Original Article

Available online at
www.heca-analitika.com/hjas

Heca Journal of Applied Sciences

Vol. 2, No. 1, 2024

In Vitro Test of Antioxidant Activity of Leilem Leaf Ethanol Extract (*Clerodendrum minahassae*) Using DPPH and FRAP Methods

Angeline Priscillia Malino [1], Billy Johnson Kepel [2,*], Fona Dwiana Hermina Budiarso [2], Fatimawali Fatimawali [2], Aaltje Ellen Manampiring [2] and Widdhi Bodhi [2]

1 Medical Education Study Program, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia; angelinemalino011@student.unsrat.ac.id (A.P.M.)

2 Chemistry Department, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia; billy.kepel@unsrat.ac.id (B.J.K.); fonabudiarso@unsrat.ac.id (F.D.H.B.); fatimawali@unsrat.ac.id (F.F.); aldamanampiring@unsrat.ac.id (A.E.M.); widdhi_bodhi@unsrat.ac.id (W.B.)

* Correspondence: billy.kepel@unsrat.ac.id

Article History

Received 19 January 2024
Revised 3 March 2024
Accepted 16 March 2024
Available Online 22 March 2024

Keywords:
Clerodendrum minahassae
Free radicals
Phytochemical screening
Antioxidant assay

Abstract

The risk of degenerative diseases is increasing due to unhealthy lifestyles. Many people consume junk food, smoke, and do not protect themselves from sun exposure and pollution, exposing their bodies to free radicals. Free radicals can cause oxidative stress, necessitating the need for antioxidants to neutralize them. The leilem plant (*Clerodendrum minahassae*) is an endemic plant from North Sulawesi that is often used in traditional dishes and medicine. Moreover, leilem leaves are believed to be a source of natural antioxidants. This study aimed to determine the efficacy of leilem leaf ethanol extract as a source of antioxidants. The antioxidant potential was evaluated using phytochemical screening and antioxidant assays such as 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) methods, with ascorbic acid as a comparison. The level of antioxidant activity was measured by determining the IC_{50} value, which corresponds to the concentration of extract required to inhibit 50% of free radicals. The results of phytochemical screening showed that the ethanol extract of leilem (*Clerodendrum minahassae*) leaves contains active compounds such as phenols, flavonoids, saponins, tannins, steroids/triterpenoids, and alkaloids, indicating a high potential for antioxidant activity. The IC_{50} value for the DPPH method was 78.799 ppm, and the IC_{50} value for the FRAP method was 92.127 ppm. These results demonstrate that the ethanol extract of leilem leaves has strong antioxidant activity in both the DPPH and FRAP methods.

1. Introduction

Every year, around 41 million people die from degenerative diseases worldwide (WHO, 2022) [1]. The risk of these diseases is increasing due to unhealthy lifestyles such as eating junk food, smoking, drinking alcohol, and not protecting themselves from excessive exposure to air pollution and sunlight so that the body is exposed to free radicals [2, 3].

Free radicals are molecules that are highly reactive and unstable because they have unpaired electrons on their outer layer [4]. If excessive free radicals are not neutralized, this can lead to oxidative stress which causes damage to cells and tissues. Antioxidants are needed by the body to prevent oxidative stress. Antioxidants donate electrons to neutralize free radicals [5]. Antioxidants can be found in vegetables, fruits, nuts, chocolate, tea, coffee, and certain herbs [6, 7].

Indonesia is rich in plants that can be used for medicine [8–10]. One notable medicinal plant in Indonesia is the leilem plant (*Clerodendrum minahassae*), which is endemic to North Sulawesi. This plant is believed to be a source of antioxidants and has been traditionally used to treat stomach pain, worms, and mouth ulcers. In addition to its medicinal properties, the leaves of the leilem plant are often used as vegetables and seasonings in local cuisine.

Antioxidant activity assessment can be done by various methods [11]. However, in this study, DPPH and FRAP methods were used. The principle of the 2,2-Difenil-1-Pikrilhidrazil (DPPH) method is that the DPPH radical will react with hydrogen atoms and be reduced. The advantages of this method are that it requires low cost, simple procedures, high sensitivity, and only requires a few samples [12]. The Ferric Reducing Antioxidant Power (FRAP) method principle is that antioxidants from plant extracts will reduce ferric complexes (Fe^{3+}) to ferrous complexes (Fe^{2+}). This method has advantages such as easy-to-prepare reagents, fairly simple procedures, fast and not requiring special equipment [13].

Several studies have been conducted on the antioxidant activity of leilem leaf extract using DPPH and FRAP methods. Research has been conducted by Rachmatia et al. on the antioxidant test of methanol extracts with the DPPH method. The study results were weak antioxidant activity with an IC_{50} value of methanol extract of 300.57 g/ml [14]. Another study was conducted by Pratiwi et al. using the DPPH method, which obtained an IC_{50} value of ethanol extract of leilem leaves of 13.8 ppm, which means very strong antioxidant activity [15]. Research on antioxidant testing of leilem leaves was also conducted by Kairupan et al. using the DPPH and FRAP methods. The results of this study obtained the IC_{50} value of leilem leaf ethanol extract for the DPPH method of 565.45 g/ml and the FRAP method of 123.62 g/ml [16]. Based on several existing studies, there is a gap in the IC_{50} value in the DPPH method, and research on the antioxidant test of leilem leaves with the FRAP method is still very limited. Therefore, this study aims to identify active compounds and the antioxidant activity of ethanol extract of leilem leaves using DPPH and FRAP methods.

2. Materials and Methods

This research was conducted at the Laboratory of the Faculty of Medicine, Sam Ratulangi University, and the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia. The samples used were leilem leaves obtained from Rumoong Atas Village, Tareran District, Minahasa Regency at an altitude of 600 meters above sea level located between 1.2210676 north latitude and 124.7367846 east longitude and Kayuuwi Village, West Kawangkoan District, Minahasa Regency at an altitude of 700 meters above sea level located between 1° 12' 35.2656'' north latitude and 124° 46' 28.1892'' east longitude.

2.1. Sample Preparation

The young leaves of the leilem plant are picked and then cleaned with running water until clean and drained. After that, leilem leaves are dried by aerating indoors for a week. The dried leaves were then pulverized with a blender to obtain dry simplisia powder and sieved using a mesh sieve to obtain a fine and uniform simplisia powder. Then, the weight of the simplisia powder was measured using an analytical balance.

2.2. Extract Preparation

The extraction method used in this study is maceration. 400 g of simplisia powder was put into a glass jar, and 2000 ml of 96% ethanol solution was added. The glass jar was then closed and left for three days at room temperature while occasionally stirring. After three days, filtering was carried out using filter paper to obtain filtrate I. Then, remaceration was carried out by mixing the dregs with 500 ml of new 96% ethanol solution, and then the dregs were allowed to stand for two days while occasionally stirring. After two days, filtration was carried out to obtain filtrate II. Filtrate I and II were combined and evaporated using an oven. The thick extract was then allowed to stand at room temperature until the solvent evaporated. After that, the thick extract was weighed using an analytical balance and stored in a closed container.

2.3. Qualitative Phytochemical Screening

The sample extract solution was made by mixing 2 g of thick extract of leilem leaf with 20 ml of 96% ethanol.

2.3.1. Triterpenoids and Steroids

Add 2 ml of sample extract into a test tube and 2 ml of glacial CH_3COOH to it. After that, add 2 ml of H_2SO_4. For a few minutes, positive steroids will form a green-blue-black color, and positive triterpenoids will form a purple-red color [17].

2.3.2. Phenols and Tannins

add 2 ml of sample extract to the test tube with 5-10 drops of FeCl3 1%. Phenols and tannins are positive if a blue-black or green color is formed [17].

2.3.3. Flavonoids

Add 2 ml of sample extract into a test tube and then heat it. Furthermore, five drops of HCL and 0.1 g of magnesium powder were added to the solution. The solution will form a dark red color, which indicates that it is positive for flavonoids [17].

2.3.4. Saponins

2 ml of extract solution in a test tube was heated, and then 20 drops of water were added to it and homogenized for approximately 1 minute. A stable foam formed indicates positive saponins [17].

2.3.5. Alkaloids

add 2 ml of sample extract into the test tube, then add 5 ml of chloroform, 5 ml of ammonia, and three drops of H_2SO_4. After that, the test tube was homogenized and allowed to stand for 3 minutes. The top layer was then pipetted and put into three test tubes. Each test tube was dripped with ten drops of Mayer, Wager, and Dragendorff reagents. Alkaloids are positive if a white or yellow precipitate is formed for the Mayer reagent, a brown precipitate for the Wager reagent, and an orange precipitate for the Dragendorff reagent [17].

2.4. DPPH Reagent Preparation

DPPH solution was prepared by mixing 4 mg of DPPH powder with 100 ml of 96% ethanol.

2.4.1 Sample Solution Preparation

The 1000 ppm stock solution was made by weighing 10 mg of leilem leaf extract and then dissolved with 10 ml of 96% ethanol. From 1000 ppm stock solution, a sample solution was made with concentration variations of 20 ppm, 40 ppm, 80 ppm, and 100 ppm. The 1000 ppm stock solution was pipetted 0.2 ml, 0.4 ml, 0.8 ml, and 1 ml, respectively, in a 10 ml volumetric flask, then sufficed with 96% ethanol until the limit line.

2.4.2 Antioxidant Activity Test Using 2,2-Difenil-1-Pikrilhidrazil (DPPH) Method

Sample solutions with various concentrations (20 ppm, 40 ppm, 80 ppm, 100 ppm) were taken as much as 2 ml each and added with 2 ml DPPH solution into 5 test tubes, then vortexed to make it homogeneous. The mixture was then incubated for 30 minutes at room temperature. The sample solution that changes color is then measured for absorbance at a wavelength of 517 nm using a UV-Vis spectrophotometer and recorded as the absorbance of the sample. The absorbance of control was obtained by measuring the absorbance of the DPPH solution. The measurement was done three times with ascorbic acid as the standard, and the same treatment was given to the sample. The absorbance value obtained was used to find the % inhibition value (Equation 1). Making a curve where x as concentration (ppm) against y as % inhibition, then obtained a linear regression equation y = ax + b. From the linear regression equation, the IC_{50} value was obtained [18].

$$\% \, Inhibition = \frac{Ac - As}{Ac} \, x \, 100\% \quad (1)$$

where Ac represents the absorbance of the control and As represents the absorbance of the sample

2.5. FRAP Reagents Preparation

The FRAP reagents were prepared as follows: a phosphate diluent solution was made by dissolving 0.8 g of NaOH and 2.72 g of KH2PO4 in distilled water to 100 ml each, then 39.1 ml of NaOH solution and 50 ml of KH2PO4 solution were combined in a 200 ml volumetric flask and filled with distilled water; a 1% potassium ferricyanide solution was made by dissolving 1 g of potassium ferricyanide in distilled water to 100 ml; a 0.1% iron (III) chloride solution was made by dissolving 0.1 g of FeCl3 in distilled water to 100 ml; a 10% trichloroacetic acid solution was made by dissolving 10 g of TCA in distilled water to 100 ml; and a 1% oxalic acid solution was made by dissolving 1 g of oxalic acid in distilled water to 100 ml.

2.5.1. Sample Solution Preparation

The 1000 ppm stock solution was made by weighing 10 mg of leilem leaf extract and then dissolved with 10 ml of 96% ethanol. From 1000 ppm stock solution, a sample solution was made with concentration variations of 20 ppm, 40 ppm, 60 ppm, and 80 ppm. The 1000 ppm stock solution was pipetted 0.2 ml, 0.4 ml, 0.6 ml, and 0.8 ml, respectively, in a 10 ml volumetric flask, then sufficed with 96% ethanol to the limit line.

2.5.2 Antioxidant Activity Test Using Ferric Reducing Antioxidant Power (FRAP) Method

A total of 1 ml of sample solution of each concentration (20 ppm, 40 ppm, 60 ppm, 80 ppm) was added to 1 ml of phosphate buffer solution and 1 ml of $K_3Fe(CN)_6$. The mixture was then homogenized and incubated for 20 minutes at 50°C. After that, the solution was added with 1 ml of TCA and centrifuged for 10 minutes at 3000 rpm. The top layer (supernatant) was then taken as much as 1 ml, mixed with 1 ml of distilled water and 0.5 ml of $FeCl_3$ 0.1%, and allowed to stand for 10 minutes. The sample solution that changes color is then measured for

Table 1. Phytochemical screening test results of *Clerodendrum minahassae.*

No.	Phytochemical Tested	Reagents	Results	Description
1	Phenol	$FeCl_3$	+	Blue-black color formed
2	Flavonoids	HCL Magnesium powder	+	Brick red color formed
3	Saponins	Aquades	+	There is a little forth
4	Tannins	$FeCl_3$	+	Blue-black edge
5	Steroids/ Triterpenoids	H_2SO_4 Acetic Acid	+	Purple-brown color formed
6	Alkaloids	Wagner Mayer Dragendorff	+	There is a red-brown sediment There is a white sediment There is an orange sediment

"+" indicates a compound contained in the extract.

absorbance at a wavelength of 720 nm using a UV-Vis spectrophotometer. Measurements were taken three times. In the FRAP method, ascorbic acid is used as a standard that is given the same treatment as the sample. The absorbance value obtained is used to find the % reducing power value (Equation 2). Making a curve where x as concentration (ppm) against y as % reducing power (%RP), then obtained a linear regression equation y = ax + b. From the linear regression equation, the IC_{50} value was obtained [19].

$$\% \ Reducing \ Power = \left[1 - \left(1 - \frac{As}{Ac}\right) x \ 100\%\right] \qquad (2)$$

where As represents the absorbance of the sample and Ac represents the absorbance of the standard (ascorbic acid).

3. Results and Discussion

3.1. Leilem Leaf Extraction Result

The extraction process is carried out so that the compounds contained in the plant can be separated from the mixture or simplisia. The extraction process uses the maceration method because the solvent will penetrate the cell wall of the material and then enter the cell cavity containing active compounds. The difference in concentration between the active compounds inside and outside causes the concentrated solution to be pushed out [20]. For solvent selection, ethanol is used because it is semi-polar, which can dissolve polar and nonpolar compounds and attract secondary metabolite compounds [21]. 400 grams of leilem leaf simplisia powder was extracted by maceration using 96% ethanol solvent. Then, the filtrate was evaporated using an oven for three days. This process obtained 28.84 g of thick extract with a yield of 7.21%.

3.2. Phytochemical Screening Test Results

Based on the results of phytochemical screening (Table 1), ethanol extract of leilem leaves contains active compounds such as phenols, flavonoids, saponins, tannins, steroids/triterpenoids, and alkaloids where these compounds can act as antioxidants. Each compound has its mechanism for reducing free radicals. Phenol can donate hydrogen atoms, reducing free radicals to a more stable form. Tannins have hydroxyl groups whose hydrogen atoms can be donated to free radicals so that they become more stable forms. Steroids/triterpenoids play a role in the primary antioxidant mechanism of action, which can break the chain reaction and convert free radicals into a more stable form. Saponins can reduce superoxide by forming hyperoxide intermediates so that biomolecular damage by free radicals can be prevented [22]. Flavonoids act as antioxidants because the aromatic ring carbon has a hydroxyl group, so free radicals generated from fat peroxidation reactions can be captured [23]. Alkaloids act as antioxidants because they contain nitrogen atoms with free electron pairs to reduce free radical activity [24].

3.3. Analysis of Antioxidant Activity Using 2,2-Difenil-1-Pikrilhidrazil (DPPH) Method

An antioxidant activity test using the DPPH method uses the principle that antioxidant compounds will react with DPPH by donating hydrogen atoms to get a pair. The color change that occurs is then measured using a UV-Vis spectrophotometer with a wavelength of 517 nm to obtain the absorbance value. The absorbance value obtained is then entered into the formula for the percent inhibition [18].

Table 2 shows the absorbance value and % inhibition of ethanol extract of leilem leaves, and Table 3 shows the absorbance value and % inhibition of ethanol extract of ascorbic acid as a control. Based on the measurement

Table 2. Absorbance value of leilem leaf extract with DPPH method.

Concentration (ppm)	Ethanol Extract of Leilem Leaves	
	Absorbance (Mean ± SD)	% Inhibition
20	0.485 ± 0.004	41.425
40	0.473 ± 0.006	42.874
80	0.450 ± 0.013	45.652
100	0.352 ± 0.012	57.488

Table 3. Absorbance value of ascorbic acid with DPPH method.

Concentration (ppm)	Ethanol Extract of Leilem Leaves	
	Absorbance (Mean ± SD)	% Inhibition
0.5	0.516 ± 0.044	37.681
1	0.436 ± 0.056	47.343
6	0.343 ± 0.023	58.575
9	0.331 ± 0.009	60.024

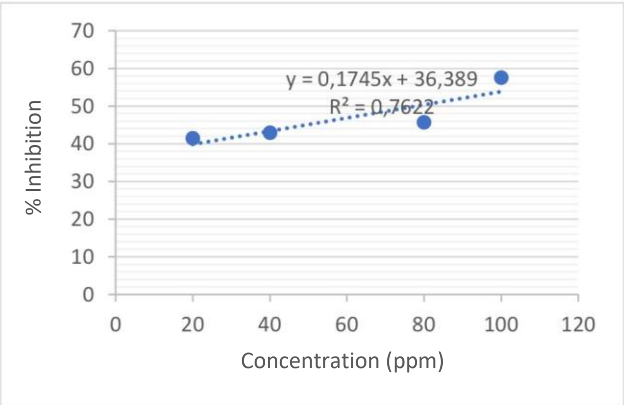

Figure 1. Relationship graph of leilem leaf ethanol extract concentration and % inhibition.

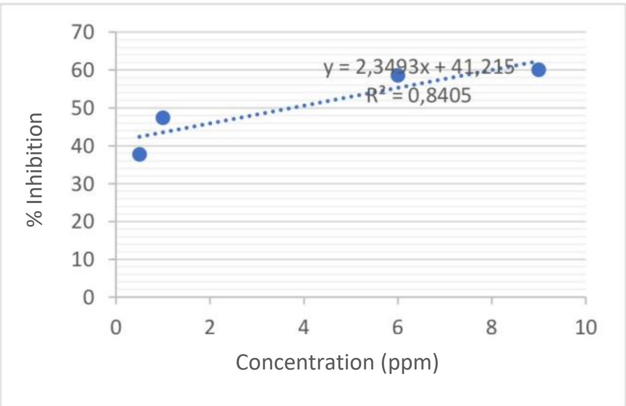

Figure 2. Relationship graph of ascorbic acid concentration to % inhibition.

results for both leilem leaf ethanol extract and ascorbic acid, it can be seen that the greater the concentration of the test solution, the lower the absorbance value and the higher the percent inhibition, indicating antioxidant activity in trapping DPPH free radicals.

The linear regression equation is obtained by plotting the concentration of the test solution with % inhibition, where concentration is the abscissa and % absorbance is the ordinate. Figure 1 is a linear regression equation of leilem leaf ethanol extract. The equation obtained is y = 0.1745x + 36.389 with a correlation coefficient value of 0.7622. Figure 2 is the linear regression equation of ascorbic acid as a control. The equation obtained is y = 2.3493x + 41.215 with a correlation coefficient value of 0.8405.

In the linear regression equation y = ax + b, y is the % antioxidant activity, a is the slope constant, b is the intercept constant, and x is the concentration of the test solution. The IC_{50} value is obtained through the linear regression equation, with the y variable worth 50 and the x variable being the IC_{50} value, which is interpreted as the amount of solution concentration that can reduce 50% of DPPH free radicals. The determination of the IC_{50} value is based on the concentration of the solution that can reduce 50% of DPPH free radicals. If the IC_{50} value is less than 50 ppm, it indicates a very strong antioxidant, IC_{50} 50-100 ppm (strong antioxidant), IC_{50} 100-150 ppm (moderate antioxidant), and IC_{50} 150-200 ppm (weak antioxidant), and IC_{50} more than 200 ppm indicates a very weak antioxidant [25].

Based on the results of these calculations, the IC_{50} value of ethanol extract of leilem leaves of 78.799 ppm is included in the strong category and the IC_{50} value of ascorbic acid of 3.739 ppm is included in the very strong category.

3.4. Analysis of Antioxidant Activity Using Ferric Reducing Antioxidant Power (FRAP) Method

Antioxidant activity test with the FRAP method uses the principle that the antioxidant will reduce the ferric

Table 4. Absorbance value of leilem leaf extract with FRAP method.

Concentration (ppm)	Ethanol Extract of Leilem Leaves	
	Absorbance (Mean ± SD)	% Reducing Power
20	0.498 ± 0.031	44.785
40	0.505 ± 0.009	45.414
60	0.530 ± 0.019	47.622
80	0.547 ± 0.034	49.191

Table 5. Absorbance value of ascorbic acid with FRAP method.

Concentration (ppm)	Ethanol Extract of Leilem Leaves	
	Absorbance (Mean ± SD)	% Reducing Power
0.5	0.516 ± 0.044	37.681
1	0.436 ± 0.056	47.343
6	0.343 ± 0.023	58.575
9	0.331 ± 0.087	60.024

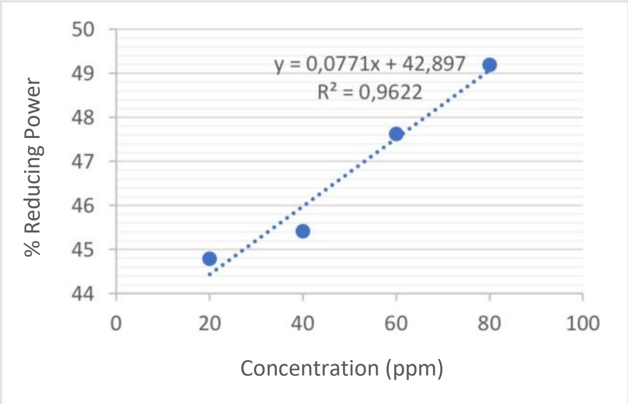

Figure 3. Relationship graph of leilem leaf ethanol extract concentration and % reducing power.

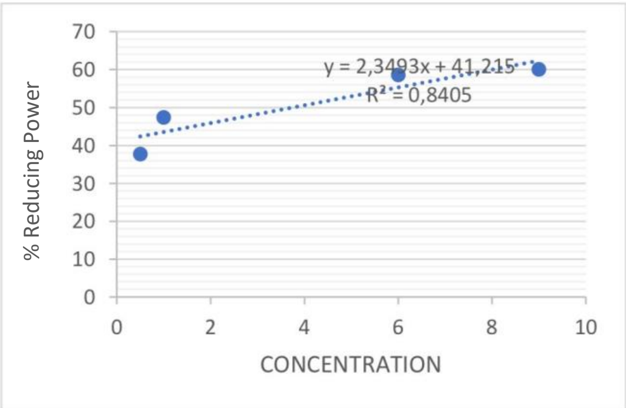

Figure 4. Relationship graph of ascorbic acid concentration and % reducing power.

complex (Fe3+), which is yellow, to ferrous complex (Fe2+), which is bluish green due to electron donors from antioxidant compounds. The color change that occurs is then measured using a UV-Vis spectrophotometer with a wavelength of 720 nm to obtain the absorbance value.16 The absorbance value obtained is then entered into the formula with the absorbance of the ascorbic acid standard at the maximum concentration tested (6 ppm), which is 1.112, to obtain the % reducing power (%RP) [19].

Table 4 is the absorbance value and % reducing the power of leilem leaf ethanol extract, and Table 5 is the absorbance value and % reducing the power of ascorbic acid as control. Based on the measurement results for both leilem leaf ethanol extract and ascorbic acid, it can be seen that the greater the concentration of the test solution, the more the absorbance value increases and the more the % reducing power increases.

The linear regression equation is obtained by plotting the concentration of the test solution with the % reducing power, where concentration is the abscissa and % reducing power is the ordinate. Figure 3 is a linear regression equation of leilem leaf ethanol extract. The equation obtained is y = 0.0771x + 42.897, with a correlation coefficient value of 0.9622. Figure 4 is the linear regression equation of ascorbic acid as a control.

In the linear regression equation y = ax + b, y is the % antioxidant activity, a is the slope constant, b is the intercept constant, and x is the concentration of the test solution. The IC_{50} value of ethanol extract of leilem leaves is obtained through a linear regression equation, with the y variable being 50 and the x variable being the IC_{50} value, which is interpreted as the amount of solution concentration that can reduce 50% of Fe^{3+} ions to Fe^{2+}. The determination of the IC_{50} value is based on the concentration of solution that can reduce 50% of Fe^{3+} ions to Fe^{2+}. If the IC_{50} value is less than 50 ppm, it indicates a very strong antioxidant, IC_{50} 50-100 ppm (strong antioxidant), IC_{50} 100-150 ppm (moderate antioxidant), IC_{50} 150-200 ppm (weak antioxidant), and IC_{50} more than 200 ppm indicates a very weak antioxidant [19].

Based on the results of these calculations, the IC_{50} value of ethanol extract of leilem leaves is 92.127, which is included in the strong category, and the IC_{50} value of ascorbic acid is 4.614 ppm, which is included in the very strong category.

3.5. DPPH and FRAP methods

Based on the results, both with DPPH and FRAP methods, the antioxidant activity of ethanol extracts of leilem leaves is included in the strong category. The results of this study are in line with several studies that have been conducted previously that there is an antioxidant activity in the leilem leaf extract. The research conducted by Maesaroh et al. shows that the DPPH method is the most effective and efficient compared to the FRAP and FIC methods, but the DPPH and FRAP methods have a very high correlation (R>0.98). This shows that free radical silencing and reduction of iron ion compounds are closely related, thus increasing the possibility that these two methods can influence each other and even replace each other [26].

However, the results of the antioxidant activity of ethanol extracts of leilem leaves can be influenced by several factors, such as the flavonoid content contained in the extract is still in an impure state; it is recommended that fractionation be carried out in the hope of obtaining the IC_{50} value of specific compounds such as flavonoids that have antioxidant activity [27]. The viscous extract is not used directly; it is recommended that it be used directly for better results. In this study, the thick extract was not used directly; it is recommended to be used directly for better results. In addition, the study used the indoor wind to dry the leaves, so the time required was very long; it is recommended to use an oven. This study also used an oven to evaporate the solvent, so it is recommended to use a rotary evaporator for a shorter time.

4. Conclusions

Based on the research that has been done, ethanol extract of leilem leaves contains active compounds such as phenols, flavonoids, saponins, tannins, steroids/triterpenoids, and alkaloids that can act as antioxidants and are classified as "strong" antioxidant activity, both using the DPPH and FRAP methods as indicated by the IC_{50} values of 78.799 ppm and 92.127 ppm, respectively. However, further research is needed, such as fractionation, to obtain the IC_{50} value of specific compounds with stronger antioxidant activity.

Author Contributions: Conceptualization, A.P.M., B.J.K., and F.D.H.B.; methodology, A.P.M.; software, F.F.; validation, F.F., A.E.M., and W.B.; formal analysis, A.P.M., B.J.K., and F.F; investigation, A.P.M.; resources, F.F.; data curation, A.P.M.; writing—original draft preparation, A.P.M., B.J.K., and F.D.H.B.; writing—review and editing, A.P.M., B.J.K., and F.D.H.B; visualization, A.E.M.; supervision, F.F.; project administration, F.F. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Ethical Clearance: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: The data used in this study are available upon request from the corresponding author in accordance with applicable data protection and privacy regulations.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

References

1. World Health Organization. (2023). Noncommunicable Diseases, from https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases, accessed 20-7-2023.
2. Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. El, Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., Setzer, W. N., Calina, D., Cho, W. C., and Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases, *Frontiers in Physiology*, Vol. 11. doi:10.3389/fphys.2020.00694.
3. Maulana, A., Faisal, F. R., Noviandy, T. R., Rizkia, T., Idroes, G. M., Tallei, T. E., El-Shazly, M., and Idroes, R. (2023). Machine Learning Approach for Diabetes Detection Using Fine-Tuned XGBoost Algorithm, *Infolitika Journal of Data Science*, Vol. 1, No. 1, 1–7. doi:10.60084/ijds.v1i1.72.
4. Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., and D'Alessandro, A. G. (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health, *Oxygen*, Vol. 2, No. 2, 48–78. doi:10.3390/oxygen2020006.
5. Sies, H. (2020). Oxidative Stress: Concept and Some Practical Aspects, *Antioxidants*, Vol. 9, No. 9, 852. doi:10.3390/antiox9090852.
6. Rahaman, M. M., Hossain, R., Herrera-Bravo, J., Islam, M. T., Atolani, O., Adeyemi, O. S., Owolodun, O. A., Kambizi, L., Daştan, S. D., Calina, D., and Sharifi-Rad, J. (2023). Natural Antioxidants from Some Fruits, Seeds, Foods, Natural Products, and Associated Health Benefits: An Update, *Food Science & Nutrition*, Vol. 11, No. 4, 1657–1670. doi:10.1002/fsn3.3217.
7. Sari, N. W. S., Tallei, T. E., and Kolondam, B. J. (2023). Benefits of Green Tea Polyphenols for Kidney Health: A Literature Review, *Grimsa Journal of Science Engineering and Technology*, Vol. 1, No. 2, 60–70. doi:10.61975/gjset.v1i2.13.
8. Khairan, K., Arini, M., Idroes, R., Awang, K., and Jacob, C. (2023). Antibacterial Activity of n-Hexane Dragon's Blood Resin Extract (Daemonorops draco wild Blume) from Bener Meriah, Aceh Province, Indonesia, *Malacca Pharmaceutics*, Vol. 1, No. 1, 22–29. doi:10.60084/mp.v1i1.29.
9. Imelda, E., Fitria, U., Mutia, U. P., Syahrul, S., Sari, M. D., Adev, S. M., Adev, A. M., Zakiaturrahmi, Z., and Toshniwal, N. S. (2023). Hippobroma longiflora L Leaves as a Natural Inhibitor of Cataract Progression: A Comprehensive Study Integrating Ethanol Extract, HPLC, and Molecular Docking Approaches, *Grimsa Journal of Science Engineering and Technology*, Vol. 1, No. 2, 40–51. doi:10.61975/gjset.v1i2.10.

10. Ningsih, D. S., Celik, I., Abas, A. H., Bachtiar, B. M., Kemala, P., Idroes, G. M., and Maulydia, N. B. (2023). Review of the Ethno-dentistry Activities of Calotropis gigantea, *Malacca Pharmaceutics*, Vol. 1, No. 1, 8–15. doi:10.60084/mp.v1i1.31.

11. Dolongtelide, J. I., Fatimawali, F., Tallei, T. E., Suoth, E. J., Simbala, H. E. I., Antasionasti, I., and Kalalo, M. J. (2023). In Vitro Antioxidant Activity of Chrysanthemum indicum Flowers Extract and Its Fraction, *Malacca Pharmaceutics*, Vol. 1, No. 2, 43–47. doi:10.60084/mp.v1i2.26.

12. Christodoulou, M. C., Orellana Palacios, J. C., Hesami, G., Jafarzadeh, S., Lorenzo, J. M., Domínguez, R., Moreno, A., and Hadidi, M. (2022). Spectrophotometric Methods for Measurement of Antioxidant Activity in Food and Pharmaceuticals, *Antioxidants*, Vol. 11, No. 11, 2213. doi:10.3390/antiox11112213.

13. Munteanu, I. G., and Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity: A Review, *International Journal of Molecular Sciences*, Vol. 22, No. 7, 3380. doi:10.3390/ijms22073380.

14. Rachmatiah, T., Daud, J. J., and Artanti, N. (2022). Aktivitas Antioksidan, Toksisitas, Kandungan Senyawa Fenol dan Flavonoid Total dari Daun Leilem (Clerodendrum minahassae Teijsm. & Binn), *Sainstech Farma*, Vol. 15, No. 1, 35–43. doi:10.37277/sfj.v15i1.1103.

15. Pratiwi, D., and Siampa, J. P. (2023). Analisis Aktivitas Antioksidan Ekstrak dan Fraksi Daun Leilem (Clerodendrum minahassae Teijsm. & Binn.) dengan Metode DPPH (2, 2-difenil-1-pikrihidrazil), *Jurnal Lentera Farma*, Vol. 2, No. 1, 8–14.

16. Kairupan, C. F., Mantiri, F. R., and H Rumende, R. R. (2019). Phytochemical Screening and Antioxidant Activity of Ethanol Extract of Leilem (Clerodendrum minahassae Teijsm. & Binn) as an Antihyperlipidemic and Antiatherosclerotic Agent, *IOP Conference Series: Earth and Environmental Science*, Vol. 217, 012016. doi:10.1088/1755-1315/217/1/012016.

17. Shaikh, J. R., and Patil, M. (2020). Qualitative Tests for Preliminary Phytochemical Screening: An Overview, *International Journal of Chemical Studies*, Vol. 8, No. 2, 603–608. doi:10.22271/chemi.2020.v8.i2i.8834.

18. Esati, N. K., Jawa La, E. O., and Lestari, G. A. D. (2022). Uji Aktivitas Antioksidan Ekstrak Etanol Daun Rosemary (Rosemarinus officinalis L.) dengan Metode DPPH dan FRAP serta Pengaplikasiannya sebagai Zat Aktif dalam Losion, *Jurnal Sains Dan Kesehatan*, Vol. 4, No. 4, 363–369. doi:10.25026/jsk.v4i4.1129.

19. Gashahun, G. S., and Solomon, L. B. (2018). Study of the Antioxidant Activities of Avocado (Persea americana Mill.) and Three Banana (Musa paradisiac L.) varieties by FRAP and Rancimat Assays, *Anatomy Physiology & Biochemistry*, Vol. 6, 555678.

20. Rizkita, A. D., Maulana, I., and Dewi, S. A. (2023). Detection of Flavonoid Compounds of Daruju Root Extract (Acanthus ilicifolius Linn) using Thin Layer Chromatography and UV-Vis Spectrophotometry, *Jurnal Sains Dan Kesehatan*, Vol. 5, No. 1, 1–5. doi:10.25026/jsk.v5i1.1185.

21. Kepel, B. J., and Bodhi, W. (2020). Standarisasi Parameter Spesifik dan Non-Spesifik Ekstrak Rimpang Lengkuas Merah (Alpinia Purpurata K. Schum) sebagai Obat Antibakteri, *EBiomedik*, Vol. 8, No. 1.

22. Hasan, H., Ain Thomas, N., Hiola, F., Nuzul Ramadhani, F., and Ibrahim, A. S. (2022). Skrining Fitokimia dan Uji Aktivitas Antioksidan Kulit Batang Matoa (Pometia pinnata) Dengan Metode 1,1-Diphenyl-2 picrylhidrazyl (DPPH), *Indonesian Journal of Pharmaceutical Education*, Vol. 2, No. 1, 67–73. doi:10.37311/ijpe.v2i1.10995.

23. Hernández-Rodríguez, P., Baquero, L. P., and Larrota, H. R. (2019). Flavonoids: Potential Therapeutic Agents by Their Antioxidant Capacity, *Bioactive Compounds*, Elsevier, 265–288. doi:10.1016/B978-0-12-814774-0.00014-1.

24. Kumaradewi, D. A. P., Subaidah, W. A., Andayani, Y., and Al-Mokaram, A. (2021). Phytochemical Screening and Activity Test of Antioxidant Ethanol Extract of Buni Leaves (Antidesma bunius L. Spreng) Using DPPH Method, *Jurnal Penelitian Pendidikan IPA*, Vol. 7, No. 2, 275–280. doi:10.29303/jppipa.v7i2.675.

25. Budaraga, I. K., and Putra, D. P. (2021). Analysis Antioxidant IC50 Liquid Smoke of Cocoa Skin with Several Purification Methods, *IOP Conference Series: Earth and Environmental Science*, Vol. 757, No. 1, 012053. doi:10.1088/1755-1315/757/1/012053.

26. Maesaroh, K., Kurnia, D., and Al Anshori, J. (2018). Perbandingan Metode Uji Aktivitas Antioksidan DPPH, FRAP dan FIC Terhadap Asam Askorbat, Asam Galat dan Kuersetin, *Chimica et Natura Acta*, Vol. 6, No. 2, 93. doi:10.24198/cna.v6.n2.19049.

27. Hasanuddin, A. R. P. (2023). Analisis Kadar Antioksidan Pada Ekstrak Daun Binahong Hijau (Anredera cordifolia (Ten.) Steenis), *Bioma: Jurnal Biologi Makassar*, Vol. 8, No. 2, 66–74.