

Full Length Research Paper

Analysis of the antimicrobial action of copaiba oil and endodontic substances against anaerobic bacteria

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Endodontic infections are polymicrobial and predominantly caused by anaerobic bacteria and some facultative bacteria. The list of microorganisms involved in endodontic infections keeps expanding and has the potential to become increasingly more accurate during the next few years. Copaiba oil is an important Amazonian herbal medicine commercialized worldwide. In this study, we evaluated the antimicrobial activity and minimum inhibitory concentration (MIC) of copaiba oil and substances used in the treatment of endodontic infections against anaerobic microorganisms such as *Prevotella melaninogenica*; *Prevotella intermedia*; and *Clostridium acetobutylicum*. The MIC was determined by thioglycollate broth dilution. The data were statistically analyzed by Tukey's parametric and non-parametric methods of Cochran and Kruskal-Wallis test with a confidence level of 99%. The analysis of the antimicrobial activity showed that the samples of Copaiba oil, Sodium Hypochlorite, Otosporin, Tricresol formalin, Chlorhexidine and PMCC showed high antimicrobial activity ($p < 0.01$). However, different copaiba samples presented different activities. The results reveal Copaiba I sample was the most effective against anaerobic bacteria.

Key words: Essential oil, endodontic therapy, anaerobic microorganisms.

INTRODUCTION

Periapical lesions are diseases resulting from microbial contamination, necrosis of the pulp tissue, and infection progression toward the periodontal ligament and alveolar bone (Lucisano et al., 2014). The microorganisms, in particular Gram-negative, predominantly anaerobic, bacteria, that are able to initially colonize the dental pulp tissue cause primary infection, whereas those present in

the treated root canal system are the etiological agents responsible for secondary infections (Arias et al., 2016; Pourhajbagher et al., 2017). Among the manoeuvres of endodontic therapy, only mechanical instrumentation is not able to effectively and/or permanently reduce a load of bacteria present in the root canal system. Therefore, antibacterial agents for irrigation and medication have

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been used to aid in the reduction of these microorganisms. However, there are studies demonstrating that despite the use of such agents, there may still be bacterial resistance (Al-Ahmad et al., 2014; Cavalcante et al., 2017). Because of this, much has been investigated on the antimicrobial action of substances used in endodontics.

These infections occur due to the presence of opportunistic microorganisms or resistance to conventional treatment, with a predominance of Gram-negative bacteria and strict anaerobes (Pan et al., 2014; Armalytê et al., 2019). The presence of strict anaerobes belonging to the genus *Prevotella spp* was investigated and evidenced in bacterial communities in infected root canals (Gomes and Herrera, 2018). There is a study showing of 43% of *Prevotella* in orofacial infections (Chundurin et al., 2012). It is known that microorganisms can gain resistance against disinfecting agents and endodontic medicaments, increasing the challenge to completely eliminate them during root canal treatment (Chavez de Paz et al., 2010, Tennert et al., 2014). The use of medicinal plants has been investigated in search of new sources of pharmacologically active principles more effective, in order to scientifically validate popular empirical knowledge (Leitão et al., 2010; Cavalcante et al., 2016). Effective medicinal plant use has contributed to disseminating information about their therapeutic importance and medicinal effects, validating therapeutic knowledge that has been accumulated for centuries (Vieira et al., 2018).

The economic and ecological relevance of the species belonging to the genus *Copaifera* has aroused researchers' interest (Mattos et al., 2010; Masson et al., 2013; Dias et al., 2015). Thus, *in vivo* and *in vitro* evaluation has demonstrated that oils obtained from various *Copaifera* species have anti-inflammatory, healing, anti-edematogenic, antitumor, trypanocidal, and bactericidal activities (Mendonça and Onofre, 2009; Abrão et al., 2015). Because of that the therapeutic properties of *Copaifera* oil could be beneficial in odontological products such as intracanal pastes used for direct dental pulp capping as bacterial resistance. Their presence in inaccessible areas of the pulp canal may enable their presence even after rigorous endodontic chemical-mechanical preparation (Alvares and Junior, 2009). Investigating natural products is clearly essential to the search for new molecules with antibacterial activity. In this sense, this work shall contribute to research into the potential use of *Copaiba* oil and endodontic substances against bacterial strains involved in pulp and periapical diseases.

MATERIALS AND METHODS

Plant material

The choice of *Copaifera spp.* (*Copaiba*) is due to its antimicrobial action in previous studies that used plants of the same genus (Faria

et al., 2017; Vieira et al., 2018). Three samples of *Copaiba* oil, commercially available were obtained: *Copaiba* 1 from Santarem (2°30'17.19"S, 54°56'52.249"W); *Copaiba* 2 from Monte Alegre (1°45'27.018"S 55°51'42.89"W) and *Copaiba* 3 from Oriximiná (1°45'27.018"S, 55°51'42.89"W), all from the municipalities in the state of Pará. In the selection of endodontic substances, commercial formulations of topical chemical substances were used in endodontic therapy, besides the standard antimicrobials Metronidazole and Clindamycin.

Microbiological methods

Prevotella intermedia (ATCC 00463), *Prevotella melaninogenica* (ATCC 25845) and bacterial cultures were obtained from the Oswaldo Cruz Foundation's (National Institute of Quality and Health Control -INCQS) and *Clostridium acetobutylicum* (ATCC 4259) cultures were obtained from the Biochemistry and Physiology of microorganisms Laboratory (Department of Antibiotics), Federal University of Pernambuco (UFPE), Recife (PE), Brazil.

Bacterial strains were plated on Sodium Thioglycolate (BBL) broth supplemented with Hemin + Vitamin K (5µg / ml) (LABORCLIN, Brazil). The plates containing bacterial strains were incubated overnight at 37°C and subsequently stored at 4°C. The test cultures were prepared by inoculating 5 ml of BBL broth supplemented with Hemin (5 µg/ml) + Vitamin K (5 µg / ml). The tubes were incubated under anaerobiosis for 48 h at 37°C.

Sample preparation

Samples were prepared by solubilizing 200 µg of Metronidazole in 2 ml of distilled water to obtain a solution with a concentration of 0.1 mg / ml. Clindamycin disks commercially obtained (LABORCLIN, Pinhais - PR, Brazil) with a standard concentration of 2 µg/mL were used. For Calcium Hydroxide [Ca(OH)₂] with and without PMCC, 2.4 mg of the calcium hydroxide slurry (CALEN®- SSWhite São Cristovão - RJ) was diluted in 2 ml of distilled water to give a concentration of 1.2 mg/ml solution in each aliquot. The concentration used in this study for the different *Copaiba* oils was 20µg / mL. The other substances used in endodontic therapy were used according to their commercial formulations: Paramonochlorophenol camphorated® (Biodynamics Chemicals and Pharmaceuticals LTDA®, PR, Brazil), Tricresol formalin (Biodynamics Chemicals & Pharmaceuticals LTDA®, PR, Brazil), Sodium Hypochlorite 2.5% (Q-Boa®, Indústrias Anhembi S/A, Osasco – SP), Otosporin® (Farmoquímica S.A. Rio de Janeiro, RJ, Brazil) and 2% Chlorhexidine (Chlorhexidine®-Maquirá Indústria de Produtos Odontológicos S.A.Maringá, PR/ Brazil). These substances had their antimicrobial activity tested in this study by the broth diffusion method, a methodology recommended by Konemam (1997).

Minimal inhibitory concentration (MIC) determination

This assay consists in the determination of chemical agent spectrum of action, according to resistance of studied microorganisms. The minimum inhibitory concentration (MIC) for every chemical agent was evaluated through the classic method of successive dilution. MIC was performed using the liquid dilution method recommended (Punjabi et al., 2018). Once the disks were prepared, the subsequent step was to set up the experiment by introducing one disk of each drug into each tube containing thioglycollate broth inoculated with the respective microorganism. These tubes were hermetically sealed and incubated at 37°C for 24 h under anaerobic conditions. After the incubation period, the reading was performed by checking the presence of growth of the

Table 1. Antibacterial activity of substances and drugs against microorganisms tested (p <0.01).

Sample	Microorganisms		
	<i>P. intermédia</i>	<i>C. acetobutylicum</i>	<i>P. melaninogenica</i>
Calen® with PMCC	+	+	+
Calen®	+	+	-
Metronidazole	+	+	-
Clindamycin	+	+	-
Chlorhexidine 2%	-	+	-
Copaíba 1	-	-	-
Copaíba 2	-	+	-
Copaíba 3	-	+	+
Otosporin®	-	-	+
PMCC	-	+	-
Tricresol formalin	-	-	+
Sodium hypochlorite	-	+	+

+ : (There was bacterial growth), - : (There was no bacterial growth).

microorganism, visualized with the naked eye through the turbidity of the medium. This assay was performed on 03 sterile 96-well microtiter plates, 11 wells from each row of one plate being used for 11 substances and the other wells of its last column and the first column of the other plate for the culture medium with and without microbial inoculation. Microbial inoculums were standardized to 10^4 CFU of the Mc Fallard scale. A volume of 200 μ l/ml of the substance was placed in the 11 wells of the first top line and 100 μ l / ml of microbial inoculum diluted in the 11 wells of the remaining lines of a plate. Then, 100 μ l of the first-line substance was withdrawn and placed in the corresponding well of the second line, successively being repeated until reaching the last line of the plate, obtaining a serial dilution ranging from 200 μ l / ml to 0.78 μ l / ml for each substance. Afterward, all microplates were incubated at 37°C for 24 h in a stove under anaerobic atmosphere. After this time, 20 μ l of a 5% aqueous solution of Triphenyl Tetrazolium (VETEC® Rio de Janeiro, Brazil) was added to each well and the plates were again incubated for 24 h under the same conditions.

Analysis of data

Statistical results were previously evaluated by normality tests and afterward analysed by Tukey's parametric and non-parametric methods of Cochran and Kruskal-Wallis, considering 95% and 99.99% confidence intervals and a level of significance (p) ranging from 0.05 to 0.01. The analysis of the results was performed by the Tukey parametric and non-parametric statistical tests of Cochran and Kruskal-Wallis.

RESULTS

The antimicrobial activity of the studied samples of copaiba oils and endodontic substances tested are shown in Table 1. Minimum Inhibitory Concentration (MIC) values are shown in Table 2. In terms of sensitivity and resistance of microorganisms, the results obtained for growth or not in the culture medium were transformed

into percent and statistically evaluated and expressed in Figure 2.

In this study, Calen® with PMCC was not effective against any tested strain and Calen® without PMCC only showed action against *P. melaninogenica*. The Chlorhexidine 2% solution showed a better efficiency against *P. intermedia* when compared to the antimicrobial action of the 10% solution of calcium hydroxide and PMCC. The experiments were conducted in triplicate (Figure 1). In the current research, three different samples of Copaíba oils were used, which obtained varied performances in the biological activity test. Sample 1 of Copaiba oil was effective against all microorganisms tested. Nevertheless, samples 2 and 3 showed no effectiveness against *C. acetobutylicum* and Gram-positive organisms. Clindamycin and Metronidazole are drugs indicated for the control of endodontic infections, however, in the present study, such medications did not show effectiveness against *P. intermedia*. This can be explained by the various resistance mechanisms of Gram-negative bacteria, including the presence of the Lipopolysaccharide molecule (LPS) present in its outer membrane according to Vianna in 2005. The experiments were performed in triplicate. Figure 2 shows information on the resistance of the strains used. *P. intermedia* showed the most sensitive microorganism in relation to the tested active substances, presenting a MIC value of 0.78 μ l / ml for 75% of the substances tested.

DISCUSSION

The aim of endodontic treatment is the elimination of infection; therefore, the mechanical instrumentation associated with antimicrobial chemical substances is

Table 2. Minimal inhibitory concentration (MIC) ($p < 0.01$).

Substances	Microorganisms		
	<i>C. acetobutylicum</i>	<i>P. melaninogenica</i>	<i>P. intermédia</i>
Calen®	X	X	50 µl/ml
Metronidazole	X	X	200 µl/ml
Clindamycin	X	X	3,12 µl/ml
Chlorhexidine 2%	0.78 µl/ml	X	0.78 µl/ml
Copaiba 1	6.25 µl/ml	0.78 µl/ml	200 µl/ml
Copaiba 2	12.5 µl/ml	X	200 µl/ml
Copaiba 3	0.78 µl/ml	X	X
Otosporin®	0.78 µl/ml	6.25 µl/ml	X
PMCC	0.78 µl/ml	X	0.78 µl/ml
Tricresol formalin tricresol	0.78 µl/ml	0.78 µl/ml	X
Sodium hypochlorite	0.78 µl/ml	X	X

X= means any antimicrobial action.

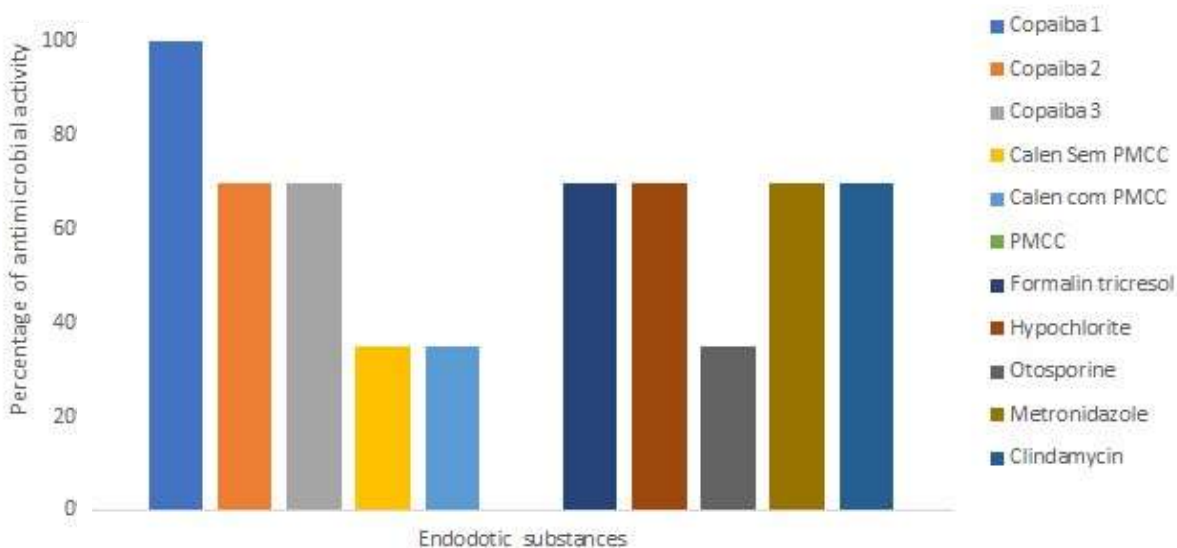


Figure 1. Percentage of antimicrobial activity efficacy of tested substances and drugs ($p > 0.05$).

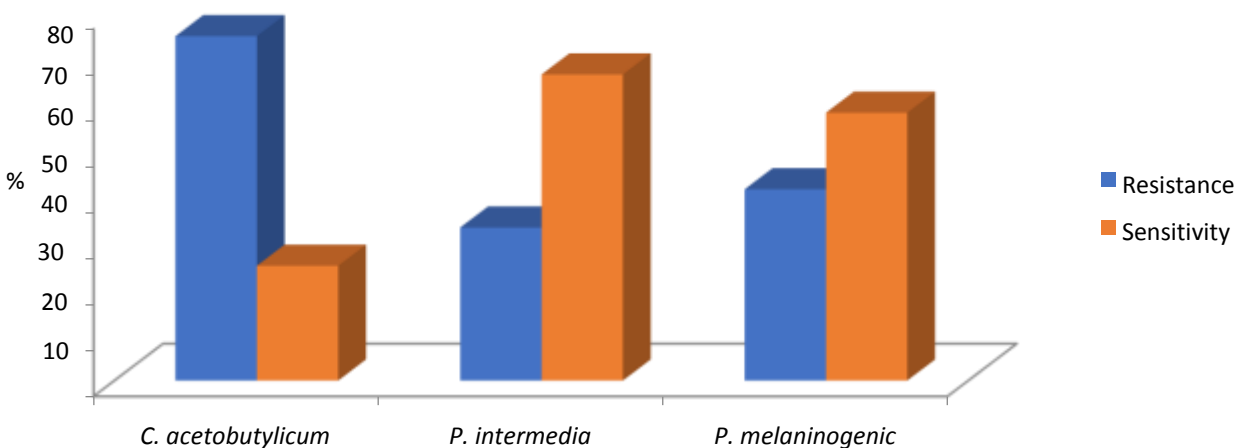


Figure 2. Percentage of sensitivity and resistance of microorganisms to substances and drugs tested ($p < 0.01$).

used to eliminate bacteria that can remain viable after root canal preparation, multiplying in the period between treatments (Rahimi et al., 2014).

Gram-negative anaerobes predominate in primary endodontic infections, while facultative Gram-positive tend to become prevalent in secondary endodontic infections (Andrews, 2001). As a result, the present study tested strains of two Gram-negative anaerobes (*P. intermedia* and *P. melaninogenica*) and one Gram-positive anaerobic (*C. acetobutylicum*). Currently, substances with antimicrobial activity, such as Calcium Hydroxide with and without PMCC, Tricresol formalin, Paramonochlorophenol Camphor, Sodium Hypochlorite, Otosporin®, Chlorhexidine, Metronidazole and Clindamycin (Santos et al., 2008; Rajasekharan et al., 2018; Nopnakepongsa et al., 2019).

In this study, Calen® with PMCC was not effective against any tested strain and Calen® without PMCC only showed action against *P. melaninogenic*. Chlorhexidine 2% solution presents better efficiency against *P. intermedia* when compared to the antimicrobial activity of the Ca(OH)₂ 10% solution and of PMCC which showed high significant difference ($p < 0.001$). In the present investigation, in the 24 h interval, 2% Chlorhexidine showed activity against *P. melaninogenica* and *P. intermedia*, whereas Metronidazole was effective only against *P. melaninogenica*. *Clostridium perfringens* was more resistant, in agreement with the results obtained by Ferreira (2010) and Matos et al. (2010), who, when evaluating these chemical substances, observed that they had no antimicrobial action against strains of *C. perfringens* and *C. difficile*. This is in line with the studies of Nisengard and Newman (1994) that Gram positive microorganisms have greater antimicrobial resistance due to the composition of their cell wall which, despite being simple, consists of a thick layer, which has peptidoglycan responsible for its maintenance and stiffness.

The minimum inhibitory concentrations (MICs) of chlorhexidine (CHX) and Paramonochlorophenol (PMC) were investigated using solid-state dilution tests against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Candida albicans*, *P. intermedia*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella denticola* and *Prevotella melaninogenic* and it was detected that even at low concentrations PMC and CHX showed antimicrobial activity against several microorganisms commonly found in endodontic infections (Lima et al., 2006). In the same way, in this study, CHX presented a high action spectrum; such antimicrobial activity is explained by the interaction of its cationic molecule with the anionic cell wall, altering the surface structures and increasing the permeability of the bacterial membrane.

The antimicrobial action of camphorated Paramonochlorophenol was evaluated in another research, through the agar diffusion method, on *Staphylococcus aureus*, *Pseudomonas aeruginosa*,

Bacillus subtilis, and *C. albicans*. This confirms its effectiveness (Oliveira et al., 2010). Likewise, in the current study, PMCC also showed antimicrobial activity against *Prevotella intermedia* and *P. melaninogenica*. Also in the present study, Tricresol formalin showed activity against *P. intermedia* and *C. acetobutylicum*. In another work, when evaluating the antimicrobial activity of Tricresol formalin and Paramonochlorophenol camphorated against *F. nucleatum* and *Clostridium difficile*, authors evidenced a high level of antimicrobial activity (Panzarini et al., 2006). The main antimicrobial action of Tricresol formalin is, according to Siqueira Jr. and Lopes (2010), the formaldehyde portion of the drug, with alkylating action on proteins and nucleic acids of microorganisms.

When comparing Metronidazole, Calcium Hydroxide and the association of these as a delay dressing in endodontic therapy, researchers concluded that the use of Metronidazole or its association with calcium hydroxide did not favour endodontic treatment when compared to the use of Calcium hydroxide alone (Packer and Luz, 2007). However, another study compared endodontic treatments performed with Calen® Paste without PMCC and Metronidazole in the form of a gynecological gel and verified that both medications showed good results, indicating Metronidazole as a possible alternative in endodontic therapy (Montero-Miralles and Martín González, 2018). On the other hand, this conclusion differs from the current investigation, since Metronidazole proved to be ineffective against *P. intermedia*.

Clindamycin and Metronidazole are drugs used for the control of endodontic infections (Siqueira and Lopes, 1999); however, in the present study, such medications did not show effectiveness against *P. intermedia*. This can be explained by the different mechanisms of resistance that certified strains (ATCC) have in relation to clinical isolate strains (CI), not used in the present study.

Our results showed that Otosporin® was effective against *C. acetobutylicum* and *P. intermedia*, a result equivalent to the one which showed the antimicrobial action of Otosporin® against *Enterococcus faecalis* and *Klebsiella pneumoniae*, both facultative anaerobic microorganisms (Mattos et al., 2010). Otosporin® is the combination of hydrocortisone, a corticosteroid, and antibiotics, Polymyxin B Sulfate and Neomycin Sulfate. The antimicrobial spectrum of Polymyxin B encompasses only gram-negative germs (Soares et al., 2010), and Neomycin is effective against Gram-positive and particularly Gram-negative bacteria (Tortamano et al., 2008), possibly explaining the action spectrum of Otosporin® in the current research.

The antimicrobial activity of plants used in folk medicine has been analyzed on microorganisms present in root canal infections, and its antimicrobial activity has obtained satisfactory results (Arévalo-Híjar et al., 2018; Babaji et al., 2016). The oil of Copaiba has medicinal properties quite widespread among Latin American Indians since the first European explorers arrived in the

16th century (Leitão et al., 2010).

In the current research, three different samples of Copaíba oils were used, which obtained varied performances in the biological activity test. These results can be explained by the different chemical compositions that each species of *Copaifera* can present (Leitão et al., 2010). The presence of terpenoids in this mixture is also recognized, as is the endowment of antimicrobial properties (Veiga et al., 2005).

Sample 1 of Copaiba oil was effective against all microorganisms tested. There are several results in the literature when evaluating the antimicrobial activity of Copaíba oil as well as cement associated with dental use against Gram-positive and Gram-negative bacteria (Santos et al., 2008; Dias et al., 2015; Simões et al., 2016).

However, Samples 2 and 3 of Copaiba oils showed no effectiveness against *Clostridium acetobutylicum*, a Gram-positive microorganism. However, there is a report where Copaiba oil was effective only against Gram-positive microorganisms (Vianna, 2005). In the present evaluation, the resistance of *C. acetobutylicum* may be related to the composition of the samples of the tested oils, which may present distinct components with or without antimicrobial activity. How else can we explain the fact that these differences in the activity of oils assessed can be attributed to factors such as the location of the collection, seasonality, problems in the extraction and synergism.

Conclusions

In this study, 11 endodontic substances were assessed for their antibacterial activities. The results indicated that samples of Copaiba 1, Copaiba 2, PMCC, Formocresol and Otosporin have potential antibacterial effects on bacterial strains tested. This was confirmed by determination of both diameters of inhibition zones and minimal inhibitory concentrations. Copaíba I was the most effective. This indicates that these endodontic substances potentially have antibacterial properties. The Calen paste with PMCC proved to be ineffective against all microorganisms. According to the MIC obtained, *Clostridium acetobutylicum* was the most resistant of the tested bacteria.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Abrão F, Alves JM, Costa LDA, Senedese JM, de Castro PT, Ambrósio SR, Veneziani RC, Bastos JK, Tavares DC, Martins CH (2015). *Copaifera langsdorffii* oleoresin and its isolated compounds: antibacterial effect and antiproliferative activity in cancer cell lines. *BMC Complementary and Alternative Medicine* 15(1):443-453.
- Al-Ahmad A, Ameen H, Pelz K, Karygianni L, Wittmer A, Anderson AC, Spitzmüller B, Hellwig E (2014). Antibiotic resistance and capacity for biofilm formation of different bacteria isolated from endodontic infections associated with root-filled teeth. *Journal of Endodontics* 40(2):223-230.
- Alvares DC, Junior JCA (2009). Endotoxina na endodontia. *Scientific Journal of UFPA* 7(1):1-25.
- Andrews JM (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* 48(1):5-16.
- Arévalo-Hijar L, Aguilar-Luis MA, Caballero-García S, Gonzáles-Soto N, Del ValleMendoza J (2018). Antibacterial and Cytotoxic Effects of *Moringa oleifera* (Moringa) and *Azadirachta indica* (Neem) Methanolic Extracts against Strains of *Enterococcus faecalis*. *International Journal of Dentistry* 2018:1-5. .
- Arias MP, Maliza AG, Midená RZ, Graeff MS, Duarte MA, Andrade FB (2016). Effect of ultrasonic streaming on intra-dental disinfection and penetration of calcium hydroxide paste in endodontic treatment. *Journal of Applied Oral Science* 24(6):575-581.
- Armalytė J, Skerniškytė J, Bakienė E, Krasauskas R, Šiugzdinienė R., Karevieniė V, Kerzienė, S, Klimienė I, Sužiedėlienė E, Ružauskas M (2019). Microbial diversity and antimicrobial resistance profile in microbiota from soils of conventional and organic farming systems. *Frontiers in Microbiology* 10(1):892-904.
- Babaji P, Jaqtaq K, Lau H, Bansal N, ajuraj S, Sondhi P (2016). "Comparative evaluation of antimicrobial effect of herbal root canal irrigants (*Morinda citrifolia*, *Azadirachta indica*, *Aloe Vera*) with sodium hypochlorite: an in vitro study," *Journal of International Society of Preventive and Community Dentistry* 6(3):196-199.
- Cavalcante AM, Leitao CS, Porfírio Z, Santana AEG, Santos LBO, Silva MAB (2016). Antimicrobial analysis of Copaíba oil extract from *Passiflora cincinnata* and endodontic substances. *Journal of Microbiology and Antimicrobials* 8(6):34-38.
- Cavalcante AM, Ribeiro JKAL, Ximenes ECPA, Porfírio Z, Reis JI, Santana AEG (2017). Evaluation of the antimicrobial activity of *Annona crassiflora* Mart against *Candida albicans*. *Journal of Medicinal Plants Research* 11(13):253-259.
- Chavez de Paz LE, Bergenholtz G, Svensater G (2010). The effects of antimicrobials on endodontic biofilm bacteria. *Journal of Endodontics* 36(1):70-77.
- Chundurin NS, Madasu K, Goteki VR, Karpe T, Reddy H (2012). Evaluation of bacterial spectrum of orofacial infections and their antibiotic susceptibility. *Annals of Maxillofacial Surgery* 2(1):46-50.
- Dias FGG, Casemiro LA, Martins CHG, Dias LGGG, Pereira LF, Nishimura LT, Souza FF, Honsho CS (2015). Endodontics pastes formulated with copaiba oil: action on oral microbiota and dentin bridge formation in dogs. *Rural Science* 45(6):1073-1078.
- Faria MJM, Braga CASB, Paula JR, André MCDPB, Vaz BG, Carvalho TC, Romão W, Costa HB, Conceição EC (2017). Antimicrobial activity of *copaifera* spp. against bacteria isolated from milk of cows with mastitis. *Brazilian Animal Science* 18(1):1-14
- Gomes BPFA, Herrera DR (2018). Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology. *Brazilian Oral Research* 32(1):82-110.
- Konemam EW, Allen SD, Janda WM, Schreckenberger PC, Winn (1997). *Collor atlas and textbook of diagnostic microbiology*. Philadelphia: Lippincott-Raven Publishers 5:363-393.
- Leitão C, Santos L, Jacó LB, Soares IBL, Cavalcante AM, Silva ZP (2010). Analysis of the antimicrobial action and minimal inhibitory concentration of copaiba oils and endodontic substances. *Brazilian Research in Pediatric Dentistry and Integrated Clinic* 10(1):75-87.

- Lima MRF, Ximenes Eulália CPA, Luna JS, Santana AEG (2006). The antibiotic activity of some Brazilian medicinal plants. *Brazilian Journal of Pharmacognosy* 16(3):300-306.
- Lucisano MP, Nelson-Filho P, Silva RAB, Silva LAB, Rossi A (2014). Role of endotoxin in the etiology of periapical lesions: molecular mechanisms involved in endotoxin's recognition and cell activation. *Brazilian Journal of Dentistry* 62(3):289-298.
- Masson DS, Salvador SL, Polizello ACM, Frade MAC (2013). Antimicrobial activity of copaiba oil-resin (*Copaifera langsdorffii*) in bacteria of clinical significance in cutaneous ulcers. *Brazilian Journal of Medicinal Plants* 15(4):664-669.
- Mattos ECG, Chain MC, Santos ARS, Smânia Jnr EFA, Rdrigues FR (2010). Antimicrobial activity of endodontic paste prepared with tetracycline, thiamphenicol and zinc oxide assessed using agar diffusion method. *Oral Sciences* 2(1):13-18.
- Mendonça DE, Onofre SB (2009). Atividade antimicrobiana do óleo-resina produzido pela copaiba – *Copaifera multijuga* Hayne (Leguminosae). *Brazilian Journal of Pharmacognosy* 19(2):577-581.
- Montero-Miralles P, Martín-González J, Alonso-Ezpeleta O, Jiménez-Sánchez MC, Segura-Egea JJ, Velasco-Ortega E (2018). Effectiveness and clinical implications of the use of topical antibiotics in regenerative endodontic procedures: a review. *International Endodontic Journal* 51(1):981-988.
- Nisengard RJ, Newman MG (1994). *Oral Microbiology and Immunology* 2:360-384.
- Nopnakeepongsa W, Jantarit J, Surarit R, Smutkeeree A (2019). Assessment of root dentin pH changes in primary and permanent molars with different types of calcium hydroxide intracanal medication. *Pediatric Dental Journal* 29(1):23-29.
- Oliveira EPM, Irala LED, Santos AR, Melo TAF (2010). Evaluation of the antimicrobial action of four formulations based on calcium hydroxide used as intracanal medication. *Journal of the Faculty of Dentistry* 15(1):35-39.
- Packer JF, Luz MMS (2007). Method for evaluation and research of the antimicrobial activity of products of natural origin. *Revista Brasileira de Farmacognosia* 17(1):102-107.
- Pan J, Zhao J, Jiang N (2014). Oral cavity infection: an adverse effect after the treatment of oral cancer in aged individuals. *Journal of Applied Oral Science* 22(4):261-267.
- Panzarini SR, Souza V, Holland R, Dezan-Junior E (2006). Association of calcium hydroxide and metronidazole in the treatment of dog's teeth with chronic periapical lesion. *Journal of Applied Oral Science* 14(5):334-340.
- Pourhajibagher M, Ghorbanzadeh R, Bahador A. (2017). Culture-dependent approaches to explore the prevalence of root canal pathogens from endodontic infections. *Brazilian Oral Research* 31(108):1-7.
- Punjabi K, Mehta S, Chavan R, Chitalia V, Deogharkar D, Deshpande S (2018). Efficiency of Biosynthesized Silver and Zinc Nanoparticles against Multi-Drug Resistant Pathogens. *Frontiers in Microbiology* 9(2207):1-9.
- Rahimi S, Janani M, Lotfi M, Shahi S, Aghbali A, Vahid Pakdel M (2014). A review of antibacterial agents in endodontic treatment. *Iranian Endodontic Journal* 9(1):161-168.
- Rajasekharan S, Verbruyse C, Martens L, Verbeeck R (2018). Effect of exposed surface area, volume and environmental pH on the calcium ion release of three commercially available tricalcium silicate based dental cements. *Materials (Basel)* 11(1):123.
- Santos AO, Ueda-Nakamura T, Dias Filho BP, Veiga Jr VF, Pinto AC, Nakamura CV (2008). Antimicrobial activity of Brazilian copaiba oils obtained from different species of the *Copaifera* genus. *Journal of Microbes and Their Vectors Causing Human Infections* 103(3):277-281.
- Simões CA, Conde NC, Venâncio GN, Milério PS, Bandeira MF, da Veiga Júnior VF (2016). Antibacterial Activity of Copaiba Oil Gel on Dental Biofilm. *The open Dentistry Journal* 10(6):188-195.
- Siqueira Jr JF, Lopes HP (1999). Comparative study of the antimicrobial activity of intrachannel drugs through direct contact and distance. *Brazilian Journal of Health Research* 1(2):60-64.
- Siqueira Jr JF, Lopes HP (2010). Endodontics: Biology and Art 3:57-58.
- Soares JA, Carvalho, MAR, Santos SMC, Mendonça RMC, Ribeiro-Sobrinho AP, Bito Júnior M, Magalhães PP, Santos MH, Farias LM (2010). Effectiveness of chemomechanical preparation with alternating use of Sodium Hypochlorite and EDTA in eliminating intracanal *Enterococcus faecalis* biofilm. *Journal of Endodontics* 36(5):894-898.
- Tennert C, Feldmann K, Haamann E, Al-Ahmad A, Follo M, Wrbas KT, Hellwig E, Altenburger MJ (2014). Effect of photodynamic therapy (PDT) on *Enterococcus faecalis* biofilm in experimental primary and secondary endodontic infections. *BMC Oral Health* 14(132):1-8.
- Tortamano IP, Horliana ACRT, Costa CG, Romano MM, Soares MS, Rocha RG (2008). Antibiotic therapy in the treatment of acute periapical abscess: when to indicate and how to proceed? *Odonto Magazine* 32(1):1-12.
- Veiga J, Valdir F, Pinto, AC, Maciel MAM (2005). Plantas medicinais: cura segura?. *New Chemistry* 28(3):519-528.
- Vianna ME, Gomes BPFA, Sena NT, Zaia AA (2005). In vitro evaluation of the susceptibility of endodontic pathogens to calcium hydroxide combined with different vehicles. *Brazilian Dental Journal* 16(3):175-180.
- Vieira R, Moraes T, Silva LO, Bianchi TC, Veneziani R, Ambrósio SR, Bastos JK, Pires RH, Martins C (2018). In vitro studies of the antibacterial activity of *Copaifera* spp. oleoresins, sodium hypochlorite, and peracetic acid against clinical and environmental isolates recovered from a hemodialysis unit. *Antimicrobial Resistance and Infection Control* 7(1):14-27.