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Identification of Antioxidant Activity of *Bridelia Micrantha* Bark Using the DPPH Method

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Abstract

This research aims to identify the antioxidant activity of *Bridelia micrantha* stem bark using the DPPH method. This research is a laboratory experimental research. Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Melandean Bark Extract is made using the maceration method with 80% methanol solvent. Identification of antioxidant activity was carried out at concentrations of 20 ppm, 40ppm, 60ppm, 80ppm, and 100ppm. Vitamin C was used as a positive control. Absorbance measurements were carried out using a UV-Vis spectrophotometer at a wavelength of 517nm. The test results in this research showed that the IC50 value of melandean bark extract was 85.54 ppm. Melandean bark extract is classified as a strong antioxidant.

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INTRODUCTION

Antioxidants are compounds that help protect the body's cells from damage caused by free radicals. Free radicals are molecules that can cause damage to the body's cells, which in turn can lead to various health problems, including heart disease, cancer, and premature aging. Antioxidants work by capturing free radicals and stopping the chain reactions that can damage cells (Antolovich et al., 2002; Ikhrar et al., 2019; Shahidi & Zhong, 2015). Antioxidant compounds can be found in a variety of foods, such as fruits, vegetables, grains, nuts, and plants that grow in specific regions and conditions (Gülçin, 2012). Some common examples of antioxidants include vitamin C, vitamin E, beta-carotene, selenium, and flavonoids. Consuming antioxidant-rich foods can help maintain overall body health and reduce the risk of chronic diseases (Kumar et al., 2021; Michalak, 2022; Sen & Chakraborty, 2011).

Oxidative stress occurs when there is an imbalance between free radicals and antioxidants in the body. Free radicals are highly reactive molecules that can cause damage to cells, proteins, and DNA. Antioxidants help neutralize these free radicals, thereby reducing oxidative stress and the risk of cell damage (Chatterjee, 2016; Hussain et al., 2016; McGarry et al., 2018; Sen & Chakraborty, 2011). Oxidative stress is associated with the development of various chronic diseases, including heart disease, cancer, diabetes, and neurodegenerative disorders such as Alzheimer's and Parkinson's. By combating oxidative stress, antioxidants can help reduce the risk of these conditions and contribute to overall health and longevity. Antioxidants are needed to support the immune system by protecting immune cells from oxidative damage. This can help enhance the body's ability to fight infections and diseases (Hajian, 2014; Li et al., 2020; Reda et al., 2020; Wu & Meydani, 1999).

(Dubois-Deruy et al., 2020; Pignatelli et al., 2018) reported that oxidative stress plays a role in the development of cardiovascular diseases such as atherosclerosis, hypertension, and stroke. Antioxidants can help prevent oxidative damage to blood vessels, reduce inflammation, and improve overall cardiovascular function. Overall, antioxidants are crucial for maintaining health and well-being by protecting cells and tissues from oxidative damage, reducing the risk of chronic diseases, and supporting various bodily functions. Incorporating antioxidant-rich foods into your diet and, in some cases, taking antioxidant supplements can help ensure an adequate intake and maximize their health benefits (Hussain et al., 2016).

Exploration of plants containing high levels of antioxidants is important to continue. The emergence of various diseases resulting from weakened immunity underscores the importance of antioxidant-rich medicinal plants. A weakened immune system can increase the risk of various diseases and conditions. When the immune system is compromised, the body is more susceptible to bacterial, viral, fungal, and parasitic infections. Common infections such as colds, flu, Respiratory tract infections, skin infections, and other infections may occur more frequently (Hajian, 2014; Li et al., 2020). An overly active or imbalanced immune system can cause attacks on the body's own tissues and organs. This can lead to autoimmune diseases such as lupus, rheumatoid arthritis, and autoimmune thyroid disease. Diseases like COVID-19 are also reported to be caused by immune system disturbances within the body (Gracia-Ramos et al., 2021). Indonesia, rich in diverse medicinal plants, has great potential in exploring plants rich in antioxidants. One plant that has been rarely studied is the melandean. Melandean, scientifically known as Bridellia Micrantha, is utilized by the people of Lombok as a medicinal plant with various benefits. One of the perceived benefits by the people of Lombok is its ability to treat symptoms of lymphoma.

Cancer is a disease that arises from weakened immunity. A weakened immune system can also increase the risk of cancer. A healthy immune system helps the body recognize and destroy cancer cells. However, if the immune system is disrupted, the risk of cancer can increase. (Adika et al., 2012; Asumang et al., 2021; Bayani et al., 2023) reported that Bridelia Micrantha contains high levels of flavonoid compounds. Flavonoids are a group of compounds that have been shown to have high antioxidant activity (Kevin et al., 2023). Empirically, melandean is one of the plants found in certain areas of West Nusa Tenggara, especially East Lombok. Melandean is commonly used by local residents as a traditional medicine for various ailments, with the bark of the melandean tree being a frequently used part. The dried bark of the melandean tree is made into a drink by local residents. Based on interviews conducted with a resident who underwent therapy using melandean bark, it was found that a child, after undergoing a biomedical lab check in 2022, had a positive result for lymph node glands measuring 0.8 cm x 2cm. After undergoing therapy by consuming melandean bark, subsequent lab checks showed that the symptoms disappeared completely. Currently, a pharmacy student at a campus in Lombok who experienced swollen lymph nodes on her shoulder is undergoing therapy by consuming melandean bark. Based on interviews with residents who use therapy from melandean bark extract, it is found that melandean bark has the potential as an anti-cancer agent.

Cancer is one of the non-communicable diseases that is a global health burden. Cancer is characterized by the presence of abnormal cells that can grow uncontrollably and have the ability to invade and spread between cells and tissues of the body. The World Health Organization (WHO) refers to cancer as one of the leading causes of death worldwide. Data from the Global Burden of Cancer (GLOBOCAN) released by the WHO stated that the number of cancer cases and deaths up to 2018 amounted to 18.1 million cases and 9.6 million deaths in 2018. Cancer deaths are estimated to continue to increase to more than 13.1 million in 2030 (Ministry of Health, 2019). One common type of cancer is lymphoma.

Lymphoma is a general term for various types of blood cancers that arise in the lymphatic system, causing enlargement of the lymph nodes. Lymphoma is caused by B or T lymphocyte cells, which are white blood cells that normally help maintain our body's immunity to fight bacterial, fungal, parasitic, and viral infections, becoming abnormal by dividing faster than normal cells or living longer than usual. The lymphatic system itself is a network of vessels with valves and glands in specific locations that circulate lymph fluid through nearby muscle contractions to glands. Lymph nodes filter foreign substances from lymph fluid and also transport fat absorbed from the small intestine to the liver. Lymphoma is one of the top ten most common cancer diseases in the world in 2012. Deaths from Non-Hodgkin's Lymphoma and Hodgkin's Lymphoma are quite high, reaching half of the percentage of new cases (Infodatin, 2015). Cancer is caused by free radical compounds that are uncontrollable within the body's cells (Hidayati et al., 2020).

Increased free radicals can cause oxidative damage from the cellular tissue level to the body's organs. Therefore, antioxidant compounds are needed to stabilize free radicals (Amiani et al., 2022). Radical compounds in the body are highly reactive to cells, which can cause various diseases. Compounds capable of neutralizing free radicals are called antioxidants (Hidayati et al., 2020). In research, an Antioxidant is any substance that, when in low concentration compared to the oxidized substrate, can significantly delay or inhibit the oxidation of the substrate (Andriani and Murtisiwi, 2020). Antioxidants are chemical substances naturally present in the human body that can donate hydrogen atoms to free radicals, thereby stopping chain reactions and converting free radicals into stable forms (Kamoda et al, 2021). Characterizing compounds with antioxidant activity is very interesting because of their ability to reduce the production of reactive oxygen species and, therefore, prevent several age-related diseases (Tore, 2019). Antioxidants are compounds that donate electrons (reducers) that can neutralize free radicals by sacrificing themselves to oxidized, stabilizing free radical atoms or molecules (Andry et al., 2022). Based on the above description, exploration of local Lombok Bridelia Micrantha is important to test its antioxidant activity. Analysis of the Identification of Antioxidant Compounds in Melandean Bark Extract (Bridelia Micrantha) is important to test the benefits of melandean bark that has been widely used by residents.

METHOD

This type of research is a quantitative study. The quantitative research method is a way used to address research problems related to numerical data and statistical programs. This research is a laboratory experimental study in identifying the antioxidant activity of Melandean Bark (Bridelia Micrantha) using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The sample used is dried Melandean bark. The samples were obtained from Gunung Sepang village, Selong District, East Lombok Regency. The independent variable in this study is the extract of Melandean bark (Bridellia Micrantha). The dependent variable in this study is the IC50 value, which indicates the antioxidant ability to inhibit free radicals. The research process can be seen in Figure 1.

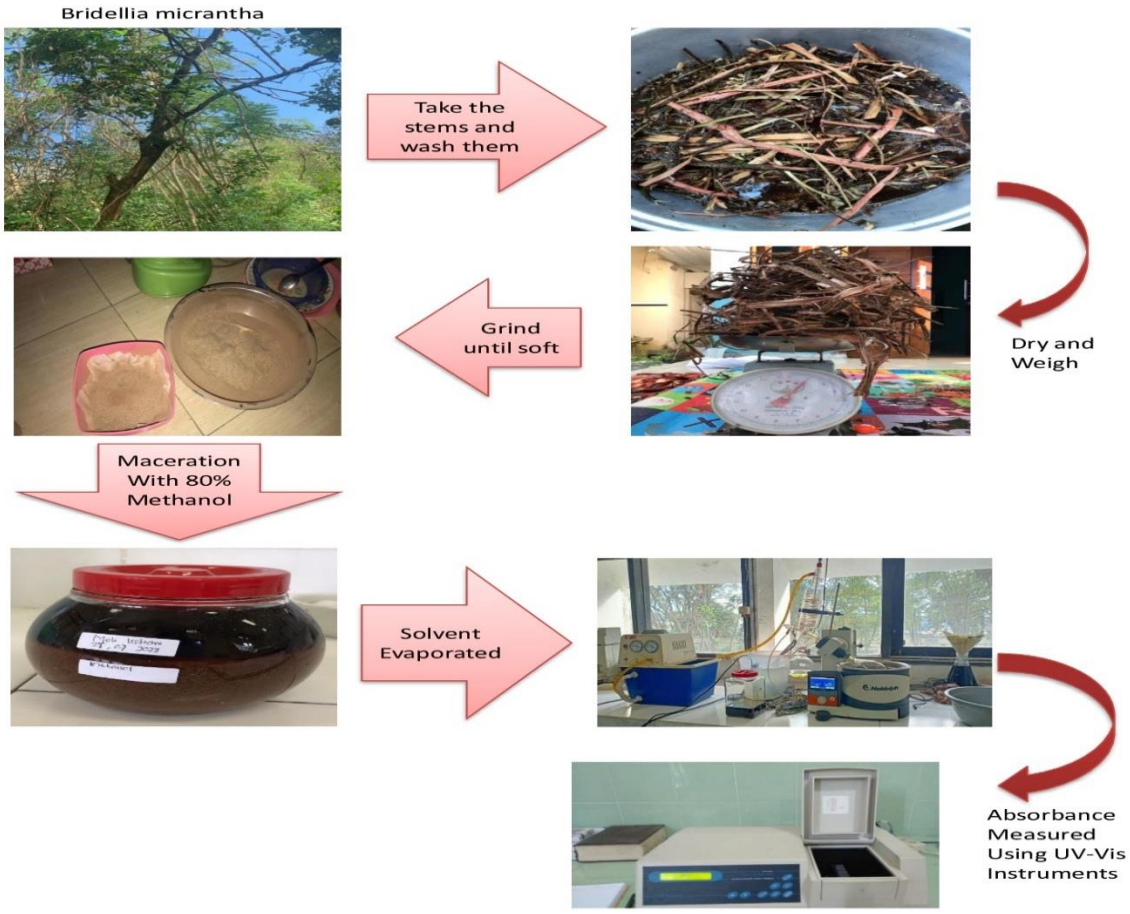

Figure 1. Process of Antioxidant Activity Identification of Bridellia Micrantha

Research Instruments

Equipment

The tools used in the research are UV-Vis Spectrophotometry (Model UV-200-RS), micropipettes (socorex), blender (Philips), stopwatch (timer joyko), Rotary evaporator (Heidolph), analytical balance (A&D model HR-200, Japan), laboratory glassware (iwaki pirex), aluminum foil, cuvettes (Model UV-200-RS), and filter paper (Whatman).

Materials

The materials used in this research include Melandean Bark, 80% Methanol, Vitamin C, and DPPH compound (2,2-diphenyl-1-picrylhydrazyl).

Data Collection Method

Preparation of Simplisia and Melandean Bark Powder

Melandean Bark weighing 1,500g was cleaned from adhering dirt, then washed with running water until clean, drained, sorted, then weighed and recorded as wet weight. Next, it was dried by airing in open air protected from direct sunlight. Melandean Bark was considered dry when it was brittle when broken, after which it weighed 1,200g. It was then powdered using a blender, and the weight of the simplified powder was recorded. The powdered simplisia was stored in a tightly closed container protected from sunlight.

Preparation of Melandean Bark Extract

The extraction of Melandean bark was done by the maceration method using 80% methanol solvent. The extraction procedure involved macerating 200g of simplified powder with 80% methanol at a ratio of 1:5, placed in a closed container, and left at room temperature for 3 days protected from light while frequently stirred. After 3 days, the maceration result was filtered and squeezed. The filtrate was combined and then concentrated using a rotary evaporator until a thick extract was obtained. Evaporation was carried out at a temperature of 50°C (Andry, et al., 2017).

Identification of Antioxidant Activity using DPPH Method

1. Preparation and measurement of standard curve (blank)

2 mL of 40 ppm DPPH solution was pipetted into a reaction tube and added with 1 mL of 80% methanol then shaken until homogenous, incubated for approximately 30 minutes, then the absorbance value was measured at a wavelength range of 400 nm-800 nm. The result obtained will give the maximum wavelength of 517 nm. (Indrawati, 2022)

2. Preparation and measurement of vitamin C reference solution

The preparation of a 1000 ppm vitamin C reference solution involved weighing 100 mg and dissolving it in 80% ethanol up to 100 mL. Dilution of the vitamin C solution from the stock solution was made to obtain concentrations of 10, 20, 30, 40, and 50 ppm. Each solution was pipetted 1 mL, placed in reaction tubes covered with aluminum foil, and added with 4 mL of 40 ppm DPPH solution. The solution was incubated for 30 minutes, then the absorbance was measured at the maximum wavelength using UV-Vis spectrophotometry (Indrawati, 2020).

3. Identification of DPPH Control Solution and Antioxidant Activity with DPPH Method

The steps for identifying antioxidant activity are as follows:

a. Creating identification samples by diluting each to 20, 30, 40, 60, and 100 ppm.
b. 1 mL of each solution above was taken and added with 2 mL of DPPH solution. After 30 minutes, the absorbance of the solution was measured at 517 nm.
c. Ascorbic acid (vitamin C) was used as a positive control created with concentration variations of 10, 20, 30, 40, 50 ppm. Each was treated like the sample treatment (Bayani, 2016).

The free radical scavenging activity (percent inhibition) was calculated as the percentage decrease in DPPH color using the formula:

$$\%\ \text{inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \text{x}100$$

(Ikhrar et al. 2019).

Data analysis and presentation

Data obtained from identification using UV-Vis spectrophotometer consists of spectra and maximum wavelength, after which the data obtained will be analyzed. Data analysis in this study is conducted by calculating the percentage (%) of antioxidant activity obtained from the absorbance data of each concentration. Once the percentage (%) of antioxidant activity for each sample absorbance is obtained, the IC50 value is calculated using a non-linear regression equation by substituting y = ax + b representing the relationship between the log concentration and the percentage (%) of antioxidant activity (inhibition). Then, the obtained IC50 is observed, where a compound is considered highly active if the IC50 value is less than 50 ppm, active if the IC50 value ranges from 50-100 ppm, moderate if the IC50 value is 101-250 ppm, and weak if the IC50 value ranges from 250-500 ppm. Data analysis is conducted descriptively, considering the spectra and maximum wavelength obtained (Kamoda et al., 2021).

RESULTS AND DISCUSSION

Identification of Melandean Bark (Bridellia Micrantha)

The plants to be studied were first identified before being collected as samples. The identification was carried out to ascertain the accuracy of the plants to be studied, to avoid errors in collecting materials, and to prevent the possibility of mixing the plants to be studied with other plants (Klau & Hesturini, 2021). Thus, errors in collecting the materials to be studied can be avoided. The Melandean bark (Bridellia Micrantha) used for this research was identified in the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) at the University of Mataram (UNRAM).

The plant used in this study is the Melandean bark. This bark was collected in June 2023 from one of the villages in East Lombok, amounting to 1,500g. The collected bark was sorted. Sorting was done to remove soil, gravel, grass, and other parts of the plant that were not used in the study, thus reducing contaminants. It was then washed thoroughly with running water and sliced before being dried under indirect sunlight. This was done to avoid altering the chemical content of the sample (Prasetyo et al., 2021). After drying, 1,100g of dried Melandean bark was obtained, resulting in a moisture content of 15%. The drying process is useful for reducing the moisture content of the raw material, preventing fungal growth. The sample was then ground to obtain 200g of crude drug. Grinding the sample facilitates the extraction process. The extraction process aims to dissolve all substances contained in the crude drug using an appropriate solvent to obtain a concentrated extract (Prasetyo et al., 2021).

The sample was then extracted using the maceration method. The maceration method was chosen for its simple and fast processing, yet it could extract chemical compounds from the sample maximally. The main advantage of this method is that no heating is involved, thus preventing the possible decomposition of active substances in the sample due to temperature and compounds that are heat-sensitive (Sa'adah et al., 2015).

Absorbance and Percentage of Inhibition

The results of the extraction of Melandean bark were then identified for their antioxidant activity using the DPPH method. After the identification solution with various concentrations was mixed with DPPH, incubation was carried out for 30 minutes. The purpose of incubating for 30 minutes is to ensure that the reaction between the sample solution and the DPPH solution occurs perfectly before measurement.

Then, after incubation for 30 minutes, the color change in the identification solution of Melandean bark and the positive control solution, namely vitamin C, was observed. The principle of the DPPH method is that antioxidant compounds will donate their hydrogen atoms to the DPPH radical, causing DPPH to become a reduced non-radical form. DPPH in the non-radical form loses its purple color (Puspitasari, and Ningsih 2016). Then, after incubation for 30 minutes, the absorbance was measured using a spectrophotometer at a wavelength of 517 nm because DPPH gives strong absorption at that wavelength (Damanis et al., 2020).

According to Apak et al., and Malik et al., DPPH will react in two ways: hydrogen atom donation mechanism and electron donation mechanism, where the radical DPPH will take hydrogen atoms from the antioxidant compounds to obtain electron pairs (Aryanti et al., 2021).

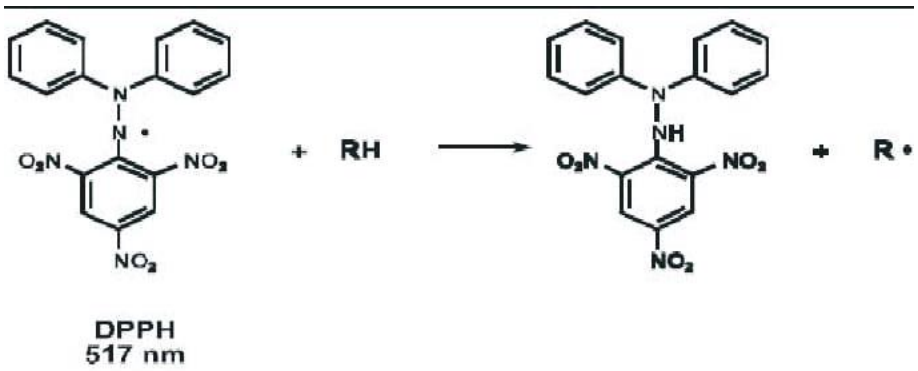

Figure 2. DPPH Working Mechanism

After measuring the wavelength using a spectrophotometer, the absorbance of each identification solution will be obtained. Then, from the known absorbance, calculations are performed to determine the percentage of inhibition.

Table 1. Absorbance, percentage of inhibition, and IC50 Value of Melandean Bark

Sample Code	C mg/L	Abs sample	Abs DPPH	Inhibition%	IC-50 (mg/L)
M1	20.08	1.210	1.429	15.33	
M2	40.15	1.077	1.429	24.63	
M3	60.23	0.911	1.429	36.25	85.54
M4	80.30	0.758	1.429	46.96	
M5	100.38	0.594	1.429	58.43	

Table 2. Absorbance, percent inhibition, and IC50 value of vitamin C

No.	Sample Code	C mg/L	Abs sample	Abs DPPH	Inhibition %	IC-50 (mg/L)
1	C1	10,00	0,799	0,879	9,10	
2	C2	20,00	0,662	0,879	24,69	
3	C3	30,00	0,491	0,879	44,14	35,22
4	C4	40,00	0,357	0,879	59,39	
5	C5	50,00	0,253	0,879	71,22	

Tables 1 and 2 show that the higher the concentration of Melandean bark solution or vitamin C, the lower the absorbance of the solution. Meanwhile, as the concentration of the solution increases, the percentage of inhibition will also increase. This is consistent with the research conducted by Hanani et al. in (Moniung et al., 2022), which states that the percentage of inhibition against free radical activity will increase with increasing concentration. The higher the concentration of the extract, the higher the percentage of inhibition of the extract against free radical activity.

After obtaining the percentage inhibition values, the regression equation for the sample against the percentage inhibition is determined and calculated. The equation is obtained based on the concentration of the solution and the percentage of inhibition graph. The graph and regression equation Y = ax + b for Melandean bark and vitamin C can be seen in figure 3 and 4

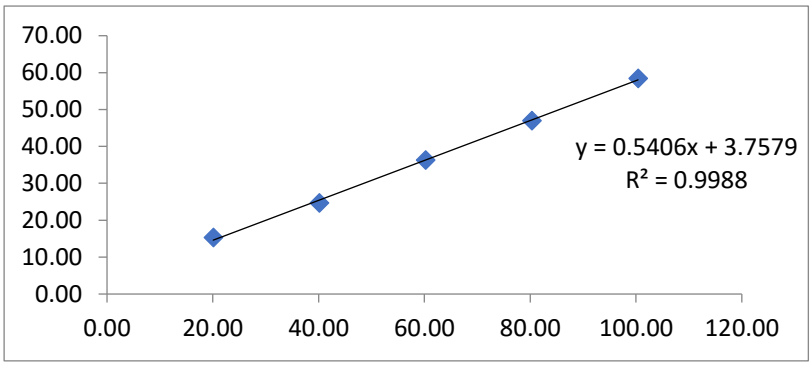

Figure 3. Linear regression equation graph of Melandean bark

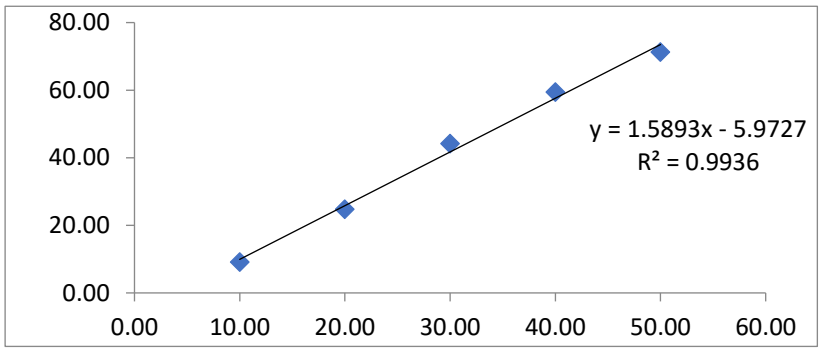

Figure 4.persamaan regreasi linier Vitamin C sebagai kontrol positif

IC50 value is determined based on the linear regression equation obtained earlier. The IC50 value is obtained from the X value in the equation Y = ax + b. Meanwhile, the Y value is the IC value that has been set at 50. The smaller the IC50 value, the greater the antioxidant activity (Widyasanti et al., 2016). Identifying antioxidant activity in a plant is crucial to determine whether the plant indeed exhibits binding activity against free radicals. The method used in antioxidant activity identification is the DPPH (1,1-diphenyl-2-picrylhydrazyl) method because it is the most effective and efficient among others (Maesaroh, et al., 2018).

Antioxidant activity measurement is conducted using UV-Vis spectrophotometry at a wavelength of 517 nm, which is the maximum wavelength for DPPH. The principle of the DPPH method is that antioxidant compounds will donate their hydrogen atoms to the DPPH radical, causing DPPH to become a reduced non-radical form. DPPH in the non-radical form loses its purple color (Puspitasari, and Ningsih 2016).Vitamin C is a natural antioxidant compound often used as a reference compound in antioxidant activity identification. This is because natural antioxidant compounds are relatively safe and do not cause toxicity. Based on research, vitamin C is more commonly used as a reference compound compared to vitamin A and vitamin E because it is cheaper and more readily available (Lung, and Destiani 2017).

The magnitude of antioxidant activity is indicated by the IC50 value, which is the concentration of the sample solution needed to inhibit 50% of the DPPH free radicals. The smaller the IC50 value in scavenging free radicals, or it can be said to have stronger antioxidant activity (Maryam 2015). A compound is considered a very strong antioxidant if the IC50 value is less than 50, strong (50-100), moderate (100-150), and weak (151-200). The smaller the IC50 value, the higher the antioxidant activity (Tristantini, 2016). Identification of antioxidant activity using the DPPH method on Melandean bark obtained an IC50 value of 85.54 ppm, indicating strong antioxidant activity. Antioxidant activity using DPPH vitamin C obtained an IC50 value of 35.22 ppm. From the results of antioxidant activity identification of Melandean bark with vitamin C, it is concluded that the antioxidant activity of vitamin C is higher than Melandean bark. This is because Vitamin C is a pure compound.

CONCLUSION

Based on the research conducted on Melandean bark (Bridelia Micrantha), it is concluded that:
1. Melandean bark has the potential as an antioxidant.
2. The antioxidant activity of Melandean bark has an IC50 value of 85.54 ppm (strong).

RECOMMENDATIONS

Further research on the antioxidant activity of Melandean bark extract with methanol solvent using other methods is needed, and research using other solvents should also be conducted.

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