Capsaicin's Inhibition Effects on Biofilm Aerococcus Viridans

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Capsaicin's Inhibition Effects on Biofilm Aerococcus Viridans

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Abstract

Introduction: Aerococci has colony shape with *Streptococcus viridans*, so that it is usually missdiagnosed between of them. It has high difficulty on its identification, because it is rarely to be found and resistant of Antibiotic grup, called penicilin and vancomycin.

Material and Method: This research used two kinds of samples, such as: fried food sold on the road and toothbrush obtained from ten respondens. Then, it needs inducing its biofilm and measuring by *Tissue Culture Plate* (TCP) methode and ELISA.

Result: ANOVA one-way revealed that extract capsaicin has anti biofilm effect on 12.5% concentration with p=0,0000 (p<0,05) among each group.

Conclusion: Extract capsaicin can be used as an alternative herbal agent to treat infection caused by *Aerococcus viridans* because it has antibiofilm effect. As known before, biofilm causes antibiotic resistance to treat *Aerococcus viridans*'s infection.

Keywords: *UTI; antibiotic resistance; Bioterrorism; ELISA; traditional herbal medicine.*

Introduction

Aerococcus viridans is a bacteria that found in 1953, rarely described on the literature. It has some abilities to infect and cause some diseases for human being. [1,2,3] Its infection is usually treated by antibiotic, namely: trimethoprim, sulphametoxazole, and penicillin. *Aerococcus viridans* is able to form biofilm, so that it triggers its resistantcy, especially since it has been known that *Aerococcus viridans* can live on the root canal of the tooth. [4] It needs searching an alternative therapy to treat biofilm *Aerococcus viridans*. One of the traditional herbals that can be used as an alternative therapy to treat *Aerococcus viridans*'s infection is capsaicin. Capsaicin is an alcaloid crystal which formula is $C_{18}H_{27}NO_3$. Indonesian civilization commonly use capsaicin as a mixing substance on their food and medicine. [5,6] Capsaicin has some effects, such as strong stimulant on blood and heart, and anti-bacteria. It is agonist of *Transient Receptor Potential Vanilloid-1* (TRPV-1) which functioning as vanilloid receptor, that can be found on every sensory nerve ending and release neuropeptides, such as Substance P (SP) and *Calcitonine Gene Related Protein* (CGRP). [7,8]

Aerococcus viridans has a strong relationship in denstistry, especially with decayed tooth and supragingival plaque. [4,9] Another reason is *Aerococcus viridans* relates to the toothbrush, or everything that is usually used in oral cavity, antibiotic which can trigger its resistance, and hospitalized patients. As mentioned before, it is possible that using *Aerococcus viridans* as a bioterrorism agent which can endanger human civilization. [8,9]

Material and Method

Experimental design

This was an experimental laboratory research which was done on Research Centre Laboratorium of Faculty of Dentistry Universitas Airlangga. There were two kinds of samples used in this research, such as: sample that was obtained from fried food sold on the road and sample was obtained from toothbrush's. This research have been approved by the ethical commite of Faculty of Medicine Airlangga University 288/EC/KEPK/FKUA/2019.

Experimental procedures

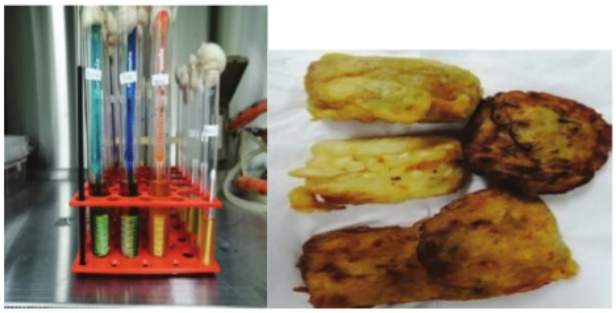

Figure 1. Samples that obtained from two kinds of samples, such as: toothbrush and food.

This research was conducted by using two kinds of samples come from fried food sold on the road and the toothbrush's respondents after being used for ten days as shown on Figure 1. There were some criterias of the respondents, 18-35 years old and did not eat pig as their food. Methode used in this research was an experimental laboratory, post test only group control design and its replication were counted by Federrer. Samples both from food and toothbrush were swabbed and cultured in Blood Agar Plate (BAP) media (Oxoid). BAP then incubated 1x24 hours at 37°C. The suspect isolate bacterias were sent to the Balai Besar Laboratorium Kesehatan (BBLK) to be Vitec tested.

The induction process was done by culturing *Aerococcus viridans* into BHIB inserted to anaerobic jar and incubator which was given gas kit, so that it could be in anaerobic condition, and incubated 2x24 hours. The speciment then was sentrifuged (Thermo Scientific), collected using micropipet 50-250 µL (Titertek) and cultured to the ELISA. There were two times and materials of staining, the first was Crystal Violet 0,1 % 50 µl. After being given Crystal Violet, it was washed using PBS 200µl and replied two times. The biofilm speciment wes fixated using ethanol 50 µl and transfered to the new ELISA. The capsaicin extract then was diluted from 100% until 0,78125% on the test tube.[10,11]

Test of antibiofilm effect of extract capsaicin against *Aerococcus viridans*'s biofilm

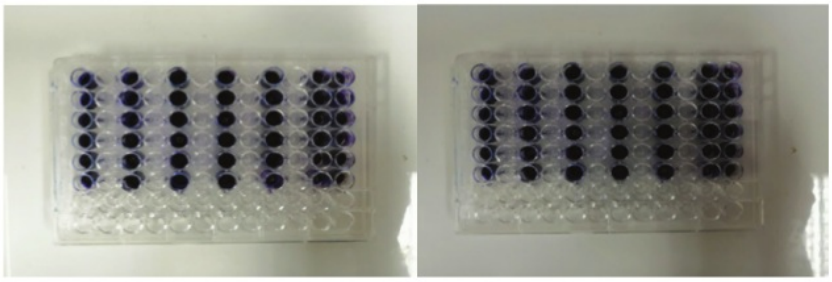

Figure 2. The test of biofilm *Aerococcus viridans* using ELISA and extract capsaicin from the concentration 100% - 0,78125%.

After the biofilm of *Aerococcus viridans* formed, it then inserted to the tube together with the capsaicin extract to be tested using TCP methode shown Figure 2. This methode was to test the Optical Density (OD). [10,11,12]

Analysis data

The data obtained was statistically analyzed using ANOVA one-way with post-hoct from SPSS (Statistical Package for the Social version 25 and EZR commander). The differences between group and pairwise comparison

was conducted using ANOVA one-way and continued with post hoc to know the difference with significant level of 0,05.

Results

Figure 3. The suspect of colony *Aerococcus viridans* and proven using Vitek Test.

Figure 3 showed that it could be seen that there were some growth of *Aerococcus viridans* colony on BAP and its proven was done by using VITEK which was proven by Kementrian Kesehatan Balai Besar Laboratorium Kesehatan (BBLK) on Jalan Karang Menjangan. We had to test its normality of the data using Kolmogorrov-Smirnow which was shown both on table 1.

Table 1. Statistical Normality check using both SPSS for Windows version 25 and EZR among each group.

Group	P value (p>0,05)	Skewness
100%	0,8491	-0,397
50%	0,9772	-0,279
25%	0,9941	0,173
12,5%	0,9762	0,0201
6,25%	0,9167	-0,287
3,125%	0,9888	0,20
1,5625%	0,6509	0,747
0,78125%	0,6657	-0,542
Positive Control	0,9266	0,069
Negative Control	0,732	0,537

Table 1 showed about the normality check using Kolmogorrov-Smirnov test done by two kinds of statistic program, namely: SPSS (Statistical Package for the Social Sciences, version 25) and EZR commander. The distribution among each group was normal, so that the next step was to examine the different among each group using ANOVA one-way which was also shown on the table 2. The p value and skewness of each group has been shown on the table 1.

Table 2. Average and Weighted Standard Deviations Biofilm Aerococcus viridans Among Each Group.

Capsaisin Extract (%)	Mean ± SD	P value									
		100%	**50%**	**25%**	**12,5%**	**6,25%**	**3,125%**	**1,5625%**	**0,78125%**	**KP**	**Kn**
100%	0,484±0,032	0,000a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	1,00 a
50%	0,891±0,007	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
25%	1,290±0,005	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
12,5%	1,624±0,032	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
6,25%	2,253±0,013	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
3,125%	2,625±0,038	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
1,5625%	3,713±0,035	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
0,78125%	4,213±0,093	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
Positive Control	4,410±0,031	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b
Negative Control	0,471±0,029	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b	0,000 a,b

Anova-One way showed significant difference between group, $p<0.05$. a,b denotes significant differences between groups (post-hoc test)

Table 2 showed that there were significant differences among each group with the concentration of capsaicin extract using both ANOVA one-way from SPSS for Windows version 25 and EZR commander and the mean and standard deviation of each group. A substance that could inhibit the growth of biofilm, such as: the cut off value between 0 until 2 showed that weak biofilm, 2 until 4 showed moderate biofilm, and over the 4 showed that strong biofilm.

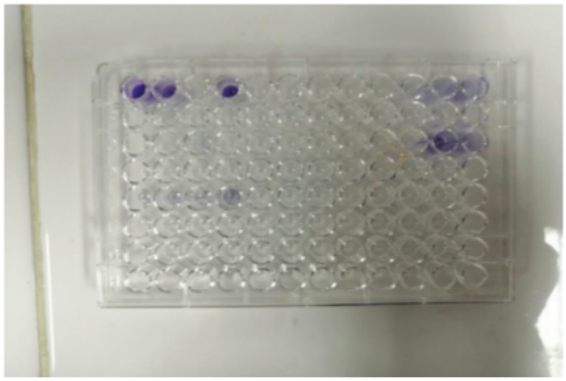

Figure 4. The staining process of the *Aerococcus viridans* biofilm using TCP method.

The minimum extract capsaicin which could inhibit the growth of *Aerococcus viridans* biofilm was 12,5% using TCP method. *Aerococcus viridans* biofilm qualification using TCP method can be seen on Figure 4. [13]

Discussion

This study confirmed that extract capsaicin can be used as an antibiofilm traditional herbal to treat *Aerococcus viridans* infection which has two common life cycle, such as: planktonic and biofilm, which biofilm is a community of the bacteria which they are embedded in a extracelullar polymeric substances. Bacteria which living on biofilm will be more resistant for any kinds of antibiotic. [14]

This result has revealed that extract capsaicin has some active compounds, so that it can easily penetrate to the peptidoglycan layer of *Aerococcus viridans* layer. Observation of biofilm OD values in each study group was carried out using the TCP method which is quantitative method biofilms in vitro. The discussion in the 1st group, namely in the 100% capsaicin extract concentration group with Aerococcus viridans bacterial biofilms that had an OD cut value of 0.484, the 2nd group with a 50% capsaicin extract concentration that had an OD cut value of 0.891, 3rd group with 25% capsaicin extract concentration which has a cut off value of OD 1,290, 4th group with capsaicin extract concentration 12.5% which has a cut-off OD value 1,624, 5th group with capsaicin extract concentration 6, 25% who have a cut-off value of OD 2.253, the 6th group with a concentration of capsaicin extract 3.125% which have a cut-off value of 2,625, the 7th group with a capsaicin extract concentration of 1.5625% which has an OD cut-off value 3,713, the 8th group with a concentration of capsaicin extract 0.78125% which has a cut-off value of OD value 4,213. For the positive control group it has an OD cut value of 4,410 and a negative control group that has an OD cut value of 0.471. [10,11]

Analysis conducted on several OD cut-off values with SD values for each group is in accordance with the TCP method theory, namely: the cut off value of biofilm OD in the concentration group of 100% to 12.5% has a range between 0 to 2 which means weak. Capsaicin extract concentration group of 6.25% to 1.5625% has a range between 2 to 4 which means moderate. The capsaicin extract concentration group of 0.78125% has an OD value of 4. It reflects the strongest biofilm strength. The difference in the OD cut-off value between each group is significantly different and supported by the ANOVA-one way test value between each group, namely: p = 0,000 which means that the p value is below 0.05 so that there are Significant differences between each study group. [12,13]

From the results of this research, capsaicin extract has an effect or inhibitory effect on Aerococcus viridans bacterial biofilms at a concentration of 12.5. This is consistent with the proven theory that capsaicin extract contains several active components, namely: carotenoids, phenols, flavonoids, alkaloids, and vitamin C. Flavonoids are chemical components explored in capsaicin, because they contain antibacterial compounds and removal of bacterial virulence. Flavonoids are also the largest and most polar phenol group, so they can easily penetrate into the peptidoglycan layer in both Aerococcus viridans and cause damage to cell walls. Peptidoglycan is an important component of gram-positive bacteria, especially Aerococcus viridans which functions as a protective barrier and material required for the attachment process of cleavage, morphogenesis, and bacterial pathogenesis. [14]

Phenol is a compound for membrane damaging activity, activating enzymes, and denaturating proteins so that cause decrease in permeability to the cell membrane. Carotenoids, bind to porins which are transmembrane proteins found in the outer membrane of bacterial cell walls which resulting in damage to the porin and decreasing membrane permeability. Alkaloids inhibites the synthesis of cell membranes in bacteria and causes bacterial cells to be more permeable and its cytoplasmic contents easily leaving out. [14]

Based on some of the things discussed above, it can be attributed to the bacteria Aerococcus viridans, biological bombs (bioterrorism), and the ability of capsaicin extract as an alternative ingredient in herbal medicine. Bioterrorism is the use of biological material as a weapon that threats to health or death to the civilization. Bioterrorism has three types of categories, namely categories A, B, and C with the difference in each of these categories is for category A can cause high mortality rates, category B does not cause death but only causes high morbidity and category C consists of materials that can be designed to cause high levels of morbidity and effects on health care facilities. Based on the data presented above, the Aercoccus viridans bacteria can be a category B bioterrorism material because these bacteria can be easily obtained in nature and can cause high levels of morbidity. [15]

Conclusion

Based on this research, the extract capsaicin can be used as an alternative herbal agent against the *Aerococcus viridans* biofilm.

Conflict of Interests: The authors declare that they have no conflict of interest in publishing this article.

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Ethics Approval and Consent to Participate

This study has been agreed by ethical commite of Faculty of Medicine Airlangga University 288/EC/KEPK/FKUA/2019

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List of Abbreviations

UTI : Urinary Tract Infection

TCP : Tissue Culture Plate

OD : Optical Density

ANOVA: Analysis of Varians

PBS: Phosphate Buffer Saline

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7