

The Effectiveness of *Channa striata* Extract Antimicrobial Effect on Periopathogen Bacteria (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*)

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ABSTRACT

Introduction: Periodontal disease involves polymicrobial which is a multifactorial disease and many host factors are involved in determining an individual's susceptibility. Clinical and experimental evidence has confirmed that certain strains of bacteria in the periodontal environment can cause inflammation and bone damage. This bacterial strain is identified as a periodontal pathogen (periopathogen). Periodontal infection is associated with the presence of periopathogen bacteria, such as *Porphyromonas gingivalis* (Pg), and *Aggregatibacter actinomycetemcomitans* (Aa). *Channa striata* (cork fish) contains a lot of amino acids, fatty acids, and albumin. This species is known to has beneficial aspects which are anti-inflammatory, antimicrobial, antinociceptive, and anticancer properties.

Objective: To determine the effectiveness of *Channa striata* extract as an antimicrobial agent in periopathogen bacteria Pg dan Aa.

Materials and Methods: Bacterial samples were collected from patients with periodontitis. The samples were collected using a paper point/curette and kept it in the transport medium. The Identified Pg and Aa, were divided into 3 groups. Those groups were tested with

channa striata extract with 100% concentration, chlorhexidine, and aquadest. After that, the inhibition zone was measured using the calipers.

Results: The inhibition zone in the group tested with *channa striata* extract was higher for Pg and Aa bacteria compared to the chlorhexidine inhibition zone. The highest inhibition zone is seen in the effect of *channa striata* extract on Pg bacteria, this means that Pg bacteria are more sensitive to *channa striata* extract compared to Aa bacteria.

Conclusions: *Channa striata* extract was effective as antimicrobial therapy in periopathogenic bacteria Pg dan Aa

Keywords: *Aggregatibacter actinomycetemcomitans*, *Channa striata*, Periodontitis, *Porphyromonas gingivalis*.

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INTRODUCTION

Periodontitis is an infectious disease in the tooth-supporting tissue caused by several microorganisms that cause progressive damage to the periodontal ligament and alveolar bone with pocket formation, recession, or both.¹ Periodontal disease involves polymicrobial which is multifactorial diseases and many host factors are involved in determining individual susceptibility to disease.² Like other infectious diseases, the identification of pathogenic bacteria that become the etiology of periodontitis is the first step to the development of an effective therapeutic approach.³ When a specific bacterium overgrows in the subgingival space, this will cause periodontal infections and attachment and bone loss.^{1,2}

Clinical and experimental evidence has confirmed that certain strains of bacteria in the periodontal environment can cause inflammation and bone damage. This bacterial strain is identified as a periodontal pathogen (periopathogen). Only a small fraction of dental biofilm bacteria are identified as pathogens for periodontal tissue. Although the presence of these bacteria in small amounts, it can damage the periodontal structure. Most of the periodontal pathogens are anaerobic bacteria.²

Several experimental evidence has shown that the main etiological agents of periodontal diseases are generally Gram-negative bacteria such as *Aggregatibacter actinomycetemcomitans* (Aa), *Tannerella forsythia* (formerly

called *Bacteroides forsythus*), *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* (Pg). It was also mentioned that periodontal infection was associated with the presence of Pg and Aa periopathogenic bacteria because these bacteria were found in small numbers in healthy subjects, while in periodontal disease, they were large in number.^{4,5}

Among the major periopathogenic bacteria, Pg is one of the main etiological bacteria in pathogenesis and the development of inflammation in periodontal disease because of its ability to attach to the oral epithelium, attack the oral epithelium, and have virulence factors. These periopathogenic bacteria are usually found 10% – 25% in healthy subjects and 79% – 90% in periodontitis subjects.^{4,5,6} Aa are exogenous bacteria, which are also one of the periopathogenic bacteria that cause "true infections" associated with periodontitis in young individuals because it also has virulence factors.^{7,8}

Periodontitis treatment can be surgical or non-surgical. Scaling and root planing (SRP) is a non-surgical treatment which is the gold standard given in treating periodontal diseases. Because this mechanic debridement has limitations, therefore, additional antimicrobial therapy is used to eliminate or reduce the number of pathogenic bacteria. Additional antimicrobial therapy can be given both systemically and locally.^{9,10} About 80% of the world's population uses herbal products for their basic health care

(primary care). Herbal products are preferred over chemical drugs because of their extensive biological activity, higher safety, and lower costs. Side effects of chemical drugs and continuous intake have resulted in antibiotic resistance. Thus, herbal medicines are increasingly being used as food supplements to fight or prevent disease.^{11,12}

Fish is a food that contains good quality protein because of its complete essential amino acid content. Fish can be extracted to get plasma proteins (sarcoplasm) that contain albumin and other nutrients. The most widely used fish extract is currently obtained from cork fish (*Channa striata*).¹³

Channa striata is a freshwater fish, which is famous in the Southeast Asian region because of its protein content and is used as traditional medicine. *Channa striata* has several names depending on location. *Channa striata* is a good source of healthy food among Asians because it contains amino acids, fatty acids, high albumin. Many scientific studies have revealed the biomedical potential of *channa striata*. This species is known to have beneficial anti-inflammatory, antimicrobial, antinociceptive, and anticancer properties.¹⁴

There are more development of treatment using herbal ingredients, this made us interested in knowing the effectiveness of *Channa striata* extract as an antimicrobial agent in periopathogenic bacteria (Pg and Aa)

- patients who continue treatment until the surgical stage
- aged 25 – 45 years
- has no systemic disease
- patients not taking antibiotics in the past 6 months.

Bacterial samples were taken from patients who had signed informed consent that they agreed to be involved in this study. When patients receive surgical treatment (flap opening), bacterial samples are harvested using a paper point/curette in the area of surgery and kept in the transport medium. Within 24 hours, bacterial samples were transported to the laboratories. The identification of PG and AA bacteria was carried out using Vitek system.

The results of bacterial culture were divided into 12 petri dishes with Pg bacteria and 12 petri dishes with Aa bacteria. Each petri dish is marked at the bottom which is divided into 3 regions. Each area was labeled with S (*channa striata* extract concentration of 100%), Ch (positive control /chlorhexidine), and Pg or Aa (negative control / aquadest).

In each bacterial media that has been inoculated with bacteria, 3 holes were made in the S, Ch and Pg or Aa areas with a diameter of 5 mm and a depth of 4 mm using a rigid straw as a substitute for sterile borer. Then in each well, 5 µL of extract, positive control (chlorhexidine), and negative (aquadest) were added. Next, the media is put in a desiccator and placed in an incubator at 37°C for 24 hours. After that, observation and measurement of the inhibition zone were carried out, namely the clear area around the wellbore. By using calipers, inhibition zones are measured in diameter. Measurements were taken by 4 observers and taken an average of the four.

RESULTS

Channa striata extract antibacterial tests have been carried out against Pg and Aa bacteria. Twenty four inhibitory zone measurement data on bacteria were obtained. The data were then analyzed with IBM SPSS Statistics version 25.

Table 1: Comparison of the average inhibition zones of *Channa striata*, chlorhexidine extract bacteria and against PG and AA bacteria

BACTERIA GROUP	CHANNA STRIATA	MEAN	CHLORHEXIDINE	MEAN	AQUADEST	MEAN
PG	U1	24,13	15,61		7	
	U2	20,83	15,14		7	
	U3	22,11	15,89		7	
	U4	23,18	15,23		7	
	U5	22,17	16,23		7	
	U6	18,85	17,46		7	
	U7	21,29	16,99	15,91	7	7
	U8	19,57	16,44		7	
	U9	20,69	15,75		7	
	U10	20,15	16,43		7	
	U11	20,35	14,58		7	
	U12	18,33	15,3		7	
AA	U1	20,55	17,75		7	
	U2	19,62	19,2		7	
	U3	19,1	19,58		7	
	U4	18,55	19,18		7	

U5	23,73		20,9		7
U6	21,8	20,56	19,81	19,33	7
U7	21,76		19,83		7
U8	22,02		18,22		7
U9	18,21		19,58		7
U10	21,72		18,77		7
U11	20,18		20,32		7
U12	19,63		18,92		7

Table 1 shows that the mean number of inhibition zones of the *channa striata* extract group in Pg and Aa bacteria was higher compared to the inhibition zone of the chlorhexidine and aquadest groups. Among the mean inhibition zones of *channa striata* extract inhibition against Pg and Aa bacteria,

it was seen that the inhibition zone of *channa striata* extract was higher in Pg bacteria compared with Aa bacteria. The mean bacterial inhibition zone is also displayed in figure 1.

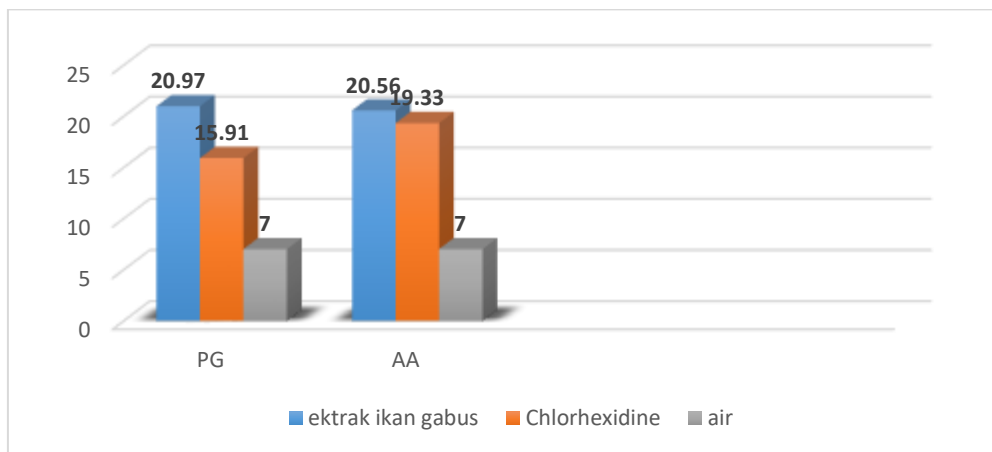


Figure 1: Comparison diagram of the average inhibition zone of *channa striata* extract bacteria and control of Pg and Aa bacteria

From the diagram above it can be seen that the inhibition zone in the group tested with *channa striata* extract was higher for Pg and Aa bacteria compared to the chlorhexidine inhibition zone. The highest inhibition zone

is seen in the effect of *channa striata* extract on Pg bacteria, this means that Pg bacteria are more sensitive to *channa striata* extract compared to Aa bacteria.

Table 2: Post-hoc analysis of the comparison of mean inhibition zones of *channa striata* extract against Pg and Aa bacteria

BACTERIA	INTERVENSION		MEAN DIFFERENCES	P
PG	<i>Channa striata</i>	Chlorhexidine	5,13	0,000
		Aquadest	14,05	0,000
AA	<i>Channa striata</i>	Chlorhexidine	1,31	0,005
		Aquadest	13,57	0,000

In table 2 with post-hoc analysis, it can be seen that the difference in inhibition zone in the treatment group against Pg and Aa bacteria compared to the control group (+) and control (-), which shows a significant value with $P \leq 0.05$.

This shows that a significant difference in the average inhibition zone occurred in the treatment group with *channa striata* extract on Pg and Aa bacteria.

Table 3: Kruskal wallis test results of different inhibition zone tests between groups with *channa striata* extract

	PG	AA
KRUSKAL-WALLIS H	32,32	25,94
DF	2	2
P	0,000	0,000

In table 3, the results of the kruskal wallis different test of inhibition zone test between groups with *channa striata*

extract and in Pg and Aa bacteria obtained $P \leq 0.05$ values. This shows that *channa striata* extract can inhibit bacteria against Pa and Aa bacteria.

DISCUSSION

Periodontal disease has several stages of treatment: the preliminary phase, the nonsurgical phase, the evaluation phase of the nonsurgical phase, the surgical phase, and the restorative phase.¹⁵ Various surgical and non-surgical treatments can be performed in the treatment of periodontal disease depending on the severity of the disease. Scaling and root planning is a non-surgical treatment which is the gold standard given in treating periodontal diseases. In this case, biofilm and sub-gingival calculus are removed mechanically by the dentist using various instruments. However, this mechanical debridement has several disadvantages such as the inability to reach deep pockets and furcation areas. Therefore, additional antimicrobial therapy is used to eliminate or reduce the number of pathogenic bacteria in these difficult places.¹⁶ This additional therapy was carried out so that the pathogenic organisms that cannot be accessed by mechanical debridement can be reduced in number or eliminated by antimicrobial therapy.^{9,10} Additional antimicrobial therapy can be given both systemically and locally. Both therapies have their limitations and strengths.

In this study, patients with a diagnosis of periodontitis undergo surgical treatment by an open flap to reach the areas that cannot be reached by SRP.^{17,18} Pg and Aa bacteria that have been taken from patients were tested for sensitivity by giving *channa striata* extract (treatment) and chlorhexidine (control +) and aquadest (control -)

In this study, the one way ANOVA test showed a significant difference between the average inhibition zones between the *channa striata* extract treatment groups against the bacteria with the chlorhexidine group and the negative control with a value ($P \leq 0.05$). This shows that *channa striata* extract has the ability as an antibacterial against Pg and Aa bacteria.

From the test, *channa striata* extract contains a protein with mostly albumin, fat, and some minerals such as Zn, Cu, and Fe. *Channa striata* meat contains up to 25.1% protein and 6.224% of the protein is albumin. The glycoprotein on *channa striata* has antibacterial properties. The bioactive of peptide also has antibacterial properties. The albumin compounds, minerals, and zinc can inhibit the growth of pathogenic bacteria. The arginine amino acid on *channa striata* extract has anti-bacterial activity.¹⁹ Arginine has a positive charge that can be bound with the negative surface of bacterial cells such as lipopolysaccharide (LPS), teichoic acid and phospholipids. Arginine amino acids can inhibit bacterial coaggregation, change cell communication and alter bacterial cell metabolism in a broad spectrum of the oral cavity.

The amino acid glutamine plays an important role in the body's defense system. Amino acids can limit the availability of nutrients containing nitrogen so that they affect microorganisms.²⁰ Our results were in agreement with research conducted by Aurelio Rosa et al. They stated that the administration of glutamine can prevent the

development of periodontitis in a group of mice with periodontitis.^{21,22}

CONCLUSION

Channa striata extract was effective as antimicrobial therapy in periopathogenic bacteria (*Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*).

CONFLICT OF INTEREST

There was no conflict of interest

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