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Antibacterial Effectivity of Javanese Turmeric (*Curcuma zanthorrhiza)* Compared to Red Ginger (*Zingiber officinale var. rubrum*) Extracts against *Enterococcus faecalis*

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Abstract: *Enterococcus faecalis* contributes to a number of dental infections, including root canal infections, periodontitis, and peri-implantitis and shows antibiotic resistance. Javanese turmeric (*Curcuma zanthorrhiza*) and red ginger (*Zingiber officinale var. rubrum*), rich in antibacterial compounds, offer potential alternatives. This study explores their effectiveness at two concentrations against *E. faecalis*. This experimental study analyzed the phytochemical compounds qualitatively, which play an important role as antibacterial against *Enterococcus faecalis*. Afterwards, both extracts at 50% and 75% concentrations were prepared, tested on bacterial cultures, and compared to a 2.5% NaOCl solution. Inhibition zones were measured, and data were analyzed statistically to compare the effectivity of each extract group. The results showed that Javanese turmeric extract (JTE) (50% and 75%) had higher effectiveness, averaging 12.20±0.23 and 14.44±0.39, respectively. Red ginger extract (RGE) (50% and 75%) inhibited *Enterococcus faecalis* with a lower inhibition zone of 10.76±0.38 and 13.64±0.55. The 2.5% NaOCl control group had an inhibition zone of 13.38±0.79. When using the ANOVA and post-hoc Tukey statistical analysis, JTE 50% and RGE 50% showed the most significant differences compared to other groups ($p<0.05$). The inhibition power increased in higher concentration groups. In conclusion, Javanese turmeric extract and red ginger extract 75% are the most significant inhibitors of *Enterococcus faecalis*. The control group does not has significant differences with RGE 75% treatment groups, suggesting similar effectivity to the control.
Keywords: *Curcuma zanthorrhiza*; *Zingiber officinale var rubrum*; *Enterococcus faecalis*; antibacterial effect

INTRODUCTION

The bacterium *Enterococcus faecalis* is one of the bacteria commonly found in root canal infections. This bacterium is responsible for 80-90% of root canal infections, where it is typically the only species present. Another study also stated that 63% of root canal treatment failures with reinfection are caused by *Enterococcus faecalis*.[1]

Although generally considered a harmless part of the human body's microbiome, *Enterococcus faecalis* has the potential to cause serious infections in people with weakened or compromised immune systems.[2] Studies have shown a positive relationship between biofilm formation in *E. faecalis*. One of the mainstays of therapy for anaerobic bacterial infections, including those caused by *E. faecalis*, is antibiotics.[3] However, sodium hypochlorite (NaOCl) remains the most commonly used endodontic irrigant due to its excellent antimicrobial and tissue-dissolving properties.[4]

Various natural ingredients such as turmeric, garlic, eucalyptus and various types of ginger, especially red ginger, have shown the ability to kill bacteria in pathogens found in the root canal. Turmeric contains a phenolic compound called curcumin, which has been proven to have antibacterial properties in the root canal area. Red ginger (*Zingiber officinale var rubrum*), a member of the *Zingiberaceae* family, can also be used as another alternative ingredient. As a spice and traditional medicine, ginger rhizomes are commonly used to reduce various complaints such as nausea, diarrhoea, and coughs.[5]

Javanese turmeric and red ginger extracts have strong antibacterial properties. Both extracts contain antibacterial properties in the form of xanthorrhizol. The hydroxyl functional group (-OH) contained in phenol compounds interacts with bacterial cells through an absorption mechanism involving hydrogen bonds and is able to modify cell membrane permeability.[6] When phenol penetrates cells at high concentrations, this can cause proteins in the cell membrane to coagulate and lyse. In contrast, the formation of hydrogen bonds between cell membrane proteins and phenol compounds disrupts membrane permeability. As a result, important components of the cell will come out, which eventually causes bacterial death.[7]

Red ginger has various benefits, including its ability to be an antioxidant, antiviral, antifungal, antiemetic, anti-inflammatory, anticancer, and antibacterial.[8] Antibacterial activity against Gram-positive and Gram-negative bacterial pathogens has been proven by *Z. officinale* rhizomes, with antioxidant activity measured using two different methods and varying concentrations.[9] Anthraquinones, terpenoids, glycosides, saponins, alkaloids, and tannin compounds were extracted from red ginger, which showed antioxidant activity. Other components extracted, like gingerol, limonene, zingiberol, linalool, geraniol and citral, revealed its antimicrobial activity. The study found that the antimicrobial compounds found in red ginger (*Zingiber officinale var. rubrum*) also provide antibacterial properties against *S. pyogenes*.[10] The terpenoid components contained in essential oils work by disrupting the structure of the cell membrane. Meanwhile, the mechanism of action of gingerol occurs through the process of protein denaturation and destruction of bacterial membranes. The protein denaturation process is also produced by linalool and geraniol. Unlike other compounds, citral inhibits bacterial enzyme activity through two main mechanisms, namely alkylation and protein denaturation.[11] Literature review from various previous studies indicates that red ginger nanoparticles can inhibit the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Rhizopus sp*, *Aspergillus nige*, and *Candida albicans* bacteria.[12]

Various previous studies have utilized extracts with concentrations ranging up to 50%, while studies using concentrations higher than 50% are still limited in number, possibly due to toxicity considerations. Based on this, researchers are interested in analyzing the efficacy comparison of Javanese turmeric and red ginger extracts at two concentrations, i.e. 50% and 75%. The main objective of this study was to analyze the comparative antibacterial effectiveness of Javanese turmeric extract (*Curcuma zanthorriza*) and red ginger extract (*Zingiber officinale var. rubrum*) in inhibiting the growth of *Enterococcus faecalis*.

METHODS

This research was a descriptive study with an experimental approach. The research samples consisted of five treatment groups: Javanese turmeric extract with concentrations of 50% and 75%, red ginger extract with the same concentration, and 2.5% NaOCl as a positive control.

Two kilograms of each rhizoma were harvested and identified as Javanese turmeric (*Curcuma zanthorriza*) and red ginger (*Zingiber officinale var rubrum*). The process of making the extract begins by slicing the rhizomes into thin slices around 1 mm, then air drying for five days. The dried slices were then crushed using a blender and soaked in six litres of ethanol in a closed container to prevent evaporation. Both test tubes containing maceration solutions were stirred using Ose for 15 minutes every 24 hours for five days and then filtered. Then, both extracts were processed using a rotary evaporator and water bath until they reached a thick consistency. The thick extracts were diluted by mixing five grams of each extract with 10 mL of 10% DMSO separately to obtain a concentration of 50%.

Phytochemical tests were conducted to identify compounds in the extract. Alkaloids were found, showing an orange or reddish colour when heating 1 gram of extract 0.5 mL of 2% HCl and adding Dragendorff reagent. Flavonoids were detected by adding hot water, Mg ribbon, and concentrated HCl to the extract, then heating in a water bath for 15 minutes, with a yellow or red colour indicating a positive result. Saponins were tested by adding 2 mL of boiling water to the extract, shaking for ten seconds, and adding 1 mL of 2N HCl. Tannins were identified by boiling the extract with hot water and adding FeCl3, producing a blue-green colour for catechol tannins and a blue-black colour for tannins. Triterpenoids and steroids were tested by shaking the extract with ethyl acetate, drying the layer, and adding anhydrous acetic acid or H2SO4, with a reddish yellow or green colour indicating a positive result.[13]

Agar media was made by mixing three grams of nutrient agar in 120 mL of distilled water, heating to boiling, then sterilized using an autoclave at 121°C for 15 minutes and poured into sterile Petri dishes. The pure culture was inoculated on nutrient agar using a sterilized loop in a petri dish, then divided into five segments, i.e. Javanese turmeric extract/JTE (50% and 75%), Red ginger extract/RGE (50% and 75%), and 2.5% NaOCl as a positive control. The procedure was repeated five times. The incubation process was carried out at a temperature of 37°C for 24 hours to obtain a pure culture of *Enterococcus faecalis*. The next step required taking one loop from the pure culture, which is then dissolved in Natrium Chloride (0.9% saline), resulting in a cloudy white appearance according to the McFarland standard, which ensures a standardized bacterial concentration for accurate and reproducible experimental results. The antibacterial activity was carried out by soaking disc paper in the solutions, placing it on the bacterial culture, and then incubating it at 37°C for 24 hours before measuring the inhibition zone with a calliper. The data were then collected and processed using SPSS.

RESULTS

Table 1 showed the qualitative phytochemical screening test revealing that the JTE and RGE contained alkaloids, flavonoids, triterpenoids/steroids, and glycosides. Javanese turmeric extract also contained saponin. Both extracts did not have tannin properties.

Measurement of the clear zone that appeared around the disc paper using a digital calliper was carried out to assess the inhibition zone of JTE and RGE, as well as NaOCl 2.5% (as the positive control) against *Enterococcus faecalis*. This measurement process is an important part of antibacterial testing to determine the effectiveness of each of these ingredients. Table 2 showed that JTE 50% had an average of 12.20±0.23 mm inhibition. In comparison, JTE 75% showed higher effectiveness with an average of 14.44±0.39 mm inhibition against *Enterococcus faecalis* bacteria. The RGEt 50% and 75% showed the ability to inhibit *Enterococcus faecalis* bacteria with an average value of 10.76±0.38 mm and 13.64±0.55 mm, respectively. The control group (NaOCl 2.5%) recorded an average of 13.38±0.79 mm against the same bacteria. The test results showed that all sample groups had relatively strong inhibition power with a range of 10-20 mm.[14] The data

collected were homogenous and normally distributed (p>0.05) based on the Shapiro-Wilk analysis. There were significant differences (p<0.05) between the JTE groups (50% and 75%), the RGE groups (50% and 75%), and the control group (NaOCl 2,5%) based on ANOVA analysis.

Table 1. Phytochemical screening test results of Javanese turmeric and red ginger

Type	Section	Solvent	Phytochemical Screening Test Results							
			Alkaloid			F	T	G	S	T
			B	M	D	MgHCl + H_2SO_4	L-B	M + H_2SO_4	A	$FeCl_3$
Javanese turmeric	Rhizome	Etanol 70%	+	+	+	+	+	+	+	-
Red ginger	Rhizome	Etanol 70%	+	+	+	+	+	+	-	-

Alkaloids: F, flavonoid; T, Triterpenoid/steroid; G, Glycoside; S, Saponin; T, Tannin
Reagents: B, Bouchardat; M, Meyer; D, Dragendorff; L-B, Lieberman-Bouchardat; M, Molish; A, Aquadest

Table 2. The inhibition power of Javanese turmeric extracts, red ginger extracts (50%, 75%) and NaOCl 2.5% against *Enterococcus faecalis*.

Group	*Enterococcus faecalis Inhibition Zone*	
	Mean±SD	p-value
JTE 50%	12.20±0.23	0.000*
JTE 75%	14.44±0.39	
RGE 50%	10.76±0.38	
RGE 75%	13.64±0.55	
Control (NaOCl 2.5%)	13.38±0.79	

The post-hoc Tukey test shown in Table 3 was used to determine the difference between all groups within the ANOVA. Both JTE and RGE at 50% concentration had significant difference with all groups. However, the antibacterial efficacy of RGE 75% was similar to the positive control (NaOCl 2.5%), in which there was no significant difference between both groups. In addition, there was also no difference between RGE 75% with JTE 75%.

Table 3. Post-hoc Tukey test performed on all groups against *Enterococcus faecalis*

Material testing	JTE 50%	JTE 75%	RGE 50%	RGE 75%	NaOCl 2.5%
50% JTE		0.000*	0.002*	0.002*	0.011*
75% JTE	0.000*		0.000*	0.127	0.025*
50% RGE	0.002*	0.000*		0.000*	0.000*
75% RGE	0.002*	0.127	0.000*		0.922
2.5% NaOCl	0.011*	0.025*	0.000*	0.922	

*Tukey post-hoc test, * value of significance based on p<0.05. RGE: red ginger extract, JTE: Javanese turmeric extract

DISCUSSION

The compounds found in Javanese turmeric and red ginger extracts play an important role as inhibitors of bacterial growth and development. Alkaloids are known to have analgesic, anti-inflammatory, and adaptogenic properties. Flavonoids play a role in various biological activities that are beneficial to health, such as preventing atherosclerosis and protecting the cardiovascular system. Meanwhile, saponins have anti-inflammatory effects.[15] Meanwhile, Khoir et al[16] found in their study that red ginger extract contains terpenoid and flavonoid compounds that contribute to increasing its antibacterial effectiveness.

A study was conducted on Javanese turmeric extract and red ginger extract to evaluate their

ability to inhibit the growth of *Enterococcus faecalis* bacteria at two different concentration levels, namely 50% and 75%. When antibacterial testing was carried out, the results showed that the antibacterial power of extracts increased in higher concentrations (75%) compared to 50% in both extracts. From all groups, the 75% concentrations showed the higher inhibition power, i.e. average value of 14.44 ± 0.39 mm and 13.64 ± 0.55 mm for Javanese turmeric and red ginger, respectively. The effectivity is similar to the positive control (NaOCl 2.5%) of 13.38 ± 0.79 mm against the same bacteria, *Enterococcus faecalis*. In the study, 2.5% NaOCl was chosen as the positive control because this concentration has been proven effective in killing bacteria within biofilms in dentinal tubules while posing minimal risk of damage to root dentin and periapical tissues compared to higher concentrations such as 5.25%. At the same time, it still produced a significant inhibition zone (13.38±0.79 mm), as demonstrated in the antibacterial test.

Curcumin and xanthorrhizol are the primary active compounds in the Javanese turmeric rhizome, exhibiting a broad-spectrum antibacterial effect against both gram-positive and gram-negative bacteria. Curcumin functions by inhibiting cell proliferation and targeting bacterial cell walls or membranes, altering their permeability.[17] This disruption leads to uncontrolled substance transport in and out of the cell, causing essential compounds like organic ions, enzymes, amino acids, and nutrients to escape. The loss of enzymes, along with water and nutrients, hampers metabolism and reduces ATP production, which is crucial for bacterial growth and proliferation. Consequently, bacterial cell growth is suppressed, ultimately leading to cell death.[18]

Additionally, essential oils containing phenols and terpenoids can inhibit bacterial growth by disrupting the cytoplasmic membrane. Tannin compounds also function as growth inhibitors, preventing the proliferation of microorganisms. Flavonoids in the Javanese turmeric rhizome are known for their ability to damage cell walls and hinder protein formation, which is essential for bacterial growth. Furthermore, the alkaloid content in Javanese turmeric interferes with peptidoglycan synthesis, making the bacterial cell wall structure unstable.[19]

Previous research results revealed that phenol isolates from red ginger ethyl acetate extract in the form of yellow solids are thought to be phenol derivative compounds. The test results showed that the isolate had antibacterial properties against *S. aureus* with a minimum inhibitory concentration of 6.25% and a minimum bactericidal concentration of 25%. A study comparing the antimicrobial potential of red ginger and elephant ginger revealed variations in their ability to inhibit different bacterial strains. Red ginger (*Zingiber officinale var. rubrum*) exhibited the highest inhibition zone against *S. aureus* (15.83 mm).[20]

Essential oils and oleoresins contain chemical compounds that inhibit bacterial growth and eliminate bacteria by disrupting the plasma membrane, interfering with cellular processes, and leading to bacterial cell lysis. Additionally, they alter the three-dimensional structure of proteins, resulting in protein denaturation. Although the amino acid sequence remains unchanged after denaturation, the protein loses its functionality.[18]

In this study, it was found that a concentration of 50% Javanese turmeric extract could inhibit *Enterococcus faecalis*, but different from the study by Bachtiar et al, who studied ethanol extract of torch ginger leaves against *Enterococcus faecalis*, it was found that a concentration of 80% the extract could significantly inhibit the bacteria.[17] Furthermore, in this study, it was found that a concentration of 75% red ginger extract has the highest inhibition zone towards Enterococcus faecalis (13,64±0,55) but different from the study by Khoir et al[16] who studied extract of red ginger against Enterococcus faecalis, it was found that 15% concentration has the highest inhibit zone (9.48±0.28). The difference in results is most likely due to several variables, such as different extraction methods and solvents, different harvesting times, cultivation environmental conditions (including altitude, rainfall, and soil type), and geographical position which affect the number of active substances contained in each extract.[21]

CONCLUSION

Javanese turmeric extract at concentrations of 50% and 75% has higher effectiveness in

inhibiting the growth of *Enterococcus faecalis*, compared to red ginger extract at same concentration level. However, the red ginger extract 75% shows no significant difference with NaOCl 2.5% in inhibition power against these bacteria. To sum up, both Javanese turmeric and red ginger extracts in 75% concentration can be used as an alternative such as oral health mouthwash to inhibit the growth of *Enterococcus faecalis* mostly due to its phytochemical compounds.

Conflict of Interest

The author declares no conflict of interest in this study.

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