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COMPARISON OF THE ANTIBACTERIAL EFFECTIVENESS OF RED BETEL EXTRACT WITH BLACK BETEL EXTRACT ON *Porphyromonas gingivalis* BACTERIA

(PERBANDINGAN EFEKTIVITAS ANTIBAKTERI EKSTRAK SIRIH MERAH DENGAN EKSTRAK SIRIH HITAM TERHADAP BAKTERI Porphyromonas gingivalis)

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ABSTRACT

Periodontitis is a chronic inflammatory disease of the tooth-supporting structures of the teeth caused by a group of specific microorganisms in dental plaque. *Porphyromonas gingivalis* (Pg) is a bacterium that causes periodontitis found in subgingival plaque. Antibacterial agents are needed as additional therapy to complete mechanical treatment, the main therapy for periodontitis treatment. Red betel (*Piper crocatum*) and black betel (*Piper betle L. var nigra*) are herbal plants with active compounds of flavonoids, alkaloids, saponins, tannins, phenols, and essential oils. These compounds are known to act as antibacterial and anti-inflammatory agents. This study aimed to compare the inhibition of red betel leaf extract and black betel leaf extract on the growth of Pg

bacteria. This research method is experimental with an in vitro Post Test Only Control Group design. The study was conducted using the disc diffusion method in 10 groups, namely red betel leaf extract concentrations of 25%, 50%, 75%, 100%, black betel leaf extract concentrations of 25%, 50%, 75%, 100%, metronidazole as a positive control, and sterile distilled water as a negative control. Repetition was done thrice in each group on Mueller-Hinton Agar (MHA) media. The results showed that both types of betel leaf extract at all concentrations had antibacterial effectiveness against Pg bacteria. The higher the extract concentration, the more effective bacteria inhibition is. Betel leaf extract with a concentration of 100% had the highest inhibition zone, which is 25.16 mm for red betel and 19.65 mm for black betel. These results indicate that red betel leaf extract has better effectiveness than black betel leaf extract.

Keywords: antibacterial; black betel leaf extract; *Porphyromonas gingivalis*, red betel leaf extract

ABSTRAK

Periodontitis adalah penyakit inflamasi kronis pada struktur pendukung gigi yang disebabkan oleh sekumpulan mikroorganisme spesifik dalam plak gigi. Porphyromonas gingivalis (Pg) merupakan bakteri penyebab periodontitis yang ditemukan di plak subgingiva. Diperlukan agen antibakteri sebagai terapi tambahan untuk melengkapi perawatan mekanis yang merupakan terapi utama perawatan periodontitis. Sirih merah (Piper crocatum) dan sirih hitam (Piper betle L. var nigra) merupakan tanaman herbal yang mengandung senyawa aktif flavonoid, alkaloid, saponin, tanin, fenol, dan minyak atsiri. Senyawa-senyawa tersebut diketahui berperan sebagai agen antibakteri dan antiinflamasi. Tujuan penelitian ini adalah untuk membandingkan daya hambat ekstrak daun sirih merah dan ekstrak daun sirih hitam terhadap pertumbuhan bakteri Pg. Metode penelitian ini adalah eksperimental murni dengan desain Post Test Only Control Group secara in vitro. Penelitian dilakukan dengan metode difusi cakram pada 10 kelompok

yaitu ekstrak daun sirih merah konsentrasi 25%, 50%, 75%, 100%, ekstrak daun sirih hitam konsentrasi 25%, 50%, 75%, 100%, metronidazol sebagai kontrol positif, serta akuades steril sebagai kontrol negatif. Pengulangan dilakukan sebanyak 3 kali pada setiap kelompok di media Mueller-Hinton Agar (MHA). Hasil penelitian menunjukan bahwa ekstrak kedua jenis daun sirih pada semua konsentrasi memiliki efektifitas antibakteri terhadap bakteri Pg. Semakin tinggi konsentrasi ekstrak maka semakin efektif daya hambat bakterinya. Ekstrak daun sirih dengan konsentrasi 100% memiliki zona hambat yang paling tinggi yaitu sebesar 25,16 mm untuk sirih merah dan 19,65 mm untuk sirih hitam. Hasil ini menunjukkan bahwa ekstrak daun sirih merah memiliki efektivitas yang lebih baik dibandingkan ekstrak daun sirih hitam.

Kata kunci: *antibakteri; ekstrak daun sirih hitam; ekstrak daun sirih merah; Porphyromonas gingivalis*

INTRODUCTION

It is necessary to pay attention to the health of the supporting structures of the teeth so that the teeth can function properly and be free from dental and oral diseases. Basic Health Research (RISKESDAS) results in 2018 showed the % of dental and oral diseases in Indonesian society was 57.6%. One of the dental and oral diseases that still has a high incidence in Indonesia is periodontitis, with a percentage of 74.1%.[1] A survey conducted by the Global Burden of Disease Study (2016) reported that the prevalence of severe periodontal disease was ranked 11th in the world. The world's average prevalence of periodontal disease is around 20-50%.[2]

Periodontitis can be defined as an inflammatory disease of the supporting structures of the teeth caused by specific microorganisms. The main etiology of periodontitis is supragingival and subgingival plaques, also known as microbial biofilms.[4.5] One of the subgingival bacteria that causes periodontitis is the bacterium *Porphyromonas gingivalis.* These bacteria are gram-negative pathogenic, obligate anaerobes, *nonmotile*, and rod-shaped. This bacterium is found in approximately 87.5% of chronic periodontitis subgingival plaque patients. The pathogenicity of Pg bacteria depends on its virulence factors.[5-8]

Mechanical measures such as

scaling and root planing as periodontitis treatment are essential. In certain conditions, it is sometimes necessary to provide additional antibacterial agents to increase the effectiveness of plaque bacteria elimination.[9] Effectively, antibacterial drugs containing chemical substances inhibit bacterial growth but often cause side effects.[10,11] Therefore, other alternatives for antibacterial therapy can use natural ingredients and come from herbal plants that are well known to the people of Indonesia. This herbal therapy is considered relatively safer and does not cause side effects to the body. One of these herbal plants is betel leaf.[12,13]

Many types of betel leaf grow in Indonesia, including red betel leaf (*Piper crocatum*) and black betel leaf (*Piper betle L. var nigra*). Betel leaf contains flavonoids, saponins, tannins, alkaloids, terpenoids, and phenols. These active compounds are known to be effective as antibacterial and anti-inflammatory.[14,15] Although each type of betel leaf generally has the same content, its effectiveness as an antibacterial may differ according to the levels of each active compound contained in each type of betel leaf.[16,17]

Several previous studies prove the effectiveness of betel leaf in inhibiting bacterial growth. Sendy et al. (2014)

researched the antibacterial power of red betel leaf extract against Pg bacteria using the good diffusion method with betel leaf extract concentrations of 25%, 50%, and 100%. The results showed that there was an inhibition zone of Pg bacteria at each concentration with the highest value at 100% extract concentration of 10.7638 mm.[18]

The authors have not found research on black betel leaf extract against Pg bacteria. However, there was research conducted by Saputri (2018) regarding the antibacterial activity of the ethanol extract of black betel leaves on *Staphylococcus aureus* bacteria. The study used the well diffusion method with betel leaf extract concentrations of 5%, 10%, 20%, and 25%. This study's results indicate that there are bacterial inhibition zones at concentrations of 10%, 20%, and 25%. The largest inhibition zone was found at a concentration of 25%, which was 6.89 mm.[16] Although there have been previous studies on the two types of betel leaf, there has been no research comparing the effectiveness of the two against Pg bacteria.

Based on this background, the authors are interested in research to compare the effectiveness of red betel leaf extract with black betel leaf at concentrations of 25%, 50%, 75%, and 100% in inhibiting the growth of

Porhpyromonas gingivalis (Pg) bacteria.

METHOD

This research was carried out after obtaining ethical approval from KEP (Research Ethics Commission) at Padjadjaran University with ethical approval number 1331/UN6.KEP/EC/2022. This research is an experimental laboratory with a posttest-only control group design. The research was conducted using the disc diffusion method to see the inhibition of each extract.

The research subjects were red betel leaf extract and black betel leaf extract, which had been determined, and the object of this study was *Porphyromonas gingivalis* ATCC 33277. Inclusion criteria included colonies of *Porphyromonas gingivalis bacteria* that grew purely on MHA media without contaminating other microorganisms, red betel leaves, and black betel leaves free from pests. Exclusion criteria included colonies of the bacteria *Porphyromonas gingivalis* ATCC 33277, which had died and could not be used because they were expired, and red and black betel leaves were too old or too young.

In this study, there were ten research groups, namely red betel leaf extract concentrations of 25%, 50%, 75%, and 100%, black betel leaf extract concentrations of 25%, 50%, 75%, 100%, control (+) with metronidazole, and control (-) with sterile distilled water. In each of the test groups, three repetitions were carried out.

The tools used in this study were osseous, gigaskrin, bunsen, blender, oven, glass objects, test tubes, scales/balances, erlenmeyer tubes, microscopes, loops, beaker glass, centrifuge, caliper with a degree of accuracy of 0.5 mm, micropipette, syringe, marker, laminar flow, incubator, autoclave, desiccator, rotary evaporator, petridishes, pipettes, label stickers, stationery, bins, and a spectrophotometer for the McFarland test.

The materials used in this study were *Porphyromonas gingivalis* ATCC 33277 bacteria, red betel leaf extract concentrations of 25%, 50%, 75%, and 100%, black betel leaf extract concentrations of 25%, 50%, 75%, and 100%, paper filter, 96% ethanol, sterile distilled water, metronidazole, Brain Heart Infusion Broth (BHI-B), Mueller Hinton Agar (MHA), sterile cotton swabs, and disc paper.

The research procedure was started by sterilizing the media using an autoclave and letting it stand for 15 minutes at 121oC, the tools used the oven for 2 hours at 170oC, and the ose using a bunsen flame passed it over the fire.[19]

Red and black betel leaf extracts were made at the Biochemistry Laboratory of Universitas Jenderal Achmad Yani. Each type of betel is weighed 1 kg, cleaned, dried using an oven at 40oC for 5 hours, then put in a blender to form a powder. The extraction was carried out by the maceration method using 1500 ml of 96% ethanol and allowed to stand for 72 hours, and then the macerate was evaporated.

A Porphyromonas gingivalis antibacterial test was conducted at the Microbiology Laboratory, Airlangga University. Preparation for the antibacterial test begins with preparing the tools and materials needed, then making a suspension of *Porphyromonas gingivalis bacteria*. Put 2 ml of sterile BHI-B solution into a test tube and add 1 ose of bacteria. The treatment was carried out by passing the test tube over the spirit lamp, then homogenizing it over *the centrifuge*.

Incubation for 24 hours was at 37°C in an incubator. The presence of turbidity indicates the presence of *Porphyromonas gingivalis bacteria.* Perform dilution by adding sterile distilled water and homogenize over *the centrifuge*. The absorbance measurement used an Mc Farland 0.5 standard with a wavelength of 560nm and an absorbance of 0.05 using *a spectrophotometer.*[18,20]

The media preparation for the antibacterial inhibition test incorporated 38 grams of MHA (*Mueller Hinton Agar*) *media and dissolved it in 1 liter of sterile distilled water.* Sterilize MHA media in an autoclave, pour 15 ml into a petri dish, and let it solidify.

Streak method on MHA media using *a cotton swab,* scratch *zigzag*, and do the streaking three times with 60o until the inoculum is spread evenly. Next, 20 μl of red and black betel leaf extract with a concentration of 25%, 50%, 75%, and 100% were dripped on the sterile disc paper, then incubated for 24 hours at 37oC. The next step is measuring the zone using a vernier caliper to hold down the clear colored area around the disc from edge to opposite edge and through the center of the disc.[21]

The data obtained is then processed using the SPSS application. Normality test using Shapiro-Wilk and homogeneity test using Levene-test. Furthermore, the One-Way ANOVA test is if the distribution is normal and homogeneous. Still, if the data is not normal and not homogeneous, then the Kruskal-

Wallis test is performed. The test used to determine whether there is a significant difference in each group is the T-Independent test if the data is homogeneous or the Mann-Whitney test if the data is not homogeneous.

RESULT

Inhibition zone measurements were carried out using a digital caliper. The size of the inhibition zone in each repetition was processed using the SPSS application to obtain the average value for each group.

According to David and Stout (1971), the antibacterial power category of a component is divided into three, including an inhibition zone size that does not exceed 5 mm is considered a weak criterion, an inhibition zone size of 5-10 mm is considered a medium criterion, an inhibition zone size of 11-20 mm is classified as strong criteria, as well as the size of the inhibition zone that exceeds 20 mm including very strong criteria. [22] Results of the mean inhibition zone measurements formed in the antibacterial test of red betel and black betel extracts against *Porhpyromonas gingivalis bacteria* can be seen in Table 1.

Table 1. Mean inhibition zones of red betel extract and black betel extract against *Porhpyromonas gingivalis* (Pg)

bacteria

Group		n	*Means (mm)*	Category Antibacterial Power
Aquades		3	0.00	None
25%	Black betel	3	8.53	Medium
	Red betel	3	17,62	Strong
50%	Black betel	3	13,13	Strong
	Red betel	3	20,92	Very strong
75%	Black betel	3	17,77	Strong
	Red betel	3	23.53	Very strong
100%	Black betel	3	19.65	Strong
	Red betel	3	25,16	Very strong
Metronidazole		3	27.50	Very strong

Table 1 shows that black betel extract can inhibit the growth of *Porphyromonas gingivalis* bacteria with a concentration of 100% (19.65 mm) having the largest average inhibition zone. In comparison, a concentration of 25% (8.53 mm) has the largest average inhibition zone. Small Red betel extract also inhibited the growth of *Porphyromonas gingivalis bacteria* with the result that a concentration of 100% (25.16 mm) had the largest average inhibition zone. In comparison, a concentration of 25% (17.62 mm) had the smallest average inhibition zone.

Sterile distilled water as a negative control showed no antibacterial activity, as indicated by the average inhibition zone size of 0.00 mm. Measurements on the positive control using metronidazole had an average inhibition zone of 27.50 mm. It shows that the mean of the inhibition zone formed by metronidazole is still higher than

that of red betel leaf and black betel leaf extracts.

Data analysis regarding comparing red betel extract in inhibiting the growth of *Porphyromonas gingivalis bacteria* based on concentration began with normality and homogeneity tests in the red betel group. Then one-way ANOVA statistical test was continued to determine whether there was an effect on various red betel treatments. One-way ANOVA was chosen because the data is normally distributed and homogeneous. The statistical test to determine whether there is a significant difference in comparing each red betel concentration is using the independent T-test. The results of the data analysis are presented in Table 2.

Table 2. Comparison test of inhibitory power of red betel extract as antibacterial based on the concentration

Test Group	(-)	25%	50%	75%	100%	(+)
(-)		0.000*	0.000*	0.000*	0.000*	0.000*
25%	0.000*		0.002*	0.001*	0.000*	0.000*
50%	0.000*	0.002*		0.042*	0.005*	0.001*
75%	0.000*	0.001*	0.042*		0.159	0.012*
100%	0.000*	0.000*	0.005*	0.159		0.040*
(+)	0.000*	0.000*	0.001*	0.012*	0.040*	

*Independent T- test $p<0.05$ (there is a significant difference)

Porphyromonas gingivalis bacteria based on concentration begins with tests of normality and homogeneity in the red betel group. Furthermore, the Kruskal Wallis statistical test was continued to

determine whether there was an effect or not on various treatments of black betel. Kruskal Wallis was chosen because the data were normally distributed but not homogeneous. The statistical test to determine whether there is a significant difference in comparing each black betel concentration is the Mann-Whitney test. The results of the data analysis are presented in Table 3.

Table 3. Comparison test of inhibitory power of black betel extract as antibacterial based on the concentration

Test Group	(-)	25%	50%	75%	100%	(+)
(-)		0.037*	0.037*	0.037*	0.037*	0.037*
25%	0.037*		0.050*	0.050*	0.050*	0.050*
50%	0.037*	0.050*		0.050*	0.050*	0.050*
75%	0.037*	0.050*	0.050*		0.050*	0.050*
100%	0.037*	0.050*	0.050*	0.050*		0.050*
(+)	0.037*	0.050*	0.050*	0.050*	0.050*	

*Mann Whitney test $p\leq0.05$ (there is a significant difference)

The results of data analysis regarding the comparison of the effectiveness of the two types of betel leaf extract in inhibiting the growth of *Porphyromonas gingivalis bacteria* are presented in table 4. Statistical analysis was used to determine whether there was a significant difference using the *Mann-Whitney test* because one data variant was not homogeneous.

Table 4. Comparison of the effectiveness of the two types of betel leaf in inhibiting the

growth of *Porphyromonas gingivalis* (Pg) bacteria

Test Group		MERAH					
		(-)	25 %	50 %	75 %	100 %	(+)
	(-)	■	0.037*	0.037*	0.037*	0.037*	0.037*
H	25 %	0.037*	0.050*	0.050*	0.050*	0.050*	0.050*
I T	50 %	0.037*	0.050*	0.050*	0.050*	0.050*	0.050*
A M	75 %	0.037*	0.513	0.050*	0.050*	0.050*	0.050*
	100 %	0.037*	0.050*	0.127	0.050*	0.050*	0.050*
	(+)	0.037*	0.050*	0.050*	0.050*	0.050*	■

*Mann Whitney test $p \leq 0.05$ (there is a significant difference)

Based on the table presented above, several groups have significant differences, but several other groups do not have significant differences. The results of the test groups that did not have significant differences showed that the average size of the inhibition zones formed was not far from one group to another. Meanwhile, if there is a significant difference, it means that the size of the inhibition zone formed in the group is quite influential.

DISCUSSION

Based on the research that has been done, the results shown in Table 1 show that red betel extract and black betel extract with concentrations of 25%, 50%, 75%, and 100% effectively inhibit bacterial growth.

Porphyromonas gingivalis. It is in line with research conducted by Sendy et al. (2014), and Pujiastuti et al. (2015) that red betel leaf extract (*Piper crocatum*) has antibacterial power against *Porphyromonas gingivalis*.[17,20] The antibacterial effectiveness of black betel leaf extract in inhibiting the growth of *Porphyromonas gingivalis bacteria* has not been found, but there is research conducted by Saputri (2018) that black betel extract can be antibacterial against *Staphylococcus aureus*.[16]

The presence of a clear zone formed around the disc paper is thought to be due to the presence of secondary metabolites that function as antibacterials, causing the growth of *Porphyromonas gingivalis bacteria* to be disrupted. Previously, the authors conducted a phytochemical test first and found that red betel and black betel extracts contain alkaloids, flavonoids, polyphenols, saponins, and quinones.

Alkaloids can interfere with the growth activity of bacteria by damaging the constituent components of peptidoglycan so that bacteria cannot grow properly. Flavonoids are also known as antibacterial compounds because they can interfere with the metabolic processes of bacteria.[18,23] Polyphenols could damage bacterial cells,

resulting in cell leakage, protein denaturation, and disrupting the enzymes these bacteria possess. Saponins can cause hemolysis of bacterial cells.[23]

Quinones work as antibacterial agents by forming compounds such as cell wall polypeptides, enzymes on the surface of cell membranes, and nucleophilic amino acid residues in transmembrane proteins. [24] It is also known that red and black betel leaves contain essential oils which can interfere with forming membranes or cell walls so that bacteria will not form completely.[18]

In the negative control (-), i.e., distilled water did not show any ability to inhibit *Porphyromonas gingivalis bacteria*. Sterile distilled water is a neutral compound, so it cannot inhibit bacterial growth, which is why the authors chose distilled water as a negative control.[25]

Unlike the control (+), metronidazole effectively inhibited the growth of *Porphyromonas gingivalis bacteria*. Metronidazole is an effective antibiotic against anaerobic bacteria. This drug works by interfering with DNA synthesis. In forming deoxyribonucleic acid (DNA) graise, metronidazole causes the superhelix in DNA to open, inhibiting DNA replication.[26]

Based on the data presented in Table 2. and Table 3. it can be concluded that most of the data analysis regarding the comparison of red and black betel extracts based on concentration has a significant difference; this indicates that the concentration of a component affects inhibiting bacterial activity, especially Porphyromonas *gingivalis*. The increase in concentration is proportional to the increase in the diameter of the inhibition zone formed. The average size of the inhibition zone seems to be getting higher, starting from a concentration of 25% to 100%. These results are consistent with Sendy et al. (2014) research that the optimal concentration of betel leaf extract as an antibacterial is 100%. [18] The difference in the inhibition zones formed is thought to be due to the amount of antibacterial active substance in the betel leaf extract, which varies according to the concentration.

At a concentration of 100%, a very pure betel leaf extract was extracted without adding pure aqua dest diluents, such as at concentrations of 25%, 50%, and 75%. It shows that the secondary metabolites possessed by betel leaf extract with a concentration of 100%, namely alkaloids, flavonoids, polyphenols, saponins, tannins, and essential oils, are higher, thus increasing the ability to damage or disrupt bacterial cells. Meanwhile, the smaller the

concentration of the extract, the more diluent is needed; therefore, the active substances contained in these components also decrease.

Compounds can be bactericidal, namely killing bacteria, or bacteriostatic, namely temporarily stopping the growth of bacteria. It can be concluded that a component is antibacterial depending on the active compound and its concentration. [27]

The data analysis presented in Table 4 shows that red and black betel extracts have significant differences in inhibiting the growth of *Porphyromonas gingivalis bacteria*. It can be seen in Table 1 that red betel extract has a larger average inhibition zone than black betel extract. Such results state that red betel leaf extract has a higher antibacterial effectiveness in inhibiting the growth of *Porhyromonas gingivalis bacteria.*

Phytochemical tests conducted by Rahmawati et al. (2016) showed that there are active compounds in red betel leaves that are stronger than black betel leaves. These compounds include phenol hydroquinone, saponins, and steroids.[28] This causes red betel leaf extract to be more effective than black betel leaf extract.

Phenol can damage enzymes and bacterial cell walls.[29] Saponins can lyse bacterial cells by destroying the permeability of the cell wall.[30] Steroids can interact with cell membrane phospholipids towards lipophilic compounds so that the shape of the cell membrane and its strength decreases. This steroidal, antibacterial agent can weaken and lyse cells.[24]

Differences in where the betel leaf is taken may influence its content. The author obtained samples of black betel from Cihideung and red betel from the Manoko Lembang plantation. Two factors affect crop yields, namely internal factors and external factors.[31]

Internal factors are derived from genetic factors. In addition, external factors include temperature, air humidity and water supply, soil system characteristics, solar systems, gas components in the soil, soil pH, nutrient supply, and biotic factors.[32] Based on research conducted by Darmawan et al. (2019), planting media can also affect the nutrients, water, and oxygen plants receive.[33]

Metronidazole antibiotics were still more effective than betel nut extracts, judging by the inhibition zones formed. Metronidazole is often used as the drug of choice for periodontitis because it inhibits bacterial growth, especially anaerobic bacteria. Metronidazole can penetrate the gingival sulcus fluid, especially the

subgingival and saliva, through oral preparations or gels.[34]

Despite these results, administration of metronidazole in the long term can affect the balance of normal oral flora microorganisms that can become pathogens; besides that, it can damage the digestive organs, causing dizziness, skin redness, and depression so that the use of drugs made from herbs such as betel leaf is a choice. Treatment as it has minimal side effects.[35,36]

CONCLUSION

Based on the results of the antibacterial test of red betel leaf extract and black betel leaf extract against *Porphyromonas gingivalis*, it can be concluded that extracts of red betel leaves and black betel leaves at all concentrations have effectiveness in inhibiting the growth of *Porphyromonas gingivalis* (Pg) bacteria. Comparison of the effectiveness of red and black betel leaf extract at all significantly different concentrations. The higher the concentration, the higher the effectiveness in inhibiting the growth of *Porphyromonas gingivalis* (Pg) bacteria. Red betel leaf extract has significantly better effectiveness than black betel leaf extract in inhibiting the growth of *Porphyromonas gingivalis* (Pg) bacteria.

CONFLICT OF INTEREST

There is no conflict of interest in the scientific articles we write.

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