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The Effectivity of Lemongrass (Cymbopogon Citratus) Extract Against Porphyromonas Gingivalis ATCC® 33277™ (IN-VITRO)

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***Abstract*−Lemongrass extract contains citral and geranial that act as the antibacterial property. The mechanism of citral that acts as antibacterial agent is the "hydrophobicity" characteristic that able to rip apart the mitochondria's lipid layer of the membrane cell. The alcohol compound can also cause the denaturation and coagulates the Porphyromonas gingivalis' cell's protein that caused disruption in bacterial metabolism so that Porphyromonas gingivalis stopped growing and become lysis. Porphyromonas gingivalis is one of causes in tissue's destruction in periodontitis. Periodontitis is an inflammation that can cause damage to the tissue and alveolar bone resorption. The purpose of the study is to find out the MIC and MBC levels of the lemongrass extract in 5 different concentration levels (50%, 25%, 12,5%, 6,125%, and 3,125%) against Porphyromonas gingivalis ATCC® 33277™. The method of this study is experimental laboratories with Posttest only control group design. The sample is the stamp of Porphyromonas gingivalis ATCC® 33277™. The effectivity test used dilution method using Mueller Hinton Broth (MHB) and subculture method using Nutrient Agar. The result of the subculture test showed that for MIC level 25%, while for the MBC level is 50%. The result of Anova test showed that there's significance between the levels of the extract concentration and bacterial colonies. The conclusion of this study is 25% of the concentration levels of extract are already effective enough against Porphyromonas gingivalis ATCC® 33277™. It is true that lemongrass has effectiveness in Porphyromonas gingivalis ATCC® 33277™.**

***Keywords*−lemongrass, effectivity, MIC, MBC, Porphyromonas gingivalis ATCC® 33277™**

I. INTRODUCTION

Lemongrass (Cymbopogon citratus) is one of the most commonly grown crops in tropical climates and is commonly used as a cooking herb or as an alternative herbal remedy in treating or preventing some diseases because it has antibacterial, antifungal, antioxidant, antiseptic, anti-inflammatory, analgesic and antipyretic properties [1].

Lemongrass (Cymbopogon citratus) is a perennial plant with a height of 50 - 100 cm having a fringed single leaf length can reach up to 1 m and width of 1.5 - 2 cm. The leaf bone is parallel while the texture of the top and bottom leaf surfaces is rather rough. The stems are not woody and purplish-white, have fibrous roots and grow gradually. Lemongrass is a type of plant that grows fast and grows optimally at an altitude of 50 - 2700 meters above sea level. This tropical plant can grow well at a temperature of 10 – 33^{o}C with enough sunlight [2,3]. The best lemongrass growth can be obtained in 700 - 3000 mm rainfall areas with the rain frequencies spread evenly throughout the year [4].

Lemongrass thrives on soil with 5-7 pH and has good drainage. These are the ideal condition for lemongrass. Harvest period is done when the plant height has reached 1 - 1.5 meters; the first harvest is done after 6-9 months. The next harvest interval 3-4 months due to the harvesting age greatly affect the content contained in it. Others studies reported that lemongrass plants harvested at age 5.5 months is the most appropriate age to obtain essential oils with the optimum amount. While at 6.5 months of age is the age where the content of citral and geranial most will be found on the lemongrass so that the best harvest period is 6.5 - 7 months [2,3].

The use of lemongrass essential oils as oral health care, especially chronic periodontitis, makes lemongrass extract widely used because of its anti-bacterial and non-toxic properties so it is safe to be used and has been proven for years in mouthwash used for the treatment and prevention of various oral diseases especially periodontal diseases [5]. As Indonesia is one of the developing countries located tropical areas, the existence of lemongrass plants are abundant because it is very easy to grow on various types of soil and does not require special care.

Lemongrass contains many useful chemical compounds, such as saponins, flavonoids, polyphenols, alkaloids and essential oils in which citral, citronellal, geraniol, mirsena, nerol, farsenol, methylheptenone, dipentene, eugenol methyl ether, kadinen, kadinol and limonene [6].

The content of lemongrass which has antibacterial properties is citral and geranial. Citral has antibacterial properties because it destroys bacterial cells by increasing bacterial cell permeability activity, alter cell morphology and reduce ATP synthesis because membrane potential is the primary key in order to

synthesize ATP. Furthermore, the reduction in internal production of ATP occurs along with the loss of potential bacterial cell membranes, causing the synthesis of enzymes and proteins do not occur continuously to cause bacteria to lysis or die [7].

Studies on the antibacterial power of lemongrass extract and the study showed the effect of inhibitory effect and bactericidal effect from lemongrass extract to various microbes as conducted by Goyal and Ananad, lemongrass essential oil extract showed antibacterial effectivity against Staphylococcus aureus, Streptococcus mutans, Porphyromonas gingivalis and Prevotella intermedia by diffusion method in 100%, 50%, 0.2%, 0.1%, 0.05% and 0.025% concentrations [8]. The classification of Lemongrass is in Plantae kingdom, Magnoliophyta division, Liliopsida class, Poales ordo, Poaceae family, Cymbopogon genus and Cymbopogon citratus species (Figure 1) [9].

Figure 1. Lemongrass.

Human's oral cavity contains various bacteria, normal flora such as Streptococcus mutans, Streptococcus oralis, Streptococcus sanguis and Streptococcus salivarius and pathogenic bacterials such as Porphyromonas gingivalis, Prevotella spp, Fusobacterium nucleatum, Actinomyces naeslundii, Actinomyces viscosus, Veilonella spp dan Actinobacillus actinomycetemcommitans [10,11].

Porphyromonas gingivalis is coccobaccillus with short characteristics, pleomorphic, and aerotoleran which means that it can grow in anaerobic hood and has characteristics assacarolytic which means the source of the energy for the growth and development come from the catabolism of amino acid (Figure 2). Porphyromonas gingivalis can grow in MacConkey media culture with colony diameter 1-2 μm, smooth and shiny, while center part shows a darker image because of protoheme production which is a substance that responsible for its characteristic color. Porphyromonas gingivalis can be found in oral cavity, gingival infection and also chronic periodontitis [12-14].

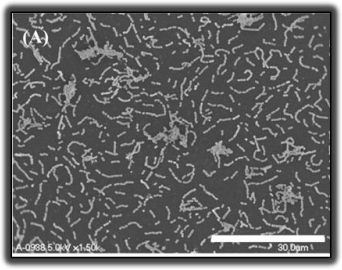

Figure 2. Porphyromonas gingivalis.

Porphyromonas gingivalis is one of the pathogenic bacteria that caused the periodontitis in which the amount of Porphyromonas gingivalis will increase significantly in periodontitis. Porphyromonas gingivalis was detected 25% in healthy subjects (46 of 181) and about 79% in periodontitis samples (103 of 130) [14,15]. The other bacteria that caused gingival inflammation in periodontitis is obligate anaerobic gram negative such as Prevotella intermedia, Bacteroides forsythus, Fusobacterium nucleatum, Selenomonas and Campylobacter, as well as facultative anaerobe gram negative bacteria such as Actinobacillus actinomycetemcommitans, Capnocytophaga and Eikenella corrodens [16].

Mechanism of Porphyromonas gingivalis invading the tissue starts from epithelial cells disruption because of bacterial penetration are the first steps initiation in inflammatory process and immune response that caused damage to the tissue and the surrounding supportive teeth that generates loss of attachments and end up with the loss of the teeth. Porphyromonas gingivalis attacks the periodontal tissue and inhibits the host defense mechanism. Porphyromonas gingivalis takes advantage of toxin/virulence factors, such as Arg-gingipain and Lys-gingipain that leads to deregulation of immune and inflammation responses, also produce collagenase enzyme that functions in degradation of the connective tissue, protease causes disruptions in host's cell membranes, phospholipase A inducts the loss of the bones, IgA and IgG that able to degrade the immunoglobulin [17-19].

PMN as one of the host defenses mechanism in gingival gap not able to prevent the plaque invasion on the pocket walls as the results of Porphyromonas gingivalis evasion capability to host immune response, so that the subgingival bacteria including Porphyromonas gingivalis can penetrate to the gingival epithelium. Penetration and the inclusion of Porphyromonas gingivalis into the connective tissue adding a magnification to the epithelial tissue because of Arg-gingipain and Lys-gingipain involved in peptide of connective tissue degradation and protein cellular matrix in cell host. Porphyromonas gingivalis can be found in periodontitis that had the loss of attachment [12,20-22].

The engagement of Porphyromonas gingivalis in periodontitis encouraged the scientists to look for the alternative remedy in preventing and treating oral diseases, alternative remedy chosen the least probability of its side-effects. As known that chlorhexidine which is

a gold standard that has effectiveness has been tested as anti-plaque, but after further investigation there are side effects caused by Chlorhexidine gluconate mouthwash which is the discoloration of teeth, restorations and dorsal tongue, loss of taste sensitivity, ulceration of the oral mucosa, swelling of unilateral or bilateral parotid glands and also accelerate the formation of supra gingival calculus on long-term use. So that alternative remedy that are useful as antibacterial, antifungal, and antiseptic among them contained in lemongrass [23].

II. MATERIALS AND METHODS

The design of this study was laboratory experimental, with a Posttest Only Control Group Design. Research subject is Porphyromonas gingivalis ATCC® 33277™. The selected lemongrass meets the inclusion criteria (healthy, pest-free, non-rot or wet, fresh, freshly harvested and non-dried) and exclusions (citronella, purple root grass.

Lemongrass was collected randomly, selected by following exclusion and inclusion criteria at Traditional Market of Medan. Extracts preparation is done in Laboratory of Traditional Medicines Faculty of Pharmacy USU, starting from making simplisia to making extract with maceration technique using ethanol 96% for five days to get 100% lemongrass extract.

The extract was further diluted with aquabidest at each concentration of 50%, 25%, 12.5%, 6.125%, and 3.125%. This study used two controls, namely chlorhexidine 0.1% as a positive control and aquadest as a negative control, with five repetitions which produced total of 35 tubes. To the reaction tube containing MHB media was added lemongrass extract in various concentrations as well as positive and negative controls and subsequently added Porphyromonas gingivalis ATCC® 33277™ suspension with turbidity of 0.5 McFarland. Then vortex the tubes and incubated in an incubator for 24 hours in an anaerobic hood (anaerobic jar) at 37°C (after which observation of the presence or absence of sediment on all tube base. The clear-sighted tube and no precipitate with smallest concentration of lemongrass extract is the KHM value of the lemongrass extract on the growth of Porphyromonas gingivalis ATCC® 33277™.

After observation of the MIC level was completed, the study continued by sub culturing all test tubes in nutrient agar and incubated in a 24 hour incubator in an anaerobic hood at 37°C. After 24 hours observation is the presence or absence of bacterial colonies that grow on nutrient agar that already incubated. Nutrient agar that there is no bacterial growth with the smallest concentration of lemongrass extract is the MBC levels of lemongrass extract against the growth of Porphyromonas gingivalis ATCC® 33277™.

III. RESULTS

After the test of lemongrass extract against the growth of Porphyromonas gingivalis ATCC® 33277 ™ was done, look for the average number of colonies after 5 repetitions to find MIC and MBC levels. In Table I, it is seen that the observed dilution results are biased because all the concentrations that have been incubated are turbid and sediment presence after 24 hours. Subculture test is done at all concentrations.

TABLE I. RESULTS OF BACTERIAL DILUTION TEST OF PORPHYROMONAS GINGIVALIS ATCC® 33277™

Group	Concentrations	N	Results
1	50%	5	Turbid
2	25%	5	Turbid
3	12,5%	5	Turbid
4	6,125%	5	Turbid
5	3,125%	5	Turbid
6	Control(+)	5	Clear
7	Control (-)	5	Turbid

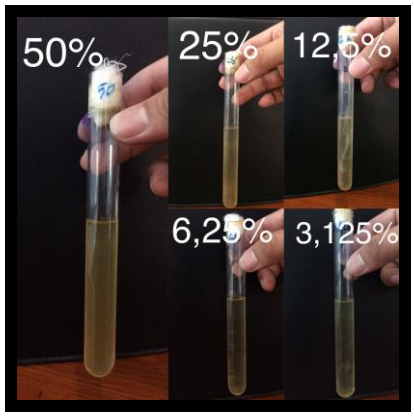

Figure 3. Dilution test results after 24 hours.

In table II, it can be seen that the results of subculture test, MBC levels at 50%, as seen from the average value of bacterial colony is 0. MIC value was at 25% concentration where the average value of bacteria is below the significant value of bacterium (300).

TABLE II. MEAN NUMBER OF BACTERIAL COLONIES OF PORPHYROMONAS GINGIVALIS ATCC® 33277™ ON SUBCULTURE TEST

Group	Concentration	N	Σ	SD	P
1	50%	5	0.00	0.000	0,000
2	25%	5	219.00	94.366	
3	12,5%	5	321.20	8.983	
4	6,125%	5	350.00	8.916	
5	3,125%	5	385.60	6.427	
6	Control (+)	5	0.00	0.000	
7	Control (-)	5	399.60	6.189	

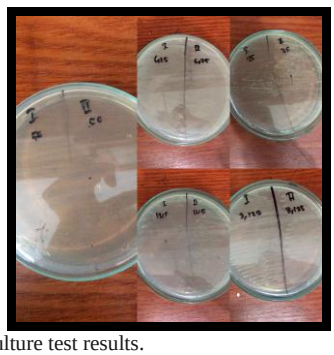
Figure 4. Subculture test results.

Based on ANOVA one way test (table 3), it is obtained that p value = 0,000 <0.05, hence there is

significant influence of concentration value to number of bacterial colony.

TABLE III. ANOVA TEST OF EFFECT OF CONCENTRATION OF LEMONGRASS EXTRACT ON THE MEAN NUMBER OF BACTERIAL COLONIES OF PORPHYROMONAS GINGIVALIS ATCC® 33277™

Concentration levels	F	Sig.
Total bacterial colonies	115.459	0.000

IV. DISCUSSION

This research was conducted by dilution method using aquabidest as diluent to get five concentrations of lemongrass extract. In each test tube was added Mueller Hinton Broth liquid medium (MHB) and Porphyromonas gingivalis ATCC® 33277™ bacterial suspension with turbidity of 0.5 McFarland and incubated for 24 hours in anaerobic hood. Observation of MIC concentration was performed after test tube was incubated in incubator for 24 hours in anaerobic hood at 37^{o}C to see if there was any turbidity indicating bacterial growth or clear indicated that there is no bacterial growth.

Based on the observations made by tubes containing lemongrass extract in various concentrations, seen the turbid impurities around the tube, so observation made becomes rather difficult. The use of new test tubes is preferred to make it easier to observe. The clear-sighted tube is a positive control tube containing chlorhexidine that indicates the absence of bacterial growth. The presence of turbidity is considering the concentration of the lemongrass extract. The clear-sighted tube is a positive control tube containing chlorhexidine indicating the absence of bacterial growth (Table I).

Then subculture method was done on solid medium Nutrient Agar then incubated at incubator for 24 hours at temperature 37^{o}C in anaerobic hood. After 24 hours the bacteria were cultured on Nutrient agar that has been incubated there were no bacterial colonies representing MBC values that exhibit a bactericidal effect. The smallest concentration with no colony growth is the concentration of MIC. This research is done with five times repetition and calculated average of MIC and MBC concentration from lemongrass extract. The average number of bacterial colonies was seen to rise significantly starting from concentrations of 50% to 3.125% and dropping on positive controls and again rising on negative controls (Table II).

As a conclusion, the lemongrass extract has effectiveness in inhibiting (MIC) at 25% and killing (MBC) at 50% concentration of Porphyromonas gingivalis ATCC® 33277™ growth. Application of other methods with better tools and materials is expected for further research on the looking for MIC and MBC levels of lemongrass extract against Porphyromonas gingivalis or other bacteria that cause periodontitis such as Fusobacterium nucleatum.

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