

## Antibacterial activity of *Zingiber Officinale Roscoe* extract as a potential root canal irrigation solution against *Enterococcus faecalis*

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### ABSTRACT

**Introduction:** The aim of this study was to determine and formulate antibacterial activity of *Zingiber Officinale Roscoe* (*Z. officinale*) extract against *Enterococcus faecalis* (*E. faecalis*) as a potential irrigating solution which are easily available and cost-effective. **Methods:** Extract of *Z. officinale Roscoe* was yielded through soxhlet technique. Samples divided into two groups, i.e, the extract of *Z. officinale Roscoe* and chlorhexidine 2% as control. The antimicrobial activity was observed using diffuse agar method. Minimum inhibitory concentration (MIC) was determined by serial microdilution method and minimum bactericidal concentration (MBC) was determined by cultured samples in blood agar. Data were analyzed using Anova and Kruskal-Wallis test. **Results:** Extract of *Zingiber officinale Roscoe* has antimicrobial activity againsts *E. faecalis* with the MBC of 15.625 mg/mL. ( $p < 0,05$ ). **Conclusion:** Extract of *Zingiber officinale Roscoe* have shown antibacterial activity againsts *E. faecalis* and can be further developed as a potential root canal irrigation solution.

**Keywords:** Antibacterial activity, *Enterococcus faecalis*, extract of *Zingiber officinale Roscoe*, root canal irrigating solution.

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### INTRODUCTION

Bacteria are among the main causes of periapical infections.. It has been reported that success rate of root canal treatment was higher when teeth were free of bacteria after chemomechanical

instrumentation<sup>1</sup>. While instruments are important in removal of the infected dentine from the main root canal, irrigants play an indispensable role in areas inaccessible to instruments, such as lateral and accessory canals as well as fins and webs throughout the canal<sup>2</sup>. Irrigation of the root

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canals with antibacterial solutions is a mandatory step to reduce or eliminate microorganisms or their byproducts from the root canal system<sup>3</sup>. Considering the ineffective, potential side effects and safety concerns of synthetic drugs, the herbal alternatives may prove to be advantageous for endodontic usage<sup>4</sup>. Natural products have been used in dental and medical practice for thousands of years and have become even more popular nowadays because of their high antimicrobial activity, biocompatibility, antioxidant and anti-inflammatory properties<sup>4</sup>.

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is widely used spice in the daily diet in many Asian countries<sup>5</sup>. It is a rhizomatous plant grown throughout South-eastern Asia, China and in parts of Japan, Austria, Latin America, Jamaica and Africa. It has been used as a spice and medicine in India and China since ancient times. It was known in Germany and France in the ninth century and in England in 10th century for its medicinal properties<sup>6</sup>.

Over three quarters of the world population still rely on plants and plant extracts for health care<sup>6</sup>. Ginger compounds are active against specific type of diarrhea which is leading to cause death in infant in developing countries. Moreover, it has been found that ginger is effective in treating nausea caused by sea sickness, morning sickness and chemotherapy, though it was found superior over a placebo for post operative nausea<sup>7</sup>. In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antibacteria, antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic, and anti-tumor<sup>5-15</sup>.

Ethanol and *n*-hexane fractions of ginger exhibited antibacterial activities against anaerobic bacteria associated with periodontitis of the human oral cavity<sup>16</sup>. Extract of its leaves and roots could be used alongside with conventional antibiotics to fight against infections<sup>17</sup>. Moreover, it was found that the extract was effective against *Candida albicans*<sup>18</sup>. Furthermore, ginger extract was found to be active *Enterococcus faecalis* compared with sodium hypochlorite<sup>19</sup>. In this study the possible antibacterial activity of *Z. officinale* extract is to be compared with 2% chlorohexidine as the gold standard for antibacterial activity against a wide range of Gram positive and Gram negative

organisms as well as yeast, facultative anaerobes and aerobes<sup>20</sup>. In addition to its ability to be adsorbed by dental tissue and mucous membrane with prolonged gradual release at therapeutic level (substantivity)<sup>21</sup>.

The aim of this study was to determine and formulate antibacterial activity extract of *Zingiber officinale* Roscoe to *E. faecalis* as potential root canal irrigating solution which easily available, cost effective, increased shelf life, low toxicity compare to the gold standard of irrigation solution.

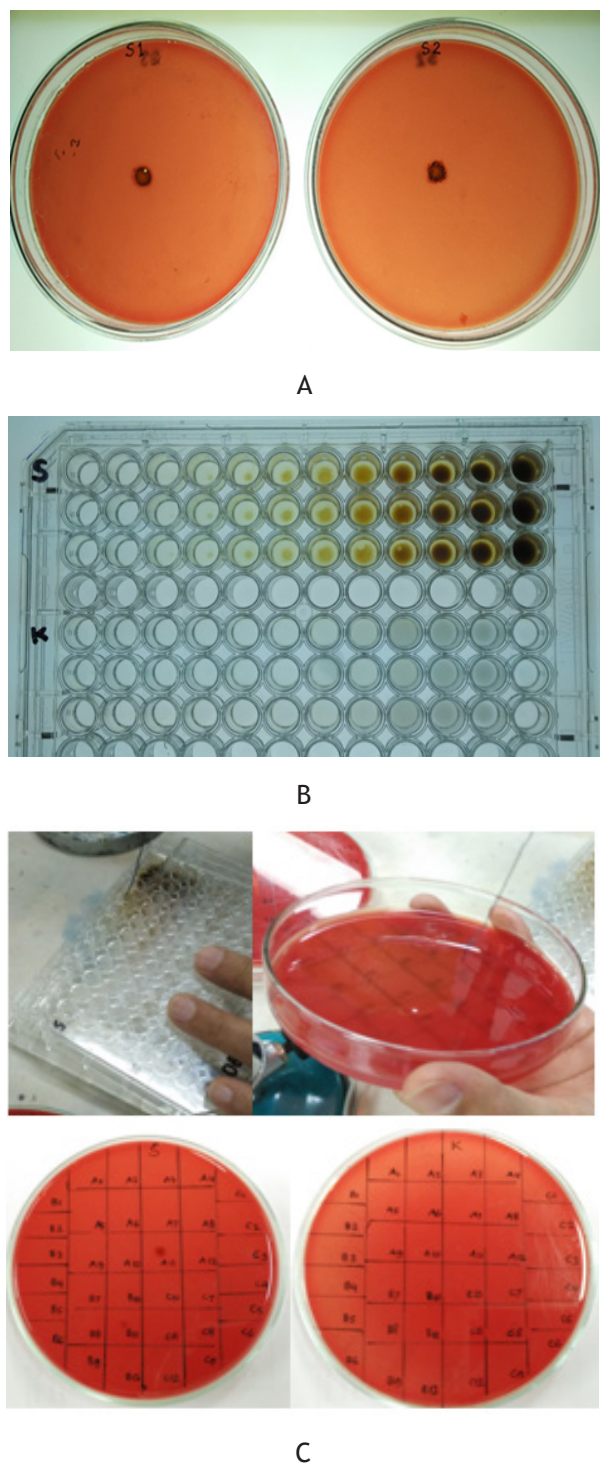
## METHODS

Preparation of the irrigating solution *Zingiber officinale* Roscoe, *Zingiber officinale* Roscoe was originally from Ciwidei, Bandung determined by Bandungense Herbarium Laboratory, Bandung Institute of Technology. Five hundreds grams of sliced fresh rhizomes were extracted with 95% ethanol for 72 hour at room temperature. The extract was then filtered through Whatman No. 1 filter paper, concentrated with a rotary evaporator (Büchi Company, Flawil, Switzerland) and kept in a desiccator overnight. The residue was then dissolved in aquadest in order to bring to a concentration of 1000 mg /mL.

Evaluation of the antibacterial activity using agar well diffusion test, Lawn culture of *E. faecalis* was prepared on a TSA plate. Wells of 6,5mm depth and 6 mm diameters were prepared, which were filled with 40 µL of 1000 mg/mL of the extracts. Two percents of chlorhexidine was used as the positive control. Plates were incubated at 37°C for 24 hours. Interpretation of diffusion results was carried out by noting the presence or absence of zone of inhibition around the wells.



Picture 1. Extract of *Zingiber Officinale* Roscoe



**Picture 2.** A. *E faecalis* was cultured on TSA plate with the sample disc in the center, zone of inhibition was then observed for their antibacterial activities; B. Microplate used for determining the minimum inhibitory concentration (MIC) Of *Zingiber officinale* Roscoe extract and clorhexidine against *E faecalis*; C. Procedure of determining the minimum bactericidal concentration (MBC) of *Zingiber officinale* Roscoe extract and 2% clorhexidine.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), Extract of *Zingiber officinale* Roscoe were dissolved in aquadest and diluted with culture broth to a concentration of 500 mg/mL. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 500 to 0.977 mg/mL; 100  $\mu$ L of each dilution were distributed in 96-well plates, as well as a sterility control and a growth control (pure culture broth). Each test and growth control well was inoculated with 10  $\mu$ L of *E. faecalis* suspension in TSB with 0.5 McFarland solution. All experiments were performed in triplicate and the microdilution trays were incubated at 37°C for 24 hour. After the incubation, the MIC values were determined by visual inspection of the wells. In each series of wells, the last wells with clear supernatant was considered to be without any growth and taken as MIC value. Turbidity in the MIC tube indicated growth of bacterial strain implying that *E. faecalis* was resistant to ethanolic extract of *Zingiber officinale* Roscoe.

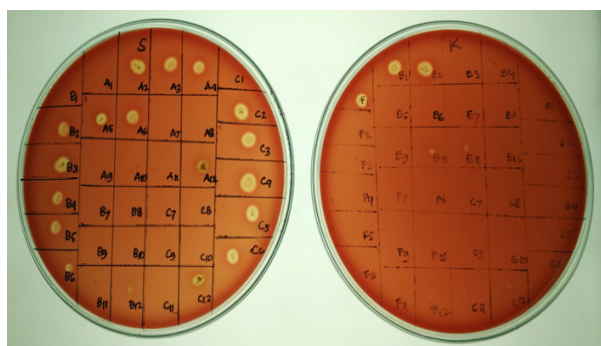
MBC (Minimum Barcterical concentration), was determined by sub culturing the contents of selective wells into blood agar. Each of the selected sample in well in the microplate was inoculated in to blood agar containing petri plate by 0.1 mL sterile tips in drop method. The plates were incubated at 37°C for 24 hours.

Results were expressed as mean value  $\pm$  standard error of the mean (SEM) of growth inhibition zones diameters obtained by extracts of *Zingiber officinale* Roscoe. Statistical differences between extract of *Zingiber officinale* Roscoe and clorhexidine were detected by Kruskal Wallis analysis and analysis of variance (ANOVA) to compare diameters of inhibition ethanolic extract of *Zingiber officinale* Roscoe and clorhexidine. P values lower than 0.05 ( $p < 0.05$ ) were considered significant.

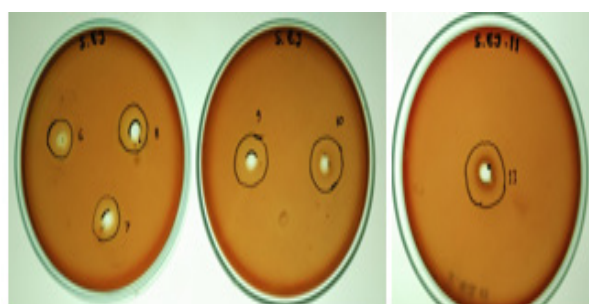
## RESULT

According to antibacterial activity tested by agar diffusion method in this study, 100% extract of *Zingiber officinale* Roscoe had a mean zones of inhibition growth for *E. Faecalis* of 31,40 mm ( $p < 0.01$ ) while 2% clorhexidine was 19,24 mm. This preliminary screening test showed that *E. faecalis*

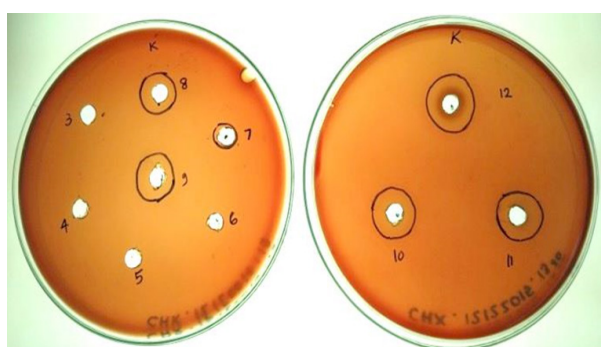




A



B



C

**Picture 5.** A. Observation of blood agar cultured to determine MBC; B. linhibition zone of extract of *Zingiber officinale*; C. linhibition zone of Ch-lorhexidine

is densitive to the extract of *Zingiber officinale* Roscoe and the study continues.

The MIC of the extract of *Zingiber officinale* Roscoe could not be clearly observed visually. The MBC for *Zingiber officinale* Roscoe extract was 15.625 mg/mL, while clorhexidine was 1.25 mg/mL.

## DISCUSSION

In this study, the MIC could not be determined by visual inspection as all wells in the microplate was muddy except negative control. Therefore, the MIC was eliminated from the study and to evaluate antibacterial activity, MBC were used as the indicator.

MBC for extract of *Zingiber officinale* Roscoe was 15.625 mg/mL which is equivalent to 1.56%. This shows that extract of *Zingiber officinale* Roscoe starts to work as a bactericide to *E. faecalis* at the concentration 15.625 mg/mL, whereas the MBC for clorhexidine as positive control was 1.25 mg/mL which is equivalent to 0.125%. The highest mean of diameter of inhibition zone of extract of *Zingiber officinale* Roscoe

Extract of *Zingiber officinale* Roscoe has an antimicrobial activity against *E. faecalis*. This may be caused of the presence of gingerol and shogaol as active ingredient within ginger, as mentioned in many studies indicated that the antimicrobial potency of ginger mainly caused by the presence of oxygenated mono- and sesquiterpenes, phenolic compounds (shogaol, gingerol), which are lipid-soluble phenol compounds primarily isolated from the root of ginger. Gingerol and shogaol was bactericidal and bacteriostatic components

**Table 1.** Means of diameters inhibition zone of extract Etanol *Zingiber officinale* Roscoe which concentration which no growth bacteriy

Concentration (mg/mL)	15,625	31.25	62.5	125	250	500
Mean Inhibition Zone (mm)	13.87	15,23	16,20	18,22	18,35	20,48

**Table 2.** Result of determine MBC extract Etanol *Zingiber officinale* Roscoe to *E. faecalis*

	KM	KB	0.977	1.953	3.906	7.813	15.625	31.25	6.,5	125	250	500
I	-	+	+	+	+	+	-	-	-	-	-	-
II	-	+	+	+	+	+	-	-	-	-	-	-
III	-	+	+	+	+	+	-	-	-	-	-	-

could break the phospholipid membrane became permeable due to released main components of cell and inhibit membrane function (electron transport, nutrient supply, synthetic of nucleat acid and enzym activity). Some of that components had invasive target due to bacterial pathogenity.

Mean of diameters of inhibition zone at MBC was similar. Analysis of Anova had confirmed that MBC of the extract of *Zingiber officinale* Roscoe and clorhexidine had significant differences. It showed that result of MBC was valid. It explained that to begin killed *E. Faecalis*, extract of *Zingiber officinale* Roscoe need ten times higher concentration than clorhexidine. It showed that to achieve antibacterial activity of clorhexidine as gold standard of root canal irrigant, a concentration of extract of 15.625 mg/mL *Zingiber officinale* Roscoe is needed.

## CONCLUSSION

Extract of *Zingiber officinale* Roscoe had antibacterial activity againts *E. Faecalis* possibly to its phenolic compound which soluble in lipid and has the ability to rupture the phospholipid membrane of the bacteria. To achieve an equal bactericidal activity as chlorhexidine, a concentration of 15.625 mg/ml is needed.

## ACKNOWLEDGMENTS

The authors deny any conflicts of interest related to this study.

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