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RESEARCH ARTICLE

Antimicrobial Efficacy of Various Concentrations of Bamboo Salt against *Enterococcus faecalis* and *Candida albicans*: An *in vitro* Study

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ABSTRACT

Sodium hypochlorite (NaOCl) remains the gold standard against which any new endodontic irrigant is compared. But, its inadvertent extrusion beyond the confines of the root canal can be caustic to vital periapical or periodontal tissues. There has been an increase in the use of herbal medicines as irrigants over the last two decades. Bamboo salt is a Korean folk medicine, which shows promising antimicrobial, antioxidant, and anti-inflammatory properties. The aim of this *in vitro* study was to comparatively evaluate the effectiveness of 1, 3, and 5% bamboo salt against *Enterococcus faecalis* and *Candida albicans* using agar diffusion test; 4% NaOCl was used as control. The experiment was performed in triplicate and the zone of inhibition (ZOI) was measured. The results of the present study showed that 4% NaOCl and 5% bamboo salt showed significantly higher mean ZOI than the other groups against *E. faecalis*; 4% NaOCl showed significantly higher mean ZOI than the other groups against *C. albicans*, followed by 5 and 3% bamboo salt. Hence, it can be concluded that 4% NaOCl proved to be the most effective antimicrobial against both the species; 5% bamboo salt was as effective as 4% NaOCl against *E. faecalis*, but significantly less effective against *C. albicans*.

Keywords: Antimicrobial, Bamboo salt, *Candida albicans,* Endodontic irrigants, *Enterococcus faecalis*, Sodium hypochlorite.

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INTRODUCTION

Bacteria play a pivotal role in the development of pulpal and periapical lesions.[1,2] Endodontic infection involves multiple bacterial species, with *E. faecalis* being the most commonly associated strain in persistent endodontic infection and failed endodontic treatment.[3] *E. faecalis*, a gram-positive, facultative anaerobic cocci can withstand very harsh intracanal conditions, such as an extreme pH of 9.6 and high temperatures of about 60°C.[4] It possesses several other virulence factors, which make it the star survivor of the root canal.[5] Previous studies have shown that <1% of root canal flora in persistent cases of apical periodontitis is composed of yeast.[6] Though yeasts comprise a smaller proportion, their eradication is challenging as they are resistant to most of the currently available antimicrobials. Among the yeasts, *C. albicans* is the most commonly detected species in infected root canals.[6,7] Chandra et al[7] observed that *C. albicans* was more resistant to irrigating solutions in the presence of a smear layer.

The main goal of endodontic therapy is to reestablish a healthy status for the tooth by eliminating microbes and their by-products.[8] Instrumentation of the root canal results in reduction of microbes to an extent but leaves many areas unaffected. Hence, the use of an irrigant becomes an indispensable part of cleaning and shaping of the root canal.[9] Most commonly used irrigants are NaOCl and chlorhexidine. Various studies have proven their effectiveness against root canal flora, including *E. faecalis* and *C. albicans*.[8]

The irrigants used in contemporary dental practice are associated with some kind of allergic or toxic reactions.[10] Sodium hypochlorite is reported to cause adverse reactions like chemical burns and necrosis of the periapical tissues.[11-13] Mohammadi and Abbott[14] reported that chlorhexidine causes allergic reactions like desquamative gingivitis and discoloration of teeth and gums. Hence there is a constant quest for an ideal root canal irrigant with no or minimal adverse effect.

Various natural products with a wide range of therapeutic properties have been experimented as endodontic irrigants and medicaments. Azadirachta indica, aloe vera, and garlic are some of them.[15,16] Bamboo salt is one such product, which is derived by burning natural salt obtained from yellow sea in bamboo sticks using pinewood as fuel. The salt from yellow sea is rich in mineral content. It is not only used as taste enhancer in Korean cuisine but is also reported to have antimicrobial, antioxidant, and anti-inflammatory properties.[17,18] Shin et al[19] and Moon et al[20] have reported that bamboo salt is

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effective against *S. mutans* and *S. enteritidis* respectively. However, its efficacy against endodontic pathogens is not yet known. Hence, the aim of this *in vitro* study was to evaluate the effectiveness of three different concentrations of bamboo salt against *E. faecalis* and *C. albicans* using agar diffusion test.

MATERIALS AND METHODS

Preparation of Microbial Inoculum

Pure cultures of test strain *E. faecalis* ATCC 29212 and *C. albicans* ATCC 10231 were grown in Mueller-Hinton broth (Himedia Laboratories, Mumbai, India) and Sabouraud dextrose broth (Himedia Laboratories, Mumbai, India) respectively. The microbial load was standardized to an optical density of approximately 0.5×10^8 colony forming units (CFU)/mL for *E. faecalis* and 1.5×10^8 CFU/mL for *C. albicans* by comparing its turbidity to a McFarland 0.5 scale.

Preparation of Solutions and Grouping

In a sterile beaker, 1, 3, and 5% bamboo salt solutions were prepared by mixing 1, 3, and 5 gm of bamboo salt (Koreasalt Co. Ltd., Gyeongsangnam, Korea) in 100 mL of distilled water respectively. The sterility check was done for the solutions. The experimental groups were as follows: Group I – 4% NaOCl (Prime Dental Products Pvt. Ltd., Mumbai, India), group II – 1% bamboo salt solution, group III – 3% bamboo salt solution, and group IV – 5% bamboo salt solution.

Agar Diffusion Test

In agar plates 8 mm diameter wells were punched. Three such wells were used for each group. Mueller-Hinton agar (Himedia Laboratories, Mumbai, India) was selected for *E. faecalis* and Sabouraud dextrose agar (Himedia Laboratories, Mumbai, India) for *C. albicans.* The microbial streaking was done on the respective culture plates. In each well, 100 µL volume of the experimental solution was deposited. The entire procedure was carried out in a biosafety cabinet. The plates thus prepared were incubated at 37°C for 24 hours in an incubator. The ZOI was measured and expressed in millimeters. The mean of the three ZOIs was calculated for each group. Statistical analysis was done by one-way analysis of variance and Kruskal–Wallis test ($p < 0.05$).

RESULTS

The mean ZOI of the experimental groups against *E. faecalis* and *C. albicans* is given in Table 1. Group I (4% NaOCl) showed the highest ZOI followed by group IV (5% bamboo salt), group III (3% bamboo salt), and group II (1% bamboo salt) against both *E. faecalis* and *C. albicans*.

Table 1: The ZOI (mean ± standard deviation) of the experimental agents against *E. faecalis* and *C. albicans*

Groups	*E. faecalis*	*C. albican*
I	17.30 ± 0.503[a]	20.20 ± 0.721[a]
II	11.67 ± 0.577[b]	10.33 ± 1.528[c]
III	13.33 ± 0.577[b]	13.80 ± 1.058[b]
IV	16.33 ± 1.155[a]	16.33 ± 1.155[b]

Under each microbe, different superscript letters (a,b,c) indicate statistically significant difference between the groups ($p < 0.05$).

Against *E. faecalis*, groups I and IV showed significantly higher mean ZOI than the other groups ($p < 0.05$). There was no significant difference between groups II and III against *E. faecalis* ($p > 0.05$). Against *C. albicans*, group I showed significantly higher ZOI than the other groups. Groups IV and III showed significantly higher ZOI than group II, but lesser than group I ($p < 0.05$).

DISCUSSION

Enterococcus faecalis is the most commonly found bacteria in persistent periradicular lesions and root filled teeth.[3] The myriad virulence factors associated with *E. faecalis* make it resistant to most of the currently used antimicrobial agents.[21] It possesses lytic enzymes like cytolysin, which lyse other bacteria. It adheres to host cells by aggregation substance and surface adhesins.[5] The proton pump in *E. faecalis* provides an additional means of pH homeostasis.[22] It enters a viable but noncultivable state, which helps it to survive in harsh conditions or environmental stress.[23]

Among the yeast species, *C. albicans* is the most commonly isolated and is more commonly found in root canals with failed endodontic treatment.[6,24] Though few studies have shown the presence of *C. albicans* in root canals, their role in establishing and/or progression of endodontic infection is still uncertain.[25] Waltimo et al[26] observed that *C. albicans* was highly resistant to the most commonly used intracanal medicament, calcium hydroxide. Radcliffe et al[27] showed that *E. faecalis* was highly resistant to NaOCl. The increasing resistance of these microbes to contemporary antimicrobials reinforces the need to explore newer materials, which could effectively eradicate them from the root canal.

In this study, 4% NaOCl was effective against both the microbes and showed the highest ZOI. These results are supported by several other previous studies.[16,26] NaOCl exerts its antimicrobial action by saponification, chloramination, and denaturation of bacterial proteins.[28] Waltimo et al[6] inferred that eradication of *C. albicans* from the root canal under clinical conditions was an achievable goal with the routine disinfection protocol using NaOCl, iodine compounds, or chlorhexidine. In a study by Radcliffe et al,[27] *C. albicans* proved highly susceptible to the action

of NaOCl, whereas *E. faecalis* was found to be resistant. While lesser concentration (0.5% NaOCl) required long contact times of up to 30 minutes to achieve zero viable counts for *E. faecalis*, the same was achieved in 2 minutes with a higher concentration (5.25% NaOCl). But, such higher concentrations are associated with cytotoxicity.[12]

When NaOCl is extruded beyond the apex, it causes chemical burns leading to localized or extensive tissue necrosis. It results in acute inflammatory reactions, which lead to swelling associated with bleeding into interstitial tissues, bruising and ecchymosis of the surrounding mucosa. When NaOCl comes in contact with the nerve, neurological complications like paresthesia ensue.[29] The most life-threatening complication of NaOCl is upper airway obstruction, when NaOCl leaks into the oral cavity leading to ingestion or inhalation by the patient.[30] Another drawback of NaOCl is that it oxidizes the root dentin substrate, thereby inhibiting polymerization of resin sealers used subsequently during obturation of root canal. This results in reduced bond strength of resin sealers to NaOCl treated dentin.[31,32]

There has been an increase in the use of herbal medicines over the last 15 to 20 years as they are easily available, inexpensive, lack microbial resistance, and have no adverse effects.[33,34] Hence, bamboo salt, a natural herbal medicine, was selected for the study. The results of the present study showed that 5% bamboo salt was equally effective as 4% NaOCl against *E. faecalis* and next best to 4% NaOCl against *C. albicans*. Bamboo salt causes hypersomatic shock to the microbes by reducing the water content in the microbial cell. In addition, it has metals like Zn, Mn, Ca, P, K, Mg, and Fe, which further boost its antimicrobial property.[20] These metals cause discrete and distinct injuries to microbial cells resulting in oxidative stress, protein dysfunction, and membrane damage.[35] This might be the reason for the antimicrobial activity of bamboo salt. The results of the present study are in accordance with previous studies by Shin et al[19] and Moon et al.[20] Shin et al[19] showed that 1% bamboo salt was effective against *S. mutans* and Moon et al[20] observed that 5% bamboo salt caused complete inhibition of *S. enteritidis*. This is a preliminary study to assess the antimicrobial effect of bamboo salt against *E. faecalis* and *C. albicans*. Further studies using increasing concentrations of bamboo salt are required to explore its antimicrobial potential and subsequent use in dentistry.

CONCLUSION

From the results of this *in vitro* study, it can be concluded that 4% NaOCl proved to be the most effective antimicrobial agent against both the species; 5% bamboo salt was as effective as 4% NaOCl against *E. faecalis*, but significantly less effective against *C. albicans*.

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