The effectiveness of white turmeric (Curcuma zedoaria) extracts as root canal irrigation alternative material on Streptococcus viridans

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Abstract. Irrigation is one of the important principles of triad endodontic to eradicate microorganisms in root canal infections. The rhizomes of white turmeric (Curcuma zedoaria) contain compounds that have antibacterial activity and has a potential to be used as an alternative for irrigation solution. The study was done to evaluated the antimicrobial activity of white turmeric extracts againts Streptococcus viridans. This experimental in vitro study used four extracts of white turmeric with various concentrations (100%, 50%, 25%, 12.5%) as tested groups, 2% Chlorhexidine as positive control and distilled water as negative control group. Solid BHI medium containing Streptococcus viridans 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 efficacy. Data were analyzed by One Way ANOVA and continued by Post-hoc Bonferroni. The results showed that all groups had significant differences. The largest growth inhibition zone was 100% white turmeric extracts group and had strong antibacterial effect, followed by 50% and 2% chlorhexidine, while 25% and 12.5% extrac ts had moderate antibacterial effect. Based on the results of this study it can be concluded that white turmeric extracts can inhibit the growth of Streptococcus viridans.

1. Introduction
Elimination of irritants and prevention of recontamination of the root canal are the essential elements for successful outcomes of root canal treatment. The complexity of the root canal system, presence of numerous dentinal tubules in the roots, invasion of the tubules by microorganisms, formation of smear layer during instrumentation are the major obstacles in achieving the primary objectives of complete cleaning and shaping of root canal systems. Intracanal irrigants and medications are used during root canal treatment to reach the natural complexities and remove the smear layer [1].

During and after instrumentation, the irrigants facilitate removal of tissue remnants, dissolving either organic or inorganic tissue in the root canal, and removing microorganism from the root canal [2]. Studies have reported that Gram-positive bacteria are more prevalent in primary root canal
infections, and the two most prevalent species among the collected samples were Peptostreptococcus and Streptococcus spp. [3] Sanchez et al. reported that the most frequently isolated organisms were different of viridans streptococci, and this is in coincidence with the observations of other authors [4,5].

Several irrigating substances have been recommended for use in combination with canal preparation. Chlorhexidine digluconate (CHX) is widely used in disinfection in dentistry because of its good antimicrobial activity. However, CHX has no tissue-dissolving capability. Moreover, it has in vitro cytotoxic effect on human osteoblast, eventhough it seems to be dose dependent [6]. There is a growing interest on the importance of medicinal plants throughout the world, including in Indonesia, as a natural alternative to synthetic chemicals with lesser side effects. Some medicinal plants have been studied to investigate its antimicrobial activity, to be developed as an alternative for chemical endodontic irrigants. Mariyatin et al. reported that red and green piper betle leaf extract has antibacterial effect against S. viridans, and it is related with its content i.e essential oils containing phenolic compounds. These compounds are also found in white turmeric (Curcuma zedoaria) [7]. Curcuma zedoaria has been used traditionally in many countries especially in South-East Asia as a folk medicine for many centuries. The dried rhizomes were used to make drinks or extracted as traditional medicine to treat stomach diseases, blood stagnation, diarrhea, and during menstruation. This perennial plant is a rich source of essential oils, starch, curcumin, arabin, gums, etc and exhibit antimicrobial activity [8]. Previous study reported antimicrobial activity of the extract of Curcuma zedoaria against Gram positive and Gram-negative bacteria such as Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus [9].

A study evaluated the cytotoxic effects of fluid extract of Curcuma zedoaria through cell culture, and the findings suggest that the extract is not immediately toxic to the cells, although significant differences were found between concentrations [10]. In dentistry, its clinical use and studies are still limited. Bugno et al. compared the in vitro antimicrobial efficacy of Curcuma zedoaria fluid extract and commercial mouthrinses on microorganisms frequently related to oral problems. The results indicated that antimicrobial efficacy of Curcuma extract were similar to that of commercial products against all microorganisms studied [11]. It may also have a potential to be used as an alternative for irrigation solution. The aim of this study was to evaluated the antimicrobial activity of white turmeric extracts against Streptococcus viridans, compared with 2% chlorhexidine irrigation solution.

2. Methods
This is an experimental study. The white turmeric extract was prepared in Integrated Testing Laboratory of Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sriwijaya University. Fresh plants were peeled and washed thoroughly, cut into small pieces and dried in microwave at 60°C for 10 min and then pulverized using a blender. The powder were prepared with ethanol 96% for 3 days, and then the solvents were removed by using rotary evaporator at 40°C for 3 hrs to get 100 % extract concentration. The extract was diluted to 50, 25, and 12.5 % concentrations for anti-microbial evaluation. Aquadest and 2% Chlorhexidine (CHX) were used as control group.

![Figure 1 Illustration of labelled petri dish.](image)
This study used agar well diffusion method to evaluate antimicrobial activity. *Streptococcus viridans* suspension was made according to 0.5 McFarland standard. The microorganism suspension was put into tube containing 2 cc Brain Heart Infusion Broth (BHI-B), and incubated for 1x24hrs at 37°C. The suspension were taken using a syringe and then mixed in Brain Heart Infusion Agar media (BHI-A) in a previously labelled petri dish, and waited until solidified. Five petri dish were used, each contain six wells of 6mm diameter made in the solid agar surfaces using a cork borer. Each wells dropped by 5 mL of all test compounds, positive control 2 % CHX, and distilled water as negative control in accordance to the label underneath the petridish (Figure.1), then incubated at 37 ° C for 24 hours.

![Figure 2. Measurement of inhibition zone.](image)

The test compounds were evaluated for the zones of inhibition by measuring the diameter of inhibition zone that showed no visual turbidity around each wells by using a caliper (Fig.2). The procedure was repeated two times to avoid mistake in readings for each extract. The data were analyzed statistically for normality with the Shapiro-Wilk, and tests of homogeneity with Levene test. Analytical results are normal and homogenous hence continued with oneway ANOVA test, and followed by Post Hoc Bonferroni test to evaluate the significant differences among groups.

### 3. Results

Table 1 show that the largest mean diameter of zone of inhibition is 18.665±0.655 mm in experimental group with 100 % concentration, and the smallest diameter is 0 mm in negative control group. All groups had significant differences, as showed in Table 2.

| Test compound                  | Mean ± SD (mm) |
|-------------------------------|----------------|
| White turmeric extracts 12,5% | 6.032±0.608    |
| White turmeric extracts 25%   | 7.874±0.624    |
| White turmeric extracts 50%   | 11.764±0.907   |
| White turmeric extracts 100%  | 18.668±0.655   |
| Chlorhexidine 2%              | 13.405±0.809   |
| Aquadest                      | 0.000± 0.000   |

### 4. Discussion

White turmeric (*Curcuma zedoaria*) is medicinal plant that cultivated and grows mainly in the East-Asian countries including China, Vietnam, India, Bangladesh, Malaysia, Japan and Indonesia. Different parts of this plants such as rhizome, fresh roots, tuber, and also leaf have been reported to posses antibacterial, anticancer, antifungal, antimutagenic, antiamoebic, antiinflammation, antioxidant, and antidiabetic activities [8].
This in-vitro study evaluates antibacterial activity of the rhizomes of *Curcuma zedoaria* in ethanol extracts with various concentration, i.e 100%, 50%, 25%, 12.5%. Two percent Chlorhexidine was used as positive control and distilled water as negative control. Our findings suggest that each concentration of the tested turmeric extract showed inhibition zone. The inhibition zone is related to active compounds contained within each concentration of the extract. Phytochemical investigation of the ethanolic rhizome extract of *Curcuma zedoaria* indicates the presence of tannins, alkaloids, flavonoids, gum and carbohydrates, reducing sugar and terpenoids [12].

In the present study every tested group showed significant differences, and the antibacterial activity was directly proportional to the concentration tested compounds (Table 2). The strength of antibacterial activity is determined following Davis and Stout category: inhibition ≥ 20 mm is very strong, 10-20 mm is strong, 5-10 mm is moderate, and ≤ 5 mm is very weak [13]. Based on the diameter of inhibition zone showed in Table 1, white turmeric extract with 100% and 50% concentration as well as 2% Chlorhexidine exhibit strong antibacterial activity. However, 100% white turmeric extract showed larger inhibition zone (18.668 mm) compared with 2% Chlorhexidine (13.4056 mm). Previous study investigated antibacterial activity of white turmeric rhizome against other Gram positive facultative anaerobic bacteria which are often found in infected root canal, *Staphylococcus aureus*. The results showed that the inhibition zone of rhizome extract was 13 mm. Meanwhile, other study reported growth inhibition zone when the test bacteria were in contact with 2% chlorhexidine gluconate gel was lower (11.79 mm). Furthermore, the 25 % and 12.5 % extract exhibit moderate antibacterial activity, while distilled water showed no inhibition zone.

| Test compound                  | White turmeric extracts 12.5% | White turmeric extracts 25% | White turmeric extracts 50% | White turmeric extracts 100% | CHX % | Aquadest |
|-------------------------------|-------------------------------|-----------------------------|-----------------------------|-----------------------------|-------|----------|
| White turmeric extracts 12.5%| -                             | 0.003*                      | 0.000*                      | 0.000*                      | 0.000*| 0.000*   |
| White turmeric extracts 25%  | 0.003*                        | -                           | 0.000*                      | 0.000*                      | 0.000*| 0.000*   |
| White turmeric extracts 50%  | 0.000*                        | 0.000*                      | -                           | 0.000*                      | 0.010*| 0.000*   |
| White turmeric extracts 100% | 0.000*                        | 0.000*                      | 0.000*                      | -                           | 0.000*| 0.000*   |
| *Curcuma zedoaria* 2%         | 0.000*                        | 0.000*                      | 0.010*                      | 0.000*                      | -     | 0.000*   |
| Aquadest                      | 0.000*                        | 0.000*                      | 0.000*                      | 0.000*                      | -     |          |

*significantly p<0.050

Natural active compounds contained in medicinal plants is important factor in their pharmacological actions. As mentioned previously, *Curcuma zedoaria* possesses a very complex chemical composition. It is rich in tannin that have antimicrobial action by inhibition of extracellular microbial enzymes, and have an action to polypeptide on the membranes of the microorganisms, leading to cell lysis [14]. Flavonoid mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth [15]. Terpenoid commonly used as aromatic compound that give a distinctive taste and smell, it is known to have antibacterial effect by disrupting the process of membrane cell formation [16]. In addition to essential oils, *Curcuma* contains curcuminoids including Curcumin, that can be defined as phenolic compounds derived from the roots of *Curcuma spp*. It is a low molecular weight polyphenol, that is generally regarded as the most active constituent and comprises 2–8% of most extract preparations [8]. These and other compounds contained in *Curcuma zedoaria* may explain the greater inhibition zone when compared to 2% Chlorhexidine.
The chemical composition of the herbal extracts can be variable, considering the influence of cultivation conditions, soil quality, season and procedure of harvesting [10]. Christiane et al. described the seasonal variation of curcumenol and dihydrocurcudione, two active terpenoids from roots and rhizome of *Curcuma zedoaria* grown in Brazil. The analysis was carried out by high resolution gas chromatography, and the results showed that both terpenoids are present in all the parts studied. However, *Curcuma zedoaria* exhibited about three times more terpenoids in the mother rhizome than in other parts and seasonal studied [17]. Dewi et al. investigated the effect of age harvest and maceration levels on curcumin content of turmeric extract. The results showed that age of harvest did not significantly affect the curcumin content, however, they found that age of harvest 11 months produced the highest yield of turmeric extract. The longer turmeric rhizome planted in the soil, the more active substances that are formed. In our study we used white turmeric rhizome that harvested at the age or harvest 9-11 month [18].

The findings of this study may be used as preliminary indication that rhizome extract of *Curcuma zedoaria* may have potential to be further developed as irrigation solution in endodontic treatment. Further in vitro and in vivo studies are required to test the antimicrobial activity against other specific microorganisms in infected or necrotic pulp, and also to investigate the most effective and optimal concentration of the extract.

Based on the results and statistical analysis of this study, it is concluded that white tumeric extracts can inhibit the growth of *Streptococcus viridans*. The largest growth inhibition zones were produced when the test bacteria were contact with extract concentrations 100%.

5. References

[1] Endodontists A A 2011 Am Assoc Endodontists. 1–8
[2] Haapasalo M, Shen Y, Qian W and Gao Y 2010 Dent Clin N Am. 54 291–312
[3] Gajen E B, Aghazadeh M, Abashov R, Milani A S and Moosavi Z 2009 J Dent Res Dent Clin Dent Prospect 3 (1), 24-27
[4] Sánchez R, Mirada E, Arias J, Paño J R and Burgueño 2011 M Med Oral Patol Oral Cir Bucal 16(5), 670-6
[5] Krmpotić M, Macan D, Škrlin J and Perić B 2002 Acta Stomatol Croat. 36, 375-379
[6] Gomes B P F A, Vianna M E, Zaia A A, Almeida J F A, Souza-Filho F J and Ferraz C C R 2013 Brazilian Dental Journal. 24(2), 89-102
[7] Mariyatin H, Widyowati E and Lestari S 20’12 Artikel Ilmiah UNEJ. 1-4
[8] Tholkappiyavathi K, Selva K M, Nayanila S K and Yoganandam G P 2013 Inter J of Phytotherapy 3 (1), 1-4
[9] Philip K, Malek S N A, Sani W, Shin S K, Kumar S, Lai H S, Serm L G and Rahman S N S A 2009 Am J Appl Sci. 6(8), 1613–7
[10] Fernandes J P, Mello-Moura A C V, Marques M M and Nicoletti M A 2012 Acta Odontol Scand. 70(6), 610–4
[11] Bugno A, Nicoletti M A, Almodovar A A B, Pereira T C and Auricchio M T 2007 Brazilian Journal of Microbiology. 38, 440-45
[12] Ullah H M A, Zaman S, Juhara F, Akter L, Tareq S M, Masum E H and Bhattacharjee R 2014 BMC Complementary&Alternative Medicine. 14, 346
[13] Das K and Rahman M A 2012 International Journal of Pharmacy and Pharmaceutical Sciences. 4(5), 322-8
[14] Akiyama H, Fujii K, Yamasaki O, Oono T and Iwatsuki K 2001 Journal of Antimicrobial Chemotherapy. 48, 487-91
[15] Kumar S and Pandey A K 2013 The Scientific World Journal. Article ID 162750. doi:10.1155/2013/162750
[16] Darsana I G O, Besung I N K and Mahatmi H 2012 Indonesia Medicus Veterinus 1(3), 337-351
[17] Pamplona C R, de Souza M M A, Machado M S, Filho V C, Navarro D Yunes R., Monache F D and Niero R 2006 Z Naturforsch 61, 6–10
[18] Dewi P J N, Hartiati A and Mulyani S 2016 *Jurnal Rekayasa dan Manajemen Argoindustri* **4**(2),101-1

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