Antifungal effect of calcium hydroxide and cresotin liquid against *Candida albicans* as root canal treatment materials

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ABSTRACT

Introduction: *Candida albicans* can cause quite a high prevalence (35%) of root canal treatment failure. *Candida albicans* are difficult to eliminate and resistant to calcium hydroxide. Therefore the research was conducted to determine sterilization material which is effective as antifungal material to eliminate *Candida albicans* so it can minimize the possibility of failure in root canal treatment. Purpose of this research is to analyze the optimum concentration of paste that consist of calcium hydroxide powder combined with cresotin liquid against the *Candida albicans*. Method : This study divided sample into 4 groups, namely P1 (Ca(OH)2), P2 (Cresotin), P3 (Ca(OH)2)+Cresotin = 1:1), and P4 (Ca(OH)2)+Cresotin = 1:2) Data analyzed using the *One-way* ANOVA test *Least Significant Difference* test. Result : The combination of caoh and cresotin obtained averaged inhibition zone diameter in the groups P1:7.34mm, P2:0.07mm, P3:8.78mm, P4:10.65mm. Normality test this research group P1, P2, P3, and P4 distributed normally ($p > 0,05$). The result of the One-way ANOVA test showed a significant value of $p= 0,000$ ($p<0,05$). The result of Least Significant Difference test showed that each group had a significant difference because of the value of $p=0$ ($p<0,05$) due to the combination calcium hydroxide and cresotin component. **Conclusion:** The combination of calcium hydroxide and cresotin liquid has an antifungal effect against Candida albicans as a root canal treatment material, the most optimal combination is the combination of CaOH: Cresotin liquid is 1: 2 because it has the greatest average inhibition of 10.65mm

Keywords: *Candida albicans*, calcium hydroxide, cresotin, antifungal.

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INTRODUCTION

Necrosis teeth need be treated by root canal treatment, which is dental treatment by removing all pulp tissue both in the pulp chamber and also in the root canal.[1] Root canal treatment will work well if it is able to eliminate the source of infection which is eliminating microorganisms in the root canal system.[2] Clinically, the failure or success is determination by the absence of

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sign and symptom apically periodontitis. From the technical and pharmacological aspects of prevention and treatment primarily aimed at controlling the infection. Application of root canal sterilization drugs is at once with the root canal treatment and be one of successful factors of root canal treatment.

Beside bacteria there are also other microorganisms which is take effect in endodontic infection, such as (1) Fungi, some species of fungus that often involved in endodontic infection is *Candida* especially*Candida albicans*. There was a revealed that there was *Candida albicans* in the sample with primary root canal infection, (2) *Archae*, a Prokaryotic group of bacteria and (3) Virus, several studied revealed that virus in the root canal would only infect vital oot canals and in HIV patient. Another study revealed that HCMV and EBV are the cause of apical periodontitis infection.[2]

The most common microbiota which cause failure of root canal treatment is *Entereococcus faecalis*, but in a recent study it was found that about 35% failure of root canal treatment was caused by *Candida albicans*. *Candida albicans* forms a thin biofilm layer which can survive from the toxic liquid in the inorganic area of the tooth. Some factors that allow *Candida albicans* to survive is ability to attach on the dental tissue, using hyphae to penetrate to dentinal tubules (*Thigmotropism*).

Candida Albicans and several other type of fungi are also known to be more resistant to calcium hydroxide compared to *Enterococcus faecalis.*[3] Cresotin also known as methacresyl acetate, this material is a clear, oily and nonvolatile liquid. It has antiseptic and pain relief effects. Antimibrobial effect of cresotin is less than formocresol or para-chlorophenol, this drug also less irritating for the tissue.[1]

calcium hydroxide is preferred intercanal medicament and commercially available as a paste or pure powder which mixed with water or saline to make the paste with desire consistency. The characteristic of calcium hydroxide is having very high pH (12), it will degrades the remining pulp tissue (it works in synergy with sodium hypochlorite), broad spectrume antimicrobial agent, antimicrobial works in long term by contrilling serous exudate. calcium hydroxide weakens dentin and if it left in root canal for a long time.[4] During root canal treatment with multiple visits, root canal should be given an antimicrobial medicament between the time of the visit. Calcium hydroxide is first choice medicament in multiple visits root canal treatment. Root canal system that infected is hard to disinfect, especially because of root canal in teeth are easily infected by microbes that permanent and difficult to remove.[5]

METHODS

The type of this research is laboratory experimental study. The sampling technique is *simple random sampling*. The research tools used in this study is test tubes, inoculating loop, inoculating needle, burner, measuring pipettes, incubator, autoclaves, becker glass, erlenmeyer, petri dish. Meanwhile, the materials used were Cresotin liquid, calcium hydroxide powder, etanol 96%, *Candida albicans*.

The sample used in this study is the concentration of mixture of $Ca(OH)_2$ (powder) and Cresotin (liquid). group experiment divided into 4 group P1 (Ca(OH)2), P2 (Cresotin), P3 (Ca(OH)2)+Cresotin = 1:1), and P4 (Ca(OH)2)+Cresotin = 1:2) The initial antifungal activity was carried out using the *disc diffusion* with *spread plate* technique. The medium used in this method is Medium SGA (Saburoud's Glucose Agar). The liquid SGA medium is placed into a petri dish, the medium is left into solid. The petri dish cover was labeled according to the isolate and concentration of extract that was tested.

The inoculum suspension of *Candida albicans* fungi which was adjusted to the 0,5 McFarland turbidity standard was prepared, then 200 µL of inoculum fungal suspension was taken, and it was dropped onto the SGA media which has solidified in the petri dish, then the inoculum was flattened on the SGA media surface use sterile L stems. Disc paper was soaked into every concentration for 1 minute and pasted on the surface of the medium which has been mixed with fungal cultures using sterile tweezers.

The disc is pressed gently to make sure that the disc is attached to the medium. The culture was incubated at 37^0C for 24 hours. After incubation, the medium plate is observed for Inhibition of the bacterial growth by measuring the diameter of the clear area around the disc.[6]

RESULTS

The result of the research data were analized descriptively statistic to obtain a distributive and summarized data in order to clarify the result. Normality test this research data used *Shapiro-Wilk* test, because the subject is less than 50 subjects. The normality test using *Shapiro-Wilk* test in table showed that all groups, namely group P1, P2, P3, and P4 distributed normally (p > 0,05). From the result of the homogeneity test is known that the significance value p = 0,069 (p>0,05), it can be concluded that the data in all groups have homogeneous variances. The Parametric test used is *One-way* ANOVA because data was normal and homogeneous with an error rate of 5%.

Table 1. The result of inhibition between calcium hydroxide and cresotin and combination of both in the growth of *Candida albicans* (in mm)

Group	N	Mean	Std. Deviation
P1	4	7.34	0.206
P2	4	0.07	0.044
P3	4	8.78	0.435
P4	4	10.65	0.342

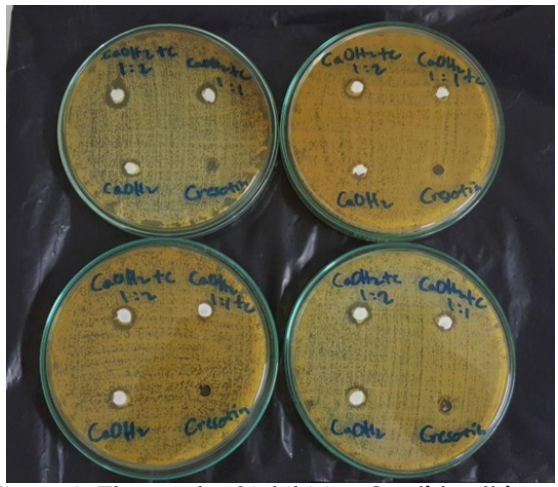

Figure 1. The result of inhibition *Candida albicans*

Table 2. The result of One-way ANOVA of inhibition of *Candida albicans* (mm)

Group	Shapiro-Wilk		
	Statistic	df	Sig.
P1	.925	4	0.564
P2	0.864	4	0.274
P3	.958	4	0.764
P4	.971	4	0.85

Table 3. The result of One-way ANOVA of inhibition of *Candida albicans* (mm)

ANOVA					
Bacteria inhibition (mm)					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	260.817	3	86.939	998.939	.000
Within Groups	1.044	12	.087		
Total	**261.861**	**15**			

Based on table 3, the result of the *One-way* ANOVA test showed a significance value p= 0,000 (p < 0,05), therefore it was concluded that there were at least 2 groups with significant differences, and analysis continued with The result of *Least Significant Difference* test showed that each data

Table 4. The result of Least Significant Difference test of inhibition of Candida albicans (mm)

	P1	P2	P3	P4
P1	.000	.000	.000	.000
P2		.000	.000	.000
P3			.000	.000
P4				.000

group with another had significant difference because p=0 (p<0,05). There were significant differences between group P1 with P2, P1 with P3, P1 with P4, P2 with P3, P2 with P4, P3 with P4.

DISCUSSION

Fungi can be found in root canal infection and periapical disease. Prevalence of fungi is more common in chronic root canal infection and reinfection especially in open cavities and in immunocompressive patients.[7] *C. albicans* is the most common fungal pathogen in humans causing both superficial and systemic infections. The ability of *C. albicans* to form biofilms is a major virulence factor and approximately 65% of microbial infections in humans are biofilm-related . This mode of growth protects *Candida* spp. against endogenous and exogenous inhibitory substances. High antifungal tolerance is a well-known factor of *Candida* biofilms and a challenge for treatment. The surrounding environment and its cues have a major impact on *C. albicans* biofilm formation. Hyphal growth is an essential element of *C. albicans* biofilms that provides integrity within these complex and dense structures . Multiple studies have shown that environmental pH, oxygen levels and nutritional status impact on the morphogenesis of *C. albicans*.[8]

According to Kumar et al.[9] study using *gem tube test* method found a prevalance of *yeast Candida albicans* is 1- 17 % with CFU more than 400/ml saliva in failure root canal treatment cases. This can be caused, inadequate isulation when root canal treatment, irrigation fliud and also sterilization drugs that less effective in antifungal power.[9] Considered as one of the most resistant species in the oral cavity, C.albicans is a possible cause of root canal system treatment failure. According to the research of Delgado et.al[10] intracanal medication significantly reduced C. albicans CFUs at both depths investigated.

The most commonly used as sterilization drugs in endodontic treatment and has antimicrobial qualities is calcium hydroxide. Calcium hydroxide is effective for eliminating the most pathogen bacteria in the root canal, but the studies have shown that *E. faecalis* dan *Candida albicans* are resistant to calcium hydroxide. Cresotin also known as methacresyl acetate, this material is a clear, oily and nonvolatile liquid. It has antiseptic and pain relief effect. Antimibrobial effect of cresotin is less than formocresol or para-chlorophenol, this drug also less irritating for the tissue.[11]

In this study, the combination of calcium hydroxide and cresotine solution which has an antifungal effect against Candida albicans is the most optimal combination of CaOH: Cresotine liquid is 1: 2 because it has the largest average inhibition of 10.65 mm. Mixture of $Ca(OH)_2$ with metacresylacetate produce a chemical reaction namely calcium cresilate and Acetic acid. calcium cresilate is strong disinfectant. Acetic Acid dissociates and releases hydroxyl ions ($H+$)[13]. Peracetic acid and hydrogen peroxide also suppressed biofilm formation, and the former inhibited Candida orthopsilosis and C. albicans biofilm formation. Acid sanitizers are generally utilized at a concentration of 100 ppm or 0.1 g/ liter.[14]

The antifungal activities of acetic acid as organic acid influences the transportation of important nutrient through the damaged cytoplasmic membrane due to increased acidity. The toxic effect of acetic acid refers to the low acids penetration into microbes so that it stops the nuclet acids and protein synthesis. The

antifungal activities of acetic acid refers to the presence of potassium hydoxide which prevent water absorbtion of water by microorganisms so it stops the growth. The changes in pH cause transformation of *Candida albicans* form Y form into M form so that is inhibits the process of cell division[16].With the increase of initial m-cresol concentration, the cell growth underwent a longer lag phase, and the specific growth rate decreased.

It demonstrated the presence of inhibitory effects, and higher substrate concentration brought about stronger inhibition on cell growth. However, at high substrate concentration, the inhibitory effects on cell growth were stronger and more energy was required to maintain the cell activity, but not to synthesize new cells. Another possible reason for the decreased cell mass was the production and accumulation of various intermediates. And a much higher specific growth rate and higher ultimate cell concentration were observed in phenol solution, indicating that the inhibitory effect on cell growth of m-cresol was stronger than that of phenol.[11]

Antifungal activity of natural phenolic compounds against resistant Candida strains depends on the presence of a free hydroxyl group on the aromatic ring. Some phenolic compounds may be oxidized to quinones methylene (QM) during the metabolic processes. These QM are structures that are highly toxic in varying biological systems. The rate of formation and stability of these QM are related to the substituents of the aromatic ring. The increased length of the alkyl group or volume allows for greater QM stability and thus enough time to reach the place where QM will interact, generating a toxic phenomenon. The alkylphenols with isopropyl substituents give rise to a QM with a higher cytotoxic activity than those with methyl groups. This would explain the highest anti-Candida activity of carvacrol and thymol and the low activity of phenol Cresol isomers, which only differ in the different positions of a methyl substituent, have the following order of antifungal activity, m-cresol>p-cresol>o-cresol.[12]

CONCLUSION

The results showed The combination of calcium hydroxide and cresotin liquid has an antifungal effect against Candida albicans as a root canal treatment material, the most optimal combination is the combination of CaOH: Cresotin liquid is 1: 2 because it has the greatest average inhibition of 10.65 mm

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