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EFFECTIVENESS INHIBITION OF BLACK CUMIN EXTRACT (*Nigella sativa*) AGAINST *Staphylococcus aureus* (IN VITRO)

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ABSTRACT

Staphylococcus aureus is one of the bacteria identified from a tooth root canal which is necrotic. A thorough knowledge of bacteria in the root canal associated with pulp disease such as necrotic teeth is the basis for achieving successful endodontic treatment, namely to eliminate bacteria so as not to re-infect the tissues around the root canals of teeth. Staphylococcus aureus are resistant to several antibiotics, one of which is tetracycline. High levels of resistance to tetracycline result in the ineffective use of antibiotics. Black cumin is often used as an herbal medicine because in the black cumin seed extract there is a content of essential oils that function as antibacterial. The purpose of this study is to determine the inhibition of black cumin extract (Nigella sativa) on the growth of Staphylococcus aureus in vitro. This research method used an experimental posttest only control group design laboratory in vitro with the Kirby Bauer antibacterial test. The research used black cumin extract with a concentration of 15%, 30%, 45% dissolved with sterile aquadest, blank disk dipped in each concentrationsolution black cumin extract, aquadest negative control and Cresotin positive control, put the blank disk that already contained the solution of each concentration and control group on the MHA media which was inoculated with Staphylococcus aureus, incubated for 24 hours at 37°C. Repetition in the study was conducted 5 times each. The results showed that a concentration of 15% had an average inhibition zone of 9.8 mm, at a concentration of 30% there was an average inhibition zone of 11.6 mm, whereas at a concentration of 45% there was an average inhibition zone of 15 mm. Cresotin has the greatest inhibition with an average inhibition zone of 56.8 mm. In conclusion, black cumin extract (Nigella sativa) can inhibit the growth of Staphylococcus aureus.

Keywords: Staphylococcus aureus, Black Cumin Extract, Antibacterial

Introduction

Based on Riskesdas data in year 2007 and 2013 the prevalence of dental and mouth problems in Indonesia rose from 23.4% to 25.9%.[1] Pulp disease basically begins because of bacterial invasion of pulp tissue due to caries. Bacterial invasion that has reached the pulp will cause the pulp tissue to become inflamed

but remain vital for some time or will quickly become necrotic. Dental caries will cause a more severe infection to the root canal. Almost 90% - 95% infections that occur in the orofacial region are odontogenic infections, and about 70% are periapical lesions, especially acute dental alveolar abscesses and periodontal abscesses. One of the bacteria that plays a role in this infection is Staphylococcus aureus.[2]

Knowledge about bacteria is very important in endodontic treatment. It shows that a thorough knowledge of bacteria in the root canal associated with pulp disease, is the basis for the achievement of endodontic treatment success. The dominant type of bacteria in pulp disease, especially tooth necrosis through bacterial identification from the root canal of the tooth identified is Staphylococccus aureus.[3]

Bacterial resistance to antibiotics causes infections are difficult to treat, therefore many studies have been conducted to find other antibacterial drugs, one of which is from herbal ingredients. The use of natural ingredients as traditional medicine in Indonesia has been carried out by our ancestors since centuries ago. Medicinal plants or herbal plants are considered safer because of relatively small side effects and relatively lower prices. Herbal medicine is widely accepted in almost all countries in the World. According to WHO, many countries in Africa, Asia and Latin America use herbal medicines as a complement to the primary treatment they receive.[4]

According to Randhawa (2005), medicinal plants have a variety of uses in the health field, one example is the black cumin plant or Nigella sativa. The black cumin plant is also known by the name Kalonji in the Southeast Asian region,

Habbah-Al-Sauda in Saudi Arabia, and Black Cumin in the United Kingdom.[5] Black cumin is a plant found in the Mediterranean region and desert regions such as the Middle East, Eastern Europe and Central Asia.[6] Part of the black cumin plant that is often used for treatment is the seeds. Nigella sativa seeds are essential oils, fatty oils, thymoquinone, dithymoquinone, thymohydroquinone, thymol, carvacrol, alkaloids, saponins, tannins, nigellicine, nigellimines, dithymoquinone, thymohydroquinone, thymol, carvacrol, alkaloids, saponins, tannins, nigellicine, nigellimines, nithellimine, nigellimine-n-oxide, nigellidine, nigellid, nigellid and alpha hedrin, while the main components in the essential oil Nigella sativa are p-cymene, thymol, carvacrol and thymoquinone.[5]

Various studies have shown that linolenic acid in black cumin has efficacy as an anti-inflammatory and as an immunomodulator.[7] Thymoquinone contained in black cumin functions as an anti-inflammatory by inhibiting the cyclo-oxygenase and lipooksigenase pathways that function as mediators of allergies and inflammation. In a scientific study, black cumin or Nigella sativa seed extract was proven to be able to improve polymorphonuclear (PMN) cell function. Other research also proves the effect of Nigella sativa in stimulating Macrophage Activating Factor (MAF) cytokines thus increasing the function of macrophages that play a role in the cellular immune system.[8] Black cumin oil can also be used as an antiseptic and local anesthetic.[9] This study aims to determine the inhibition of black cumin extract (Nigella sativa) on the growth of Staphylococcus aureus bacteria.

Samples and Research Methodology

The research was designed using Posttest Only Control Group Design research. The sample used in this study was the Staphylococcus aureus ATCC 25923 bacteria obtained from bacterial stock cultures stored at the Microbiology Laboratory of the Faculty of Medicine, Udayana University. The size of the samples in this study used general formula (Federer, 1997) that is (n-1) (t-1)> 15. In this study the concentration of sampleswas divided into 5 groups, such as:

(1) Group I : negative control solution with sterile aquadest
(2) Group II : black cumin extract solution with a concentration of 15%
(3) Group III : 30% black cumin extract solution
(4) Group IV : 45% black cumin extract solution
(5) Group V : positive control solution with Cresotin

Based on the calculation of the Federer formula, the result is 4.75. To make a real number it is rounded into 5 which meansthe number of repetition is done 5 times each.

Research Protocol

The inhibitory test of black cumin seed extract against Staphylococcus aureus was carried out by the diffusion method or Kirby Bauer. Test solutions were made with concentrations of 15%, 30%, and 45%. The 15% solution means that the solution consists of 0.15 ml of black cumin extract and 0.85 ml of aquadest. The 30% solution consisting of 0.30 ml of black cumin extract and 0.70 ml of aquadest, while the 45% solution means that the solution consists of 0.45 ml of black cumin extract solution and 0.55 ml of distilled water. Sterile disc paper (blank disk) is dipped into a test tube containing a solution of black cumin seed

extract with various concentrations, then dried. Suspension of Staphylococcus aureus bacteria that has been adjusted to turbidity level is bred to a petri dish containing Mueller Hinton Agar using a certain spreading technique. The colonies on the bacterial suspension which is taken with sterile cotton sticks dipped in suspension and squeezed in the tube wall. Sterile cotton sticks containing test bacteria are then inoculated on the entire surface of the MHA media. Paper disks (blank disks) are placed on the surface of the MHA that contains the bacterium Staphylococcus aureus, and are incubated for 24 hours at 37ºC. Measuring zone of inhibition is done to see the inhibition zone that is the clear area around the paper disc and then measured by the calipers.

Result and Discussion

In this study, some treatments in various concentration are held, such as in 15%, 30% and 45%, also controlled (control+) and uncontrolled (control-). The result of inhibition zone of bacteriaStaphylococcus aureus displayed in the following table.

Table 1

Result of the inhibition zone diameter of bacteria Staphylococcus aureus (mm)

Repetition	**Treatment in various concentration**				
	15%	**30%**	**45%**	**+Controlled**	**-Controlled**
1.	10 mm	11 mm	15 mm	53 mm	0
2.	13 mm	13 mm	15 mm	58 mm	0
3.	9 mm	10 mm	15 mm	61 mm	0
4.	9 mm	11 mm	14 mm	54 mm	0
5.	8 mm	13 mm	16 m	58 mm	0
Average	9,8 mm	11,6 mm	15 mm	56,8 mm	0

Normality test is done to identify the significant difference. This test is done using *Saphiro-Wilk* test since the sample for this research <30. The result of the test is shown in Table 2 below.

Table 2

The result of normality testof bacteria Staphylococcus aureusinhibition

Concentration	*Shapiro-Wilk*		
	Statistic	Df	Sig.
15%	0.859	5	0.223
30%	0.852	5	0.201
45%	0.883	5	0.325
Controlled +	0.992	5	0.544

Table 2 informed us that the significance for concentration value of black cumin extract (*Nigella sativa*) in concentration of 15% is 0.223, while in

concentration of 30% showed (*Nigellasativa*) 0.201. Theconcentration of *Nigellasativa* 45% showed value of 0.325. In the controlled condition it showed value of 0.544. The study showed none significant value in noncontrolled (-) because it has constant data from the beginning to the end of the repetition. It can be concluded that the data in table 2 is normally distributed because the significance value >0.05.The analysis test uses the *One Way Anova*

Table 3

Result of *One way Anova* of bacteria*Staphylococcus aureus*

Variables in Each Group	N	F	Sig.(P)
Concentration 15%	5	727,766	0,000
Concentration 30%	5		
Concentration 45%	5		
Control (+) *Cresotin*	5		
Control(-) *Aquadest*	5		

Based on Table 3,it showed us that result data of *One Way Anova* est is 0,000 where the value of significant $\rho < 0{,}05$. It means there is a significant difference among groups of black cumin concentration in inhibition the bacteria. Furthermore, to identify the difference among those groups the researcher used *Games-Howell Post HocTest* and get the result as shown in Table 4 below.

Table 4

The Result of Games-Howell Post Hoc Test

Groups	Beda rerata	Sig.
Consentration 15% and 30%	1,800	0.480
Consentration 15% and 45%	5,200	0,012
Consentration15% and Control (+)	47,000	0,000
Consentration 15% and Control (-)	9,800	0,002
Consentration30% and 45%	3,400	0,013
Consentration 30% and Control (+)	45,200	0,000
Consentration 30% and Control (-)	11,600	0,000
Consentration 45% and Control (+)	41,800	0,000
Consentration 45% and Control (-)	15,000	0,000
Controlled (+) andControl (-)	56,800	0,000

Based on the results obtained as shown in table 4, it can be seen the difference between one group with another group by identifying the value of sig. (ρ). Significant group differences obtained sig. (ρ) <0.05. The results showed that black cumin extract at a concentration of 15% with a concentration of 30% there was no significant difference because ρ> 0.05, while other groups had a significant difference because ρ <0.05.

black cumin extract concentration of 15%, 30%, 45% effectively inhibits the growth of Staphilococcus aureus bacteria when compared with negative controls. Between groups 15% and 30% there were no significant differences, while concentrations of 15% and 45%, and concentrations of 30% and 45% there were significant differences, this means that 45% concentration is more effective in inhibiting the growth of Staphylococcus aureus bacteria compared to concentrations of 15% and 30%.

These results also in accordance with research conducted by Morsi (2000), who reported that gram-positive bacteria such as Streptococcus and Staphylococcus can be inhibited by the use of black cumin extract, because gram-positive bacteria have a simpler cell wall structure that only consists of cell peptidoglycan and teikhoat acid, so that gram-positive bacteria are more easily inhibitedits growth by antimicrobials.[10] The results of test for antibacterial activity showed that the greater the concentration used, the greater the diameter of the inhibition produced. This indicates a dose-response relationship. Inhibitory zone diameters are categorized by their antibacterial power strength based on Davis and Stout classification, as follows.[11]

a. Clear zone diameter of 21 mm or more means that the drag is very strong.

b. Clear zone diameter of 11-20 mm means strong inhibition.

c. Clear zone diameter of 6-10 mm means medium inhibitory power.

d. Clear zone diameter of 2-5 mm means weak inhibition.

Staphylococcus aureus is a gram-positive bacterium that has a round shape with diameter of 0.7-1.2 μm, consist of jumbled or irregular form, form no spores, and did not move. Gram-positive bacteria have a low lipid content that is only equal to 1-4% when compared with gram-negative bacteria. Gram-positive bacteria have only one layer of a thick peptidoglycan membrane, which causes the growth of Staphylococcus aureus bacteria can be inhibited by black cumin extract containing antibacterial substances. According to Jawetz (2005) differences in the structure of cell walls determine the penetration, bonding and activity of antibacterial compounds. Staphylococcus aureus has a cell wall structure with more peptidoglycan, 2% lipids, and cell walls contain polysaccharides

(teikhoatacid). Teikhoat acid is a water-soluble polymer, which functions as a transport of positive ions to get in or out. It is this water soluble nature that shows that the cell wall of gram-positive bacteria is polar while the compound in black cumin extract is a polar part so that it is easier to penetrate polar peptidoglycan layers and cause bacterial inhibitory activity.[12]

The ability of black cumin extract to inhibit the growth of Staphylococcus aureus is influenced by the active content of essential oils in black cumin extract, namely thymoquinone, thymol, carvacrol and p-cymene which function as antibacterial. Bacteria treated with essential oils show an increase in the permeability of bacterial cell membranes to protons, so that it can interfere with intracellular pH balance which results in disruption of protein synthesis and ATP.[13]

Thymoquinone can form complexes that are irreversible with nucleophilic amino acids in bacterial proteins, causing protein inactivation. Carvacrol and thymol have effects to damage lipid bilayer. Carvacrol can increase cell membrane permeability. The phenolic hydroxyl group will bind to the hydrophilic part and the benzene ring will bind to the hydrophobic part of the lipid bilayer of the cytoplasmic membrane so that it will disrupt the colloidal system and result in clumping and deposition of proteins, in addition, carvacrol can also interfere with membrane depolarization by lowering the membrane potential, this will disturb the colloidal system and cause clumping and deposition of proteins. thought to be caused by the hydroxyl group that works as a protonophore which will insert into the cytoplasmic membrane then change the physical structure and chemical structure of the membrane and ultimately affect the composition and stability of

the bilayer layer on the membrane so that protons exit the membrane, whereas thymol substances can bind to membrane proteins hydrophobically by binding with hydrogen thus changing the permeability of the membrane, protein damage will decrease surface tension and trigger leakage of cell matter such as ions, ATP and nucleic acids.[13]

p-cymene contained in black cumin extract is a precursor of carvacrol which is hydrophobic in nature so that it can cause swelling of the cytoplasmic membrane, this substance is also effective when combined with carvacrol by facilitating the transport of carvacrol through the cytoplasmic membrane.[13]

The tannin content in black cumin extract works by interfering with the synthesis of peptidoglycan so that the formation of cell walls becomes imperfect. This situation will cause bacterial cells to become lysis due to osmotic pressure and physical pressure so that bacterial cells will die. According to Naim (2004), the mechanism of action of tannins as an antibacterial is related to the ability of tannins to inactivate adhesin bacterial cells (molecules that attach to host cells) found on the cell surface. Tannins that have targets on cell wall polypeptides will cause damage to cell walls because tannins are one of the phenol compounds. In cell membrane damage, H + ions from phenol compounds and their derivatives (flavonoids) will attack the polar group (phosphate group) so that the phospholipid molecule will break down into glycerol, carboxylic acid and phosphoric acid, this will cause phospholipids to be unable to maintain the shape of the cell membrane, consequently membranes will leak and bacteria will experience growth inhibition even bacteria will die, tannin compounds can also denaturate cell proteins, inactivate adhesin, enzymes, inhibit cell membrane

function (transport substances from cells to one cell to another) and inhibit the synthesis of nucleic acids thereby interfering with growth Staphylococcus aureus bacteria.[14]

Conclusion

Based on the results of research held, it can be concluded that black cumin extract (Nigella sativa) concentrations of 15%, 30% and 45% can inhibit the growth of Staphylococcus aureus bacteria in vitro. The greater the concentration used, the greater the diameter of the resulting obstacle. It indicates a dose-response relationship (dose-response relationship). The ability of black cumin extract to inhibit the growth of Staphylococcus aureus is influenced by the active content of essential oils in black cumin extract, namely thymoquinone, p-cymene, carvacrol and thymol which function as antibacterial.

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