Minimum Inhibitory Concentration Assay of Ethyl Acetate Fraction of Walay Rhizome (*Meistera chinensis)* Against The Inhibition of *Staphylococcus aureus*

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ABSTRACT

Infectious diseases are usually caused by bacteria, one of which is *Staphylococcus aureus.* The use of traditional medicine is currently more clinically effective because there are fewer side effects. One of the plants whose habitat is often found in South Konawe district, Southeast Sulawesi Province is walay rhizome (*Meistera chinensis*). This research aims to determine the minimum inhibitory concentration assay against *Staphylococcus aureus* bacteria and secondary metabolite compound of the ethyl acetate fraction of walay rhizome (*Meistera chinensis*). This research is experimental research. The sample was extracted using the maceration method using 96% ethanol, then fractionated using liquid-liquid partition using distilled water, methanol and ethyl acetate in a ratio of 1:1. The ethyl acetate fraction was separated and the minimum inhibitory concentration assay was using turbidumetry and UV-Vis Spectrophotometry methods. The data obtained was analyzed using statistical tests *One Way Anova.* The results of the minimum inhibitory concentration assay using the turbidumetry method at concentrations of 1.563%, 3.125%, 6.25%, 12.5%, 25% and 50% were clearer than the negative control tube. In the test using the Uv-Vis Spectrophotometry method, the smallest concentration of 1.563% to the largest concentration of 50% showed a decrease in the absorbance value. Results of phytochemical screening of the ethyl acetate fraction of walay rhizome (*Meistera chinensis*) positively compounds such as flavonoid, tannins and saponins as antibacterial compounds.

Keywords: Ethyl acetate fraction, Minimum inhibitory concentration, *Meistera chinensis, Staphylococcus aureus*

INTRODUCTION

Infectious diseases caused by bacteria are still the main cause of health problems in Indonesia, one of the bacteria that causes infectious diseases is *Staphylococcus aureus* (Dewi et al., 2021). *Staphylococcus aureus* is a bacterial pathogen that causes antibiotic resistance due to increasing clinical use. Symptoms of this bacterial infection are stomach cramps, vomiting and severe diarrhea. These bacteria can cause various diseases, ranging from mild skin infections, poisoning, and systemic infections. Hamzah's research results (2021) reported that infection *Staphylococcus aureus* has a death rate of 25%.

Currently, traditional medicines are clinically effective and preferred because they have fewer side effects than synthetic medicines. One plant that is known to have antibacterial activity is walay rhizome (*Meistera chinensis)* which is one type of plant that can be used in traditional medicine. Empirically, walay rhizomes (*Meistera chinensis)* used to improve the taste of food, relieve pain, and strengthen the immune system (Musdalipah et al., 2021).

In previous research, ethanol extract of walay rhizomes (*Meistera chinensis)*. It is known to contain secondary metabolites such as flavonoids, saponins, alkaloids, steroids and phenols. The extract has been shown to inhibit growth *Escherichia coli* with an average inhibitory zone diameter respectively at concentrations of 10%, 20% and 30% of 6.08 ± 1.79; 8.16 ± 0.11 and 10.57 ± 1.34 mm. On *Staphylococcus aureus*, the inhibition zone for each concentration of 10%, 20%, and 30% is 5.02 ± 0.79; 6.01± 0.69; 8.03 ± 0.76 mm, which indicates that the ethanol extract of walay rhizomes (*Meistera chinensis)* has antibacterial activity (Karmilah, 2023).

Several methods can be used to test the activity of an antimicrobial, including the tube dilution technique. This technique is used to determine the smallest amount of antimicrobial substance needed to inhibit the growth of microorganisms *in vitro*. This amount is known as MIC (Minimum Inhibitory Concentration) which is able to inhibit bacterial growth after 24 hours of incubation by observing the number of bacterial colonies that grow using the dilution method (Tortora et al., 2010).

Based on plant research *Meistera chinensis* Some research is still needed to reveal the antimicrobial activity of the rhizome. This study aims to determine the secondary metabolite content and Minimum Inhibitory Concentration of the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) against bacteria *Staphylococcus aureus* by dilution.

METHODOLOGY

Tools and materials

The tools used in this research were an autoclave, stirring rod, *beaker glass* (pyrex^{R}), porcelain cups, funnels, split funnels (pyrex^{R}), desiccator, Erlenmeyer (pyrex^{R}), measuring cup (pyrex^{R}), *heating mantle, hot plate*, incubator, tube needle, filter paper, label paper, oven, dropper pipette, *rotary evaporator*, horn spoon, spirit, spoit, test tube (pyrex^{R}), tweezers, analytical balance, maceration vessel.

The materials used in this research were distilled water, 96% ethanol, ethyl acetate, HCl 2N, Mayer's reagent, Dragendorf's reagent, NaOH 10%, Mg, $FeCl_3$,

CH_3COOH reagent, H_2SO_4, pure culture of bacteria *Staphylococcus aureus*, walay rhizome (*Meistera chinensis)*, NaCl, *Nutrient broth* (NB), and *Nutrient Agar* (NA).

Procedure of Research

1. Determination

Determination of plants is carried out by comparing the morphological characteristics of plants including shape, size, number, parts of leaves, flowers, fruit, seeds and others. Compare and equate the characteristics of the plants that will be used in this research, namely walay rhizomes (*Meistera chinensis*). Sample determination was carried out at the Pharmacognosy-Phytochemistry Laboratory, Mandala Waluya University.

2. Sample Processing

Prepared walay rhizomes (*Meistera chinensis*) 1000 g of fresh fruit is cleaned, then cut into thin pieces, dried at room temperature or dried in the sun without being exposed to direct sunlight in an open room, until dried simplicia is obtained. Walay rhizome (*Meistera chinensis)* which has been dried is then sorted dry and then ground with a blender until it turns into a coarse powder, then weighed.

3. Extraction of Walay Rhizomes (*Meistera chinensis)*

Extraction is carried out using the maceration method, namely soaking the walay rhizomes (*Meistera chinensis)* with 96% ethanol solvent. This process is carried out by soaking walay rhizome powder (*Meistera chinensis)* for 3 times 24 hours in a maceration vessel while stirring occasionally, and filtering every 1 time 24 hours. The filtrate is collected, then concentrated using *rotary evaporator* at a temperature of 50^0C to produce a thick extract (Reymon, 2021).

Thick extract from walay rhizomes *(Meistera chinensis)* calculated percent (%) of soaking using the formula:

$$\text{Yield}=\frac{Total\ weight\ of\ extract}{Dried\ simplicia\ weight}\times 100\%$$

4. Fractionation of Walay Rhizome (*Meistera chinensis)*

20 grams of the ethanol extract obtained was taken to be extracted with ethyl acetate solvent using liquid-liquid partitioning. A total of 20 grams of ethanol extract of walay rhizomes (*Meistera chinensis)* dissolved in 200 mL each of ethyl acetate and methanol. Put it in a separating funnel and add 200 mL of distilled water for separation by partitioning the extract and solvent. Shake the separating funnel vigorously until

mixed and wait a few minutes until it separates into the methanol and ethyl acetate fractions. Then it is removed from the funnel and stored in a different container. The residue is repartitioned according to the method above, repeated until clear. The ethyl acetate fraction layer is collected and evaporated to obtain the ethyl acetate fraction (Reymon, 2021).

5. Identification of the Chemical Compounds of the Ethyl Acetate Fraction of Walay Rhizomes (*Meistera chinensis)*

a. Alkaloid Identification

The test solution was put into a test tube and then added with HCl 2N. Mayer's reagent was added to the first tube and Dragendorf's reagent to the second tube. Positive if a white precipitate forms in the first tube and an orange precipitate in the second tube (Suhaenah, 2023).

b. Tannins Identification

The test solution was put into a test tube, then a few drops of $FeCl_3$ solution were added. Tannin is positive if a blackish green/blue color forms (Suhaenah, 2023).

c. Flavonoid Identification

The dissolved fraction is put into a test tube, concentrated Mg+HCl powder is added, containing flavonoids if foam forms and the color changes to yellow, orange or red (Suhaenah, 2023).

d. Saponin Identification

A total of 1 mL of the test solution was put into a test tube and 10 mL of hot distilled water was added. The mixture is shaken until foam appears and left for 1 minute. Then add 2 drops of HCl 2 N and shake again until foam forms. The presence of saponin compounds is indicated by the formation of stable foam for 10 minutes with a height of 3 cm (Suhaenah, 2023).

e. Identification of Steroids and Triterpenoids

The test solution was put into a tube, then was CH_3COOH reagent and H_2SO_4 through the tube wall. If a brownish/violet ring forms, it indicates that the sample is positive for containing triterpenoids, and the formation of a greenish blue ring indicates that it is positive for the presence of steroids (Suhaenah, 2023).

6. Minimum Inhibitory Concentration Assay Against *Staphylococcus aureus*

a. Sterilization

Materials and equipment used in the laboratory must be sterile. Sterilize tools such as vials, petri dishes and test tubes in hot air (oven) at a temperature of 180^0C for 2 hours. Bacterial growers (ose) are sterilized by burning, that is, burning them until they are red hot with an alcohol lamp. The media was sterilized using an autoclave at a temperature of 121^0C and pressure 1-2 atm for 15 minutes.

b. Procedure for Making Bacterial Suspensions

Pure culture *Staphylococcus aureus* Take one loop and then etch it on the media *Nutrient Agar* (NA) tilted in a tube which was then incubated for 1x24 hours at a temperature of 25^0C. The NA media that has been incubated is then taken and put into a test tube containing 1 ml of sterile NB then put into the incubator and incubated anaerobically for 1×24 hours at 37ºC. Next, carry out the dilution by adding sterile distilled water and homogenizing until the turbidity is comparable to the standard *Mc Farland* 0,5 ($1,5\times10^8$).

c. Preparation of Turbidity Standards (Solution *Mc. Farland* 0,5)

Solution H_2SO_4 1% as much as 9.95 mL is mixed with $BaCI_2$ solution 1.175% as much as 0.05 mL in a test tube. The mixture is then shaken until it forms a cloudy solution. This turbidity is used as a standard for the turbidity of the test bacterial suspension (Pinta et al., 2017).

d. Preparation of Positive Control Solution

The positive control solution used was clindamycin® 10%, made by weighing it to 1 g according to calculations, and dissolving it in 10 ml of distilled water.

e. Bacterial Minimum Inhibitory Concentration Assay

8 test tubes are provided. Each is labeled 1-8, then the processing stage is carried out. A total of 10 mL of distilled water is filled in tube 1 and 5 mL of sterile distilled water is filled in tubes 2-10 and 11, then in tube No. 1 is added 5 g of walay rhizome (*Meistera chinensis)* ethyl acetate fraction, for 50% sterile extract. Then, the concentration was reduced by transferring 5 mL of the fraction suspension from tube No.1 to tube No.2. Then, 5 mL from tube 2 is transferred to tube 3 and so on until tube No. 6, so that the concentration of the walay rhizome ethyl acetate

fraction is obtained (*Meistera chinensis)* respectively, tube No.1 to Tube No.6, namely 50%, 25% 12.5%, 6.25%, 3.125% and 1.563%.

In the next stage, 5 mL of liquid NB containing the test bacterial suspension was added into tubes 1-6 until the tube volume was 10 mL. Tube No. 7 was filled with 5 mL of the test bacterial suspension (negative control), tube No. 8 was filled with 10 mL of 10% Clindamycin® (positive control). Measured visually and measured absorbance using a UV-Vis spectrophotometer with a wavelength of 285 nm for each tube before incubation and after incubation, incubation was carried out for 24 hours at a temperature of 37^0C (Munier, 2021).

Data analysis

Data from research results of the Minimum Inhibitory Concentration (MIC) assay of ethyl acetate fraction of walay rhizomes (*Meistera chinensis)* against *Staphylcoccus aureus* analysis was carried out using the SPSS program to see the effect of the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) has minimum inhibitory concentration against *Staphylcoccus aureus.*

RESULTS AND DISCUSSION

In this research, the samples used were walay rhizomes (*Meistera chinensis*) taken in Alangga Village, Andolo District, South Konawe Regency, Southeast Sulawesi Province. Then, the sample is wet sorted to separate foreign objects contained in the sample, after that it is washed to remove dirt contained in the sample, then the sample is chopped with the aim of making the drying process easier. After chopping, a drying process is carried out on the samples. The dried sample was then crushed until a fairly smooth simplicia weighing 1000 grams was obtained.

Plant determination was carried out at Pharmacognosy-Phytochemistry Laboratory, Mandala Waluya University. The results of plant determination prove that the samples used in this study were walay rhizomes (*Meistera chinensis).* The determination of walay plants in this research was first tested for correctness to avoid errors in the research (Ramadhan, 2024).

Simplicia which has gone through the drying process is then extracted using 96% ethanol solvent using the maceration method. In the maceration process, the sample powder is first soaked using 96% ethanol solvent for 3x24 hours, every 1x24 hours the solvent is replaced. The filtrate obtained is filtered and collected then

concentrated using *rotary evaporator*. After concentration, ethanol extract of walay rhizome (*Meistera chinensis*) is dark brown in color with an extract weight of 46 grams.

The resulting yield was 4.6%. According to Cahyadi *et al*, (2018) the quality of the extract is inversely proportional to the amount of yield produced, the higher the yield value the lower the quality obtained so that the ethanol extract of walay rhizomes (*Meistera chinensis*) produced meets the requirements.

The reason for using the maceration method is that the advantage of this method is that it is easy and does not require heating so there is little chance of the natural ingredients being damaged or decomposed. Cold extraction in principle does not require heating. This is intended for natural materials that contain chemical components that are not resistant to heating. The advantages of this method are that it is simple, does not require complicated tools, and is relatively cheap (Amelia *et al*., 2021).

The reason for choosing 96% ethanol solvent is because it is universal, polar and easy to obtain 96% ethanol was chosen because it is selective, non-toxic, has good absorption and high filtering ability so it can filter non-polar, semi-polar and polar compounds. The 96% ethanol solvent penetrates more easily into the cell walls of the sample than the ethanol solvent with a lower concentration, resulting in a concentrated extract (Novira *et al*., 2021).

After the extraction process, the extract is diffraction using the liquid-liquid partition method using distilled water, methanol and ethyl acetate as solvents. The fractionation process was carried out by dissolving 20 grams of the extract in 200 mL each of methanol and ethyl acetate solvents. The solution was put into a separating funnel and 200 mL of distilled water was added to partition the extract and solvent. The separating funnel was shaken vigorously and left for a few minutes then the methanol and ethyl acetate fractions were separated, the ethyl acetate fraction was concentrated using *rotary evaporator* and get 15 grams of ethyl acetate fraction. The soaking yield of the ethyl acetate fraction (table 1) was 32%, according to Whika *et al*. (2017) the greater the yield produced, the more bioactive components it contains.

Fractionation uses a separating funnel with the working principle of separating certain compounds contained in the sample due to differences in specific gravity from the use of two solvents that do not mix with each other. The use of solvents in the

fractionation process is in accordance with the requirements, namely first, the solvent used must not mix with water, the specific gravity of the solvent is lower than water so that the top layer of solvent can form, thus separation is easy to carry out. Second, solvent what is used must be safe and not damage the environment, one of which is the solvent ethyl acetate. The semipolar nature of the ethyl acetate solvent is capable of dissolving polar and non-polar substances (Rusli, 2023).

The next stage is the identification of the chemical compound groups contained in the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) carried out using chemical reactions (color and precipitate). The results of phytochemical screening (table 1) show that the ethyl acetate fraction of walay rhizomes (*Meistera chinensis)* contains flavonoids, tannins and saponins. According to Musdalipah *et al.* (2021) fraction of walay rhizome (*Meistera chinensis*) contains secondary metabolites that are similar to the fruit content such as phenolics and flavonoids. The results on *Zingiberaceae* has antioxidant, toxicity, immunomudolator, anticancer and antibacterial properties (Haleagrahara et al., 2010; Juwita et al., 2018). The secondary metabolites in each plant mostly contain phenolic and flavonoid compounds, and have various pharmacological activities (Sungthong and Srichaikul, 2018).

Table 1. Identification Test Results for the Chemical Compounds of the Ethyl Acetate Fraction of Walay Rhizomes (*Mastera Chinensis*)

Chemical Compounds	**Reagent**	**Results**	**Information**
Alkaloid	Mayer	A white precipitate is formed	(-)
	Dragendorf	An orange precipitate is formed	
Flavonoid	Concentrated Mg+HCl powder	A yellow color forms	(+)
Tannin	$FeCl_3$	A blackish green color forms	(+)
Saponin	Hot distilled water + drops of HCl 2N	A persistent foam/foam is formed	(+)
Steroid and Triterpenoid	Glacial acetic acid + sulfuric acid	A brownish ring forms	(-)

Information:
(+): positive for containing chemical compounds
(-): negative contains chemical compounds

In identifying flavonoids, after the fraction was added Mg powder and continued with the addition of concentrated HCl. The addition of Mg powder is used as a reducing agent where the reduction process is carried out in an acidic atmosphere with the addition of concentrated HCl. The reduction process with magnesium and concentrated

HCl produces a red color (Agustina *et al*., 2017). The results obtained are in accordance with the literature where when identifying flavonoids the reaction that occurs is the formation of a yellow color (Julianto, 2019).

Identification of saponins includes a simple test where 10 ml of hot water is added then cooled, then shaken vigorously for 10 seconds then HCl 2N is added and foam will form on the surface. From the identity results obtained, foam was formed which remained for 10 minutes with a foam height of 6 cm. The foam produced in the saponin test is caused by the presence of glycosides which can form foam in water and hydrolyze into glucose and other compounds (Agustina *et al*., 2017). The results obtained are in accordance with the literature, where when identifying saponin compounds, the reaction that occurs is the formation of persistent foam (Julianto, 2019).

Results of tannin testing on the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) shows positive as indicated by a change in color to blackish green. The tannin obtained in this test is condensed tannin which reacts with $FeCl_3$ produces a blackish green color (Harbone, 1996). From the identification of tannin compounds, the results obtained are in accordance with the literature where the reaction that occurs changes the extract to a blackish green color where the tannin contained in the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) is condensed tannin (Julianto, 2019).

Test of the antibacterial activity of the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) was carried out to determine the antibacterial activity of the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) against *Staphylococcus aureus,* The method used is the dilution method. The liquid dilution test is a method that aims to measure the Minimum Inhibitory Concentration (MIC) of the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) (Pratiwi, 2008). According to Results *et al.* (2022) The serial dilution method has the advantage that the contact between the test sample and bacteria is higher because the media surface is large, bacteria can be tested using one point, this method is more economical and easy to implement. This dilution test was carried out using the turbidimetric method and UV-Vis spectrophotometer.

Staphylococcus aureus are gram-positive bacteria arranged in irregular groups like grapes, facultative anaerobes, do not form spores, and do not move, round in shape with a diameter of 0.7-1.2 µm. According to Sihombing (2022), this bacteria is also a major pathogen for humans which can cause various cases of diseases such as skin

infections, food poisoning, endocarditis, pneumonia, osteomyolitis, sepsis, arthritis and encephalitis (Agustin, 2023).

Clindamycin® was used as a positive control because clindamycin is mainly used in the treatment of infections caused by aerobic bacteria and is very effective against gram-positive bacteria such as *Staphylococcus aureus*. The mechanism of clindamycin as an antibacterial works by inhibiting the reproduction or growth of bacteria, namely by inhibiting bacterial protein synthesis according to Yasir (2021), while the K (-) used is a bacterial suspension. *Staphylococcus aureus* grown in NB media whose turbidity is comparable to standard solutions *Mc Farland* 0,5 sothat the bacterial suspension will be within the range given to standardize the testing microbes (Rosmania and Fitri, 2020).

Reasons for choosing media *Nutrient Broth* Because In this MIC test using the dilution method. *Nutrient Broth* included in the liquid media used as a bacterial growth medium, consisting of yeast extract as a protein source and peptone as a nitrogen source (Wahyuningsih, 2018).

The next stage is testing the minimum inhibitory content of the ethyl acetate fraction of walay rhizomes (*Meistera chinensis)* against bacteria *Staphylococcus aureus* using the turbidimetric method or visually by comparing the turbidity of each concentration tube of 1.563%, 3.125%, 6.25%, 12.5%, 25% and 50%, as well as K (+) with K (-) tube after 1x24 hour incubation. The results obtained can be seen in table 2.

Table 2. Test Results of the ethyl acetate fraction of Walay rhizomes (*Mastera* chinensis) against bacteria *Staphylococcus aureus* with the turbidimetric method.

Tube Number	Concentration of Fraction Ethyl Acetate Walay Rhizome (*Mastera Chinensis)*	The Results		
		Treatment 1	**Treatment 2**	**Treatment 3**
1	1,563%	-	-	-
2	3,125%	-	-	-
3	6,25%	-	-	-
4	12,5%	-	-	-
5	25%	-	-	-
6	50%	-	-	-
7	K-	+	+	+
8	K+	-	-	-

Information:
Sign (+): the solution in the tube looks cloudy, meaning there is bacterial growth.
Sign (-): the solution in the tube starts to clear, which means the growth of bacteria is starting to be inhibited

In testing the tirbudimetry method, it shows that there is no turbidity similar to the K (-) tube, which means that there is bacterial inhibition at each concentration, this is in accordance with the literature according to Lolongan *et al*., (2016), if the turbidity of each tube is still equal to or more turbid than the K (-) tube, it means bacteria can still grow, but when the solution in the tube looks clearer than the K (-) tube, it means bacterial growth is starting to be inhibited. Turbidimetric measurements have drawbacks because the human eye cannot differentiate dead bacterial cells from living bacterial cells so it can be subjective. So, testing was continued using a UV-Vis spectrophotometer at a wavelength of 285 nm (Wiharningtias, 2016).

Table 3. The results of MIC assay of the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) against bacteria *Staphylococcus aureus* using a UV-Vis Spectrophotometer

Tube Number	Concentration of Fraction Ethyl Acetate of Walay Rhizome (*Mastera Chinensis)*	Value Results Average ± Standar of Deviation		Information
		Before Incubation	**After Incubation**	
1	1,563%	3,459 ± 0,014	3,329 ± 0,067	Down
2	3,125%	3,494 ± 0,0255	3,467 ± 0,034	Down
3	6,25%	3,526 ± 0,027	3,507 ± 0,046	Down
4	12,5%	3,645 ± 0,088	3,636 ± 0,087	Down
5	25%	3,716 ± 0,049	3,677 ± 0,035	Down
6	50%	3,944 ± 0,058	3,720 ± 0,018	Down
7	K-	2,245 ± 0,001	2,367 ± 0,039	Go on
8	K+	1,068 ± 0,000	1,068 ± 0,000	Still

The test results (table 3) using a UV-Vis spectrophotometer show that at the smallest concentration, namely 1.563%, to the largest concentration, namely 50%, there was a decrease in the absorbance value so that it was stated that the Minimum Inhibitory Concentration value was at a concentration of 1.563%. This is in accordance with the Howling theory *et al*., (2016), if the final absorbance value (after incubation) of each tube is greater than the initial absorbance value (before incubation), then it is concluded that bacterial growth is still occurring, but if there is no change in the absorbance value between the final absorbance value and the initial absorbance, or the final absorbance value is smaller than the initial absorbance value, then it is concluded that bacterial growth is inhibited. MIC is determined by the concentration of the smallest fraction in the treatment tube which has begun to inhibit bacterial growth (Lolongan *et al*., 2016).

In this research, the data collected was analyzed using the SPSS For Windows program. The results of the normality test used in this research were the Shapiro-Wilk test. So it is proven that the data is normally distributed. Next, a homogeneity test was carried out, so that it was proven that the data was homogeneous, and the data could be analyzed using a parametric test (Anova). The data was normally distributed and homogeneous so it was continued with the One Way Anova test.

Based on table 4, statistical test results for the ethyl acetate fraction of walay rhizomes (*Meistera chinensis)* against bacteria *Staphylococcus aureus* at concentrations of 1.563%, 3.125%, 6.25%, 12.5%, 25% and 50%, and K (+) shows a difference with K (-) this shows that the treatment shows that the ethyl acetate fraction of walay rhizomes (*Meistera chinensis)* has antibacterial activity which can inhibit bacterial growth *Staphylococcus aureus*, this is in accordance research of Musdalipah *et al.* (2021) fraction of walay rhizome (*Meistera chinensis*) contains antibacterial compounds, namely flavonoids, which are one of the secondary compounds that have an antibacterial function. Flavonoid compounds can inhibit bacterial growth by damaging cell walls, inactivating enzyme action, binding to adhesins, and damaging cell membranes (Nugraha et al., 2017).

CONCLUSION

Based on the results of research that has been carried out, it can be concluded that the class of chemical compounds contained in the ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) such as flavonoids, tannins and saponins. Ethyl acetate fraction of walay rhizomes (*Meistera chinensis*) has antibacterial activity against *Staphylococcus aureus,* Minimum Inhibitory Concentration (MIC) of walay rhizome ethyl acetate fraction (*Meistera chinensis*) can be determined at a concentration of 1.563% which is indicated by a decrease in the absorbance value at a wavelength of 285 nm after 1x24 hours of incubation.

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