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In Vitro analysis of the Antibacterial action of the Extract of Costus Spiralis (Costaceae) on Enterococcus Faecalis

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Abstract**—** *Enterococcus faecalis is an important cause of endodontic and nosocomial infections, making the search for new antimicrobial drugs important. To analyze in vitro the antibacterial action of Costus spiralis extract on E. faecalis. The antimicrobial sensitivity test was performed using the well agar diffusion method. E. faecalis strains (NEWProv-0012) were revitalized in BHI broth and incubated at 37 ° C for 18 h. Subsequently, the microbial suspension was adjusted to a concentration of 5x105 CFU mL-1 and sown on Mueller Hinton agar, after which 6 mm wells were made. The crude alcoholic extract paste of C. spiralis was used as a positive control 2.5% sodium hypochlorite and as a negative control 96% ethanol and BHI broth. The halos of minimal inhibitory inhibition (MIC) using the crude extract paste and solutions prepared with BHI broth in different concentrations. The viability and microbial quantification of E. faecalis in culture with MIC were determined by serial dilution after 24, 48 and 72 h. Results: The crude extract of C. spiralis has an effective action in inhibiting the growth of E. faecalis. MIC was observed at a concentration of 25% of the extract in solution and promoted the inhibition of E. faecalis growth by 50% after 24 h and 98% in 48 h of incubation. Conclusion: The C. spiralis extract has antibacterial action and can be a therapeutic alternative for infections caused by E. faecalis.*

Keywords**— Costus spiralis. Medicinal plants. Antibacterials.**

I. INTRODUCTION

Enterococcus are characterized as gram-positive cocci arranged in a single form, in pairs or in short chains. They are facultative anaerobic microorganisms, producers of lactic acid and negative catalase (IKE, 2017). It has the ability to form biofilm, which can be found in the gastrointestinal tract, oral cavity and vagina (LEE D. et. Al., 2019; GARCÍA-SOLACHE et.al., 2019). Harmless commensal microorganisms have already been considered, however, research increasingly shows the potential of Enterococcus to survive in a hospital environment, colonize patients and cause human infections such as bacteremia, peritonitis, endocarditis, urinary tract infection, with E. faecalis responsible for more than 90% of enterococcal infections (SAVA et al., 2010; POCHHAMMER et.al, 2017; KAYAOGLU; ORSTAVIK, 2004).

Enterococcus faecalis seem to have acquired over time an ability to accumulate and share chromosomal elements that have characteristics to encode both virulence and antimicrobial resistance genes, perhaps this explains their increasing presence in hospital environments, being the main bacterial group related to infections nosocomial (LEBRETON et al., 2017; SHARIFI et al., 2013). The species stands out for presenting new phenotypes resistant to important antibiotics, both those of conventional use and against the latest generation antibiotics, such as vancomycin, and transmitting this capacity to other gram-positive and gram-negative species, leading to serious public health problems and economic expenses (COURVALIN, 1994; LEBRETON et al., 2017; NUÑEZ et al., 2016; WILLEMS; BONTEN, 2007).

In dental research, the survival mechanisms of E. faecalis in the root canals and the methods used by the bacteria to

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establish themselves in these sites have expanded the studies with the species (ALSHWAIMI et al., 2016; HAAPASALO; ORSTAVIK, 1987; ORSTAVIK; HAAPASALO , 1990; SANTA-ROSA et al., 2019;). Visa which are the main microbial agents responsible for secondary endodontic infections, stand out for their high prevalence and causes of failure in dental treatments (DELBONI et al., 2017; NACIF; ALVES, 2010; SIQUEIRA JUNIOR; RÔÇAS, 2004).

Due to the appearance of microorganisms resistant to the action of the most used drugs, there was a growing search for new agents, aiming at alternatives to control the spread of these strains (FRIERI et al., 2017). Historically, many drugs come from natural products, and these are being increasingly researched, in search of improving existing drugs and developing new, more efficient classes (BOLDI, 2004; BRAGA; SILVA, 2015; PREETHA, 2017).

The marsh cane, Costus spiralis (Jacq.) Roscoe, widely found in tropical South America, has been used as a medicinal plant, mainly in communities in the Amazon region (DUARTE et al., 2017; LORENZI; MATOS, 2002). With leaf infusions, communities have used it as a diuretic, healing, antidiabetic and against infections (ALBUQUERQUE, 1989; BRAGA et al., 2007; FERREIRA et al., 2015; HABSAH et al., 2000; MARTINS et al., 2003). In view of these considerations, it is necessary to investigate and develop new alternative antimicrobial options to those already used, and it is relevant to verify the effectiveness of medicinal plants found in our territory. Therefore, the present study sought to analyze the antibacterial action and the determination of the Minimum Inhibitory Concentration (MIC) in vitro of the crude ethanolic extract of the Costus spiralis plant on Enterococcus faecalis.

II. MATERIALS AND METHODS

The leaves and branches of caninha-do-brejo (Costus spiralis [Jacq.] Roscoe) were collected, following the herborization techniques of Fidalgo&Bononi (1984), at the Sítio Novo farm, municipality of Divinópolis do Tocantins, in the western region of state of Tocantins, Brazil, between the geographical coordinates 9 ° 54'32 ”south latitude and 49 ° 09'27” west longitude. The species was identified by the botanist responsible for the herbarium of the Federal University of Tocantins campus Porto Nacional.

The exsiccates are incorporated into the collection of the referred herbarium, under registration number 12098 HTO. The leaves were washed with water and dried, then crushed and subjected to maceration with 96% ethanol for five days. After that period the macerate was filtered on filter paper and the ethanolic solution was concentrated on a rotary evaporator, with temperature controlled at 40 ° C, to recover the solvent and obtain the crude extract, with 6.44% yield.

Antimicrobian activity

A commercial strain of Enterococcus faecalis (NEWProv-0012) was used, revitalized in BHI broth, incubated at 37 ° C ± 2 ° C in a bacteriological oven until visible turbidity (18 h). After the microorganisms were inoculated in a plate of non-selective nutrient medium (Nutrient agar, KASVI) by the depletion technique and they were again incubated at 37 ° C ± 2 ° C for 24 h. In order to carry out the tests, the microbial concentration was adjusted to the concentration of 5x105 CFU / mL of BHI broth, controlled by reading the turbidity in a spectrophotometer with a wavelength of 600 nm and later counting of plate colonies by performing serial dilutions. in 0.95% saline solution.

To determine the antibacterial action on plates, Mueller Hinton agar (MH, KASVI) was prepared, previously melted, sterilized and cooled to 45-50 ° C, then distributed in 150 mm diameter Petri dishes until reaching a thickness of approximately 4 mm. After obtaining solid consistency of the MH agar, sterile swabs were used to collect microorganisms in the bacterial suspension (≈ 5x105 CFU / mL) and the surface seeding technique was performed in three directions on each plate, paying attention to uniform distribution, avoiding the growth of isolated colonies. Then, 6 mm diameter wells were made, with four perforations in each plate.

The MIC determination was performed by diluting the crude extract in brain heart infusion broth (BHI, KASVI), to prepare solutions in different concentrations: 100%, 50%, 25%, 12.5%, 6.25%, 3, 1%, 1.5% and 0.7%, to determine the MIC. As a positive control, 2.5% sodium hypochlorite was used and as a negative control, 96% ethanol and BHI broth. The inhibition halos were measured with the aid of a caliper, the results being expressed in millimeters. MIC was considered to be the lowest concentration of the extract solution that enabled the formation of an inhibition halo. The wells were completely filled with sufficient quantities of crude extract or controls and incubated at 37 ° C ± 2 ° C.

After MIC determination, the microorganisms (≈ 5x105 CFU mL-1) were grown in a bacteriological incubator at 37 ° C ± 2 ° C, in the presence or absence of the crude extract of C. spiralis at 25%. For the analysis of viability and microbial

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quantification, after 24, 48 and 72 h of culture, the counting of colonies in the plate was carried out by performing serial dilutions in 0.95% saline solution.

All experiments were carried out in triplicates. To determine the antimicrobial action of the C. spiralis extract, the final result was the arithmetic mean of the inhibition halos or the number of CFU / mL obtained from triplicates of three experiments carried out consecutively.

III. RESULTS AND DISCUSSION

The results obtained show that the vegetable preparation of the leaves of Costus spiralis has potential antimicrobial activity against the tested strain of Enterococcus faecalis. The crude extract was able to inhibit microbial growth after 24 h of incubation, with inhibition halos with an average diameter of 13 mm (Figure 1), which were maintained even after 30 days of culture.

Figure 1. Sensitivity test of E. faecalis (NP 0012) to the C. spiralis Crude Extract paste. Microorganisms at a concentration of 5x105 UFC mL-1 were seeded in a confluent manner on the surface of Mueller-Hinton agar (KASVI). Well made in the center of the plate was completely filled with the paste of the crude extract of C. spiralis and after 2 h of pre-diffusion at room temperature the plate was incubated at 37 ° C. The diameter of the inhibition zone was measured in millimeters, with the aid of a caliper, after 24 h and 30 days of incubation, keeping the same.

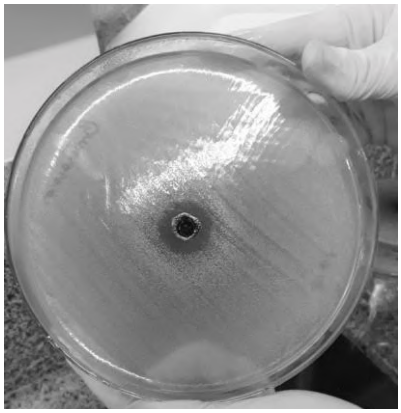

Fig.1: Sensitivity test of E. faecalis culture (NP 0012) to the Crude Extract of C. spiralis paste.

The determination of the Minimum Inhibitory Concentration (MIC) was performed with the extract of C. spiralis in paste and with solutions prepared in concentrations 100%; 50%; 25%; 12.5%; 6.2% 3.1%, 1.5% and 0.7%, (Table 1). Growth inhibition was directly proportional to the concentration of the extract, with MIC being fixed in the dilution of the crude extract in 25% solution in BHI medium, with the formation of an inhibition halo of 7 mm in diameter.

Table 1. Minimum Inhibitory Concentration (MIC) in a solid medium of C. spiralis Extract, on E. faecalis.

Extract concentration (g ml-1) (%)	measured between the edge of the well and the inhibition zone (mm)	Inhibition halo diameter (mm)
Crude paste extract	3,5	13
Crude extract in solution 100%	2,5	11
Crude extract in solution 50%	1,5	9
Crude extract in solution 25%	0,5	7
Crude extract in solution 12,5%	0	0
Crude extract in solution 6,2%	0	0
Crude extract in solution 3,1%	0	0
Crude extract in solution 1,5%	0	0
Crude extract in solution 0,7%		
Sodium hypochlorite 2,5%	6,5	19
Alcohol 96%	0	0
Medium BHI	0	0

Source: own authorship.

The evaluation of the in vitro growth curve of E. faecalis in the presence or absence of the crude extract of C. spiralis in the MIC 25% identified an effective reduction in microbial growth in the presence of the plant extract after 24 h of culture and that there was no more growth colonies visible after 48 h of incubation (Figure 2).

Figure 2. Growth curve of in vitro E. faecalis in the presence and absence of Crude Extract of C. spiralis.

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Microorganisms at a concentration of 5x105 CFU mL-1 were grown in BHI broth or in BHI broth + C. spiralis Crude Extract paste, forming a 25% solution (EB C. spiralis), corresponding to CIM. After 24, 48 and 72 h of incubation in a bacteriological oven at 37 ° C, serial dilutions (10-1 to 10-9) and respective sowing on non-selective agar for colony counting and determination of the number of CFU mL-1 . Source: prepared by the researchers.

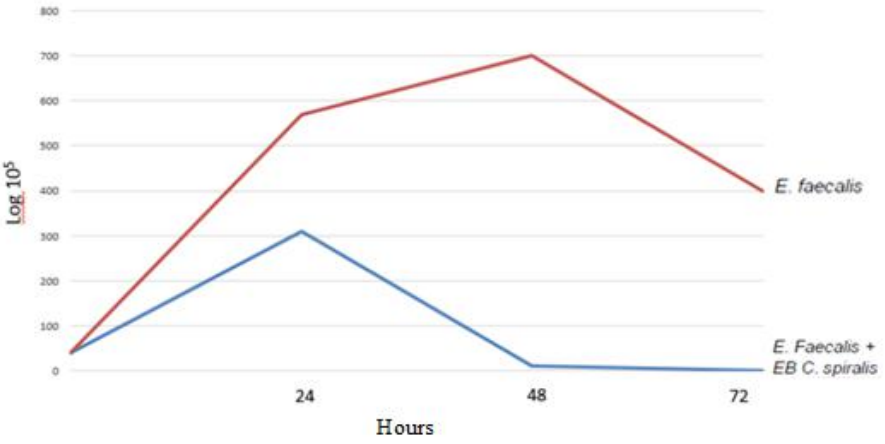

Fig.2: Growth curve of in vitro E. faecalis in the presence and absence of Crude Extract of C. spiralis

The rate of inhibition of microbial growth in the presence of the crude extract of C. spiralis at MIC 25% was compared with microbial growth free of any limiting factor. The extract at MIC 25% was able to inhibit microbial growth by 54% in 24 h, 98% after 48 h and in 72 h it inhibited 100% (Figure 3).

Figure 3. In vitro growth inhibition rate of E. faecalis by the crude extract of C. spiralis. After evaluating the in vitro growth curve of E. faecalis in the presence and absence of the 25% crude extract of C. spiralis (EB), the inhibition of microbial growth by the presence of the plant extract was calculated.

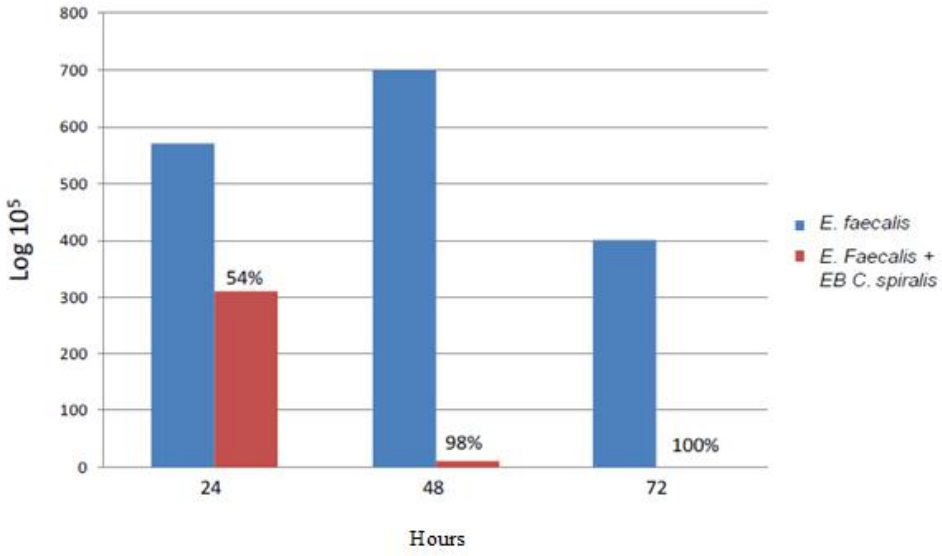

Fig.3: In vitro growth inhibition rate of E. faecalis by the crude extract of C. spiralis

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The formulations with concentrations of the crude extract of caninha do-brejo from 25% analyzed in this study showed action against E. faecalis. Previous research using plant extract of Costus spiralis shows antibacterial activity in vitro against different gram-negative bacteria Pseudomonas aeruginosa, Shigella sonnei, Salmonella sp., Vibrio cholerae and against gram positive bacilli, Bacillus cereus, (BOUZADA et al., 2009; PÉREZ et al., 2008). In none of these studies, the species E. faecalis was included among the strains analyzed, not allowing the comparison of results with the present study.

Uliana et al., (2015) when researching the antibacterial activity of Costusspicatus revealed that such action could be related to the presence of phenolic and flavonoid compounds identified in the extract. This potential may also be related to the composition of the methanol extracts of C. spiralis, holders of both substances, considering that phytochemical studies have identified its main

components, especially alkaloids, flavonoids, steroids, saponins, inulin, tannins, sitosterol, mucilages, sapogenins, pectins and calcium oxalate (ALBUQUERQUE, 1989; BRAGA et al., 2007; DUARTE et al., 2017; SILVA; PARENTE, 2004). Although the exact nature of compounds that have an antimicrobial effect on the extract of C. spiralis are unknown, these results can be useful forfuture isolation tests of the active ingredients that act on the strains.

In the experiments carried out by Bouzada et al. (2009), using plant extract of C. spiralis, obtained as a result inhibition halos of 10 mm against Shigella sonnei, 10 mm against Klebsiella pneumoniae and 9 mm against Pseudomonas aeruginosa and 15 mm against Bacillus cereus. In the present study, the inhibition halos recorded in the tests against E. faecalis were 13 mm, suggesting that gram positive bacteria may be more sensitive to the active principles present in the plant.

Sodium hypochlorite at 2.5% is a substance of global use, mainly in endodontic treatments, being the first option for irrigation of root canals, with capacities beyond the antibiotic, antifungal and antiviral effects, still allows to lighten, deodorize, dissolve tissues organic, having low surface tension and alkaline pH (BORIN et al., 2007; GOMES et al., 2018; TARTARI et al., 2016;). Despite being the substance of choice because it has versatile

capacities, hypochlorite, due to its denaturing effect on proteins, is considered a substance that causes cytotoxicity when in contact with tissues adjacent to the root canal space, which can cause hemolysis, ulceration and inhibit the migration of neutrophils (FIDALGO et al., 2009; HAND et al., 1978; SERMEÑO, et al., 2009). On the other hand, Braga et al. (2007), when studying the effects of Costus spiralis extract at a concentration of 250 µg ml-1, revealed non-significant cytotoxicity in mammalian cells.

The caninha-do-brejo, due to its wide use as a medicinal plant, was researched in other segments that have confirmed its therapeutic effects using its plant extracts, corroborating its medicinal properties. The hypoglycemic, anti-lipid and antioxidant actions in experiments with rats (DUARTE et al., 2019), action against Leishmania chagasi and Leishmania amazonenses (BRAGA et al., 2007), anti-inflammatory capacity, inhibition of vascular permeability, protection stand out cardiovascular and anti-atherosclerotic (SILVA; PARENTE, 2004), and antiurolytic activity in kidney stones formed by calcium oxalate crystals in living experimental models (VIEL et al., 1999), validating the different medicinal functions present in the same plant, and with potential for new discoveries, such as those described in the present study.

IV. FINAL CONSIDERATIONS

It was found in in vitro studies that Costus spiralis leaf extracts have antimicrobial activity against Enterococcus faecalis from concentrations of 25% crude extract. The evidence from the present study indicates a relevant contribution of C. spiralis as a raw material for new arsenals of antimicrobial substances that will combat the feared infections by E. faecalis. The results encourage the development of new studies to prove its effectiveness, isolation of active ingredients, synergism with other substances and clinical applicability, enabling its use as an auxiliary substance in future treatments.

REFERENCES

[1] ALBUQUERQUE, J. M. Plantas medicinais de uso popular. **Associação Brasileira de Educação Agrícola Superior (ABEAS)**. Brasília: Ministério da Agricultura, 1989. 96p.

[2] ALSHWAIMI, E.; BOGARI, D.; AJAJ, R.; AL-SHAHRANI, S.; ALMAS, K.; MAJEED, A. In vitro antimicrobial effectiveness of root canal sealers against *Enterococcus faecalis*: a systematic review. **Journal of Endodontics**, [S.l.], v.42, n.11, p.1588-1597, 2016.

[3] BOLDI, A. M. Libraries from natural product-like scarffolds. **Current Opinion in Chemical Biology**, [S.l.], v.8, n.3, p.281-286, 2004.

[4] BORIN, G.; BECKER, A. N.; OLIVEIRA, E. P. M. A história do hipoclorito de sódio e a sua importância como substância auxiliar no preparo químico mecânico de canais radiculares. **Rev Endod Pesq Ens on-line**, [S.l.], n.5, p.1-5, 2007.

[5] BOUZADA, M. L.; FABRI, R. L.; NOGUEIRA, M. M.; KONNO, T. U.; DUARTE, G. G.; SCIO, E. Antibacterial, cytotoxic and phytochemical screening of some traditional medicinal plants in Brazil. **Pharmaceutical biology**, [S.l.], v.47, n.1, p.44-52, 2009.

[6] BRAGA, F. G.; BOUZADA, M. L. M.; FABRI, R. L.; MATOS, M. D. O.; MOREIRA, F. O.; SCIO, E.; COIMBRA, E. S. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. **Journal of Ethnopharmacology**, [S.l.], v.111, n.2, p.396-402, 2007.

[7] BRAGA, M. D.; SILVA, C. M. Atividade antimicrobiana do extrato aquoso de *Copaiferalangsdorffii*Desf. sobre *Staphylococcus aureus*. **Unimontes Científica**, [S.l.], v.9, n.1, p.91-98, 2015.

[8] COURVALIN, P. Transfer of antibiotic resistance genes between gram-positive and gram-negative bacteria. **Antimicrob. Agents Chemother**, [S.l.], v.38, n.7, p.1447–1451, 1994.

[9] DELBONI, M. G.; GOMES, B. P.; FRANCISCO, P. A.; TEIXEIRA, F. B.; DRAKE, D. Diversity of *Enterococcus faecalis* genotypes from multiple oral sites associated with endodontic failure using repetitive sequence-based polymerase chain reaction and arbitrarily primed polymerase chain reaction. **Journalofendodontics**, [S.l.], v.43, n.3, p.377-382, 2017.

[10] DUARTE, R. C.; TALEB-CONTINI, S. H.; PEREIRA, P. S.; OLIVEIRA, C. F.;MIRANDA, C. E. S.; BERTONI, B. W.; ... PEREIRA, A. M. S. Effect of *Costus spiralis* (Jacq.) Roscoe leaves, methanolicextract and guajaverino blood glucose and lipid levels in a type II diabetic rat model. **Chemistry&Biodiversity**, [S.l.], v.16, n.1, e1800365, 2019.

[11] DUARTE, R.; ANDRADE, L.; OLIVEIRA, T. Revisão da planta *Costusspiralis*(Jacq.) Roscoe: Pluralidade em propriedades medicinais. **Revista Fitos**,[S.l.], v.11, n.2, p.119-249, 2017.

[12] FERREIRA, A. L. S.; BATISTA, C. A. S.; PASA, M. C. Uso de plantas medicinais na comunidade quilombola Mata Cavalo em Nossa Senhora do Livramento – MT, Brasil. **Biodiversidade**, [S.l.], v.14, n.1, p.151-160, 2015.

[13] FIDALGO, O.; BONONI, V. L. Técnicas de coleta, preservação e herborização de material botânico. **São Paulo: Instituto de Botânica**, São Paulo, 1984.

[14] FIDALGO, T. K. D. S.; BARCELOS, R.; PETRÓPOLIS, D. B.; AZEVEDO, B. R.; PRIMO, L. G.; SILVA FILHO, F. C. Citotoxidade de diferentes concentrações de hipoclorito de sódio sobre osteoblastos humanos. **Revista Gaúchade Odontologia**, [S.l.], v.57, n.3, p.317-321, 2009.

[15] FRIERI, M.; KUMAR, K.; BOUTIN, A. Antibioticresistance. **Journal of Infection and Public Health**, [S.l.], v.10, n.1, p.369-378, 2017.GARCÍA-SOLACHE, M., & RICE, L. B. The Enterococcus: a Model ofAdaptability to Its Environment. **ClinicalMicrobiology**. [S.l.], v.32. 2019.

[16] GOMES, F. A.; SANTIAGO, N. S.; SENA, F. M.; DIAS, R. A.; FERREIRA, C. M.; ROCHA, V. G. A.; PAPPEN, F. G. Capacidade da dissolução tecidual do hipoclorito de sódio em diferentes concentrações Capacity of sodium ypochloritetissuedis solution in different concentrations. **RSBO**, [S.l.], v.15, n.2, p.101-107, 2018.

[17] HAAPASALO, M., ORSTAVIK, D. In vitro infection and desinfection of dentinal tubules. **Journal of Dental Research**, [S.l.], v.66, n.8, p.1375-1479, 1987.

[18] HABSAH, M.; AMRAN, M.; MACKEEN, M. M.; LAJIS, N. H.; KIKUZAKI, H.; NAKATANI, N.;.ALI, A. M. Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. **Journal of Ethnopharmacology**, [S.l.], v.72, n.3, p.403-410, 2000.

[19] HAND, R. E.; SMITH, M. L.; HARRISON, J. W. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. **JEndod**., [S.l.], v.4, n.2, p.60-64, 1978.

[20] IKE, Y. Pathogenicity of enterococci. Nihon saikingakuzasshi. **Japanese journal of bacteriology**, [S.l.], v.72, n.2, p.189–211, 2017.

[21] KAYAOGLU G.; ORSTAVIK D. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. **Crit Rev Oral Biol Med**, [S.l.], v.15, p.308-20, 2004.

[22] LEBRETON, F.; MANSON, A. L.; SAAVEDRA, J. T.; STRAUB, T. J.; EARL, A. M.; GILMORE, M. S. Tracing the enterococci from Paleozoic origins to the hospital. **Cell**, [S.l.], v.169, n.5, p.849–61, 2017.

[23] LEE, D., IM, J., NA, H., RYU, S., YUN, C.-H., & HAN, S. H. The novel enterococcus phage vb_efas_hef13 has broad lytic activity against clinical isolates of *Enterococcus faecalis*. **Front microbiol**., [S.l.], v.10, n.2877,2019.

[24] LORENZI, H.; MATOS, F. J. A. Plantas medicinais no Brasil: Nativas e exóticas. **Nova Odessa: Instituto Plantarium**, [S.l.], 511 p. 2002.

[25] MARTINS, E. R.; CASTRO, D. M.; CASTELLIANI, D.; DIAS, **J. E. Plantas medicinais. 5° Reimpressão**,Viçosa: Editora UFV, v.220, 2003.NACIF, M. C. A. M.; ALVES, F. R. F. *Enterococcusfaecalis*na Endodontia:um desafio ao sucesso. **Rev. Bras. Odontol**., [S.l.], v.67, n.2, p.209-214, 2010.

[26] NUÑEZ, L.; TORNELLO, C.; PUENTES, N.; ESPIGARES RODRÍGUEZ, E.; MORENO ROLDÁN, E.; ESPIGARES GARCÍA, M.; MORETTON, J. Hospital effluentconstitutes a sourceofvancomycin-resistantenterococci. **Ars Pharm**, [S.l.], v.57, n.3, p.121-126, 2016.

[27] ORSTAVIK, D.; HAAPASALO, M. Disinfection by endodontic irrigants and dressing of experimentally infected dentinal tubules. **Endod. Dent. Traumatol**, [S.l.], v.6, n.4, p.142-149, 1990.

https://dx.doi.org/10.22161/ijaers.79.4 *ISSN: 2349-6495(P) | 2456-1908(O)*

[28] PÉREZ, C.; FALERO, A.; HUNG, B. R.; LEDÓN, T.; FANDO, R. Antibacterial effect of *Costus spiralis* leaves extract on pathogenic strains of *Vibriocholerae*. **Revista CENIC CienciasBiológicas**, [S.l.], v.39, n.1, p.70-72, 2008.

[29] POCHHAMMER, J., KRAMER, A., & SCHÄFFER, M. Enterokokken und postoperative Wundinfektionen. **Der Chirurg**, [S.l.], *v.88*, p.377–384. 2017.

[30] PREETHA, S. P. In silico methods for herbal research. **Tanuvas**, [S.l.], p.133- 137, 2017.

[31] SANTA-ROSA, C. C.; ARAÚJO, C. V.; DE CARVALHO, M. F. F.; ARAÚ- JO, P. V. Estudo piloto da análise comparativa da atividade antimicrobiana da PDT e agentes irrigantes em canais radiculares inoculados com *Enterococcusfaecalis*"in vitro". **Arquivosem Odontologia**, [S.l.], v.55, 2019.

[32] SAVA, I. G.; HEIKENS, E.; HUEBNER, J. Pathogenesis and immunity in enterococcal infections. **Clinical Microbiology and Infection**, [S.l.], v.16, n.6, p.533-540, 2010.

[33] SERMEÑO R. F.; BEZERRA, A.; HERRERA, H.; HERRERA, H.; SILVA, R. A. B.; LEONARDO, M. R. Tissue damage after sodium hypochlorite extrusion during root canal treatment. **Oral Surg Oral Med Oral Pathol OralRadiolEndod**, [S.l.], v.108, n.1, p.46-49, 2009.

[34] SHARIFI, Y.; HASANI, A.; GHOTASLOU, R.; NAGHILI, B.; AGHAZADEH, M.; MILANI, M.; BAZMANY, A. Virulence and antimicrobial resistance in enterococci isolated from urinary tract infections. **Advanced PharmaceuticalBulletin**, [S.l.], v.3, n.1, p.197–201, 2013.

[35] SILVA, B. P.; PARENTE, J. P. New steroidal saponins from rhizomes of *Costusspiralis*. ZeitschriftfurNaturforschung, **Section C: Journal of Biosciences**, [S.l.], v.59, n.1-2, p.81-85, 2004.

[36] SIQUEIRA JUNIOR, J. F.; RÔÇAS, I. N. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. **OralSurg. Oral Med. Oral Pathol. Oral Radiol. Endod**, [S.l.], v.97, n.1, p.85-94, 2004.

[37] TARTARI, T.; BACHMANN, L.; MALIZA, A. G. A.; ANDRADE, F. B.; DUARTE, M. A. H.; BRAMANTE, C. M. Tissue dissolution and modifications in dentin composition by different sodium hypochlorite concentrations. **J Appl.Oral Sci**., [S.l.], v.24, n.3, p.291-298, 2016.

[38] ULIANA, M. P.; DA SILVA, A. G.; FRONZA, M.; SCHERER, R. In vitro antioxidant and antimicrobial activities of *Costusspicatus*swartz used in folk medicine for urinary tract infection in Brazil. **Lat Am J Pharm**, [S.l.], v.34, p.766-72, 2015.

[39] VIEL, T. A.; DOMINGOS, D. C.; SILVA, A. P. M.; LIMA-LANDMAN, M. T. R.; LAPA, A. J.; SOUCCAR, C. Evaluation of the antiurolithiatic activity of the extract of *Costus spiralis* (Jacq.) Roscoe in rats. **Journal of Ethnopharmacology**, [S.l.], v.66, n.2, p.193-198, 1999.

[40] WILLEMS, R. J. L.; BONTEN, M. J. M. Glycopeptide-resistant enterococci: deciphering virulence, resistance and epidemicity. **Curr. Opin. Infect. Dis**.,[S.l.],v.20, n.4, p.384-390, 2007.