

Research Report

Inhibition Activity of Black Cumin Toothpaste Contain With Detergent Compared with Black Cumin Extract Non Detergent to the Growth of Supragingiva Plaque Bacteria

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ABSTRACT

Background: Plaque can inhibit the antibacterial activity of salivary function, which can damage the periodontal tissue. Several preventive measures can be done to control plaque, mechanically or chemically. Mechanical plaque control in combination with chemical plaque control such as toothpaste can reduce plaque. The role of SLS in toothpaste which can facilitate plaque detachment from the tooth surface. Herbal toothpaste has been developed using herbal ingredients that have antibacterial properties as black cumin (*Nigella sativa*). The contents of black cumin, thymoquinone, thymol, and tannins, have been proven to be effective in inhibiting the growth of plaque bacteria. **Purpose:** This study aims to identify the inhibitory ability of black cumin toothpaste extract containing detergent compared to black cumin extract toothpaste without detergent on the growth of supragingival plaque bacteria. **Methods:** This research method of diffusion of wells on BHIB media was conducted in four research groups namely negative control, cumin paste with SLS, non SLS cumin paste, and SLS and carried out bacterial planting on MHA media. Next measure the diameter of the inhibition zone around the well. **Results:** There was a significant difference in each group against the inhibitory growth of supragingival plaque bacteria on the Oneway ANOVA test results ($p < 0.05$) **Conclusion:** Black cumin extract toothpaste containing 2% of detergent has better inhibition against supragingival plaque bacteria than non cumin detergent 3% black cumin toothpaste.

Keywords: toothpaste; black cumin; *Nigella sativa*; Sodium Lauryl Sulfate (SLS); supragingiva plaque

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INTRODUCTION

The prevalence of periodontal disease in Indonesia is still classified as one of the main focuses of public health problems. According to the survey, periodontal diseases are prevalent both in developed and developing countries and affect about 20-50% of global population.¹ Periodontal disease is thought to be due to accumulation of dental plaque. Most plaque accumulation is caused by improper brushing techniques, and irregular routine dental visits that can cause inflammation of the gingiva. Poor oral hygiene is a risk factor for periodontal disease.²

Plaque can inhibit the function of saliva to carry out antibacterial activity. Dental plaque is classified as supragingival and subgingival based on its location on the tooth surface.³ Several preventive measures that can

be done to control plaque, mechanically or chemically. Mechanical plaque control such as toothbrushes, dental floss and interdental brushes which are also used together with chemical plaque control. Although toothbrushes are the most widely used oral hygiene tool, most people cannot effectively control mechanical plaque. Therefore, chemical plaque control is needed to help reduce plaque such as toothpaste and mouthwash, which can provide significant benefits.⁴

Toothpaste formulations usually containing a combination of fluoride and SLS (*Sodium Lauryl Sulfate*) the role of SLS that can facilitate plaque detached from the tooth surface. Herbal toothpaste has been developed using herbal ingredients that have anti-inflammatory properties, as a natural antiseptic ingredient, and analgesia.^{5,6} Black cumin is one of the herbal ingredients that can be developed into

toothpaste. The contents of black cumin are *thymoquinone*, *thymol*, and *tannins* which have been proven effective in inhibiting the growth of subgingival and supragingival plaque bacteria due to periodontitis.⁷

This study aims to identify the inhibitory ability of black cumin toothpaste extract containing detergent compared to black cumin extract toothpaste without detergent on the growth of supragingival plaque bacteria.

MATERIALS AND METHODS

This research was approved by the Ethics Review Committee of the Faculty of Dental Medicine, Airlangga University, Surabaya, No. 661/HRECC.FODM/X/2019. The type of research employed an experimental laboratory methodology with post test only control group design. This research was conducted by testing the inhibition of bacteria using the diffusion method with 6 replications. There are 4 groups: (1) control, (2) black cumin toothpaste with detergent, (3) black cumin toothpaste non detergent, (4) SLS. This study uses a stock of supragingival plaque bacteria. The preparation of supragingiva plaque bacteria has put the bacteria into a test tube containing liquid media Brain Heart Infusion Broth (BHIB). After that, supragingiva plaque culture was put into an anaerobic jar in the anaerobic atmosphere and incubated in an incubator at room temperature 37°C for 48 hours, then turbidity was observed to be synchronized with the 0.5 Mc Farland standard (1.5 x 10⁸ CFU / ml). The supragingiva plaque bacterial culture was planted on the Mueller Hilton Agar. An agar plate sterile filled with 0.1 ml supragingival plaque bacterial suspension which is in

accordance with the 0.5 McFarland standard leveled using a spreader. In the diffusion method, by making a hole in the plate to use a ring with a diameter of approximately 0.5 cm by 4 holes in different places in the same agar plate . After that, the agar plates were incubated at 37°C for 48 hours. The inhibition of bacteria is determined by measuring the inhibition zone around the wells. Statistical analysis uses the one-way ANOVA test because the test carried out is to compare 2 (two) or more groups using free samples.

RESULTS

The results of the inhibition of detergent black cumin toothpaste compared to non-detergent black cumin toothpaste against supragingival plaque bacteria were carried out using 4 groups consisting of black cumin toothpaste containing detergent, black cumin toothpaste without detergent, detergent and control group. Inhibition can be shown by the presence of inhibition zones, namely clear areas around the wells that have been treated. The results of each treatment are explained in Table 1.

The results of the data distribution test using the Kolmogorov-Smirnov Test show that the value of p = 0.222 indicates that the data distribution is normal because p > 0.05. Data were performed Levene test to see the homogeneity of the data, Levene test results showed p = 0.315 (p > 0.05) which indicates that the data is homogen. Test differences between groups were analyzed using the test one-way ANOVA. The results of this test have a significant value that is p = 0.000 (p < 0.05) so we get a difference from the treatment group.

Table 1. Average inhibition zone of suprgingival plaque bacteria

Group	N	Mean	Std. Deviation
Control	6	0.000	0.000
Black cumin toothpaste with detergent	6	15.1416	0.231120459
Black cumin toothpaste non detergent	6	11.283	0.262043253
SLS	6	8.2416	0.106848803

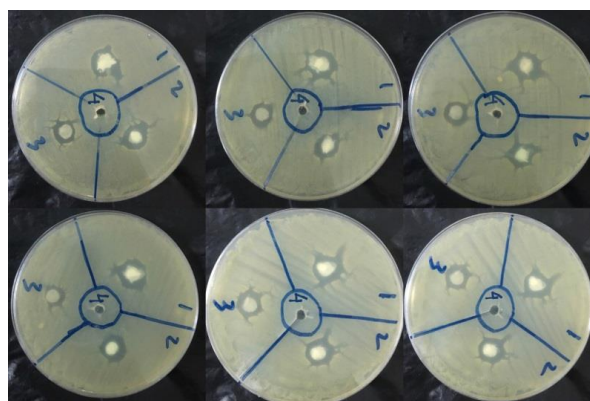


Figure 1. The results of inhibition zone.

Table 2. The result of one-way ANOVA test

Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
				Lower Bound
Black cumin + SLS	-15.14167*	.11685	.000	-15.4687
Black cumin non SLS	-11.35000*	.11685	.000	-11.6770
SLS	-8.24167*	.11685	.000	-8.5687
control	15.14167*	.11685	.000	14.8146

DISCUSSION

This research was conducted to compare the antibacterial power of black cumin detergent extract toothpaste with non-detergent black cumin extract toothpaste as an appropriate alternative to inhibit the growth of plaque bacteria *supragingival* to improve oral hygiene and prevent periodontal disease because herbal toothpaste can effectively help eliminate debris and plaque as pathogenic agents from periodontal tissue damage. To find out the antibacterial power of black cumin extract paste, laboratory experimental research was carried out by observing the growth of supragingival plaque bacteria obtained from the *Research Center* of the Airlangga Faculty of Dentistry, Surabaya. This study used 4 groups consisting of control, black cumin toothpaste with SLS, black cumin toothpaste non detergent, and SLS. To compare the inhibitory power of supragingival plaque bacteria in this study, it was carried out by the diffusion method. The diffusion method was chosen because of its superiority which is easy to do at a relatively low cost. This method is done by making a well by making a hole in the plate to use a ring with a diameter of approximately 0.5 cm. In the wells filled with black cumin toothpaste with SLS black cumin toothpaste non detergent, SLS, and untreated control. Bacterial inhibition is determined by measuring the clear zone around the well. This diffusion research was conducted 6 times by replication. In this study using black cumin herbal ingredients, because in previous studies known to be safe to use and contain ingredients that can inhibit the activity of bacteria in the oral cavity. This study shows black cumin extract toothpaste at a concentration of 3% with SLS can inhibit the growth of supragingival plaque bacteria because of the activity of the thymoquinone content with SLS. Black cumin has been used in Indonesia as a natural alternative health product from medicinal plants. This plant has potential as a treatment ingredient in dentistry, one of which is plaque control.

In the inhibitory zone calculation results, it was found that black cumin paste containing SLS has an average inhibition zone measurement results higher than non cumulative black cumin paste. This shows the content of black cumin with a concentration of 3% coupled with SLS can inhibit bacteria better, so that this research is in accordance with the hypothesis. Thymoquinone, thymol, and tannin in black cumin is known to be effective in inhibiting the growth of gram-negative and gram-positive bacteria. THQ is a derivative of quinon that contains the THY element in it. Quinon has strong bactericidal power. Thymoquinone is known to form *irreversible complexes* with nucleophilic amino acids in proteins that can inactivate proteins resulting in malfunctioning and targets in bacterial cells are the surface of adhesin, polypeptide cell walls, and membrane bound enzymes. The antibacterial activity of THQ from black cumin extract is effective in inhibiting the growth of supragingival and subgingival plaque bacteria that cause periodontitis.^{8,9} While the SLS content is a synthetic detergent with the ability to reduce surface tension and emulsion of plaque remnants. The results showed that

SLS was effective as an inhibitor of supragingival plaque bacteria, which is known to be a pathogenic agent of tooth surfaces also disrupting the integrity of microbiota cells and reducing plaque acidity. SLS can also prevent plaque adhesion to tooth enamel because the action is electrostatically adhered to the tooth surface.¹⁰

Plaque cleaning can be improved by abrasive and detergent content. The main purpose of toothpaste is to provide a thorough cleaning effect in the oral cavity. In general, the detergent content used in toothpaste is SLS, which can produce foam and increase the effect of a fresh taste sensation. SLS can have side effects such as thickening and proliferation of epithelial cells in the oral cavity if the concentration exceeds 0.15%, but in America and Europe many kinds of toothpaste contain SLS with a concentration of 0.5% to 2%.¹¹ According to previous research, SLS binds to bacterial proteins, causing inhibition of bacterial attachment to teeth, so that plaque formation decreases. SLS can reduce surface tension thus creating a clean impression. Also, it can dissolve active ingredients and have antimicrobial properties and inhibit plaque. SLS also helps in the dispersion of intra-oral toothpaste and inhibits the growth of several microorganisms. The antimicrobial activity of SLS is related to adsorption and penetration through porous cell walls followed by interactions with cell membrane components, lipids and proteins. Penetration of SLS into the membrane causes an increase in bacterial cell permeability, through denaturation of the oral mucosa which can lead to leakage of intracellular components and cell lysis.¹²

CONCLUSION

It can be concluded that 3% black cumin extract toothpaste containing 2% detergent has a better inhibitory effect against supragingival plaque bacteria than non-detergent 3% black cumin toothpaste.

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