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# Effectiveness of Okra Fruit (*Abelmoschus esculentus*) Extract Against *Aggregatibacter actinomycetemcomitans* (*Aa*) as a Bacterium that Causes Aggressive Periodontitis

Muhammad Luthfi<sup>1</sup>, Yuliati Yuliati<sup>1</sup>, Aqsa S. Oki<sup>1</sup>, Agung Sosiawan<sup>2</sup>, Bella P. Cida<sup>3</sup>

<sup>1</sup>Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, <sup>2</sup>Department of Dental Public Health, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, <sup>3</sup>Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

## Abstract

**Aims and Objectives:** The aim of this study was to determine that okra fruit extracts are effective in inhibiting growth and killing the *Aggregatibacter actinomycetemcomitans* (*Aa*) bacteria that cause aggressive periodontitis. **Materials and Methods:** *Aa* ATCC 4371 strain Y3 serotype b bacteria obtained from the Stock Research Center of the Faculty of Medicine, Airlangga University, Jawa Timur, Indonesia, were bred on the Mueller Hinton media with the inclusion criteria that identification of bacteria from the stock shows that the bacterium is *Aa*, and the growth of bacteria in the Mueller Hinton media is with a number of colonies between 30–300 colony forming units (CFU)/mL. Culture media containing *Aa* bacteria were incubated for 1 × 24 h at 37°C, after it was diluted according to McFarland standard 0.5 (1.5 × 10<sup>8</sup> CFU/mL). Fresh okra fruit derived from Materia Medica was prepared for extract. Serial dilution or dilution methods of 1:2 (wt/vol) are used for the detection of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). **Results:** One-way analysis of variance test showed a difference with significance ( $P = 0.000$ ), whereas, Tukey honestly significant difference (HSD) test showed a significant difference between okra fruit extract group with positive control concentrations of 100%, 3.125%, and 1.565%. **Conclusion:** The okra fruit extract effectively kills the *Aa* bacteria that causes aggressive periodontitis, as indicated by MIC at a concentration of 3.125% and MBC at a concentration of 6.25%.

**Keywords:** *Aggregatibacter Actinomycetemcomitans*, Aggressive Periodontitis, Minimal Bactericidal Concentration, Minimal Inhibitory Concentration, okra fruit (*Abelmoschus esculentus*) extract

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## INTRODUCTION

Periodontitis is an inflammation that affects the supporting tissues of teeth, which is caused by microorganisms, and can cause progressive damage to the periodontal ligament, alveolar bone, and is accompanied by pocket formation. Periodontitis causes permanent tissue destruction, characterized by chronic inflammation, migration of the fused epithelium to the apical, loss of connective tissue, and loss of alveolar bone.<sup>[1]</sup>

Aggressive periodontitis (AP) is a complex disease, which is caused by microbial changes and cellular dysfunction, and is characterized by a rapid loss of attachment and bone damage to the tooth surface.<sup>[2]</sup> The majority of

periodontal pathogens are Gram-negative anaerobes and *Aggregatibacter actinomycetemcomitans* (*Aa*), which has often been associated with AP.<sup>[3]</sup> The role of this bacterium in the pathogenesis of periodontitis is due to its ability to attach to epithelial cells and produce many virulent factors such as extracellular matrix proteins, proteases, collagenase, endotoxin (LPS), bacteriocins, hemotactic inhibitors, leukotoxins, cytotoxins, toxic metabolic substances, and immunosuppressive proteins.<sup>[4]</sup>

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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 new drug alternatives to treat a disease. Herbal alternatives have enormous potential to develop new drugs that are very useful for treatment and are strong and effective antibacterial agents.<sup>[5]</sup>

*Abelmoschus esculentus* (okra) has many benefits. This is because okra contains secondary metabolite components, such as alkaloids, terpenoids, and flavonoids.<sup>[6]</sup> Flavonoids found in plants are known for their antibacterial effects because of their ability to reduce the permeability of bacterial cell walls.<sup>[7]</sup>

Because of the explanation of aforementioned fact, the researchers decided to prove that okra fruit extract was effective in inhibiting and killing *Aa* bacteria that cause AP. From the results of this research, it is expected to be used as a therapy for AP.

## MATERIALS AND METHODS

### Setting and design

This was an experimental laboratory using a posttest only control group design. Ethical clearance test at Faculty of Dental Medicine, Universitas Airlangga, Indonesia was performed with Health Research Ethical Clearance Commission (approval number 112/HRECC. FODM/VII/2018).

### Sampling criteria

This study uses *Aa*, ATCC 4371 strain Y3 serotype b bacteria, obtained from the Stock Research Center of the Faculty of Medicine, Airlangga University, Jawa Timur, Indonesia, with specification of ATCC 43718, which were bred on the Mueller Hinton media with the inclusion criteria that the identification of bacteria from the stock shows that the bacterium is *Aa* and the bacterial growth in the Mueller Hinton media is with a number of colonies between 30 and 300 colony forming units (CFU)/mL.

### Study method

*Aa* ATCC 4371 strain Y3 serotype bacterial stock was inoculated in the brain heart infusion broth (BHIB) culture media. Culture media containing *Aa* bacteria was incubated for 1 × 24 h at 37°C, after which it was diluted according to McFarland standard 0.5 (1.5 × 10<sup>8</sup> CFU/mL). Furthermore, the bacteria were ready to be tested.

### Okra fruit extract making

Fresh okra fruit derived from *Materia Medica* for extract was prepared.<sup>[7]</sup> Samples of okra fruit were cut into pieces and weighed 200 g, then put into a jar, and 70% of ethanol was added to make the volume to 300 mL. Maceration was carried out for 24 h at room temperature. After 24 h, the solution was filtered or separated using a Buchner

filter. Filtering residue was aerated, and maceration was done up to three times. The sieve 1–3 was mixed and concentrated with a rotary vacuum evaporator at 40°C until a concentrated extract was obtained. To obtain various concentrations, serial dilution or dilution methods of 1:2 (wt/vol) were used.

### Antibacterial test using the serial dilution method

Preparation of *Aa* bacteria stored in BHIB media in an incubator at 37°C was obtained with a sterile Ose needle.<sup>[8]</sup> The Mueller Hinton media was embedded by scratching. The bacteria that had been scratched on Mueller Hinton media were incubated in an incubator at 37°C for 1 × 24 h. The scratched bacteria were obtained from the Mueller Hinton media using a sterile Ose needle. It was put in the BHIB until the turbidity was the same as the McFarland 0.5 standard. Eleven sterile test tubes were prepared. Each test tube was labeled 1–9 (concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.78%, and 0.39%, respectively), then tube 10 was given K(+) label, which was a positive control. Tube 10 contained the bacterial suspension, which was equivalent to McFarland 0.5 turbidity standard. Tube 11 was labeled with K(-), which was a negative control. This tube contained okra fruit extract with a concentration of 100%. The tube 1 was filled with 4 mL concentration of 100% okra fruit extract. The tubes 2–9 were filled with 2 mL of BHIB liquid media. Two milliliter of solution from the tube 1 was put in tube 2. It was mixed until homogeneous, so that the concentration of 50% was obtained. The same thing was carried out up to tube 9 until all extract concentrations were obtained with a ratio of 1:2 (wt/vol). To test turbidity, bacterial suspension media were taken, which had been equalized with McFarland 0.5 turbidity standard of 0.1 mL and put into test tubes in 1–9 labels (concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.78%, and 0.39%, respectively). Then, all the tubes that were put in an airtight anaerobic container were then incubated at 37°C for 1 × 24 h. After one incubation, turbidity was observed. If the turbidity of the tube was still equivalent or more turbid than the positive control (K+) tube containing the bacterial suspension McFarland 0.5, it meant that bacteria can still thrive. However, when the solution in the tube appeared to be clearer than the K (+) tube, it meant that the growth of bacteria began to be inhibited. This was what showed the minimum inhibition concentration (MIC). After observing turbidity, a total plate count (TPC) test was conducted to determine bacteriostatic and bactericide properties. The TPC test was carried out on Mueller Hinton agar media containing concentrations of extracts from tubes that looked the clearest. Furthermore, each petri dish was incubated at 37°C for 1 × 24 h. The number of colonies was then counted.

### Statistical analysis

The data obtained were the number of bacterial colonies measured in CFU. Data were then tabulated and analyzed



using the Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM, New York).

The data distribution was carried out with the Kolmogorov–Smirnov test to determine whether the data could be normally distributed. To identify whether the collected data were homogeneous, a variance homogeneity test was performed using the Levene test with  $\alpha > 0.05$ . Furthermore, the parametric test using the analysis of variance (ANOVA) was used to identify the significance of differences in the number of bacterial colonies between the study groups. All analyses were tested at the significance level of 0.05.

## RESULTS

From the three treatments, the number of *Aa* bacterial colonies from the positive control tube TPC test, negative control, tube 4, tube 5, tube 6, and tube 7 were obtained as shown in Figures 1 and 2.

Table 1 shows that the MIC of okra fruit extract on *Aa* bacteria is on the sixth tube at a concentration of 3.125%, and the minimal bactericidal concentration (MBC) is on the fifth tube at a concentration of 6.25%.

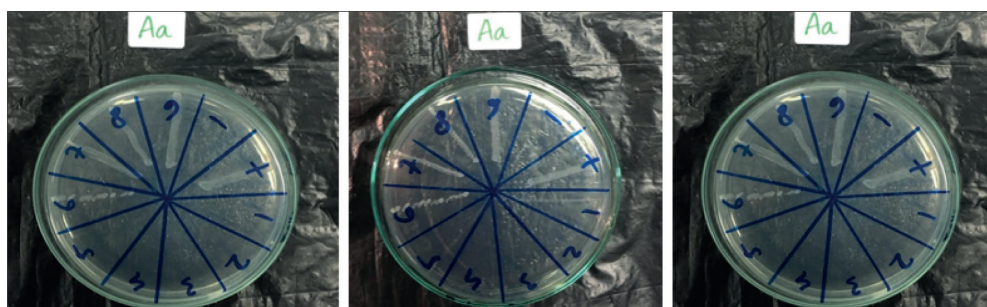
Data obtained showed that they were normally distributed based on the Kolmogorov–Smirnov test normality test, then Levene homogeneity analysis test showed that data were homogeneous with  $P = 0.215 (>0.05)$  [Table 2]. The

results of the research data were analyzed using one-way ANOVA statistical test [Table 2], the results showed that there was a significant difference ( $P = 0.000$ ) between the control group compared to the treatment group giving okra fruit extract (*Abelmoschus esculentus*). While the statistical analysis using the Tukey HSD test showed that a significant difference occurred between the control group and the treatment group in the administration of okra (*A. esculentus*) fruit extracts at concentrations of 3.125%, and 1.565%. This means that there are significant differences in inhibiting or killing the *Aa* bacteria [Table 3].

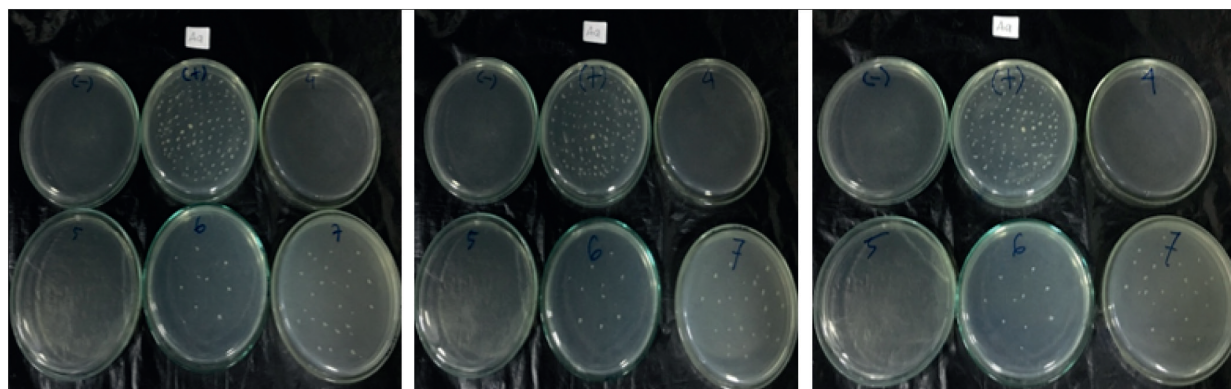
## DISCUSSION

On the basis on the results of data analysis from the one-way ANOVA test in Table 1, the  $P$  value was found to be 0.000, indicating that if  $P < 0.05$ , it means that there is a significant difference between the control group and the treatment group. The results obtained indicate that the administration of natural okra (*A. esculentus*) extracts is effective in inhibiting or killing the *Aa* bacteria, which are predominant bacteria causing AP.

Figure 3 is the observation of 11 *Aa* bacteria tubes containing okra fruit extracts showing that MIC is in tube number 6 with okra fruit extract concentration of 3.125% which is marked by a slight turbidity in the tube and which shows MBC in tube number 5 with extract concentration



**Figure 1:** Results of scratches from 11 test tubes that showed the presence of *Aggregatibacter actinomycetemcomitans* bacteria growth in Mueller Hinton media from three replications



**Figure 2:** Total plate count test on Mueller Hinton media from positive control tube, negative control tube, number 4 tube, number 5 tube, number 6 tube, and number 7 tube from three replications

okra fruit of 6.25% which is marked by the absence of turbidity in the tube. The next step is to find out the presence or absence of bacterial growth in each tube, then the scraping method of the 11 tubes of the serial results of the *A. actinomycetemcomitans* bacterial dilution on the media so that Mueller Hinton uses the streaking method.

From previous studies it was said that phytochemical ingredients such as quercetin have antimicrobial activity against gram-positive and gram-negative bacteria.<sup>[9]</sup>

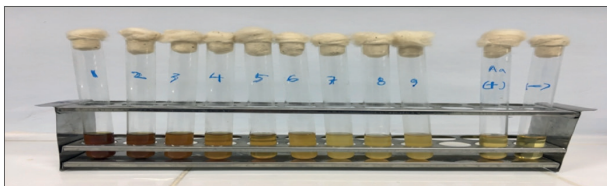
The effectiveness of the extract of okra fruit (*A. esculentus*) is caused by its content in the form of secondary metabolite components such as flavonoids, alkaloids, terpenoids, and quercetin.<sup>[6]</sup>

The antibacterial effect resulting from the extraction of okra against *Aa* is due to the presence of active substances that are soluble and contained in 70% ethanol solvent, which is a flavonoid and is a polar compound, which is generally soluble in polar solvents, namely phenols and

quercetin.<sup>[10]</sup> During the extraction process of okra fruit (*Abelmoschus esculentus*) ethanol solvent is used because ethanol is a polar solvent that has a hydroxyl group (OH) that participates in the formation of hydrogen bonds which is the cause of the liquid is difficult to evaporate when compared with other organic compounds.<sup>[11]</sup>

Quercetin has many biological properties such as antioxidants, nerve protection, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, and anti-obesity.<sup>[12]</sup> It has been widely used in herbal medicine as traditional medicine for hundreds of years.<sup>[13]</sup>

The antibacterial potential of quercetin against the *Aa* bacteria is caused because quercetin has the ability to react to form complex components with metals such as Ag, Au, and Fe.<sup>[12]</sup> This is one reason that quercetin has potential antimicrobial activity.<sup>[14]</sup> Antibacterial activities of quercetin are mechanism against the cytoplasmic membrane of the bacteria, which is damaged through the perforation action of the quercetin. The inhibition of both energy metabolism and the synthesis of nucleic acids is another mechanism.<sup>[15]</sup> Flavonoids as antimicrobials, which are one of the active ingredients of okra fruit extract,



**Figure 3:** Results of serial dilution of okra fruit extract on *Aggregatibacter actinomycetemcomitans* bacteria. The first tube contains 100% okra fruit extract. Tube 2 contains 50% okra extract concentration. Tube 3 contains 25% okra extract concentration. Tube 4 contains 12.5% okra extract concentration. Tube 5 contains 6.25% okra extract concentration. Tube 6 contains 3.125% okra extract concentration. Tube 7 contains 1.563% okra extract concentration. Tube 8 contains 0.78% okra extract concentration. Tube 9 contains 0.39% okra extract concentration. The tube (+) is a positive control. Tube (-) is a negative control

**Table 3: Tukey honestly significant difference test for bacterial *Aggregatibacter actinomycetemcomitans* between concentration**

Group	N	Subset for alpha = 0.05			
		1	2	3	1
Concentration of 3.125%	3		13.0000		
Concentration of 1.565%	3			26.3333	
Positive control	3				120.6667
Sig.			1.000	1.000	1.000

**Table 1: Results of the number of *Aggregatibacter actinomycetemcomitans* bacterial colonies**

Tube	Concentration of okra fruit extract	Number of <i>Aa</i> bacterial colonies (CFU/mL)		
		Treatment 1	Treatment 2	Treatment 3
4	12.5%	-	-	-
5	6.25%	-	-	-
6	3.125%	11	15	13
7	1.565%	25	28	26
(+)	100% + bacteria	116	126	120
(-)	100% without bacteria	-	-	-

Table 1 shows that the minimal inhibitory concentration of okra fruit extract on *Aggregatibacter actinomycetemcomitans* bacteria is on the sixth tube at a concentration of 3.125% and the minimal bactericidal concentration is on the fifth tube at a concentration of 6.25%

**Table 2: One-way analysis of variance test for bacterial *Aggregatibacter actinomycetemcomitans* between groups**

	Sum of squares	df	Mean square	F	Sig.
Between groups	20,668.667	2	10,334.333	979.042	0.000*
Within groups	63.333	6	10.556		
Total	20,732.000	8			

\*Significant

have three mechanisms of action in killing microbes, the first possibility is to inhibit the synthesis of nucleic acids, the second is to inhibit the function of cell membranes, and the third is to inhibit the metabolism in bacterial cells, from all three aspects, flavonoids can cause damage to permeability in bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial deoxyribonucleic acid. The mechanism of action of flavonoids inhibits the function of cell membranes to form complex compounds with extracellular proteins that can damage bacterial cell membranes and is followed by the release of intracellular compounds.<sup>[16]</sup> Flavonoids have the ability to inhibit cell membrane function by interfering with the permeability of cell membranes and inhibiting the binding of enzymes, such as ATPase and phospholipase. The correlation between antibacterial activity and membrane disorders supports the theory that flavonoids can show antibacterial activity by reducing the fluidity of bacterial cell membranes.

Therefore, the results showed that there was a significant decrease in the number of Aa colonies in the administration of okra fruit extract with a concentration of 3.125%, while in the administration with a concentration of 6.25% there was no growth of Aa bacteria. On the basis of the role of the flavonoid content of okra fruit extract as aforementioned, okra fruit extract had the power to kill Aa bacteria, which was shown by the MIC in the administration of 3.125%, whereas the MBC was at 6.25%.

The okra fruit extract effectively kills the Aa bacteria, which is the bacterium that causes AP as indicated by MIC at a concentration of 3.125% and MBC at a concentration of 6.25%.

### Acknowledgement

The authors would like to thank Bela P. Cida for the help in conducting this research.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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# EFFECTIVENESS OF OKRA FRUIT (ABELMOSCHUS ESCULENTUS) EXTRACT ON AGGREGATIBACTER ACTINOMYCETEMCOMITANS (Aa) **GROWTH**

(in vitro laboratory experimental study)

**Running title:** Effect Okra fruit(Abelmoschus esculentus) extract against aggregatibacter actinomycetemcomitans (Aa)bacteria

## **Abstract:**

**Background:** Aggressive periodontitis (AP) is a complex disease caused by microbial changes and cellular dysfunction which is characterized by rapid loss of attachment and bone damage to the tooth surface. The majority of periodontal pathogens are Gram-negative anaerobes and Aggregatibacter actinomycetemcomitans (Aa) which has often been associated with aggressive periodontitis. Abelmoschus esculentus (okra) contains secondary metabolite components, such as alkaloids, terpenoids, flavonoids, flavonoids found in plants are known for their antibacterial effects because of their ability to reduce the permeability of bacterial cell walls.

**Aim:** To determine that okra fruit extracts are effective in inhibiting growth and killing the Aa bacteria which are bacteria that cause aggressive periodontitis.

**Materials and Method:** To detect minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) used serial dilution test.

**Results:** One Way Anova test showed a difference with significance  $p = 0.000$ . Whereas, Tukey HSD test showed a significant difference between okra fruit extract group with positive control concentrations of 100%, 3.125%, 1.565%.

**Conclusion:** The okra fruit extract effectively kills the Aa bacteria which is the bacterium that causes aggressive periodontitis as indicated by MIC at a concentration of 3.125% and MBC at a concentration of 6.25%.

## Abstract

**Aim:** To determine that okra fruit extracts are effective in inhibiting growth and killing the Aa bacteria which are bacteria that cause aggressive periodontitis. **Materials and Method:** Aggregatibacter actinomycetemcomitans (Aa) ATCC 4371 strain Y3 serotype b bacteria taken from the Stock Research Center of the Faculty of Medicine, Airlangga University which were bred on the mueller hinton media with the inclusion criteria that Identification of bacteria from the stock shows that the bacterium is Aa and showed the growth of bacteria in the Mueller Hinton media with a number of colonies between 30- 300 colony forming units (CFU) / ml. Culture media containing Aa bacteria was incubated for 1 x 24 hours at 37°C after it was diluted

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**Commented [MOU2R1]:**  
(in vitro laboratory experimental study)

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•it is not novel topic so remove Context / Background and Redefine Materials and Method and prepare abstract upto 250 words.  
•In abstract, material method section need to highlight, type of study, sampling method, no. of samples, grouping, brief idea of study method and applied statistical test.

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1 according to McFarland standard 0.5 (1.5x10<sup>8</sup> CFU/ml).. Fresh okra fruit derived from *Materia*  
2 *Medica* for extract was prepared. Serial serial dilution or dilution methods of 1: 2 (w / v) are  
3 used for detection of minimal inhibitory concentration (MIC) and minimal bactericidal  
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8 causes aggressive periodontitis as indicated by MIC at a concentration of 3.125% and MBC at  
9 a concentration of 6.25%.

10 **Key-words:** *Aggregatibacter actinomycetemcomitans* (Aa), aggressive periodontitis, minimal  
11 bactericidal concentration (MBC), minimal inhibitory concentration (MIC), okra fruit extract

#### 12 **Key Messages**

13 Ekstrak buah okra (*Abelmoschus esculentus*) has many benefits. This is because okra contains  
14 secondary metabolite components, such as alkaloids, terpenoids, flavonoids. Flavonoids found  
15 in plants are known for their antibacterial effects because of their ability to reduce the  
16 permeability of bacterial cell walls. Aggressive periodontitis (AP) is a complex disease caused  
17 by microbial changes and cellular dysfunction which is characterized by rapid loss of  
18 attachment and bone damage to the tooth surface. The majority of periodontal pathogens are  
19 Gram-negative anaerobes and *Aggregatibacter actinomycetemcomitans* (Aa) which has often  
20 been associated with aggressive periodontitis.  
21

#### 22 **Introduction:**

23 Periodontitis is an inflammation that affects the supporting tissues of teeth caused by  
24 microorganisms and can cause progressive damage to the periodontal ligament, alveolar bone  
25 and is accompanied by pocket formation. Periodontitis causes permanent tissue destruction  
26 characterized by chronic inflammation, migration of the fused epithelium to the apical, loss of  
27 connective tissue and loss of alveolar bone.<sup>[1]</sup>

28 Aggressive periodontitis (AP) is a complex disease caused by microbial changes and cellular  
29 dysfunction which is characterized by rapid loss of attachment and bone damage to the tooth  
30 surface.<sup>[2]</sup> The majority of periodontal pathogens are Gram-negative anaerobes and  
31 *Aggregatibacter actinomycetemcomitans* (Aa) which has often been associated with aggressive  
32 periodontitis.<sup>[3]</sup> The role of this bacterium in the pathogenesis of periodontitis is due to its  
33 ability to attach to epithelial cells, produce many virulent factors such as extra-cellular matrix  
34

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1 proteins, proteases, collagenase, endotoxin (LPS), bacteriocins, hemotactic inhibitors,  
2 leukotoxins, cytotoxins, toxic metabolic substances, immunosuppressive proteins, etc., The  
3 leukotoxin produced by *A. actinomycetemcomitans* (and the JP2 genotype where clinical  
4 evidence support a causative role in disease progression).<sup>[4]</sup>

5 The use of synthetic drugs is not only expensive for the treatment of a disease, but also has  
6 toxicity and adverse side effects. This type of situation causes the need to look for new drug  
7 alternatives to treat a disease. Herbal alternatives have enormous potential to develop new  
8 drugs that are very useful for treatment and strong and effective antibacterial agents.<sup>[5]</sup>

9 *Abelmoschus esculentus* (okra) has many benefits. This is because okra contains secondary  
10 metabolite components, such as alkaloids, terpenoids, flavonoids, etc..<sup>[6]</sup> Flavonoids found in  
11 plants are known for their antibacterial effects because of their ability to reduce the  
12 permeability of bacterial cell walls.<sup>[7]</sup>

13 Because of the explanation above, the researchers decided to prove that okra fruit extract was  
14 effective in inhibiting and killing Aa bacteria which are the bacteria that cause aggressive  
15 periodontitis. From the results of this research, it is expected that it can be used as a therapy  
16 for aggressive periodontitis.

17

## 18 **Materials and Methods:**

### 19 **Setting and Design:**

20 This was an experimental laboratory experiment using a post-test only control group design  
21 that had been conducted by ethical clearance test at Universitas Airlangga, Faculty of Dental  
22 Medicine with Health Research Ethical Clearance Commission number of 112/HRECC.  
23 FODM/VII/2018.

### 24 **Sampling criteria:**

25 This study uses *Aggregatibacter actinomycetemcomitans* (Aa) (Aa) ATCC 4371strain Y3  
26 serotype b bacteria taken from the Stock Research Center of the Faculty of Medicine, Airlangga  
27 University with spesification ATCC 43718, which were bred on the Mueller Hinton media with  
28 the inclusion criteria that Identification of bacteria from the stock shows that the bacterium is  
29 Aa and showed the growth of bacteria in the Mueller Hinton media with a number of colonies  
30 between 30- 300 colony forming units (CFU) / ml.

### 31 **Study Method:**

32 *Aggregatibacter actinomycetemcomitans* (Aa)ATCC 4371strain Y3 serotype bacterial stock  
33 was taken into the Brain Heart Infusion Broth (BHIB) culture media. Culture media containing

1 Aa bacteria was incubated for 1 x 24 hours at 37°C after it was diluted according to McFarland  
2 standard 0.5 (1.5x10<sup>8</sup> CFU/ml). Furthermore, the bacteria were ready to be tested.

3 Okra Fruit Extract Making.<sup>[7]</sup>, Fresh okra fruit derived from Materia Medica for extract was  
4 prepared. Samples of okra fruit were cut into pieces and weighed 200 grams then Put into a jar  
5 and add 70% of ethanol as much as 300 ml. Maceration for 24 hours at room temperature. After  
6 24 hours, the solution is filtered or separated using a Buchner filter. Filtering residue was  
7 aerated and maceration was done up to 3 times. The sieve 1-3 is mixed and concentrated with  
8 a Rotary Vacum Evaporator at 40°C until a concentrated extract is obtained. To obtain various  
9 concentrations, serial serial dilution or dilution methods of 1: 2 (w / v) are used.

10 Antibacterial Test Using the Dilution Serial Method.<sup>[8]</sup>, Preparation of Aa bacteria stored in  
11 BHIB media in an incubator at 37 °C was taken with a sterile ose needle was taken with sterile  
12 ose needles. The Mueller Hinton media was embed by scratching. The bacteria that had been  
13 scratched on Mueller Hinton media were incubated in an incubator at 37°C for 1 x 24 hours.  
14 The scratched bacteria were taken from the Mueller Hinton media using a sterile ose needle. It  
15 was put in the BHI-B until the turbidity was the same as the McFarland 0.5 standard. Eleven  
16 sterile test tubes were prepared. Each test tube was labeled 1-9 (concentrations of 100%, 50%,  
17 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.78%, 0.39%), then tube 10 was given K(+) label which  
18 was a positive control. Tube 10 contained the bacterial suspension which was equivalent to  
19 McFarland 0.5 turbidity standard. Tube 11 was labeled with K(-) which was a negative control.  
20 This tube contained okra fruit extract with a concentration of 100%. Tube 1 was filled with 4  
21 ml concentration of 100% okra fruit extract. Tubes 2-9 were filled with 2 ml of BHI-B liquid  
22 media. Two ml of solution from tube 1 was put in tube 2. It was mixed until homogeneous so  
23 that the concentration of 50% was obtained. The same thing was done up to tube 9 until all  
24 extract concentrations were obtained with a ratio of 1:2 (w/v). To test turbidity, bacterial  
25 suspension media was taken which had been equalized with Mc. Clarland 0.5 turbidity standard  
26 of 0.1 ml and put into test tubes in 1-9 labels (concentrations of 100%, 50%, 25%, 12.5% ,  
27 6.25%, 3.125%, 1.563%, 0.78%, and 0.39%). Then, all the tubes were put into Anaerobic jars  
28 are an airtight sealed container used for the cultivation of anaerobic microorganismsthen  
29 incubated at 37°C for 1 x 24 hours with 3 times the incubation repetition. After one incubation,  
30 turbidity was observed. If the turbidity of the tube was still equivalent or more turbid than the  
31 positive control (K+) tube containing the bacterial suspension Mc. Farland 0.5, it means that  
32 bacteria can still thrive. However, when the solution in the tube appeared to be clearer than the  
33 K (+) tube, the growth of bacteria began to be inhibited. This was what showed the Minimum  
34 Inhibition Concentration (MIC). After observing turbidity, a Total Plate Count (TPC) test was

1 conducted to determine bacteriostatic and bacteriocide properties. The TPC test was carried  
2 out on Mueller Hinton agar media containing concentrations of extracts from tubes that looked  
3 the clearest. It was made 3 times of treatment or 3 petri dishes. Furthermore, each petri dish  
4 was incubated at 37oC for 1 x 24 hours. The number of colonies was then counted.

#### 5 **Statistical analysis**

6 The data obtained were the number of bacterial colonies with the Colony Forming Unit (CFU).  
7 Data were then tabulated and analyzed using the SPSS 20 (IBM, New York, USA).

8 Test the data distribution was done with the Kolmogorov-Smirnov test to determine whether  
9 the data could be normally distributed. To identify whether the collected data was  
10 homogeneous, a variance homogeneity test was performed using the Levene test with  $\alpha > 0.05$ .  
11 Furthermore, the parametric test using Anova Test was used to identify the significance of  
12 differences in the number of bacterial colonies between the study groups. All analyses were  
13 tested at the significance level of 0.05.

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#### 17 **Results:**

18 From the three treatments, the number of Aa bacterial colonies from the positive control tube  
19 TPC test, negative control, tube 4, tube 5, tube 6 and tube 7 were obtained as shown in Figures  
20 2 and 3.

21 Table 1 shows that the MIC of okra fruit extract on *Aggregatibacter actinomycetemcomitans*  
22 (Aa) bacteria is on the 6th tube at a concentration of 3.125% and the MBC is on the 5th tube at  
23 a concentration of 6.25%.

24 Data obtained showed that data were normally distributed based on the Kolmogorov-Smirnov  
25 Test normality test, then Levene homogeneity analysis test showed that data were  
26 homogeneous with  $p = 0.215 (> 0.05)$

27 Table 2. The results of statistical data analysis using the One Way Anova test (table 2) obtained  
28 significance with ( $p = 0.000$ ) then if  $p < 0.05$  indicates a significant difference from the positive  
29 control group, 1.565% concentration and 3.125% concentration.

30 Table 3. Data from statistical analysis using the Tukey HSD test can be seen the difference that  
31 there is a significant significant difference between the okra fruit extract groups with positive  
32 control concentrations of 100%, 3.125%, 1.565%. This means that there are significant  
33 differences in inhibiting / killing A.a

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**Discussion:**

Based on the results of data analysis from the One Way Anova test in Table 1, the p value is 0,000, indicating that if  $p < 0.05$ , it means that there is a significant difference between the control group and the treatment group. Based on the results obtained indicate that the administration of natural okra (*Abelmoschus esculentus*) extracts is effective in inhibiting or killing the Aa bacteria which are predominant bacteria as a cause of aggressive periodontitis.

From various studies, phytochemicals are materials that can inhibit and kill microbes both in vitro and in vivo. Phytochemicals such as quercetin have been widely studied as antimicrobial agents against Gram-positive and Gram-negative.<sup>[9]</sup>

The effectiveness of the extract of okra fruit (*Abelmoschus esculentus*) is caused by its content in the form of secondary metabolite components such as flavonoids, alkaloids, terpenoids, and Quercetine.<sup>[6]</sup>

The antibacterial effect resulting from the extraction of okra against Aa is due to the presence of active substances that are soluble and contained in 70% ethanol solvent, which is a flavonoid is a polar compound which is generally soluble in polar solvents, namely phenols and quercetin.<sup>[10]</sup> Ethanol solvents are used in the process of extracting natural materials because ethanol is a polar solvent that has a hydroxyl group (OH), in which the hydroxyl group in ethanol is participating in the formation of hydrogen bonds which is the cause of the liquid is difficult to evaporate when compared to other organic compounds which have molecular mass same.<sup>[11]</sup>

Quercetin has many biological properties such as antioxidants, nerve protection, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, and anti-obesity.<sup>[12]</sup> Quercetin has been widely used in herbal medicine for traditional medicine for hundreds of years.<sup>[13]</sup>

The antibacterial potential of quercetin against the Aa bacteria is caused because Quercetin has the ability to react to form complex components with metals such as Ag, Au, and Fe<sup>[12]</sup> This is a cause that Quercetin has very strong antimicrobial activity.<sup>[14]</sup> Antibacterial activities of quercetin are mechanism against the cytoplasmic membrane of the bacteria is damaged through the perforation action of the quercetin. The inhibition of both energy metabolism and the synthesis of nucleic acids is another mechanism,<sup>[15]</sup> Flavonoids as antimicrobials, which are one of the active ingredients of okra fruit extract, have three mechanisms of action in killing microbes, the first possibility is to inhibit the synthesis of

1 nucleic acids the second is to inhibit the function of cell membranes and the third is to inhibit  
2 metabolism in bacterial cells, from all three aspects flavonoids can cause damage to  
3 permeability in bacterial cell walls, microsomes, and lysosomes as a result of interactions  
4 between flavonoids and Bacterial DNA. The mechanism of action of flavonoids inhibits the  
5 function of cell membranes is to form complex compounds with extracellular proteins that can  
6 damage bacterial cell membranes and are followed by the release of intracellular  
7 compounds.<sup>[16]</sup>Flavonoids have the ability to inhibit cell membrane function by interfering with  
8 the permeability of cell membranes and inhibiting the binding of enzymes, such as ATPase and  
9 phospholipase. The correlation between antibacterial activity and membrane disorders supports  
10 the theory that flavonoids can show antibacterial activity by reducing the fluidity of bacterial  
11 cell membranes.

12 Therefore, the results of the study showed a decrease in the number of Aa colonies by giving  
13 concentration of okra fruit extract 3.125% even did not show any growth in the administration  
14 of concentration of okra fruit extract (*Abelmoschus esculentus*) 6.25%.Based on the role of the  
15 flavonoid content of okra fruit extract above, okra fruit extract had the power to kill Aa bacteria  
16 which was shown by the minimum inhibitory concentration (MIC) in the administration of  
17 3.125%, while the minimum bactericidal concentration (MBC) at 6.25%.

18 **Conclusion:** The okra fruit extract effectively kills the Aa bacteria which is the bacterium that  
19 causes aggressive periodontitis as indicated by MIC at a concentration of 3.125% and MBC at  
20 a concentration of 6.25%

21  
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24 **Conflict of interest:** There is no conflict of interest in this research

25

#### 26 **Abbreviations**

27 Aa : *Aggregatibacter actinomycetemcomitans*

28 MIC : Minimal inhibitory concentration

29 MBC : Minimal bactericidal concentration

30 AP : Aggressive periodontitis

31 DNA : Deoxyribonucleic acid

32 ATP-ase : ATP synthase

33 CFU : Colony forming unit

1 ATCC : American Type Culture Collection

2

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22 Table 1. Results of the number of *Aggregatibacter actinomycetemcomitans* (Aa) bacterial  
 23 colonies.

24 Table 1 shows that the MIC of okra fruit extract on *Aggregatibacter actinomycetemcomitans*  
 25 (Aa) bacteria is on the 6th tube at a concentration of 3.125% and the MBC is on the 5th tube at  
 26 a concentration of 6.25%.

Tube	Concentration of Okra fruit extract	Number of Aa Bacterial Colonies (CFU/ml)		
		Treatment 1	Treatment 2	Treatment 3
4	12.5 %	-	-	-
5	6.25 %	-	-	-
6	3.125%	11	15	13
7	1.565 %	25	28	26



(+)	100% bacteria	+ 116	126	120
(-)	100 % without bacteria	-	-	-

1  
2 Table 2. One Way Anova test for bacterial A. actinomycetemcomitans between groups

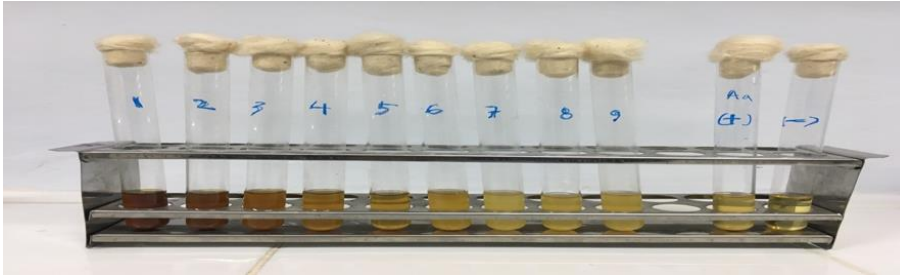
	Sum of Squares	df	Mean Square	F	Sig.
<b>Between Groups</b>	20668.667	2	10334.333	979.042	.000*
<b>Within Groups</b>	63.333	6	10.556		
<b>Total</b>	20732.000	8			

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4 \*Significant

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11 Table 3. Tukey HSD test for bacterial A. actinomycetemcomitans between concentration

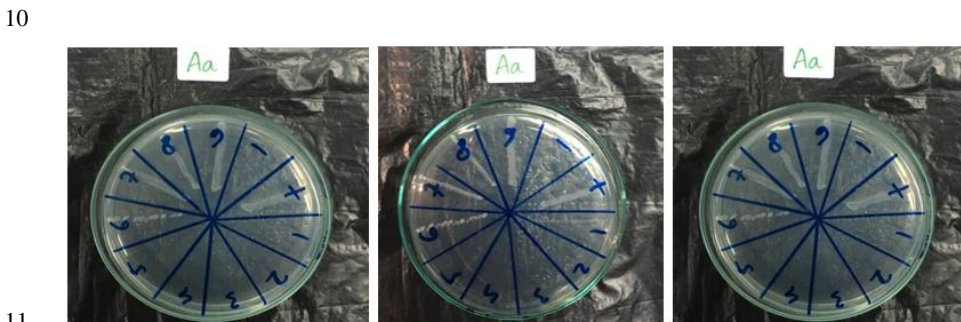
group	N	Subset for alpha = .05		
	1	2	3	1
<b>kons.3.125%</b>	3	13.0000		
<b>kons.1.565%</b>	3		26.3333	
<b>kontrol pos</b>	3			120.6667
<b>Sig.</b>		1.000	1.000	1.000

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2 Figure 1. Results of serial dilution of okra fruit extract on Aa bacteria.

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4 The first tube contains 100% okra fruit extract. Tube 2 contains 50% okra extract concentration.  
5 Tube 3 contains 25% okra extract concentration. Tube 4 contains 12.5% okra extract  
6 concentration. The 5 contains 6.25% okra extract concentration. Tube 6 contains 3.125% okra  
7 extract concentration. Tube 7 contains 1.563% okra extract concentration. Tube 8 contains  
8 0.78% okra extract concentration. Tube 9 contains 0.39% okra extract concentration. The tube  
9 (+) is a positive control. Tube (-) is a negative control.



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12 Figure 2. Results of scratches from 11 test tubes that showed the presence of Aa bacteria growth  
13 in Mueller Hinton media from 3 replications.

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'jioh\_294\_18'

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