



Antioxidant Activity Of Leilem Leaf Extract Fractions *Clerodendrum minahassae* Teijsm. and Binn. Using The DPPH Method (2,2-Diphenyl-1-Picrylhydrazil)

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Abstract

Clerodendrum minahassae Teijsm. and Binn plant is included in the *Clerodendrum* genus which contains chemicals such as alkaloids, flavonoids, phenols, tannins and steroids which have an important role in the development of medicine, including as antioxidants, anti-inflammatory, anti-diabetic and antibacterial. This study aims to determine the antioxidant activity of leilem leaf fractions using the DPPH method. Leilem leaves were extracted using the maceration method using 70% ethanol, then partitioned using the liquid-liquid extraction method using three different solvents, namely n-hexane, ethyl acetate and water. The fraction of the partition was then tested for antioxidant activity using the DPPH method and its absorbance was measured using a UV-VIS spectrophotometer with a wavelength of 515 nm. The research results showed that the n-hexane fraction had an IC₅₀ value of 185.704 ppm, the ethyl acetate fraction had an IC₅₀ value of 105.357 ppm and the water fraction had an IC₅₀ value of 549.258 ppm with the IC₅₀ value of the quarcetin comparison being 8.86 ppm. It can be concluded that the highest antioxidant activity is found in the ethyl acetate fraction, which is included in the moderate antioxidant category.

Keywords: *Clerodendrum Minahassae* Teijsm. and Binn. leaf, Antioxidant, DPPH

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1. Introduction

Nowadays, antioxidants have become an important topic in various scientific disciplines. Especially in the fields of medicine and health, theories about radical compounds, free radicals and antioxidants are increasingly developing. This is based on the fact that it is increasingly understood that most diseases are initiated by excessive oxidation reactions in the body (Sayuti and Yenrika, 2015).

Clerodendrum minahassae Teijsm. and Binn is a plant that grows a lot in the Minahasa area. Minahasa people generally use leilem leaves as a cooking spice and use it to treat diseases such as stomach aches, worms and lung diseases. This leilem plant belongs to the genus *Clerodendrum* and the family Verbenaceae (Wiar, 2002).

Based on an ethnopharmacological approach, it is known that the *Clerodendrum* genus has various important roles in the development of medicine, including anti-inflammatory, anti-diabetic and anti-bacterial (Shrivastava and Patel, 2007). (Adam *et al.*, 2013) reported that the methanol extract of leilem leaves had antioxidant activity ranging from 64.83 - 70.12%. According to (Bontjura, 2015) conducted research on testing the antibacterial effect of leilem leaf extract (*Clerodendrum minahassae* l.) against *Streptococcus mutans* bacteria, showing that the phenolic compounds in leilem leaf extract had an antibacterial effect against *Streptococcus mutans* bacteria. Another study reported that ethanol extract of leilem leaves can inhibit the growth of *Escherichia coli* bacteria at concentrations of 5%, 10% and 15% because the flavonoids contained therein can damage bacterial cell membranes (Tangka *et al.*, 2016).

Apart from that, standardization testing of raw materials has been carried out including the compound content of leilem leaf extract, namely the extract contains alkaloid, steroid, flavonoid and tannin compounds which have many pharmacological effects (Utami *et al.*, 2017). Research has been carried out on the isolation and characterization of leilem leaf extract using UV-Vis spectrophotometric analysis. Isolate 2 has maximum absorption at wavelengths of 269.0 nm and 333.0 nm⁻¹. FT-IR data shows the presence of O-H, C-H,





C=C and C-O functional groups which are thought to be flavonoid compounds (Utami, Imrawati and Rasyid, 2018).

Based on the above background, researchers conducted research on testing the antioxidant activity of *Clerodendrum minahassae* Teijsm. and Binn. leaf extract fractions using the DPPH (2,2-Diphenyl-1-Picrilhydrazil) method.

2. Research Method

This type of research is scale experimental research. This research was carried out at the Pharmaceutical Biology Laboratory and Pharmaceutical Chemistry Laboratory, Faculty of Health Sciences, Almarisah Madani University.

a. Materials

The tools used are glassware (pyrex), maceration tool, separating funnel, porcelain cup, beaker, measuring flask 5 ml, 10 ml, 50 ml, micropipette, analytical balance, coarse scale, UV-VIS spectrophotometer (Shimadzu®).

The materials that will be used are ethanol p.a, 70% ethanol, 96% ethanol, n-hexane, ethyl acetate, sterile distilled water, methanol, Wagner reagent, Mayer reagent, Dragendrof reagent, 2N HCl, chloroform, 2 N H₂SO₄, ammonia, magnesium powder, FeCl₃, concentrated HCl, ethanol p.a, DPPH, leilem leaves, quercetin.

b. Sample preparation

Leilem leaf samples were obtained from Kairagi Dua Village, Mapanget District, Manado City, North Sulawesi Province.

Samples of leilem leaves that have been harvested first are then sorted wet, washed with running water until clean, chopped and dried by air-drying without being exposed to direct sunlight. Next, the sample is ready to be extracted (Utami, 2020).

c. Extraction process

Three hundred and fifty grams of leilem leaves were macerated using 3 liters of 70% ethanol solvent at room temperature and protected from light for 5 days, stirring occasionally. Then the residue is filtered and the filtrate is separated, then the dregs are re-macerated using the same treatment, the filtrate obtained is collected with filtrate 1





then evaporated in the evaporator until a thick extract of leilem leaves (*Clerodendrum minahassae* Teijsm. and Binn.) is obtained (Utami, Mubarak and Rahman, 2023).

d. Separation Process

Fractionation of the ethanol extract of leilem leaves using a separating funnel. Each ethanol extract of leilem leaves was suspended using 100 mL water as a solvent. Then 100 mL of n-hexane solvent was added 4 times until the solvent was clear in color. Then 100 mL ethyl acetate solvent was added 5 times until the solvent was clear in color. The fractionation process was carried out 4 times, the resulting n-hexane, ethyl acetate and water fractions were collected and then evaporated until a thick fraction was obtained (Annisa, Yuniarti and Sunardi, 2018).

e. Antioxidant Activity Test

1. Preparation of leilem leaf fraction stock solution (Utami, 2021)

A stock solution of 1000 ppm was prepared by weighing 50 mg of each fraction, namely the n-hexane fraction, ethyl acetate and leilem leaf water and dissolving it with ethanol p.a while homogenizing, the final volume was made up with ethanol p.a to 50 ml in a volumetric flask.

2. Preparation of quercetin stock solution

A stock solution of 1000 ppm was prepared by weighing 50 mg of quercetin and then dissolving it with ethanol pa solvent while homogenizing, the final volume was made up to 50 ml with ethanol p.a.

3. Preparation of 0.4 mM DPPH stock solution

Weighed DPPH as much as 0.01577 g then dissolved using ethanol solvent while homogenizing. The final volume was filled with ethanol p.a to 100 mL in a volumetric flask to obtain a 0.4 mM DPPH solution (Molyneux, 2004).

f. Measurement of antioxidant activity using the DPPH method

1. DPPH blank absorbance measurement





One milliliters of 0.4 mM DPPH solution was pipetted and the volume was made up to 5 ml with absolute ethanol in a volumetric flask. This solution was then measured with a UV-VIS spectrophotometer at a wavelength of 515 nm.

2. Measurement of DPPH free radical binding activity on the fractions

Measurement of DPPH free radical binding activity with a stock solution of 1000 ppm n-hexane fraction of leilem leaves was pipetted into 500 μL , 750 μL , 1000 μL and 1250 μL , 1500 μL each, the mixture was added with 1 mL of 0.4 mM DPPH solution then the volume was made up. up to 5 ml with absolute ethanol to obtain solutions with concentrations of 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm. Next, it was homogenized and left for 30 minutes, then the absorbance was measured with a UV-VIS spectrophotometer at a wavelength of 515 nm.

Measurement of DPPH free radical binding activity with a stock solution of 1000 ppm leilem leaf ethyl acetate fraction was pipetted into 125 μL , 250 μL , 375 μL , 500 μL and 625 μL each, the mixture was added with 1 mL of 0.4 mM DPPH solution then the volume was increased to 5 ml with absolute ethanol to obtain solutions with concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm. Next, homogenize and leave for 30 minutes, then absorbance is measured with a UV-VIS spectrophotometer at a wavelength of 515 nm.

Measurement of DPPH free radical binding activity with a stock solution of 1000 ppm leilem leaf water fraction samples was pipetted into 750 μL , 1500 μL , 2250 μL , 3000 μL and 3750 μL each, the mixture was added with 1 mL of 0.4 mM DPPH solution then the volume was made up to 5 ml with absolute ethanol to obtain solutions with concentrations of 150 ppm, 300 ppm, 450 ppm, 600 ppm and 750 ppm. Next, it was homogenized and left for 30 minutes, then the absorbance was measured with a UV-VIS spectrophotometer at a wavelength of 515 nm.

3. Measurement of DPPH free radical binding activity on quercetin

Measurement of DPPH free radical binding activity with 1000 ppm quercetin pipetted into 12.5 μL each; 25 μL ; 37.5 μL ; 50 μL and 62.5 μL , the mixture was





added with 1 mL of 0.4 mM DPPH solution then the volume was increased to 5 ml with absolute ethanol to obtain a solution with a concentration of 2.5 ppm; 5ppm; 7.5 ppm; 10 ppm and 12.5 ppm. Next, it was homogenized and left for 30 minutes, then the absorbance was measured with a UV-VIS spectrophotometer at a wavelength of 515 nm.

g. Data analysis

The percentage of DPPH reduction produced by each concentration of leilem and quercetin leaf fractions is calculated using the formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs Blank} - \text{Abs sample})}{\text{Absorbansi Blank}} \times 100\%$$

The IC₅₀ (50% Inhibitory Concentration) value to obtain the strength of the antioxidant activity of the leilem leaf fraction (*Clerodendrum minahassae* Teijsm. and Binn.) was calculated using a linear regression equation.

3. Results And Discussions

a. Result

Table 1.

Yield Results of Leilem Leaves (*Clerodendrum minahassae* Teijsm. and Binn.).

Simplicity weight (g)	Extract weight (g)	Yield (%)
350	35,68	10,19

Table 2.

Results of liquid-liquid extraction of leilem leaf extract

<i>Clerodendrum minahassae</i> Extract	Extract liquid-liquid partition results		
	n-heksan fraction	etil asetat fraction	Water fraction
20 gram	2,4 gram	3,4 gram	12,67 gram

Table 3.





Calculation results of testing the antioxidant activity of the n-hexane fraction of leilem leaves

Concentration (ppm)	Absorbance	Average	% Inhibition	Line equation	IC ₅₀ Value (ppm)
100	0,491	0,487	24,496	Y= 0,3019x – 5,5963	184,704
	0,484				
	0,487				
150	0,389	0,386	40,155		
	0,394				
	0,375				
200	0,303	0,295	54,263		
	0,284				
	0,3				
250	0,187	0,195	89,767		
	0,203				
	0,196				
300	0,082	0,095	85,271		
	0,098				
	0,106				
Blank	0,7	0,645			
	0,677				
	0,56				

Table 4.

Calculation results of testing the antioxidant activity of the etil acetat fraction of leilem leaves

Concentration (ppm)	Absorbance	Average	% Inhibition	Line equation	IC ₅₀ Value (ppm)
25	0,558	0,562	8,766	y = 0.5092x - 3.627	105,357
	0,564				
	0,566				
50	0,473	0,478	22,40		
	0,483				
	0,480				
75	0,403	0,402	34,740		
	0,398				
	0,407				
100	0,325	0,328	46,753		
	0,328				
	0,332				
125	0,249	0,245	60,227		
	0,241				
	0,246				
Blank	0,621	0,616			
	0,618				





	0,611			
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Table 5.

Calculation results of testing the antioxidant activity of the water fraction of leilem leaves

Concentration (ppm)	Absorbance	Average	% Inhibition	Line equation	IC ₅₀ Value (ppm)
150	0,651	0,657	13,666	$y = 0.0898x + 1.116$	549,258
	0,662				
	0,658				
300	0,55	0,543	28,646		
	0,548				
	0,532				
450	0,444	0,441	42,049		
	0,448				
	0,431				
600	0,344	0,339	55,453		
	0,343				
	0,33				
750	0,243	0,246	67,674		
	0,249				
	0,246				
Blank	0,753	0,761			
	0,764				
	0,768				

Table 6.

Calculation results of testing the antioxidant activity of quarcetin

Concentration (ppm)	Absorbance	Average	% Inhibition	Line equation	IC ₅₀ Value (ppm)
2,5	0,646	0,682	10,62	$y = 5.6951x - 0.4587$	8.86 ppm
	0,587				
	0,813				
5	0,535	0,523	31,50		
	0,464				
	0,569				
7,5	0,480	0,434	43,08		
	0,334				
	0,489				
10	0,369	0,328	57,06		
	0,250				
	0,364				
12,5	0,304	0,236	69,03		





	0,135			
	0,270			
Blanko	0,763	0,763		

b. Discussion

The samples used in this research were leilem leaves (*Clerodendrum minahassae* Teijsm. and Binn.) taken from Kairagi Dua Village, Mapanget District, Manado City, North Sulawesi Province. The part of the plant used in this research is leilem leaves in the form of dried simplicia because the water content contained in simplicia is generally less, making it easier for the filter fluid to enter the plant cells and draw out the active substances contained perfectly.

The filtering (extraction) process uses the maceration method with 70% ethanol. The maceration method is used because it has the advantage of being a fast processing method, the equipment used is simple, relatively easy and cheap. In this research, 70% ethanol solvent was used because it is a universal solvent, it can dissolve almost all organic compounds in the sample, both polar and non-polar compounds (Munte, 2015).

The results of the maceration of leilem leaf simplicia (*Clerodendrum minahassae* Teijsm. and Binn.) resulted in a thick extract of 35.68 grams and a yield of 10.19%. Yield is the percentage result between the parts that can be extracted from the raw material. Ethanol extract of leilem leaves (*Clerodendrum minahassae* Teijsm. and Binn.) followed by liquid-liquid extraction (ECC) using three solvents with different levels of polarity, namely n-hexane, ethyl acetate and water. From the liquid-liquid extraction results, the weight of the fraction is obtained as follows table 2.

The results of this fraction were then tested for antioxidant activity using the DPPH method. The DPPH method is a method for measuring antioxidants that is simple, fast and does not require as many reagents as other methods. The calculation used to calculate the amount of antioxidant activity is by determining the IC₅₀ value. The IC₅₀ value was obtained using a linear regression equation which states the relationship between sample concentration and the percentage of DPPH radical reduction.





Quantitative antioxidant activity tests were carried out using a UV-Vis spectrophotometer to determine the absorbance of DPPH remaining after adding the extract. If a compound has antioxidant activity, there will be a decrease in the DPPH absorbance value at a wavelength of 515 nm. The decrease in DPPH absorbance was measured against the control absorbance, namely the absorbance of DPPH in ethanol p.a. without the addition of the test substance. The decrease in DPPH absorbance is indicated by the degradation of DPPH color from purple to yellow which is directly proportional to the concentration of the added extract. From the DPPH absorbance value obtained, the percentage value of DPPH radical reduction (% inhibition) can be determined. From the % inhibition value, the IC_{50} (inhibitory concentration) value can be determined.

From the calculated data, it is known that the ethyl acetate fraction provides the greatest reduction as indicated by the smallest IC_{50} value, namely 105,357 ppm. These results indicate that the ethyl acetate fraction is included in the moderate antioxidant category because it is in the range of 100-150 ppm. The n-hexane fraction and water fraction respectively had IC_{50} values of 184,704 ppm and 549,258 ppm, where the n-hexane fraction was included in the weak antioxidant category with a range of 151-200 ppm and the water fraction did not have potential as an antioxidant because the resulting IC_{50} value was > 200 ppm, you can see table 3 and 4.

Quercetin is used as a comparison because quercetin is a natural antioxidant. The concentration used for quercetin is 2.5 ppm; 5ppm; 7.5 ppm; 10 ppm and 12.5 ppm. Based on the results of antioxidant activity tests that were carried out on the n-hexane, ethyl acetate and leilem leaf water fractions. The IC_{50} value of quercetin is smaller (the IC_{50} is very strong) you can see table 6, compared to the three leilem leaf fractions because quercetin is a pure compound. Quercetin has very strong antioxidant activity because it has 5 hydroxyl groups which makes it easier to donate hydrogen. According to the classification carried out by Molyneux, the IC_{50} value of the ethyl acetate fraction is a moderate antioxidant, namely in the range of 101-150 ppm, the n-hexane fraction





has weak antioxidant activity, namely in the range of 151-200 ppm, and the water fraction has no antioxidant activity. because it has an IC_{50} value above 200 ppm, namely 549,258 ppm, you can see table 5.

4. Conclusion

Based on the results of the research conducted, conclusions can be drawn, namely:

- a. The research results showed that the n-hexane fraction had an IC_{50} value of 185,704 ppm, the ethyl acetate fraction had an IC_{50} value of 105,357 ppm and the water fraction had an IC_{50} value of 549,258 ppm with the IC_{50} value of the quarcetin comparison being 8.86 ppm.
- b. The most powerful fraction in providing antioxidant effects is the ethyl acetate fraction with an IC_{50} value of 105,357 ppm, which is included in the moderate antioxidant category.

5. Compliance with ethical standards

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Disclosure of conflict of interest

This research collaboration is a positive thing for all researchers so that conflicts, problems and others are absolutely no problem for all writers.

Statement of informed consent

Every action we take as authors is a mutual agreement or consent

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