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Antibacterial Activity Test of Sambung Nyawa Leaf Extract (Gynura procumbens L.) Against Klebsiella pneumonia bacteria

Faelga Sara Rosiana*[a]| Dewi Natalia Sri Harmony [a] | Munawir [a] | Adieba Warda Hayya [b]
[a] Study Program of Pharmacy, Universitas Nahdlatul Ulama Nusa Tenggara Barat, Indonesia
[b] Study Program of Biology Education, Institut Agama Islam Negeri Kudus, Indonesia
*Corresponding Author: *sararosian@gmail.com*

No HP Author: +62 81999921482

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ABSTRACT

Introduction*: Infectious diseases are one of the health problems that have developed over time. Infections are generally caused by various microorganisms, such as bacteria, viruses, fungi, and protozoa. One of the bacteria that causes infection is Klebsiella pneumonia. Gram-negative bacteria can cause urinary tract infections and respiratory infections, especially in individuals with weak immune systems. Based on previous research, one traditional medicine used to relieve pneumonia symptoms is the 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 Klebsiella pneumonia. 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 60% of 12,9 mm, a concentration of 50% of 11,7 mm, a concentration of 40% of 9,8 mm, a concentration of 30% of 9,0 mmm. The MIC test obtained the minimum inhibition ability at a concentration of 40%. Results: of the Kruskall-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 40%. However, no MBC has been found against Klebsiella pneumonia.*

Introduction

Infectious diseases are one of the health problems that have developed over time. Infections are generally caused by various microorganisms, such as bacteria, viruses, fungi, and protozoa (Tika & Lanny, 2021). One of the bacteria that causes infection is Klebsiella pneumonia. Gram-negative bacteria can cause urinary tract infections and respiratory infections, especially in individuals with weak immune systems (Schroll et al., 2010). The process by which infectious diseases begin when an organism enters the host's body and then reproduces. Pathogenic bacteria must be able to enter the host's body to cause disease; infection describes the development or reproduction of microorganisms there. Approximately 8% of healthy individuals

have Klebsiella pneumoniae in their respiratory tract and feces (Marfanny, 2017), one of the Klebsiella species. In dealing with these infection problems, the use of antibacterials is essential. However, its continued use can cause various problems. So, a safer and more effective alternative treatment is needed, namely utilizing natural ingredients (Putri et al., 2019). With compounds in plants that have antibacterial activity, it is possible to develop antibiotics with fewer side effects.

Indonesia is one of the countries with a very high level of flora diversity. Indonesia is estimated to have 25% of the world's flowering plant species. It is the seventh largest country, with several species reaching 20,000, of which 40% are endemic or native to Indonesia (Kusmana & Hikmat, 2015). The high level of biodiversity gives Indonesia a variety of medicinal plants. The potential of native Indonesian medicinal plants can be seen from their contribution to world drug production. As many as 45 types of essential drugs produced by the United States come from tropical medicinal plants, 14 species of which come from Indonesia (Novianti, 2017). Around 27% of the 30,000–40,000 types of plants in Indonesia have been utilized by the population; the remaining 75% are found in the forest. Currently, traditional medicine is used by individuals in underdeveloped countries, especially to overcome public health problems (Murdopo, 2014).

Medicinal plants are one plant used by the community to overcome health problems (Pranaka et al., 2020). The Gynura procumbens plant is a famous traditional medicinal plant often used in the area. In the leaves of this plant, there are various chemical substances such as alkaloids, essential oils, flavonoids, phenolics, saponins, terpenoids, polyphenols, steroids, chlorogenic acid, caffeic acid, vanillic acid, coumaric acid, parahydroxy benzoic acid (Siregar, 2021). The community believes that the sambung nyawa plant contains these chemical substances and can treat various diseases such as fever, hypertension, shortness of breath, boils, scabies, and itching both from within and outside the body. (Simamora, 2021).

Many of the sambung nyawa leaves' benefits and properties are used to cure various diseases. The antibacterial properties of the extract of the sambung nyawa leaves (Gynura procumbens L.) against Klebsiella pneumoniae have piqued the interest of researchers, who decided to pursue this topic further..

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 sambungjiwa leaf extract and determines the effective dosage for further applications in the medical or health sector.

Results and Discussion

Tabel 1. Hasil Skrining Fitokimia Ekstrak Daun Sambung Nyawa

Senyawa Metabolit Sekunder	Pereaksi	Hasil
Alkaloid	Bouchardart	+
	Mayer	+
	Dragendroff	+
	Wagner	+
Steroida dan Triterpenoid	Lieberman-Burchad	+
Flavonoida	FeCl3 5%	+
Tanin	FeCl3 1%	+

Tes skrining fitokimia adalah prosedur pengujian pendahuluan yang digunakan dalam analisis kualitatif untuk mengidentifikasi jumlah bahan kimia aktif yang ada dalam tanaman, yang memungkinkannya digunakan sebagai obat untuk mengobati berbagai gangguan. Untuk memastikan jumlah sebaran metabolit sekunder yang terdapat pada tumbuhan tersebut yang dapat dimanfaatkan sebagai obat, maka perlu dilakukan deteksi komponen berbagai jenis tumbuhan dengan uji fitokimia. Alkaloid, flavonoid, steroid, terpenoid, dan tanin adalah contoh metabolit sekunder yang umum ditemukan pada tumbuhan. (Botahala, 2020). Jika dua hingga tiga reagen berubah, uji alkaloid dianggap berhasil; jika hanya satu perubahan yang terjadi, ekstrak tidak dapat dinyatakan mengandung alkaloid. Sebagian besar alkaloid memiliki pH basa, yang merupakan fitur yang bergantung pada keberadaan pasangan elektron komponen nitrogen. Pada tumbuhan, alkaloid biasanya bergabung dengan asam organik untuk menghasilkan garam. Dengan pelarut organik yang tepat, garam ini diekstraksi. Karena sifat semipolarnya, pelarut etil asetat dan kloroform secara efektif melarutkan alkaloid. (Kapondo, 2020). Ketika ada keberadaan triterpenoid, reaksi Lieberman-Bouchard akan menghasilkan perubahan warna dari hijau menjadi hijau kebiruan, sementara reaksi Salkowsky menghasilkan perubahan warna dari hijau menjadi merah bata (Hidayah, 2016). Biasanya, tanaman mengandung flavonoid yang memiliki aglikon (tanpa gula terikat) atau glikosida yang terikat gula. Flavonoid mudah larut dalam pelarut polar seperti aseton, etanol, metanol, butanol, dimetilformamida, dimetilsulfoksida, dan air karena adanya gula yang terkait dengan strukturnya (Henaulu, 2020). Melalui uji fitokimia dengan FeCl3, ekstrak daun sambung nyawa menunjukkan hasil positif mengandung komponen tanin yang ditandai dengan perubahan warna menjadi hitam kehijauan (Halimu, 2017). Menurut

Setyowati et al., (2014) penambahan ekstrak tanin ke dalam larutan FeCl3 akan menghasilkan berbagai perubahan warna seperti hijau cerah, merah, ungu, dan hitam.

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 2.

Table 2. Results of Antibacterial Activity using the Well Diffusion Method

Treatment	Diameter (mm)				Mean (mm)
	I	II	III	IV	
Concentration 60%	14,6	12,3	10,4	14,4	12,9
Concentration 50%	13,8	11,8	9,9	11,3	11,7
Concentration 40%	10,0	10,2	9,4	9,7	9,8
Concentration 30%	9,3	9,4	8,7	8,9	9,0
Positive control	30,1	29,6	29,8	30,3	29,95
Negative Control		-	-	-	-

In research on the antibacterial potential of sambung nyowo leaf extract against Klebsiella pneumoniae, 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 Klebsiella pneumoniae. The test results showed that chloramphenicol, with an average inhibitory zone diameter of 29.95 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 60% produces an average inhibitory zone diameter of 12.09 mm, placing it in the practical category with a standard value of 11-20 mm. This shows that sambung nyowo leaf extract significantly inhibits Klebsiella pneumoniae'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 3. Results of the Well Diffusion Kruskall-Wallis Test

	diameter of the inhibition zone
Chi-Square	15.675
df	5
Asymp.sig	.005

a. Kruskal Wallis Test

Furthermore, statistical analysis using the Kruskall-Wallis test strengthened these findings by showing a significant difference in the effect of the extract on based on different concentrations, with a significance value of 0.005 ($p < 0.05$). 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 40%, 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 4. Kruskall-Wallis Dilution Test Results Minimum Inhibitory Concentration (MIC)

	minimum inhibitory concentration
Chi-Square	18.000
df	5
Asymp.sig	.006

a. Kruskal Wallis Test

The results of the Kruskall-Wallis test showed a significant difference in the sambung nyowo leaf extract group against Klebsiella pneumoniae bacteria, with a significance value of 0.006 ($p < 0.05$). These results show fundamental differences in the treatment groups (60% concentration, 50% concentration, 40% concentration, 30% 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 60% 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 5. Results of the Kruskall-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 Kruskall Wallis test showed a significant difference in the sambung nyowo leaf extract group against Klebsiella pneumoniae, with a significance value of 0.007 ($p < 0.05$). These results show fundamental differences in the treatment groups (60% concentration, 50% concentration, 40% 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. sambung nyowo leaves have antibacterial activity through healthy diffusion testing with an average inhibition zone at 60% concentration of 12.09 mm, included in the strong inhibition zone category (11-20 mm). sambung nyowo leaves have a minimum inhibitory capacity at a concentration of 40%. The results of the Kruskall-Wallis test in the three tests showed significant differences in each treatment group. sambung nyowo leaves have antibacterial activity against Klebsiella pneumoniae with a MIC value at a concentration of 40%, but no KBM has been found against Klebsiella pneumoniae.

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