Potency of Okra Fruit Extract (*Abelmoschus esculentus*) Against *Porphyromonas Gingivalis* as the Cause of Chronic Periodontitis

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Abstract

Porphyromonas gingivalis (P. gingivalis) is an etiological agent of chronic periodontitis which is the main component of oral cavity microorganism. Porphyromonas gingivalis correlates with chronic periodontitis because it has the ability to avoid host immunity without inhibiting the inflammatory response that occurs which greatly benefits the presence of P. gingivalis and other bacteria while gingival crevicular fluid is an important source of nutrition because it contains peptides and iron.

To prove that okra (Abelmoschus esculentus) fruit extract has the potential to inhibit and kill P. gingivalis which is a microorganism that causes chronic periodontitis.

Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) were determined by using a serial dilution test.

One Way Anova test showed a significant difference (p = 0.000) then based on the Tukey HSD test showed a significant difference between the okra fruit extract (100%, 3.125%, 1.565% concentration) group with positive control.

Okra fruit extract have potency to eliminate in P.gingivalis that causes aggressive periodontitis indicated by MIC at concentrations 3.125% and MBC at concentrations of 6.25%

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Introduction

Porphyromonas gingivalis (*P. gingivalis*) is an anaerobic Gram-negative bacterium in the oral cavity that plays an important role in the pathogenesis of periodontitis as a cause of inflammation which can damage the tissue that supports the teeth which can eventually cause tooth loss.[1] *P. gingivalis* as the etiological agent of chronic periodontitis which is the main component of oral microorganism.[2]

Porphyromonas gingivalis correlated with chronic periodontitis because it has the ability to avoid host immunity without inhibiting the inflammatory response that occurs so it is beneficial for the presence of *P. gingivalis* and other periodontal bacteria where gingival crevicular fluid is an important source of nutrition because it contains peptides and iron. One of the virulency of *P. gingivalis* is Adhesin, *P. gingivalis* retention is facilitated by adhesion repressors such as fimbriae, hemagglutinin and proteinase.[3] Histatin is a peptide originating from the human salivary gland with antimicrobial and anti-inflammatory activity. Histatin 5 binds to hemagglutinin B (HagB) found in *P. gingivalis* and decreases the role of HagB chemokine induced in human dendritic cells, in addition histatin 5 is able to reduce chemokine responses that play a role in helping control inflammation in the oral cavity.[5] There are periodontal status differences between chronic periodontitis patient with and without Type 2 Diabettes mellitus (T2DM) , with findings on deeper pocket depth, bigger gingival recession, and greater attachment lost on subjects with T2DM compared with subjects without T2DM.[6] The IL-1α periodontitis is higher than that in mild– moderate chronic periodontitis.[7] Periodontitis is a disease that causes inflammation of the supporting tissues of

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teeth that are due to bacteria of specific microorganisms such as anaerobic Gram-negative bacteria. Periodontitis can cause progressive damage to the all supportive tissue around the teeth like ligament periodontal, cementum, gingiva and alveolar bone then eventually become pocket and some recession.[8]

Porphyromonas gingivalis (Pg) produces a large amount of cysteine arginine and lysine proteinase which is specific in the form of cells and secretions.[5] The use of synthetic drugs is not only expensive for the treatment of a disease but also has toxicity and adverse side effects. This type of situation causes the need to look for alternative new drugs to treat an illness. Herbal alternatives have enormous potential to be developed to a new drugs that are very beneficial for treatment and powerful and effective antibacterial agents.[9]

Abelmoschus esculentus (Okra) has many benefits, this is because okra contains secondary metabolite components such as alkaloids, terpenoids, flavonoids, etc.[10] Flavonoids are found in plants known for their antibacterial effects because of their ability to reduce bacterial cell wall permeability.[11]

Based on the background above, this study wants to prove that okra fruit extract is effective in inhibiting and eliminate *P.Gingivalis* that causes chronic periodontitis.

Materials and methods

This was an experimental laboratory (In vitro) study by means of post-test only control group designs that have been carried out with ethical clearance tests at Airlangga University, Faculty of Dental Medicine, Heath Research Ethical Clearance Commission (112 / HRECC. FODM / VII / 2018)

Sample Preparation

P. Gingivalis (Pg) sample was obtained and cultured in the Brain Heart Infusion Broth (BHIB). BHIB that containing Pg were incubated 1 x 24 hours at 37oC after being diluted according to 0.5 McFarland standard ($1.5x10^8$ CFU/ ml), then the bacteria were ready for testing.

Okra Fruit Extract Preparation

Prepare fresh okra fruit originated from Materia Medica, Batu- Malang for extract. Okra fruit samples were cut into pieces and weighed as much as 200 grams then put in a jar container and 70% ethanol was added as much as 300 ml and macerated for 24 hours at room temperature After 24 hours, the solution was filtered or separated using a Buchner filter. The filtering residue is aerated and maceration process was repeated up to 3 times. All distilates are mixed and concentrated with the Rotary Vaccum Evaporator at a temperature of 40ºC until a concentrated extract is obtained. To obtain various concentrations, the serial dilution method or multilevel dilution is used with a ratio of 1: 2 (w/v).[12]

Antibacterial Test Using the Serial Dilution Method

Preparations of bacteria *P.Gingivalis* stored in BHIB media are taken with sterile ose needles. Then the bacteria sample were inoculated in Mueller Hinton agar with streak method. The samples in Mueller Hinton agar were incubated in an incubator at 370C for 1 x 24 hours. The colonies were obtained from the Mueller Hinton agar using sterile ose needles. Then the bacteria samples were inoculated to BHI-B until the turbidity fullfill the 0.5 the McFarland 0.5.[12] 11 sterile test tubes were prepared. Each test tube is labeled 1-9 (concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.78%, 0.39%), then the tube 10 is given the K (+) label that act as positive control, this tube is containing a bacterial suspension that is equivalent to the 0.5 McFarland turbidity standard. Tube 11 is labeled K (-) which is a negative control, the tube containing 100% okra fruit extract. Tube 1 is filled with 4 ml of 100% concentration of okra fruit extract. Tubes 2-9 are filled with 2 ml of BHI-B liquid media. Take 2 ml of solution from tube 1, put in tube 2, mix until homogeneous so that the concentration is 50%. The same procedure was done until tube 9, until all extract concentrations are obtained with a ratio of 1: 2 (w / v). For turbidity test, bacterial suspension with 0.5 McFarland standard were taken as much as 0.1 ml, then put the suspension (1ml) into the treatment group in tube 1-9 (concentration of 100%, 50%, 25%, 12.5% , 6.25%, 3.125%, 1.563%, 0.78%, 0.39%). Then all the tubes were inserted into the anaerobic jar and then incubated at 370 C for 1 x 24 hours with 3 repetitions of incubation. Each after 1 incubation the turbidity was observed. If the turbidity of the tube is still as turbid as or more turbid than the tube K (+) which contains a bacterial suspension with 0.5 McFarland standard) it means that

bacteria still colonized, but when the solution in the tube is more clearer than tube K (+) it means that bacterial growth begins to be inhibited, this is shows the Minimum Inhibitory Concentration (MIC). After observing turbidity, a Total Plate Count (TPC) test was carried out to determine bacteriostatic and bacteriocid properties of the extract. The TPC test was carried out on Mueller Hinton agar media, the bacteria will be inoculated from the tube that looks more clearer than the other tube and cultured 3 times in 3 petri dishes. Then each petri dish was incubated at 370 C for 1x24 hours. then the number of colonies wil be counted.

Statistical analysis

The data obtained is the number of bacterial colonies in the form of the Colony Forming Unit (CFU), then tabulated and analyzed using the SPSS program. Data distribution test is done by Kolmogorov-Smirnov test to find out whether the data can be normally distributed. To see whether the data collected is homogeneous, the variance homogeneity test is performed using the Levene test with α> 0.05. Furthermore, a parametric test was performed using the ANOVA test to see the significance of differences in the number of bacterial colonies between study groups, then followed by HSD Tukey test.

Results

From the three treatments, the number of colonies of *P. gingivalis* could be calculated from the TPC test positive control tube, negative control tube, tube number 4, tube number 5, tube number 6 and tube number 7 (Table 1). The following is the calculation results regarding the number of colonies in each treatment, namely in the Figure 1, 2 and 3.

Tube No-	Okra Fruit Extract Concentration	Number of Pg colonies (CFU)		
		Treatment 1	Treatment 2	Treatment 3
4	12,5 %	-	-	-
5	6,25 %	-	-	-
6	3,125%	11	17	13
7	1,565 %	26	29	27
(+)	100% + bacteria	118	128	123
(-)	100 % without bacteria	-	-	-

Table 1. Results of the number of colonies of *P. gingivalis* bacteria after administration of various concentrations of okra fruit extract.

Observation result of *P. gingivalis* shows that the MIC was achieved in tube number 6 with okra fruit extract concentration of 3.125% and MBC was achieved in tube number 5 with okra fruit extract concentration of 6.25%. (Figure 3)

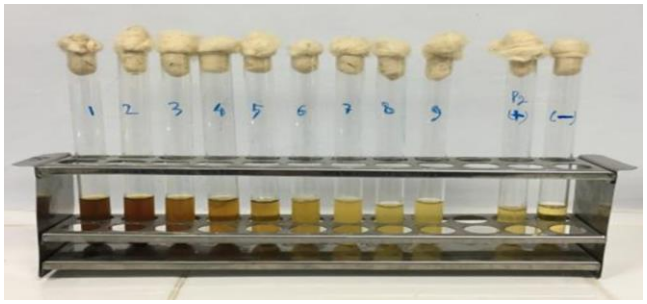

Figure 1. Results of serial dilution of okra extract on P. gingivalis bacteria by observing the turbidity and obtained MIC on the 6th tube and MBC on the 5th tube.

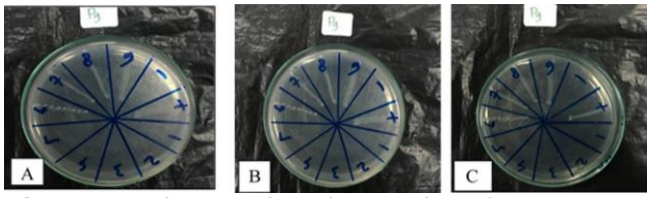

Figure 2. The results of scratches from 11 test tubes showing the presence or absence of P. gingivalis bacterial growth on Mueller Hinton's media from 3 replications, (A). first replication, (B). second replication, (C). third replication.

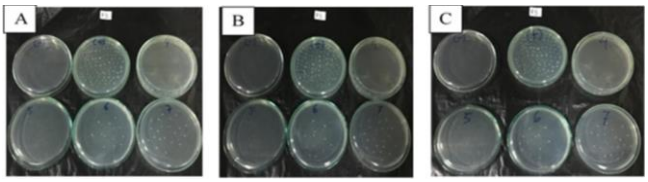

Figure 3. TPC test of *P. gingivalis* on Mueller Hinton media from positive control, negative control, tube number 4, tube no 5, tube no 6, and tube no 7 with 3 replication, (A). first replication, (B). second replication, (C). third replication.

Then to determine whether there is bacterial growth in each tube, inoculation was carried out in Mueller Hinton agar with 3 replications using the streak method.

The Kolmogorov-Smirnov normality test results shows that positive controls had p value of 1000 (p = 1,000), concentration of 3.125% had p value (p = 0.991), and concentration of 1.565% had p value (p = 0.991). The results above showed that the antibacterial power of okra fruit extract in *P. gingivalis* bacteria between each concentration was normally distributed with (p> 0.05). The homogeneity analysis was carried out by using Levene test.

The homogeneity test results show that p value is above 0.05, namely (p = 0.411). The results of the analysis show that the data variance is homogeneous so that the data analysis meets the requirements to be continued using the One Way Anova parametric test.

The results of statistical data analysis using the One Way Anova test shows significant differences (p = 0.000). P value (p<0,05) showed a significant difference for positive control group, concentration of 1.565% and concentration of 3.125%. (Table 2).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21292.667	2	10646.333	871.064	.000*
Within Groups	73.333	6	12.222		
Total	21366.000	8			

Table 2. One Way Anova test Results. *significant

The results of statistical analysis using the Tukey HSD test shows that there is a significant difference between the group of okra fruit extract (100%, 3,125%, 1,565%) with positive control. This means that there are significant differences in antibacterial propertioes of okra fruit extract. (Table 3).

Group	N	Subset for alpha = .05		
	1	**2**	**3**	**1**
Concentration of 3.125%	3	13.6667		
Concentration of 1.565%	3		27.3333	
Kontrol pos	3			123.0000
Sig.		1.000	1.000	1.000

Table 3. Turkey HSD test results between control group and treatment group.

Discussion

Research that has been done serially dilution on P. gingivalis bacteria, with fruit extracts of Abelmoschus esculentus (okra) using concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563 %, 0.78%, 0.39% showed that A. actinomycetemcomitans and P. gingivalis bacteria had MIC at a concentration of 3.125% and MBC at a concentration of 6.25%.

The antibacterial effects from the extraction of okra fruit against *P.Gingivalis* can occur due to the presence of active substances which was dissolved and contained in 70% ethanol solvents.

The action interactions of phenols are divided into 5, the first is action on the external membrane of the bacterial wall. Bacteria are protected against the surroundings by membranes, which is integration which is very important for bacterial survival. This membrane consists of basic compositions such as phospholipids and lipopolysaccharides, and is made stable by cations Mg ++ and Ca ++. Thus, if the ionizing disinfectant molecule is absorbed or rejected by an electric charge on the contact pedestal and the initial displacement, then the next action is non-polar molecules can dissolve and enter into the driving system special parts supermolecules can be changed - different molecules and will ready to be allocated membrane organization by staying in a certain place. The second is action on the bacterial wall. The bacterial wall is an important part, because this section provides very different rigidity between Gram-positive and Gram-negative bacteria. It is this diversity that causes large variations in the hydrophilic antibacterial response. The third is action on the cytoplasmic membrane. An active molecule of phenol, it can penetrate the cytoplasmic membrane by means of diffuse passive which is not specific and slow, also active transport that is specific, this method can collect products in bacteria after transforming or binding to protein membranes. The fourth interaction is in action on the cytoplasm and nucleus. This method of phenol can be activated at the cytoplasm and nucleus at the chromosome level. Interaction between actions in bacterial spores. The presence of dipikolinic acid in the spores of microorganisms builds up these forms are far more resilient against antibacterial than the vegetative form. Phenol disinfectants actively bind oxidative goods, similar to oxides and gases, which can destabilize this structure in spores.[13]

Flavonoids are polar compounds, so flavonoids generally dissolved in polar solvents such as ethanol, as well as phenol and quercetin.[14] The ethanol solvent in the extraction process of natural ingredients has the highest content because water and ethanol is a polar solvent that has a hydroxyl (OH) group, where hydroxyl groups in water and ethanol can participate in hydrogen bonds so that the liquid become harder to evaporates from other organic compounds that have the same molecular mass, so the natural substance will be extracted properly.[15]

Flavonoid as an antimicrobial agent which is one of the active ingredients of okra fruit extract has three working mechanisms for eliminating microorganism, namely 1. inhibiting nucleic acid synthesis, 2. inhibiting cell membrane function, and 3. inhibiting energy metabolism. Flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA. The mechanism of action of flavonoids inhibits cell membrane function is to form complex compounds with extracellular proteins that can damage bacterial cell membranes and are followed by the release of intracellular compounds.[16]

Flavonoids are chemically based on fifteen carbon structure consisting of two benzene rings (A and B) which are connected through a pyramid heterocyclic ring (C). In inhibiting nucleic acid synthesis, ring A and B of flavonoid compounds play an important role in the process of hydrogen interconnection or bonding, by accumulating nucleic acid bases thus inhibiting DNA and RNA formation. While the work of flavonoids that cause damage to the permeability of bacterial cell walls, microsomes and lysosomes, is the result of interactions between flavonoids and bacterial DNA.[17]

Flavonoids have the ability to inhibit cell membrane function by disrupting the permeability of cell membranes and inhibiting enzyme for bonding such as ATPase and phospholipase. The correlation between antibacterial activity and membrane disruption supports the theory that flavonoids can show antibacterial activity by reducing the fluidity of bacterial cell membranes.[18]

The bacterial plasma membrane is responsible for osmoregulation, respiration and transport processes, biosynthesis and cross-linking of peptidoglycan, as well as biosynthesis of lipids. For performing all of these functions, membrane integrity is a prerequisite, and its disruption can directly or indirectly cause metabolic dysfunction and finally lead to bacterial death.[19] To date, flavonoids, especially catechins, have been widely studied for their antimicrobial properties in both Gram-positive and Gram-negative bacteria. The interactions of flavonoids with lipid bilayers involve two mechanisms.[20]

Based on the role of the flavonoid of the okra fruit extract above shows that okra fruit extract has the ability to eliminate *P.Gingivalis* which is indicated by the presence of minimal inhibitory concentration (MIC) at 3.125% while minimizing bactericidal concentration (MBC) at 6.25%.

Conclusions

Okra fruit extract effectively eliminate *P. gingivalis* which is a bacteria that causes chronic periodontitis indicated by MIC at concentrations of 3.125% and MBC at concentrations of 6.25%

Ethical Approval

This study was approved by the Health Research Ethical Clearance Commission (HRECC) with certificate number 112 / HRECC. FODM / VII / 2018

Declaration of Interest

No conflict of interest associated with this work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

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