Effectivity Test of Black Cumin Extract (*Nigella Sativa)* on Growth of *Staphylococcus Aureus* Isolates Odontogenic Infection Pus

Rahmi Syaflida[1], Ahyar Riza[1], Abdullah Oes[1], Ricky Rianto[1]
[1]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry Universitas Sumatera Utara, Universitas 9 street Padang Bulan, Medan, Indonesia*

Keywords: Odontogenic infection, *Staphylococcus aureus*, Black cumin, Inhibition zone

Abstract : The majority of infection that manifest in the oral cavity are odontogenic. Based on research, *Staphylococcus aureus* is the most dominant bacteria. Antibiotic administration is one of the treatment planning, but inappropriate antibiotics administration results in resistance. In recent years, many research showed resistance of antibiotics. Based on this, a new alternative from another material is needed to overcome this problem like black cumin. This study aims to determine the effect of black cumin extract (*Nigella sativa)* concentration on the growth of *Staphylococcus aureus.*This is a laboratory experimental study with a "post-test with control group" design. Black cumin (*Nigella sativa*) extract was prepared at concentrations of 50%,75%,and 100% and controlled with aquadest. The first step of the trial was bacteria rejuvenation. Furthermore the antimicrobial test were tested with disc diffusion technique, and the data were analyzed with Kruskkal Wallis.The result of inhibition zone measurement at 50% concentration (6.08 ± 0.48 cm), 75% concentration (6.65±1.07 cm), at 100% concentration (9.47 ± 0.88 cm), and there was no inhibition zone of control group. The average percentage at 50% concentration (56.95 ± 4.24%), 75% concentration (65.55 ± 9,11%), and 100% (92.09 ± 5,87%).

1 INTRODUCTION

The majority of infection that manifest in the oral cavity are odontogenic. The infections caused by both aerobic and anaerobic bacteria comprised about 60% of all odontogenic infections. (Fragiskos, 2007) Based on research, *Staphylococcus aureus* is the most dominant bacteria. (Kohli, 2009) Bacterial virulence can move bacteria freely in all directions. Antibiotic administration can be used to overcome this, but inappropriate antibiotic administration results in resistance. (Syed, 2011) A research of Benardo and Ueno showed almost all *Staphylococcus aureus* strains were resistant to amoxicilin and amphicilin in Brazil. (Foday, 2010) Based on this, a new alternative from another material is needed to overcome this problem. Black cumin (*Nigella sativa)* is cultivated in many countries in the world like Middle Eastern, Mediteranian region, India, and Saudi Arabia. Black cumin grows at 20-90 cm tall. The flowers are white, yellow, pink, with 5-10 petals. Black cumin has 4 important active ingredients, they are thymoquinone, tanin, thymol, and dithymoquinone. This ingredients have antibacterial effect. An experiment of Wasim Raja showed about antibacterial effect of black cumin extract to gram positive bacteria like *Enterobacter* showed the average measurement of inhibition zone at 100% concentration is 11.15 cm. (Raja, 2016)

2 MATERIAL AND METHOD

This study was an experimental study with post-test with control group design. The research was conduct in the Laboratory of Microbiology Faculty of Mathematics and Natural Science Sumatera Utara University, Medan, Indonesia. The sampling calculation in this study use Federer formula, in which sample in this study was 28 petri dishes. Sample was take from Laboratory of Microbiology Faculty of Health Sumatera Utara University. Maceration technique is used to produce black cumin (*Nigella sativa*) extract. Production black cumin extract is carried out using 96% ethanol solution. Black cumin extract was prepared at concentrations of 50%,75%,and 100%. Odontogenic infection pus

Syaflida, R., Riza, A., Oes, A. and Rianto, R.
Effectivity Test of Black Cumin Extract (Nigella Sativa) on Growth of Staphylococcus Aureus Isolates Odontogenic Infection Pus.
DOI: 10.5220/0010099909040907
In *Proceedings of the International Conference of Science, Technology, Engineering, Environmental and Ramification Researches (ICOSTEERR 2018) - Research in Industry 4.0*, pages 904-907
ISBN: 978-989-758-449-7

has been previously isolated from Laboratory of Microbiology Faculty of Health Sumatera Utara University and from the result of isolation *Staphylococcus aureus* is dominant bacteria. Antimicrobial test started from bacteria rejuvenation. The bacteria rejuvenation tarted by streaking 1-3 ose bacteria in Nutrient Agar then incubate for 24 hours in incubator.

Antimicrobial test was performed on the media under the following procedures. First step is taken 3 ose from rejuvenated bacteria then put the bacteria in test tube that contain 5-10 milimeter aquadest, and vortex. After the vortex process, the absorbance of suspension was measured with spectrophotometry at wavelenght of 600 nm until the absorbance value of 0,5 is obtained. Dip the sterile cotton bud into bacterial suspension, then streak on the surface of Mueller Hinton Agar. Take the disc paper that has been soaked in each concetration for 1 hour then placed on the part that has been marked, then incubate the petri dish in incubator for 24 hours on 37^0C.

Table 1: The result of inhibition zone percentage

Group	Concentration			Contr ol
	50%	75%	100%	
I	57.42 %	46.21%	98.03%	0%
II	57.42%	72.82%	98.97%	0%
III	56.02%	66.29%	88.23%	0%
IV	63.02%	66.76%	94.30%	0%
V	60.22%	66.76%	94.77%	0%
VI	55.08%	66.29%	86.36%	0%
VII	49.48%	73.76%	84.03%	0%

The inhibition zone known based on the measurement of the clear zone diameter around disc paper. Measurement performed with caliper. Measurements were carried out 3 times by measuring the largest diameter of the inhibitory zone at each concentration. Data obtained from this experiment was put into a table. Data processing was done with computer and analyzed with SPSS using Kruskall-Wallis test.

3 RESULT

The result of inhibiton zone measurement at 50% concentration are 6.15 cm, 6.15 cm, 6 cm, 6.75 cm, 6.45 cm, 5.90 cm, and 5.30 cm. Then at 75% concentration are 4.95 cm, 7.80 cm, 7.10 cm, 7.15 cm, 7.15 cm, 7.10 cm, and 7.90 cm. The biggest concentration in this study is 100%. The result of the inhibition zone measurement are 10.50 cm, 10.60 cm, 9.45 cm, 10.1 cm, 10.15 cm, 9.25 cm, and 9 cm, and there was no inhibition zone of control group. (Table 1)

The result of inhibition zone percentage at 50% concentration are 57.42%, 57.42%, 56.02%, 63.02%, 60.22%, 55.08%, and 49.48%. Then at 75% concentration are 46.21%, 72.82%, 66.29%, 66.76%, 66.76%, 66.29%, and 73.76%. The inhibition zone percentage at 100% concentration are 98.03%, 98.97%, 88.23%, 94.30%, 94.77%, 86.36%, and 84.03%. (Table 2)

The result of average measurement showed at table 3, the smallest inhibition zone at 50% concentration (6.08 ± 0.48 cm) and the largest inhibition zone was found at 100% concentration (9.47 ± 0.88 cm) and there was no inhibition zone of control group. (Figure 1,2,3,4) The average percentage at 50% concentration (56.95 ± 4.24%), 75% concentration (65.55 ± 9,11%), and 100% (92.09 ± 5,87%). (Table 3)

Table 2: The result of the inhibition zone measurement

Group	Concentration			Contr ol
	50%	75%	100%	
I	6.15 cm	4.95 cm	10.05 cm	0 cm
II	6.15 cm	7.80 cm	10.60 cm	0 cm
III	6.00 cm	7.10 cm	9.45 cm	0 cm
IV	6.75 cm	7.15 cm	10.10 cm	0 cm
V	6.45 cm	7.15 cm	10.15 cm	0 cm
VI	5.90 cm	7.10 cm	9.25 cm	0 cm
VII	5.30 cm	7.90 cm	9.00 cm	0 cm

Table 3: The average measurement and percentage of inhibition zone.

Concentration	Inhibition Zone	Percentage of Inhibition Zone	p-value
	Mean±SD (cm)	Mean±SD (%)	
50%	6.08±0.48	56.95±4.24	0.000
75%	6.65±1.07	65.55±9.11	
100%	9.47±0.88	92.09±5.87	
Control	0	0	

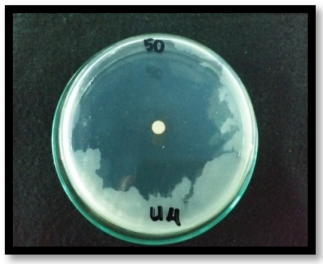


Figure 1: Petri dish at 50% concentration.

Figure 2: Petri dish at 75% concentration.

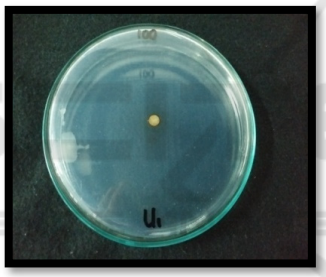


Figure 3: Petri dish at 100% concentration.

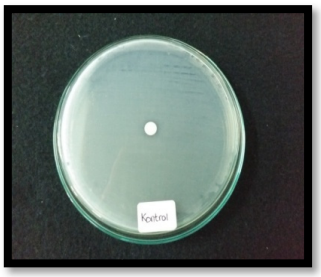
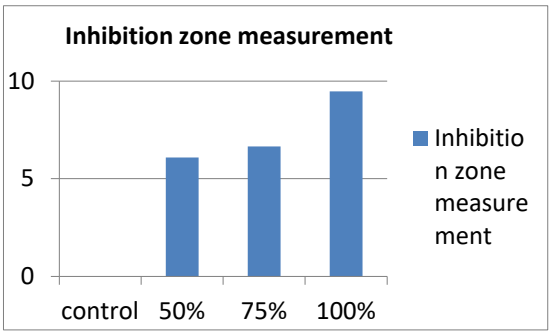


Figure 4: Petri dish for control.

The result of kruskall wallis statistical test was significant (p=0.000; p< 0.05). This means black cumin extract can inhibit *Staphylococcoccus aureus* growth. Beside that, there is an increase inhibition zone value as the concentration value increase.

Graphic 1: The average measurement and percentage of inhibition zone.



4 DISCUSSION

The inhibition zone is a clear area that appears on media in petri dish after the disc has placed. This clear area indicates an inhibition of the growth of microorganism by antimicrobial agents on media. (Pratiwi, 2008) The result of this study is accordance with the Morsi's research who reported Black Cumin (*Nigella satia)* extract can inhibit gram positive bacteria growth such as streptococcus and staphylococcus. (Asniyah, 2009) Beside that, Wasim Raja who reported black cumin extract can inhibit gram positive bacteria like *Bacilus subtilis* with the result of inhibition zone measurement is 9 cm. (Raja, 2016)

There is an increase inhibition zone value as the concentration value increase. This result is as same as the results of the Arici study, that showed there is an unidirectional relationship between both. (Arici, 2007) An experiment of Asniyah about antibacterial effect of black cumin extract to gram negative bacteria like *Escherichia coli* showed the average measurement of inhibition zone at 50% concentration is 0.9 cm, then at 100% concentration is 1.3 cm. (Asniyah, 2009) The other experiment of Wasim Raja about antibacterial effect of black cumin extract to gram positive bacteria like *Enterobacter* showed the average measurement of inhibition zone at 50% concentration is 5.6 cm, then at 100% concentration is 11.15 cm. (Raja, 2016) There is a difference in the average inhibition zone value between gram positive and negative bacteria. The average value in gram positive bacteria is greater than gram negarive bacteria. Etiology of this situation is the cell wall structure of gram positive bacteria is more simple than gram negative. The cell wall structure of gram positive consists of peptidoglycan and teichoic acid. In conclusion, gram positive bacteria is easier inhibited by antibacterial substances than gram negative bacteria.

5 CONCLUSION

Based on the results of research and data analysis, it can be concluded the lowest inhibition zone value at 50% concentration and the highest value at 100% concentration. The p-value from Kruskall wallis statistical test is $p<0.05$, it means black cumin is effective in inhibiting the growth of *Staphylococcus aureus* bacteria. The content of thymoquinone, tanin, thymol, and dithymoquinone in black cumin having a function as an antibacterial. This inhibition zone is formed caused by the active ingredient of black cumin. They are thymoquinone, tanin, thymol, and dithymoquinone. Thymoquinone and dithymoquinone can form irreversible complexes with bacterial proteins and resulting in protein inactivation. While tanin and thymol worked by inactivating enzymes and cell wall proteins that disrupt bacterial growth. (Aprilia, 2016)

REFERENCES

Aprilia E, Setiawati EM, Kurnia S. 2016 The effectiveness of nigella sativa extract to reduce subgingival plaque bacteria. *Indonesian Dent J* 23(3) 60-2.Fragiskos D 2007 *Oral surgery* (Leipzig: Springer) p. 205-20

Arici M, Sagdic O, Gecgel U 2007 Antibacterial of turkish black cumin (*Nigella sativa)* oils. *J Of Plant Develop Sci* 3(4) 259-62.Kohli M, Mathur A, Siddiqui SR 2009 In vitro evaluation of microbiological flora of orofacial infection. *J Maxillofac Oral Surg* 8(4) 329-33.

Asniyah 2009 Efek antimikroba minyak jintan hitam terhadap pertumbuhan *Escherchia coli* in vitro. *J of Biomedic* 1(1) 25-9.Syed R, Prasad G, Deeba F, Rani D, Jamil K, Alshatwi AA 2011 Antibiotic drug resistance of hospital acquired *Staphylococcus aureus* in Andra Pradesh: A Monitoring Study. *Afr J Microbiol Res* 5(6) 671-4.

Kohli M, Mathur A, Siddiqui SR 2009 In vitro evaluation of microbiological flora of orofacial infection. *J Maxillofac Oral Surg* 8(4) 329-33.

Pratiwi ST 2008 *Mikrobiologi farmasi* (Jakarta: Erlangga) p.188-91

Raja W, Dewangan T 2016 Determination of antibacterial activity of nigella sativa seed extract against some human pathogenic bacteria. *American J of Bio and Pharm Res* 3(2) 65-69.

Syed R, Prasad G, Deeba F, Rani D, Jamil K, Alshatwi AA 2011 Antibiotic drug resistance of hospital acquired *Staphylococcus aureus* in Andra Pradesh: A Monitoring Study. *Afr J Microbiol Res* 5(6) 671-4.