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Research Article

The Effect of *Curcuma zedoaria* Extract on *Enterococcus faecalis*

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ABSTRACT

Introduction: *Enterococcus faecalis* is a microorganism that is commonly found in persistent or secondary infection of root canal treatment. Irrigation is one of the main processes of endodontic triad to eliminate microorganisms in root canal infections. *Curcuma zedoaria*, a medicinal plant with antibacterial properties, is effective against several types of microorganisms and has the potential to be used as an alternative of chemical-based irrigant. **Objective:** This study aimed to evaluate the antibacterial activity of *Curcuma zedoaria* extract against *Enterococcus faecalis*. **Methods:** This *in vitro* study used *C. zedoaria* extract with six concentrations (100%, 90%, 80%, 70%, 60%, 50%) as tested groups, 2% Chlorhexidine and distilled water were used as a positive and negative control group, respectively. Solid Mueller-Hinton Agar (MHA) medium containing *Enterococcus faecalis* ATCC 29212 was perforated with a cork borer and dropped by 5 mL solution according to each group. The inhibition zone diameter was measured to evaluate antibacterial activity. Data were analyzed using a One Way ANOVA, continued by a Post-hoc Bonferroni. **Results:** The largest growth inhibition zone was associated with the highest concentration (100%), with a mean diameter of 8.36 mm. **Conclusion:** The 100% *C. zedoaria* extract concentration had the most effective antibacterial potency against *E. faecalis*.

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INTRODUCTION

Enterococcus faecalis (*E. faecalis*) is a Gram-positive, anaerobic, facultative bacteria, with unique characteristics that allow it to survive under conditions lethal to other microorganisms.^{1,2} These conditions include a high salt concentration, various temperatures and pH levels. Furthermore, *E. faecalis* is resistant to some root canal medicaments.¹ *E. faecalis* can be persistent in primary root canal infections, despite being in low numbers.³ It has been a universal agreement that the triad of endodontic success is access preparation, shaping and cleaning the canals, and filling root canal systems.⁴ Irrigation is often regarded as the most important part of endodontic treatment, in particular for the eradication of root canal microbes. During and following instrumentation, irrigating solutions facilitate the killing and removal of microorganisms, necrotic and inflamed tissue and dentine debris.⁵

Microorganism control is one factor involved in successful root canal treatment. It includes the elimination of microorganisms using mechanical methods (i.e., endodontic instrumentation), assisted by irrigation. According to Schäfer, root canal irrigation materials must have broad-spectrum antimicrobial properties and be able to dissolve tissue.⁶ They must also have biocompatible properties for treatment support.⁶ Chlorhexidine (CHX) 2% is a widely used root canal irrigation solution. It has a high bactericidal capacity, inhibits glycosidic and proteolytic activities in a huge variety of oral bacteria.⁷ Several earlier studies that compared the antibacterial effect of sodium hypochlorite (NaOCl) and CHX 2% against intracanal infections have shown little or no difference between the two products.^{5,8}

Chlorhexidine cannot dissolve biofilm or other organic debris, but it can be combined with other irrigants, such as NaOCl. Although this combination was reported to have optimal results, it was mutagenic, forming a carcinogenic product, parachloroaniline. It is a chlorhexidine hydrolysis product in the form of a brownish precipitate that can affect the obturation seal.⁹ Other disadvantages of CHX are that its antibacterial activity depends on the pH level, and it is less active in the presence of organic material.^{8,10} The toxic properties of CHX highlight the need to use natural irrigation solutions, which can eliminate bacteria and are biocompatible with tissues.

White turmeric (*Curcuma zedoaria*), which is common throughout various regions of Indonesia, has antimicrobial properties. It belongs to the Zingiberacea family and has long been used in several Asian countries as a medicinal plant.¹¹⁻¹³ *C. zedoaria* contains various active ingredients, including curcumin, furanodiene,

zedorone, curzerone, and terpenoids.¹² Chachad et al. showed that rhizome ethanol extract of *C. zedoaria* exerted antimicrobial activity against some bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*.¹¹ This study aimed to evaluate the antibacterial activity of *Curcuma zedoaria* extract against *Enterococcus faecalis*, compared with 2% CHX irrigation solution.

MATERIALS AND METHODS

Curcuma zedoaria extract was made using the maceration method, with ethanol used as a solvent.¹⁴ A 750 grams of *C. zedoaria* rhizome, cut and dried in an oven (MSI-5, Navi Mumbai, India) at 60° C for 10 min. The dried rhizome was made into a powder using a blender. The powder was dissolved in 750 ml of ethanol 96% for 3 days and filtered. The filtrate was evaporated in a rotary evaporator (RE202, Yamato Scientific, Jepang) at 40° C for 3 hours until a 100% concentrated solution was obtained.¹⁵ The 100% extract was diluted by adding distilled water and Tween 80 Polysorbate (Citra Sari Kimia, Indonesia) to predetermined concentrations which were 90%, 80%, 70%, 60%, and 50%. Tween 80 was used to prevent clumping of the extract.

E. faecalis ATCC 29212 was inoculated in Mueller Hinton Agar media (Merck Millipore, Darmstadt). Thirty-two wells were made using a cork borer with 2 mm depth and 6 mm diameter. A 50 µl of *C. zedoaria* extract with different concentrations (100%, 90%, 80%, 70%, 60%, and 50%) was filled in each well using micropipettes (Socorex, Ecublens, Switzerland). CHX 2% (Cerkamed, Stalowa Wola, Poland), and distilled water were used as positive and negative control, respectively. The petri dishes were stored in an incubator (Incubator I, Memmert, Jerman) at 37° C for 24 hours.^{15,16} Subsequently, the dishes were removed, and the inhibition zone was measured using a sliding caliper (Sketmat, Tricle brand, Shanghai, China) (Fig.1). The procedure was repeated two times to avoid a mistake in readings. The strength of antibacterial activity can be determined using Davis and Stout category inhibition

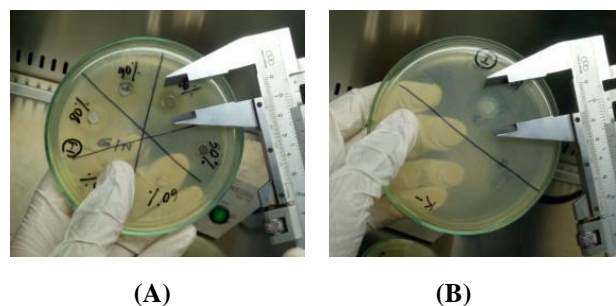


Figure 1. (A) Inhibition zone of the treatment group (B) Inhibition zone of the control group.

where ≥ 20 mm is very strong, 10–20 mm is strong, 5–10 mm is moderate, and \leq mm is very weak.¹⁷

Statistical Analysis

Data were analyzed with Shapiro-Wilk's normality test and Levene's homogeneity test. Comparative analysis was conducted with One Way ANOVA. A Post-hoc test was used to measure the statistical difference, set at $p < 0.05$. All tests were done with the SPSS Statistics for Windows software version 20 (IBM, Armonk, USA).

RESULTS

The statistical analysis showed that data were normally distributed ($p > 0.05$). Comparative analysis of the data with one-way ANOVA showed that there were differences in all concentrations compared to a positive control (CHX) as showed in Table 1 ($p < 0.05$). A post-hoc test was used to determine the statistically significant difference. The results showed that there was a significant difference between all the extract concentrations and positive control.

Table 1. Mean diameter of inhibition zone

Groups	Mean \pm SD (mm)
<i>Curcuma zedoaria</i> extract 100%	8.362 \pm 0.49
<i>Curcuma zedoaria</i> extract 90%	6.525 \pm 1.26
<i>Curcuma zedoaria</i> extract 80%	5.777 \pm 2.13
<i>Curcuma zedoaria</i> extract 70%	4.067 \pm 2.93
<i>Curcuma zedoaria</i> extract 60%	3.377 \pm 2.31
<i>Curcuma zedoaria</i> extract 50%	1.700 \pm 2.02
Chlorhexidine 2%	16.835 \pm 1.55
Distilled water	0.000 \pm 0.00

DISCUSSION

Curcuma zedoaria is a medicinal herb plant found in tropical countries such as India, Japan, and Thailand. In Indonesia, it is most likely found at Java Island and South Sumatera.¹⁸ This *in vitro* study evaluates antibacterial activity of *C. zedoaria* in ethanol extracts with various concentrations, i.e 100%, 90%, 80%, 70%, 60%, and 50%. Two percent of Chlorhexidine was used as positive control and distilled water as a negative control. We found each concentration of the tested groups showed an inhibition zone, with the highest concentration (100%) has the largest mean diameter of 8.3 mm. It indicated that the highest concentration had the greatest inhibitory effect on bacterial growth.¹⁹ A previous phytochemical study of ethanolic *C. zedoaria* rhizome extract detected the presence of terpenoids, alkaloids, saponins, flavonoids, glycosides and carbohydrates, phenolics, tannins and phytosterols, etc.²⁰

When compared with the zone of inhibition of 2% chlorhexidine, the mean diameter of the zone of inhibition of the *C. zedoaria* rhizome extract at the 100% concentration was smaller, as confirmed by the Post-hoc test which revealed a significant between group difference in the sizes of the zones of inhibition. In this study, the *C. zedoaria* rhizome extract inhibited the growth of *E. faecalis*. This finding was consistent with that reported in previous studies, which concluded that *C. zedoaria* rhizome extract had antibacterial activity.^{11,12} According to Davis and Stout, the *C. zedoaria* rhizome extract at concentrations of 100%, 90%, and 80% exhibited moderate antibacterial activity.

The antibacterial mechanisms of active compounds in medicinal plants differ. *C. zedoaria* is rich in flavonoids that inhibit the formation of DNA and RNA so that nucleic bases accumulate and damage the permeability of cell walls, lysosomes, and microsomes.²¹ It also forms complex compounds with extracellular proteins causing damage to bacterial cell membranes and the release of intracellular compounds. Tannins will damage bacterial cell membranes by changing permeability and disrupt the power of the cytoplasmic membrane protons that dissolve fat.^{13,21}

This was an *in vitro* study and further research is needed using simulated *ex-vivo* irrigation to provide more data. Further studies are required to evaluate the antimicrobial activity against other specific microorganisms in the infected pulp.

CONCLUSION

Based on the results and statistical analysis of this study, we conclude that *C. zedoaria* rhizome extract inhibited the growth of *E. faecalis*. The largest growth inhibition zones were produced at a *C. zedoaria* rhizome extract concentration of 100%.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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