Effect of essential oils of clove and cumin against the growth of staphylococcus aureus from denture stomatitis

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ABSTRACT

Background: Essential oils of clove and cumin had an inhibition effect against *Staphylococcus aureus*. Clove's essential oils has a compound named eugenol, which can directly damage the membrane cell of bacteria. Thymoquinone, the active ingredient in the black cumin's essential oils inhibits the protein synthesis and cause malfunction of the bacterial cell. The purpose of this research was to determine the differences of inhibitory effect from essential oils of cloves and cumin to the growth of *Staphylococcus aureus*. **Method:** This research was an experimental laboratory with Post-test Only Control Group Design. Sample that being used for this experiment was *Staphylococcus aureus* that had been isolated from a denture stomatitis patient. This inhibition test was determined using a Disc Diffusion Test's method with the essential oils of clove and cumin, while distilled water and 96% ethanol as a negative and positive control, respectively. Essential oils were obtained from the distillation method with water and steam and the test was done 7 times repetition with every ingredients. Inhibition zone was measured with a vernier calipers. The data were analyzed by ANOVA One-way test followed by a multiple comparison test. **Result:** The average zone of inhibition against *Staphylococcus aureus* from aquades 0 mm, 96% ethanol 13.894 mm, the essential oils of clove 14.784 mm and black cumin 11.944 mm. The multiple comparison test analysis showed a significant differences ($p < 0.05$) between the average zone of inhibition of the materials tested. **Conclusion:** Clove essential oil has a greater inhibition against *Staphylococcus aureus* than the essential oils of cumin.

Keywords : essential oils, clove, cumin, Staphylococcus aureus, denture stomatitis

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ABSTRAK

Latar Belakang: Minyak atsiri cengkeh dan jintan hitam mempunyai daya hambat terhadap Staphylococcus aureus. Minyak atsiri cengkeh memiliki senyawa eugenol yang dapat merusak langsung membran sel bakteri. Thymoquinone merupakan bahan aktif dalam minyak atsiri jintan hitam dapat menghambat sintesa protein dan menyebabkan gangguan fungsi sel bakteri. Tujuan penelitian untuk melihat perbedaan daya hambat minyak atsiri cengkeh dan jintan hitam terhadap pertumbuhan Staphylococcus aureus. Metode: Penelitian ini bersifat eksperimental laboratorium dengan desain Posttest Only Control Group Design. Sampel yang digunakan adalah bakteri Staphylococcus aureus diisolasi dari denture stomatitis. Uji daya hambat minyak atsiri cengkeh dan jintan hitam menggunakan teknik Disk Diffusion Test. Sedangkan aquades sebagai kontrol negatif dan etanol 96% sebagai kontrol positif. Minyak atsiri didapatkan dari metode punyulingan dengan air dan uap dan setiap minyak esensial diuji dengan 7 kali pengulangan. Zona hambat yang terbentuk diukur dengan kaliper sorong. Data dianalisa dengan uji Oneway ANOVA dan dilanjutkan dengan uji Komparasi Ganda. Hasil: Hasil pengukuran menunjukkan rata-rata zona hambat bakteri Staphylococcus aureus terhadap aquades 0 mm, etanol 96% 13,894 mm, minyak atsiri cengkeh 14,784 mm serta jintan hitam 11,944 mm. Hasil uji analisis Oneway ANOVA dan komparasi ganda menunjukkan terdapat perbedaan bermakna (p<0,05) antara rata-rata zona hambat kelompok perlakuan. Simpulan: Minyak atsiri cengkeh memiliki daya hambat lebih besar dibandingkan dengan jintan hitam terhadap Staphylococcus aureus.

***Kata kunci** : minyak atsiri, cengkeh, jintan hitam, Staphylococcus aureus, denture stomatitis*

INTRODUCTION

Indonesia is one of the country that populer with the highest biological mega-biodiversity in the world and have a potential to produce essential oils. The use of herbal's essential oils as a medicine are populer among people is concomitant with back to nature motion from people. WHO's data showed that herbal products demand of several countries in Europe from 1999-2004 have reached 66% from the world demand.[1]

Research's result from Rahman, et all (2010) showed that there is an antibacterial effect of clove againts *Stapylococcus aureus, Klebsiella pneumonia, Enterococcus faecalis, Mycobacterium smegmatis, Micrococcus luteus,* and *Candida albicans.* Babu et all (2011) showed that an active compound from clove named eugenol, had an ability to inhibit the growth of *Stapylococcus aureus* by directly damage the membrane cell of bacteria so it can inhibit the protein synthesis and lead to disruption of the cell function and cause the lysis of the cell.[2,3]

Salman, et all showed that there is zone of inhibition from essential oils of cumin againts the growth of *Stapylococcus aureus* bacteria.[3] Bhatia (2007) showed that cumin gave an influence to liver and blood plasma lipids' metabolism and blood plasma glucose's metabolism. Moreover, cumin had an antioxidant capacity which is 60% antioxidant capacity of vitamin C and had an antibacterial compound.[4]

Stapylococcus aureus is one of the pathogen bacteria that is a common cause to human infection. This organism can cause disease by invasion to the tissue and produce toxins (leucosidin, enterotoxin). *Stapylococcus aureus* has an important role for causing or aggravating oral disease. *Stapylococcus aureus* was found to had the most prevalence from denture stomatitis's patients aside from *Candida albicans, Streptococcus mutans,* and etc. Monroy et al (2005) stated that from denture stomatitis's patients with average pH 5,2, *Candida albicans, Stapylococcus aureus,* and *Streptococcus mutans* was found from the mucose membrane 51,4%, 52,4%, and 67,6% respectively.[5]

Essential oils of clove had farmacological effect as a stimultant, local anasthesia, carminative, antiemetic, and antispasmodic. In China, Dinasty Han periode, clove was use as mouth's deodorizer. Ayurvedic treatment in India chewing clove and cardamom wrapped in betel leaves was used to improve digestion. In Europe, leaves clove's extract was used as antiplaque

medicine. In Indonesia, clove's water was used as antiplaque medicine. In Indonesia, clove's water was used to cure sore eyes and used in balsam because of its analgesic property and can reduce the pain of rheumatism.[6]

In dentistry, eugenol was used as a temporary dental material fillings and as acute pulpitis's pain relief. As an antibiotic, eugenol is effective in vitro against several bacteria such as *Stapylococcus aureus*, *Streptococcus mutans*, *Candida albicans*, and *Escherisia coli*.[7,8]

Clove's oil in Indonesia traditionally produced from clove's flower, stalk, and leave distillation with water and steam distillation method. Water and steam distillation method can produce clove's oil with 80-85% of eugenol. The distillation's time for 500 gram of clove was among 3-4 hours.[1]

Composition of cumin seeds (*Nigella sativa*) that had been found including fixed oils, saponin, carbohydrate, water, vegetable oil, saturated fatty acids such as palmitat acid, stearat acid, and miristat acid; unsaturated fatty acids such as arachidonat acid, linoleat acid, oleat acid, and almioleat acid; essential oils that contains nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, d-limonene, d-citronellol, pcymene dan 2-(2-methoxypropyl)-5-methyl-1,4-benzenediol; amino acids such as arginin, lisin, leusin, metionin, tirosin, prolin, and treonin; alkaloid such as coumarin; nigellicine, nigellidine, and nigellimine-N-oxide; coumarin; mineral such as calcium, phosphate, natrium and iron; fiber; and water. From those severals composition, it has been reported that the main components of *Nigella sativa* seeds's extract are p-cymene (7.1%-15.5%), carvacrol (5.8%-11.6%), and the major component is thymoquinone. Analysis and publication study in several countries showed that *Nigella sativa* can be used as an antioxidant, antidiabetic, anticholesterol, anticancer, antiinflamation, antihistamine, antibronchial asthma, antibacterial infection, viral, and parasite and can be used also as an immunomodulator.[9]

Staphylococcus aureus is a gram-positive bacteria, aerobic or anaerob facultative, appeared as clusters like grapes. This bacteria didn't move, didn't have spores, had an ability to form capsules and cocci as shape. The size of this bacteria approximately 1µm. The bacteria colony are golden yellow in color, usually with haemolysis, when it is culture on Blood Agar medium. The name gold is form the bacteria name "*Staphylococcus*" which means gold in Latin. In Mannitol Salt Agar medium, characteristics of *Stapylococcus aureus* are the opaque colony, soft with yellow pigmentation.[1]

Stapylococcus aureus contains lisostphine that can cause the lysis of the red blood cells. Toxins that being produced are leucosidin, enterotocin that are found in the food usually the one that had an effect on gastrointestinal tract. Leucosidin attacks white blood cell and cause the decrease of the immune system. Exofoliatin is a toxin that attacks the skin with the signs is burn ulcer on the skin.[1] *Stapylococcus aureus* are found had a greater prevalence on denture stomatitis patient. Monroy et al (2005) reported that from 105 people that wear denture, 50 people of them were denture stomatitis's patient with average pH 5,2 and on their mucose membrane *Candida albicans*, *Stapylococcus aureus*, and *Streptococcus mutans* were found 51,4%, 52,4%, and 67,6% respectively. Meanwhile, from the denture, *Candida albicans* was found 66,7%, and *Stapylococcus aureus* and *Streptococcus mutans* were found 49,5%.[5]

The aim of this research was to found the effect of clove and cumin's essential oils againts the growth of *Stapylococcus aureus* with water and steam distillation's method.

MATERIAL AND METHODS

Type of this research is laboratorium experiment with the pretest-postest control group design, population *Stapylococcus aureus*, and sample *Stapylococcus aureus* cultured from denture stomatitis's patients. This research used four group, each of them consists of : 1.Group I : aquadest as negative control; 2. Group II : ethanol as positive control; 3. Group III : essential oils of clove; 4. Group IV : essential oils of cumin.

Number of sample (n) that had been found is five, but can be added 2-3 more sample, so sample that being used in this research is 7 times repetition respectively from group I until IV.

Antibacterial Effectivity Test With Agar Diffusion

Culture of *Stapylococcus aureus* was being done twice with round ose and placed at MHA and then the streak method with hermetically technique was done in the surface of MHA and be diffused with scatterplot tool. After soaking for 60 minutes, the discs were placed and pressed in MHA medium.

Medium in the discs then be numbered with label number 1 until 4 with treatment group and placed the medium inside the incubator with 37°C for 24 hours. After 24 hours, take out the petri dishes and see the inhibition zone on every discs and measured each of them with digital caliper with the formula (Φ horizontal + Φ vertical) and then divided by two and the calculations were placed inside the table.

RESULTS

After the placement of materials which were aquadest, ethanol 96%, essential oils of clove and cumin, observations were made after 24 hours to see the inhibition zone around the discs. The clear zone is the area that the growth *Stapylococcus aureus* was inhibited by the materials. Each of the materials were tested with seven times repetition. Observations were made to all the repetition of the materials in the same time. Results of this research showed that there were an inhibition zone of essential oils of clove and cumin, and ethanol 96%, meanwhile aquadest didn't showed any inhibition zone againts *Stapylococcus aureus*. Table 1 showed the average of inhibition zone and standard deviation of aquadest, ethanol 96%, essential oils of clove and cumin.

Table 1. Difference of the average inhibition zone showed by aquadest, ethanol 96%, essential oils of clove and cumin.

Group	Material	N	¤ (mm)	¤ (mm) ± SD	P
I	Aquadest (control)	7	0.0000	0.0000 ± 0.0000	0,00001*
II	Ethanol 96% (control)	7	13.8943	13.8943 ± 0.43408	
III	Clove's essential oil	7	14.7843	14.7843 ± 0.17587	
IV	Cumin's essentail oil	7	11.9443	11.9443 ± 0.39522	

*There is a significant difference at P < 0,05

Table 2. Results of multiple comparation test

Group	Comparation	P
I (Aquadest)	II (Ethanol 96%)	0,00001*
	III (Clove's essential oil)	
	IV (Cumin's essential oil)	
II (Ethanol 96%)	I (Aquadest)	
	III (Clove's essential oil)	
	IV (Cumin's essential oil)	
III (Clove's essential oil)	I (Aquadest)	
	II (Ethanol 96%)	
	IV (Cumin's essential oil)	
IV (Cumin's essential oil)	I (Aquadest)	
	II (Ethanol 96%)	
	III (Clove's essential oil)	

*There is a significant difference at P < 0,05 (H_0 rejected)

ANOVA one way test (table 1) showed that the value of P was 0,00001, which means that there is a significant difference (P < 0,05) within clove's essential oil, cumin's essential oil, ethanol 96%, and aquadest. To see the difference average inhibition zone within each of the materials, it can be seen from multiple comparation test. Multiple comparation test showed that each group materials had a significant difference because the value of P was 0,00001 (Table 2). This results showed that there is a significant difference (P < 0,05) between the average inhibition zone of each group materials, which are aquadest, ethanol 96%, clove's essential oil and cumin's essential oil. This results showed that hypothesis accepted which is there are a difference inhibition zone between clove and cumin's essential oils againts *Stapylococcus aureus*.

DISCUSSION

This research that being done using essential oils of clove and cumin was to proved that there are an inhibition zone againts *Stapylococcus aureus* and to showed that there are a difference in inhibition zone between essential oils of clove and cumin, and for the control groups are aquadest

and ethanol 96%. In this research, sensitivity test was done using disc diffusion test. The difference in inhibition zone were observed by the diameter of inhibition zone that formed around the discs that had been filled by group materials in Mueller Hilton Agar (MHA) medium that had been inoculation with *Stapylococcus aureus* that had been isolated from denture stomatitis's patient.

Measurement of the inhibition zone was done after 24 hours, medium that had been filled with essential oils then be taken out from incubator and the measurement was done using the digital caliper with 0,01mm precision. Inhibition zone was the area in which there was a clear zone around the disc that showed there was an inhibition zone between each group materials. In this research, essential oils of clove and cumin were made by water and steam distillation method. This method was being used because this was an easy and cheaper way to get the essential oils, and the tool's construction was simple.

Based on the results, the average inhibition zone of aquadest were 0 mm, ethanol 96% 13,894 mm, clove's essential oil 14,784 mm, and cumin's essential oil 11,994 mm (Table 1). The greatest average inhibition zone's value showed by group materials that contains essential oil of clove which was 14,784 mm. Ethanol 96%'s average inhibition zone (positive control) was 13,894 mm and aquadest as negative control didn't showed any inhibition zone. From this results, we can concluded that H_0 rejected because $P < 0,05$.

From this results, ethanol 96%, essential oils of clove and cumin had an inhibition zone againts *Stapylococcus aureus* with different ability. An active compound from clove and cumin were eugenol and thymoquinone respectively. These active compounds were showed to had inhibition effect in the growth of *Stapylococcus aureus*.

Research of Rahman (2010) showed that essential oil of clove effective againts *Stapylococcus aureus* because of antimicroba effect from eugenol and eugenol acetate that contained inside the essential oil.[2] Babu (2011) showed that essential oil of clove had an inhibition zone againts the growth of *Stapylococcus aureus* by directly damage the membrane cell of bacteria so it can inhibit the protein synthesis and lead to disruption of the cell function and cause the lysis of the cell and the results showed that the diameter of inhibition zone of clove's essential oil was 25,00 mm.[7] From this research showed that the average inhibition zone of clove's essential oil was 14,78 mm. This difference maybe because of the different method that was used to produce essential oils. The different of method may caused the different contains of active compound in essential oils. Babu's research showed that the inhibition zone was greater because the essential oil that Babu's used were made by factory, meanwhile from this research, essential oil were made by a simple tool.

Salman (2008) showed that clove's essential oil were found active againts the foodborne gram-positive bacteria, like *Stapylococcus aureus* and gram-negative bacteria like *Escherichia coli*. After that, active compound of clove, eugenol had an antibacteria activities againts bacteria *Streptococcus mutans*, *Candida albicans*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*. Besides eugenol, there was another active compound inside clove's essential oil that can inhibited the growth of bacteria like biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid, and oleanoic acid.

Marhadian's research (2005) showed that cumin's extract had an antimicroba effect againts the growth of *Stapylococcus aureus*, and also showed anticestodal and antiinflammatory effect.[8] Zuridah's research (2008) showed that cumin's essential oil had an inhibition effect againts several bacteria such as *Stapylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The average inhibition zone againts *Stapylococcus aureus* had the highest value if compared with the other gram-negative bacteria which was 25,00 mm and the lowest value was *Escherichia coli* which was 10,00 mm.[9]

According to Salman (2008), thymoquinone was an active compound inside cumin's essential oil that showed an inhibition effect againts the growth of several type of bacteria. Thymoquinone inhibited free radical sources, and known to had nucleofilic amino acid that was found inside the protein that can cause protein to inactivated and the loss of cell's function. Disruptions to bacteria's metabolism caused the inbalance of energy needs that leads to permanent's destruction of bacterial cells and the lysis of the cells.[3]

Based on ANOVA test (Table 1), showed that there is a significant difference ($P < 0,05$) between the average inhibition zone of ethanol 96%, clove's essential oil, and cumin's essential oil. From multiple comparison test (Table 2) showed that there is a significant difference ($P < 0,05$) in the average inhibition zone of each materials.

This in vivo research showed that essential oils of clove and cumin had an inhibition effect againts the growth of *Stapylococcus aureus*. Essential oils can be used as an alternative antibacterial because of the inhibition effect againts several types of bacteria, but further research was needed in order to used this materials as an antibacteria material for worldwide.

CONCLUSION

Based on this research, it can be concluded that the difference of inhibition zone by clove and cumin's essential oils were : 1. There was an inhibition effect of clove and cumin's essential oils againts *Stapylococcus aureus*. 2. Clove and cumin's essential oils and control had an inhibition effect againts *Stapylococcus aureus* with different abilities which was clove's essential oil contains eugenol that had the greatest inhibition effect if compared to the others.

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