International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

INCORPORATION OF ROSEMARY (*ROSMARINUS OFFICINALIS* LINN.) EXTRACT IN AN ENDODONTIC SEALER: ANTIMICROBIAL EVALUATION

Marcela Agne Alves Valones[1], Jalber Almeida Santos[1], Thiago Maciel Cavalcanti[1], Michelly Cauás Queiroz Gatis[1], Arnaldo França Caldas Júnior[1*], Eliziane Pereira Costa[2], Janete Magali de Araújo[2] Alessandra de Albuquerque Tavares Carvalho[1]

[1]Department of Clinical and Preventive Dentistry, Graduate Program in Dentistry, Faculty of Dentistry, Federal University of Pernambuco, Recife, Pernambuco, Brazil
[2]Department of Antibiotics, Microbial Collection Laboratory, Faculty of Pharmacy, Federal University of Pernambuco, Recife, Pernambuco, Brazil
***Corresponding author e-mail:** caldasjr@alldeia.com.br

Received on: 25-02-2015; Revised on: 10-11-2015; Accepted on: 21-12-2015

ABSTRACT

The aim of this study is to evaluate the microbial activity of an endodontic sealer containing rosemary extract in comparison to Sealer 26®. Agar well diffusion assays were used to evaluate the antimicrobial activity of the materials in the presence of the following bacterial strains: *S. mutans* (ATCC 25175), *S. aureus* (ATCC 9811) and *E. faecalis* (ATCC 51299). Bacterial growth inhibition halos were measured. The Mann-Whitney test was used to determine the statistical significance of differences between groups. Mean inhibition halos were 27.1, 26.4 and 38.6 mm for the strains of *S. aureus*, *E. faecalis* and *S. mutans* when the rosemary-based sealer was used and 16.3, 15.8 and 25.5 mm, respectively, when Sealer 26® was used. Significant differences between sealers were found for each bacterial strain ($p < 0.05$). The rosemary-based endodontic sealer demonstrated greater antimicrobial efficacy in comparison to Sealer 26®.

Keywords: *Rosmarinus officinalis;* Root canal obturation; *Staphylococcus aureus*; *Streptococcus mutans*; *Enterococcus faecalis*

INTRODUCTION

Microorganisms and their byproducts are considered the main agents of endodontic disease. Thus, the major goal of endodontic treatment is the destruction of these microorganisms in the root canal system [1]. The success of endodontic treatment depends on effective performance in all phases. While no particular phase is considered the most important, special care must be given to obturation, which involves the use of a solid material (gutta-percha) and a sealer [2].

Microorganisms can cause persistent and secondary infections of root canal systems. *Enterococcus faecalis* is an anaerobic, facultative bacterium related to the failure of root canal treatment as well as pulp necrosis and periapical lesions. Although the genus *Enterococcus* is not present in considerable amounts in the initial flora of untreated root canals, once stabilized, it becomes viable and resistant to antimicrobial treatment and medicinal interactions, persisting after the obturation [3]. *Staphylococcus aureus* is a spherical (coccus), Gram-positive bacterium that appears in pairs in short chains or bunches upon microscopic examination [4]. It is one of the main agents of hospital-acquired infection and the main cause of surgical infection throughout the world [5]. This bacterium is considered one of the most resistant species and a possible cause of the failure of root canal treatment [6]. The mutans streptococci are a group of heterogeneous cocci that make up part of the resident microbiota in the oral

cavity. Many are infectious and considered the main etiologic agent of dental caries as well as important contributors to infectious endocarditis [7].

Endodontic sealers perform a special function in filling root canals. These products have inherent antimicrobial activity, which contributes to the control of the microbial population. In typical clinical situations, the complete elimination of bacteria from root canal system appears to be impossible. However, the use of sealers with antibacterial properties helps reduce the number of microorganisms and contributes to the avoidance of infection [8]. Thus, the incorporation of antimicrobial agents in endodontic sealers can enhance these desirable properties [9].

The current trend is the development of endodontic sealers that maintain and/or enhance the properties of traditional fillers and are more biocompatible [10]. In principle, it is possible to improve the properties of these materials through the addition of antimicrobial agents. A number of such substances have been employed in dentistry in the form of mouthwashes, restorative materials and toothpastes [11].

There is an ongoing search for the development of an endodontic sealer that meets all the requirements to be considered ideal. Many researchers have investigated the use of plant extracts and phytochemicals with antimicrobial properties. In recent years, studies have demonstrated the effectiveness of these extracts, which mainly stems from the antimicrobial action of their secondary metabolites [12]. One such plant, rosemary (*Rosmarinus officinalis* Linn.), has been described to have a number of medicinal properties, which justifies its traditional use in folk remedies. The plants contain essential oil, flavonoids, phenols and terpenoids, which have antioxidant and antimicrobial properties [13].

The aim of the present study was to evaluate the microbial activity of an endodontic sealer containing rosemary extract in comparison to Sealer 26® (Dentsply Maillefer, USA).

MATERIALS AND METHODS

Production of rosemary-based endodontic sealer: *Rosmarinus officinallis* Linn. was acquired and identified through comparisons with material deposited in the herbarium of the Pernambuco Institute of Agricultural Research (Brazil). The material was washed and dried at room temperature. Leaves and stems were weighed (320 g) and macerated in 1 L of ethanol for 30 days under refrigeration. The extract was filtered and placed in a rotary evaporator at a temperature of 50°C. The dried extract was divided into portions for the microbiological, toxicological and phytochemical tests and kept under refrigeration between tests. A sealer similar to Sealer 26® (Dentsply Maillefer, USA) was mixed at a compounding pharmacy (Sensoriale® Manipulação Farmacêutica e Cosmética, Recife, Brazil). The blended sealer did not contain calcium hydroxide, which was replaced with the rosemary extract at the minimum inhibitory concentration (MIC) for the bacteria tested. The MIC of the extract for each bacterium was tested in a previous experiment [14] (Chart 1). As three different microorganisms were tested, the largest MIC was used while respecting the LD_{50} for this phytotherapeutic substance. The sealer produced exhibited similar physical and organoleptic characteristics to Sealer 26® and was compounded in the same way, following the manufacturer's instructions.

Antimicrobial evaluation of rosemary-based endodontic sealer: Agar well diffusion assays [15] were performed for the evaluation of the antimicrobial activity of the rosemary-based endodontic sealer in the presence of *S. mutans* (ATCC 25175), *S. aureus* (ATCC 9811) and *E. faecalis* (ATCC 51299). The bacterial strains were acquired from the Microbial Collection Laboratory of the Department of Antibiotics of the Federal University of Pernambuco (Brazil) and reactivated in test tubes containing Brain Heart Infusion (BHI®, DIFCO, USA). The strains were inoculated on glass dishes containing 10 ml of BHI® agar medium. The growth conditions of each bacterium were respected: aerobiotic conditions for 24 h for *S. aureus* and *E. faecalis* and anaerobiotic conditions for 48 h for *S. mutans* in a bacteriological chamber at 37 ºC. Colonies of each inoculum were diluted in test tubes with sterile water and placed in a vortex mixer for one minute to achieve turbidity similar to tube n^0 2 of the McFarland Scale®.

100µl of homogenized suspension were inoculated on sterile glass dishes containing 10 ml of BHI® medium and homogenized with the aid of sterile swabs so that the entire medium was inoculated. Wells measuring 10 mm in diameter were made with a perforator in the center of each plate and filled with the test sealer and Sealer 26® as the positive control – both blended following the manufacturer's instructions. An inoculated dish with a well filled with sterile gelose of the medium was used as the negative control. The dishes were incubated in accordance with the requirements of each microorganism. All assays were conducted in triplicate. Following incubation, the bacterial growth inhibition halos were measured with the aid of halo meter and the results were expressed as mean values in millimeters.

The normality test demonstrated the need for a nonparametric test, as no variables exhibited normal distribution. The Mann-Whitney test was used to determine the statistical significance of differences between the materials ($p < 0.05$).

RESULTS

Mean inhibition halos were 27.1, 26.4 and 38.6 mm for the strains of *S. aureus*, *E. faecalis* and *S. mutans* when the rosemary-based sealer was used and 16.3, 15.8 and 25.5 mm, respectively, when Sealer 26® was used. Significant differences between sealers were found for each bacterial strain ($p < 0.05$) (Table 1).

DISCUSSION

There has been growing interest in research into the anaerobic microorganisms that infect root canal systems, especially with regard to long-term infections [1,9,16]. Anaerobic bacteria are well adapted to survive in necrotic pulp tissue, where the blood supply is either limited or nonexistent. Facultative anaerobic microorganisms can interact with strict anaerobic microorganisms, leading to changes in the nutritional relationship and oxygen tension-reduction, which favor microbial bonding and survival [9]. Thus, the antimicrobial action of endodontic sealers may participate in the control of infection.

The ideal endodontic sealer should exhibit adequate antimicrobial activity, provide a hermetic seal and offer a low degree of toxicity to biological tissues. Antimicrobial substances can be added to enhance the properties of endodontic sealers [9,17]. Gjorgievska *et al* [11] evaluated the effect of the addition of benzalkonium chloride and cetylperydinium chloride to endodontic sealers on strains of *S. mutans*, *L. casei* and *A. viscosus* and found an increase in the antimicrobial activity of all sealers analyzed, demonstrating that these substances have potential clinical applications in root canal treatment. However, further studies are needed to evaluate whether the addition of such substances can compromise the physicochemical characteristics of endodontic sealers.

In the present study, Sealer 26® was used for the purposes of comparison to the rosemary-based sealer. Sealer 26® is composed of bismuth oxide, calcium hydroxide and epoxy resin and is known for its excellent sealing property and satisfactory biocompatibility [18]. A number of studies have evaluated the antimicrobial activity of Sealer 26® in the presence of bacterial strains or microorganisms of the oral cavity [2,3,18].

In the present study, the rosemary-based endodontic sealer demonstrated greater inhibition halos in comparison to Sealer 26®. Gomes *et al* [2] also found lesser antimicrobial activity with Sealer 26® in comparison to Endo-fill®, Endomethasone®, Endomethasone N® and AH-Plus®, but the differences did not achieve statistical significance. Cruz *et al* [19] and Kooper *et al* [20] found smaller inhibition halos with Sealer 26® for all microorganisms tested in comparison to Rickert®/N-Rickert® and Endo-Fill®. However, Kooper *et al* [20] found a larger inhibition halo for *S. aureus* using Sealer 26®, which is in agreement with findings described by Leonardi *et al* [21], who compared this substance to Endo-fill®, AH-Plus® and Acroseal® with regard to the same bacterium. Tanomaru-Filho *et al* [18] also report the adequate performance of Sealer 26®. Maia *et al.* [3] report the antimicrobial action of this product in the presence *E. faecalis*. However, this bacterium is known to be resistant to calcium hydroxide [22]. Thus, the action of Sealer 26® on *E. faecalis* may be due to the release of another substance, such as formaldehyde, which occurs after the mixture of the other components of the product [23].

Aal-Saraj *et al* [16] evaluated the antimicrobial activity of a novel endodontic sealer containing nano-hydroxyapatite epoxy resin (nanoseal) in comparison to the commercial products AH 26®, Tubliseal®, Sealapex® and Roekoseal® in the presence of the facultative anaerobic bacteria *E. faecalis*, *P. aeruginosa*, *S. mutans*, *S. sobrinus* and *E. coli*. The authors found that the nanoseal had better antimicrobial action in comparison to Roekoseal®, similar action to AH 26® and lesser action in comparison to Tubliseal® and Sealapex® in the presence of *E. faecalis*, *P. aeruginosa* and *E. coli* as well as greater action in comparison to Sealapex® and Roekoseal®, similar action to AH 26® and lesser action in comparison to Tubliseal® in the presence of *S. mutans* and *S. sobrinus*.

In the present study, agar well diffusion was used to test antimicrobial activity. This is one of the most widely used methods for this purpose [2,24,25]. However, the size of the inhibition zone does not indicate the absolute antimicrobial effect of an endodontic sealer. According to Bodrumlu & Semiz [24], the inhibition zone can be affected by the diffusibility of the sealer through the agar, the interaction between the sealer and components of the medium and the *in vivo* micro-environmental conditions.

The mean inhibition halos for *S. aureus*, *E. faecalis* and *S. mutans* were significantly larger with the rosemary-based endodontic sealer in comparison to Sealer 26®. This finding may be explained by the fact that rosemary has anti-inflammatory, antioxidant and antimicrobial properties [13]. This plant is made

up of essential oil, flavonoids, phenols and terpenoids. Klancnik *et al* [26] report greater sensitivity to rosemary extract among Gram-positive bacteria in comparison to Gram-negative bacteria.

The essential oil of rosemary is considered to have the greatest antimicrobial action. Bernardes *et al* [27] analyzed the effect of rosemary extract on *E. faecalis, S. salivarius, S. sanguinis, S. mitis, S. mutans* and *S. sobrinus*. The authors also performed both biomonitored fractioning and a chromatographic analysis of the extract to identify the main components, attributing the antimicrobial activity mainly to carnosic acid and carnosol. Moghtader & Afzali [28] report that the essential oil of rosemary has antibacterial, antinociceptive and antifungal properties.

Hofling *et al* [29] found strong antifungal activity of rosemary extract on some species of the genus *Candida*. Fungal adherence to biomaterials used in medicine, such as catheters, valves and implants, was the object of a study by Chifiriuc *et al* [30], who combined the unique properties of nanoparticles with the antimicrobial activity of the essential oil from rosemary to create a nanobiological system that could be used to cover the surface of these materials and impede both microbial colonization and the development of biofilm. The authors found that the essential oil fortified with nanoparticles was able to inhibit and control fungal adherence (*C. tropicalis* and *C. albicans*). Del-Campo *et al* [31] evaluated the effect of rosemary extract on different microorganisms and found activity against Gram-positive bacteria, such as *S. aureus, B. cereus* and *S. mutans*. According to the authors, the phenols in the plant are responsible for this activity.

CONCLUSION

In the present study, the rosemary-based endodontic sealer demonstrated greater antimicrobial efficacy in comparison to Sealer 26® with regard to *S. aureus*, *E. faecalis* and *S. mutans.* Further studies are needed to evaluate the effectiveness of rosemary-based endodontic sealers in daily clinical use.

ACKNOWLEDGEMENTS

To the Microbial Collection Laboratory of the Department of Antibiotics of the Federal University of Pernambuco to allow the experiments in their facilities; To the Sensoriale® Pharmaceutical and Cosmetics Manipulation for the creation and manipulation of the sealer technology.

Chart 1: MIC of rosemary extract for bacterial strains studied[14]

Bacterial strain	MIC
S. aureus (ATCC 9811)	6.25 mg/ml
E. faecalis (ATCC 51299)	6.25 mg/ml
S. mutans (ATCC 25175)	14.9 mg/ml

Table 1: Mean inhibition halos (in mm) using Sealer 26 and the rosemary-based endodontic sealer in the presence of *S. aureus, E. faecalis* and *S. mutans*

Bacterial strain	Sealer	Mean	Standard deviation	p-value[1]
S. aureus	Sealer 26	16.3	1.5	0.030*
	Rosemary	27.1	1.0	
E. faecalis	Sealer 26	15.8	1.0	0.013*
	Rosemary	26.4	1.3	
S. mutans	Sealer 26	25.5	1.0	0.030*
	Rosemary	38.6	2.5	

[1]*Nonparametric Mann-Whitney test*

** Statistically significant difference*

REFERENCES

1. Saha S, Samadi F, Jaiswal JN, Ghoshal U. J Ind Soc Periodont Prevent Dent, 2010; 28(4):251-7.
2. Gomes BPFA, Pedroso JA, Jacinto RC, Vianna ME, Ferraz CCR, Zaia AA, Souza-Filho FJ. Braz Dent J, 2004;15 (1):30-5.
3. Maia CCR, Filho EMM, Filho IB, Rodrigues EC, Borges KRA. RIB, 2010; 58(2):19-26.
4. Benenson AS. APHA - American Public Health Association: Control of Communicable Diseases. 16th ed., Washington; 1985 pp. 184-187.
5. Takahashi S, Tanaka T, Ashiki A. JPNJ Urol, 1990; 81(10): 1480-6.
6. Gomes BPFA, Ferraz CCR, Vianna ME, Rosalen PL, Zaia AA, Teixeira FB, Souza Filho FJ. Braz Dent J, 2002; 13(3):155-61.
7. Loesche WJ. Microbiol Res, 1986; 50(4):353-80.
8. Nawal RR, Parande M, Sehgal R, Naik A, Rao NR. Int Endod J, 2011; 44 (4): 307-13.
9. Shantiaee Y, Dianat O, Janani A, Ahari G. Iran Endod J, 2010; 5(1):1-5.
10. Pascon EA, Souza CJA, Langeland K. Rev ABO Nac, 2000; 8 (4):238-50.
11. Gjorgievska E, Apostolska S, Dimkov A, Nicholson JW, Kaftandzieva A. Dent Mater, 2013; 29 (1):29-34.
12. Smullen J, Finney M, Storey DM, Foster HA. J Appl Microbiol, 2012; 113(5): 964-73.
13. Matos FJA. Farmácias vivas. Fortaleza; UFC: 1998, pp.18-20.
14. Ellof JN. J Ethnopharmacol, 1998; 60 (1): 711-13.
15. Bauer AW, Kirby WMM, Sherris JC, Turc M. Am J Clin Pathol, 1966; 45(4): 493-6.
16. Aal-Saraj AB, Ariffin Z, Masudi SM. Aust Endod J, 2012; 38 (2):60-3.
17. Yasuda Y, Kamaguchi A, Saito T. J Oral Sci, 2008; 50 (3): 309-13.
18. Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. J Oral Sci, 2007; 49 (1): 41-5.
19. Cruz CW, Moura PPR, Habitante SM, Zolner N, Jorge AOC. Rev Biociênc, 2001; 7 (1):49-53.
20. Kopper PM, Rosa RO, Figueiredo JAP, Pereira CC, Tartarotti E, Filippini HF. J Dent Sci, 2007; 22 (56):106-11.
21. Leonardi DP, Battisti JC, Klimiont DT, Tomazinho PH, Baratto Filho F, Haragushiku GA, Tomazinho FSF. RSBO, 2009; 6 (4): 367- 73.
22. Evans M, Davies JK, Sundqvist G, Figdor D. Int Endod J, 2002; 35 (3):221-8.
23. Leonardo MR, Bezerra da Silva LA, Filho MT, Santana da Silva R. Oral Surg, Oral Med, Oral Pathol, Oral Radiol Endod, 1999; 88 (2): 221–5.
24. Bodrumlu E, Semiz M. J Can Dent Assoc, 2006; 72 (7):637-37c.
25. Farmakis ET, Kontakiotis EJ, Tseleni-Kotsovili A, Tsatsas VG. J Invest Clin Dent, 2012; 3 (4):271-5.
26. Klancnik A, Guzej B, Kolar MH, Abramovic H, Mozina SS. J Food Prot, 2009; 72 (8):1744-52.
27. Bernardes WA, Lucarini R, Tozatti MG, Souza MGM, Silva MLA, Filho AAS, Martins CHG, Crotti AEM, Pauletti PM, Groppo M, Cunha WR. Chem Biodivers, 2010; 7 (7):1835-40.
28. Moghtader M, Afzali D. Am Eurasian J Agric Environ Sci, 2009; 5 (3):393-7.
29. Hofling JF, Anibal PC, Obando-Pereda GA, Peixoto IAT, Furletti VF, Foglio MA, Conçalves RB. Braz J Biol, 2010; 70(4):1065-8.
30. Chifiriuc C, Grumezescu V,Grumezescu AM, Saviuc C, Lazar V, Andronescu E. Nanoscale Res Lett, 2012; 7(209):2-7.
31. Del Campo J, Amiot M-J, Nguyen-The C. J Food Prot, 2000; 63(10): 1359-68.