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Antibacterial Effectiveness of A Combination of *Anredera cordifolia* (Ten.) Steenis and *Strobilanthes crispus* Blume Extract on Inhibition of the Growth of *Streptococcus sp.* Causes of Diabetic Ulcers

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Abstract: Diabetic ulcers are open wounds on the skin surface due to macroangiopathy complications that become infected due to the entry of bacteria. Binahong and keji beling plants, can be used to treat diseases known as antibacterial and anti-inflammatory. This study aimed to determine the antibacterial effectiveness of a combination of binahong (*Anredera cordifolia* Ten. Steenis) and keji beling (*Strobilanthes crispus* Blume) extracts against *Streptococcus sp*. bacteria that cause diabetic ulcers. Samples were extracted using 96% ethanol solvent and then diluted the extract with a concentration variation of 25%, 50%, 75%, and 100%—antibacterial test using disc diffusion method (*Kirby Bauer*). Data analysis was used for the One-way ANOVA test and Duncan's Multiple Range Test further test method, which had a significance level of 0.05. Determination of antibacterial effectiveness was done by comparing the diameter of the most effective inhibition zone with the control antibiotic Ceftriaxone 30 µg. The results showed that the mean diameter of the inhibition zone of 25% concentration was 7.10 mm, and the inhibition zone of 100% concentration was 9.20 mm with moderate inhibition response. Statistical One Way Anova test showed that the combination of extracts affected the growth of *Streptococcus sp.* The most effective Concentration is at 100% concentration of 9.20 mm and antibacterial effectiveness of 92% with positive control Ceftriaxone 30 µg at 10.00 mm. It can be concluded that the combination of binahong and keji beling extracts can inhibit *Streptococcus sp.* bacteria, but its potential is not yet effective compared to *Ceftriaxone* 30 µg antibiotics.
Keywords: Antibacterial effectiveness*;* ceftriaxone; diabetic ulcers; *Streptococcus sp.*

INTRODUCTION

Diabetes mellitus (DM) is a degenerative disease that is ranked 4th in Indonesia. This disease is caused by abnormal secretion or work of insulin and causes high blood sugar levels in the body (hyperglycemia). According to the 2018 Basic Health Research, the prevalence of diabetes in adults has increased significantly in the last five years. In 2013, the prevalence reached 6.9% and continued to increase to 8.5% in 2018. According to data collected by the Samarinda City Health Office in 2021, diabetes mellitus with complications ranked 4th with 1,947 cases, while diabetes mellitus without complications ranked 7th with 741 cases. Of the ten most common diseases in Samarinda, diabetes mellitus ranked 7th[1].

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DM has a variety of chronic complications, the most common of which is diabetic ulcers. Diabetic ulcers are open wounds on the surface of the skin due to complications of macroangiopathy, leading to vascular insufficiency and neuropathy. In addition, due to the entry of germs or bacteria and because high blood sugar is a strategic location for bacterial growth, it can cause infection. One of the causes of diabetic ulcers is infection with Gram-negative and Gram-positive bacteria. Gram-positive bacteria such as *Streptococcus sp*. and *Staphylococcus aureus* are the dominant bacteria that infect the diabetic foot[2].

Handling infection problems requires proper treatment with antibiotics that can kill and inhibit the growth of bacteria. If antibiotics are given excessively over a long period, they will cause bacteria to become resistant. Ceftriaxone is a third-generation cephalosporin that is usually used to treat diabetic ulcer infections alone or in combination with metronidazole antibiotics.

Binahong plant or *Anredera cordifolia* (Ten.) Steenis) is one of the plants from the Basellacea family. Based on previous research, it has content that can be used as an antioxidant[3], antibacterial[4], antifungal[5], and antidiabetic[6]. Binahong is known among the community to traditionally treat various diseases, one of which is for wound healing. Phytochemical test screening results showed that ethanol extract of binahong leaves positively contained flavonoids, steroids, alkaloids, saponins, and phenol compounds. According to several studies, binahong leaf extract can inhibit the growth of *Shigella flexneri*[7] and *Escherichia coli*[8]. *Staphylococcus aureus*[9]. and *Propionibacterium* acnes[10].

Keji beling is planted (*Strobilanthes crispus Bl*.), a wild plant that lives by itself in the yard. Some people use the keji beling plant (*Strobilanthes crispus Bl*.) as a natural herbal medicine to treat diabetes mellitus. The results of the phytochemical test of keji beling leaf extract are favorable for the active compounds flavonoids, alkaloids, saponins, triterpenoids, steroids and tannins[11]. In previous research on the inhibition test, keji beling extract has inhibition against the growth of *S. pyogenes* bacteria[12], *S.aureus*[13], *Salmonella thypi*[14] and *Escherichia coli*[15]. Research conducted by Romantika[16]. proves that ethanol extract of keji beling leaves (*Strobilanthes crispus Bl*.) has antibacterial activity against *Streptococcus mutans* bacteria at concentrations of 55%, 35%, and 22.5%, with the diameter of the inhibition zone at a concentration of 55%, which is 19.10 ± 0.54 mm.

Currently, alternative medicine that combines several medicinal plants has emerged among the public. Previous research related to binahong (*Anredera cordifolia* Ten. Steenis) and keji beling (*Strobilanthes crispus Bl*.) leaf extracts have been carried out with a combination of other plants, such as a combination with betel crocatum leaves against *Escherichia coli* bacteria[17]. However, more research is still needed with a combination of various medicinal plants such as binahong leaves (*Anredera cordifolia* Ten. Steenis) and keji beling (*Strobilanthes crispus* Bl.) so that this study aims to determine the antibacterial effectiveness of the combination of *Anredera cordifolia* Ten. Steenis and *Strobilanthes crispus* Bl. Extracts against *Streptococcus sp.* bacteria that cause diabetic ulcers.

This study used test bacteria from clinical samples of diabetic ulcers obtained from Abdul Wahab Sjahranie Hospital and has passed the ethical feasibility process Number: DP.04.03/ F.XI.II.25/0004/2004.

MATERIALS AND METHODS

This study was conducted using a one-factor complete randomized design, a mixture of binahong and keji beling extracts (ratio 1:1) with concentrations of 25%, 50%, 75%, 100%, positive control using Ceftriaxone 30 µg antibiotics and negative control using sterile distilled water. Based on the federer formula the number of repetitions carried out six times the treatments were arranged in factorial form so that 24 treatment combinations were obtained. This research was conducted from October 2023 to April 2024; plant identification was carried out at the Anatomy and Plant Systematics Laboratory, Faculty of Mathematics and Natural Sciences, Department of Biology, Mulawarman University, Samarinda, Indonesia. The extraction process was carried out at the Pharmacology Laboratory, Samarinda College of Health Sciences, and antibacterial effectiveness testing was carried out at the bacterial laboratory of the medical laboratory technology department of the Health Polytechnic of the Ministry of Health, East Kalimantan. This study used test bacteria from clinical samples of diabetic ulcers obtained from Abdul Wahab Sjahranie Hospital and has passed the ethical feasibility process Number: DP.04.03/ F.XI.II.25/0004/2004.

The population in this study was green binahong leaves (*Anredera cordifolia* Ten. Steenis) and keji beling leaves (*Strobilanthes crispus* BI), which were obtained from hydroponic plant sellers on Apt. Pranoto, Samarinda Seberang city.

The materials and tools used in this study were fresh binahong leaves and keji beling leaves, clinical samples of diabetic ulcers. Media used for bacterial identification are Blood Agar (Oxoid), Brain Hearth Infusion (Oxoid), Sodium Agar (Oxoid), Mc Conkey Oxoid, Muller Hinton Agar (Oxoid), Ceftriaxone 30 µg antibiotic as control, sterile distilled water, physiological NaCl, 70% alcohol, sulfuric acid (H_2SO_4 1% Merck), barium chloride ($BaCl_2$ 1% Merck), hydrogen peroxide (H_2O_2 3% Merck), Gram paint (Gentian violet, lugol, acetone/alcohol, safranin) analytical scales, refrigerator, hot plate, incubator Memmert INE 400, autoclave Infitek STV-I Series Class N, rotary evaporator Buchi R-210, vortex, blender, Erlemenyer, beaker glass, test tube, micropipette, object glass, glass stirring rod, filter paper, sterile tweezers, sterile cotton stick.

Preparation of Binahong and Keji Beling Simplisia

Fresh binahong and keji beling leaves weighed as much as 2000 grams, then washed with running water, and drained by laying on a clean tray. Then binahong leaves were cut with a leaf width of ± 2 cm and dried using an oven at 40°C for 2-3 days, while keji beling leaves were dried at 50°C for 3.5 hours[18]. Furthermore, binahong leaves are mashed using a blender and filtered using a sieve, and the results are stored in a clean and tightly closed bottle or container.

Preparation of Ethanol Extract

Simplisia of binahong leaves and keji beling leaves weighed as much as 200 grams were put into Erlemenyer and extracted by maceration method using 96% ethanol solvent as much as 500 ml (1:2 b/v). The extraction process lasted 3 × 24 hours with a continuous stirring every 1 hour. After that, the results of maceration were filtered with filter paper to separate the residue from the filtrate; then the filtrate was evaporated using a rotary evaporator with a temperature of 40° C to obtain thick binahong leaf and keji beling leaf extracts, the resulting thick extracts were weighed and stored in sterile bottles[19].

Preparation of Extract Combination

A combination solution of binahong and keji beling leaf extracts was made as much as 30 mg/ml by weighing 15 mg of thick binahong leaf extract plus 15 mg of thick keji beling leaf extract (1:1) and homogenizing and making dilutions according to the desired Concentration.

Dilution of Extract Combination

Dilution of the combination of binahong leaf extract and keji beling leaves in a 1:1 ratio was made as much as 10 ml. Dilution is done using distilled water in each concentration variation made by diluting 30 mg of the combination of binahong and keji beling extracts made previously. The dilution process is carried out using the following formula equation:[20]

$$V1 \times N1 = V2 \times N2$$

Description: V1 = Initial Volume (mL), N1 = Initial Concentration (%), V2 = Volume of dilution to be made (mL), N2 = Concentration to be made (%).

Preparation of Bacterial Suspensions and McFarland Turbidity Standards

Making a suspension of *Streptococcus sp*. bacteria is done by taking one ose of bacterial colonies on Blood Agar media, suspending it in physiological NaCl, and then homogenizing it with a vortex until the turbidity is the same as the McFarland standard 0.5. If it is less turbid, add bacterial colonies; if it is more turbid, add 0.9% NaCl. The standard solution is made by pipetting 1% H_2SO_4 (sulfuric acid) as much as 9.95 ml with a measuring pipette into a test tube, then adding 1% BaCl2 solution as much as 0.05 ml Mc Farland standard is used as a basis for comparison of making bacterial suspensions[21].

Antibacterial Test

The *Streptococcus sp.* bacterial suspension that has been standardized using the Mac Farland standard is cultured as much as 100 µL, then spread using a sterile cotton stick on MHA media. Then, the disc paper that has been impregnated with 15 µL of each extract solution with a predetermined concentration is placed using sterile tweezers on Muller Hinton Agar (MHA) media and includes positive control (Ceftriaxone 30 µg antibiotic) and negative control (sterile aquadest). The distance between one disc paper and the other is not less than 21 mm; each test is done with six repetitions. Then, the MHA media was incubated at 37°C for 24 hours; inhibition zone diameter readings were taken using a dark-colored mat and measured with a ruler in millimeters.

Data Processing and Analysis

The diameter of the inhibition zone formed in each treatment was calculated as the average diameter of the inhibition zone. The mean diameter of the inhibition zone was calculated using the following formula:[12].

$$R = \frac{Dv+Dh}{2}$$

Description: Dv = Vertical diameter, Dh = Horizontal diameter, R = Average diameter.
The average diameter of the inhibition zone was then categorized based on the growth inhibition response.

The data analysis used in this study was One Way Anova analysis to determine the effect of extracts on the inhibition of *Streptococcus sp*. The next test was Duncan's to determine the most effective concentration of the antibacterial combination of binahong and keji beling extracts. The results obtained from Duncan's analysis, then the calculation of antibacterial effectiveness was carried out by comparing the diameter of the inhibition

zone produced by the positive control antibiotic Ceftriaxone 30 µg. The formula for antibacterial effectiveness is as follows: [22].

$$E = \frac{D}{Da} \times 100\%$$

Description: E = Antibacterial Effectiveness (%), D = Inhibition Zone Diameter of Extract Combination (mm), Da = Inhibition Zone Diameter of positive control antibiotic (mm).

RESULTS AND DISCUSSION

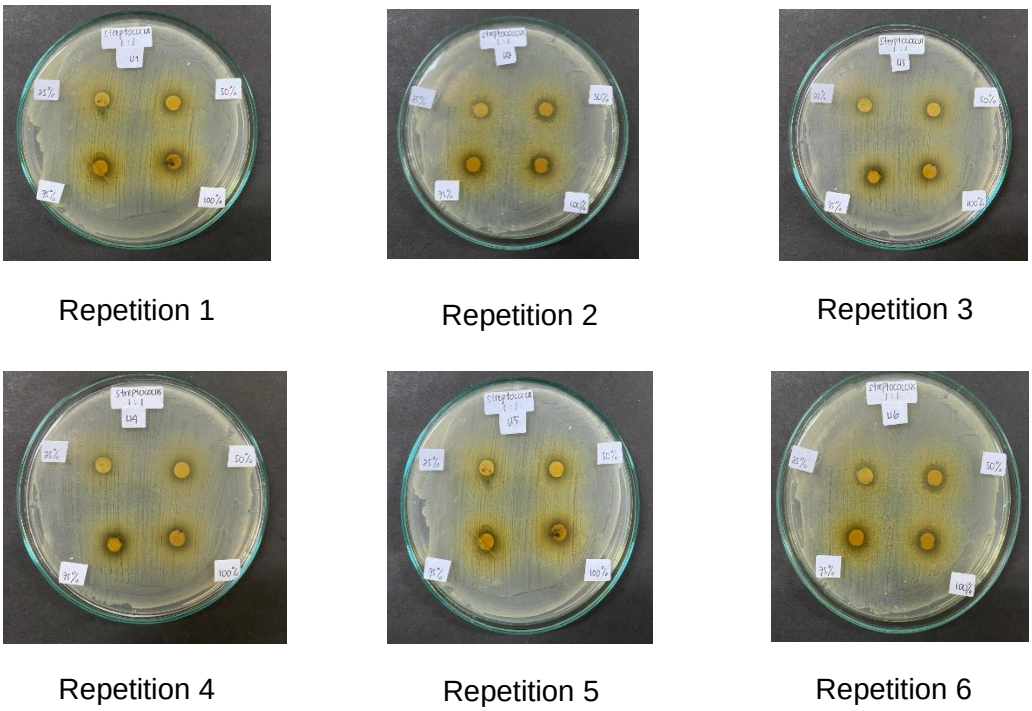
Before antibacterial testing of binahong and keji beling extracts, viscous extracts were first tested for ethanol-free; this test was carried out to ensure that there was no ethanol solvent remaining in the viscous extract content that could affect the diameter of the inhibition zone. The results obtained from the test showed that the extracts did not smell of esters, so it can be concluded that the binahong extract and keji beling extract are ethanol-free. Then, it can be continued with antibacterial tests of single extracts and combinations of extracts against *Streptococcus sp*. The results of the diameter of the inhibition zone of a single extract of binahong and a single extract of keji beling against the inhibition of bacterial growth of *Streptococcus sp.* are presented in Table 1.

Table 1. Diameter of Inhibition Zone of Binahong Single Extract and Keji Beling Single Extract Against *Streptococcus sp* Bacteria

Extract concentration	Single Extract of Binahong (mm)	Single Extract of keji beling (mm)
25%	6.00	7.00
50%	6.50	7.50
75%	7.00	8.25
100%	7.50	9.20

The diameter formed from a single extract of binahong showed the diameter of the smallest inhibition zone at a concentration of 25% of 6.00 mm and the largest concentration of 100% of 7.50 mm, while the single extract of keji beling at the smallest concentration of 25% of 7.00 mm and the largest concentration of 100% of 8.50 mm. The presence of inhibition zones formed from the extracts indicates that both extracts are able to inhibit *Streptococcus sp.* The research shows the results of the diameter of the inhibition zone of the binahong single extract and keji beling single extract (Table 1). The inhibition zone formed from every single extract shows that the single extract of keji beling leaves can better inhibit the growth of *Streptococcus sp*. due to the difference in the number of metabolite compounds in the two extracts. It is known that the diameter of a single extract of binahong and a single extract of keji beling has a different diameter of inhibition zone at each concentration.

The average diameter of the inhibition zone of the combination of binahong and keji beling extracts with a ratio of 1:1 was obtained by calculating the diameter of the clear zone formed around a 6 mm disc. The average diameter of the combination of binahong and keji beling extracts is presented in Figure 1.



Repetition 1 Repetition 2 Repetition 3

Repetition 4 Repetition 5 Repetition 6

Figure 1. Zone of Inhibition of Binahong and Keji Beling Extract Combination against *Streptococcus sp.*

Table 2. Diameter of Inhibition Zone and response barriers of Binahong and Keji Beling Extract Combination against *Streptococcus sp* bacteria.

Extract concentration	Mean Diameter of Binahong and Keji Beling Extract Combination (mm)	Positive Control Inhibition zone diameter (mm)	Negative Control Zone of Inhibition Diameter (mm)	Response Barriers
25%	7.10	10.00	0.00	Medium
50%	8.10	10.00	0.00	Medium
75%	8,50	10.00	0.00	Medium
100%	9.20	10.00	0.00	Medium

It is known that the diameter formed from the combination of binahong leaf extract and keji beling 1:1 ratio (Table 2) with the average of the smallest inhibition zone at a concentration of 25% of 7.1 mm and the largest inhibition zone at a concentration of 100% of 9.2 mm and positive control (Ceftriaxone 30 µg) of 10.00 mm.

The research shows the average diameter of the combination of binahong and keji beling extracted in a 1:1 ratio (Table 2), indicating an increase in diameter at each Concentration. This is in line with the statement of Pelczar & Chan (1986), which says that the higher the concentration of an extract, the greater the effect it will cause. The higher the Concentration of an extract, the more compounds contained in the extract that can inhibit bacterial growth[23]. Research conducted by Usman I et al. (2019) showed that

the combination of binahong leaf extract and basil leaves was better than a single extract, with a 70:30 ratio with a diameter of 8.69 mm a comparison that has a more significant inhibition zone compared to other comparisons[24], while in this study a combination of binahong and keji beling extracts in a 1:1 ratio was obtained at the highest concentration with a diameter of 9.20 mm. The results obtained are different from this study due to the Concentration of different extract combinations and bacterial culture samples used. So, it can be concluded that the combination of binahong leaf extract and keji beling leaf has better activity than the single extract; the researcher assumes when the two extracts are combined, there are active substances that can synergize so that increasing the concentration of the extract will affect the area of the inhibition zone formed around the disc.

The average inhibition response of the combination of binahong and keji beling extracts is determined based on the results obtained from the calculation of the average diameter of the inhibition zone of the combination of binahong and keji beling extracts in a 1:1 ratio, the results of the inhibition response are presented in Table 2.

The research shows the average inhibition response of the combination of binahong and keji beling extracts to inhibit *Streptococcus sp*. (Table 2). determined based on the results obtained from the calculation of the average diameter of the inhibition zone of the combination of binahong and keji beling extracts 1:1, it is known that the results of measuring the average diameter of the inhibition zone of binahong and keji beling extracts at each Concentration are included in the moderate category, the resulting inhibition response is included in the criteria based on David and Stout (1971) which states that the category of inhibition strength with an inhibition zone diameter <5 mm means weak, 5-10 diameter is moderate when 10-20 diameter is substantial, and >20 mm diameter is solid. The inhibition formed from the combination of binahong and keji beling extracts occurs due to the mechanism of action of the active compounds contained in the two extracts; one of the active compounds contained in binahong and keji beling leaves is a flavonoid compound that works by denaturing bacterial cell proteins and damaging cell membranes that can interfere with the growth of *Streptococcus sp*[25]. The difference in the average diameter of the inhibition zone of the combination of extracts at each Concentration may be due to the amount of extract absorbed on the sterile disc. There are differences in the soaking time of the disc by the extract; there are factors that can affect the size of the diameter of the inhibition zone in the diffusion method, namely the density of the inoculum, disc mounting time, incubation temperature, incubation time, plate size, thickness of agar media, disc spacing, and media composition[26].

The results of the One Way Anova statistical test show that the value of F Count (113.153) > F Table (0.05) = 0.00, which means that there is an effect of the variation of concentration of binahong and keji beling extracts 1:1 on inhibiting the growth of *Streptococcus sp*. This shows that the variation in the Concentration of the combination of different binahong and keji beling extracts has an actual or significant effect on inhibiting the growth of *Streptococcus sp*.

Determination of the most effective extraction concentration of the combination of binahong and keji beling extracts using Duncan's post hoc test. Duncan's results show that 100% extract concentration produces more significant inhibition compared to other concentrations; this means that the combination of binahong and keji beling extracts of

the highest Concentration has the best antibacterial effect in inhibiting bacterial growth of *Streptococcus sp.*

Antibacterial effectiveness was determined using the formula of comparing the diameter of the best inhibition zone with the diameter of the positive control inhibition zone. The positive control used the third-generation cephalosporin antibiotic Ceftriaxone 30 µg because this antibiotic has good sensitivity and is often used to treat diabetic ulcers. International Disease of America (IDSA) also recommends Ceftriaxone antibiotics as empirical antibiotics for the preferred therapy of diabetic ulcer infections caused by MSSA (Methicilin *Staphylococcus Streptococcus aureus*) infecting bacteria, *Streptococcus spp*: *Enterobacteriaceae*, *Pseudomonas sp*, and *anaerobic* bacteria[27]. Based on the results of this study, the diameter of the inhibition zone of the positive control of Ceftriaxone 30 µg antibiotic against *Streptococcus sp*. was 10 mm. The diameter of the inhibition zone of the most practical combination of binahong and keji beling extracts was obtained at a concentration of 100% of 9.20 mm so that the antibacterial effectiveness of the combination of binahong and keji beling extracts against *Streptococcus sp*. was 92%.

The limitations of this study include the fact that planting bacteria should be carried out in Laminar Air Flow to ensure its sterilization to avoid contamination by fungi or other bacteria. Still, the limited tools used in this study, which did not use Laminar Air Flow tools, affected the occurrence of other bacterial contamination.

CONCLUSION

The combination of binahong and keji beling leaf extracts has antibacterial activity that can inhibit the growth of *Streptococcus sp*. Combining the two extracts with a ratio of 1:1 can produce an inhibition zone around the disc. Moreover, it has an antibacterial effectiveness of 92% in inhibiting the growth of *Streptococcus sp*. but is not yet effective when compared to the antibiotic Ceftriaxone 30 µg. Suggestions for future researchers: research can be conducted on the antibacterial effectiveness of binahong and keji beling extracts of bacterial species with different bacterial cultures and extract comparisons.

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CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Djamil R, Kartika Pratami D, Vidia Riyantika L. Quality Parameters Test and α-Glukosidase Enzyme Inhibitory Activity of 70% Ethanol Extract Keji Beling Leaves (*Sericocalyx Crispus (L.) Bremek*). Jornal of Herbal Medicine. 2020;5(1):1–8.
2. Aryzki S, Alicia M, Rahmah S. Overview of Antibiotic Use in Diabetic Ulcer Patients in the Internal Medicine Outpatient Installation of Rsud Ulin Banjarmasin Period July - December 2018. Manuntung Scietific Journal. 2020;6(2):265–72.
3. Anwar TM, Soleha TU. Benefits of Binahong Leaf (*Anredera cordifolia*) as a therapy for Acne Vulgaris. Majority. 2016;5(5):179–83.

4. Halim HA, Ratnah S, Abdullah T. Phytochemical Screening and Potential Antibacterial Activity of Binahong (*Anredera Cordifolia* (Ten.) Steenis) Leaf Extract against *Staphylococcus aureus* and *Escherichia coli*. Journal of Labora Medika 2022;6(2):49–52. http://jurnal.unimus.ac.id/index.php/JLabMed
5. Wisudawan, Arsal ASF, Imron A, Bamahry A, Makmun A. Test of Inhibitory Effectiveness of Binahong Leaf Extract against the Growth of *Candida albicans*. Fakumi Med J. 2021;3(5):254–60.
6. Rani A, Haris NM, Faisal TA. Activity Test of Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis) Against Blood Sugar Levels of Mice. In: Proceedings of the National Seminar on Research Results. 2020. p. 484–90:
7. Ainurrochmah A, Ratnasari E, Lisdiana L. Effectiveness of Binahong Leaf Extract (Anredera cordifolia) on Growth Inhibition of *Shigella flexneri* Bacteria by the Well Method. Journal of LenteraBio. 2013;2(3):233–7.
8. Darsana, I.G., Besung, I.N., & Mahatmi, H. The potential of Binahong Leaf (*Anredera cordifolia* (Tenore) Steenis) in Inhibiting the Growth of *Escherichia coli* Bacteria In Vitro. Indones Med Veterinus. 2012;1(3):337–51.
9. Mengga C, Rampe M, Sangande F. Antibacterial Effectiveness Test of Binahong Leaf Extract (Anredera cordifolia (Tenore) Steenis) Against *Staphylococcus aureus* Bacteria. Biofarmasetikal Trop. 2022;5(1):60–5.
10. Sasebohe VY, Prakasita VC, Aditiyarini D. Antibacterial Activity of Binahong Leaf Ethanol Extract Against *Staphylococcus aureus* and *Propionibacterium acnes* that Cause Acne. Sciscitatio. 2023;4(1):1–10.
11. Roring N, Yudistira A, Lolo WA. Standardization of Specific Parameters and Anticancer Activity Test against T47D Breast Cancer Cells from Ethanol Extract of Keji Beling Leaf (*Strobilanthes Crispa (L.) Blume*). Pharmacon. 2017;6(3):176–85.
12. Rawung I, Wowor PM, Mambo C. Inhibition Test of Keji Beling Leaf Extract (Sericocalyx crispus (L). Bremek) against the Growth of *Streptococcus pyogenes*. J e-Biomedik. 2019;7(2):125–9.
13. Dita Artanti SF. Effectiveness of Keji Beling (*Sericocalyx crispus Linn*) Leaf Juice in Inhibiting the Growth of *Staphylococcus aureus*. J Muhammadiyah Med Lab Technol. 2017;1(1):78–83.
14. Benigna M. Inhibition Test of Keji Beling Leaf Extract (*Srobilanthes crispa* Bl.) Against the Growth of *Salmonella typhi* Bacteria In Vitro. Universitas Sanata Dharma; 2015.
15. Adibi S, Nordan H, Ningsih SN, Kurnia M, Evando, Rohiat S. Antioxidant and Antibacteria Activities Of *Strobilanthes crispus* Bl (Keji Beling) Extract Againts *Stapylococcus aureus* and *Escherichia coli.* ALOTROP Jornal of Chemical Education and Science. 2017;1(2):148–54.
16. Helianto RL. Antibacterial Activity Test of Keji Beling (Strobilanthes crispus) Leaf Extract against *Streptococcus mutans* Bacteria. 2022: https://repositori.usu.ac.id/handle/123456789/50291
17. Charismawati NA. Antibacterial Effectiveness Test of Combination of Binahong Leaf Extract (*Basella cordifolia* Lam.) and green betel leaf extract (*Piper betel* L.) against *Escherichia coli* bacteria by diffusion method. Stikes Bhakti Husada Mulia Madiun; 2019.
18. Fitriana A, Harun N, Yusmarini Y. The Quality of Herbal Tea Leaves Keji Beling With

Drying Time Treatment. J Sagu. 2017;16(2):34–41.
19. Samirana PO, Swastini DA, Ardinata IPR, Suarka IPSD. Determination of Chemical Content Profile of Binahong Leaf Ethanol Extract (*Anredera scandens* (L.) Moq.). Journal of Udayana Farmasi. 2017;6(1):23–5.
20. Afrida Amaliah LL. Antibacterial Activity of Ethanol Extract Combination of Binahong and Basil Leaves to The Growth of *Escherichia coli*. Journal of Lentera Bio. 2022;11(3):603–10: https://journal.unesa.ac.id/index.php/lenterabio/index603
21. Pancawati Ariami, Igan Danuyanti BRA. Effectiveness of Mangosteen Fruit Peel Tea (*Garcinia mangostana L*) as an Antimicrobial Against the Growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Bacteria. Journal of Laboratory Technology. 2017;3(6):3–8.
22. Harlita TD, Anggrieni N, Rahmawati AAW. Antibacterial Activity and Effectiveness of Ciplukan Leaf Extract (*Physalis angulata L*.) Against the Growth of *Bacillus cereus*. Husada Mahakan Health Journal. 2019;V(1):51–60.
23. Harlita TD, Oedjijono, Asnani A. The Atibacterial Activity of Dayak Onion (*Eleutherine palmifolia (L.) merr*) Towards Pathogenic Bacteria. Trop Life Sci Res. 2018;29(2):39–52.
24. Usman I, Rambung JS, Hijriah ER, Ismail. Antibacterial Activity Of Extracts Of Basil Leaves And Binahong Leaves On *Streptococcus mutans*. Journal of Pharmaceutical Media. 2019;15(2):107–11.
25. Tilarso DP, Muadifah A, Handaru W, Pratiwi PI, Khusna ML. Antibacterial Activity of Combination of Betel Leaf and Belimbing Wuluh Extracts by Hydroextraction Method. Chempublish J. 2021;6(2):63–74.
26. Herrialfian, Lubis MMN, Darmawi, Dewi M, Erina, Hennivanda AH. Inhibition Activity of Ethanolic Extract of Binahong Leaf (*Anredera cordifolia* (Ten.) Steenis) on *Staphylococcus aureus* Bacteria. Journal of Medica Veterinaria. 2022;15(1):43–55.
27. Setyoningsih H, Yudanto GP, Ismah K, Handayani Y. Evaluation of Antibiotic Use in Diabetes Patients Based on the Gyssens Method at Kudus Islamic Hospital. Cendikia J Pharm. 2015;6(2):257–69.