



Antibacterial Activity Test *Gynura procumbens* (Lour.) Merr (of Sambung Nyawa) Leaf Extract Against *Escherichia coli*

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ABSTRACT

Introduction: Diarrhea is a disease that contributes to the highest death rate in Indonesia. The number of diarrhea cases recorded in Indonesia in 2021 was 7.3 million. The most common cause of diarrhea is *Escherichia coli* bacteria. Based on previous studies, one of the traditional medicines that can treat diarrhea is sambung nyawa leaf (*Gynura procumbens* (Lour.) Merr.), which has various secondary metabolite compounds that can kill bacteria.

Objectives and Methods: This study aimed to determine the antibacterial activity of sambung nyawa leaf against *Escherichia coli*. The method used for extracting sambung nyawa leaf was the ultrasonic method. Antibacterial activity testing uses the well-diffusion method and the determination of the Minimum Inhibitory Concentration (MIC) and the determination of Minimum Bactericidal Concentration (MBC) by the dilution method. Test results data were analyzed using the Kruskal-Wallis Test.

Results: of this research showed that sambung nyawa leaf has antibacterial activity through well-diffusion testing with an average inhibition zone at a concentration of 100% of 18.215 mm, a concentration of 75% of 16.02 mm, a concentration of 50% of 14.445 mm, a concentration of 25% of 13.33 mmm. The MIC test obtained the minimum inhibition ability at a concentration of 50%.

Results: of the Kruskal-Wallis test in the three tests showed significant differences in each treatment group.

Conclusions: This research concludes that sambung nyawa leaf has antibacterial activity against *Escherichia coli* with a MIC value at a concentration of 50%. However, no MBC has been found against *Escherichia coli*.

Introduction

Indonesia, with its abundant natural wealth, is known as an archipelagic country that has high biological diversity, including various types of unique flora and fauna. Of these many natural riches, there are around 9,600 types of plants that have been recognized as having medicinal properties by local communities and researchers. Ironically, only around 200 types of these plants have been officially used in the traditional medicine industry. This shows the great potential that can still be explored further for the development of traditional medicines (Badan Pusat Statistik Indonesia, 2021; Susidarti, 2017).

The advantages of traditional medicines compared to chemical medicines are many, including having relatively lower side effects, the ability of some plants to provide a variety of pharmacological effects, and their effectiveness in treating several metabolic and degenerative diseases such as diabetes mellitus, stroke and hypertension. This advantage is in sharp contrast



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to chemical drugs, which often only focus on reducing the symptoms of the disease without treating the leading cause and have the potential to cause dangerous side effects, mainly when used over a long period (Retnaningsih et al., 2019).

One example of a plant used in traditional medicine is the life-giving leaf (*Gynura procumbens* (Lour.) Merr.), which has become popular among Indonesians to treat various diseases, including diarrhoea, hypertension and fever. Using it is simple: boiling the leaves and drinking the boiled water. Based on research conducted by Lau in 2018, it is known that the splicing life leaf extract contains various active compounds such as alkaloids, phenolics, flavonoids, saponins, steroids, tannins and terpenoids which have the potential as antibacterial agents (Lau et al., 2018).

In Indonesia, diarrhoea is still a serious public health problem, being one of the leading causes of death, especially among toddlers, with the prevalence of diarrhoea reaching quite significant figures in various age groups at 8%, toddlers 12.3%, and infants 10.6%. In 2021, the number of recorded cases of diarrhoea will be around 7.3 million (Kementerian Kesehatan Republik Indonesia, 2021). *Escherichia coli* is the main causative bacteria, and the importance of developing effective traditional medicines to overcome this health problem is becoming increasingly apparent. However, there are still limitations in research regarding the effectiveness of sambung jiwa leaf extract against *Escherichia coli* bacteria, so further research is needed to explore this potential in more depth. The development of traditional medicines from Indonesia's natural wealth will not only help overcome various health problems but will also contribute to preserving this rich biological diversity.

Material and Methods

1. Tools and Materials

The tools used in this research are incubator, biological safety cabinet (BSC), hot plate (thermo scientific-care), magnetic stirrer, vortex, stirring rod, Erlenmeyer (Pyrex), test tube (Pyrex), rack, glass measuring (Pyrex), dropper pipette, arlogy glass, analytical balance (Acis AD-600i), petri dish, beaker glass (Pyrex), horn spoon, gloves, mask, spatula, tissue, lighter, autoclave (GEA YX-280D), tube needle, spirit lamp, funnel (Pyrex), aluminium foil, plastic wrap, ultrasonic cleaner, scissors, rotary evaporator, caliper, refrigerator, sieve, micropipette, blender, and porcelain cup.

The materials used in this research were the leaves of life-giving (*Gynura procumbens* (Lour.) Merr.) as research samples, sterile distilled water, 70% ethanol, the drug chloramphenicol, *Escherichia coli* bacteria ATTC 25922, Nutrient Agar (NA), Nutrient Broth (NB), DMSO 10%, label paper, cotton, BaCl₂, H₂SO₄, and NaCl.

2. Methods

a. Extraction of Sambung Nyawa Leaves (*Gynura procumbens* (Lour.) Merr.)

Making luciferous leaf extract (*Gynura procumbens* (Lour.) Merr.) is a method that prioritizes caution and precision. In the initial stage, 100 grams of sambung nyawa leaves collected and cleaned were weighed accurately using a sensitive analytical balance. The leaves are then carefully placed into a clean and sterile glass beaker. To dissolve the sambung nyawa leaves, 70% ethanol was used as a solvent, with a ratio of 1 part leaf to 5 parts solvent. The effectiveness of this dissolving process is then increased by using ultrasonic extraction techniques, which are carried out with an Ultrasonic Cleaner. This tool works at a frequency of 40 KHz and carries out extraction for 20 minutes at a controlled temperature of 45°C. After extraction, the resulting liquid extract is filtered carefully using Whatman No. Filter paper. 1 to separate solid particles from liquid. The filtered liquid is then concentrated using a Rotary Evaporator. This concentration process was carried out at a temperature of 60°C with a rotational speed of 60 rpm until a thick extract was finally obtained from the sambung nyawa leaves, which was ready for further testing.





b. Test of the Antibacterial Activity of Sambung Nyawa Leaves

Testing the antibacterial activity of sambung nyawa leaf extract is very important to determine the potential of the extract as an antibacterial agent. Testing begins with the healthy diffusion method, carried out under sterile conditions in the Bio Safety Cabinet (BSC). The main aim of this test is to identify the presence of clear zones that form around the wells in the bacterial growth medium, which indicates the antibacterial activity of the extract. In this case, the bacteria tested is *Escherichia coli*, which often causes infections. After the clear zone is identified, more in-depth testing uses the dilution method. This test aims to determine the extract's Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (KBM). MIC is the minimum dose of extract that can inhibit bacterial growth, while KBM is the minimum dose that can kill the bacteria. This entire process reveals the antibacterial potential of sambung nyawa leaf extract and determines the effective dosage for further applications in the medical or health sector.

Results and Discussion

The first test was an excellent diffusion test to determine whether there was antibacterial activity. The results of the well diffusion test are shown in Table 1.

Table 1. Results of Antibacterial Activity using the Well Diffusion Method

Treatment	Diameter (mm)			Mean (mm)
	I	II	III	
Concentration 100%	18,12	19,40	19,52	19,01
Concentration 75%	16,45	16,73	17,63	16,94
Concentration 50%	14,80	14,73	13,52	14,35
Concentration 25%	12,45	15,11	13,45	13,67
Positive control (Kloramfeniko I)	24,6	26,5	25,3	25,47
Negative Control (DMSO 10%)	-	-	-	-

The next test carried out was the Minimum Inhibitory Concentration (MIC) dilution test to determine the inhibitory power value of the life-saving leaves. The MIC test results are in Table 2.

Table 2. Results of Antibacterial Activity using the Minimum Inhibitory Concentration (MIC) Dilution Method

Treatment	Replication		
	I	II	III
Concentration 100%	Clear	Clear	Clear
Concentration 75%	Clear	Clear	Clear
Concentration 50%	Clear	Clear	Clear
Concentration 25%	Cloudy	Cloudy	Cloudy
Positive control (Kloramfeniko I)	Clear	Clear	Clear
Negative Control (DMSO 10%)	Cloudy	Cloudy	Cloudy

The final test was the Minimum Kill Concentration (KBM) dilution test to see the value of the killing power of life-saving leaves. The KBM test results can be seen in Table 3.

Table 3. Results of Antibacterial Activity using the Minimum Kill Concentration (KBM) Dilution Method

Treatment	Concentration		
	I	II	III
Concentration 100%	Growing colonies	Growing colonies	Growing colonies
Concentration 75%	Growing colonies	Growing colonies	Growing colonies



Concentration 50%	Growing colonies	Growing colonies	Growing colonies
Positive control (Kloramfeniko l)	Colonies do not grow	Colonies do not grow	Colonies do not grow
Negative Control (DMSO 10%)	Growing colonies	Growing colonies	Growing colonies

In research on the antibacterial potential of sambungjiwa leaf extract against *Escherichia coli*, the healthy diffusion method was used as an initial technique to assess the inhibitory ability of the extract on bacterial growth. In this test, chloramphenicol was used as a positive control because of its well-known ability to inhibit various types of bacteria, including *Escherichia coli*. The test results showed that chloramphenicol, with an average inhibitory zone diameter of 25.47 mm, was included in the very effective category, with a standard value of ≥ 21 mm according to the assessment criteria. On the other hand, sambung nyowo leaf extract at a concentration of 100% produces an average inhibitory zone diameter of 19.01 mm, placing it in the practical category with a standard value of 11-20 mm. This shows that sambung nyowo leaf extract significantly inhibits *Escherichia coli*'s growth, although not as strong as chloramphenicol. The effectiveness of this inhibition appears to increase as the extract concentration increases, indicating a positive correlation between extract concentration and inhibition ability (Bakhtra et al., 2018).

Table 4. Results of the Well Diffusion Kruskal-Wallis Test

	diameter of the inhibition zone
Chi-Square	15.876
df	5
Asymp.sig	.006

a. Kruskal Wallis Test

Furthermore, statistical analysis using the Kruskal-Wallis test strengthened these findings by showing a significant difference in the effect of the extract on *Escherichia coli* based on different concentrations, with a significance value of 0.006 ($p < 0.05$). This confirms that variations in extract concentration have different effects on the bacterial inhibitory ability, most likely caused by differences in secondary metabolite content at each extract concentration.

In this study, the Minimum Inhibitory Concentration (MIC) was found at 50%, which indicates a transition from turbidity to clarity in the bacterial growth medium. This indicates the extract's effectiveness at this concentration in inhibiting bacterial growth (Djarot et al., 2019).

Table 5. Kruskal-Wallis Dilution Test Results Minimum Inhibitory Concentration (MIC)

	minimum inhibitory concentration
Chi-Square	17.000
df	5
Asymp.sig	.005

a. Kruskal Wallis Test

The results of the Kruskal-Wallis test showed a significant difference in the sambung nyowo leaf extract group against *Escherichia coli* bacteria, with a significance value of 0.005 ($p < 0.05$). These results show fundamental differences in the treatment groups (100% concentration, 75% concentration, 50% concentration, 25% concentration, positive control, and negative control). The differences due to the different concentrations in each treatment group will make the secondary metabolite content different.

However, although the MIC was found, this research could not determine the Minimum Kill Concentration (KBM) because bacteria could grow at all tested, including the 100% concentrations, except in the positive control sample, which used chloramphenicol. This situation shows that the succinct leaf extract could not wholly kill bacteria at the concentration tested, even





though it had shown significant ability to inhibit its growth. The limited literature discussing the KBM of sambung nyowo leaf extract makes interpreting the results more difficult, but based on theory, this could mean that the secondary metabolite content in sambung nyowo leaves requires an even higher concentration to be able to kill bacteria effectively (Hasanah & Gultom, 2020).

Table 6. Results of the Kruskal-Wallis Dilution Test for Minimum Kill Concentration (KBM)

	minimum kill concentration
Chi-Square	14.000
df	4
Asymp.sig	.007

a. Kruskal Wallis Test

The results of the Kruskal Wallis test showed a significant difference in the sambung nyowo leaf extract group against *Escherichia coli* bacteria, with a significance value of 0.007 ($p < 0.05$). These results show fundamental differences in the treatment groups (100% concentration, 75% concentration, 50% concentration, positive control, and negative control). The differences due to the different concentrations in each treatment group will make the secondary metabolite content different.

Sambung nyowo leaves, known for their extraordinary properties, contain various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and steroids. These components have been shown to have strong antibacterial properties, making them potential agents in alternative medicine to treat bacterial infections. In particular, alkaloids and flavonoids are known for their ability to kill bacteria directly, while tannins and saponins work by inhibiting the growth of bacteria, not killing them directly. Steroids, however, also contribute to antibacterial activity, although the mechanism of action is different (Endarini, 2016).

In research on Sambung nyowo leaf extract, the MIC (Minimum Inhibitory Concentration) value was found, indicating the minimum dose of extract needed to inhibit bacterial growth. However, the KBM (Minimum Kill Concentration) value, namely the minimum dose required to kill bacteria, has not been identified. This difference may be caused by the predominance of tannin and saponin content in Sambung nyowo leaves, which inhibit rather than kill bacteria. Therefore, to achieve a practical KBM value, increasing the concentration of sambung nyowo leaf extract is necessary. This increase is expected to increase the amount of active secondary metabolites, especially alkaloids, flavonoids, and steroids, thereby allowing the extract to inhibit bacterial growth and kill bacteria effectively. Further research is needed to optimize extract concentrations and identify the most practical combination of secondary metabolites for antibacterial activity (Endarini, 2016).

Conclusion

The conclusion of this research is based on the results of the research. Langkawi leaves have antibacterial activity through healthy diffusion testing with an average inhibition zone at 100% concentration of 19.01 mm, included in the strong inhibition zone category (11-20 mm). Samun's life leaves have a minimum inhibitory capacity at a concentration of 50%. The results of the Kruskal-Wallis test in the three tests showed significant differences in each treatment group. Langkawi leaves have antibacterial activity against *Escherichia coli* with a MIC value at a concentration of 50%, but no KBM has been found against *Escherichia coli*.

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