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Test of Antioxidant and Antibacterial Activity of the Extract of SambungNyawa (*Gynura procumbens* [Lour.] Merr.) Against *Streptococcus pyogenes* Bacteria

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Abstract:
Background: Upper respiratory tract infections, one of which is pharyngitis, is increasing worldwide caused by infection with the Streptococcus pyogenes bacteria. The sambungnyawa has been tested, and the results can be used as an antioxidant and antibacterial because it has phenolic and flavonoid compounds
Materials and Methods:The purpose of this study was to examine the antioxidant and antibacterial activity of 70% ethanol extract, ethyl acetate extract and sambungnyawa chloroform extract. The method used to test the antioxidant activity was the DPPH method, and the antibacterial activity test using microdilution was performed on Streptococcus pyogenes bacteria.
Results: The results obtained showed that the IC50 values of 70% ethanol extract, ethyl acetate extract and sambungnyawa chloroform extract had antioxidant activity values, respectively, namely 135.0479; 116.8404; 122.1700. The total phenol and flavonoid content of 70% ethanol extract, ethyl acetate and chloroform were 23.73 ± 0.33 %, respectively; 37.42 ± 2.07%; 23.12 ± 0.93% and 0.98 ± 0.04%; 7.28 ± 0.12 %; 9.57 ± 0.33%. All sambungnyawa extracts had MIC at a concentration of 256 ppm.
Conclusion: Based on the results of the study, it can be concluded that the sambungnyawa extract has the highest antioxidant activity in ethyl acetate extract, the highest total phenolic content, namely ethyl acetate extract and total flavonoid content, namely chloroform extract, and can inhibit the growth of Streptococcus pyogenes bacteria.
Key Word:antioxidant; antibacterial; sambungnyawa; Streptococcus pyogenes

I. INTRODUCTION

Acute Respiratory Tract Infection (ISPA) is a disease that attacks the respiratory tract which is contagious, especially through droplets caused by infectious agents(1). ISPA is a major global health problem and a major cause of death in children in developing countries(2). The prevalence of ISPA in 2013 was 25%, in 2018 as many as 20.06% of Indonesian people experienced acute respiratory infections, the amount of data was almost the same as the previous year, namely 20.56%. This data has indeed decreased, but the ARI that occurs is still widespread in parts of Indonesia, from 2009-2019 this disease was controlled throughout Indonesia (3).

Gyunara procumbens [Lour.] Merr. discovered centuries ago on the African continent. The genus Gynura (Asteraceae-Senecioneae) consists of 44 species and is distributed from tropical Africa to South and East Asia and Australia with one species in tropical Australia. The highest species diversity is found in Southeast Asia. Various leaf extracts (Gynura procumbens [Lour.] Merr.) contain several active chemical elements such as flavonoids, saponins, tannins, terpenoids and sterol glycosides. The sambungnyawa plant (Gynura procumbens [Lour.] Merr.) also contains rutin, kaempferol and antioxidant components, namely kaempferol-3-O-rutinoside and astragalin. Flavonoids are polyphenolic compounds with potential beneficial effects on human health with anti-inflammatory, antibacterial and antioxidant activities(4).

The antibacterial activity of the sambungnyawa plant (Gynura procumbens [Lour.] Merr.) has been tested against gram-positive and gram-negative bacteria(5). Sambungnyawa plants (Gynura procumbens [Lour.] Merr.) also contain antioxidants (6), a number of studies have shown that flavonoids and phenolic content contribute to the antioxidant activity of natural compounds(7). Potential anti-inflammatory effects have been attributed to antioxidant activity, evidence suggests that oxidative stress plays a pathogenic role in chronic inflammatory diseases (8). Antioxidants can reduce reactive oxygen species (ROS) and nitric oxide (NO) production(9). So that researchers are interested in conducting research on the antibacterial and antioxidant activity of chloroform, ethyl acetate, ethanol extracts of sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) possess antibacterial activity in acute respiratory infections.

II. MATERIAL AND METHODS

Work procedures

Extraction

Researchers acquired sambungnyawa plants (Gynura procumbens [Lour.] Merr.) from the ManokoLembang Experimental Plantation and then made the determination at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung.The researcher washed the leaves of sambungnyawa (Gynura procumbens [Lour.] Merr.) until clean, then sliced them thinly and weighed them. The leaves of sambungnyawa are then stored under the sun. The researchers then blended and sifted them until they formed a fine powder.The extract of sambungnyawa leaves was acquired (Gynura procumbens [Lour.] Merr.) through the cold method, namely maceration by means of 160 grams of crushed leaves of sambungnyawa macerated into chloroform, ethyl acetate and 70% ethanol solvent for 24 hours separately with occasional stirring. Maceration process was carried out for 3 days. The extract was collected through filtering with filter paper and the extract was concentrated to dryness under reduced pressure using a rotary evaporator (10).

Determination of Antioxidant Activity Test

DPPH Solution Preparation

The solution was prepared by weighing 5 mg of DPPH and adding 100 mL of methanol in a volumetric flask, to obtain a concentration of 50 ppm (11).

Sample Solution Preparation

A sample solution of 500 ppm was prepared by extracting chloroform, ethyl acetate and 70% ethanol from sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) which were weighed as much as 5 mg, incorporated with methanol and mixed until homogeneous. A total of 10mL of methanol was added. Furthermore, the concentration series is made, namely 60; 100; 140 and 160 ppm (11).

Comparison Solution Preparation

A comparison solution of 100 ppm was prepared by weighing 1 mg of vitamin c, dissolved with methanol, stirred until homogeneous and added up to 10 mL in volume. Then the researchers made a series of concentrations, namely 2; 4; 6; 8 and 10 ppm (11).

Preliminary Test

Extracts of chloroform, ethyl acetate, 70% ethanol of sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) were dissolved in methanol. Furthermore, spotting was carried out on the silica gel F254 plate using a capillary tube. The silica gel F254 plate was eluted using eluent, after that it was sprayed with the previously prepared DPPH solution. Leave it alone and observe the appearance of spots under UV light at 254 nm and 366 nm (11).

Antioxidant Power Measurement (Blank)

A total of 4 mL of DPPH solution was vortexed and incubated at 37°C. Absorbance measurements were carried out at a wavelength of 517 nm (11).

Measurement of Antioxidant Power of Chloroform, Ethyl Acetate and Ethanol Extracts of DaunSambungNyawa (Gynura procumbens [Lour.] Merr.)

Sample solution of 0.5 mL was taken from concentration 0; 60; 100; 140 and 160 ppm. Then 3.5 mL of DPPH solution was added and vortexed and incubated at 37°C. Absorbance measurements were carried out at a wavelength of 517 nm (11).

Measurement of Antioxidant Power of Vitamin C Comparison Sample

Vitamin C solution was taken from several concentrations (0; 2; 4; 6; 8 and 10 ppm) as much as 0.5 mL, to which 3.5 mL of DPPH was added. Then it was vortexed and incubated at 37°C. Absorbance measurements were carried out at a wavelength of 517 nm (11). Antioxidant activity is calculated by the following equation:

$$\%\ \text{Inhibition} = \frac{Abs.\ blanko - Abs.sample}{Abs.blanko} \times 100\%$$

Information:

Abs. Blangko = Absorbansiblanko DPPH

Abs. Sampel = Extract sample absorbance (*Gynura procumbens* [Lour.] Merr.)

Determination of Total Phenol Levels by Folin-Ciocalteu

The test solution was prepared by weighing approximately 0.2 grams of the extract which was put into an erlenmeyer, incorporated with 25 mL of methanol P, which was then stirred for 30 minutes using a magnetic stirrer. Then it was put into a 25 mL volumetric flask while being filtered and methanol P was added through a filter until it reached the limit mark.The comparison solution was prepared by weighing an amount of 10 mg put into a 25 mL volumetric flask, dissolved in methanol P and added up to the limit mark. A series of concentrations of the comparison solution was made with successive levels, namely 100; 70; 50; 30; 15; 5 g/mL.The procedure for determining the total phenolic content by means of each 1 mL of the solution and a series solution of the concentration of the comparison solution is put into a suitable container. Add 5 mL of

dilute Follin-Ciocalteu LP made up to 7.5% in water, wait for 8 minutes. Then, 4 mL of 1% NaOH was added and incubated for 1 hour. Absorbance measurements were carried out at a wavelength of 730 nm. Calculate the concentration of the test solution (12).

Determination of Total Flavonoid Level

Preparation of the test solution by weighing approximately 0.5 grams of extract, put into a 10 mL volumetric flask, then add 10 mL of ethanol P, sonicated until all extracts are dissolved. Next, the solution was put into a 10 mL volumetric flask while filtering, the filter paper was rinsed with ethanol P and ethanol P was added until it reached the limit mark. Preparation of the quercetin comparison solution by weighing 4 mg of quercetin which was then put into a 10 mL volumetric volumetric flask, dissolved and added with ethanol P to the limit mark. A series of dilutions were carried out with successive levels of 2; 1; 0.75; 0.30; 0.10 and 0.05 mg/mL.The procedure for determining the levels of flavonoids by pipetting 0.40 mL of the test solution and each concentration series of the comparison solution into the appropriate container. Each solution was added as much as 1.5 mL of ethanol P; aluminum chloride P 10% 0.1mL; 0.1 mL 1M sodium acetate and 2.8 mL water. Shaking was carried out and left for 30 minutes at room temperature. Absorption was measured at a maximum wavelength of approximately 420 nm. The blank measurements were carried out in the same way, without adding aluminum chloride. After that, a calibration curve is made (12).Calculate the total flavonoid content as quercetin in the extract using a standard curve or with (12):

$$\% = \frac{C_p \times \frac{A_u}{A_p} \times V \times f}{W} \times 100$$

Information:
C_p = Level of comparison solution
A_u = Test solution absorption
A_p = Absorption of comparison solution
V =Volume of test solution before dilution
f = Test solution dilution factor
W = Test material weight

Bacteria Identification

Tools and Materials Sterilization

The utensils are washed and dried, before being put into the autoclave, the glassware is covered with cotton and wrapped using sterile gauze and finally wrapped in aluminum foil, the petri dish is wrapped in aluminum foil. All tools and materials were sterilized by autoclaving within 15 minutes at a temperature of 121°C, while tweezers and ossicles were burned on a spiritus lamp.

Media Creation

***Muller Hinton Agar* (MHA)**

The MHA media was weighed as much as 9.5 grams, mixed with 250 mL of distilled water into an erlenmeyer, heated until dissolved. Then sterilized by autoclaving at 121 °C for 15 minutes. The sterile media was put into a sterile petri dish, allowed to solidify(13).

***Mueller Hinton Broth* (MHB)**

A total of 21 grams of MHB was weighed and 500 mL of distilled water was added into a beaker glass, then heated until dissolved and homogeneous. Which is then put into an erlenmeyer and sterilized by wet heat using an autoclave at 121°C for 15 minutes (14).

Blood Agar Medium

A total of 4 grams of Blood Agar Based was weighed and then added 100 mL of distilled water, heated until dissolved and sterilized in an autoclave at 121°C for 15 minutes. The media was removed from the autoclave and then allowed to warm, then add 5 mL of sheep blood. The media is poured into sterile petri dishes (15).

Bacterial Rejuvenation

Bacteria from pure cultures were taken, etched onto agar media. Incubated within 18 - 24 hours, temperature 37°C. The process is carried out aseptically.

Gram stain

Gram stain was used to identify bacteria. The glass preparation to be used is cleaned with 70% alcohol and heated near a flame. The glass slide was dripped with bacterial culture and heated near a flame until the bacterial culture was dry. After that, dripped purple crystal solution on the bacterial culture on the glass slide, let stand 1 minute. Cleaning is done using distilled water and dried again near the flame. The results of staining with purple crystals are then dripped with iodine solution, wait 1 minute and wash with distilled water. The preparations were dried near a flame. The results of the iodine staining were dripped again with 70% ethanol, allowed to stand for 30 seconds, washed again with distilled water and dried near a flame. The results of staining with ethanol, dripped with safranin, let stand 30 seconds, wash using distilled water and dried near a flame. After the stained bacterial culture was dry, then the staining results were observed under a microscope. If the result is purple it means gram positive bacteria, if red means gram negative bacteria (16,17).

Antibacterial Activity Test

Production of Bacterial Inoculum and Suspension

One ose of pure bacterial culture was taken, then suspended in 10 mL of MHB media in a test tube and vortexed until homogeneous. Then incubated for 24 hours at 37°C, producing inoculum.

Bacterial suspension was made by taking 1 mL of inoculum added to a test tube containing 9 mL of MHB. Then the researchers read the absorbance on a spectrophotometer at a wavelength of 625 nm, to produce an absorbance in the range of 0.08 – 0.1. Equivalent to the Mc Farland standard (18).

Preparation of Test and Comparison Sample Solutions

The extract/comparison of amoxicillin was weighed as much as 100.24 mg and then dissolved in 10 mL of 5% dimethyl sulfoxide (DMSO) and obtained a concentration of 10240 g/mL. The mother liquor was then taken 1 mL and added 10 mL of 5% DMSO, the concentration was 1024 ppm (19).

Microdilution Method Antibacterial Testing

Negative control was made using Mueller Hinton Broth (MHB) as much as 100 L which was inserted into the first column microplate. Column microplates 2 to 12 were filled with MHB media and bacterial suspension. A total of 100 L of leaf extract solution of sambungnyawa (Gynura procumbens [Lour.] Merr.) was added to column 12 and homogenized. A total of 100 L from column 12 was added to column 11 to column 3 (the concentration is getting smaller). Then incubated at 37°C for 24 hours, observing the clear part. Minimum Inhibitory Concentration (MIC) is shown as the smallest concentration of bacterial growth (20).

Determining the Minimum Kill Concentration (KBM) by taking 5 L of the concentration into MIC into NA medium, incubating for 24 hours at 37°C. Determination of KBM occurs when at the lowest concentration where there is no bacterial growth (20).

III. Result and Discussion

Results of Determination Test

Sambungnyawa plants were determined at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung. The purpose of this determination is to obtain the validity of the plant, avoiding errors from morphologically similar plants (21). Identification of plants seen from the roots, stems and leaves. The results show that this plant is a species of Gynura procumbens (Lour.) Merr issued with letter number 42/HB/03/2021.

Results of Material Preparation

The SambungNyawa plant (Gynura procumbens [Lour.] Merr.) was obtained from the ManokoLembang Experimental Plantation. The leaves are dried by aerating, stored in the sun until they become dry simplicia. The dried simplicia was determined by drying shrinkage. Shrinkage is a non-specific parameter that determines the evaporation of a substance, so the purpose of shrinkage is to determine the maximum (range) of compounds lost during the drying process. Drying shrinkage is carried out at the setting temperature of 105°C (21). The drying shrinkage parameter will produce a percent value after being dried on a moisture balance device at a temperature of 105°C until it reaches a constant weight (22). The mean of sambungnyawa drying shrinkage (Gynura procumbens [Lour.] Merr.) was 3,731 (Table 1). In accordance with the provisions according to the Indonesian Ministry of Health (2017) that dry simplicia from sambungnyawa (Gynura procumbens [Lour.] Merr.) must be less than 10%.

Table no 1: Drying shrinkage test results

No. Samplel	SambungNyawa Simplicity Weight(*Gyunara procumbens* [Lour.] Merr.)	%Drying Loss
1	2,003	3,748
2	2,027	3,890
3	2,024	3,556

Average	3,731 ± 0,168

Extract Production Results

Extraction is the most important thing to extract bioactive constituents from plants. The choice of extraction method is very important, because it is used to extract the required constituents with the help of a solvent. The most commonly used extraction methods are maceration, percolation, infusion, decoctation, reflux, soxhlet and others (23). The leaves of sambungnyawa (Gynura procumbens [Lour.] Merr.) were extracted by maceration method. Maceration is a simple extraction process and is referred to as cold extraction because the process does not go through heating (24). The advantage of this maceration method is that it uses simple equipment, the procedure is easy by immersing the sample with stirring for one hour (25). The maceration method was chosen because of the secondary metabolites contained in the leaves of sambungnyawa (Gynura procumbens [Lour.] Merr.) can be extracted selectively. As research conducted by Ashraf et al., (2020) the method used for the extraction of sambungnyawa (Gynura procumbens [Lour.] Merr.) is maceration(10).

The simplicia was macerated with three solvents, namely 70% ethanol, ethyl acetate and chloroform which were added to each different container. Simplicia immersed in 70% ethanol, ethyl acetate and chlorform solvents will undergo membrane and cell wall breakdown. This is because there is a pressure difference between the outside and inside of the cell, causing the cell and the walls to break. Then the secondary metabolites contained in the sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) will come out and dissolve into organic solvents (26). The maceration process uses 70% ethanol as a polar solvent, and ethyl acetate, chloroform is a semi polar solvent. Ethanol solvent will dissolve polar bioactive compounds from sambungnyawa (Gynura procumbens [Lour.] Merr.) and ethyl acetate and chloroform solvents will dissolve semipolar bioactive compounds. The use of solvents that have different polarities aims to identify differences in the yield of extracts and obtain active compounds from sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) based on their level of polarity (27).

The extract from the maceration process was then evaporated with a vacuum rotary evaporator to separate the solvent from the extract. The working principle of the vacuum rotary evaporator itself is to evaporate the extraction solvent (28). The extract obtained is then concentrated by means of water bathing to create a thick extract. The thick extract was then weighed to calculate the yield. The yield obtained, namely 70% ethanol extract is shown in table 2. The use of different solvents can affect different results, because the solvent that diffuses into the sample will dissolve components with the same polarity during the extraction process (29).

Table no 2: Extract yield results

Sampel	Simplicia Weight(g)	Thick Extract Weight (g)	Extract Yield (%)
Ethanol Extract 70% of SambungNyawa	160	16,88	10,55 %
Ethyl Acetate Extract SambungNyawa	160	12,84	8,025%
SambungNyawa Chloroform Extract	160	13,25	8,28%

Phytochemical Screening Results

Phytochemical screening is intended to determine or to provide an overview of what compounds are contained in the extract. The results of the phytochemical screening showed that the sambungnyawa extract (Gynura procumbens [Lour.] Merr.) contained metabolites such as flavonoids, saponins, tannins and steroids. The results of this study are in line with research conducted by Rahmi (2019) that the extract of sambungnyawa (Gynura procumbens [Lour.] Merr.) contains flavonoid compounds, saponins, tannins and steroids(30). However, it does not contain any alkaloids. Environmental conditions can affect differences in compound yields, causing differences in the types and amounts of metabolites in plants (31). Metabolite compounds such as flavonoids, saponins, tannins and steroids are attracted to 70% ethanol, while ethyl acetate and chloroform are attracted to flavonoids and steroids. Flavonoid compounds can be attracted to polar solvents as mentioned by Nugraha et al., (2017) in their research that ethanol solvents are polar solvents so they can attract flavonoids(32). Flavonoids can also be attracted by semipolar solvents, as in the research of Putu Sri Dia et al., (2015) that flavonoids can also be attracted to semipolar solvents(33). According to Markham (1988) in Putu Sri Dia et al., (2015) aglycone flavonoids (isoflavones, flavanones, flavones, and flavonols) tend to be attracted to semi-polar solvents such as chloroform, ethyl acetate, n-butanol and ether, while flavonoid glycosides tend to soluble in polar solvents. Meydia et al., (2016) stated that steroids can be dissolved in ethyl acetate and chloroform solvents because they are semi-pattern compounds, steroids are also soluble in polar solvents such as ethanol but the solubility is less perfect than semi-polar solvents(34). The results of phytochemical screening on the extract of sambungnyawa (Gynura procumbens [Lour.] Merr.) are shown in table 3.

Table no 3: Phytochemical screening results

Test Type	Ethanol Extract 70%	Ethyl Acetate Extract	Chloroform Extract
Alkaloid	-	-	-
Flavonoid	+	+	+
Saponin	+	-	-
Tanin	+	-	-
Steroid/ Triterpenoid	+	+	+

Information: - = There are no compounds
+ = Compounds confirmed

Gram Stain Results

Gram staining is one of the bacterial identification techniques using a microscope so that it can see bacteria, see the size and shape of the bacteria (35,36). The results of this gram stain can see gram-positive and gram-negative bacteria. Gram staining itself uses crystal violet which gives a purple color as the primary color for microbes, while safranin is used as a secondary color and will produce a red color. The binding of the color will be strengthened by the iodine solution. Alcohol is used to rinse the primary color on the bacteria. The distilled water rinse is intended to rinse crystal violet, safranin and iodine (35). The results of the Gram staining of Streptococcus pyogenes (ATTC 19615) showed that this bacterium was a gram positive bacterium.

The difference between gram positive and gram negative bacteria lies in the structure, cell wall permeability and cell wall composition. Gram-positive bacteria will retain the purple color (primary color) caused by the addition of alcohol which will cause the peptidoglycan pores to shrink and crystal violet will stick, fade or dissolve. The addition of alcohol to gram-positive bacteria produces crystal violet which will adhere. Gram-positive bacteria have lower lipids than gram-negative bacteria, so that when ethanol (alcohol) is added, the lipids are hydrated lower. The permeability is reduced, and the pore size will be smaller(37).

Antioxidant Activity Test Results

Antioxidant activity was tested quantitatively using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). The purpose of testing the antioxidant activity using the DPPH method is because the measurement time of antioxidant activity is fast, the cost is affordable and simple (38). The DPPH free radical has unpaired electrons so it is unstable and will give a purple color, then it will turn yellow when the free electrons are paired to produce a stable radical. Therefore, the maximum wavelength absorbance changes in DPPH as measured by a UV-Vis spectrophotometer. The change in absorbance results in a damping activity value (39).

Antioxidant activity of 70% ethanol extract, ethyl acetate and sambungnyawa chloroform (Gynura procumbens [Lour.] Merr.) was measured using a UV-Vis spectrophotometer with a wavelength of 517 nm. The amount of antioxidant activity can be seen from the IC_{50} value, where IC_{50} is the concentration of the extract inhibiting 50% of DPPH free radicals. The IC_{50} value of 70% ethanol extract, ethyl acetate and sambungnyawa chloroform (Gynura procumbens [Lour.] Merr.) was obtained from the linear regression results from the relationship curve of the sample concentration to the percent inhibition value (inhibition) with the equation $y= ax + b$, where the x-axis is the concentration (ppm) and the y-axis is the percent inhibition. Based on table 4, respectively, the IC_{50} value was 135.0479 ppm for 70% ethanol extract; 116,8404 ppm for ethyl acetate extract and 122.1700 ppm for chloroform extract.

The results of this study indicate that the solvent used in the extraction process affects the antioxidant yield. The highest and lowest antioxidant activities were ethyl acetate, chloroform and ethanol extracts, respectively. The results of this study are different from the research conducted by Ashraf et al., (2020) which stated that the sambungnyawa extract (Gynura procumbens [Lour.] Merr.) with polar solvents had higher antioxidant activity than semi-polar solvents(10). Likewise with research conducted by Teoh et al., (2016) that the highest antioxidant activity is in polar solvents(40). This is presumably due to differences in the amount and content of active compounds in the extracts which result in differences in antioxidant activity, besides that it can be influenced by the method during the extraction process, planting location and the type of solvent used.

Table no 4: Data on antioxidant activity testing of samples of 70% ethanol extract, ethyl acetate and sambungnyawa chloroform (Gynura procumbens [Lour.] Merr.)

Sample	Concentration	Absorbance Value	Inhibitory Value (%)	IC_{50} Value	Category
Vitamin C	0	0,788	0,000	6,6451	Very Strong
	2	0,667	15,355		
	4	0,533	32,360		
	6	0,402	48,985		
	8	0,321	59,264		
	10	0,219	72,208		
Ethanol Extract 70%	0	0,925	0,000	135,0479	Medium
	60	0,677	26,811		

	100	0,567	38,703		
	140	0,455	50,811		
	160	0,391	57,730		
Ethyl Acetate Extract	0	0,922	0,000	116,8404	Medium
	60	0,64	30,586		
	100	0,515	44,143		
	140	0,373	59,544		
	160	0,319	65,401		
Chloroform Extract	0	0,912	0,000	122,1700	Medium
	60	0,647	29,057		
	100	0,519	43,092		
	140	0,397	56,469		
	160	0,339	62,829		

Antioxidant activity is divided into several categories, namely, if the IC_{50} value is less than 50 ppm, it means that it has very strong antioxidant activity, the IC_{50} value is in the range of 50 ppm - 100 ppm, which means it has strong antioxidant activity, and the IC_{50} value between 100 ppm - 150 ppm means moderate. , if the IC_{50} value is 150 ppm - 200 ppm it means that the antioxidant activity is weak and if the IC_{50} value is more than 200 ppm it means it has very weak antioxidant activity (41). The data from the research showed that the antioxidant activity of 70% ethanol extract, ethyl acetate and sambungnyawa chloroform (Gynura procumbens [Lour.] Merr.) had moderate antioxidant activity. The comparison used in testing this antioxidant activity is ascorbic acid or called vitamin c. The resulting IC_{50} value is 6.6451%, which means it has a higher value than the extract.

Results of Determination of Total Phenolic and Flavonoid Levels

Determination of total phenolic and flavonoid levels was carried out on viscous extracts of 70% ethanol extract, ethyl acetate and sambungnyawa chloroform (Gynura procumbens [Lour.] Merr.). Determination of total phenolic content using gallic acid as a comparison, because gallic acid is a derivative of hydroxybenzoic acid, one of the phenolic compounds. Phenol positive is indicated by a green-black color that occurs when gallic acid reacts with Folin-Ciocalteu which is reacted in an alkaline environment. The goal is to react in the alkaline atmosphere so that proton dissociation occurs into phenolic ions (42). Determination of flavonoid levels with AlCl3 reagent using quercetin as a comparison. Quercetin is a flavonoid of the flavonol group, having a keto group at C-4 and a hydroxyl group at C-3 and C-5. AlCl3 reagent will form a complex with a keto group at C-4 and a hydroxyl group at C-3 and C-5 (43).

The highest total phenolic content was in the ethyl acetate extract, which was 37.42 ± 2.07%, while the highest total flavonoid content was in the chloroform extract (9.57 ± 0.33%) which is shown in table 5. Most of the total content of Phenolics obtained from sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) are very semipolar compounds, so the compounds will be attracted to semipolar compounds such as ethyl acetate (44). Compounds that have low polarity such as flavonoid aglycones can be dissolved in chloroform, while ethyl acetate solvent can extract moderately polar flavonoids and glycosides. The difference in the results of total phenolic levels with total flavonoid levels is due to not all phenolic levels being flavonoid compounds, such as phenolic acids, melanin, coumarins, flavonoids, lignin and tannins which are the largest polyphenol compounds in plants(45). Phenolic compounds have an important role for human health, because they have benefits as antioxidants, anticarcinogenic and anti-inflammatory (6).

Based on the results obtained in line with the measurement of antioxidant activity, where the best IC_{50} value is ethyl acetate extract, the greater the content of phenolic compounds, the greater the antioxidant activity will be(46).

Table no 5: Results of determination of total phenolic and flavonoid levels

Sample	Total Phenolic Content	Total Flavonoid Level
Ethanol Extract 70%	23,73 ± 0,33 %	0,98 ± 0,04 %
Ethyl Acetate Extract	37,42 ± 2,07 %	7,28 ± 0,12 %
Chloroform Extract	23,12 ± 0,93 %	9,57 ± 0,33 %

Antibacterial Activity Test Results

Antibacterial activity testing was carried out using the microdilution method, where the smallest concentration that did not show bacterial growth was determined as the Minimum Inhibitory Concentration (MIC). Researchers chose to use the microdilution method for testing because this method has a higher sensitivity, which is 30 times higher than the diffusion method(47). Antibacterial activity test of 70% ethanol extract, ethyl acetate and sambungnyawa chloroform was carried out on Streptococcus pyogenes bacteria. Streptococcus pyogenes is a group A Lancefield (GAS) Streptococcus bacteria that can cause throat infections such as pharyngitis (48). The results of the antibacterial activity test are shown in table 6.

Table no 6: Antibacterial activity test results

Sample	KHM	KBM
SambungNyawa Ethanol Extract 70%	256 ppm	-
SambungNyawa Ethyl Acetate Extract	256 ppm	-
SambungNyawa Chloroform Extract	256 ppm	-
Amoxicillin Comparator	8 ppm	64 ppm

Note: - = There is no minimum kill concentration (KBM)

Researchers used MHB media for negative control, to ensure zero contamination and sterilize the microplate. The positive control contained media and bacterial suspension, aiming to see that the bacteria grew in the test, indicated by the presence of turbidity. This antibacterial activity test used amoxicillin as a comparison, the aim was to compare the MIC results with the extract solution.

Based on table 6, 70% ethanol extract, ethyl acetate and sambungnyawa chloroform inhibited at a concentration of 256 ppm. It is suspected that there are other compounds found in the ethyl acetate and chloroform extracts. According to Silva et al., (2015) stated that if the MIC is less than 100 ppm it means it has strong antimicrobial activity, if the MIC with a concentration of 100 ppm - 500 ppm, it has moderate antimicrobial activity, if the MIC has a concentration of 500 ppm - 1,000 ppm, it means it has antimicrobial activity. Weak antimicrobial and if the MIC with a concentration of more than 1,000 ppm is considered the extract is inactive(49). Based on the above categories, it can be stated that the extract of sambungnyawa (Gynura procumbens [Lour.] Merr.) has moderate antibacterial activity. Comparative test of amoxicillin inhibited at a concentration of 8 ppm, this means 70% ethanol extract, ethyl acetate extract and sambungnyawa chloroform extract (Gynura procumbens [Lour.] Merr.) had weak antibacterial activity inhibition compared to amoxicillin. A similar study conducted by Gurning et al., 2019 stated that the extract of sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) could inhibit bacteria and its inhibitory power was lower than the comparison (50). The next test after the MIC test is the minimum kill concentration (KBM) test.

The minimum killing concentration (MBC) is a measure of an antimicrobial that can kill 99% of the inoculum that can be seen after incubation for 24 hours, this test can be carried out after the microdilution test (51). The results of the data obtained that 70% ethanol extract, ethyl acetate and sambungnyawa leaves chloroform (Gynura procumbens [Lour.] Merr.) could not kill Streptococcus pyogenes bacteria. 70% ethanol extract, ethyl acetate and chloroform of sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) were suspected to have MBC at concentrations of more than 512 ppm. The comparison of amoxicillin has a MBC value at a concentration of 64 ppm, because amoxicillin has bactericidal properties and works against gram-positive and gram-negative microorganisms by inhibiting the biosynthesis and repair of bacterial mucopeptide walls (52).

Antibacterial activity in plants has been associated with the presence of secondary metabolites. Secondary metabolites from these plants can provide protection for the plant itself against bacterial, fungal and viral infections (53). Phytochemical activity of sambungnyawa extract (Gynura procumbens [Lour.] Merr.) contained secondary metabolites of flavonoids, saponins, tannins and steroids. Flavonoids are the largest group of phenolic compounds, it is suspected that these bioactive compounds have potential antibacterial activity where the alcohol group in flavonoids will react to damage bacterial cell walls (lipids and amino acids). Flavonoids have an antimicrobial mechanism by inhibiting nucleic acid synthesis, cytoplasmic membrane function and energy metabolism (54). The antimicrobial properties of saponins can cause leakage of certain proteins and enzymes from cells (55). Tannins possess a mechanism of action as an antibacterial by causing Streptococcus pyogenes bacteria to lyse because tannins will interfere with the polypeptide wall of the bacteria and will cause the formation of the bacterial cell wall to be less than perfect so that the bacterial cell will die. In addition, tannins can also interfere with cell protein pathways and inactivate enzymes from bacteria(56). Steroids are reported to have antibacterial activity, which can cause leakage from liposomes because the correlation between membrane lipids and sensitivity to steroids suggests a mechanism by which steroids specifically associate with membrane lipids and exert their action by causing leakage from liposomes (55).

IV. CONCLUSION

Based on the research conducted, it can be concluded that sambungnyawa (Gynura procumbens [Lour.] Merr.) has antioxidant activity with IC50 values of 70% ethanol extract, ethyl acetate extract and chloroform extract respectively 135.0479; 116.8404; 122.1700. The sambungnyawa (Gynura procumbens [Lour.] Merr.) has moderate antioxidant activity. The total febolic content of 70% ethanol extract, ethyl acetate extract and sambungnyawa chloroform extract (Gynura procumbens [Lour.] Merr.) was 23.73 ± 0.33 %; 37.42 ± 2.07%; 23.12 ± 0.93%. The total flavonoid content of 70% ethanol extract, ethyl acetate extract and sambungnyawa chloroform extract (Gynura procumbens [Lour.] Merr.) was 0.98 ± 0.04%; 7.28 ± 0.12 %; 9.57 ± 0.33%.

The extract of sambungnyawa leaves (Gynura procumbens [Lour.] Merr.) possessed antibacterial activity with MIC of all extracts (70% ethanol extract, ethyl acetate extract and chloroform extract) was 256 ppm and the minimum killing concentration (MBC) in all extracts was >512 ppm.

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