The Effectiveness Of Black Cumin Nanoemulsion Gel Concentration 10% And 15% On Staphylococcus Aureus Biofilm Thickness (In Vitro)

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ABSTRACT

Background: *Periodontal disease is a disease that often occurs in the oral cavity with prevalence in Semarang City increasing in 2016 by 67.18% to 89.53%. One of the causes of periodontal disease is the buildup of Stapylococcus aureus bacterial biofilm during the initial colonization of the tooth pellicle formation. One herbal ingredient that can be used as an antibacterial is black cumin (Nigella sativa). Gel nanoemulsion technology has the advantage of increasing material stability. This research aims to determine the effectiveness of black cumin gel nanoemulsion on the growth of Stapylococcus aureus biofilm thickness.*

Methods:*The treatment group consisted of black cumin gel nanoemulsion with a concentration of 10% and a concentration of 15% and the control group, namely 0.2% chlorhexidine and distilled water. Stapylococcus aureus biofilm thickness was measured using an ELISA-reader. Statistical tests were carried out using the One Way Anova test.*

Results: *The average thickness of Stapylococcus aureus with the addition of black cumin gel nanoemulsion with a concentration of 15% was the lowest, namely 0.046, while the thickness of Stapylococcus aureus with the addition of distilled water was the highest, namely 0.158. The One Way Anova test obtained a significance figure of 0.000 (p<0.05) so it could be concluded that there were significant differences in the 4 Stapylococcus aureus thickness test groups.*

Conclusion: *Black cumin gel nanoemulsion concentrations of 10% and 15% have antibacterial properties which can reduce the thickness of Stapylococcus aureus biofilm.*

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INTRODUCTION

Periodontal disease has the main etiology, namely plaque on the teeth (1).Plaque on teeth is a collection of biofilms consisting of a population of bacteria that grow on the surface of the teeth trapped in a polysaccharide matrix. Dental plaque is an accumulation of biofilm communities that occurs through sequential and regular colonization of several normal flora in the gingival sulcus. Normal bacterial flora in the oral cavity is generally good if there is a balanced relationship between normal flora and the immune host. Collection of biofilm can cause dental and oral diseases such as tissue infections due to the accumulation of biofilm on dental biomaterials in the oral cavity, gingivitis, periodontitis, and tissue necrosis. The gingival sulcus area has many blood vessels, which makes it very easy for bacteria to enter the blood vessels and cause systemic disorders because bacteria can spread throughout the body. This condition is called bacteremia. Severe cases of bacteremia can even be life-threatening if the bacteria enter the blood vessels of the heart and cause infective endocarditis. These conditions will not appear in individuals with good dental biofilm management.

One of the bacteria found in dental biofilm is Staphylococcus aureus bacteria which is known as a primary colonizer on the tooth surface. This bacteria is an acidogenic bacteria which will produce fermentation and change the atmosphere of the oral cavity to acidic. The prevalence of Staphylococcus aureus in periodontal pockets is quite high, namely 13.4% and the overall prevalence in the oral cavity is 15.8%. Staphylococcus aureus is a Gram-positive bacterium which has several mechanisms for forming biofilms by producing polysaccharide matrices, adhesin proteins, enzymes, toxins which can make it easier for bacterial aggregation, avoid the host's immune response from antimicrobial agents, and can form biofilms in human blood vessels. According to(1)the invasion of gram-positive bacteria such as Staphylococcus aureus can be found at the second stage of plaque formation, namely at the primary colonization stage of the dental pellicle (1).

Mouthwash containing chlorhexidine functions to reduce and inhibit plaque growth and can prevent periodontal disease. This can be caused by chlorhexidine which has bacteriostatic and bactericidal properties against various bacteria, one of which is bacteria in dental plaque (2). Long-term use of chlorhexidine can cause various negative impacts such as desquamation of the oral mucosa, hypogeusia, glossodynia, xerostomia, oral paresthesia, discoloration of the teeth and tongue, as well as the potential for type I and type IV hypersensitivity reactions.

Previous research stated that seagrass extract using an incubation time of 48 hours with a concentration of 15% was proven to have the highest inhibitory power compared to using treatments with an incubation time of 24 hours and 72 hours(3). Black cumin in toothpaste preparations with a concentration of 15% is the concentration with the best activity capacity with a clear zone diameter of 8.22 mm, compared to concentrations of 6% and 12% which only have a zone diameter. clear 3.99 mm and 5.67 mm. Antibacterial effect of Nigella sativa seeds was studied using gram-positive bacteria, namely Staphylococcus aureus, using the paper disc method. Black seed extract using the agar well diffusion method showed extraordinary effects against gram-positive bacteria with the largest zone of inhibition for Staphylococcus aureus ATCC 29213. Many studies have reported the antibacterial activity of Nigella sativa which has antibacterial activity against most bacteria, especially Gram-positive bacteria, one of which is Staphylococcus aureus and Staphylococcus epidermidis (4).

Existing research finds evidence that black cumin extract can inhibit Gram-positive bacteria, Staphylococcus aureus, more effectively than Gram-negative bacteria. This is due to the components that make up the cell membrane of Gram-positive and Gram-negative bacteria. The cell membrane of Gram-positive

bacteria has 50% peptidoglycan and does not contain lipopolysaccharide, the cell membrane of Gram-negative bacteria consists of 2–10% lipopolysaccharide (5)

The development of science and increasingly advanced technological developments make it possible to stabilize dispersions and produce gel nanoemulsion dosage forms. Nanoemulsion gel preparations can be used effectively to increase the stability and bioavailability of the active ingredient itself (6). Nanoemulsion is a drug delivery system consisting of an oil phase and a water phase that can be stabilized by surfactants, and increases the solubility and pharmacological activity of herbal ingredients. Nanoemulsions have many advantages such as their very small size of around 50-1000 nm, facilitating the absorption of lipophilic drugs into the body, and the oil-in-water dosage form avoiding oxidation and hydrolysis. In addition, to reduce side effects from drug intake, nanoemulsions can combine lipophilic drugs and hydrophilic drugs. Previous research did not use black cumin extract preparations in the form of gel nanoemulsions (7).

METHODS

Type of in vitro laboratory experimental research with a posttest-only control group design. The research population was S. aureus ATCC 25923 bacteria and the research sample was S. aureus ATCC 25923 biofilm grown in microplates. The samples were divided into 4 groups, namely the group applying black cumin gel nanoemulsion (Nigella sativa) with concentrations of 10% and 15%, positive control (chlorhexidine gluconate 0.2%), negative control (aquades) on S. aureus bacteria located in sterile incubation well plates for 4 hours. Each group of samples had 3 repetitions in one microplate. This number of repetitions is adjusted to the acceptable and common number of repetitions in cell-based experiments including anti-biofilm experiments, namely a minimum of 3 repetitions per group, a higher number is permitted to see the consistency of treatment results and if resources are adequate. The research tools used are digital balances, Erlenmeyer tubes, test tubes and racks, petri dishes, plastic wrap, ose needles, handscoons, microplates, microtiter plate readers, incubators, filter paper, ovens, rotary vacuum evaporators, water baths, micropipettes, microtips, object glass, light microscope, and autoclave. The materials used in this research include the bacterial culture S. aureus ATCC 25923, shallot skin, 96% ethanol, 1% DMSO, 0.2% CHG, H2SO4, potassium dichromate, chloroform, magnesium metal, 1% FeCl3, cotton, activated charcoal, acetic acid anhydride, ammonia chloroform, Mayer's reagent, Congo red agar, trypticase soy broth, glucose, distilled water, PBS, isopropanol, 1% crystal violet, Lugol's solution, acetone alcohol, safranin, NaCl, and acid glacial acetate.

The research began with making extracts using the maceration method. Making black cumin extract can be done using the maceration method. A total of 250 grams of Nigella sativa had been washed thoroughly and then ground using a pollinator machine. Extract black cumin using the 96% ethanol solvent maceration method within 24 hours. Black cumin was mixed manually by stirring every day for 3 days at room temperature until the solution became clear. Filter the solution and evaporate using a rotary evaporator at a temperature of 40–50 °C until the extract becomes thick. After black cumin extract (Nigella sativa) is made, the active ingredients are black cumin extract, palm oil (oil), Tween 80 (surfactant), propylene glycol (co-surfactant), methylparaben, propylparaben, and ethanol. Black cumin extract was mixed with palm oil, 10 grams of propylene glycol, 0.1 grams of methylparaben, and 0.02 grams of propylparaben at a speed of 1000 rpm for 2 hours using a stirrer as the oil phase. Then, using a homogenizer, mix 3.6 grams of Tween 80 as a surfactant, distilled water as a solvent, and ethanol as the water phase at a temperature of 30 °C and a speed of 2000 rpm

for 2 hours. The temperature of the water phase was reduced to 30°C by adding the oil phase at a rate of 2000 rpm for 60 minutes using a titration method using a mechanical stirrer. Then stir in an ultrasonic device for 25 minutes until a homogeneous mass is formed. Next, making the base gel begins by mixing 0.1 g of methylparaben and 0.05 g of propylparaben in distilled water. Mix 2 g Carbopol 940 in a measuring cup and add TEA with a pipette. Add black cumin extract nanoemulsion to the gel base of the gel nanoemulsion preparation and stir for 15 minutes using a stirrer at 500 rpm. Research samples were prepared by growing S. aureus ATCC 25923 bacteria in BAP (Blood Agar Plate) and incubated for 24 hours in an incubator at 37ºC to see the formation of biofilm (8).

The results of the subculture were subjected to Gram staining to confirm the S. aureus bacteria through its morphological characteristics, namely the bacteria are in the form of cocci in clusters like grapes and are colored purple by crystal violet when observed in a microscope. The subculture results were made into a bacterial suspension according to the McFarland standard 0.5 (~1.5 x 108 CFU/mL) by dilution using a physiological solution of 0.9% NaCl. Well plate that has been coated with 200 µL of saliva. Then add 110 µl of the centrifuged Staphylococcus aureus bacterial suspension. then incubated at room temperature for 4 hours, then washed with Phosphate Buffer Saline (PBS) with pH 7.5 using an Eppendorf Multi Channel and Shaker for 15 minutes at a speed of 500 rpm (9). Insert it into the microplate well in a different well, according to the position

RESULTS

Table 1. The group mean reading result of the Staphylococcus aureus biofilm optical density value with the mean value of the biofilm thickness of the distilled water negative control group was the highest, namely 0.158, while the black cumin gel nanoemulsion (Nigella sativa) concentration of 15% had the lowest mean value, namely 0.046. Then proceed with the Shapiro-Wilk test (sample size n<50), to determine normality.

No.	Groups	Mean±SD
1.	Black cumin gel nanoemulsion 10% concentration	0,049 ± 0,007
2.	Black cumin gel nanoemulsion 15% concentration	0,046 ± 0,006
3.	Positive Control Chlorhexidine 0.2%	0,067 ± 0,002
4.	Aquades Negative Control	0,158 ± 0,002

Table 2. Shows that the results of the normality test all data are normally distributed (p>0.05). The data was then tested for homogeneity using the Levene Statistics test. The homogeneity test in table 4.2 shows a value of 0.131 (p<0.05), indicating that the data for all groups is homogeneous. Once it is known that the data is normally distributed and homogeneous, the next stage is the One Way Anova test to determine differences in the thickness of Staphylococcus aureus in each group.

Groups	Saphiro-Wilk	Levene
	Sig.	Sig.
Black cumin gel nanoemulsion 10% concentration	,537	,131
Black cumin gel nanoemulsion	,144	

15% concentration	
Positive Control Chlorhexidine 0.2%	,363
Aquades Negative Control	,363

Table 3. The significance figure shown in the table is 0.000 (p<0.05) so it can be concluded that there is a significant difference in the Staphylococcus aureus thickness test group. Then a post hoc LSD follow-up test was carried out to determine the differences in influence between each group.

Antar Kelompok	Sig
	0.000

Table 4. Based on the LSD post hoc follow-up test, there were significant differences in Staphylococcus aureus thickness values (p<0.05) in several groups. In the 10% concentration group there was a significant difference between 0.2% chlorhexidine and distilled water. In the 15% concentration group there was a significant difference between 0.2% chlorhexidine and distilled water.

Comparison Between Groups		**p value**
Concentration 10%	Concentration 15%	0,601
	*Chlorhexidin*e 0,2%	0,003*
	Aquades	0,000*
Concentration 15%	*Chlorhexidin*e 0,2%	0,001*
	Aquades	0,000*
***Chlorhexidin*e 0,2%**	Aquades	0,000*

Figure 1. The results of the Gram staining test on subcultured bacteria were observed using a light microscope with 100x magnification. Observation of the preparations showed the presence of coccus-shaped and clustered bacterial colonies that matched the morphology of S. aureus bacteria.

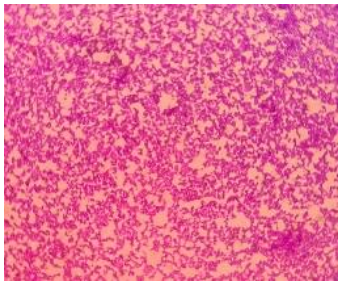

figure 1. Subculture of S. aureus on BAP Media

DISCUSSION

The results of subculture of S. aureus bacteria on Blood Agar Plate (BAP) media for 24 hours incubation showed the formation of colonies with a small, round, white pigmented consistency with a smooth surface. These colonies indicate that the visible S. aureus bacteria are related to the production of polysaccharide

intercellular adhesin (PIA) which functions as an adhesive for each bacterial coccus so that it becomes a colony (8).

The results of measuring the thickness of the Staphylococcus aureus biofilm were obtained using the Microtiter Plate Assay (MPA) method with 1% crystal violet staining using a wavelength of 630 nm. The MPA method was chosen because this method is the gold standard for testing bacterial biofilms. Microplate reading using the MPA method produces data in the form of OD values to measure the thickness of the Staphylococcus aureus bacterial biofilm. Optical density is a standard measurement for testing microbial concentrations in a medium, including biofilm testing. The OD value is obtained from the amount of absorption or absorbance of a mass (biofilm) in light of a certain wavelength.

The results of the average optical density (OD) value of research on the antibacterial effectiveness of black cumin (Nigella sativa) gel nanoemulsion with 0.2% chlorhexidine gluconate and distilled water on Staphylococcus aureus bacterial biofilms showed that the concentration value of 15% black cumin (Nigella sativa) nanoemulsion was the smallest, namely 0.046 compared to other concentration groups. It can be concluded that a 15% concentration of black cumin gel nanoemulsion (Nigella sativa) is able to inhibit Staphylococcus aureus bacterial biofilm. Meanwhile, the average optical density (OD) value of the positive control with 0.2% chlorhexidine, namely 0.067, shows a value greater than the black cumin (Nigella sativa) nanoemulsion concentration of 10% and 15%. The results of the analysis showed that there was a significant difference between the thickness of the Staphylococcus aureus biofilm and the administration of black cumin gel nanoemulsion ($p<0.05$). This indicates that there is an effect of administering black cumin gel nanoemulsion on the thickness of the Staphylococcus aureus biofilm. The results of the average optical density value of the research showed a reduction in the blue color of the well-plate media with each increase in concentration of the black cumin gel nanoemulsion formulation (Nigella sativa). Research on black cumin gel nanoemulsion (Nigella sativa) with a concentration of 15% had a lighter color intensity than the research group with a concentration of 10%. It was concluded that the 15% concentration of black cumin gel (Nigella sativa) nanoemulsion gel research group had the greatest antibacterial power compared to other concentration research groups. This is because the more secondary metabolites contained in the extract (high concentration at a certain limit), the higher the antimicrobial effect provided(10). Previous research showed that black cumin extract (Nigella sativa) at a concentration of 10% was effective in inhibiting the growth of Staphylococcus aureus bacteria. Previous research has proven that the greater the concentration given, the greater the inhibition zone formed against Staphylococcus aureus bacteria. Black cumin (Nigella sativa) extract with high concentrations has great efficiency in inhibiting the growth of bacteria forming biofilms (2). Previous research is known to have used an extract formulation, but this research used a nanoemulsion gel formulation. The nanoemulsion gel formulation is in the form of small particles measuring 1-100 nm which is effective in increasing the stability of the active ingredient and is easy to apply(6).

Significant differences occurred in all concentrations of black cumin gel nanoemulsion and distilled water ($p<0.05$). This ability is due to the phytochemical compounds contained in black cumin, including flavonoids, saponins, tannins, terpenoids, alkaloids and phenols, each of which has a role as both an antibiofilm and antibacterial against Staphylococcus aureus. This can be strengthened by several previous studies which stated that black cumin extract contains alkaloids, such as nigelidin, nigelisimin, nigelisin, essential oils and others. Black cumin essential oil is also known to contain saponins, nigellon, thymoquinone, dithymoquinone,

hymohydroquinone, and thymol (11). Apart from alkaloids, black cumin also contains flavonoids, tannins, saponins, phenols and terpenoids (12).

Variations in extract concentration play a role in the formation process of Staphylococcus aureus biofilm. The value of S. aureus biofilm thickness increased at a concentration of 10% to a concentration of 15%. The greater the concentration of the extract, the more secondary metabolite compounds it contains, so the role of the extract in inhibiting biofilm thickness will be greater. However, in the positive control, namely chlorhexidine 0.2% liquid preparation, the percentage obtained actually decreased. This is likely caused by the reaction of the active compound in chlorhexidine 0.2% liquid preparation in binding to the receptor, resulting in saturation. One of the processes of quorum sensing or signaling that occurs in bacterial gene expression in biofilm formation is played by receptors. Quorum-sensing is a communication process for bacteria to aggregate to form a biofilm. The quorum-sensing process of the Staphylococcus aureus bacteria is initiated by transcription of the accessory gene regulator (Agr) which is activated by glucose to become autoinducing peptide (AIP), and then AIP activates another Agr to carry out the quorum-sensing process. Secondary metabolite compounds contained in the extract can bind to the active site of Agr and can inhibit the performance of Agr to induce the quorum-sensing process. The concentration of the extract can affect the affinity of secondary metabolites to penetrate receptors on the cell walls of bacteria. The amount of secondary metabolites that can be dissolved in the extract must be balanced with the number of receptors contained therein, so that penetration of secondary metabolites into the active side of the receptor takes place optimally and can provide a good inhibitory effect on biofilm thickness. This is because when there is too much dissolved substance in the extract, it will be difficult for the substance to penetrate the limited number of receptors because there are crowded substances. Too few substances dissolved in the extract are not optimal for providing medicinal effects because the amount of substances that penetrate the receptors is less (13).

The results of this study showed that there was a decrease in biofilm thickness from a nanoemulsion gel concentration of 10% to a nanoemulsion gel concentration of 15%, but there was an increase in the thickness of the biofilm in the liquid preparation of 0.2% chlorhexidine. These results are in accordance with the nanoemulsion gel drug delivery system which is an appropriate dosage form to deliver and increase the bioavailability of hydrophobic drugs and drugs that have high first-pass metabolism(6). Nanoemulsion gel is a lipid-based formulation system that can increase the solubility and bioavailability of hydrophobic drugs and bioactive components. This nanoemulsion system has a high interfacial area and stability, protecting the compound from adverse environmental conditions and increasing its stability. Nanoemulsion systems can be used to deliver drugs via transmucosal and transdermal routes. Therefore, this system can effectively increase bioavailability (14).

Plaque formation has 4 stages, namely the initial attachment stage, initial colonization, secondary colonization, and plaque maturation. Initial colonization begins with the formation of a thin film, namely contamination of a biofilm layer of saliva (glycoprotein) on the surface of the tissue in the normal flora of the oral cavity, as well as debris from food remains attached to the surface of the teeth, called the pellicle (9). Secondary colonization can be characterized by a decrease in the number of aerobic gram-positive bacteria and an increase in the number of anaerobic gram-negative bacteria (5). The plaque maturation stage is a continuous metabolic process of bacteria which will increase in number with the number of bacterial colonies from sessile to motile. If this process occurs over a long period of time and occurs continuously with poor oral hygiene and

an inadequate immune response, it can cause an inflammatory process in the tissues supporting the teeth which can be damaged.

This study used a saliva incubation time of 4 hours, because biofilm formation from Staphylococcus aureus bacteria was still in the initial colonization stage which could be inhibited by antibacterial secondary metabolite compounds from black cumin (Nigella sativa) gel nanoemulsion formulations. This study was to compare the antibacterial effect of black cumin gel nanoemulsion (Nigella sativa) with 0.2% chlorhexidine as a positive control and distilled water as a negative control on Staphylococcus aureus bacterial biofilm which can be seen from the results of the average optical density (OD) value. Chlorhexidine is a broad-spectrum antimicrobial substance which is the gold standard which functions to prevent plaque formation in the field of dentistry (9).

In the black cumin (Nigella sativa) gel nanoemulsion group treatment with a concentration of 10% and a concentration of 15%, each 4 hour incubation had an average optical density (OD) value of 0.049 and 0.046 compared to chlorhexidine 0.2% as a group. positive control and distilled water as a negative control group with 4 hours of incubation respectively, namely 0.067 and 0.158. The results obtained can be concluded that chlorhexidine 0.2% and distilled water have a thicker biofilm thickness compared to the black cumin gel nanoemulsion group with concentrations of 10% and 15%.

The decrease in biofilm thickness from black cumin gel nanoemulsion comes from the secondary metabolite content in it. Specific research regarding the anti-biofilm effect of flavonoid compounds on Staphylococcus aureus biofilms has been investigated. Flavonoid derivative compounds in the form of quercetin, myricetin, and scutellarein with the same concentration of 10 µg/mL have been proven to strongly inhibit the formation of Staphylococcus aureus biofilm thickness. Quercetin compound has the best inhibitory effect on Staphylococcus aureus biofilm thickness than myricetin and scutellarein. This derivative compound can inhibit the process of thickness formation in Staphylococcus aureus biofilms through a physical interference process. These compounds can physically disrupt the anchoring process (Biofilm Associated Protein) of protein components in the polysaccharide matrix during biofilm formation and can interfere with BAP polymerization (5).

Specific research regarding the effect of tannin compounds on inhibiting the formation of S. aureus biofilm thickness has also been carried out. Tannin derivative compounds such as epigallocatechin gallate, tannic acid, rosmarinic acid, and hamamelitanin have been proven to inhibit the formation of Staphylococcus aureus biofilm thickness without affecting bacterial cell growth. Epigallocatechin gallate inhibits the formation of biofilm thickness by reducing the production of biofilm slime. Tannic acid has a role in inhibiting the formation of biofilm thickness by influencing the expression of the icaA gene and the expression of the icaD gene which play a role in biofilm production. Hamamelitanin functions to inhibit biofilm formation by inhibiting the quorum-sensing regulator RNAIII. Rosmarinic acid can inhibit the formation of biofilms by suppressing the activity of bacteria in the early stages of biofilm formation.

The inhibitory effect of Staphylococcus aureus biofilm formation by phenolic compounds contained in the ethanol extract of Hungarian propolis is said to be related to the ability of phenol to disrupt bacterial cell metabolism and is not related to phenol's disruption of the icaABCD gene. Metabolic disturbances by phenol include protein denaturation and destruction of bacterial cell membranes which result in the production of extracellular matrix polysaccharides being hampered or unable to take place (3).

Saponins consist of two different groups, namely steroidal saponins and triterpenoidal saponins. Previous research shows that black cumin contains steroid-type saponins. The ability of saponin to inhibit the formation of bacterial biofilm thickness is related to the detergent-like activity of saponin. Saponins can disrupt the stability of the extracellular polysaccharide matrix which is a component that forms biofilms. The anti-biofilm power of saponin is also related to its ability to increase the permeability of bacterial walls so that bacteria will lyse (11). Other research states that saponins can inhibit the formation of Staphylococcus aureus biofilms by binding to the active site of the mannitol dehydrogenase and eDNA enzymes which function during alginate synthesis, the main components of the polysaccharide extracellular matrix (13).

Chlorhexidine has a bactericidal effect and bacteriostatic effect against gram-positive and gram-negative bacteria. Chlorhexidine also has a high activity effect against gram-positive bacteria. Chlorhexidine has a large inhibition zone value against Staphylococcus aureus bacteria (3). Chlorhexidine has an effective working mechanism to inhibit and kill the growth of gram-positive bacteria and gram-negative bacteria because chlorhexidine consists of mostly negatively charged molecules (anions) and positively charged molecules (cations) (5). This can result in strong adhesion between chlorhexidine and the bacterial cell membrane, resulting in changes in the permeability of the cytoplasmic membrane environment in bacteria, which can cause the release of intracellular compounds. This can cause bacterial cells to be damaged, so that chlorhexidine easily penetrates the entire surface of the plaque and can result in the proliferation of new organisms, and can cause osteolysis. Chlorhexidine is a drug that has low toxicity and has a high therapeutic index.

Chlorhexidine used continuously over a long period of time can cause many side effects, such as discoloration of the teeth and tongue, changes in the taste of taste, dry mouth, mucosal irritation, disturbed balance of the normal flora of the oral cavity. This can be minimized using traditional plants such as black cumin (Nigella sativa) which has minimal side effectsn (7). Based on the ANOVA test, the results obtained were p = 0.000 so that the p value was <0.05 so there was a significant difference in the 4 test groups for Staphylococcus aureus biofilm thickness. Next, in order to find out significant differences between each group, the LSD post hoc test was carried out. Based on the LSD post hoc test, it showed that there were significant differences in Staphylococcus aureus biofilm thickness values with a p value <0.05 in several groups. The 10% concentration group showed a significant difference to 0.2% chlorhexidine and distilled water. There was a significant difference in the 15% concentration group compared to 0.2% chlorhexidine and distilled water. This is because it is influenced by the quality of the black cumin gel nanoemulsion. The quality of the nanoemulsion can be influenced by the ratio of the extract and gel emulsion which has an influence on the viscosity of the gel nanoemulsion, storage time and storage location. Test methods, culture media, and the speed of diffusion of bacterial substances are things that can influence the inhibition zone.

Previous research has proven that the secondary metabolite content in plants has the potential to be a natural anti-biofilm agent against Staphylococcus aureus bacteria. This research also shows the presence of the same secondary metabolites contained in black cumin gel nanoemulsion which can have potential as an anti-biofilm agent in the process of forming the thickness of Staphylococcus aureus biofilm. Limitations in this research are the use of chlorhexidine 0.2% liquid preparation, the absence of a secondary metabolite level test in black cumin gel nanoemulsion, a specific potency test to determine the secondary metabolites that play the most role in inhibiting biofilm formation, a molecular test to determine the mechanism of secondary metabolites in inhibiting biofilm formation. Staphylococcus aureus biofilms.

CONCLUSION

The formulation of black cumin gel nanoemulsion (Nigella sativa) concentrations of 10% and 15% was effective in reducing the thickness of the Staphylococcus aureus bacterial biofilm in vitro. There was a significant change in the thickness of the black cumin gel nanoemulsion biofilm with concentrations of 10% and 15% with 0.2% chlorhexidine and distilled water. The antibacterial effect of black cumin gel nanoemulsion (Nigella sativa) at concentrations of 10% and 15% is greater than the antibacterial effect of chlorhexidine 0.2% liquid preparation.

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