

## The Antibacterial Potential of Ethanol Extracts of Torch Ginger Leaves (*Etlingera elatior* (Jack) R.M.Sm.) Against *Enterococcus faecalis* as an Alternative Irrigation Material in Root Canal Treatment

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### ABSTRACT

Root canal infection is a polymicrobial infection that is a progression of chronic caries affecting the dental nerve tissue and surrounding root tissue, which can be accompanied by extremely uncomfortable sharp pain. *Enterococcus faecalis* is one of the infecting agents that can be found in primary root canals experiencing endodontic infections and is resistant to root canal irrigation materials, thus leading to root canal treatment failure. Herbal plants have been used since ancient times by the Indonesian people to address health issues due to the believed beneficial properties of their constituents. Research on torch ginger reports pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, and anti-aging effects. Torch ginger (*E. elatior*) is a spice plant reported to have antibacterial bioactive compounds. This study aims to determine the antibacterial activity of ethanol extract from torch ginger leaves (*E. elatior*) against *Enterococcus faecalis* bacteria. The Kirby-Bauer disk diffusion method was used to determine the minimum inhibitory concentration (MIC) value and the streaking method from MIC testing to determine the minimum bactericidal concentration (MBC) with 8 concentrations and 2 controls. Data analysis of the MIC and MBC values was performed using One-Way ANOVA and Kruskal-Wallis parametric tests. The ethanol extract of torch ginger leaves (*E. elatior*) has minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against *Enterococcus faecalis* bacteria. The minimum inhibitory concentration of the ethanol extract of torch ginger leaves is at a concentration of 3.125%, while the minimum bactericidal concentration is at a concentration of 65%.  
Keyword: *Enterococcus faecalis*, extract of torch ginger leaf, Minimum bactericidal concentration, Minimum inhibitory concentration

### Introduction

Dental and oral diseases are among the most common complaints worldwide.[1]. According to the World Health Organization (WHO), nearly 3.5 billion people (about 50% of the global population) are known to suffer from one or more dental and oral diseases, making it a significant global health burden. The diseases that contribute to this global burden include chronic decay of the hard tissues of

the teeth (chronic caries), periodontal disease, tooth loss, and oral cancer. Indonesia is reported to rank fifth among the countries with the highest prevalence of dental and oral diseases, particularly in the categories of chronic caries and periodontal disease.[2].

Root canal infection is a polymicrobial infection that arises as a progression of chronic caries, affecting the dental nerve tissue (pulp) and the surrounding tissue near the tooth root

(periradicular), often accompanied by sharp, uncomfortable pain.[3]. Root canal infection begins with chronic caries, which causes the decay of the tooth's hard layers, eventually exposing the pulp to the oral cavity environment, which is filled with infectious agents.[4]. Root canal infection leads to pulp and periapical diseases. The management of root canal infections is carried out through root canal treatment (RCT), which aims to eliminate and inhibit the growth of infectious agents.[1].

Root canal infections are caused by a variety of microbes that accumulate and grow within the biofilm of the root canal system. The group of bacteria responsible for root canal infections is dominated by anaerobic and facultative anaerobic bacteria.[5]. *Enterococcus faecalis* (*E. faecalis*) is one of the bacterial species involved in root canal infections, with a prevalence of 77%.<sup>6</sup> The eradication of *E. faecalis* during root canal treatment (RCT) is essential to prevent secondary root canal infections and ensure successful treatment outcomes. The elimination of these infectious bacteria is achieved through root canal disinfection using antiseptic irrigation solutions.[1,6].

The gold standard irrigation solution for root canal treatment is a combination of sodium hypochlorite (NaOCl), which can dissolve necrotic pulp tissue, and chlorhexidine, which has antibacterial properties.[7]. However, NaOCl has the drawback of being toxic. Additionally, some studies have shown that the combination of these two irrigation solutions can interact and produce parachloroaniline, which is difficult to remove, cytotoxic, and carcinogenic to the tissues surrounding the tooth root.[8,9]. Therefore, research is needed to explore the potential of antibacterial agents from natural resources as root canal irrigation solutions.

According to the Indonesian Institute of Sciences (LIPI), Indonesia is reported to have around 7,500 medicinal plants with pharmacological potential.[10]. Medicinal plants have been used by the Indonesian people for generations to address health issues, due to their active compounds believed to have medicinal properties.[11]. These medicinal plants can come from various parts of the plant, such as the rhizome, stem, leaves, flowers, or fruit. One of Indonesia's distinctive medicinal plants is kecombrang (torch ginger), which offers numerous health benefits.[12].

The torch ginger plant (*Etlingera elatior* (Jack) R.M.Sm or *E. elatior*) is a native flora of Indonesia.[13]. It is known by different names in

various regions, such as cekala, kincung, or rias in North Sumatra.[14] Research on torch ginger has revealed several pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and anti-aging effects.[15] These benefits are derived from the secondary metabolites of *E. elatior*, which contain bioactive compounds such as phenols, flavonoids, tannins, saponins, alkaloids, steroids, and triterpenoids. Flavonoids, in particular, are polar compounds that are more dominant in ethanol extracts of torch ginger and have potential antibacterial properties.[16] Polar compounds are highly effective and relatively penetrate the polar cell walls of gram-positive bacteria by breaking peptidoglycan cross-links and inhibiting cell metabolism.[17]

This study aims to explore the antibacterial properties of natural resources with the ability to inhibit and kill bacteria responsible for root canal infections. One such resource is torch ginger (*E. elatior*), an indigenous Indonesian plant known for its antibacterial properties, which derive from secondary metabolites such as flavonoids, tannins, and saponins. These secondary metabolites are abundant in ethanol extracts of torch ginger leaves and possess both bactericidal (killing) and bacteriostatic (inhibiting) effects on bacteria. The concentrations of ethanol extracts from the flowers and leaves used in this study were 3.125%, 6.25%, 12.5%, 25%, 50%, 65%, 75%, and 80%. Chlorhexidine 0.2% was used as a positive control, and Dimethyl sulfoxide (DMSO) as a negative control, to evaluate antibacterial activity based on Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *E. faecalis*. The antibacterial effect of torch ginger shows potential as an alternative root canal irrigation solution, as it is derived from non-toxic and biocompatible natural resources.

Based on the aforementioned description, this study aims to determine the antibacterial activity of ethanol extract from the leaves of torch ginger (*Etlingera elatior* (Jack) R. M. Sm) against *Enterococcus faecalis*, a bacterium often responsible for failures in root canal treatment.

## Methods

This study is an experimental laboratory research using a post-test only with controlled group design. The research was conducted by administering treatments, observing, and measuring the outcomes after the treatment was given. The

antibacterial activity of ethanol extract from torch ginger (*E. elatior*) leaves at varying concentrations was tested against the growth of *Enterococcus faecalis*. The torch ginger leaves used in this study were collected from Sukamandi Village, Merek District, Karo Regency. Sample plant identification was carried out at the Herbarium Medanese, University of North Sumatra, while phytochemical screening and extraction of bioactive compounds from the torch ginger leaves were performed at the Phytochemistry Laboratory of the Faculty of Pharmacy, University of North Sumatra. The antibacterial activity testing of the ethanol extract was conducted at the Microbiology Laboratory, Faculty of Medicine, University of North Sumatra.

The extraction of torch ginger (*E. elatior*) leaves was performed using a stepwise maceration method. First, the leaves were cleaned. The extraction process began by preparing a glass vessel to mix and soak 208 grams of dried, powdered torch ginger leaves (*simplisia*) in 2000 ml of 70% ethanol. Stirring was carried out during the first 6 hours, and the mixture was left for 24 hours, with occasional stirring. The mixture was then filtered using filter paper to obtain the first filtrate (macerate 1). The extraction was repeated with 1000 ml of 70% ethanol for 3 × 24 hours to obtain the second macerate. Both macerates were combined and evaporated using a rotary evaporator at 40°C to remove the solvent, resulting in a thick extract.

The process begins by preparing 20 grams of *Mueller Hinton Agar* (MHA) media, which is dissolved in 500 ml of distilled water in an Erlenmeyer flask. The mixture is stirred to ensure the media powder dissolves well and is then covered with aluminum foil. The bacterial growth media solution is sterilized in an autoclave for 15 minutes at 121°C. After sterilization, the bacterial growth media is left to cool to 40°C or room temperature before being poured into petri dishes. For the *Mueller Hinton Broth* (MHB) media, 5 grams are weighed and dissolved in 250 ml of distilled water

in an Erlenmeyer flask. The solution is stirred until the media is fully dissolved and appears clear, then covered with aluminum foil. The bacterial growth broth is also sterilized in an autoclave for 15 minutes at 121°C. Once sterilized, the broth is left to cool to room temperature and poured into test tubes.

The test bacteria, which have been inoculated using a sterile inoculating loop, are then dissolved in 10 ml of 0.9% NaCl in a test tube. The bacterial suspension is homogenized using a vortex mixer until it reaches a turbidity equivalent to 0.5 McFarland, indicating a bacterial concentration of approximately 10<sup>8</sup> CFU/ml. Dilution is performed by mixing 0.1 ml of the bacterial suspension with 9.9 ml of MHB in a test tube, then homogenized with a vortex mixer, resulting in a bacterial inoculum with a turbidity level of 10<sup>6</sup> CFU/ml.

The Minimum Inhibitory Concentration (MIC) is determined at the lowest concentration of the extract that is capable of inhibiting bacterial growth from the initial colony count. The Minimum Bactericidal Concentration (MBC) is determined at the lowest concentration of the extract that can reduce the initial bacterial colony by 99%.

Data obtained from all treatments in the tests are processed and analyzed using IBM SPSS software. An initial examination is conducted using the Shapiro-Wilk normality test (for samples <50) to determine whether the data is normally distributed. If the results indicate that the data is normally distributed ( $p > 0.05$ ), data analysis will proceed with One-Way ANOVA to assess the relationships between the tested variables. Subsequently, a Post Hoc test (LSD) will be performed to identify which variables show significant differences between groups. However, if the data is found to be not normally distributed ( $p < 0.05$ ), a non-parametric test using the Kruskal-Wallis test will be conducted, followed by the Mann-Whitney test to examine the relationships and significance differences among the tested variables.

## Results and Discussion

**Tabel 1. Results of Phytochemical Screening of Ethanol Extract of Kecombrang Leaves**

No	Secondary Metabolites	Reagent	Results
1.	Alkaloid	Dragendroff	-
		Bouchardat	-
		Meyer	-
2.	Flavonoid	Serbuk Mg+Amil Alkohol+HCL	+
3.	Glikosida	Molish+H <sub>2</sub> SO <sub>4</sub>	+
4.	Saponin	Air Panas/dikocok	+
5.	Tanin	FeCL <sub>3</sub>	+
6.	Triterpenoid/steroid	Ieberman-Bourchat	+

Exp: (-) = The extract does not contain secondary metabolite compounds.  
 (+) = The extract contains secondary metabolite compounds.

**Tabel 2.**  
**Results of the Inhibition Zone Measurement**

No	Concentration of extract (%)	Results (mm)			Inhibition zone (Average±SD)
		Replication			
		I	II	III	
1	80%	15,9	14,7	14,2	14,93±0,87
2	75%	14,5	15,5	13,2	14,40±1,15
3	65%	14	14,2	13,6	13,93±0,30
4	50%	12,9	13,5	12,3	12,90±0,60
5	25%	9,5	9,6	9,4	9,50±0,10
6	12,5%	7	8,2	8,2	7,80±0,70
7	6,25%	6,5	7,7	8	7,40±0,80
8	3,125%	6,4	7	7,3	6,90±0,46
9	K (+)	16,6	15,7	15,6	15,97±0,55
10	K (-)	0	0	0	0,00±0,00

**Tabel 3.**  
**Hasil Perhitungan Daya Bunuh**

No	Concentration of extract (%)	Number of colonies ( $\bar{x}$ ±SD) (CFU/ml)	Difference (B-A)	% Reduction
1	80%	0,67±1,15	1060	99,93%
2	75%	8,67±8,32	1052	99,18%
3	65%	10,00±7,00	1050,67	99,05%
4	50%	50,00±38,35	1010,67	95,28%
5	25%	184,00±144,59	876,64	82,65%
6	12,5%	451,67±158,53	609	57,41%
7	6,25%	819,33±7,76	241,34	22,75%
8	3,125%	843,33±26,50	217,34	29,49%
9	K (+)	0±0,00	1060,67	100%
10	K (-)	1060,67±41,23	0	0%

**Tabel 4. Results of Inhibition Activity Test with oneway ANOVA**

No	Concentration of extract (%)	n	p-value Inhibition Activity
1	80%	3	0,000*
2	75%	3	
3	65%	3	
4	50%	3	
5	25%	3	
6	12,5%	3	
7	6,25%	3	
8	3,125%	3	
9	K+	3	
10	K-	3	

\* p value <0,05 = significant

**Tabel 5. Results of Killing Test with Kruskal-Wallis**

No	Concentration of extract (%)	n	p-value Killing Activity
1	80%	3	0,002*
2	75%	3	
3	65%	3	
4	50%	3	
5	25%	3	
6	12,5%	3	
7	6,25%	3	
8	3,125%	3	
9	K+	3	
10	K-	3	

\* Kruskal-Wallis test significant at  $p < 0.05$

The results of the phytochemical screening of torch ginger leaf extract in this study indicate that the extract possesses antibacterial properties, as shown in Table 1. Research conducted at the Microbiology Laboratory of the Faculty of Medicine, University of North Sumatra (USU), demonstrated that the torch ginger leaf extract (*E. elatior*) effectively inhibits the growth of *Enterococcus faecalis* (Table 2). The antibacterial activity against *E. faecalis* was assessed by observing the inhibition zone formed around the antibiotic discs.

Based on the observations and calculations of the inhibition zones, the Minimum Inhibitory Concentration (MIC) of the torch ginger leaf extract (*E. elatior*) was found to be at a concentration of 3.125%, with an average inhibition zone diameter of  $6.90 \pm 0.46$  mm. This indicates that a concentration of 3.125% is the lowest concentration of the torch ginger leaf extract that has the ability to inhibit the growth of *E. faecalis*.

The inhibition activity of torch ginger leaf extract (*E. elatior*) at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, 65%, 75%, and 80% against the growth of *Enterococcus faecalis* was analyzed using One-Way ANOVA statistical testing, which yielded a significant value of  $p = 0.000$  ( $p < 0.05$ ). The significance results from the parametric One-Way ANOVA test indicate a meaningful difference among the various concentrations in this study, demonstrating the antibacterial activity of torch ginger leaf extract (*E. elatior*) in inhibiting *E. faecalis*.

This study is consistent with research conducted by Kusumawati et al. (2015), which used different solvents and bacterial types to investigate the extract of torch ginger leaves at concentrations of 20%, 40%, 60%, 80%, and 100% against *Salmonella typhi*. [18] The results of that study

reported the highest inhibition zone at the 100% concentration, while the smallest inhibition zone was observed at the 20% concentration. [18]

A similar study conducted by Binugraha et al. (2020) indicated that torch ginger leaf extract possesses antibacterial activity against *Staphylococcus aureus*. The 100% ethanol extract

concentration was found to be effective in inhibiting *Staphylococcus aureus*. [19] All of the research results presented demonstrate that torch ginger leaf extract has antibacterial activity that can inhibit bacterial growth, even with different test bacteria and solvents. Previous studies have concluded that the greater the amount of active antibacterial compounds contained in the extract, the larger the inhibition zone produced.

The inhibition activity of torch ginger leaf extract (*E. elatior*) at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, 65%, 75%, and 80% against the growth of *Enterococcus faecalis* was analyzed using One-Way ANOVA statistical testing, which yielded a significant value of  $p = 0.000$  ( $p < 0.05$ ). The significance results from the parametric One-Way ANOVA test indicate a meaningful difference among the various concentrations in this study, demonstrating the antibacterial activity of torch ginger leaf extract (*E. elatior*) in inhibiting *E. faecalis*. Further statistical analysis using the Post Hoc Test (LSD) was performed to identify which groups showed significant differences at one concentration compared to the others. Based on the Post Hoc Test (LSD).

The Minimum Bactericidal Concentration (MBC) was determined from the tested torch ginger leaf extract at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, 65%, 75%, and 80% based on the inhibition zones formed. According to the calculation of colony counts assisted by a colony counter, an MBC value was obtained. Research

conducted by Nasri et al. (2021) indicated that a good MBC value is derived from the lowest extract concentration that can reduce bacteria by 98%-99%.<sup>20</sup> The concentrations that were able to reduce bacteria by 98%-99% from the initial colony count (negative control) of the torch ginger leaf extract in this study were 65%, 75%, and 80%. The 65% concentration was able to reduce the initial colony count by 99.05%, while the 75% concentration reduced the initial colony count by 99.18%, and the 80% concentration reduced the initial colony count by 99.93%. Therefore, the MBC obtained in this study is at a concentration of 65%.

The Kruskal-Wallis statistical test was conducted to determine the Minimum Bactericidal Concentration (MBC) of the torch ginger leaf extract (*E. elatior*) at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, 65%, 75%, and 80% against *Enterococcus faecalis*, yielding a significance value of  $p = 0.002$  ( $p < 0.05$ ). This result indicates a significant difference among the various concentrations and demonstrates the antibacterial activity of torch ginger leaf extract (*E. elatior*) in killing *E. faecalis*. The Mann-Whitney statistical test was conducted to identify groups that showed significant differences at one concentration compared to the others.

This study demonstrates that there is antibacterial activity in torch ginger leaf extract against *Enterococcus faecalis*, as evidenced by the presence of clear zones around the discs at each tested concentration. According to the Indonesian Pharmacopoeia Book, 6th edition, the antibacterial activity of an extract is considered effective if it has a zone of inhibition of approximately 14-16 mm. The results of this study show that the inhibition zones at various concentrations were within the range of 14-16 mm, specifically at  $14.40 \pm 1.15$  mm for the 75% concentration and  $14.93 \pm 0.87$  mm for the 80% concentration. Referring to the classification table for bacterial growth inhibition responses by David and Stout (1971), the inhibition zones are categorized based on their diameters. If the inhibition zone is  $\geq 20$  mm, it is classified as having very strong antibacterial activity; 10-20 mm is classified as strong antibacterial activity; 5-10 mm as moderate antibacterial activity; and  $\leq 5$  mm as weak antibacterial activity. In this study, the concentrations of 3.125%, 6.25%, 12.5%, and 25% fall into the moderate category, while the concentrations of 50%, 65%, 75%, and 80% are classified as having strong antibacterial activity.

The concentrations of torch ginger leaf extract in this study that exhibited strong inhibitory

activity against *Enterococcus faecalis* based on reference standards were at 50%, 65%, 75%, and 80%. The study also found that the bactericidal activity against *Enterococcus faecalis* occurred at concentrations of 65%, 75%, and 80%, as these concentrations were able to reduce the initial bacterial colonies. However, when compared to the positive control group, chlorhexidine demonstrated superior bactericidal activity, reducing the initial bacterial colonies by 100%.

Considerations regarding the potential of torch ginger leaf extract as an alternative root canal irrigant include various aspects, such as viscosity and flow rate. The concentration of torch ginger leaf extract affects its viscosity and flow rate; higher concentrations lead to increased viscosity, which results in decreased flow rate. Flow rate is crucial for root canal irrigants to ensure that the solution can thoroughly clean the entire surface of the root canal walls and penetrate the dentin and its tubules.[22] Therefore, further research is needed to evaluate the formulation of torch ginger leaf extract as a root canal irrigant. Another factor to consider regarding the use of torch ginger leaf extract as an alternative root canal irrigant is the complexity of the extraction process and its relatively high cost. In terms of cost, NaOCl, which is the standard irrigant for root canal treatment, is more affordable than torch ginger leaf extract.[23] However, when considering the advantages of torch ginger leaf extract, it has non-toxic properties, is safe, and has minimal side effects due to its natural origin. In contrast, NaOCl has toxic properties, can irritate periapical tissues, possesses a bitter taste and odor, and can reduce the strength and elastic modulus of dentin.[23]

## Conclusion

The ethanolic extract of torch ginger leaves (*Etlingera elatior* (Jack) R. M. Sm) has the ability to inhibit and kill *Enterococcus faecalis* bacteria. The minimum inhibitory concentration (MIC) in this study was found at a concentration of 3.125%, with an inhibition zone width of  $6.90 \pm 0.46$  mm, while the minimum bactericidal concentration (MBC) was determined at a concentration of 65%, as it was able to reduce the initial bacterial colonies by 99%.

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