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Potential Antibacterial of Binahong Leaf Extract Against Bacteria *Enterococcus faecalis* ATCC® 29212™ (in vitro)

Potensi Antibakteri Ekstrak Daun Binahong Terhadap Bakteri *Enterococcus faecalis* ATCC® 29212™ (in vitro)

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Abstract

Binahong is a plant widely found in Indonesia and has been used for generations, but only by inheritance without much optimal research. *Enterococcus faecalis* is bacteria that cause many problems in the oral cavity such as periradicular lesion. The purpose of this study was to determine the zone of inhibition, Minimum Bactericidal Concentration (MBC), and Minimum Inhibitory Concentration (MIC) of Binahong leaf extract against bacteria *Enterococcus faecalis* ATCC® 29212™ concentrations of 100%, 90%, 80%, 70%, and 60%, *chlorhexidine* 0,2% as positive control and DMSO as a negative control. The experimental method used for the research with a post-test only control group design, pure bacteria prepared in the microbiology laboratory, and Binahong leaf extract used in the chemical laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) at the University of North Sumatera. Five treatments of Binahong leaf extract concentration were tested as samples four times. MBC and MIC measurements of Binahong leaf extract against *Enterococcus faecalis* ATCC® 29212™ were exchanged for each concentration. Mueller Hinton Agar (MHA) and Nutrient Broth incubated for 24 hours at 37°C. The ANOVA test data is analysed, followed by the Post Hoc test. The average inhibition zone of 100% concentration was 14.19 mm, 90% was 12.25 mm, 80% was (10.19 mm), 70% was 8.81 mm, 60% was 0.00 mm, *chlorhexidine* 0,2% was 20.88 mm, and DMSO was 0.00 m). The conclusion is that the best inhibition zone has a concentration of 100%, MBC was at 100%, and MIC was at 60%.

Keywords: Binahong leaf extract, antimicrobial activity, *Enterococcus faecalis* ATCC® 29212™.

Abstrak

Tanaman binahong banyak ditemukan di Indonesia dan digunakan secara turun-temurun, namun hanya berdasarkan empiris peninggalan pendahulu karena belum banyak dilakukan penelitian ilmiah. *Enterococcus faecalis* merupakan bakteri penyebab berbagai permasalahan di rongga mulut seperti lesi periradikular. Penelitian ini bertujuan untuk mengetahui zona hambat, Kadar Hambat Minimum (KHM) dan Kadar Bunuh Minimum (KBM) ekstrak daun Binahong terhadap pertumbuhan bakteri *Entercoccus faecalis* ATCC® 29212™ (*in vitro*) dengan menggunakan konsentrasi 100%, 90%, 80%, 70%, 60%, klorheksidin 2% sebagai kontrol positif dan DMSO sebagai kontrol negatif. Metode penelitian adalah *Post Test Only Control Group Design*. Bakteri merupakan isolat ATCC yang disubkultur di laboratorium Mikrobiologi Rumah Sakit Universitas Sumatera Utara (RS.USU), sedangkan ekstrak daun Binahong dibuat di Laboratorium kimia FMIPA Universitas Sumatera Utara. Sampel diuji terhadap 5 perlakuan konsentrasi ekstrak daun binahong dan 2 perlakuan kontrol dengan empat kali pengulangan. Pengukuran Zona Hambat, penentuan KHM dan KBM ekstrak daun Binahong terhadap bakteri *Entercoccus faecalis* ATCC® 29212™ menggunakan media *Mueller Hinton Agar* dan media *Nutrient Broth*, diinkubasikan selama 24 jam pada suhu 37°C. Analisis data menggunakan uji ANOVA dan dilanjutkan dengan uji *Post-Hoc* untuk mengetahui perbedaan yang signifikan pada setiap konsentrasi. Hasil penelitian rata-rata zona hambat ekstrak daun binahong pada konsentrasi 100% (14,19 mm); 90% (12,25 mm); 80% (10,19 mm); 70% (8,81 mm); 60% (0,00 mm); DMSO (0.00 mm) dan klorheksidin (20,88 mm). Nilai KHM dan KBM ekstrak daun Binahong terhadap bakteri *Entercoccus faecalis* ATCC® 29212™ adalah 100% dan 80%. Kesimpulan, ekstrak daun

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Binahong memberikan efek antibakteri dimulai dari konsentrasi 80% terhadap bakteri *Entercoccus faecalis* ATCC® 29212™.

Kata Kunci: Ekstrak daun Binahong, Zona Hambat, KHM, KBM, *Entercoccus faecalis* ATCC® 29212™.

INTRODUCTION

Indonesia is an agricultural country that has a lot of potential plants that can be used as medicine. Binahong (*Anredera cordifolia*) is one of the plants found in Indonesia among 30 thousand types of plants and 950 types of plants that function as medicine for curing various kinds of mild and severe diseases, including as a wound medicine. The benefits of binahong as medicine are appropriate to observe.[1,2]

Binahong (*Anredera cordifolia*) is a species of rhizome-shaped plant that grows creepingly, has a long trunk, and can reach heights of 5 meters or more. The trunk is soft, cylindrical, intertwined, red, and smooth. A tuber grows beneath the leaf. The leaves are numerous single, very short stems, arranged alternately, green, -a shaped heart (cordata), 5-10 cm in length. The leaves are thin and limp, with pointed tips, a notched base, flat edges, and a smooth and shiny surface. The flowers are long-stemmed, a compound in the form of bunches, and appear in the leaf axils; the crown is composed of five strands that are not attached. 0.51 cm strands, whitish cream colour, and fragrant. The stamens have three shorter branches in the middle.[3,4,5]

Almost all Binahong plant parts such as tubers, stems, flowers, and leaves can be used for medical therapy. The secondary metabolite content of Binahong leaves includes flavonoids, saponins, alkaloids, polyphenols and ancordin.[6,7,8]

Enterococcus faecalis is a persistent microorganism that is a leading cause of periradicular lesions following root canal therapy. These bacteria can survive in the root canal, resulting in root canal failed treatment.[9]

Enterococcus faecalis is a gram-positive, ovoid in size 0.6-2.0×0.6-2.5µm which can colonize in pairs like chains that do not spore and sometimes move. Its non-haemolytic nature grows at a temperature of 0-44°C, with optimal growth at a temperature of 37°C. The nature of this bacterium is facultative anaerobic meaning it grows well in an atmosphere without oxygen.[10,11,12]

Many studies have found that the Binahong plant can aid in the healing process of a variety of diseases, including internal and external wounds, colitis, diabetes, stroke prevention, gout treatment, and energy vitality maintenance.[5,13] Recent research found that extract of Binahong leaf (*Anredera cordifolia* (*Tenore*) *Steenis*) affected wound healing caused by infection *Staphylococcus aureus* in mice.[14]

Based on the problems above, Binahong is a plant that is widely found in Indonesia and has been used for generations, but that just only by inheritance without much optimal research. *Enterococcus faecalis* is bacteria that cause many problems in the oral cavity such as periradicular lesion. The research must be done to know the antibacterial potential of Binahong leaf extract (*Anredera cordifolia* (Ten.) *Steenis*) against *Enterococcus faecalis* ATCC® 29212™. The binahong leaf could become an irrigation material, mouthwash and oral cream that can be used for dental treatment of lesions in the future.

MATERIALS AND METHODS

The materials used include, binahong leaf, Ethanol 70%, *Mueller Hinton Agar*, *Nutrient Broth*, *Enterococcus Faecalis* ATCC® 29212™, and DMSO. The binahong leaves were collected by purposive sampling and obtained from the Binjai city. Digital Weigh, paper disk, filter paper, cotton, plastic pot, percolator, Erlenmeyer, vial, vacuum rotavapor, vortex, incubator, micropipette, ose, spiritus, Petri disk, lab coat, mask and gloves.

The type of research is in vitro experimental laboratory with a post-test only control group design. This research was conducted at the Microbiology Laboratory of the University of North Sumatra Hospital from January 2021 to April 2021.

Binahong leaves weighing 2 kg were washed with water and dried in a drying cabinet for one week at temperatures 50-60ºC. The dried binahong leaves are then crushed into small, smooth pieces (simplicia).

As much as 200 g of siplacia was added to 2 litres of 70% ethanol and stirred for the first 6 hours. Refrigerate for 18 hours, stirring occasionally. The extract was then filtered and evaporated using a rotary vacuum Rotavapor at a temperature of 40ºC, yielding a concentrated extract, which was then placed in a vial.

This study used 7 treatment groups, each consisting of Group I (4 samples, 100% extract), Group II (4 samples, 90% extract), Group III (4 samples, 80% extract), Group IV (4 samples, 70% extract), Group V (4 samples, 60% extract), Group VI (4 samples,

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Chlorhexidine 2% as positive control), Group VII (4 samples, DMSO as negative control).

The used samples were a pure culture of *Enterococcus faecalis* ATTC® 29212™ obtained from the Microbiology Laboratory of the University of North Sumatra Hospital. An ose of *Enterococcus faecalis* ATCC®29212™ was taken then cultured and suspended in 0.85% NaCl solution until the turbidity is the same as 0.5 McFarland standard. Put as much as 1 ose into the media and then streak/scrape tightly on the surface of the MHA on the Petri disk.

Antibacterial test using the diffusion method disc to determine the zone of inhibition (Kirby-Bauer). The principle of this method is to determine the agent's antimicrobial ability by measuring the diameter, which results in the antimicrobial strength inhibiting and even killing the test microbes. The inhibition zone formed is a colony-free area measured with a calliper that computes the vertical and horizontal diameters.[15]

Set sterile paper disc and dip them with binahong leaf extract with concentrations (100%, 90%, 80%, 70%, and 60%), while positive control with 2% chlorhexidine and negative control with DMSO. Place an ose of *Enterococcus faecalis* ATCC® 29212™ on the Petri disk and streak/scrape tightly on the surface of the MHA. Using tweezers, gently press the prepared paper disks onto the MHA medium in a petri dish. Incubate at 37°C for 24 hours. The results of the culture can be seen after 24 hours.

Prepare test tubes by labelling them and adding 1 ml of NB media to each tube. Drop 1 ml of binahong leaf extract at various concentrations (100%, 90%, 80%, 70%, and 60%) into each tube using a micropipette. in addition to 1 ml of each positive and negative control solution Using a cotton swab, insert one bacterial streak in the inhibition zone of each concentration on a petri dish into each tube that has been vortexed to see the MBC. The tube is then incubated for 24 hours. Remove the rack and record the amount of cloudiness in the liquid media on the tube. Tubes infected with *Enterococcus faecalis* ATCC® 29212™ become cloudy, whereas those with stunted growth become clear.

Each test solution was planted on MHA media and incubated for 24 hours to ensure the MIC and MBC values. The number of colonies was then determined using a colony counter. The lowest concentration in media that is not overgrown with bacteria is referred to as MBC. The data that have been obtained were analysed using Statistical Packaging for Social Sciences (SPSS) with the descriptive test, one-way ANOVA and post hoc LSD.

RESULTS

Measurement of antibacterial potency of Binahong leaf extract against *Enterococcus faecalis*: The antibacterial potential of Binahong leaf extract against bacteria was tested in *Enterococcus faecalis* ATCC® 29212™ using the diffusion method for 15 minutes, over a medium that has been inoculated with bacteria. Incubated for 24 hours at 37°C, the inhibitory zone formed around discs was measured using a calliper with a precision of 0.02 and the average is obtained.

The results showed that the average diameter of the Binahong leaf extract inhibition zone at 100% was 14.19 ± 0.24 mm, 90% was 12.25 ± 0.20mm, 80% was 10.19 ± 0.13 mm, 70% was 8.81 ± 0.38 mm, and was no inhibition at 60% concentration against *Enterococcus faecalis* ATCC® 29212™). The average inhibitory diameter in the 0.2% *chlorhexidine* as a positive control group was 20.88 ± 0.48 mm. There was no inhibition zone in the DMSO as a negative control group. The best inhibition zone produced by Binahong leaf extract against *Enterococcus faecalis* ATCC® 29212™ was at a concentration of 100 per cent, according to the findings of this study.

All research data on the antibacterial potential of Binahong leaves using the diffusion method were tested for normality using the statistical test Shapiro wilk. Based on the results of the normality test, the data were normally distributed. So, the data analysis that can be used in this study is one-way ANOVA and post hoc LSD.

The results of this statistical test obtained a p-value = 0.001 ($p<0.05$). This value proves that there is a significant difference in the inhibition diameter of the Binahong leaf extract concentrations of 100%, 90%, 80%, 70%, 60%, *chlorhexidine* and DMSO in inhibiting the growth of *Enterococcus faecalis* ATCC® 29212™. From the statistical results, it can be stated that there is the antibacterial potential of Binahong leaf extract in inhibiting bacteria *Enterococcus faecalis* ATCC® 29212™ which means that the higher the concentration of Binahong leaf extract, the greater the inhibition against *Enterococcus faecalis* ATCC® 29212™. After the ANOVA test one-way, post hoc LSD was used to see the difference in the antibacterial potency of the Binahong leaf extract with concentrations of 100%, 90%, 80%, 70%, 60%, *chlorhexidine* 0,2% and DMSO in inhibiting bacteria. *Enterococcus faecalis* ATCC® 29212™.

In this study, Binahong leaf extract gave a thick colour so it was difficult to assess the level of clarity of liquid media, as a result, the MIC value could not be determined. The researcher then continued planting on solid media (MHA) to be able to see several colonies that grew and calculated from each concentra-

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tion of the tested extract to get the value of MBC. The results of planting on solid media showed that the Binahong leaf extract with a concentration of 100% did not find the growth of bacteria *Enterococcus faecalis* ATCC® 29212™.

According to Table 2, the average number of bacterial colonies that grew after being given Binahong leaf extract concentrations of 60% was 312.50 ± 11.09 CFU, 70% was 263.25 ± 15.95 CFU, 80% was 106.75 ± 17.06 CFU, 90% was 52.25 ± 7.14 CFU, while no bacterial colonies grew at 100% concentration. According to the findings of this study, the MBC value of Binahong leaf extract against bacteria *Enterococcus faecalis* ATCC® 29212™ was at 100% concentration. There were no colonies that grew in the positive control group (*chlorhexidine*), but colonies were grown in the negative control group (DM SO) with an average of 334.75 ± 9.43.

All research data on the antibacterial potential of Binahong leaves using the dilution method were tested for normality using the statistical test Shapiro wilk. Based on the results of the normality test, the data were normally distributed. So, the data analysis that can be used in this study is one-way ANOVA and post hoc LSD. The Statistical test one-way ANOVA used in this dilution method aims to see the difference in the antibacterial potential of the test material in killing the growth of bacteria *Enterococcus faecalis* ATCC® 29212™.

DISCUSSIONS

Antibacterial activity can be assessed by two methods, namely diffusion and dilution methods. The research used diffusion and dilution methods to determine the antibacterial potential of Binahong leaf extract (*Anredera cordifolia* (Ten.) *Steenis*) against *Enterococcus faecalis* ATCC® 29212™. The diffusion method is one of the most frequently used methods by researchers to analyse antibacterial activity.[16,17]

The working principle is the diffusion method of antibacterial compounds in a solid medium in which microbes have been inoculated. The results obtained were in the form of the presence or absence of a clear area formed around the paper disc which indicated an inhibition zone for bacterial growth.[18,19] Researchers used the disc method to determine the inhibitory diameter of Binahong leaf extract (*Anredera cordifolia* (Ten.) *Steenis*) against *Enterococcus faecalis* ATCC® 29212™.

Based on the results of the test using the diffusion method, it was found that the average inhibition diameter of the Binahong leaf extract was 100%, 90%, 80%, 70%, 60% against *Enterococcus faecalis* ATCC® 29212™ was; 12.25 ± 0.20 mm; 10.19 ± 0.13 mm; 8.81 ± 0.38 mm, while the concentration of 60% had no inhibition. In the *chlorhexidine* group, the average inhibitory diameter was 20.88 ± 0.48 mm. For the DMSO group, no inhibited activity was found. This means that Binahong leaf extract concentrations of 100%, 90%, 80%, and 70% were able to inhibit the growth of *Enterococcus faecalis* ATCC® 29212™. With various inhibition zone diameters. The higher the concentration, the wider the resulting inhibition zone.[20]

The recent research stated that the pure extract of Binahong leaves was efficacious in inhibiting the growth of bacteria Streptococcus mutans with an average diameter of 8.32 mm.[21] The antimicrobial inhibitory activity was expressed based on the clear zone produced around the paper disc. The diameter of the zone of inhibition of bacterial growth was measured in mm. According to Datta et al (2019), the antibacterial inhibitory zone activities were grouped into four categories, namely: weak activity (<5 mm), moderate (5-10 mm), strong activity (>10-20 mm), very strong (>20- 30mm).[22] In this study, it was seen that the antibacterial potential of Binahong leaf extract concentrations of 100%, 90%, and 80% in inhibiting the growth of *Enterococcus faecalis* ATCC® 29212™ was included in the category of strong inhibitory power, while the concentration of 70% had moderate antibacterial inhibition.[22]

Based on the results of testing the antibacterial potential of Binahong leaf extract (Anredera cordifolia (Ten.) *Steenis*) against *Enterococcus faecalis* ATCC® 29212™ was seen that the higher the concentration of the extract, the greater the diameter of the inhibition and the greater the number of colonies of bacteria. *Enterococcus faecalis* ATCC® 29212™ which is lysed. This is due to the higher the concentration of Binahong leaf extract, the more antibacterial active ingredients contained therein. The addition of the concentration of antibacterial active compounds can increase the penetration of antibacterial compounds into the inside of microbial cells which will damage the cell metabolism system and can result in cell death.[23,24]

One of the active substances contained in Binahong leaf flavonoid. Flavonoids are a group of phenolic phytochemicals that function as antimicrobials. Flavonoids also play a role in inhibiting energy metabolism where these compounds will interfere with energy metabolism in a way similar to inhibiting the respiratory system because sufficient energy is required for the active absorption of various metabolites and the biosynthesis of macromolecules. The damage caused by flavonoids is damage to the permeability of bacterial cell walls, microsomes and ly-

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sosomes. Damage to cell membranes and walls will cause important metabolites in the cell to be released, resulting in cell death.[25,26,27]

Apart from being antibacterial, the flavonoid compounds in Binahong leaf extract also have antioxidant properties.[28] Saponins are surface-active compounds and are soap-like. If the extraction uses 70% ethanol solvent, saponins will give better results as antibacterial. Other compounds, namely alkaloids, are the largest group of secondary plant substances. Alkaloids include basic compounds containing one or more nitrogen atoms, usually in combination as part of a cyclic system. Furthermore, polyphenols are compounds with more than one benzene nucleus. Polyphenol compounds are easily soluble in water because they are polar.[29,30,31]

Therefore, the presence of flavonoids, alkaloids, saponins, tannins, and polyphenols contained in Binahong leaves causes antibacterial potential against *Enterococcus faecalis* ATCC® 29212™ although must use a sufficiently concentrated concentration to give an antibacterial effect.

The results of the study can be concluded that based on the results of the diffusion test the inhibition zone formed by giving Binahong leaf extract on the growth of *Enterococcus faecalis* ATCC® 29212™ bacteria starting at a concentration of the strongest 80%, 90%, and 100% (Kirby Bauer). MBC at concentration 100% and MIC at concentration 60%. Therefore, this study supports the use of the Binahong leaf as an antibacterial plant. Thus, a lot of research must be conducted to help discover drugs from plant sources.

TABLES

Table 1. The average diameter of the inhibition zone of Binahong leaf extract with concentrations of 100%, 90%, 80%, 70%, 60%, *chlorhexidine* and DMSO against *Enterococcus faecalis* ATCC® 29212™

Group	Inhibitory Diameter (mm) / Deuteronomy				
	I	II	III	IV	$\underline{x} \pm SD$
Extract 100%	14.5	14	14	14.25	14.19 ± 0.24
Extract 90%	12.5	12	12.25	12.25	12.25 ± 0.20
Extract 80%	10	10.25	10.25	10.25	10.19 ± 0.13
Extract 70%	8.5	9	8.5	9.25	8.81 ± 0.38
Extract 60%	0	0	0	0	0
Control +	20.50	21	21.50	20.50	20.88 ± 0.48
Control -	-	-	-	-	-

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Table 2. The average number of bacterial colonies of *Enterococcus faecalis* ATCC® 29212™ in each treatment group.

Group	Number of Colonies (CFU/ml)				
	Repetition				
	I	II	III	IV	$\underline{x} \pm SD$
Extract 100%	0	0	0	0	0
Extract 90%	50	45	52	62	52.25 ± 7.14
Extract 80%	127	89	97	114	106.75 ± 17.06
Extract 70%	282	270	246	255	263.25 ± 15.95
Extract 60%	302	315	327	306	312.50 ± 11.09
Control +	0	0	0	0	0
Control -	326	334	331	348	334.75 ± 9.43

FIGURES

Figure 1. Binahong Leaf (Documentation)

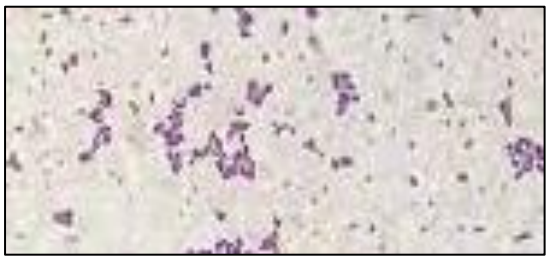

Figure 2. *Enterococcus faecalis*

REFERENCE

1. Rusli Z, Sari BL, Utami NF, Sabila S. Optimization of microwave-assisted extraction of flavonoids from binahong (*Anredera cordifolia*) leaves using respon surface methodology. J Phyto chem Ind. 2020; 7(3): 10–9.
2. Aruperes GY, Pangemanan DHC, Mintjelungan CN. Inhibitory of binahong leaf extract (*Anredera cordifolia Steenis*) against the growth of *Streptococcus mutans* bacteria. e-GiGi. 2021; 9(2): 250.
3. Restykania, Suratman, Pitoyo A, Suranto. Morphology and isozyme variation among madeira vine (*Anredera cordifolia*) accessions from southeastern part of Central Java, Indonesia. Biodiversitas. 2019; 20(10): 3024–32.
4. Dwitiyanti, Harahap Y, Elya B, Bahtiar A. Impact of solvent on the characteristics of standardized binahong leaf (*Anredera cordifolia* (Ten.) *Steenis*). Pharmacogn J. 2019;11 (6): 1463–70.

Doi: 10.32734/dentika.v25i1.7800
Received Date: 13 December 2021, Accepted Date: 25 May 2022

5. Samirana PO, Swastini DA, Ardinata IPR, Suarka IPSD. Determination of chemical content profile of binahong leaf ethanol extract (*Anredera scandens* (L.) *Moq*.). J Farm Udayana. 2017; 23.
6. Cahyanta AN, Ardiyanti NY. Activity test of anti acne ointment ethanol extract of binahong leaf (*Anredera cordifolia* (Ten) *Steenis*) against *Propionibacterium acnes*. J Pharm sci. 2018; 7(2): 239.
7. Betriksia D, Syahrial I, Suyatmiatun L. Potential test of binahong leaf extract (*Anredera cordifolia* (Ten.) *Steenis*) against increased granulation tissue thickness and healing time of burns in rats. J Pharm Sci Pract. 2018; 5(1): 11–7.
8. Tjahjani NP, Yusniawati. Description of bioactive compounds in binahong (*Anredera cordifoli*a (Ten) S*teenis*) dip preparations. Cendekia J Pharm. 2017; 1(1): 2013–5.
9. Harseno S, Mooduto L, Prasetyo EP. Antibacterial potency of kedondong bangkok leaves extract (*Spondias dulcis Forst*.) against *Enterococcus faecalis*. Conserv Dent J. 2016; 6(2): 110.
10. Radeva E. Importance of Enterococci (*Enterococcus faecalis*) for dental medicine? Micro biological characterization, prevalence and resistance. Int J Sci Res. 2017; 6(7): 1970–3.
11. Asmah HN. Molecular aspects of *Enterococcus faecalis* virulence. J Dent Syiah Kuala. 2020; 5(2): 89–94.
12. André AC, Debande L, Marteyn BS. The selective advantage of facultative anaerobes relies on their unique ability to cope with changing oxygen levels during infection. Cell Microbiol. 2021; 23(8): 1–8.
13. Purwasih R, Safitri FA. The potency of Binahong leaves (*Anredera cordifolia* (Ten.) *Steenis*) to recovery process of wound in the livestock. 2018;5(Icoh 2017): 211–5.
14. Shobib A, Kusumo P, Millah N. Characterization test of binahong (*Anredera Cordifolia* (Ten.) *Steenis*) leaves and *Aloe vera* leaves extracts using infudation method in making liquid for external wound healing. 2022; (1): 28–38.
15. Stratton CW. Susceptibility testing revisited. Prog Clin Pathol. 2019; 9: 65–100.
16. Kebede T, Gadisa E, Tufa A. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLOS One. 2021; 16(3): 1–16.
17. Nurhayati LS, Yahdiyani N, Hidayatulloh A. Comparison of testing the antibacterial activity of yogurt starter with the well diffusion method and the disc diffusion method. J Eng Farm. 2020; 1(2): 41.
18. Nassar MSM, Hazzah WA, Bakr WMK. Evaluation of antibiotic susceptibility test results: How guilty a laboratory could be? J Egypt Public Health Assoc. 2019; 94(1): 1–5.
19. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, et al. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Front Microbiol. 2018; 9(1): 1–9.
20. Makarewicz M, Drożdż I, Tarko T, Duda-Chodak A. The interactions between polyphenols and microorganisms, especially gut microbiota. J Antiox. 2021; 10(2): 1–70.
21. Prasetyaningsih Y, Kurniati E, Setiarini D. Effect of binahong (*Anredera cordifolia* (Ten.) *Steenis*) leave extract on the growth of *Streptococcus pyogenes* (in vitro). J Med. 2017; 4(1).
22. Datta FU, Daki AN, Benu I, Detha AIR, Foeh NDFK, Ndaong NA. The antimicrobial activity of rumen fluid lactic acid bacteria was tested against the growth of *Salmonella enteritidis, Bacillus cereus, Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. 7th National Seminar Veterany Faculty Universitas Nusa Cendana. Kupang, 2019; 66–85.
23. Berthold-Pluta A, Stasiak-Różańska L, Pluta A, Garbowska M. Antibacterial activities of plant-derived compounds and essential oils against *Cronobacter strains*. Eur Food Res Technol. 2019; 245(5): 1137–47.
24. Khameneh B, Iranshahy M, Soheili V, Sedigheh B, Bazzaz F. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019; 8: 1–28.
25. Luthfi M, Yuliati, Oki A, Sosiawan A, Cida B. Effectiveness of okra fruit (*Abelmoschus esculentus*) extract against *Aggregatibacter actinomycetemcomitans* (Aa) as a bacterium that causes aggressive periodontitis. J Int Oral Heal. 2020;1 2(6): 556–60
26. Žádníková P, Šínová R, Pavlík V, Šimek M, Šafránková B, Hermannová M, et al. The degradation of hyaluronan in the skin. Biomolecules. 2022; 12(2): 1–17.
27. Indrayudha P. Antibacterial activity of combination of ethanol extract of pepermine leaves (*Mentha piperita* L.) and Amikacin against *Klebsiella pneumonia, Staphylococcus aureus*, and *Escherichia coli*. J Nutraceuticals Herb Med. 2021; 4(1): 12–29.
28. Leliqia NPE, Sukandar EY, Fidrianny I. Antibacterial activities of *Anredera Cordifolia* (Ten.) V. *Steenis* leaves extracts and fractions. Asian J Pharm Clin Res. 2017; 10(12): 175–8.
29. Flom MS, Doskotch RW, Beal JL. Isolation and characterization of alkaloids from *Caulophyllum thalictroides*. J Pharm Sci. 1967; 56(11): 1515–7
30. Awuchi CG. The Biochemistry, Toxicology, and Uses of the Pharmacologically Active Phytochemicals: Alkaloids, Terpenes, Polyphenols, and Glycosides. J Food Pharm Sci. 2019; 7(1): 2
31. Feriyani F, Darmawi D, Balqis U, Lubis RR. The analysis of binahong leaves potential (*Anredera cordifolia*) as an alternative treatment of anticataractogenesis. Maced J Med Sci. 2020; 8(B): 820–4.

Doi: 10.32734/dentika.v25i1.7800
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