concentration of the extract that did not show turbidity. For the CMM determination, 10 µL of the extract at MIC value was seeded onto Brain Heart Infusion Brot (BHI) agar, as well as 10 µL of a concentration above and below MIC value. After 48h of incubation, CMM was determined at the lowest sown concentration that did not show colony growth.

3. RESULTS

The analysis of the soluble solids content for the *P. guajava* sample, considering the hydroalcoholic vehicle was 3.35%.

The minimum inhibitory concentration and the minimum microbicide concentration (MMC) of the tested groups are found in table 1 and 2.

Table 1. The minimum inhibitory concentration of NaOCl, Saline solution and *P. guajava* extract against ATCC and clinical strains of *E. faecalis* and *C. albicans.*

	NaOCl	Saline solution	*P. guajava* extract
E. faecalis ATCC	0.31 %	Not effective	Unreadable
E. faecalis (clinical)	0.15%	Not effective	Unreadable
C. albicans ATCC	0.07%	Not effective	Unreadable
C. albicans (clinical)	0,07%	Not effective	Unreadable

Source: Elaborated by the authors (2023).

Table 2. The minimum microbicide concentration of NaOCl, Saline solution and *P. guajava* extract against ATCC and clinical strains of *E. faecalis* and *C. albicans.*

	NaOCl	Saline solution	*P. guajava* extract
E. faecalis ATCC	0.31%	Not effective	0.20%
E. faecalis (clinical)	0.31%	Not effective	0.41%
C. albicans ATCC	0.01%	Not effective	Not effective
C. albicans (clinical)	0.03%	Not effective	Not effective

Source: Elaborated by the authors (2023).

4. DISCUSSION

E. faecalis is one of the main bacteria related to endodontic failure. It is a facultative anaerobic, Gram-positive bacterium that has ability to compete with other root canal bacterial cells and promote infection (SIDDIQUI; AWAN; JAVED, 2013). In the present study, the extract of *P. guajava* showed bactericidal action against *E. faecalis*, in concentrations very similar and even lower than NaOCl. Thus, the null hypothesis of this study was rejected.

NaOCl is one of the most clinically used irrigants for canal disinfection, however, this substance has low biocompatibility, and its chemical instability has been debated in the literature (REGINA et al., 2023). New substances that can be used as irrigants need to be investigated. In the present work, *P. guajava* extract shows promising effects against *E. faecalis*. Our findings are in line with the study by Chandran et al. (2021) that found that the ethanolic extract of *P. guajava* exhibited antibacterial activity against *Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus* and *E. faecalis*. The antimicrobial action of the *P. guajava* extract is reported as a broad-spectrum action, as it has action against Gram-positive and Gram-negative bacteria such as *Bacillus cereus, Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* (BISWAS et al., 2013; SHETTY et al., 2020).

In a phytochemical analysis of the *P. guajava* extract, phenolic compounds, tannins, terpenoids, flavonoids and glycosides were identified (TOUSIF et al., 2022). The presence of different compounds is also related to used vehicles. The ethanolic extract of *P. guajava* contains tannins and flavonoids, unlike the aqueous extract that contains

tannins, but does not contain flavonoids. This phenomenon occurs due to the difference in solubility of various components of guava leaves in organic solvents and water (SHETTY et al., 2020). Tannins have been described as capable of inactivating microbial adhesions and enzymes (DHIMAN et al., 2011). In the present work, the hydroethanolic extract was evaluated, which favors the extraction of compounds with affinity for both types of vehicles.

Fungi are also frequently isolated from infected root canals, with a prevalence of 3–18% of isolates. *C. albicans* is the most common fungal species that presents dimorphism, that is, it can assume the form of yeast or hyphae (YOO et al., 2020). In the present study, the hydroethanolic extract of *P. guajava* did not show antifungal action against *C. albicans*. As described earlier, the type of solvent and extraction method can play a role in the antimicrobial activity of the natural product. In an analysis of the performance of the essential oil extracted from the guava leaf, it was verified that it was effective against Gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*), Gram-negative bacteria (*Citrobacter freundii, Escherichia coli* and *Pseudomonas* spp.) and fungi (*Candida albicans, Candida tropicalis and Cryptococcus neoformans*) (LAHLOU et al., 2022). The hypothesis is that biomolecules from the *P. guajava* leaf capable of promoting action on *Candida* spp. undergo extraction only in oily vehicles.

This study indicates the use of *P. guajava* plant extract as endodontic irrigant because of its antimicrobial action. However, as a limitation of in vitro study, this extract should be evaluated in clinical studies, besides, its cytotoxicity and genotoxicity should be evaluated before being used clinically.

Lastly, the present study reveals the activity of *P. guajava* plant extract against bacteria. It showed a lower bactericidal concentration than NaOCl. To promote the bactericidal effect against *E. faecalis*, a concentration of 0.20% of the extract was necessary, on the other hand, a concentration of 0.31% of NaOCl was necessary. Such results demonstrate that the microbicidal potential of the extract can be compared to the potential of the currently used irrigant in dental clinics.

5. CONCLUSION

The hydroethanolic extract of *P. guajava* was effective against *E. faecalis* and can be considered as a possible product for the formulation of future endodontic irrigants.

REFERENCES

ABU HASNA, A. et al. Effect of sodium hypochlorite solution and gel with/without passive ultrasonic irrigation on *Enterococcus faecalis*, *Escherichia coli* and their endotoxins. **F1000Research 2020 9:642**, v. 9, p. 642, 24 jun. 2020.

ABU HASNA, A. et al. Effect of Passive Ultrasonic Irrigation over Organic Tissue of Simulated Internal Root Resorption. **International Journal of Dentistry**, v. 2021, 2021.

AL-ABDULLAH, A. et al. The Effect of Aloe vera and Chlorhexidine as Disinfectants on the Success of Selective Caries Removal Technique: A Randomized Controlled Trial. **International Journal of Dentistry**, v. 2022, 2022.

BISWAS, B. et al. Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative and Gram-Positive Bacteria. **International journal of microbiology**, v. 2013, 2013.

CARVALHO, C. A. T. et al. Clinical Study of Sodium Hypochlorite, Polymyxin B And Limewater Effect on MMP-3,-8,-9 In Apical Periodontitis. **Brazilian Dental Journal**, v. 31, n. 2, p. 116–121, 12 jun. 2020.

CESTARI, T. N. et al. Atividade antimicrobiana de agentes fitoterápicos e químicos utilizados em odontologia. **Arquivos de Ciências Da Saúde Da UNIPAR**. v. 17, n. 1, p. 19-22, 08 abr. 2013.

CHANDRAN, K. D. et al. Antibacterial evaluation of guava leaves extract and its effect on reactive oxygen species formed by calcium hydroxide and chlorhexidine mixture. **Journal of conservative dentistry : JCD**, v. 24, n. 4, p. 389–392, 1 jul. 2021.

DE, R.; LEONARDO, T.; LEONARDO, M. R. Aspectos atuais do tratamento da infecção endodôntica. **Revista da Associacao Paulista de Cirurgioes Dentistas**, v. 66, n. 3, p. 174–181, 2012.

DE SÁ ASSIS, M. A. et al. Antimicrobial and Antibiofilm Effect of Brazilian Green Propolis Aqueous Extract against Dental Anaerobic Bacteria. **Molecules 2022, Vol. 27, Page 8128**, v. 27, n. 23, p. 8128, 22 nov. 2022.

DHIMAN, A. et al. In vitro antimicrobial activity of methanolic leaf extract of Psidium guajava L. **Journal of pharmacy & bioallied sciences**, v. 3, n. 2, p. 226–229, abr. 2011.

DOMINGUES, N. et al. Antimicrobial action of four herbal plants over mixed-species biofilms of Candida albicans with four different microorganisms. **Australian Endodontic Journal**, 2022.

DOS SANTOS LIBERATO, S. F. et al. Antibiofilm action of Persea americana glycolic extract over Acinetobacter baumannii and absence of toxicity in Galleria mellonella. **Journal of Complementary and Integrative Medicine**, v. 19, n. 4, p. 905–911, 1 dez. 2022.

DUBEY, S. Comparative antimicrobial efficacy of herbal alternatives (Emblica officinalis, Psidium guajava), MTAD, and 2.5% sodium hypochlorite against Enterococcus faecalis: An in vitro study. **Journal of Oral Biology and Craniofacial Research**, v. 6, n. 1, p. 46–49, 1 jan. 2016.

FERREIRA, E. C. C. et al. The biocompatibility of Achyrocline satureioides plant extract over human gingival fibroblasts. **Research, Society and Development**, v. 10, n. 1, p. e37610111902–e37610111902, 18 jan. 2021.

HASNA, A. A. et al. Nonsurgical endodontic management of dens invaginatus: a report of two cases. **F1000Research 2019 8:2039**, v. 8, p. 2039, 2 dez. 2019.

LAHLOU, Y. et al. Chemical composition, antioxidant and antimicrobial activities of Moroccan species of Psidium guajava extracts. **Roczniki Panstwowego Zakladu Higieny**, v. 73, n. 1, p. 65–77, 2022.

MARQUES MECCATTI, V. et al. Curcuma longa L. Extract and Photodynamic Therapy are Effective against Candida spp. and Do Not Show Toxicity In Vivo. **International Journal of Dentistry**, v. 2022, 2022.

MECCATTI, V. M. et al. The biocompatibility and antifungal effect of Rosmarinus officinalis against Candida albicans in Galleria mellonella model. **Scientific Reports 2022 12:1**, v. 12, n. 1, p. 1–8, 16 set. 2022.

MECCATTI, V. M. et al. Antifungal Action of Herbal Plants' Glycolic Extracts against Candida Species. **Molecules 2023, Vol. 28, Page 2857**, v. 28, n. 6, p. 2857, 22 mar. 2023.

MOHAMMADI, Z. et al. A Review Over Benefits and Drawbacks of Combining Sodium Hypochlorite with Other Endodontic Materials. **The Open Dentistry Journal**, v. 11, n. 1, p. 661–669, 26 dez. 2017.

REGINA, S. et al. AVALIAÇÃO DO POTENCIAL HIDROGÊNIO (PH) DO HIPOCLORITO DE SÓDIO EM TRÊS DIFERENTES MOMENTOS ANTES DO SEU VENCIMENTO. **RECIMA21 - Revista Científica Multidisciplinar - ISSN 2675-6218**, v. 4, n. 5, p. e453110, 4 maio 2023.

SHETTY, S. et al. Efficacy of Psidium guajava and Allium sativum Extracts as Antimicrobial Agents against Periodontal Pathogens. **Journal of pharmacy & bioallied sciences**, v. 12, n. Suppl 1, p. S589–S594, 1 ago. 2020.

SIDDIQUI, S. H.; AWAN, K. H.; JAVED, F. Bactericidal efficacy of photodynamic therapy against Enterococcus faecalis in infected root canals: a systematic literature review. **Photodiagnosis and photodynamic therapy**, v. 10, n. 4, p. 632–643, dez. 2013.

SPENCER, H. R.; IKE, V.; BRENNAN, P. A. Review: the use of sodium hypochlorite in endodontics — potential complications and their management. **British Dental Journal 2007 202:9**, v. 202, n. 9, p. 555–559, 12 maio 2007.

TOUSIF, M. I. et al. Psidium guajava L. An Incalculable but Underexplored Food Crop: Its Phytochemistry, Ethnopharmacology, and Industrial Applications. **Molecules (Basel, Switzerland)**, v. 27, n. 20, 1 out. 2022.

YOO, Y. J. et al. Candida albicans Virulence Factors and Pathogenicity for Endodontic Infections. **Microorganisms**, v. 8, n. 9, p. 1–18, 1 set. 2020.