DENTINO
JURNAL KEDOKTERAN GIGI
Vol IX. No 1. March 2024

ANTIBACTERIAL EFFECTIVENESS TEST OF KECAPI LEAVES EXTRACTS (*Sandoricum koetjape*) AGAINST THE GROWTH OF *Porphyromonas gingivalis*

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ABSTRACT
Background: *Chronic periodontitis is a disease of the oral cavity that is influenced by the presence of plaque bacteria caused by* Porphyromonas gingivalis *which can cause chronic infection of the periodontal tissue. Kecapi leaf (*Sandoricum koetjape*) extract contains active compounds in the form of flavonoids, saponins, alkaloids, steroids, phenolics, and triterpenoids which function as antibacterial against the growth of* Porphyromonas gingivalis*. Kecapi leaf extract is used as an alternative to using herbal gluconate to prevent infection caused of* Porphyromonas gingivalis*.* ***Purpose:*** *To determine the antibacterial effectiveness of kecapi leaf extract against bacterial growth of* Porphyromonas gingivalis*.* ***Methods:*** *This study used a pure experimental research (true experimental) with post-test only with control design, consists of 7 treatment groups, including: concentrated kecapi leaf extract 5%, 10%, 15%, 25%, 50%, chlorhexidine gluconate 0.2% as positive control, and distilled water as negative control. It was repeated 7 times, the antibacterial effectiveness was assessed from Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on BHIB and NA media by dilution method.* ***Results****: MIC on kecapi leaf extract at a concentration of 5% showed the smallest result of -0.147 and the MBC value at a concentration of 50% did not show the growth of bacterial colonies.* ***Conclusion****: Kecapi leaf extract with concentrations of 5%, 10%, 15%, 25%, 50%, and positive control was effective in inhibiting and killing bacterial growth of* Porphyromonas gingivalis*.*

Keywords: *Antibacterial, Kecapi leaf extract, Porphyromonas gingivalis*
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INTRODUCTION

Periodontal disease includes a high level of dental and oral problems in Indonesia, with the prevalences of 96.58% in all age groups. Based on the report by Basic Health Research (RISKESDAS) in 2018, periodontal disease in Indonesia was quite high, with a prevalence of 74,1%.[1]

South Kalimantan has quite serious dental diseases problems, in which 60% of diseases that are frequently found are periodontitis.[1] Periodontitis disease that often occurs is chronic periodontitis. Chronic periodontitis is an infectious disease of the gingiva, which causes damage in the periodontal tissues due to the accumulation of plaque and bacteria, resulting in loss of clinical attachment of periodontal tissues.[2,3]

Dominant bacteria in chronic periodontitis with prevalences of 96.2% is *Porphyromonas gingivalis*. *Porphyromonas gingivalis* is an anaerobic negative-gram bacteria with complex cell walls and a quite large crevicular epithelium attachment of 5-20 times larger than other bacteria.[4]

Periodontitis treatment that can be performed is mechanical and supporting treatments. Mechanical treatments to inhibit the formation and development of subgingival plaque bacteria causing periodontitis are scaling and root planing.[4] Supporting treatment for chronic periodontitis is administered antiseptic in the form of mouthwash to control plaque and prevent the formation of new biofilm caused by *Porphyromonas gingivalis*. The current gold standard mouthwash is chlorhexidine

gluconate 0.2%. This mouthwash has an antimicrobial effect with a broad spectrum and is effective against gram-positive bacteria.[5]

Chlorhexidine gluconate 0.2% mouthwash used for a long period has side effects of causing resistance to bacteria, discoloration of teeth, irritation of oral mucosa, and loss of the sense of taste. The use of chlorhexidine gluconate 0.2% mouthwash causes side effects, so an alternative with herbal ingredients as natural mouthwash is required.[6,7]

South Kalimantan is rich in plants that can be used as herbal medicine. One of the typical plants is kecapi (*Sandoricum koetjape)* from the family Meliaceae that has many benefits and is easy to find. Kecapi plants (*Sandoricum koetjape*) are believed to cure infections caused by bacteria.[8] Kecapi leaves contain flavonoids, saponins, alkaloids, steroids, phenolics, and triterpenoids compounds that function as antibacterial. Triterpenoid compounds are the most abundant compounds in kecapi leaves of 26.5±0.1, phenolics of 22.6±0.2 µg/ml, flavonoids of 20.0±0.2 µg/ml, saponins of 13.2±1.0 µg/ml, alkaloids of 12.4±0,1 µg/ml, and steroids of 6.0±1.9 µg/ml, and each extract concentrations in 100 µg/ml.[9,10]

The study conducted by Nikmah B (2017) regarding the antibacterial test of Sentul kecapi leaves extracts (*Sanduricum koetjape* (Burm.f.) Merr showed that a concentration of 50% is the most optimal concentration in inhibiting the growth of *Staphylococcus aureus*. Based on the results of the study conducted by Fatmalia N (2019), antimicrobial activity test on kecapi leaves with various concentrations against the growth of *Staphylococcus aureus* and *Escherichia coli* showed that the optimal concentration that could inhibit the growth of *Staphylococcus aureus* is in the concentration of 5%. Meanwhile, the concentration of 15% can inhibit the growth of *Escherichia coli*.[11,12] Based on the results above, this study aims to find out and analyze the antibacterial effectiveness of kecapi leaves (*Sandoricum koetjape*) extracts in the concentration of 5%, 10%, 15%, 25%, and 50% against the growth of *Porphyromonas gingivalis.*

MATERIALS AND METHODS

This study was conducted after getting an Ethical Eligibility Permit by the Ethics Commission of Dentistry Universitas Lambung Mangkurat No. 047/KEPKGFKGULM/EC/III /2021. This study was true experimental with Posttest Only with Control Group Design using 7 treatments and 7 repetitions including kecapi leaves (*Sandoricum koetjape*) extracts at concentrations of 5%, 10%, 15%, 25%, 50%, positive control with chlorhexidine gluconate 0.2%, and negative control with distilled water. The measurement of MIC value was used liquid dilution method with Uv-Vis Spectrophotometer to see the difference of absorbance value, and the measurement of MBC was used solid dilution method with Colony counter to count the number of colonies growing on solid media.

Equipment and Materials

The equipment of the study needed was analytical balance, Erlenmeyer, blender, oven Memmert model 30, measuring cup, petri dish, test tube, ose sterile, spectrophotometer, pipette, micro-pipette, colony counter, spirit lamp, rotary evaporator, mask, handscoon, sterile cotton, tube rack, yellow tip, blue tip, waterbath, incubator Biobase BJPX-C50 CO2, hot plate, magnetic stirrer, and autoclave.

Materials of the study needed were kecapi leaves (*Sandoricum koetjape*) and ethanol 96%. The materials for bacterial culture and bacterial test consisted of pure isolates of *Porphyromonas gingivalis* culture, chlorhexidine gluconate 0.2% as a positive control, liquid dilution media of Brain Heart Infusion Broth (BHIB), Nutrient Agar (NA) in solid dilution, and sterile distilled water.

Determination of Kecapi Leaves

Kecapi leaves were obtained in Anjir area Barito Kuala Regency, Banjarmasin, South Kalimantan. A determination test of kecapi leaves was conducted in the Basic Laboratory of the Faculty of Mathematics and Natural Sciences in Universitas Lambung Mangkurat Banjarbaru.

Kecapi Leaves Extracts

Making extracts was by a maceration method. The first step was that 1 kg kecapi leaves were washed and cut into small pieces, then dried using an oven at 40-50ºC for 4 hours. After drying, kecapi leaves were crushed with a blender until they became a powder, then it was soaked in the Erlenmeyer tube with 1-liter ethanol 96% solvent for 3 x 24 hours. During the immersion, it was stirred and filtered using filter paper 3 times until brownish-green kecapi leaves extracts were obtained. After that, it was evaporated using a rotary evaporator at 50-60ºC for 4-6 hours, then heated on waterbath at 40ºC until all the solvents evaporated. So, 63.26 grams of total thick extracts of kecapi leaves were obtained.

The ethanol-free test was conducted by adding acetic acid and sulfuric acid, and extracts were stated free from ethanol if there was no smell of ester. After that, the extracts were diluted with distilled water using formula $V_1 . C_1 = V_2 . C_2$ so that concentrations of 5%, 10%, 15%, 25% and 50% were obtained.

Bacterial Culture

Poprhyromonas gingivalis bacterial colonies were taken using sterile ose, then planted on the surface of Nutrient Agar (NA). After that, it was incubated for 24 hours at 37ºC so that pure bacterial colonies were obtained. Then, Brain Heart Infusion Broth (BHIB) media was put into an anaerobic incubator and incubated for 1 x 24 hours at 37ºC. After that, it was diluted by adding sterile distilled water and homogenized until the turbidity was equivalent to the McFarland 0.5 standard (1,5 x 10^8).[13]

Bacterial Test

Antibacterial effectiveness test of kecapi leaves (*Sandoricum koetjape*) extracts were used solid and liquid dilution method. The first step was that the mother liquor was first made and diluted with distilled water using formula $V_1.C_1 = V_2.C_2$ and made into concentrations of 5%, 10%, 15%, 25%, and 50% in the sterile test tube using a sterilized micro-pipette. Chlorhexidine gluconate 0.2% and distilled water were added into other vacuum tubes. The vacuum tubes were covered with sterile cotton and homogenized using a vortex mixer. After that, 1 ml bacterial suspension that has been standardized using the turbidity of McFarland Standard 0.5 ($1,5x10^8$) CFU/ml was put into each test tube containing 1 ml extracts with 7 different concentrations of 5%, 10%, 15%, 25%, 50%, Chlorhexidine gluconate 0.2%, and distilled water. The test tubes were then measured before and after 24 hours incubation using Uv-Vis Spectrophotometer with a wavelength of 480 nm.

Determining MIC value, delta Optical Density (OD) value or difference of absorbance value decreasing or becoming lower than delta OD in the negative control group, which can be considered that the growth of bacteria was inhibited (MIC), was by comparing absorbance after the incubation minus before incubation using Uv-Vis Spectrophotometer. If the delta Optical Density (OD) is negative, then it can be stated that the growth of bacteria was inhibited (MIC). Determining MBC value was by taking the concentration showing MBC value then put into a petri dish containing NA, then incubated for 24 hours at 37°C, after that, calculating the number of bacteria was used colony counter. If the results of the calculation for the number of colonies show 0 (no bacterial growth), then MBC was obtained.

RESULTS

The results of MIC measurement for kecapi leaves extracts at concentrations of 5%, 10%, 15%, 25%, and 50% to the growth of *Porphyromonas gingivalis* can be seen in the following table:

Table 1. MIC measurement of kecapi leaves extracts against the growth of *Porphyromonas gingivalis*.

Perlakuan	Jumlah	Sebelum Inkubasi (Absorbansi)	Setelah Inkubasi (Absorbansi)	Selisih (Absorbansi)	Standar Deviasi	Keterangan
5%	7	0,501857143	0,354	-0,14785714	0,01965052	Turun
10%`	7	0,842285714	0,659142857	-0,18414286	0,01559304	Turun
15%	7	1,084	0,878142857	-0,20757143	0,02076742	Turun
25%	7	1,318428571	1,045714286	-0,27271429	0,0113101	Turun
50%	7	2	1,724285714	-0,36242857	0,01947054	Turun
K (+)	7	0,981	0,613428571	-0,36757143	0,04573058	Turun
K (-)	7	0,675857143	1,129142857	0,453285714	0,00394606	Naik

Table 1 shows that the treatment groups of kecapi leaves extracts at concentrations of 5%, 10%, 15%, 25%, 50%, and positive control showed a decrease in average value on the difference of absorbance value or inhibition of the growth of *Porphyromonas gingivalis*. The table shows that 5% concentration was the smallest concentration that had an inhibition against the *Porphyromonas gingivalis*, and the greater the concentrations, the greater the inhibition of bacterial growth. Meanwhile, negative control showed that the average value had increase or there was no inhibition for the growth of *Porphyromonas gingivalis*.

Table 2. MBC measurement of kecapi leaves extracts against the growth of *Porphyromonas gingivalis*.

Treatment	Total	Average CFU/µL	Standard Deviation
5%	7	2.714285714	3.4503278
10%	7	1	1
15%	7	0.85714286	1.06904497
25%	7	0.714285714	0.95118973
50%	7	0	0
K (+)	7	0	0
K (-)	7	17777.142857	395.452243

Seen from table 2 above, the kecapi leaves extracts groups at concentrations of 5%, 10%, 15%, dan 25% still had the growth of *Porphyromonas gingivalis* bacteria colonies, while at a concentration of 50%, there was no growth of *Porphyromonas gingivalis* bacteria colonies. This was the same as the positive control, which did not show the growth of *Porphyromonas gingivalis* bacteria colonies. The minimum MBC value that can kill *Porphyromonas gingivalis* was at a concentration of 50%.

The results of the study regarding the difference of absorbance value (MIC) was then conducted a statistical analysis using Software Statistic. The first step was that the normality test with Shapiro-Wilk showed a sig p-value > 0.05,

which means that the data was normally distributed. After that, the homogeneity test with Levene's test showed a sig value of 0.000 ($p < 0.05$), which means that the data was not homogeneous. Then, the data of the difference of absorbance value was continued with the One Way ANOVA parametric test, which showed a significance value of 0.000 ($p < 0.005$); this means that the hypothesis was accepted and continued with Post Hoc Games Howel test.

Table 3. The results of Post Hoc Games Howel test (MIC)

	Konsentrasi Ekstrak Daun Kecap					Kontrol	
Perlakuan	5%	10%	15%	25%	50%	(+) Klor-heksidin	(-) *Aquadest*
5%		0,031*	0,002*	0,000*	0,000*	0,000*	0,000*
10%	0,031*		0,287	0,000*	0,000*	0,000*	0,000*
15%	0,002*	0,287		0,000*	0,000*	0,000*	0,000*
25%	0,000*	0,000*	0,000*		0,000*	0,012*	0,000*
50%	0,000*	0,000*	0,000*	0,000*		1,000	0,000*
Kontrol +	0,000*	0,000*	0,000*	0,012*	1,000		0,000*
Kontrol -	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	

Note:
*: There was significant difference ($p < 0.05$)

Based on the results of the Post Hoc Games Howel test in table 3, it showed that there were significant differences at concentrations of 5%, 25%, and positive control to all concentrations. However, there was no significant difference in the treatment group at a concentration of 10% to 15% and a concentration of 50% to positive control.

From the results of the statistical test on the number of colonies (MBC), the normality test showed a significant value of $p < 0.05$, which means that the data were not normally distributed. Then, the homogeneity test with Levene's test showed a significant value of 0.000 $p < 0.05$, which means that data variances were not homogeneous. After that, a nonparametric test with Kruskal Wallis showed a significant value of 0.000 ($p < 0.05$, which means that H0 was rejected or hypothesis was accepted, then continued with Man Whitney test.

Table 4. The results of Man Whitney test (MBC)

	Konsentrasi Ekstrak Daun Kecapi					Kontrol	
Perlakuan	5%	10%	15%	25%	50%	(+) Klorheksidin	(-) *Aquadest*
5%		0,355	0,262	0,144	0,003*	0,003*	0,002*
10%	0,355		0,382	0,575	0,024*	0,024*	0,002*
15%	0,262	0,386		0,214	1,000	1,000	0,002*
25%	0,144	0,575	0,214		0,061	0,061	0,002*
50%	0,003*	0,024*	1,000	0,061		1,000	0,001*
Kontrol +	0,003*	0,024*	1,000	0,061	1,000		0,001*
Kontrol -	0,002*	0,002*	0,002*	0,002*	0,001*	0,001*	

Note:
*: There was a significant difference ($p < 0.05$)

Table 4 showed that at a concentration of 5%, there was no significant difference to the concentrations of 10%, 15%, and 25%. In a concentration of 10%, there was no significant difference to the concentrations of 5%, 15%, and 25%. In the concentrations of 5% and 10%, there was a significant difference to the concentration of 50%, positive control, and negative control. Moreover, concentrations of 15%, 25% and positive control had a significant difference to the negative control. In the negative control group, there was a significant difference to all treatment groups.

DISCUSSION

The results of the study showed that Minimum Inhibitory Concentration (MIC) on kecapi leaves extracts against the *Porphyromonas gingivalis* at a concentration of 5% showed the smallest concentration in inhibiting the bacterial growth with a decrease in absorbance of -0.147, and the Minimum Bactericidal Concentration (MBC) value was in the concentration of 50% because it did not show the growth of bacterial colonies after incubated for 24 hours.

This occurred because there were several secondary metabolic compounds in kecapi leaves extracts: alkaloids, flavonoids, triterpenoids, steroids, phenolics, and saponins. The results of the study by Hamdi (2019) stated that secondary metabolite levels from ethanolic extract of kecapi leaves were phenolics 22.6±0.2 µg/ml, flavonoids 20.0±0.2 µg/ml, saponins 13.2±1.0 µg/ml, alkaloids 12.4±0.1 µg/ml, and steroids 6.0±1,9 µg/ml, and each extract concentration was in 100 µg/ml.[9,10,14]

Triterpenoids are the most dominant compounds in kecapi leaves extracts of 26.5±0.1 µg/ml. Triterpenoids have mechanisms involving lipophilic compounds in destroying cell membranes so that bacteria will lack of nutrients and the growth of bacteria will be inhibited.[11,15]

Phenolics diffuse through the outer membrane lipopolysaccharide of the *Porphyromonas gingivalis* cell wall, binds to the cytoplasmic membrane and disrupts the stability of the bacterial cell, then the cytoplasm leaks. Mechanisms of flavonoids are by inhibiting the energy metabolism process, inhibiting the function of the cell membrane, and inhibiting the nucleic acid synthesis in the *Porphyromonas gingivalis*. Saponins diffuse through cytoplasm membranes so that the stability of membranes is disrupted and results in cytoplasmic leakage causing lysis or death of bacteria. Alkaloids can disrupt the components of the peptidoglycan wall in *Porphyromonas gingivalis* bacterial cells so that the layers of the cell wall are not formed

completely and results in the death of cells. Steroids cause the integrity of membranes to decrease, and morphology on cell membranes change and form a polymeric bond and result in damage to the porin, then lysis occurs.[16,17]

Positive control of chlorhexidine gluconate 0.2% has inhibition and killing power against the growth of bacteria, which act bacteriostatically or bactericidal. Chlorhexidine has a positive cation charge, while bacteria have a negative anion charge so that chlorhexidine can attach to the bacterial cells. The attachment mechanisms are by depositing on the cytoplasmic protein on bacterial cells so that it results in permeability of the bacterial cells to leak and results in the inhibition and death of bacteria.[14,18]

The negative control group in the form of distilled water cannot inhibit or kill the growth of bacteria, which indicated that there was a growth of bacterial colonies after giving treatment. This is in line with the study by Khotimah (2017), which stated that aquadest is pure distilled water and is frequently used as a negative control on research.[19]

Other factors that can influence the ability of an extract in inhibiting the growth of bacteria are influenced by extract concentrations and properties of bacteria tested. *Porphyromonas gingivalis* is a gram-negative bacterium that has 3 cell wall layers, peptidoglycan, lipopolysaccharide, and lipoprotein. This bacteria has more complex additional outer membranes so that the active compounds will be more difficult to penetrate the bacterial cell wall.[20]

The results of the study in treatment groups with a difference of absorbance value (MIC) given by kecapi leaves extracts from concentrations of 5%, 10%, 15%, 25%, and 50% showed the inhibition against the growth of *Porphyromonas gingivalis*. However, compared to the bacterial ability resulting from chlorhexidine gluconate of 0.2% seen from the difference in absorbance value, kecapi leaves extracts have not been equivalent to chlorhexidine gluconate 0.2% in inhibiting the growth of *Porphyromonas gingivalis*. This is due to the antibacterial compound containing in kecapi leaves extracts in low concentration. Therefore, the antibacterial compounds are also less optimal in inhibiting the growth of bacteria, and the increase of kecapi leaves extracts concentration is required to obtain more compounds so that it can result in stronger inhibition. Meanwhile, chlorhexidine gluconate 0.2% has cation and anion resulting in strong attachment on bacterial cell membranes.[18,20]

Based on the results in treatment groups, the number of colonies grown (MBC) given by Kecapi leaves extracts at concentrations of 5%, 10%, 15%, and 25% still showed the growth of bacteria, while a concentration of 50% showed no growth of bacterial colonies. This is equivalent to chlorhexidine gluconate 0.2%, which also shows no growth of bacterial colonies. Moreover, the results of the Post Hoc Mann Whitney test at a concentration of 50% and positive control showed no significant differences. This is caused by all compounds in Kecapi leaves extracts at a concentration of 50% work together in doing antibacterial mechanisms so that it results in the antibacterial effect that can kill the growth of *Porphyromonas gingivalis*.[20]

Based on the results of the study, it can be concluded that there are differences in the effect of kecapi leave extract with concentrations of 5%, 10%, 15%, 25%, 50%, positive control and negative control and effective in inhibiting and killing the growth of *Porphyromonas gingivalis*.

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