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RESEARCH ARTICLE

Evaluation of the Antimicrobial Potential of Cashew Nut Shell Liquid Extract (*Anacardium occidentale*) against *Enterococcus faecalis* Strains

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ABSTRACT

The resistance of microorganisms present in the root canal system is a problem associated with the failure of endodontic treatments. Therefore, the search for treatments based on herbal extracts has been an alternative to prevent persistent endodontic infections. The aim of this in vitro study was to evaluate the antimicrobial potential of cashew nut shell liquid extract (E-CNSL) against *Enterococcus faecalis* strains. The antimicrobial activity was evaluated using the agar well diffusion method. Petri dishes containing Mueller Hinton agar were inoculated on the surface by the microorganism using a swab, then 20 μL of the E-CNSL solution at concentrations of 500 μg/mL and 1000 μg/mL was aseptically deposited in the wells of the inoculated media. The plates were incubated at 37 °C for 24 hours. All the strains studied showed an inhibition halo ≥6 mm at both concentrate ions of the extract. Therefore, the extract of cashew nut shell liquid (*A. occidentale*) showed a potential antimicrobial effect against *E. faecalis* strains.

Keywords: *Anacardium occidentale*, antimicrobial potential, *Enterococcus faecalis*, phytotherapy.

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1. Introduction

The oral cavity has a complex, dynamic and diverse microbiota, made up of microorganisms such as bacteria, archaea, fungi and viruses. Current studies suggest that there are more than 700 species of bacteria present in the mouth, which organize themselves and form the oral biofilm on different surfaces such as teeth, gingival sulcus, inserted gingiva, jugal mucosa, palate, tongue and lips [1]. However, the imbalance of this microbiome, called dysbiosis, results in oral diseases such as dental caries, periodontal disease, oral candidiasis, pulp infections and can even predispose to systemic diseases [2].

The microbiota associated with the most prevalent diseases in the oral cavity include bacteria from the genera *Streptococcus*, *Lactobaccilus*, *Porphyromonas*, *Fusobacterium*, *Actinomyces*, *Prevotella*, *Aggregatibacter* and *Enterococcus* [3], [4]. Among the dental specialties, endodontics faces the greatest difficulties in controlling bacteria in the root canal system, as it is a more complex treatment and requires correct chemical-mechanical disinfection in order to eliminate pulp tissue, dentin remains and microorganisms to achieve successful endodontic treatment [5], [6].

In this sense, the aim of endodontic treatment is to achieve maximum disinfection of the root canal system and prevent secondary infections. However, even with the use of bactericidal agents, there can still be persistent infections associated with microbial resistance, especially *Enterococcus faecalis* [7], [8]. This microorganism is a gram-positive, anaerobic, facultative bacterium and a commensal of the oral and intestinal microbiota of humans [5].

Scientific evidence points to the prevalence of this microorganism in secondary and persistent intraradicular infections [5], [9]. In addition, clinical research has shown the resistance and high prevalence of *E. faecalis* in cases of

endodontic retreatment, where even after performing the root canal disinfection, chemical-mechanical preparation and application of intracanal medication, there was little reduction in the microbial content for *E. faecalis* [10], [11].

This resistance of *E. faecalis* is associated with specific characteristics and virulence factors, such as the ability to adhere quickly to surfaces and form biofilms, to colonize in inaccessible areas of the root canal, to survive in environments with little oxygen, high pH, poor nutrients, high salinity, wide temperature range and to penetrate dentinal tubules [5], [12]–[14].

Due to the resistance mechanisms mentioned above, *E. faecalis* is able to resist the action of antimicrobial agents used during the chemical-mechanical cleaning of endodontic treatment, such as chlorhexidine, sodium hypochlorite in concentrations greater than 5%, calcium hydroxide and antibiotics [5], [15]. Thus, many studies are looking for alternative treatments with antibacterial activity to eliminate this species of bacteria more efficiently. Among the therapeutic possibilities are the use of low-power lasers, a combination of antibiotics and irrigants and extracts of natural products [16]–[18].

Therefore, alternatives based on natural products have become popular due to their benefits such as easy access, excellent cost-effectiveness, low toxicity and increased shelf life [18]. Many plant species have shown significant results with regard to antibacterial, antioxidant, anti-inflammatory and antitumor potential, such as *Anacardium occidentale*, known as the cashew tree, a plant native to northeastern Brazil and several tropical countries, which generates a large source of income through the worldwide export of cashew nuts [19].

Recent studies have observed that substances derived from cashew nuts, especially cashew nut shell liquid (CNSL), are potential therapeutic agents against infections caused by oral bacteria such as the Streptococcus genus and the *E. faecalis* species [19]. CNSL is composed mainly of anacardic acid, cardol and cardanol. Its use has been highlighted due to its biological and non-biological applications, and it can be used as a raw material for the production of medicinal products [20].

However, there are not many studies available in the literature regarding the efficacy of the antibacterial potential of cashew nut shell liquid extract (E-CNSL) against bacteria present in persistent endodontic infections. Therefore, this project aims to investigate the antimicrobial potential of E-CNSL against strains of *E. faecalis*, in order to be used as an alternative and coadjuvant therapy in the chemical-mechanical cleaning of the root canal system.

2. Materials and Methods

2.1. Year and Place of Study

The laboratory tests were carried out in the Microbiology and Biochemistry laboratories of the Federal University of Campina Grande, Patos campus (CSTR), Paraíba-Brazil, in 2023.

2.2. Cashew Nut Shell Liquid Extract (E-CNSL)

The cashew nut shell extract (*Anacardium occidentale*) was provided by Prof. Dr. Maria Denise Leite Ferreira, Faculdade Nova Esperança (Facene), João Pessoa-Paraíba (PB), from cashew nuts collected in the municipality of Serra do Mel-Rio Grande do Norte (RN). E-CNSL was obtained according to the methodology described by Rodrigues *et al*. [21].

2.3. Bacterial Species and Culture Media

Four strains of *Enterococcus faecalis* (ATCC-29212, Ef 47, Ef 48 and Ef 50) were used, provided by the Microbiology Laboratory of the Biological Sciences Academic Unit (UACB) of the Center for Rural Health and Technology (CSTR) of the Federal University of Campina Grande (UFCG). The strains were kept in Muller-Hinton Agar (MHA) medium at 4 °C, and 24-hour replicates in MHA incubated at 35 °C were used for the tests. For the antibacterial activity study, the inocula were obtained from overnight cultures in MH at 37 °C and diluted in sterile saline solution to obtain a final concentration of approximately 1.5×10^8 colony-forming units per ml (CFU/ml), adjusted for turbidity by comparing with the 0.5 tube of the McFarland scale.

2.4. Evaluation of the Antimicrobial Potential of Cashew Nut Liquid Extract

The antimicrobial potential of E-CNSL against strains of the bacterium *Enterococcus faecalis* was evaluated using the well agar diffusion technique developed according to Bona *et al*. [22], with modifications. Petri dishes containing Mueller Hinton Agar were inoculated on the surface by the microorganism using a swab; this process was carried out evenly and repeated 3 times in 3 different directions to cover the entire surface. Next, a 6 mm diameter hole was formed in each plate using an aseptic mold, creating the wells.

Finally, 20 µL of the E-CNSL solution at concentrations of 500 µg/mL and 1000 µg/mL was aseptically poured into the wells of the inoculated media using pipettes. The plates were incubated at 37 °C for 24 hours. After incubation, the plates were observed for homogeneity of bacterial growth and, in cases where growth inhibition was found, the diameter of the halo was measured using a millimeter ruler. For the agar diffusion test per well, halos with a diameter ≥6 mm were considered to have inhibitory activity [22]. The tests were carried out in duplicate and the results correspond to the average values.

3. Results and Discussion

From laboratory agar diffusion bioassays using the well method, it was possible to observe the antimicrobial effect of the cashew nut shell liquid extract at concentrations of 500 µg/mL and 1000 µg/mL against 4 types of *Enterococcus faecalis* strains (ATCC-29212, Ef 47, Ef 48 and Ef 50). The antimicrobial potential was verified in all the strains tested by measuring the diameter of the bacterial growth inhibition halos produced by the action of E-CNSL, as shown in Table I.

In line with the results obtained in this study, a laboratory study carried out by Souza *et al*. [19] verified the antimicrobial potential of the substance from the cashew nut shell liquid, both natural and technical, against strains

TABLE I: Diameter of the Inhibitory Halo of the Antimicrobian Effect of the Extract of the Cashek Liquid of Caju Nuts against *Enterococcus Faecalis*

Strains	Concentration 500 µg/mL	Concentration 1000 µg/mL
ATCC-29212	10 mm	10 mm
Ef 47	8 mm	12 mm
Ef 48	10 mm	12 mm
Ef 50	12 mm	12 mm

Source: Authors.

of oral streptococci related to dental caries and *Enterococcus faecalis*. In addition, scanning electron microscopy showed changes in cell morphology in *E. faecalis* strains in the presence of the substance studied, suggesting damage to the bacterial membrane and disruption of cell integrity, which can induce cell death.

In this perspective, according to a phytochemical analysis reported by Salehi *et al*. [23], cashew nut shell liquid is composed of small amounts of cardanol, cardol, 2-methyl cardol and 80% anacardic acid, a substance capable of permeating the lipid bilayer of bacterial cell membranes and causing them to rupture. Thus, the antimicrobial activity of cashew nut shell liquid has been associated with this compound [19].

It is worth mentioning that the antimicrobial potential of *Anacardium occidentale* has also been investigated using phytotherapeutic extracts produced from the leaves, fruit and stem of the cashew tree. An *in vitro* study by Anand *et al*. [24] evaluated the antimicrobial potential of cashew leaf extract against strains of *E. faecalis*, showing a greater zone of inhibition of bacterial growth in the action of the extract when compared to the action of mouthwashes based on povidone-iodine and 2% chlorhexidine gluconate.

Only the two studies mentioned above evaluated the antimicrobial potential of *A. occidentale* leaf and cashew nut shell liquid against strains of *E. faecalis* [19], [24]. However, other studies have investigated the plant's antibacterial activity against oral bacteria. As in the observations of de Araújo *et al*. [25] and Lima *et al*. [20], in which extracts of the cashew stem and cashew shell showed antibacterial activity against cariogenic bacteria, with promising results against strains of *Streptococcus mitis*, *S. mutans*, *S. oralis*, *S. sanguinis*, *S. sobrinus* and *S. salivarius*.

Thus, these studies show that all parts of the plant offer therapeutic potential with regard to the antimicrobial activity of *A. occidentale*, stimulating new research into the development of natural products. However, it is important to note that the research available in the literature to date does not follow a methodological pattern and there is a significant variation in the results reported using the fruits, oils, leaves and bark of *A. occidentale*. Therefore, results based on heterogeneous studies of the same plant species compromise the validity of the evidence [26].

In addition to researching the therapeutic potential, it is important that herbal products based on *A. occidentale* are also evaluated for their cytotoxicity, since some of the chemical compounds present can cause adverse effects and make it impossible for these substances to be added safely to dental materials, such as toothpastes, mouthwashes, restorative materials, intracanal medications and root canal filling materials [25]. Thus, the study by Souza *et al*. [19] evaluated the potential cytotoxic reactions of cashew nut shell liquid on different cell lines, showing a dose-and time-dependent cytotoxic effect.

Although there have been many studies evaluating the antimicrobial effect of *Anacardium occidentale*, there has not been enough research to prove the efficacy of cashew nut liquid extract against strains of *Enterococcus faecalis*, gram-positive bacteria that are frequently associated with persistent root canal infections [10]. Therefore, this study showed promising results for E-CNSL and will serve as a reference base for future academic work.

4. Conclusion

The cashew nut shell liquid extract (E-CNSL) showed an antimicrobial effect against all the clinical strains of *Enterococcus faecalis* tested in this study. However, further in vitro research is still needed in order to highlight the therapeutic efficacy and cytotoxic potential of this herbal extract, so that it can be used safely in the treatment of oral infections, more specifically, as an adjunct therapy in the chemical-mechanical cleaning of the root canal system. It is also important to carry out studies to determine the minimum inhibitory and minimum bactericidal concentrations.

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Conflict of Interest

Authors declare that they have no conflict of interest.

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