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Original Article

Antimicrobial effect of *Pentaclethra Macroloba* plant extract against *Enterococcus Faecalis*

Efeito antimicrobiano do extrato de *Pentaclethra Macroloba* contra *Enterococcus Faecalis*

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Abstract

This study evaluated the antimicrobial efficacy of a new intracanal drug based on *Pentaclethra macroloba* extract, a plant of Amazonian origin, against *Enterococcus faecalis* using macrodilution test and intratubular evaluation with Confocal Laser Scanning Microscopy (CLSM). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the pure extract of *Pentaclethra macroloba* andits association with calcium hydroxide and ultracall were determined. Then, thirty-three dentin cylinders were prepared and inoculated with *E. faecalis*, to evaluate the antimicrobial effect of the medications on the dentinal tubules with CLSM. The data was analyzed using the Kruskal-Wallis and Dunn tests. The extract in association with calcium hydroxide showed a lower CBM, and in the intratubular test all tested medications were effective against *E. faecalis* (P >0.05). The new intracanal drug based on *P. macroloba* extract has an antimicrobial effect against *E. faecalis* and further studies are needed for its clinical use.

Keywords: antimicrobial effect, plant extract, intracanal medication, *Pentaclethra macroloba*.

Resumo

Este estudo avaliou a eficácia antimicrobiana de uma nova droga intracanal à base de extrato de *Pentaclethra macroloba*, uma planta de origem amazônica, contra *Enterococcus faecalis* por meio do teste de macrodiluição e avaliação intratubular em Microscopia Confocal de Varredura a Laser (CLSM). Foram determinadas a concentração inibitória mínima (CIM) e a concentração bactericida mínima (CBM) do extrato puro de *Pentaclethra macroloba,* sua associação com hidróxido de cálcio e ultracall. Em seguida, trinta e três cilindros de dentina foram preparados e inoculados com *E. faecalis*, para avaliar o efeito antimicrobiano de medicamentos nos túbulos dentinários em CLSM. Os dados foram analisados por meio dos testes de Kruskal-Wallis e Dunn. O extrato em associação com hidróxido de cálcio apresentou menor CBM. E no teste intratubular, todas as medicações testadas foram efetivas contra *E. faecalis* (P >0,05). A nova droga intracanal à base de extrato de *P. macroloba* tem efeito antimicrobiano contra *E. faecalis* e mais estudos são necessários para seu uso clínico.

Palavras-chave: efeito antimicrobiano, extrato de planta, medicamentos intracanais, *Pentaclethra macroloba*.

1. Introduction

The chemical-mechanical procedures performed during endodontic treatment are important to obtain cleaning, shaping, debridement, and consequently, bacterial reduction for peri-radicular tissue repair to occur (Siqueira Júnior et al., 2018). However, sometimes only the action of endodontic files in association with irrigants is not enough to eliminate microorganisms from the root canal system (RCS) (Kishan et al., 2019). Thus, additional methods such as the use of intracanal medication with antibacterial action are necessary to optimize the disinfection of the RCS, mainly in cases where the infection is persistent even after the end of the endodontic therapy and there is presence of pain or constant exudation (Zancan et al., 2016).

Calcium hydroxide ($Ca(OH)_2$) is one of the most used endodontic drugs with endodontic medication (Varshini et al., 2019) due to its biological induction of mineralized tissue deposits (Mohammadi and Dummer, 2011) and its antimicrobial properties against a wide

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range of bacteria present in root canal, which makes it the medication of choice for use as an intracanal dressing (Holland et al., 1999). Medication base of ($Ca(OH)_2$) as the medication used in endodontic routine, has antimicrobial activity, induces mineralization, has activity against bacterial LPS and is biocompatible (Zancan et al., 2016). However, it is not able to eliminate all the microorganisms present in the RCS (Aguiar et al., 2015; Zancan et al., 2016).

Facultative anaerobic bacteria such as *Enterococcus faecalis* (Gram-positive bacteria) *Actinomyces naeslundii, L. rhamnosus, L. casei, Streptococcus sanguinis, S. mitis, and Candida albicans* are microorganisms present in endodontic treatment failure (Pourhajibagher et al., 2017). *E. faecalis* is observed in asymptomatic and persistent therapies, being a bacterium resistant to endodontic treatment, which becomes a challenge for success when endodontic medication is not used between sessions (Darrag, 2013).

With the increase in bacterial resistance due to the indiscriminate use of antibiotics, an increase in the development of other classes of antimicrobials for infection control can be noted. Products extracted from roots, barks, and seeds have been an important source of research to formulate new drugs with antimicrobial action (Dotta et al., 2015; Al-Ansari et al., 2019; Kishan et al., 2019; Souza et al., 2022). *Pentaclethra macroloba* is a native Amazonian tree commonly known as "pracaxi" that stands out due to its eco-sustainable exploitation (Teixeira et al., 2020), its antibacterial action against Gram-positive (Staphylococcus spp. and Enterococcus spp.) and Gram-negative (*Pseudomonas aeruginosa*, Acinetobacter spp. and *Klebsiella pneumoniae*) (Leal et al., 2011), and its healing action (Banov et al., 2014).

In the need to find new drugs from natural products, the aim of the present study was to analyze a new endodontic medication based on natural extract of Pentaclethra macroloba, pure and associated with ($Ca(OH)_2$) and compare it to UltraCall XS (UL) to verify its antibacterial activity against *E. faecalis*. The null hypothesis was that there would be no difference in antimicrobial activity between the groups.

2. Materials and Method

2.1. Extraction

Pentachletra macroloba plant extract was obtained at the Laboratory of Bioprospection and Atomic Absorption (LAAB) of the Federal University of Amapá (UNIFAP). *Pentaclethra Macroloba* peels were cleaned and dehydrated in an oven at 37 °C for 3 days. After dehydration, grinding was performed in a mechanical mill taking the sample to powder dimensions. The bark powder was covered with ethyl alcohol PA ten percent above the sample volume, and stored for four days, with homogenization movements being carried out to enhance the extraction process, four times a day. The powder and solvent set (PA ethyl alcohol) passed through a filtering system with filter paper where the solvent was eliminated and recovered by steam distillation, seeking to balance the process by optimizing the distillation with the temperature of the water bath at the lowest temperature, with a vacuum pump system to promote the drag of the solvent thus obtaining the extract at 100%.

2.2. Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

Preliminary antibacterial activity was tested by macrodilution test to determine the concentration needed to kill the reference bacterial strains of *E. faecalis* (ATCC 29212 - American Type Culture Collection). *E. faecalis* were obtained from Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. *E. faecalis* was subcultured in brain heart infusion (BHI) broth (Difco) as a facultative microorganism.

The endodontic medications tested were:

ULT (Calcium hidroxide with methylcellulose): The medication prepared with calcium hydroxide and methylcellulose (Ultracal XS, Salt Lake, USA);

CHP (calcium hidroxide associated with *Pentaclethra macroloba* extract): Calcium hydroxide PA (Synth, São, SP, BR) associated with *Pentaclethra macroloba* extract; and

PME (*Pentaclethra macroloba* extract): pure ethyl extract from the bark of the plant.

Negative control: without contamination, but with treatment (ULT, CHP and PME).

Positive control: with culture medium for bacterial growth .

For the macrodilution test (Andrade Ferreira et al., 2007), screw-capped tubes containing 3 mL of BHI broth were used, and precise volumes of antimicrobials were added to the broths and repeaters in triplicate. The inoculum was obtained after 24 hours of incubation in BHI broth at 37 °C under anaerobic conditions. Tube turbidity was read on a spectrophotometer (Ultrospec 1000; Amersham Pharmacia Biotech, Cambridge, UK) at 540 nm. Then, cultures were diluted to MacFarland 3 standard and $5x10^5$ UFC/mL and distributed in volumes of 3 mL to each tube containing diluted broth and medication.

Turbidity readings were taken on the spectrophotometer before and after anaerobic incubation to detect tubes with bacterial growth. The tube readings determined the minimum inhibitory concentration (MIC) for the tested medication. The time required for growth bacteria was 24 hours in BHI broth. Negative and positive bacterial growth controls were used.

After reading the final absorbances, 25 µL of the solution from each tube was transferred to RCM blood agar plates measuring 15 x 60 millimeters. These plates were anaerobically incubated at 37 °C for 48 hours to establish the minimum bactericidal concentration (MBC). MBC was considered the lowest concentration of the drug that was able to inhibit bacterial growth on the plates. The tests were made in triplicate.

2.3. Intratubular viability assessment

Thirty-three human teeth extracted after approval by the Human Research Ethics Committee (CAAE: 39393620.7.0000.5416) were selected from the tooth

bank and divided into the three groups of endodontic medications tested (n=10), including one tooth of negative control in each group, without contamination but with treatment, and a tooth of positive control with bacterial growth.

Sample size calculation was based on previous studies using the G* Power v 3.1 software for Mac (Heinrich Heine, University of Düsseldorf, Germany). For three experimental groups using α error probability of 0.05, a power of 0.95, and N2/N1 ratio of 1. Ten specimens per group as the optimal size. With positive controls (C+) for intratubular bacterial contamination in all groups, n=10 teeth were used.

The extracted teeth were stored for 48 hours in a 1% sodium hypochlorite solution for initial decontamination, then stored in distilled water. The crowns of the teeth were cut and patterned on a dentin cylinder using Isomet (Isome 1 standardized, IL, USA) with a diamond blade at 250 rpm, under activated irrigation.

Root canals were prepared using ProTaper F5 rotary files (Dentsply, Tulsa Especialidades Odontológicas) with an endodontic appliance (X-Smart Plus Maillefer Dentsply) according to the manufacturer's instructions. During preparation, irrigation with 2.5% hypochlorite was carried; after that, irrigation for 5 min with etilenodiaminotetraacetic acid 17% (EDTA) (Chemical and Pharmaceutical Biodynamics, Ibiporã, PR, Brazil) was carried; then, the channels were washed with deionized water and dried for 24 h before beings autoclaved at 121 °C.

E. faecalis (ATCC 29212) was reactivated in infusion broth (BHI, Brain Disc, Kansas City, MO, USA) and kept at 37°C for 24 h. The cultured bacteria were transferred to a new BHI infusion broth and cultured for 24 hours for exponential growth. This culture was adjusted to McFarland Standard Nº 3 (9x 108 UFC/mL) using an SF325NM spectrophotometer SF325NM (Bel Photonics do Brazil Ltda, Osasco, SP, Brazil).

For the intratubular contamination test (Andrade et al., 2015), 800 µL of BHI was inserted into an Axygen Scientific microtube (Axygen Scientific, Union City, CA, USA) containing the dentin cylinder. A 15-minute ultrasonic bath was performed to allow maximum penetration of the culture medium into the dentine tubes. The contamination was carried with the microorganism *E. faecalis* and endodontic medications were inserted in the teeth for seven days. The inoculum (800 µL) was inserted into the microtubes with the dentin cylinders and centrifuged on alternate days (Eppendorf 5417R centrifuge, Eppendorf, Hamburg, Germany). The inoculum was renewed at each centrifugation cycle. On day seven, the samples were taken from microtubes and observed with Confocal Laser Scanning Microscopy (CLSM), using LIVE/DEAD® BacLightTM fluorescent identification (Molecular Probes, Eugene, OR, USA) to verify bacterial penetration and viability. Four images of each half were obtained for intratubular evaluation of each tooth.

The specimens were longitudinally sectioned with a diamond disk with Isomet. Half of the dentinal tubes were stained with 30 µL of LIVE/DEAD® bacterial viability measurement kit (Eugene Molecular Probes, OR) for 20 minutes thus allowing the identification of viable bacteria. Samples were examined with a Leica Confocal Microscope in TCS-SPE (Leica, Baden-Württemberg, Germany) using a 40X ampliefied lens. Eight sequential images were taken from each dentinal tube, four from the cervical third and four from the medial third. Such images were taken in fragments using the Leica Application Suite Advanced Fluorescence Software (LAS AF, Leica, Mannheim, Baden-Würberg, Germany).

This data was statistically evaluated using the Kruskal-Wallis and Dunn tests analysis using GraphPad software. A p-value <0.05 was considered statistically significant.

3. Results

3.1. MIC and MBC

All tested substances were able to inhibit and eliminate all strains of *E. faecallis*. However, the pure extract of pracaxi required a higher concentration than the other substances tested (Table 1). The vehicle used, propylene glycol, did not influence the antimicrobial effect. The concentration necessary to eliminate *E. faecallis* was 25% for pure *P. macroloba* extract, and when associated, 3.12% for *P. macroloba* extract and 4.37% for calcium hydroxide. Ultracall was considered as a control group, as it is already a drug with proven antimicrobial action, with 6.25% of MBC.

3.2. Intratubular viability assessment

The CLSM images obtained showed that there was bacterial penetration in all groups and throughout the root canal. A higher concentration of dead than viable bacteria was observed in all groups except the control group, which had only intratubular contamination (Figure 1).

There was a statistically significant difference between the positive control group and the others in relation to total, cervical, middle, superficial, and deep areas ($p<0.05$) (Table 2). The pure extract when compared to the one associated with calcium hydroxide showed no significant difference in any analysis ($p>0.05$). And when compared to ULT, only statistical differences were observed in deep ($p<0.05$).

Table 1. MIC and MBC in percentage (%) of the extract of all endodontic medications obtained by the macrodilution method in brain-heart infusion broth in *Enterococcus faecalis*.

PME		ULT		CHP	
MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
12.5mg/mL	25.0 mg/mL	1.56 mg/mL	6.25 mg/mL	-	3.12 + 4.37 mg/mL

PME = *Pentaclethra macroloba* extract pure; ULT = Calcium hydroxide with methylcellulose; CHP = Calcium hydroxide with *Pentaclethra macroloba* extract.

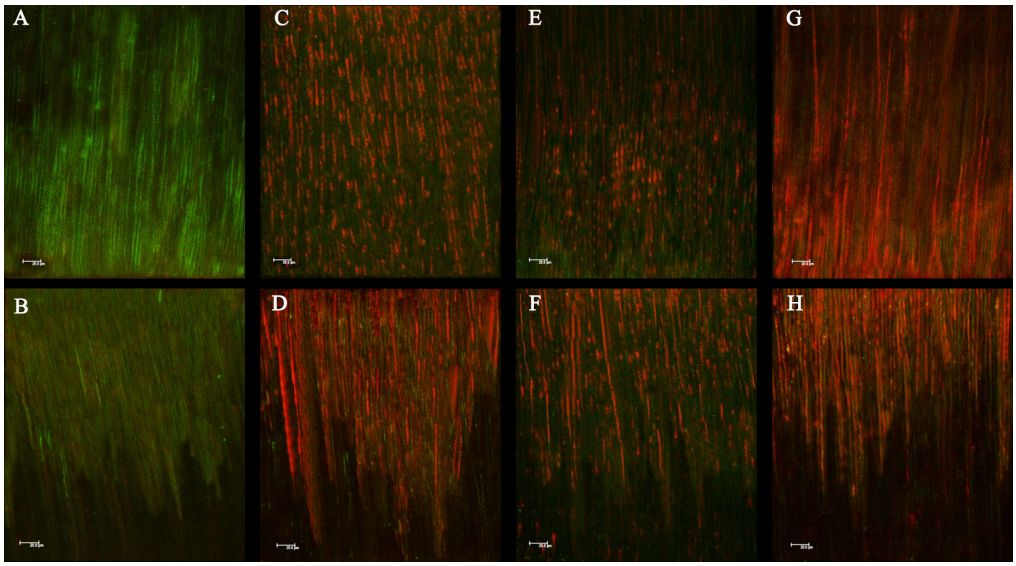

Figure 1. Confocal laser scanning microscopy (CLSM) images following contamination with *E. faecalis*. Longitudinal views of the cervical and middle third of root canal. Live bacteria can be observed in green and dead bacteria an be observed in red. (A and B) Control grup in the middle and cervical third respectively; (C and D) Ultracal group in the middle and cervical third respectively; (E and F) Pracaxi group middle and cervical third respectively; (G and H) Pracaxi with calcium hydroxide in the middle and cervical third respectively.

Table 2. Median percentage (95% confidence interval) of viable bacteria in the dentinal tubules after treatment in each group.

Group	Total	Cervical	Middle	Superficial	Deep
Control	51.64 (32.02-76.64)^{Aa}	45.39 (25.86-70.03)^{Aa}	55.67 (28.26-85.22)^{Aa}	58.23 (29.61-78.40)^{Aa}	45.39 (23.82-79.00)^{Aa}
Pracaxi 25%	32.40 (8.49-62.14)^{Ba}	41.19 (6.26-66.84)^{Aa}	25.53 (10.48-48.86)^{Ba}	23.38 (5.08-45.14)^{Ba}	38.11 (13.72-79.59)^{BCa}
Ultracal 25%	14.04 (2.31-47.53)^{Ba}	12.83 (1.34-49.44)^{Aa}	23.36 (2.24-46.33)^{Ba}	15.32 (1.80-47.57)^{Ba}	13.03 (2.49-47.02)^{Ca}
Pracaxi 6,25% + $Ca(OH)_2$ 8.75%	29.56 (10.71-63.97)^{Ba}	30.85 (6.82 – 61.20)^{Aa}	28.95 (11.55-64.15)^{Ba}	36.44 (10.38-63.97)^{ABa}	23.73 (10.71-61.49)^{BCa}

Test Kruskal-Wallis and Dunn ($p < 0,05$). Different capital letters superscript in a column represent significant differences between groups; different superscript lowercase letters in a line represent significant differences within groups.

4. Discussion

The present study was carried out using one strainof *E. faecalis* as this is the Gram-positive facultative bacterium most resistant to endodontic treatment, being frequently present when endodontic treatment fails (Almeida Gomes et al., 2002). In addition to having a capacity for deep intratubular penetration and biofilm formation in the periapical region, which contributes to its resistance to chemical-mechanical root preparation protocols (Ferrer-Luque et al., 2014), it also has the ability to proliferate after a period of incubation (Pereira et al., 2021). The bacterial reference strain ATCC 29212 *E. faecalis* used in this study has been tested in several studies because of its virulence factors that pose a challenge to current antimicrobial agents (Andrade et al., 2015; Ghoddusi et al., 2019; Cunha-Neto et al., 2021).

There are few reports in the literature on the use of *Pentaclethra macroloba*, but its antimicrobial effect was highlighted in a study by Leal et al. (2011). Due to this search for a drug with antimicrobial effect that is extracted from the Brazilian biome, a macrodilution test was performed for the use of the extract against the main microorganism resistant to endodontic treatment, *E. faecalis.* New endodontic medication was formulated where the pracaxi extract was associated with calcium hydroxide, which has proven bactericidal action effect, and was compared to two medications with Ultracal paste. In these procedures it was proved that the extract has bactericidal action on *E. faecalis*, and thus its MBC (25%) when pure and MBC (3.12%) when associated with calcium hydroxide was determined. The PCH needed a lower concentration of the extract to have the bactericidal effect; this is justified by the addition of $Ca(OH)_2$. The extract has a bactericidal

effect too, confirming what the literature has already noted about its isolated action (Bystrom et al., 1985).

The intratubular analysis was performed in order to simulate the situation that occurs in endodontic infections in the oral environment. To approximate how the procedures occur during an endodontic treatment, teeth extracted from humans were standardized at 12 mm, forming cylinders of dentin. They were prepared with ProTaper F3 rotary instrumentation and irrigated with 2.5% hypochlorite and a final irrigation with 17% EDTA. To promote an effective bacterial contamination by *E. faecalis* inside the dentinal tubules, the centrifugation protocol suggested by Ma et al. (2011) was used. This ensured better intratubular proliferation for the study than that provided by previous methods (Andrade et al., 2015).

An untreated and contaminated specimen was used as a control in each group, showing that the dentinal tubules were contaminated in the cervical, middle, superficial, and deep areas. After statistical testing, a significant difference was demonstrated between the control groups and the 3 medications under study. The Ultracal group served as a parameter, as it is an intracanal medication with proven effects against the microorganism under study. Having presented a statistically significant difference regarding the control group in all analyzes ($p<0.05$), this proves its effect and agrees with the literature (Guerreiro et al., 2021).

Several Amazonian medicinal herbs are completely unknown scientifically regarding their medicinal properties (Belhadj-Salaha et al., 2022). Nevertheless, the native population with traditional knowledge passed from generation to generation, knows how to use different herbs according to symptoms (Oliveira et al., 2013). The use of medicinal herbs has gained strength in research for new medications, as they are natural and do not cause microbial resistance (Leal et al., 2011; Dotta et al., 2015). *P. macroloba* is a plant native to riverside regions of the Amazon Rainforest, which has been little investigated in the scientific literature, but has been widely used by the native population as an antimicrobial, anti-inflammatory and healing agent. Studies have shown its effect as healing (Banov et al., 2014; Simmons et al., 2015), anti-hemorrhagic (Silva et al., 2007) and antimicrobial against *E. faecallis* (Leal et al., 2011). This agrees with the results found in the study, which confirm the antimicrobial action in both the macrodilution and intratubular assays against *E. Faecalis.*

The association with $Ca(OH)_2$ was proposed because $Ca(OH)_2$ has been widely used in endodontic therapy as intracanal medication. It is considered a root canal dressing that acts as a physical barrier, preventing RCS reinfection and thus interrupting the arrival of nutrients for the remaining bacteria (Siqueira Júnior and Lopes, 1999). Its antimicrobial effect is associated with its high pH (Bystrom et al., 1985), but no study has proven that it alone can eliminate microorganisms from the RCS. Its association with camphorated paramonochlorophenol is necessary, which is a substance toxic to living tissues (Wang et al., 2007). Searching its association with natural plants is very important, to minimize toxic effects of other products already used. A study that made an association of chitosan and propolis, herbal medicines, showed that the natural extract when associated obtained better antimicrobial results, which is in agreement with the finding that *P. macroloba* extract associated with $Ca(OH)_2$ required a lower concentration of the extract to have action against *E. faecalis* (Parolia et al., 2020).

More studies should be carried out to evaluate other characteristics of the extract in terms of in vitro and in vivo tissue repair, a property necessary for an ideal intracanal medication.

5. Conclusion

Within the limitations of our *in vitro* experiments, the null hypothesis tested was partially confirmed, as the new medication, pracaxi 25% and pracaxi 6.25% with $Ca(OH)_2$ 8.75%, presented similar results to the Ultracall group. The results showed statistically equal in total, medium, cervical, superficial and deep areas. This proves the antimicrobial effect of the tested medication and indicates the *P. macroloba* extract as a promising intracanal medication.

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References

AGUIAR, A.S., GUERREIRO-TANOMARU, J.M., FARIA, G., LEONARDO, R.T. and TANOMARU-FILHO, M., 2015. Antimicrobial activity and ph of calcium hydroxide and zinc oxide nanoparticles intracanal medication and association with chlorhexidine. *The Journal of Contemporary Dental Practice*, vol. 16, no. 8, pp. 624-629. http://dx.doi.org/10.5005/jp-journals-10024-1732. PMid:26423497.

AL-ANSARI, M., AL-HUMAID, L.A., VIJAYARAGHAVAN, P., RAVINDRAN, B., CHANG, S.W., AGASTIAN, P. and BALAMURALIKRISHNAN, B., 2019. Identification of phytochemical components from *Aerva lanata* (Linn.) medicinal plants and its in-vitro inhibitory activity against drug resistant microbial pathogens and antioxidant properties. *Saudi Journal of Biological Sciences*, vol. 26, no. 6, pp. 1129-1133. http://dx.doi.org/10.1016/j.sjbs.2019.02.010. PMid:31516340.

ALMEIDA GOMES, B.P.F., FERRAZ, C.C.R., GARRIDO, F.D., ROSALEN, P.L., ZAIA, A.A., TEIXEIRA, F.B. and DE SOUZA-FILHO, F.J., 2002. Microbial susceptibility to calcium hydroxide pastes and their vehicles. *Journal of Endodontics*, vol. 28, no. 11, pp. 758-761. http://dx.doi.org/10.1097/00004770-200211000-00003. PMid:12470019.

ANDRADE FERREIRA, F.B., TORRES, S.A., DA SILVA ROSA, O.P., FERREIRA, C.M., GARCIA, R.B., MARCUCCI, M.C. and GOMES, B.P., 2007. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. Oral Surgery. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, vol. 104, no. 5, pp. 709-716. http://dx.doi.org/10.1016/j.tripleo.2007.05.019.

ANDRADE, F.B.D., ARIAS, M.P.C., MALIZA, A.G.A., DUARTE, M.A.H., GRAEFF, M.S.Z., AMOROSO-SILVA, P.A. and MORAES, I.G.D., 2015. A new improved protocol for in vitro intratubular dentinal bacterial contamination for antimicrobial endodontic tests: standardization and validation by confocal laser scanning microscopy. *Journal*

of Applied Oral Science, vol. 23, no. 6, pp. 591-598. http://dx.doi.org/10.1590/1678-775720140261. PMid:26200524.

BANOV, D., BANOV, F. and BASSANI, A.S., 2014. Case series: the effectiveness of fatty acids from pracaxi oil in a topical silicone base for scar and wound therapy. *Dermatology and Therapy*, vol. 4, no. 2, pp. 259-269. http://dx.doi.org/10.1007/s13555-014-0065-y. PMid:25410612.

BELHADJ-SALAHA, K., SHEIKHC, H.M., AL-HASAWIA, Z.M., SELIME, E.M. and TOULIABAHF, H.E., 2022. In vitro antifungal and antibacterial potentials of organic extracts of *Avicennia marina* collected from Rabigh Lagoon, Red Sea Coasts in Saudi Arabia. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e265038. http://dx.doi.org/10.1590/1519-6984.265038. PMid:36259892.

BYSTRÖM, A., CLAESSON, R. and SUNDQVIST, G., 1985. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endodontics & Dental Traumatology*, vol. 1, no. 5, pp. 170-175. http://dx.doi.org/10.1111/j.1600-9657.1985.tb00652.x. PMid:3865763.

CUNHA-NETO, M.A., DE ALMEIDA COÊLHO, J., PINTO, K.P., CUELLAR, M.R.C., MARCUCCI, M.C., SILVA, E.J.N.L. and SASSONE, L.M., 2021. Antibacterial efficacy of triple antibiotic medication with macrogol (3Mix-MP), traditional triple antibiotic paste, calcium hydroxide, and ethanol extract of propolis: an intratubular dentin ex vivo confocal laser scanning microscopic study. *Journal of Endodontics*, vol. 47, no. 10, pp. 1609-1616. http://dx.doi.org/10.1016/j.joen.2021.07.014. PMid:34310981.

DARRAG, A.M., 2013. Antimicrobial efficacy of endodontic irrigation solutions against planktonic microorganisms and dual-species biofilm. *Tanta Dental Journal*, vol. 10, no. 3, pp. 129-137. http://dx.doi.org/10.1016/j.tdj.2013.11.005.

DOTTA, G.A., LEDIC-NETO, J.A., GONÇALVES, E.L.T.A., BRUM, A.A., MARASCHIN, M.B. and MARTINS, M.L.A., 2015. Acute inflammation and hematological response in Nile tilapia fed supplemented diet with natural extracts of propolis and *Aloe barbadensis*. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 75, no. 2, pp. 491-496. http://dx.doi.org/10.1590/1519-6984.02413. PMid:26132037.

FERRER-LUQUE, C.M., BEJARANO, I., RUIZ-LINARES, M. and BACA, P., 2014. Reduction in *Enteroccocus faecalis* counts–a comparison between rotary and reciprocating systems. *International Endodontic Journal*, vol. 47, no. 4, pp. 380-386. http://dx.doi.org/10.1111/iej.12158. PMid:23980811.

GHODDUSI, J., MOUSHEKHIAN, S., ARIAN, E., GHIASI, J. and FORGHANI, M., 2019. The effectiveness of sonic-activated irrigation in reducing intratubular Enterococcus faecalis. *Iranian Endodontic Journal*, vol. 14, no. 1, pp. 63-67. http://dx.doi.org/10.22037/iej.v14i1.22436. PMid:36879604.

GUERREIRO, J.C.M., OCHOA-RODRÍGEZ, V.M., RODRIGUES, E.M., CHAVEZ-ANDRADE, G.M., TANOMARU-FILHO, M., GUERREIRO-TANOMARU, J.M. and FARIA, G., 2021. Antibacterial activity, cytocompatibility and effect of Bio-C Temp bioceramic intracanal medicament on osteoblast biology. *International Endodontic Journal*, vol. 54, no. 7, pp. 1155-1165. http://dx.doi.org/10.1111/iej.13502. PMid:33638900.

HOLLAND, R., DE SOUZA, V., NERY, M.J., OTOBONI FILHO, J.A., BERNABÉ, P.F. and DEZAN JR, E., 1999. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. *Journal of Endodontics*, vol. 25, no. 3, pp. 161-166. http://dx.doi.org/10.1016/S0099-2399(99)80134-4. PMid:10321179.

KISHAN, K.V., SHAH, N.C., DAS, D.T. and PARIKH, M., 2019. Herbal medicaments in endodontics–Current guidelines for in vivo studies in India. *Journal of Conservative Dentistry*, vol. 22, no. 5, pp. 411-414. http://dx.doi.org/10.4103/JCD.JCD_169_19. PMid:33082653.

LEAL, I.C.R., JÚNIOR, I., PEREIRA, E.M., LAPORT, M.S., KUSTER, R.M. and SANTOS, K.R.D., 2011. Pentaclethra macroloba tannins fractions active against methicillin-resistant staphylococcal and Gram-negative strains showing selective toxicity. *Revista Brasileira de Farmacognosia*, vol. 21, no. 6, pp. 991-999. http://dx.doi.org/10.1590/S0102-695X2011005000132.

MA, J., WANG, Z., SHEN, Y. and HAAPASALO, M., 2011. A new noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. *Journal of Endodontics*, vol. 37, no. 10, pp. 1380-1385. http://dx.doi.org/10.1016/j.joen.2011.06.018. PMid:21924186.

MOHAMMADI, Z. and DUMMER, P.M.H., 2011. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *International Endodontic Journal*, vol. 44, no. 8, pp. 697-730. http://dx.doi.org/10.1111/j.1365-2591.2011.01886.x. PMid:21535021.

OLIVEIRA, A.A., SEGOVIA, J.F., SOUSA, V.Y., MATA, E.C., GONÇALVES, M.C., BEZERRA, R.M., OM JUNIOR, P. and KANZAKI, L.I., 2013. Antimicrobial activity of amazonian medicinal plants. *SpringerPlus*, vol. 2, no. 1, pp. 371. http://dx.doi.org/10.1186/2193-1801-2-371. PMid:23961431.

PAROLIA, A., KUMAR, H., RAMAMURTHY, S., DAVAMANI, F. and PAU, A., 2020. Effectiveness of chitosan-propolis nanoparticle against *Enterococcus faecalis* biofilms in the root canal. *BMC Oral Health*, vol. 20, no. 1, pp. 339. http://dx.doi.org/10.1186/s12903-020-01330-0. PMid:33238961.

PEREIRA, T.C., DIJKSTRA, R.J.B., PETRIDIS, X., SHARMA, P.K., VAN DE MEER, W.J., VAN DER SLUIS, L.W.M. and DE ANDRADE, F.B., 2021. Chemical and mechanical influence of root canal irrigation on biofilm removal from lateral morphological features of simulated root canals, dentine discs and dentinal tubules. *International Endodontic Journal*, vol. 54, no. 1, pp. 112-129. http://dx.doi.org/10.1111/iej.13399. PMid:32880989.

POURHAJIBAGHER, M., GHORBANZADEH, R., PARKER, S., CHINIFORUSH, N. and BAHADOR, A., 2017. The evaluation of cultivable microbiota profile in patients with secondary endodontic infection before and after photo-activated disinfection. *Photodiagnosis and Photodynamic Therapy*, vol. 18, pp. 198-203. http://dx.doi.org/10.1016/j.pdpdt.2017.02.013. PMid:28249746.

SILVA, J.O., FERNANDES, R.S., TICLI, F.K., OLIVEIRA, C.Z., MAZZI, M.V., FRANCO, J.J. and SAMPAIO, S.V., 2007. Triterpenoid saponins, new metalloprotease snake venom inhibitors isolated from *Pentaclethra macroloba*. *Toxicon*, vol. 50, no. 2, pp. 283-291. http://dx.doi.org/10.1016/j.toxicon.2007.03.024. PMid:17517426.

SIMMONS, C.V., BANOV, F. and BANOV, D., 2015. Use of a topical anhydrous silicone base containing fatty acids from pracaxi oil in a patient with a diabetic ulcer. *SAGE Open Medical Case Reports*, vol. 3, p. 2050313X1558967.

SIQUEIRA JÚNIOR, J.F. and LOPES, H., 1999. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *International Endodontic Journal*, vol. 32, no. 5, pp. 361-369. http://dx.doi.org/10.1046/j.1365-2591.1999.00275.x. PMid:10551109.

SIQUEIRA JÚNIOR, J.F., PÉREZ, A.R., MARCELIANO-ALVES, M.F., PROVENZANO, J.C., SILVA, S.G., PIRES, F.R. and ALVES, F.R.F., 2018. What happens to unprepared root canal walls: a correlative analysis using micro-computed tomography and histology/scanning electron microscopy. *International Endodontic Journal*, vol. 51, no. 5, pp. 501-508. http://dx.doi.org/10.1111/iej.12753. PMid:28196289.

SOUZA, L.P., ZUIM, V., STINGUEL, P., PINHEIRO, P.F. and ZAGO, H.B., 2022. Toxicity of essential oil of *Mentha piperita* (Lamiaceae) and its *Monoterpenoid Menthol* against *Tetranychus urticae* Kogan 1836 (Acari: tetranychidae). *Anais da Academia Brasileira de Ciências*, vol. 94, no. 4, suppl. 4, pp. e20200427. http://dx.doi.org/10.1590/0001-3765202220200427. PMid:36477817.

TEIXEIRA, G.L., MACIEL, L.G., MAZZUTTI, S., GONÇALVES, C.B., FERREIRA, S.R.S. and BLOCK, J.M., 2020. Composition, thermal behavior and antioxidant activity of pracaxi (*Pentaclethra macroloba*) seed oil obtained by supercritical CO2. *Biocatalysis and Agricultural Biotechnology*, vol. 24, pp. 101521. http://dx.doi.org/10.1016/j.bcab.2020.101521.

VARSHINI, R., SUBHA, A., PRABHAKAR, V., MATHINI, P., NARAYANAN, S. and MINU, K., 2019. Antimicrobial efficacy of *Aloe vera*, Lemon, *Ricinus communis*, and calcium hydroxide as intracanal medicament against *Enterococcus faecalis:* a confocal microscopic study. *Journal of Pharmacy & Bioallied Sciences*, vol. 11, no. 6, suppl. 2, pp. 256-259. http://dx.doi.org/10.4103/JPBS.JPBS_5_19. PMid:31198348.

WANG, X.L., SONG, M., LOU, J.N. and NIU, X.Y., 2007. The study of cytotoxicity of different intracanal medications and cell rehabilitation on human periodontal ligament fibroblasts. *Shanghai Journal of Stomatology*, vol. 16, no. 5, pp. 512-519.

ZANCAN, R.F., VIVAN, R.R., LOPES, M.R.M., WECKWERTH, P.H., DE ANDRADE, F.B., PONCE, J.B. and DUARTE, M.A.H., 2016. Antimicrobial activity and physicochemical properties of calcium hydroxide pastes used as intracanal medication. *Journal of Endodontics*, vol. 42, no. 12, pp. 1822-1828. http://dx.doi.org/10.1016/j.joen.2016.08.017. PMid:27776884.