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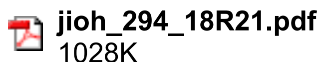
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Original Research

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

Muhammad Luthfi[1], Yuliati Yuliati[1], Aqsa S. Oki[1], Agung Sosiawan[2], Bella P. Cida[3]

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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 × 108 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.[1]

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.[2] The majority of periodontal pathogens are Gram-negative anaerobes and *Aggregatibacter actinomycetemcomitans* (*Aa*), which has often been associated with AP.[3] 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.[4]

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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.[5]

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

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 × 24h at 37°C, after which it was diluted according to McFarland standard 0.5 (1.5 × 108 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.[7] Samples of okra fruit were cut into pieces and weighed 200g, then put into a jar, and 70% of ethanol was added to make the volume to 300mL. Maceration was carried out for 24h at room temperature. After 24h, 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.[8] 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 × 24h. 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 4mL concentration of 100% okra fruit extract. The tubes 2–9 were filled with 2mL 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 0.1mL 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 bacteriocide 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 × 24h. 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

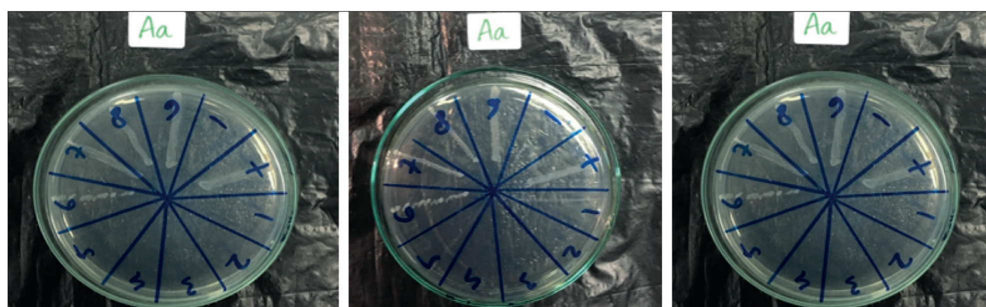

Figure 1: Results of scratches from 11 test tubes that showed the presence of *Aggregatibacter actinomycetemcomitans* bacteria growth in Mueller Hinton media from three replications

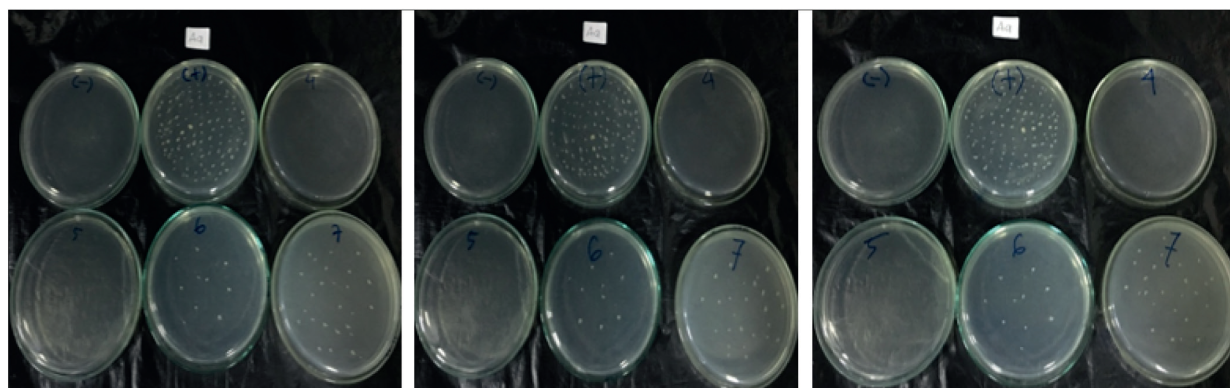

Figure 2: Total plate count test on Mueller Hinton media from positive control tube, negative control tube, number 4 tube, number 5 tube, number 6 tube, and number 7 tube from three replications

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.[9]

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.[6]

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.[10] 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.[11]

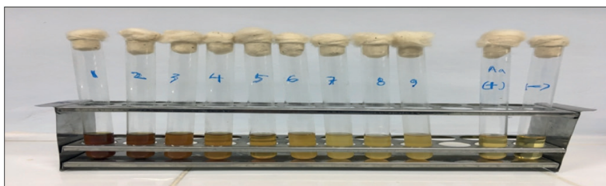

Figure 3: Results of serial dilution of okra fruit extract on *Aggregatibacter actinomycetemcomitans* bacteria. The first tube contains 100% okra fruit extract. Tube 2 contains 50% okra extract concentration. Tube 3 contains 25% okra extract concentration. Tube 4 contains 12.5% okra extract concentration. 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

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

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.[12] This is one reason that quercetin has potential antimicrobial activity.[14] 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.[15] Flavonoids as antimicrobials, which are one of the active ingredients of okra fruit extract,

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 *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	-	-	-

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.[16] 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%.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Research

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

Muhammad Luthfi[1], Yuliati[1], Aqsa S. Oki[1], Agung Sosiawan[2], Bella P. Cida[3]

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Abstract

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

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Introduction

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

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

AQ3

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 × 24h at 37°C, after which it was diluted according to McFarland standard 0.5 (1.5 × 108 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.[7] Samples of okra fruit were cut into pieces and weighed 200g, then put into a jar, and 70% of ethanol was added to make the volume to 300mL. Maceration was carried out for 24h at room temperature. After 24h, 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

AQ11

Preparation of *Aa* bacteria stored in BHIB media in an incubator at 37°C was obtained with a sterile Ose needle.[8] 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 × 24h. 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 4mL concentration of 100% okra fruit extract. The tubes 2–9 were filled with 2mL 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 0.1mL 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 bacteriocide 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 × 24h. 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 2 and 3.

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].

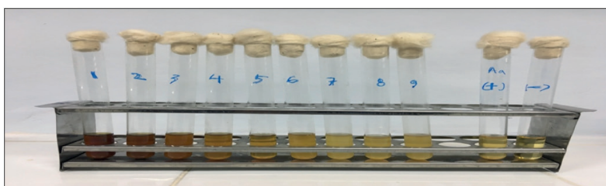

Figure 1: Results of serial dilution of okra fruit extract on *Aggregatibacter actinomycetemcomitans* bacteria. The first tube contains 100% okra fruit extract. Tube 2 contains 50% okra extract concentration. Tube 3 contains 25% okra extract concentration. Tube 4 contains 12.5% okra extract concentration. 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

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.

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

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.[6]

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.[10] 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.[11]

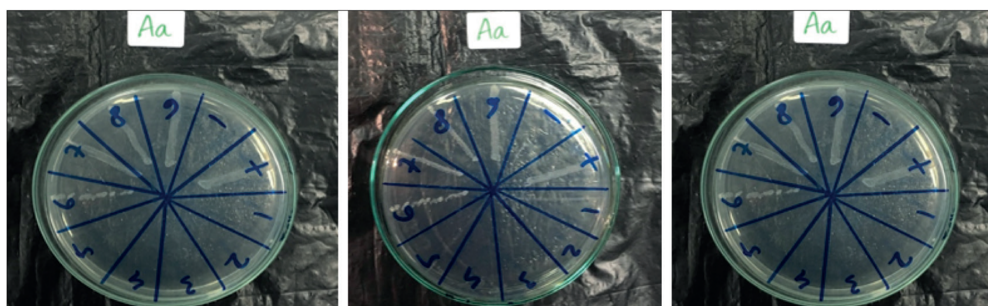

Figure 2: Results of scratches from 11 test tubes that showed the presence of *Aggregatibacter actinomycetemcomitans* bacteria growth in Mueller Hinton media from three replications

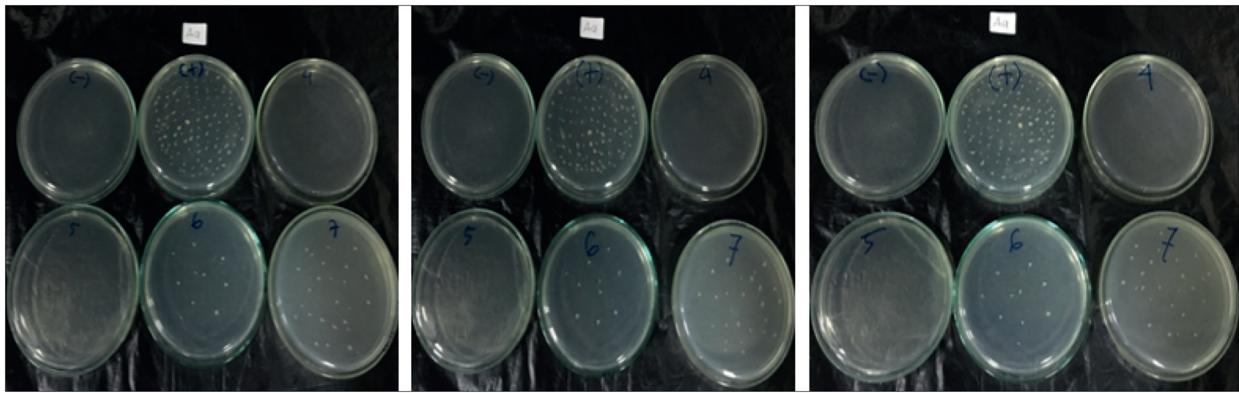

Figure 3: Total plate count test on Mueller Hinton media from positive control tube, negative control tube, number 4 tube, number 5 tube, number 6 tube, and number 7 tube from three replications

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

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	-	-	-

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

AQ5 **Table 3: Tukey honestly significant difference test for bacterial *Aggregatibacter actinomycetemcomitans* between concentration**

Group	*N*	Subset for alpha = 0.05		
	1	2	3	1
kons.3.125%	3	13.0000		
kons.1.565%	3		26.3333	
Kontrolpos	3			120.6667
Sig.		1.000	1.000	1.000

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

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.[12] This is one reason that quercetin has potential antimicrobial activity.[14] 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.[15] 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 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.[16] Flavonoids have the ability to inhibit cell membrane function by interfering with the permeability of cell membranes and

AQ7

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

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Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

AQ2

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AQ5: Please check the usage of "kons.3.125%" "kons.1.565%" and "Kontrolpos" in Table 3 for sense.

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Original Research

Effectiveness of Okra Fruit (*Abelmoschus esculentus*) Extract on *Aggregatibacter actinomycetemcomitans* Growth: *In Vitro* Experimental Study

Muhammad Luthfi[1], Yuliati Yuliati[1], Aqsa S. Oki[1], Bella P. Cida[2]

[1]Department of Oral Biology, Faculty of Dentistry, [2]Faculty of Dentistry, Universitas Airlangga, Jawa Timur, 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 × 108 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 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.[1]

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.[2] The majority of periodontal pathogens are Gram-negative anaerobes and *Aggregatibacter actinomycetemcomitans* (*Aa*), which has often been associated with AP.[3] 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. The leukotoxin produced by *Aa* (and the JP2 genotype

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where clinical evidence supports a causative role in disease progression).[4]

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.[5]

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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

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looked the clearest. It was made three times of treatment or three petri dishes. 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.

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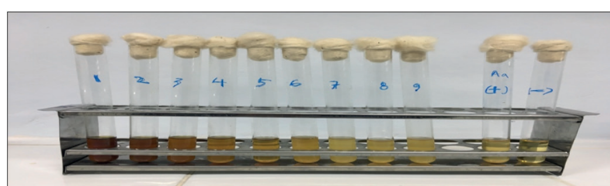

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Table 1 shows that the MIC of okra fruit extract on *Aa* bacteria is on the sixth tube at a concentration of 3.125%, and the minimal bactericidal concentration (MBC) is on the fifth tube at a concentration of 6.25%.

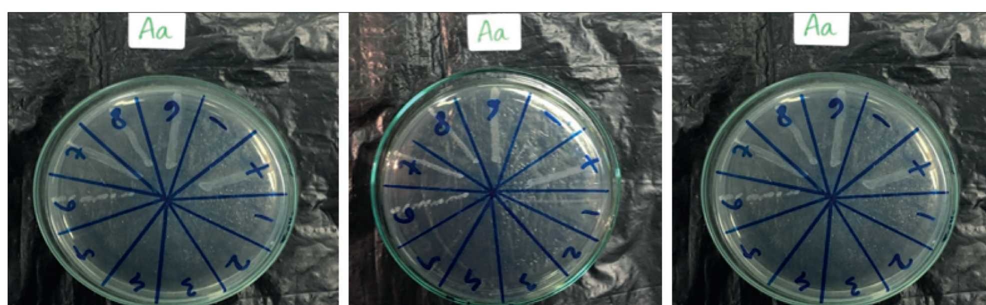
Data obtained showed that they were normally distributed based on the Kolmogorov–Smirnov test normality test, then Levene homogeneity analysis test showed that data were homogeneous with $P = 0.215$ (>0.05) [Table 2]. The results of statistical data analysis using the one-way ANOVA test [Table 2] obtained significance with ($P = 0.000$), then if $P < 0.05$ indicates a significant difference from the positive control group, 1.565% concentration and 3.125% concentration [Table 3]. Data from the statistical analysis using the Tukey HSD test can be seen the difference that there is a significant difference between the okra fruit extract groups with positive control concentrations of 100%, 3.125%, and 1.565%. This means that there are significant differences in inhibiting/killing *Aa*.

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.

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 bacteria.[9]

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.[6]



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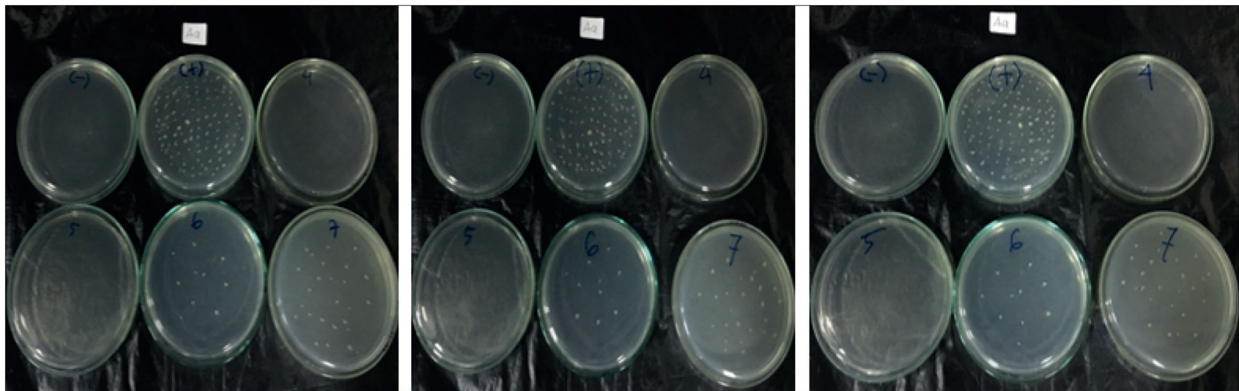

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Tube	Concentration of okra fruit extract	Number of *Aa* bacterial colonies (CFU/mL)		
		Treatment 1	Treatment 2	Treatment 3
4	12.5%	-	-	-
5	6.25%	-	-	-
6	3.125%	11	15	13
7	1.565%	25	28	26
(+)	100% + bacteria	116	126	120
(−)	100% without bacteria	-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

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.
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Within groups	63.333	6	10.556		
Total	20,732.000	8			

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

Group	*N*	Subset for alpha = 0.05		
	1	2	3	1
kons.3.125%	3	13.0000		
kons.1.565%	3		26.3333	
Kontrolpos	3			120.6667
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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.[10] 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.[11]

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Therefore, the results of the study showed a decrease in the number of *Aa* colonies by giving concentration of okra fruit extract 3.125% even did not show any growth in the administration of concentration of okra fruit extract (*A. esculentus*) 6.25%. 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

We acknowledge the Department of Oral Biology, Faculty of Dentistry, Universitas Airlangga, Jawa Timur, Indonesia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Effectiveness of Okra Fruit (*Abelmoschus esculentus*) Extract on *Aggregatibacter actinomycetemcomitans* Growth: *In Vitro* Experimental Study

Muhammad Luthfi[1], Yuliati Yuliati[1], Aqsa S. Oki[1], Bella P. Cida[2]

[1]Department of Oral Biology, Faculty of Dentistry, [2]Faculty of Dentistry, Universitas Airlangga, Jawa Timur, Indonesia

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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 × 108 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 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.[1]

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.[2] The majority of periodontal pathogens are Gram-negative anaerobes and *Aggregatibacter actinomycetemcomitans* (*Aa*), which has often been associated with AP.[3] 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. The leukotoxin produced by *Aa* (and the JP2 genotype

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where clinical evidence supports a causative role in disease progression).[4]

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.[5]

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

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 experiment using a posttest only control group design that had been conducted by ethical clearance test at Universitas Airlangga, Faculty of Dental Medicine with Health Research Ethics Clearance Commission number of 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 × 108 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.[7] 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.[8] 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 were put into anaerobic jars are an airtight sealed container used for the cultivation of anaerobic microorganisms then incubated at 37°C for 1 × 24 h with three times the incubation repetition. 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 bacteriocide properties. The TPC test was carried out on Mueller Hinton agar media containing concentrations of extracts from tubes that

looked the clearest. It was made three times of treatment or three petri dishes. 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 2 and 3.

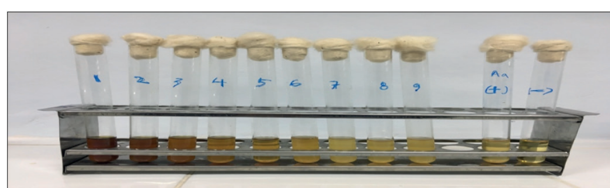

Figure 1: Results of serial dilution of okra fruit extract on *Aggregatibacter actinomycetemcomitans* bacteria. The first tube contains 100% okra fruit extract. Tube 2 contains 50% okra extract concentration. Tube 3 contains 25% okra extract concentration. Tube 4 contains 12.5% okra extract concentration. 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 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 statistical data analysis using the one-way ANOVA test [Table 2] obtained significance with ($P = 0.000$), then if $P < 0.05$ indicates a significant difference from the positive control group, 1.565% concentration and 3.125% concentration [Table 3]. Data from the statistical analysis using the Tukey HSD test can be seen the difference that there is a significant difference between the okra fruit extract groups with positive control concentrations of 100%, 3.125%, and 1.565%. This means that there are significant differences in inhibiting/killing *Aa*.

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.

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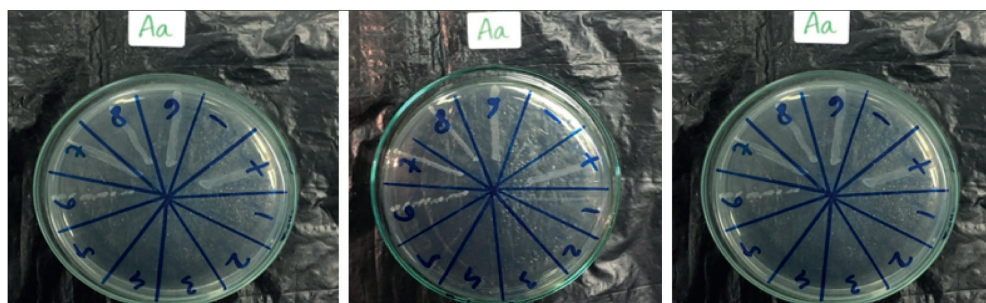

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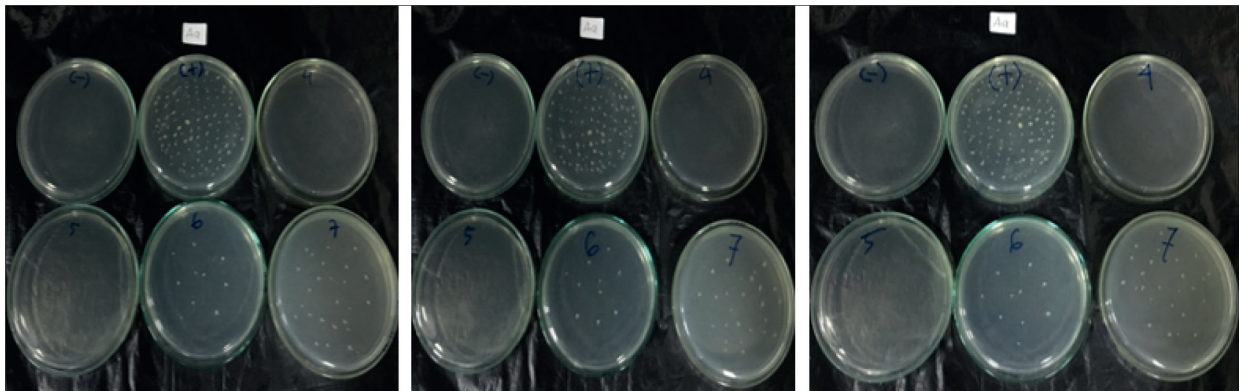

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have three mechanisms of action in killing microbes, the first possibility is to inhibit the synthesis of nucleic acids, the second is to inhibit the function of cell membranes, and the third is to inhibit the metabolism in bacterial cells, from all 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.[16] 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 of the study showed a decrease in the number of *Aa* colonies by giving concentration of okra fruit extract 3.125% even did not show any growth in the administration of concentration of okra fruit extract (*A. esculentus*) 6.25%. 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

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Research

Analysis of Interleukin-10 Anti-inflammatory Cytokines in Salivary Lymphocyte Surface: A Pilot Study

Retno Indrawati[1], Muhammad Luthfi[1], Aqsa S. Oki[1], Yuliati[1], Agung Sosiawan[2], Priyawan Rachmadi[3], Muhaimin Rifai[4]

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Abstract

Aim: The aim of this study was to analyze the expression of interleukin-10 (IL-10) in children with severe early childhood caries (S-ECC) and caries-free children. **Materials and Methods:** This was an observational analytic pilot study performed on children with social factors-ECC (S-ECC), and caries-free children as the objects of research with a cross-sectional study design. Saliva of children aged 4–6 years from the group of caries children in severe and caries-free early childhood was taken. Samples were taken by rinsing with 1.5% sterile NaCl for 30 s and then accommodated in a sterile tube, to get a 40 mL sample from the aforementioned procedure repeated four times. Flow-cytometry test was used to analyze the IL-10 expression. The results of the study were analyzed using the normality test using Shapiro–Wilk, then continued with *t* test using the Statistical Package for the Social Sciences (SPSS) software program, version 20.0 (IBM Corp., Armonk, NY, USA). The data were analyzed by independent *t* test to see the difference between caries-free children and S-ECC. **Results:** The expression of IL-10 in the saliva of children with severe ECC was 3.32 ± 0.79; meanwhile, in caries-free children it was 4.04 ± 0.65. **Conclusion:** The IL-10 expression in children with severe ECC was significantly lower than that of in caries-free children.

Keywords: Interleukin-10 Anti-inflammatory Cytokines, Lymphocyte Cells, Severe Early Childhood Caries

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Introduction

Dental caries is a multifactorial disease due to various factors, namely cariogenic microbes, carbohydrates, and social factors, whereas early childhood caries (ECC) is often found in children with low-socioeconomic conditions.[1] Several studies have recognized the importance of infection of *Streptococci mutans*.[2]

The immune system is a very varied compilation of cells, consisting of two parts of the immune system, namely innate and adaptive. The innate and adaptive immune systems are interrelated, and recognition by innate immune systems can cause the activation of the adaptive immune response.[3] The innate immune system is the first line of host defense against pathogens and recognizes molecules repeatedly against pathogens, which are called pathogen-related molecular patterns through germline-encoded pattern recognition receptors (PRRs) such as toll-like receptors (TLRs).[4]

The components that regulate the immune system, such as an immune regulator cells and regulating cytokines, both natural and acquired as induced by an antigen, plays an important role in controlling various immune responses, both physiological and pathological. Local and systemic interleukin-10 (IL-10) responses have been shown to have pathophysiological relevance in several diseases such as malignancy, infectious diseases, autoimmune diseases, and atopic disorders.[5] Because of

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this, IL-10 can activate signal transducer and activator of transcription 3 (STAT3) in macrophages and T cells to restore and respond to the presence of pro-inflammatory cytokines.[6]

Immunity in the oral cavity's immune system has an important role that is balancing the amount of microbes in the mouth. The microbial activity in oral cavity can be fluctuatives, due to pathogen situations. The mouth is the entrance and exchange with the outside environment. Therefore, homeostasis factors must be evaluated and controlled by the immune system. The immune response to pathogens involves the rapid activation of the secretion of pro-inflammatory cytokine, which functions to initiate host defenses against microbial invasion. However, excessive inflammatory cytokines in the tissues can cause systemic metabolic and hemodynamic disorders that are harmful to the host. As a result, the immune system has evolved to form anti-inflammatory functions to suppress the production of pro-inflammatory cytokines that function to limit tissue damage and to maintain tissue homeostasis.[7] IL-10 is an anti-inflammatory cytokine that plays an important role in preventing prolonged inflammation.[8]

For dental caries preventions, many efforts had been carries out. The government and supporting health organization ran some prevention programs, such as dental counseling to community. Most of the programs targeted children, pregnant woman, and elderly. They teach how to brush teeth properly, dietary that good for dental health, prevention treatment that can be applied for children, and vaccines.[9] Therefore, this study aimed to analyze the expression of IL-10 in saliva which functions as an anti-inflammatory. The results of this study are expected to be used as a marker of social factors-ECC (S-ECC).

Materials and Methods

This was an observational analytic study using children with S-ECC and caries-free children as the objects of research with a cross-sectional study design. Ethical clearance test at Faculty of Dental Medicine, Universitas Airlangga, Indonesia was performed with Health Research Ethical Clearance Commission (approval number 209/HRECC.FODM/IX/2017).

Sixteen children with S-ECC and caries-free were taken from preschool children aged 4–6 years, in the southern Surabaya region, which had previously been divided into two groups.

Group one were children with a diagnosis of S-ECC characterized by decay, extraction, and filling (def-$t > 6$), whereas, the second group were preschool children who were diagnosed with free caries marked with def-$t = 0$.

5 mL saliva is taken from preschool children with S-ECC and caries free. Sampling was carried out by researchers and trained research assistants using standard protocols. Subjects were asked not to consume food and drink, or brush their teeth for 60 min before the study was conducted. The samples obtained were stored at −80°C for analysis. IL-10 expression was analyzed using flow cytometry, according to Luthfi *et al.*[10]

Statistical analysis: The data were analyzed by independent *t* test to see the difference between caries free and S-ECC.

Results

Data from the results of the study before analysis using the t test, conducted tests of normality and homogeneity using the SPSS Shapiro–Wilk test. The results of this test showed a value of $P > 0.05$, which means that all data were normally distributed and homogeneous. Normality test using Shapiro–Wilk data showed normal distribution, whereas Levene test results showed homogeneous data.

The data obtained indicate that the average IL-10 in the caries-free group was higher than the S-ECC group, but the difference was not statistically significant between caries-free and S-ECC.

Discussion

Based on Figure 1 which is the result of examination using flow cytometry test shows that lymphocytes in saliva severe early childhood caries express IL-10 of 3.31%, while in Figure 2 which is the result of examination using flow cytometry test shows that lymphocytes in caries-free children express IL- 10 at 4.03%. This shows that the S-ECC saliva is less specialized in proinflammatory cytokines and conversely expresses inflammatory cytokines which results in chronic inflammation. The occurrence of chronic inflammation is caused because innate immunity in S-ECC is not as good as in free caries

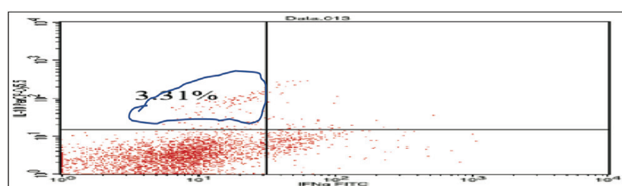

Figure 1: Expression of interleukin-10 (3.31%) from severe early childhood caries salivary after analyzed by flow-cytometry test

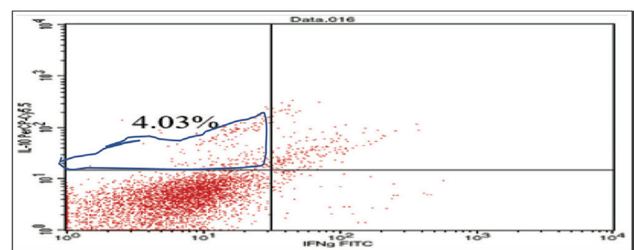

Figure 2: Expression of interleukin-10 (4.03%) from caries free salivary after analyzed by flow-cytometry test

AQ1 **Table 1: Normality test using Shapiro–Wilk interleukin-10 expression from severe early childhood caries and caries free**

Variable	Kolmogorov–Smirnov			Shapiro–Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
IL-10	143	16	200	970	16	844

IL-10 = interleukin-10, Df = degrees of freedom

AQ1 **Table 2: Mean and standard deviation of interleukin-10 expression in severe early childhood caries and caries free analyzed by flow-cytometry test, which was tested using independent *t* test**

Group	*n*	IL-10 expression (%)	
		Mean ± SD	*P*
Caries free	8	4.04 ± 0.89	0.11
S-ECC	8	3.32 ± 0.76	

S-ECC = severe early childhood caries, SD = standard deviation, IL-10 = interleukin-10

so innate immunity is not able to fight the pathogens that cause dental caries.
IL-10 is an anti-inflammatory cytokine produced by innate immunity secreted because of the response of pathogen recognition receptors (PRRs) in contact with pathogen-associated molecular patterns (PAMPs). Secretion of IL-10 during bacterial infection is the most important factor in resolution of infection. ECC has an impact on general health, ranging from local pain, infections, and abscesses.

The results showed the occurrence of decreased IL-10 expression in preschool children with S-ECC compared with in caries-free children. This may be preschool children with S-ECC responding to more antigens in the form of *S. mutans* bacteria, which are relatively high in number compared to children with free caries.[11]

Antigen structures called PAMPs, which will be recognized by PRRs, namely TLRs, are very important to trigger the effect or phase of the innate immune response.[12] TLR2 and TLR4 involved in the introduction of gram-positive and gram-negative bacteria that have been detected in the odontoblast cell membrane in healthy pulp show that odontoblasts are equipped to recognize these pathogens when they diffuse through dentinal tubules during carious infection.[13]

One of the main consequences of TLR activation is an increase in innate immune efficacy, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] One of the main consequences of TLR activation is an increase in the effectiveness of innate immunity, including antimicrobial and cytokine agents and pro-inflammatory chemokines that recruit and activate immune cells.[14] This causes a prolonged increase in inflammatory cytokines in S-ECC, increasing IFN-γ increase expression.[15] which can ultimately cause oral cavity tissue damage that affects general health, ranging from local pain, infections, abscesses, difficulty chewing, malnutrition, indigestion, and trouble sleeping.[16]

Study shows that an increase in pro-inflammatory cytokines occurs in S-ECC; this must be balanced by the host immune system by producing anti-inflammatory cytokines, IL-10. Cluster differentiation 4 ($CD4^+$) memory T cells are developed in response to pathogenic microbes. $CD4^+$ memory T cells prevent the body from fighting pathogens.[17] $CD4^+$ cells also respond as antipathogens,[18] which produce antibodies and cytotoxicity of cluster differentiation 8 ($CD8^+$) T cells,[19] but this does not occur in S-ECC so IL-10 expression in S-ECC saliva is lower than in caries-free children. This study requires larger sample size to evaluate the expression in different age groups and populations.

Conclusion

IL-10 expression in salivary lymphocytes of children with S-ECC is lower than that of caries-free children.

Data availability statement

Dataset can be made available after embargo period due to commercial restrictions.

Financial support and sponsorship

This study was supported by Directorate of Research and Community Services of Directorate General of Research and Development Strengthening from Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

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 againt 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 actinomyceetemcomitans (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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according to McFarland standard 0.5 (1.5x108 CFU/ml).. Fresh okra fruit derived from Materia Medica for extract was prepared. Serial serial dilution or dilution methods of 1: 2 (w / v) are used for detection of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). **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%.

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

~~**Key Messages**~~

~~Ekstrak buah okra (Abelmoschus esculentus)has many benefits. This is because 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.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.~~

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Introduction:

Periodontitis is an inflammation that affects the supporting tissues of teeth 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.[1]

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.[2] The majority of periodontal pathogens are Gram-negative anaerobes and Aggregatibacter actinomycetemcomitans (Aa) which has often been associated with aggressive periodontitis.[3] The role of this bacterium in the pathogenesis of periodontitis is due to its ability to attach to epithelial cells, produce many virulent factors such as extra-cellular matrix

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).[4]
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.[5]
9 Abelmoschus esculentus (okra) has many benefits. This is because okra contains secondary
10 metabolite components, such as alkaloids, terpenoids, flavonoids, etc..[6] Flavonoids found in
11 plants are known for their antibacterial effects because of their ability to reduce the
12 permeability of bacterial cell walls.[7]
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 Universitywith 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 (1.5x108 CFU/ml). Furthermore, the bacteria were ready to be tested.
3 Okra Fruit Extract Making.[7], 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 40ºC 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.[8], Preparation of Aa bacteria stored in
11 BHIB media in an incubator at 37 ^{0}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^{0}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^{0}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

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

Statistical analysis

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

Test the data distribution was done with the Kolmogorov-Smirnov test to determine whether the data could be normally distributed. To identify whether the collected data was homogeneous, a variance homogeneity test was performed using the Levene test with $\alpha > 0.05$. Furthermore, the parametric test using Anova Test 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 2 and 3.

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

Data obtained showed that data 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 statistical data analysis using the One Way Anova test (table 2) obtained significance with ($p = 0.000$) then if $p < 0.05$ indicates a significant difference from the positive control group, 1.565% concentration and 3.125% concentration.

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

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.[9]

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.[6]

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.[10]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. [11]

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

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[12]This is a cause that Quercetin has very strong antimicrobial activity.[14]Antibacterial activities of quercetin are mechanism againt 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,[15]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

nucleic acids the second is to inhibit the function of cell membranes and the third is to inhibit 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 DNA. The mechanism of action of flavonoids inhibits the function of cell membranes 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 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 of the study showed a decrease in the number of Aa colonies by giving concentration of okra fruit extract 3.125% even did not show any growth in the administration of concentration of okra fruit extract (Abelmoschus esculentus) 6.25%.Based on the role of the flavonoid content of okra fruit extract above, okra fruit extract had the power to kill Aa bacteria which was shown by the minimum inhibitory concentration (MIC) in the administration of 3.125%, while the minimum bactericidal concentration (MBC) at 6.25%.

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%

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Abbreviations

Aa	**:** Aggregatibacter actinomycetemcomitans
MIC	: Minimal inhibitory concenration
MBC	: Minimal bactericidal concenration
AP	: Aggressive periodontitis
DNA	: Deoxyribonucleic acid
ATP-ase	: ATP synthase
CFU	: Colony forming unit

1 ATCC : American Type Culture Collection

2

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

Table 1 shows that the MIC of okra fruit extract on *Aggregatibacter actinomycetemcomitans* (Aa) bacteria is on the 6th tube at a concentration of 3.125% and the MBC is on the 5th tube at 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.
Between Groups	20668.667	2	10334.333	979.042	.000*
Within Groups	63.333	6	10.556		
Total	20732.000	8			

3

4 *Significant

5

6

7

8

9

10

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

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

12

13

14

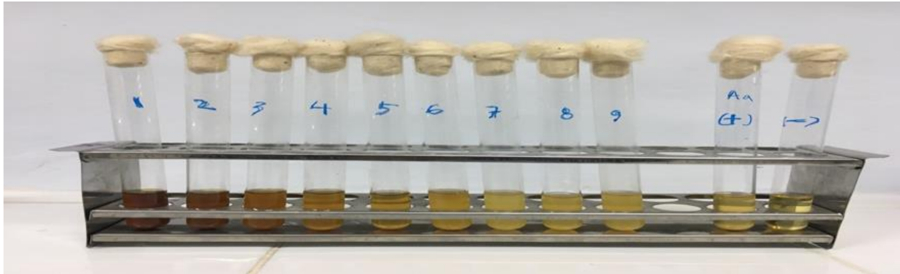

Figure 1. Results of serial dilution of okra fruit extract on Aa bacteria.

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. The 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.

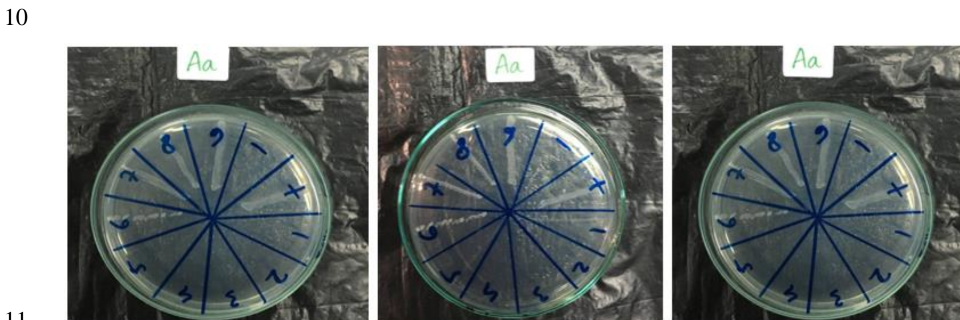

Figure 2. Results of scratches from 11 test tubes that showed the presence of Aa bacteria growth in Mueller Hinton media from 3 replications.

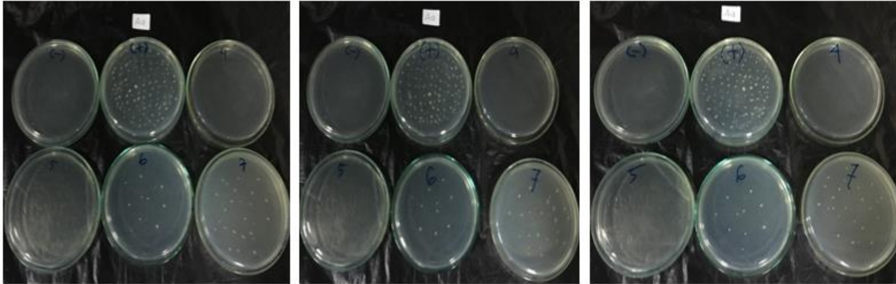

Figure 3. TPC test on Mueller Hinton media from positive control tube, negative control tube, number 4 tube, number 5 tube, number 6 tube, and number 7 tube from 3 replications.

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