Comparison of antibacterial activity inhibitory of black cumin (*Nigella sativa*) oil, Cresophene®, and Calcium Hydroxide

Fajar Dwi Anggono*, Sri Kuswandari*

***Department of Pedodontics Faculty of Dentistry Universitas Gadjah Mada**

ABSTRACT

Introduction: Black cumin *(Nigella sativa)* oil is a natural antibacterial product containing thymoquinone. *Thymoquinone* is a powerful antibacterial substance towards gram-positive bacteria. The research objective was to compare the inhibitory effects of black cumin oil, Cresophene®, and $Ca(OH)_2$ towards the growth of *Staphylococcus aureus*. **Methods:** Experimental laboratory by taking *Staphylococcus aureus* from deciduous teeth pulp necrosis that has been isolated in Brain-Heart Infusion (BHI) medium. Inhibitory of black cumin oil, Cresophene®, and $Ca(OH)_2$ were measured by making three different 6 mm diameter wells contained each substances. Data then analyzed by two-way ANOVA using statistical analysis program. **Results:** Cresophene® had the largest inhibitory zone with the average zone was 32±0.05 mm and stable from the 1st day until the 4th day then decreased on the 5th day and remain stable until the 7th day. $Ca(OH)_2$ had average inhibitory zone of 15.9±0:10 mm and remain stable from the 1st day until the 7th day. Black cumin oil had average inhibitory zone of 7.9±0.2 mm and remain stable from the 1st day until the 7th day. **Conclusion:** The inhibitory zone towards *Staphylococcus aureus* isolated from deciduous teeth pulp necrosis consecutively was Cresophene®, $Ca(OH)_2$, and black cumin oil.

Keywords: *Staphylococus aureus*, Black cumin oil, Cresophene®, $Ca(OH)_2$, Inhibition zone

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INTRODUCTION

Staphylococcus aureus is a gram-positive bacteria found on skin, nose, mouth, mucous membrane, ulcers, and wounds[1] and is one of the main causes of necrotic root canal infection. The cause of root canal infection is wide-range of bacteria, with 90 percent are anaerobic bacteria such as *Porphyromonas, Bacterioides gingivalis, Porphyromonas gingivalis, Bacterioides endodontalis, Staphylococcus aureus,* and *Prevotella Bacterioides Buccae* also known as *Bacterioides Species.*[2]

One stage of root canal treatment is root canal sterilization that's done by root canal irrigation and administration of medicaments.[3] Some medicaments often used is $Ca(OH)_2$ and Cresophene®. The use of $Ca(OH)_2$ in dentistry was first used for endodontic treatment by Hermann in 1920 as pulp capping material. Calcium hydroxide

Corresponding author: Fajar Dwi Anggono, Department of Pedodontics Faculty of Dentistry Universitas Gadjah Mada Jl. Denta no. 1, Sekip, Yogyakarta, Phone/Fax: 085236052747, Email: foxda2005@gmail.com

proven to have antimicrobial properties and ability to reduce periapical inflammation which made its use increased all the time. $Ca(OH)_2$ paste has a high pH (about 12.5 to 12.8), so it is able to kill bacteria.[4] Initially, calcium hydroxide having bacteriostatic properties that able to inhibit the growth and eventually kills the bacteria (bactericidal).[5]

Cresophene® is one of dental materials widely used as ingredients for root canal medicaments before obturation. Cresophene® contains parachlorophenol, dexamethasone, thymol, and camphor. Parachlorophenol having strong bactericidal properties; dexamethasone as anti-inflammatory, whilst thymol and camphor serves as an antiseptic. Beside as a root canal sterilization, Cresophene® also often used as a deep cavities sterilizer.[2]

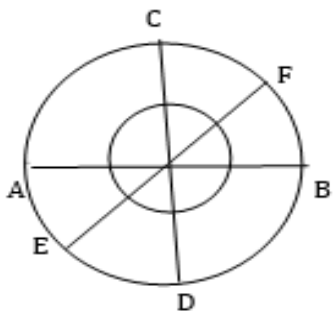
Utilization of natural ingredients as herbal medicine is a commonplace. One of the plants that can be used as antibacterial agent is black cumin *(Nigella sativa)*. The main content of black cumin oil that has been known and proven having pharmacological role as antibacterial agent is *thymoquinone*.[6] Thymoquinone has high antibacterial potency against gram-positive bacteria. The antibacterial activity of thymoquinone has proven effective on inhibiting *Streptococcus mutans* and *Streptococcus mitis*.[7]

METHODS

In this study, isolates of *Staphylococcus aureus* bacteria was taken from the necrotic root canals of anterior deciduous teeth. Retrieval of isolates in necrotic pulp of deciduous teeth caused by caries was done before root canal preparation. Teeth involved isolated in a sterile. Caries lesions were eliminated. Access to the pulp cavity using sterile round burs. After that, the sterile paper points incorporated into the root canal for 60 seconds to take the isolate of deciduous teeth necrotic pulp. Paper points contained isolate of deciduous teeth necrotic pulp put into Amyst transport medium and then sealed. The transport medium was then brought to Microbiology Laboratory of Yogyakarta Health Laboratory to be isolated.[8]

Black cumin oil was produced by using ready made black cumin oil with trademark Habasy Oil on the concentration of 100%, that was diluted into the concentration of 0.8%, and was done in Universitas Gadjah Mada Integrated Research and Testing Laboratory. Whilst Cresophene® is a trademark of root canal dressing materials composed of thymol, dexamethasone, parachlorofenol, and liquid. camphor. $Ca(OH)_2$ used was a root canal dressing materials obtained by mixing $Ca(OH)_2$ powder with glycerine solution in the ratio 1:1 until obtained consistency of paste.

Isolate of deciduous teeth necrotic pulp inside the transport medium were cultured on Brain-heart infusion (BHI) medium, incubated at the temperature of 37° C for 24 hours. The next step was the bacteria identification. Bacteria were grown in a blood agar to be isolated, then extracted the hemolytic yellowish-white areas to got a fermentation test by using mannitol, incubated at the temperature of 37° C for 24 hours and observed the color change from red to yellow. Catalase reaction then conducted with H_2O_2 and observed the agglutination. Two tests above were done to determine whether the isolated bacteria was *Staphylococcus sp*. or not. Tests with *Staphurex* then conducted with the provision that if the result was positive then the isolated bacteria was *Staphylococcus aureus*.



Note: Ø **Inhibitory zone 6 mm**

Measurement I : (AB-6 mm):2
Measurement II : (CD-6 mm):2
Measurement III : (AB-6 mm):2

Figure 1. Inhibitory zone measurement results

The next test done was the sensitivity test. Bacterial specimens diluted 0.5-1 McFarland, then swabbed with into petri dishes contained Mueller-Hinton agar (MHA) medium, then four wells were made in the media by using perforator with diameter of 6 mm, given each consecutively, the essential black cumin oil with concentration of 0.8%; Cresophene®; and $Ca(OH)_2$, incubated at the temperature of 37° C for 24 hours and then observed the inhibitory zone.[8] Inhibitory zone measurement results that was done by sliding caliper can be seen in Figure1.

RESULTS

A research about the differences between the inhibitory of black cumin oil, Cresophene®, and $Ca(OH)_2$. Preliminary research has been conducted about the inhibitory of black cumin oil at the concentration of 0.6%; 0.7%; 0.8% and 0.9% against the growth of *S. aureus* isolate of deciduous teeth necrotic pulp. The results showed that at concentration of 0.8%, black cumin oil had the largest inhibitory zone diameter mean (x) which was equal to 7.9 mm as shown in Table 1 and Figure 2.

Staphylococcus aureus colonies after diluted to 10^8 CFU/ml was cultured in MHA medium, then inoculated with black cumin oil 0.8%, Cresophene®, and $Ca(OH)_2$. Inhibitory diameter observation was done on day I to day VII. The measurement results of *Staphyococcus aureus* growth inhibitory zone from day I until day VII was shown in Table 2 and Figure 3.

Table 1. Mean (x) and Standard Deviation (SD) of black cumin oil inhibitory zone at concentration of 0.6%, 0.7%, 0.8% and 0.9% against growth of Staphylococcus aureus bacteria

Materials	N	x ± SD						
		Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII
BCO 0.6 %	3	6.8±0.02	6.9±0.03	6.8±0.01	6.8±0.01	6.9±0.02	6.9±0.02	6.9±0.03
BCO 0.7 %	3	7.8±0.02	7.9±0.02	7.9±0.02	7.9±0.02	7.7±0.05	7.8±0.02	7.7±0.01
BCO 0.8 %	3	7.8±0.02	8.0±0.01	7.9±0.03	7.9±0.01	8.0±0.03	7.9±0.01	7.7±0.01
BCO 0.9 %	3	6.5±0.01	6.5±0.02	6.5±0.01	6.2±0.02	6.2±0.02	6.3±0.01	6.2±0.01

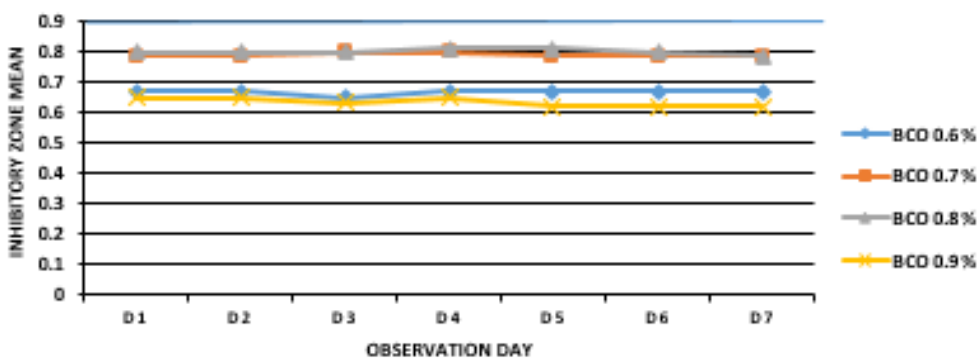


Figure 2. Black cumin oil inhibitory zone average at concentration of 0.6%, 0.7%, 0.8%, and 0.9% against Staphylococcus aureus bacteria growth

Table 2. Mean (X) and Standard Deviation (SD) of black cumin oil inhibitory zone at concentration of 0.8%, Cresophene® and $Ca(OH)_2$, against growth of *Staphylococcus aureus* bacteria

Materials	N	x ± SD						
		Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII
BCO 0.8 %	3	7.8±0.02	8.0±0.01	7.9±0.03	7.9±0.01	8.0±0.03	7.9±0.01	7.7±0.01
Cresophene®	3	31.8±0.06	32 ± 0.11	31 ± 0.05	31.8±0.04	20.4±0.02	20.4±0.02	20.0±0.07
$Ca(OH)_2$	3	15.5±0.10	16.1±0.11	15.9±0.10	15.6±0.04	16.2±0.09	15.9±0.02	16.4±0.05

Among the three materials, Cresophene® showed the largest inhibitory zone, approximately 32.4 mm until day IV, and dropped on day V into 20.4 mm and remain stable until day VII. Inhibitory zone of $Ca(OH)_2$ was below Cresophene® which was 15.5 mm and remain stable from day I to day VII. Black cumin oil at concentration of 0.8% showed the lowest results which was 7.9 mm and remain stable from day I to day VII.

Kruskal-Wallis test was used to know the difference between black cumin oil at concentration of 0.8%, Cresophene® and $Ca(OH)_2$ inhibitory zone against *Staphylococcus aureus* bacterial growth on day I to day VII, as shown in Table 3. Kruskal-Wallis test compared the inhibitory zone between each materials against *Staphylococcus aureus* bacterial growth from day I to day VII. The results showed significant difference ($p > 0.05$).

Friedman test then used to determine the effect of day or time on inhibitory zone of black cumin oil at concentration of 0.8%, Cresophene®, $Ca(OH)_2$, as shown in Table 4.

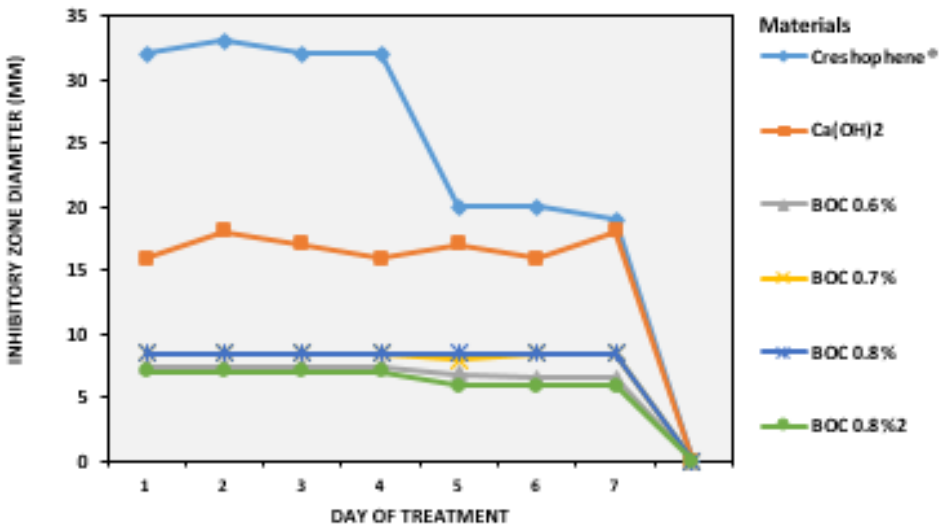


Figure 3. Mean of inhibitory zone of black cumin oil at concentration of 0.8%, Cresophene® and $Ca(OH)_2$, against *Staphylococcus aureus* bacteria growth

Table 3. Results of Kruskal-Wallis test about inhibitory zone between black cumin oil at concentration of 0.8%, Cresophene®, and $Ca(OH)_2$ against *Staphylococcus aureus* bacteria growth

Day	df	X^2	p
I	2	7.26	0.027
II	2	7.20	0.027
III	2	7.26	0.027
IV	2	7.26	0.027
V	2	7.20	0.027
VI	2	7.20	0.027
VII	2	7.20	0.027

Table 4. Friedman test results about inhibitory zone of black cumin oil at concentration of 0.8%, Cresophene®, and $Ca(OH)_2$ against *Staphylococcus aureus* bacteria growth

Materials	df	X^2	p
BCO 0.8%	2	5.16	0.52
Cresophene®	2	14.51	0.024*
$Ca(OH)_2$	2	5.27	0.51

Table 5. Wilcoxon test results about Cresophene® inhibitory zone against *Staphylococcus aureus* bacterial growth

Day	Z	p
Day I and Day II	-1.59	0:12
Day II and III	-1.62	0:11
Day III and Day IV	-1.27	0:13
Day IV and Day V	-2.22	0026 *
Day V and Day VI	-0.00	1:00
Day VI and Day VII	-0.74	0:46

Friedman test results showed that time only effected on Cresophene® inhibitory zone against *Staphylococcus aureus* bacterial growth on day I to day VII with p=0.024 (p<0.05), then proceed with the Wilcoxon test. Wilcoxon test was used to determine Cresophene® inhibitory zone against *Staphylococcus aureus* bacterial growth from day I to day VII as shown in Table 5.

Wilcoxon test results showed Cresophene® inhibitory zone on day I to day IV was not having any significant difference (p>0.05), then on the day V there was significant difference (p<0.05), whilst from day V to day VII there was no significant difference (p>0.05).

DISCUSSION

This study was done to evaluate the effectiveness of black cumin oil against *Staphylococcus aureus*. The test results showed that the largest black cumin oil inhibitory zone against *Staphylococcus aureus* of necrotic pulp of deciduous teeth isolates was at concentration of 0.8%, which was equal to 7.9±0.02 mm. It can concluded that the extract of black cumin has antimicrobial potency against *Staphylococcus aureus*. Black cumin is a natural ingredients that can be used as a therapeutic agent that will not cause microorganism resistance. Black cumin is a herbal product which active ingredient is not extracted, so the antimicrobial effect is lower than other trademarked medicine and other specific antibacterial materials.

Test results showed that the largest inhibitory zone against *Staphylococcus aureus* found in Cresophene®, compared to other materials, with inhibitory zone average was 32±0.05 mm. Cresophene® contains phenol, and has antibacterial potency, especially against gram-positive bacteria such as *Staphylococcus aureus*. Thymol as the active material has potential antibacterial effect so thymol tend to be a bactericidal against *Staphylococcus aureus*. Whilst its interaction with dexamethasone makes Cresophene® able to work as anti-inflammatory and antibacterial at once during the first 4 days. From the results showed that the inhibitory zone of Cresophene® during the first 4 days were very good, then remain stable, and decreased on day V, and remain stable again until day VII.

The results also showed the inhibitory zone of $Ca(OH)_2$ against *Staphylococcus aureus* bacteria in necrotic pulp was remain stable at 15.9±0.10 mm from day I to day VII. Whilst calcium hydroxide has a high pH that able to increases the activity of alkaline phosphatase which will also increases mineralization. Besides that, calcium hydroxide also capable to kill apical tissue-damaging microbes that can helps the formation of reparative cementum. Alkaline environment will inhibit osteoclast activity thus the reabsorption process will blocked to make tissue-repairing process keep running. Calcium hydroxide has a quite long period of pharmacology effect, with average of 14 days and works optimally in 7 days. In this study found that the inhibitory zone of $Ca(OH)_2$ against *Staphylococcus aureus* was lower than Cresophene® but tend to be stable during 7 days of observation.

CONCLUSION

The study results showed that the magnitude of inhibitory zone against the growth of *Staphylococcus aureus* in isolates of deciduous teeth necrotic pulp were consecutively Cresophene®, $Ca(OH)_2$, and black cumin oil *(Nigella sativa)*. Regarding stability, the inhibitory zone of black cumin oil *(Nigella sativa)* and $Ca(OH)_2$ were both having fixed magnitude from day I to day VII, whilst Cresophene® inhibitory zone was only having fixed

magnitude from day I to day IV, decreased on day V and remain stable again until day VII.

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