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Effect Of Avocado Seed (Persea Americana Mill.) On The Growth Of Bacteria Prevotella Intermedia (In Vitro)

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ABSTRACT

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by bacteria present in the dental plaque biofilm. Gingivitis and periodontitis are the most common periodontal diseases and are associatedbwith bacteria Prevotella intermedia. Avocado seeds are one way to inhibit this bacteria. the aim of this study is to prove that avocado seed extract (Persea americana Mill.) has an antibacterial effect on bacterial growth Prevotella intermedia. This study was a pure laboratory experimental post- test only control group design. The research sample was divided into 10 groups, namely 8 groups of avocado seed extract with different concentrations, 1 positive control group (chlorhelxidinel glulconat 0,2%), and 1 negative control group (aquades). Avocado seed extract (Persea americana Mill.) has antibacterial power against bacteria Prevotella intermedia with Minimum Inhibitory Concentration (MIC) of 3.125% and Minimum Bactericidal Concentration (MBC) of 6.25%. Avocado seed extract has antibacterial power against the bacteria Prevotella intermedia with MIC 3.125% and MBC 6.25%.

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1. INTRODUCTION

Periodontal disease is one of the diseases of the oral cavity which is ranked first as a disease that is often experienced by humans, based on world record books in 2001. And in Indonesia, periodontal disease ranks second after caries with a prevalence of 96.58% [1]. Gingivitis is the initial form of periodontal disease and if it continues, it will become periodontitis. Periodontitis is an inflammation of the supporting tissues of the teeth caused by bacteria found in the dental plaque biofilm [2]. Gingivitis and periodontitis are common periodontal diseases that are associated with the *Prevotella intermedia* bacteria [1].

Prevotella intermedia is a gram-negative, anaerobic, black-pigmented bacterium which is commonly found in subgingival plaque [3]. Prevotella intermedia has the ability to produce beta- lactamase enzymes so that it can create resistance to beta-lactane antibiotics, such as penicillin [4]. Beta-lactams are the first-choice class of antibiotics used to treat oral infections [5]. Previous studies have proven that more than half of the Prevotella intermedia bacterial isolates are resistant to penicillin, which is a beta-lactam class of antibiotics [5]. The existence of antibiotic resistance raises concerns that it requires other alternatives that have the potential to kill or inhibit the growth of bacteria, one of which is using avocado seeds.

Avocado seed was chosen as an antibacterial agent because it contains complex secondary metabolites such as saponins (51.00 ± 0.00), tannins (21.66 ± 0.00), flavonoids (21.00 ± 0.00), and alkaloids (9.43 ± 0.20) [6]. Saponin compounds can damage membrane permeability resulting in cytoplasmic release and cell death. Tannin compounds work by inhibiting the reverse transcriptase and DNA topoisomerase enzymes thereby preventing the formation of bacterial cells. Flavonoid compounds can damage cell membranes, microsomes, and lysosomes of microorganisms. And alkaloid compounds work by inhibiting the activity of enzymes used in bacterial protein synthesis thereby disrupting bacterial metabolism [7]. Supported by other

studies that the ethanol extract of avocado seeds has the ability to inhibit the growth of Streptococcus mutans biofilms with the best concentration of 12.5% [8,9]. Based on the description above, the purpose of this study is to prove whether avocado seed extract (*Persea americana* Mill.) has an antibacterial effect on the growth of *the Prevotella intermedia* bacteria.

2. METHOD

This research is a pure laboratory experimentpost test only control design. The research variables consist of independent variables namely avocado seed extract (Persea americana Mill.) and The dependent variables are Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against bacteria Prevotella intermedia. Subjects in this study namely avocado seed extract (Persea americana Mill.) and the object of research, namely the Prevotella intermedia ATCC 25611 culture obtained from the preparation of the Research Center Laboratory of the Faculty of Dentistry, Airlangga University, Surabaya, Indonesia.

The size of the study sample was calculated using the Federer formula, where the sample was divided into 10 groups, namely 8 groups of avocado seed extract treatment (Persea americana Mill.) with concentratesi 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 1 positive control group given chlorhexidine gluconat 0.2%, and 1 negative control group was given aquades. The concentration of the extract was obtained from the dilution method, namely by making serial dilutions of the antimicrobial agent in liquid medium, then adding the test microbe to obtain a solution with a multiplied level of half. The inclusion criteria in this study were non-rotten avocado seeds and avocado seed condensed extract preparations. While the exclusion criteria were avocado seeds that had rotted and were contaminated with fungi. Research done at the UPT Herbal Materia Medica Batu Laboratory for the purpose of determining avocado seeds (Persea americana Mill.) and testing for antibacterial activity was carried out at the Research Center Laboratory of the Faculty of Dentistry, Airlangga University, Surabaya, Indonesia. The research was conducted in June-July 2023.

This study uses several tools and materials to support research. The tools used are beaker glass, plastic wrap, funnel, filter paper, glass stirrer, stirrer, oven, analytical balance, rotary evaporator, measuring cup, erlenmeyer, knife, blender, sieve, pipette, aluminum foil, autoclave, loop needle, test tube rack, vortex, test tube, bunsen, incubator, petri dish, calipers, micropipette, label paper. And the material used is extract avocado seeds (Persea Americana Mill.), pure cultures of bacteria *Prevotella intermedia*, liquid media BHIB (Brain-Hearth Infosion Broth), and Nutrient Agar media (NA). Data analysis using One Way Anova because homogeneity is not fulfilled, but the data is normally distributed.

3. RESULTS AND DISCUSSION

Research results regarding The effect of avocado seed extract (*Persea americana* Mill.) on *Prevotella intermedia* bacteria was tested using the dilution method with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, positive control and negative control, the results are as shown below:

Figure 1. Antimicrobial test by dilution method

The next stage is the colony count to determine colony growth by planting bacteria on nutrient agar media with the spreading technique starting from a concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, positive control and negative control.

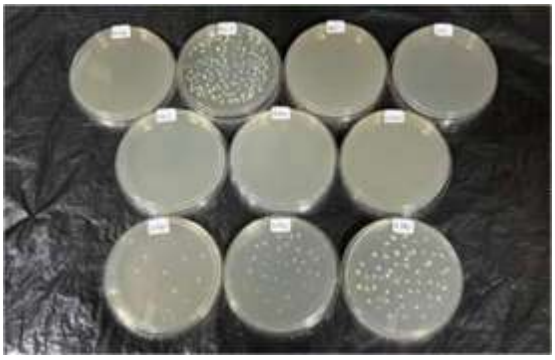

Figure 2. Counting of bacterial colonies by spreading technique

Based on the above results, a concentration of 3.125% was obtained as MIC (Minimum Inhibitory Concentration) and a concentration of 6.25% as MBC (Minimum Bactericidal Concentration), then the average results and standard deviation of the number of *Prevotella intermedia* bacteria colonies wereseen in the table below.

Table 1. Mean and standard deviation of the number of *Prevotella intermedia* bacteria colonies(CFU/ml) in various concentrations of avocado seed extract (*Persea americana* Mill.)

Concentration	N	x̄ (CFU/ml)	SD
Negative control negatif	6	160,166	14,344
Group 10 (0,78%)	6	48,666	5,316
Group 9 (1,56%)	6	32,666	5,085
Group 8 (3,125%)	6	11,000	1,414
Group 2-7 (100%-6,25%)	6	0	0
Positive control	6	0	0

Note: N, Sample size; x̄ , Average (CFU/ml); SD, Standard Deviation

Based on the results of bacterial counting carried out by three observers, the average result at aconcentration of 0.78% was 48.66 CFU/ml, at a concentration of 1.56% it was obtained 32.66 CFU/ml, at a concentration of 3.125% it was obtained 11 CFU/ml, a concentration of 6.25% - 100%, positive control had no colony growth, and negative control was 160.16 CFU/ml. Colony growth was then calculated in percentage form.

Table 2. The average number of *Prevotella intermedia* bacteria colonies at various concentrations of avocado seed extract (*Persea americana* Mill.)

Treatment Group	N	x̄ Number of Colonies (CFU/ml)	x̄ Total Colonies (%)
Positive control	6	0	0
Negative control	6	160,16	100%
Concentration 100%	6	0	0
Concentration 50%	6	0	0
Concentration 25%	6	0	0
Concentration 12,5%	6	0	0
Concentration 6,25%	6	0	0
Concentration 3,125%	6	11,00	6,8 %
Concentration 1,56 %	6	32,66	20,3%
Concentration 0,78%	6	48,66	30,3%

Based on the table above, a concentration of 3.125% is determined as MIC (Minimum Inhibitory Concentration), because it is the minimum concentration that can inhibit bacterial growth by 90% in bacteria that successfully grow in the control group and a concentration of 6.25% is determined as MBC (Minimum Bactericidal Concentration) because it is the minimum

concentration that can kill bacteria 99.9% of the bacteria that are successful in growing in the control group.

Subsequent analysis using the results of calculating the number of colonies of *Prevotella intermedia* bacteria on nutrient agar media. Data for the concentration group of 6.25% - 100% were not tested because at that concentration no bacterial growth was found.

Table 3. Shapiro Wilk Normality Test Results

Sample Group	Sig. (P)	Information
Negative control	0,458	Normal distributed data
Concentration 3,125%	0,960	Normal distributed data
Concentration 1 ,56%	0,926	Normal distributed data
Concentration 0,78%	0,960	Normal distributed data

The table above shows that the p values in all sample groups are normally distributed, with a significance value > 0.05, which indicates that the data is normally distributed. Then do the homogeneity test.

Table 4. Levene Statistical Homogeneity Test

	Results Sig.	Information
Levene test	0.000	Data variation is not homogeneous

Based on the homogeneity test using the Levene test, the data was not homogeneous with a significance value of 0.000 (p>0.05). Because the data is normally distributed but homogeneity is notfulfilled, then a test is carried out using One Way Anova.

Table 5. The results of the One Way Anova test

Group	P	Sig 0.05	Information
Negative control Concentration 3,175% Concentration 1,56% Concentration 0,78%	0,000	P<0,05	Significant differences

The results of the One Way Anova test obtained a significance value of <0.05, which is 0.000. This shows that there is a significant difference between the average number of colonies in each treatment and control group. So to find out the differences between groups, a post-hoc test was carriedout using the Games Howell method.

Table 6. Post-hoc Games Howell Test Results

Group	Negative control	Concentration 3,125%	Concentration 1,56%	Concentration0,78%
Negative control		0,000	0,000	0,000
Concentration 3,175%			0,000	0,000
Concentrationm 1,56%				0,002
Concentration 0,78%				

The results of the Games Howell post-hoc test showed that there was a significant difference between the 2 groups with a value (p <0.05), namely between the negative control group on concentrations of 3.125%, 1.56% and 0.78%. Based on the results of the analysis it was concluded that avocado seed extract (*Persea americana* Mill.) could inhibit the *Prevotella intermedia* bacteria

Discussion

The process of extracting avocado seeds (*Persea americana* Mill.) was carried out using the maceration method with 70% ethanol solvent. 70% ethanol was chosen as a solvent because it is a solvent that has a polarity that works optimally. The use of the maceration method was

chosen because it is simple in the process and is usually used for making extracts on a small scale or on an industrial scale [10]. Peliti and three observers counted the number of colonies of Prevotella intermedia bacteria that grew manually to determine MIC and MBC. The calculation results are expressed in units of CFU/ml, said to inhibit (MIC) if it is able to inhibit 90% bacterial growth at a minimum concentration and is declared to kill (MBC) if it is able to kill 99.9% of bacteria at a minimum concentration.

The concentration of 3.125% in this study has greater antibacterial power than the ability to repair bacterial cells, so that at this concentration the bacteria can be inhibited (MIC) and at a concentration of 6.25% avocado seed extract can cause bacterial lysis (MBC). In this study, the results also showed that the greater the concentration of avocado seed extract (Persea americana Mill.), the stronger the antibacterial power. This is in line with the hypothesis that there is an antibacterial effect of avocado seed extract (Persea americana Mill.) on Prevotella intermedia bacteria.

The results of the Games Howell post hoc test showed that there was a significant difference at each concentration of 0.78%, 1.56% and 3.125% of avocado seed extract (Persea americana Mill.) against the negative control group (aquades). Avocado seed extract has antibacterial properties against Prevotella intermedia bacteria. This is because the extract contains active compounds that act as antibacterial. Based on the phytochemical screening carried out qualitatively and quantitatively on avocado (Persea americana Mill.) seed extract, the results obtained were 65% antioxidant content, 0.48% flavonoids, 1.64% tannins, 2.59% phenols, alkaloids, saponins.

Phytochemical screening was carried out to identify the active compounds contained in avocado (Persea americana Mill.) seed extract. In this test is done by taking a small sample of the extract. The results of the maceration process are then added according to the compound to be identified. Flavonoid compounds have the ability to act as antibacterial by destroying the permeability of bacterial cell walls, bacterial microsomes and bacterial lysosomes, due to interactions between flavonoids and bacterial DNA [11]. Tannin compounds have an antibacterial effect by precipitating proteins, reacting with cell membranes, deactivating enzymes and deactivating the function of genetic material, after that inhibiting the reverse transcriptase and DNA topoisomerase enzymes, preventing bacterial cells from forming tannins having activities related to their ability to deactivate microbial cells [12].

Alkaloids have the ability as an antibacterial by inhibiting the activity of enzymes used in the bacterial protein synthesis process. Inhibition of this enzyme can interfere with bacterial metabolism [13]. Saponins have the ability to act as antibacterial by reducing surface tension resulting in increased permeability or cell leakage and causing intracellular compounds to come out [14]. So this study shows that avocado seed extract (Persea americana Mill.) has antibacterial power against prevotella intermedia bacteria with a MIC of 3.125% and MBC of 6.25%.

4. CONCLUSION

The concentration of avocado seed extract (*Persea americana* Mill.) of 3.125% is the Minimum Inhibitory Concentration (MIC) on the growth of *Pevotella intermedia* bacteria and the concentration of avocado seed extract (*Persea americana* Mill.) of 6.25% is the Minimum Bactericidal Concentration (MBC) on the growth of *Prevotella intermedia* bacteria Further research is needed regarding the effect of avocado seed extract (*Persea Americana* Mill.) against other bacteria that cause periodontal diseasein vitro and it is necessary to test the toxicity of avocado seeds to the mucosa or tissues of the oral cavity

REFERENCES

[1] D. Rahmawati, H. Prasetyo, and P. Santika, “Heritability and Genetic Gain of the Agronomic Characters of Crosses Between Local Black Rice and White Rice in F2 Generation,” no. 1990, pp. 113–118, 2013.

[2] Wijaya, F., *et al.* (2022). Efek Antibakteri Ekstrak Etanol Buah Asam Jawa(*Tamarindus Indica L.*) Terhadap Pertumbuhan Bakteri *Prevotella intermedia*.*Jurnal Kedokteran Gigi,*

6(2), 643–653.

[3] Cilmiaty, R., *et al*. (2013). *Prevotella intermedia* and *Porphyromonas gingivalis* indental carieswith periapical granuloma. *Dental Journal (Majalah KedokteranGigi)*, 46(4), 213.

[4] K Dayakar, M. M., *et al*. (2021). *Prevotella intermedia* - An overview and its role in periodontitis. *Journal of Advanced Clinical and Research Insights*, *8*(4), 79– 82.

[5] Y. T. Tamer *et al.*, "The highly evolvable nature of the antibiotic efflux protein TolC limits useof phages and bacterial toxins as antibacterial agents," *bioRxiv*, 2020.

[6] I. K. N, Sanjaya, *et al*. (2020). Ekstraksi katekin dari biji alpukat dengan variasi pelarut menggunakan metode maserasi. *Jurnal Kimia*, 14(1), 1-4.

[7] A. Safina *et al.*, "FACT is a sensor of DNA torsional stress in eukaryotic cells," *Nucleic AcidsRes.*, vol. 45, no. 4, pp. 1925–1945, 2017, doi: 10.1093/nar/gkw1366.

[8] P. R. Pratama, F. Isman, and A. Fadlan, "Penyelidikan Aktivitas Antikanker Payudara oleh Minyak Atsiri Bunga Michelia Alba Secara In Silico," *al-Kimiya*, vol. 9, no. 1, pp. 1–9, 2022, doi: 10.15575/ak.v9i1.17380.

[9] V, Agatha, *et al.* (2021). Aktivitas Antibakteri Ekstrak Kulit Jeruk Nipis (*Citrus Aurantifolia*) Terhadap Bakteri. *Jurnal Kedokteran Gigi Universitas Padjadjaran*, *33*(2), 167.

[10] Mukhriani. (2014). Ekstraksi, Pemisahan Senyawa, dan Identifikasi Senyawa Aktif. *Jurnal Kesehatan*, 8(2), 361-367.

[11] S, Egra, *et al*. (2019). Aktivitas Antimikroba Ekstrak Bakau (*Rhizophora mucronata*) dalam Menghambat Pertumbuhan *Ralstonia Solanacearum*Penyebab Penyakit Layu. *Agrovigor: Jurnal Agroekoteknologi*, *12*(1), 26.

[12] R, Indiyen, *et al*. (2020). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Andong Merah terhadap *Eschericia coli* dan *Staphylococcus aureus*. *Proceeding of Mulawarman Pharmaceuticals Conferences*, 22–26

[13] Fitriani, A. (2014). Aktivitas Alkaloid *Ageratum Conyzoides L*. Terhadap Pertumbuhan Bakteri *Staphylococcus Aureus* Secara *In Vitro*. *Prosiding Simposium Penelitian Bahan Obat Alami (SPBOA) XVI & Muktamar XII PERHIPBA 2014*, 1-8.

[14] Kumalasari, E., *et al*. (2020). Uji Aktivitas Antibakteri Ekstrak Etanol DaunBawang Dayak (*Eleutherine palmifolia* (L.) Merr) Terhadap Pertumbuhan *Propionibacterium acne*. *Jurnal Insan Farmasi Indonesia*, *3*(2), 261–270.