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The Difference of Biofilm Activity of Mangosteen Pericarp Extract (Garcinia mangostana L) 25% and NaOCl 2,5% Against Porphyromonas Gingivalis Biofilm

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INTRODUCTION

Root canal infection caused by some bacteria which each species has a varied virulence factor[1]. Porphyromonas gingivalis is a gram negative black-pigmented anaerob bacteria which is a primer bacteria that infected root canal. Bacterial culture test shows that P.gingivalis frequency in root canal is less than 10%, while PCR metode which is more sensitive shows that the presentation of P.gingivalis in necrotic pulp reach 28%[3]. Porphyromonas gingivalis and some bacteria in oral cavity, on in vivo studies, is not a free planktonik bacteria, it formed a biofilm in root canal surface[4]. Bacteria in biofilm has a different characteristic from the planktonic form, this bacteria has a protective barrier as a cellular matrix which allowed the biofilm formed bacteria more resistant against fagositic cells or medications[5]. Due of that reason an antibiofilm materials is needed to obliterate biofilm formed bacteria from the infected root canal. An antibiofilm agents such as NaOCl or Sodium Hypoclorite can be used as irrigation solution in a root canal treatment.

Chlorin compound in NaOCl is a biofilm dispersant which may damage extracellular matrix in biofilm6. Recommended concentration for NaOCl is between 0,5% - 5,25%. In general, the most common concentration is 2,5%, because it has the lowest toxicity but it still could maintain the tissue solubility and has an antibiofilm ability[7].

More over, a herbal agents been used and it been expected to be more compatible for root canal due to the low toxicity, and good antibiofilm ability. Mangosteen pericarp contains some active components such as alkaloid, saponin, triterpenoid, tannin, fenolic, flavonoid, glycoside, steroid and xanthone as the major component[8,9]. The active component from this extract known to have a antibiofilm ability. Saponin is a surfactant agent which act as a biofilm dispersant by disintegrate bacterial bond in biofilm[6]. In a previous study shows that mangosteen pericarp extract has a antibiofilm activity which could inhibit the Porphyromonas gingivalis biofilm in concentration 25%[10].

The aim of this study is to show the difference of antibiofilm activity between mangosteen pericarp extract and NaOCl against Porphyromonas gingivalis biofilm.

METHOD

This study is a in vitro laboratories research with a post test only control group design. This using a microtitter plate test method to know the difference of the antibiofilm activity between mangosteen pericarp extract 25% and NaOCl 2,5% against Porphyromonas gingivalis biofilm.

The sample of this study is Porphyromonas gingivalis strain ATCC 33277 from RSPTI Airlangga University and were incubated 16 days until the biofilm appear on TSB (Tripticase Soy Broth). It received an application of mangosteen extract solution 25% and NaOCl solution 2,5%.

The mangosteen pericarp prepared from balai material medika, Batu City were extracted at pharmacy laboratory Widya mandala university. The mangosteen pericarp were baked at 50oc for 24hours, and processed into powder. Then it soaked in 70% ethanol, wraped with alumunium foil and left for 24 hours before it ready to being filtered. This process repeated for few times until we get a clear filtrate then it can be evaporated to get a thick-alcohol-free extract[8].

The antibiofilm activity test performed by adding each tester material into the microtitter plate flat-bottom 96 well which contain the Porphyromonas gingivalis biofilm, before it being incubated at 37oc for 24hours. After that, the contains of each well were aspirated and washed 4 times with 200ml phosphate buffer saline. Then it ready to stained by crystal violet and incubated in room temperature for 15min and washed 3times by aquadest then let dried. 100ml DMSO 100% were added. Then tapped the microtitter plate for 1 minute and placed in a microplate reader. A quantitative analysis is performed by measuring the optical density with a microplate reader. It repeated 8times for each isolate.

The result was analyzed by non-parametric kruskal-wallis test and Mann-whitney test using SPPS 21.0 for windows.

RESULT

The results obtain from Porphyromonas gingivalis strain ATCC 33277 biofilm which formed on TSB media with addition 25% mangosteen pericarp extract and 2,5% NaOCl. The antibio-

film activity of each tester which may decrease Porphyromonas gingivalis biofilim were showed by the optical density imaging from microplate reader. Each group contains 8 samples with mean value shows on the table below.

	N	X	SD
Kontrol	8	0,190	0,026
Mangosteen pericarp extract 25%	8	0,151	0,007
NaOCl 2,5%	8	0,368	0,134

The first statistical analysis used is Komolgorof smirnov test to define the data distribution and levene test to see the homogeneity. Both test shows that the data has a normal distribution and heterogene so a non-parametric statistical analysis, kruskal-walls test, is needed to know the differences of the entire data. From the last analysis we get that signification value 0.000 which mean p is smaller than 0.05 ($p<0.05$) it shows that there is a significant differences from each group.

The statistical calculation by mann-whitney test among the group shows the signification value 0,006 between mangosteen pericarp extract 25% and the control group and 0,003 between NaOCl 2,5% and the control group. This indicates the P value is smaller that 0,05 ($P < 0,05$) which means that there is a significant differentiation between both test group and control group. Then by the statistical calculation using mann-whitney test between mangosteen pericarp extract 25% and NaOCl 2,5% obtain a signification value 0,001. So P is smaller than 0,05 and shows that there is a different between two groups.

	Group I	Group II	Group III
Group I	-	+	+
Group II	+	-	+
Group III	+	+	-

DISCUSSION

P.gingivalis is a microorganism who has a capability to form biofilm, and found in a necrotic root canal. This bacteria attached in root canal surface by some filamens called Fim A and communicate with other bacteria and form a defense called biofilm, which resistance to antimicrobe[2]. Porphyromonas gingivalis biofilm has a protective layer named extracellular polimer matrix (EPM) which stand of polisacharide, protein, and DNA. The major part of biofilm is water which take 90% of its structure[12].

In this study we know that the optical density from the mangosteen pericarp extract 25% has the smallest value among the NaOCl and Control group. It indicates that mangosteen pericarp extract has a good effectivity in degrading a biofilm. Mangosteen pericarp extract known to has some active component such as saponin, xanthone, flavonoid, and tannin which has an antibiofilm activity. Saponin is a surfactant agent who degrading biofilm matrix by disturbing biofilm metabolism then release the bond between the bacteria on the biofilm[6]. Saponin has a molecul which could attract water or hydrophilic, this thing will make extracellular matrix in Porphyromonas gingivalis biofilm dispersed. When the biofilm"s matrix dispersed the other active compound such as xanthone, flavonoid, and tannin will come in and damage the bacteria. Xanthone which is the biggest component from this extract, has a carbonil which can react with the amino acid recidu in membrane cell protein, extracellular enzyme or cell wall protein thus made them lose its function and the bacteria lysis[9]. Beside of that xanthone is also effective in eliminate anaerob gram negative bacteria which more resistant to antimicrobial agent. Flavonoid and tannin are also have a role in creating a complex compound with protein through hydrogen bond thus disturbing cell metabolism and cell permanently dmage[13]. Tanin have a role in coagulating bacteria protoplasm and make a settle bond with protein[14].

NaOCl is the most used irrigation agent in clinic. Clorin is a biofilm this person which can degrading Porphyromonas gingivalis biofilm by disturbing the metabolism and relies the bound between bacteria in biofilm[6]. The characteristic of NaOCl obtain from the Choloroamination reaction between clorin and amino acid which may interrupt the cells metabolism do to strong oxydating activity of chlor which case and oxidation of protein and cell enzyme. Chlorin could oxydating a sulphydryl compound irreversibly from the essential bacterial enzyme, cistein. This thing would inhibit enzyme work and then disturbing cell metabolism and damage the integrity of sytoplasmic membrane[15].

This study discovered that the OD value from NaOCl 2,5% group are higher comparing to the mangosteen pericarp extract and control group. It means that the number of Porphyromonas gingivalis biofilm after the adding of NaOCl solution is raising. It didn't match with the theory which said that NaOCl is the most effective solution in dismissing biofilm from root canal. As we know that in absorbancy calculation need a simple staining with crystal violet to know the OD value from each group. Meanwhile this crystal violet not only could stain the active bacteria but also death bacteria. Due to that situation the amount of the OD value from the NaOCl group has a false negative value.to prove that statement then we may perform a study to count the bacterial colony (CFU / Colony Forming Unit). From previous journals discovered that after the colony counting the NaOCl group is sterile.

Beside that, the other possibility which may case the false negative result on OD reader is the value of degradation of biofilm layer on NaOCl group is small, so that the biofilm layer which read on spectophotometric shows a high optical density value, but the active component from NaOCl can penetrate into the biofilm and adequate in killing the bacteria. Otherwise the mangosteen pericalp extract has an active component called saponin. Saponin is a surfactant agent or known as natural detergent which has a capability to disperse the biofilm layer so that the OD value whom readed on spectophotometric is smaller. This thing is match with the theory which said that an agent which has an antibiofilm activity could damage biofilm through lots of way. Such as penetrating the extra cellular matrix, dispersing cell from biofilm or interrupting the stability of EPS on biofilm[6].

The statistical analysis result from this study shows that each group has a significant differentiation. It means that there is a chance in biofilm optical density after the adding of specific antimicrobial agent. But it cannot define which group has the strongest antibiofilm activity because the varian value of the

result are not homogen. So the analysis perform in non parametric way. The unhomogen result may because of the difference of the time variable on sample preparation, such when the mixing of the testing agent and Porphyromonas gingivalis biofilm. There is possibility among the well has a few second time difference when they are start to mix. Otherwise there is a mistake in incubation process which case a variation in result due to the characteristic of P. Gingivalis which is an obligate anaerob bacteria and really hard to being incubated. Then it may also case by the washing procedure of each well before the optical density were read in a different microplate reader. A different washing result where one sample is clearer than the others were also resulting a different absorbancy reading in microplate reader. The clearer one will make the light easier to go through testing agent and make a bigger absorbancy and lower optical density value.

CONCLUSION

From this study we can conclude that there is a differences in antibiofilm activity between mangosteen pericarp extract 25% and NaOCl 2,5% against Porphyromonas gingivalis biofilm where the extract could degradating biofilm layer better than NaOCl 2,5%.