TEST THE ANTIOXIDANT ACTIVITY OF YELLOW TURMERIC (Curcuma *Domestica Val.)* AND WHITE TURMERIC *(Curcuma Mangga Val*.) RHIZOME EXTRACTS USING THE DPPH *(2,2-Diphenyl-1-Picryhydrazyl)* METHOD

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A B S T R A C T

Yellow turmeric (Curcuma domestica Val.) and white turmeric (Curcuma mangga Val.) are traditional plants widely used as herbal remedies, and they contain active compounds with antioxidant properties. Antioxidants are substances that can delay or prevent oxidative reactions of free radicals in lipid oxidation. In Kaledupa Selatan District, Southeast Sulawesi, which is known for turmeric production, samples were collected from two villages: Taou and Sandi. This study aimed to determine the antioxidant activity of yellow turmeric (Curcuma domestica Val.) and white turmeric (Curcuma mangga Val.) in these villages. The antioxidant activity was tested using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method. Antioxidant activity was assessed by inhibition percentage and IC50 (Inhibition Concentration) values. The results showed that ethanol extracts of white turmeric and yellow turmeric from the two villages had the following IC50 values: 73.92 ppm for white turmeric from Taou, 57.33 ppm for yellow turmeric from Taou, 52.61 ppm for white turmeric from Sandi, 68.83 ppm for yellow turmeric from Sandi, and 3.75 ppm for vitamin C. The conclusion is that both white and yellow turmeric from Taou and Sandi exhibit strong antioxidant activity (50-100 ppm).

INTRODUCTION

Turmeric (Curcuma domestica Val) is a popular plant among the people which is often used for medicine, the most widely used of the turmeric plant is its rhizome. People often use the benefits of turmeric, such as medicine for colds, anti-inflammatories, diarrhea, shortness of breath and medicine for itching. Other benefits of turmeric include anti-immunodeficiency and anti-inflammatory (Rani & Chandrashekar, 2018).

Of the many types of medicinal plants used as traditional medicine ingredients, the most are spices. The spice plant most often used by Indonesian people comes from the turmeric plant (Zingiberaceae) which grows widely in tropical regions such as Indonesia. Some rhizomes are useful not only as medicines but also as food coloring or cosmetic ingredients. These include yellow turmeric (Curcuma domestica Val.) and white turmeric (Curcuma mangga Val.). The distinctive yellow color of yellow turmeric is caused by the curcuminoid content which is an antioxidant which can prevent free radicals from causing cell damage (Cahya & Prabowo, 2019).

There are several types of turmeric that are known, for example yellow turmeric (Curcuma domestica) and white turmeric (Curcuma mango). There are basic differences between the two types of turmeric, namely in the color and the inside of white turmeric tends to be paler. Previous research found that turmeric contains antioxidant compounds, but it is not yet known which type has a higher antioxidant content (Siregar, 2021).

Quoted from research that has been conducted (Nurtamin, 2014), it is stated that turmeric contains compounds that function as anti-inflammatory and antioxidants, antioxidant benefits: research shows that turmeric has antioxidant properties against free radicals. Another thing shows that the ethanol extract of turmeric is richer in antioxidants than the other four types of turmeric, Curcuma amada, Curcuma angustifolia, C. zodoaria, and C. aramotica (Zamzam et al., 2023).

Antioxidants are substances that can inhibit oxidation caused by free radicals in lipid oxidation. Oxidation reactions are events that break molecular chains which produce dangerous free radicals (Fitri, 2013). One method of measuring antioxidants is the DPPDH method. The DPPH method is measured based on the ability of a sample to ward off a 2,2-diphenyl-1-picrylhydrazyl compound (Amin et al., 2022).

The UV-Vis Spectrophotometry tool has the advantage of being able to identify organic and inorganic compounds, another advantage is that with simple steps UV-Vis spectrophotometry can determine quantities even in very small amounts, the data resulting from the test is expressed accurately with graphs or digital numbers (Nurwamti et al, 2024).

Turmeric plants can live up to a height of 0-240 meters above sea level. According to previous research, turmeric can also live at an altitude of 2000 meters above sea level, where it can grow anywhere. However, optimal turmeric growth is at an altitude of 45 meters above sea level (Wahyudin et al., 2023).

In South Kaledupa District, Southeast Sulawesi, it is one of the turmeric producing areas, especially in Taou Village and Sandi Village. Therefore, researchers chose and took yellow turmeric and white turmeric rhizomes as research samples with the aim of maintaining the quality of the samples and having a different location from previous studies, this could affect the content of important nutrients such as vitamins, minerals and antioxidants in samples studied.

Given these conditions, researchers were interested in conducting research to analyze the antioxidant activity of two types of turmeric rhizomes in two villages, namely Taou Village and Sandi Village, Kaledupa District using the DPPH (2,2-diphenyl-1-picryhydrazyl) method.

Research has been carried out on the rhizomes of yellow turmeric (Curcuma domestica Val.) and white turmeric (Curcuma mangga Val.). This research is experimental research carried out at the Pharmacy Laboratory of Halu Oleo University, using the extraction principle using the maceration method, namely where the filtering of active substances is carried out by soaking simplicia powder in an appropriate filter fluid. The solvent used in this research is 96% ethanol. The choice of solvent is because it is selective, non-toxic, good absorption and the solvent's high ability to separate compounds whether they are polar, semi-polar or even non-polar.

METHODOLOGY

Time and Place of Research

This research was conducted at the Pharmacy Laboratory of Halu Oleo University from 8 July to 28 July 2024.

Tools

The equipment used includes aluminum foil, sieve, stirring rod (Rofa), blender, aluminum cup, porcelain cup, funnel, desiccator, Erlenmeyer, filter paper, measuring flask, magnetic stirrer, measuring pipette, drop pipette, plastic, test tube, shaker, rotary vacuum evaporator, Ultraviolet-Visible spectrophotometry, analytical balance (Kern) and glass jar.

Material

The ingredients used in this research were distilled water (Water one), ethnaol 96%, DPPH (1,1-diphenyl-2- picrylhydrazyl), rhizomes of yellow turmeric (Curcuma domestica Val.) and white turmeric (Curcuma mangga Val.) and vitamins C.

Research Procedures

Simple setup

The sample to be used is cleaned, then cut into small pieces to facilitate the drying process. The samples were aired until completely dry. After drying, the samples were ground with a blender and then stored in a tightly closed container.

Making yellow turmeric and white turmeric extracts

A total of 300 grams of turmeric simplicia was soaked in 1500 mL of 96% ethanol for 12 hours. After soaking, the mixture was concentrated using a rotary tool (temperature 50◦C) until a thick extract was produced.

Antioxidant Activity Test

Preparation of DPPH Solution

A standard DPPH solution was made by adding 20 mg of DPPH into a volumetric flask and then dissolving it using ethanol until a concentration of 200 ppm was obtained. After the standard solution is obtained, a solution with a concentration of 40 ppm is made from the standard solution.

Preparation of Vitamin C Mother Solution

Vitamin C stock solution was made by mixing 2.5 mg of vitamin C powder and 96% ethanol to 25 mL (100 ppm).

Preparation of Ethanol Extract Solution of Yellow Turmeric and White Turmeric

A total of 25 mg of turmeric ethanol extract was mixed with 96% ethanol until the volume was 25 mL (1000 ppm).

Measurement of Absorbance of Antioxidant Activity of Vitamin C Solution

In order to achieve the concentration for the test solution, the mother liquor of vitamin C (100 ppm) is diluted into several concentration series, namely 1 ppm; 2 ppm; 4 ppm; and 8 ppm.

Measurement of Absorbance of Antioxidant Activity of Ethanol Extract of Yellow Turmeric and White Turmeric

A concentration series solution was made from 1000 ppm turmeric mother liquor by dilution process using 96% ethanol and adding 5 mL of mother DPPH at each concentration. The series solutions made were 20 ppm, 40 ppm, 60 ppm and 80 ppm.

Absorbance Measurement

The control solution, white and yellow turmeric extract solution, Vitamin C solution were stirred with a water bath shaker then left in a dark container (covered with aluminum foil) at a temperature of 37◦ for 30 minutes. This is done with the aim that the radicals contained in DPPH are not difficult to degrade by light. After that, the absorption measurement process was carried out on a UV-Vis Spectrometer with a wavelength of 517 mm. Don't forget to also calculate the percent resistance of each solution, the formula used is:

$$\% \text{ Obstacle} = \frac{(Abs\ 0 - Abs\ sam)}{Abs\ 0} \times 100\%$$

Description:

Abs 0 = the substance or sample does not absorb light at a certain wavelength

Abs sam = measure of the quantity of light absorbed by a sample

After obtaining the % resistance activity, look for the IC50 value using the linear regression equation y = ax + b

Antioxidant Data Analysis

Analyzed and calculated IC50 value based on DPPH radical antioxidant data (% inhibition) of turmeric rhizome extract. The lower the IC50 value, this indicates that the antioxidant compound is stronger. In determining the IC50 value, a linear regression equation is used. The % resistance and solution concentration data are used to find the IC50 value with the linear regression equation y = ax + b, where y is the % resistance 50 (value 50) and x is the IC50 value. The constant value a indicates the magnitude of the value of variable y if x is 0. The value b indicates the magnitude of the change in variable y if variable x changes by one unit (Edriana, 2014).

Table 1. Classification of antioxidant values (Edriana, 2014).

No	IC_{50} Value	Antioxidant Properties
1	< 50 ppm	Very strong
2	50-100 ppm	Strong
3	100-150 ppm	Currently
4	151-200 ppm	Weak

RESULTS AND DISCUSSION

In this research, the determination of antioxidants from yellow turmeric and white turmeric rhizomes was carried out using the UV-Vis spectrophotometry method as follows.

Table 2. Results of Absorbance and percent Inhibition of Vitamin C

Concentration Sample (µg/mL)	Absorbance Control (Blank)	Absorbance	% inhibition	IC_{50} (µg/mL)
1	0,6307	0,4615	26,827	3,75
2	0,6307	0,3912	37,974	
4	0,6307	0,2976	52,814	
8	0,6307	0,1108	82,432	

Testing the quality of antioxidants is carried out based on measuring the reagent solution in the form of DPPH which is reacted with the test solution with the maximum wavelength on a spectrophotometer.

In this antioxidant test, the sample absorbance and Vitamin C absorbance are measured. The results of the sample absorbance and Vitamin C absorbance are calculated by calculating the percent inhibition. Vitamin C absorbance was measured with a value of 0.9961 (Figure 1). The antioxidant value parameter is measured based on the IC50 value obtained from the linear regression equation.

The results of the calculations carried out were using IC50 for the Vitamin C value, namely 3.75 µg/mL, which is categorized as a very strong antioxidant because it is in the <50 ppm range.

Table 3. Absorbance results and percent inhibition of Taou Village White Turmeric Extract

Concentration Sample (µg/mL)	Absorbance Control (Blank)	Absorbance	% inhibition	IC_{50} (µg/mL)
20	0,6307	0,5083	19,407	73,92
40	0,6307	0,4213	33,201	
60	0,6307	0,3651	42,112	
80	0,6307	0,2969	52,925	

The results of antioxidant testing carried out on samples of white turmeric extract from Taou Village with the DPPH reactant produced an absorbance value of 0.9925 (Figure 2). The antioxidant value parameter is measured based on the IC50 value obtained from the linear regression equation. The results of calculations carried out by IC50 for the value of Taou Village white turmeric extract with the DPPH reactant, namely 73.92 µg/mL, are categorized as a strong antioxidant because it is in the range of 50-100 ppm.

Table 3. Results of Absorbance and percent Inhibition of Sandi Village White Turmeric Extract

Concentration Sample (µg/mL)	Absorbance Control (Blank)	Absorbance	% inhibition	IC_{50} (µg/mL)
20	0,6307	0,5093	19,248	52,61
40	0,6307	0,3847	39,004	
60	0,6307	0,2829	55,145	
80	0,6307	0,1466	76,756	

The results of antioxidant testing carried out on samples of white turmeric extract from Taou Village with the DPPH reactant produced an absorbance value of 0.992 (Figure 3). Where the antioxidant value parameters are determined based on the IC50 value obtained in the linear regression equation. The results of calculations carried out by IC50 for the value of Sandi Village white turmeric extract with the DPPH reaction, namely 52.61 µg/mL, are categorized as a strong antioxidant because it is in the range of 50-100 ppm.

Table 4. Absorbance results and percent inhibition of Taou Village Yellow Turmeric Extract

Concentration Sample (µg/mL)	Absorbance Control (Blank)	Absorbance	% inhibition	IC_{50} (µg/mL)
20	0,6307	0,4789	24,068	57,33
40	0,6307	0,3766	40,289	
60	0,6307	0,2986	52,656	
80	0,6307	0,2288	63,723	

The results of antioxidant testing carried out on samples of white turmeric extract from Taou Village with the DPPH reactant produced an absorbance value of 0.9972 (Figure 4). The antioxidant value parameter is measured based on the IC50 value obtained from the linear regression equation. The results of calculations carried out by IC50 for the value of Taou Village yellow turmeric extract with the DPPH reactant, namely 57.33 µg/mL, are categorized as a strong antioxidant because it is in the range of 50-100 ppm.

Table 5. Results of Absorbance and percent Inhibition of Yellow Turmeric Extract from Sandi Village

Concentration Sample (µg/mL)	Absorbance Control (Blank)	Absorbance	% inhibition	IC_{50} (µg/mL)
20	0,6307	0,5223	17,187	
40	0,6307	0,4232	32,900	
60	0,6307	0,3454	45,235	68,83
80	0,6307	0,2770	56,081	

The results of antioxidant testing carried out on samples of white turmeric extract from Taou Village with the DPPH reactant produced an absorbance value of 0.9972 (Figure 5). The antioxidant value parameter is measured based on the IC50 value obtained from the linear regression equation. The results of IC50 calculations for the value of Taou Village white turmeric extract with the DPPH reactant, namely 68.83 µg/mL, are categorized as a strong antioxidant because it is in the range of 50-100 ppm.

After calculating the inhibition. Then a linear regression was made between concentration and percent inhibition from each table above. The linear regression equation curve can be seen in the following graph.

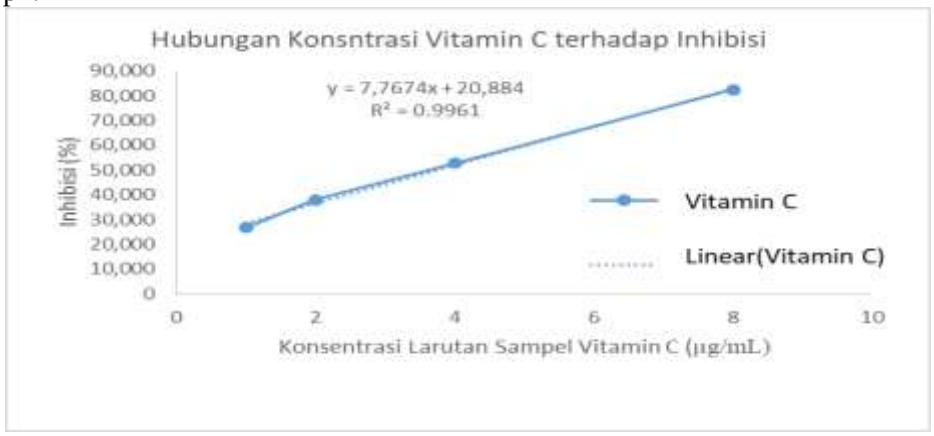

Figure 1. Inhibition curve (%) of Vitamin C

Antioxidant activity testing was then carried out on the Vitamin C solution (positive control). With concentrations of 1 ppm, 2 ppm, 4 ppm, and 8 ppm, the reaction of Vitamin C solution with additional DPPH produces an IC50 of 3.75 ppm. From the test results it can be categorized that Vitamin C has a very strong antioxidant. From the regression equation between the concentration of Vitamin C solution (x-axis) and the % inhibition of antioxidant activity (y-axis), y = 7.7674x + 20.884, with a correlation coefficient value of 0.9961. This shows that the higher the concentration of the Vitamin C solution, the more antioxidants produced.

Figure 2. Inhibition curve (%) for Taou Village White Turmeric

The results of testing the antioxidant activity of Taou Village white turmeric ethanol extract at

concentrations of 20 ppm, 40 ppm, 60 ppm and 80 ppm showed an IC50 value of 73.92 ppm. These results state that Taou Village white turmeric is a strong antioxidant because its concentration is between 50 and 100 ppm. With a correlation coefficient value of 0.9925, the regression equation for the concentration of Taou Village white turmeric extract (x-axis) and the percent inhibition of antioxidant activity (y-axis) is y = 0.5473x + 9.545. This shows that the higher the concentration of Taou Village white turmeric ethanol extract, the higher the antioxidant activity.

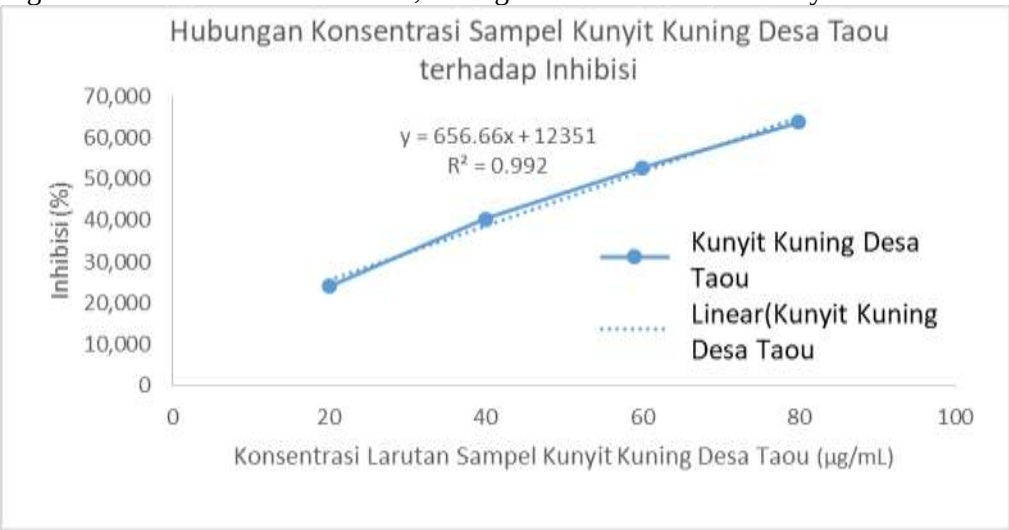

Figure 3. Inhibition curve (%) of Taou Village Yellow Turmeric

The research results found that the antioxidant activity of Taou Village yellow turmeric ethanol extract with concentrations of 20 ppm, 40 ppm, 60 ppm and 80 ppm showed an IC50 value of 57.33 ppm. These results indicate that the ethanol extract of Taou Village yellow turmeric is a strong antioxidant because it is between 50 and 100 ppm. With a correlation coefficient value of 0.992, the regression equation between the concentration of Taou Village yellow turmeric extract (x-axis) and the percent inhibition of antioxidant activity (y-axis) is y = 0.6567x + 12.351. This shows that the higher the concentration of Taou Village yellow turmeric ethanol extract, the higher the quality of its antioxidant activity.

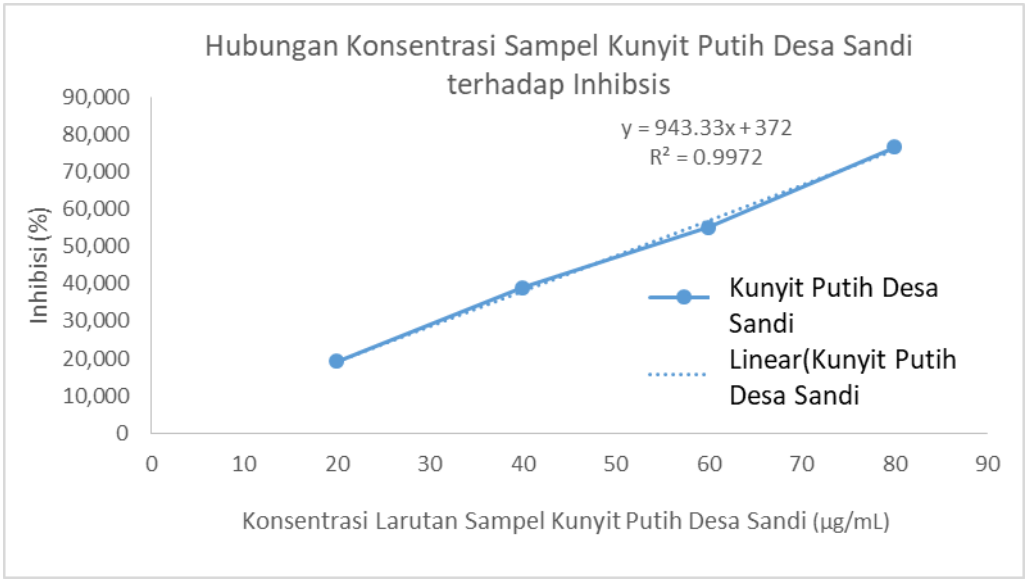

Figure 4. Inhibition Curve (%) of Sandi Village White Turmeric

The results of testing antioxidant activity on Taou Village white turmeric ethanol extract with concentrations of 20 ppm, 40 ppm, 60 ppm and 80 ppm showed an IC50 value of 73.92 ppm. These results state that Taou Village white turmeric ethanol extract is a strong antioxidant because its concentration is between 50 and 100 ppm. With a correlation coefficient value of 0.9972, the regression equation for the concentration of Sandi Village white turmeric extract (x-axis) and the

percent inhibition of antioxidant activity (y-axis) is y = 0.9433x + 0.3726. This indicates that the higher the concentration of Sandi Village white turmeric ethanol extract, the higher the quality of its antioxidative activity.

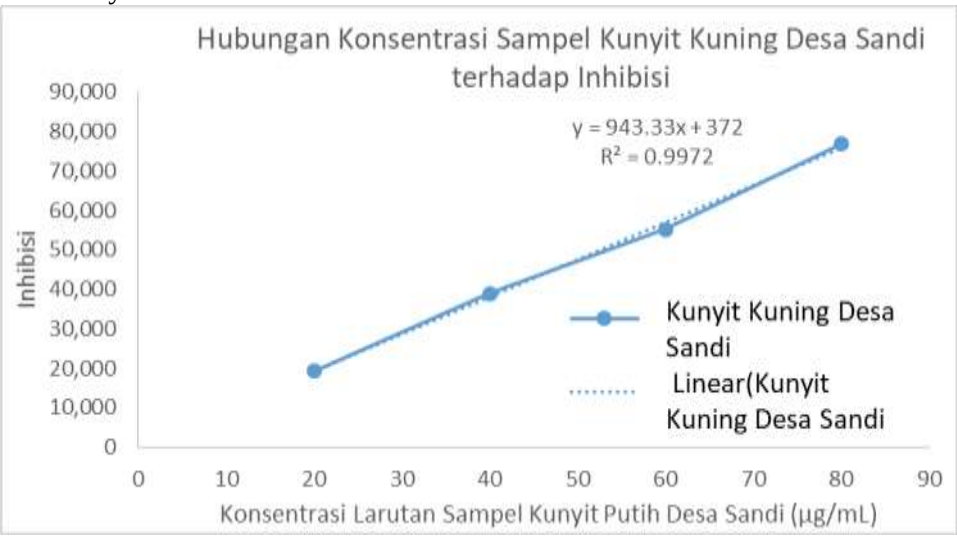

Figure 5. Inhibition curve (%) of Sandi Village Yellow Turmeric

The results of testing antioxidant activity on the ethanol extract of Sandi Village white turmeric with concentrations of 20 ppm, 40 ppm, 60 ppm and 80 ppm showed an IC50 value of 68.83 ppm. The results show that Sandi Village White Turmeric ethanol extract has relatively strong/high antioxidants because it is in the 50-100 ppm range. With a correlation coefficient value of 0.9925, the regression equation for the concentration of Taou Village white turmeric extract (x-axis) and the percent inhibition of antioxidant activity (y-axis) is y = 0.6451x + 5.597. This shows that the higher the concentration of Taou Village white turmeric ethanol extract, the better the antioxidant quality.

The antioxidant value was determined using UV-Vis spectrophotometry at a wavelength of 517 nm which is the maximum wavelength of DPPHH. DPPH (1,1-diphenyl-22 picryhydrazyl) is a purple stable free radical compound discovered in 1992 which has antioxidant properties in natural compounds such as amines, phenols, or vitamins, medicines and plant extracts which are useful for measuring. The reaction principle of this method is that DPPH is reduced through a hydrogen or electron donor process, so that the color changes from purple to yellow, the color intensity changes in proportion to the number of electron donors, and then the absorption of DPPH I will decrease. The compounds that can cause this are thought to be antioxidants or free radical scavengers. The lower the DPPH absorption, the stronger the antioxidant effect. Because vitamin C has an electron donor group, this study used vitamin C as a comparison target. This group is found in the C2 and C3 atoms. The presence of this group allows vitamin C to scavenge radicals (Kinanti ae al, 2021).

From previous research that tested the effectiveness of antioxidants in turmeric, such as that conducted by Riaminanti et al (2016). Pharmacologically, the active ingredient in turmeric, curcumin, has been widely studied as an effective anti-inflammatory, antibacterial, antioxidant and cardioprotective agent. Antioxidant properties of turmeric is widely considered to be one of the spices with the highest antioxidant activity. The antioxidant properties of turmeric can be used in a variety of applications, including in cosmetic production.

CONCLUSION

Based on the research results, it was concluded that the ethanol extract of white turmeric and yellow turmeric rhizomes is a strong antioxidant (50-100 ppm), and Taou Village has antioxidant activity with an IC50 value of 73.92 ppm. White turmeric rhizomes from Tau Village have antioxidant activity with an IC50 value of 57.33 ppm, white turmeric rhizomes from Sandy Village have antioxidant activity with an IC50 value of 52.61 ppm, and yellow turmeric rhizomes from Sandy Village have an IC50 value of 68.83 ppm.

The limitation during this research was that the research was carried out at the Halu Oleo University Laboratory, not at the Baubau Polytechnic due to limited equipment and a lack of understanding of the research material.

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