



ANTIOXIDANT POTENTIAL OF ETHANOL EXTRACT OF THE TARANTAN PLANT (*TOURNEFORTIA PUBESCENS* HOOK. F.) BY USING THE DPPH METHOD

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Abstract: This research was carried out with the aim of knowing the antioxidant activity of Tarantan (*Tournefortia pubescens*. Hook.f.) using the DPPH method. Tarantan is an endemic plant that is found in Bonelemo Village, West Bajo District, Luwu Regency, South Sulawesi Province and is used by the local community as a wound medicine. The research implementation method starts from taking and preparing samples, maceration using ethanol, phytochemical testing, flavonoid content testing, antioxidant testing and data analysis. Based on the results of research conducted, Tarantan (*Tournefortia pubescens*. Hook.f.) contains flavonoids, alkaloids, terpenoids, steroids and phenolics; The total flavonoid compound content of Tarantan (*Tournefortia pubescens*. Hook.f.) extract was 5.904 mg/g QE or 0.0005904%; The IC50 value of Tarantan (*Tournefortia pubescens*. Hook.f.) extract was 79.334 µg/mL. This value indicates that Tarantan extract (*Tournefortia pubescens*. Hook.f.) is a very strong antioxidant.

Keywords: Antioxidant; DPPH; .; ethanol; flavonoid; *Tournefortia pubescens* Hook. F.;

1. INTRODUCTION

The use of natural ingredients as traditional medicine has been carried out since time immemorial because it is believed to have lower side effects than synthetic treatments, in addition to being easy to obtain and at an affordable price. One of the plants used traditionally is the tarantan plant (*Tournefortia pubescens* Hook.f.). Tarantan leaves (*Tournefortia pubescens* Hook.f.) are widely used by the people of Bonelemo Village, West Bajo District, Luwu Regency, South Sulawesi Province.

Based on initial phytochemical tests that have been carried out, it was found that tarantan leaves (*Tournefortia pubescens* Hook.f.) contain secondary metabolite compounds such as flavonoids, saponins, steroids, tannins, alkaloids and terpenoids. This natural material containing secondary metabolite compounds is widely used in the health and pharmaceutical fields as an ingredient for making medicines. [3] suggested that medicinal plants containing flavonoids have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic and anti-cancer activities.

Apart from that, flavonoid compounds are thought to be very useful in food because they are phenolic compounds which have strong antioxidant properties and are able to ward off free radicals. Free radicals are very dangerous for the human body because they can damage cell components, tissues, and even body organs, which accelerates

the aging process and the emergence of disease. Therefore, antioxidants are needed to delay or inhibit oxidation reactions by free radicals. This antioxidant is immunomodulatory, that is, it can strengthen healthy cells to prevent cancer.

Until now, there has been no scientific research regarding the antioxidant effectiveness of the Tarantan plant (*Tournefortia pubescens* Hook.f.) using the DPPH method. It is hoped that this research will provide data on the antioxidant activity of the Tarantan Leaf plant (*Tournefortia pubescens* Hook.f.) as a basis for its development into an antioxidant product based on medicinal plants/phytopharmaceuticals.

The formulation of the problem of this research is the effectiveness of tarantan leaf extract (*Tournefortia pubescens* Hook.f.) as an antioxidant.

2. MATERIALS AND METHODS

Materials used: Tarantan plant leaves, aluminum foil, distilled water, ethanol, label paper, filter paper, plastic wrap, DPPH powder.

The research implementation method starts from taking and preparing samples, maceration using 96% ethanol, phytochemical testing, flavonoid content testing using a UV-Vis Spectrophotometer and antioxidant testing using the DPPH method.

Work procedures

1. Sample preparation

The leaves of the Tarantan plant are cleaned of adhering dirt and then washed thoroughly in running water. Next, dry it at room temperature for 7 days until the water content of the Tarantan plant leaves decreases. After that, cut it into small pieces and then mash it.

2. Maceration

150 g of Tarantan plant leaf *simplicia* was weighed and then put into a maceration container. Then add 96% ethanol until the *simplicia* is submerged and stir for 30 minutes. Next, macerate the sample for 3x24 hours. The filtrate is then concentrated to obtain a thick extract of Tarantan plant leaves.

3. Phytochemical Test

a. Flavonoids

A total of 2 mL of the extract solution was pipetted into a test tube, then heated for 5 minutes. Then add a little Mg powder and 1 mL of concentrated HCl and then homogenize. A positive test containing flavonoids is indicated by the formation of a red, yellow or orange color (Fitri, et al., 2015).

b. Alkaloids

A total of 1 mL of the extract solution was pipetted into a test tube, then added 1 mL of 2N HCl and 6 mL of distilled water then heated for 2 minutes. The filtrate was tested for the presence of alkaloid compounds using Wagner's reagent. Positive for containing alkaloids is indicated by the formation of orange, yellowish or red-brown precipitates (Jaafar, et al., 2007).

c. Terpenoid

A total of 2 mL of the extract solution was added with Libermann-Burchard reagent. A positive test for terpenoids is indicated by a color change to red or violet (Fitri, et al., 2015).

d. Steroids

A total of 5 mL of the extract sample was put into a test tube and 3 drops of concentrated H₂SO₄ were added. A positive test containing steroids is indicated by a color change to red or orange (Sukarti, 2016).

e. Phenolic

A total of 2 mL of extract was added with , where a positive reaction containing phenolic compounds was indicated by a color change to green, purple, blue or black (Jaafar, et al., 2007).

f. Saponin

A total of 2 mL of the extract solution was added with 5 drops of concentrated HCl. A positive test containing saponin is indicated by the formation of permanent foam (Jaafar, et al., 2007).

4. Determination of Total Flavonoid Content from Tarantan Leaf Extract

A total of 0.025 g or 25 mg of the ethanol extract obtained was then dissolved in a 25 mL measuring cup with 96% ethanol to the limit mark and homogenized. Pipette 0.5 mL of the solution then add 0.15 mL of 5% NaN and let sit for 6 minutes. Next, 0.15 mL of 10% AIC was added and then left for 6 minutes. React with 2 mL 4% NaOH then dilute with 2.20 mL distilled water to a volume of 5 mL and leave for 15 minutes. Next, the absorbance was measured using UV-Vis spectrophotometry.

5. Antioxidant test using the DPPH method

a. Preparation of DPPH Mother Solution

A total of 10 mg of DPPH (1,1-diphenyl-2-picrylhydrazine) was dissolved in 100 ml of methanol then homogenized until a concentration of 100 ppm was obtained.

b. DPPH Wavelength Optimization

A total of 1 ml of 100 ppm DPPH solution was put into a test tube and 5 ml of methanol was added. Homogenize then incubate in a water bath at 37o for 30 minutes. Next, measure the optimum wavelength λ , measure the absorbance at a wavelength of 510-525 nm.

c. Preparation of Blank Solution

A total of 5 ml of 100 ppm DPPH solution was put into a volumetric flask and then 25 ml of methanol was added.

d. Preparation of Tarantan plant leaf extract test solution

10 mg of Tarantan plant leaf extract was dissolved using 20 ml of ethanol to obtain a concentration of 500 ppm as a stock solution. Make standard solutions with concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm respectively. Next, the standard solution was added to each 25 ml volumetric flask and ethanol was added to the mark. 4 ml of the standard solution from the concentration series was pipetted into a test tube and 1 mL of 100 ppm DPPH was added. Homogenize the mixture then incubate at room temperature for 30 minutes so that the reaction between the sample and the DPPH solution is complete. Measure the absorbance using UV-Vis.

3. RESULTS AND DISCUSSION

Maceration

150 g of Tarantan plant leaf *simplicia* was weighed and then put into a maceration container. The extraction method used is direct maceration with 96% ethanol. Maceration was carried out for 3x24 hours with stirring once every 24 hours. Maceration is a cold extraction method by immersion using a suitable solvent to attract compounds in the sample. This method does not require heating so there is little chance of damage to a compound. Apart from being cheap and easy to do, through maceration there is a difference in

pressure inside and outside the cell so that the cell walls and membranes break and the secondary metabolite compounds in the cytoplasm are dissolved in the organic solvent. The duration of maceration can be adjusted to maximize macerate (Hartini, Nurwasliah, 2020). In the maceration process, simplicia is mixed with a solvent in a tightly closed container at room temperature. To extract compounds in the sample, the solvent is chosen based on polarity and solubility (Sauhoka, et al., 2019).

Tabel 1. Results of Simplicia Maceration of Tarantan Plant Leaves (*Tournefortia pubescens* Hook.f.)

Sample (g)	Maserasi Day to	Volum solvent (mL)	Volum filtrate (mL)	Condensed Extract (g)
	I	400	230	
57,85	II	250	207	24,58
	III	200	180	
Totally 57,85		850	617	24,58

The solvent in the maceration process uses 96% ethanol because it has a fairly high polarity so it easily dissolves the appropriate compounds. The higher the solvent polarity level, the greater the yield obtained (Agustien & Susanti, 2021). The results of maceration of the leaves of the Tarantan Plant (*Tournefortia pubescens* Hook.f.) are then filtered using filter paper with the aim of separating the filtrate and residue. The macerate obtained was 617 mL.

Tabel 2. Yield results of ethanol extract of Tarantan leaves (*Tournefortia pubescens* Hook.f.)

Sample (g)	Sample Weight (g)	Extract Results (g)	Extract Yield (%)
EEPL	57,85	24,58	42,48

a. Phytochemical Test

This phytochemical test is carried out using several reagents according to the compounds that we will identify qualitatively. The results of the phytochemical test of tarantan leaves (*Tournefortia pubescens* Hook.f.) can be seen in table 3.

Tabel 3. Phytochemical test results of tarantan leaves (*Tournefortia pubescens* Hook.f.)

No	Compound class	Positive (+) / Negative (-)
1	Flavonoids	+++
2	Alkaloids	++
3	Terpenoids	+
4	Steroids	+

5	Phenolic	+
6	Saponin	-

Source (Research data)

Berdasarkan tabel hasil uji fitokimia yang telah dilakukan diperoleh data kandungan senyawa kimia daun tarantan (*Tournefortia pubescens* Hook.f.) yaitu flavonoid, alkaloid, terpenoid, steroid, dan fenolik.

b. Analysis of flavonoid levels using UV-Vis spectrophotometry

. Maximum wavelength (λ) Quercetin

The standard quercetin solution that had been prepared with a concentration series of 1, 5, 10, 15 and 20 ppm was then measured for its maximum wavelength between 290-415 nm. The maximum wavelength obtained was 300 nm after running with UV-Vis spectrophotometry. The wavelength obtained from absorbance and the wavelength relationship can be seen in Figure 1.

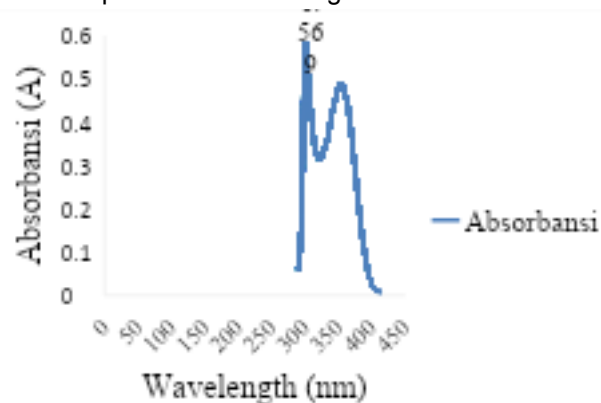


Figure 1. Absorption wavelength of quercetin

Based on the quercetin wavelength absorption image, it can be concluded qualitatively that quercetin contains aromatic compounds. It has a sharp absorption band at a wavelength of 300 nm and then forms a widening band and forms a sharp absorption again. This shows the presence of aromatic compounds such as flavonoids (Ekawati, et al., 2017).

The absorbance values obtained from concentrations of 1 ppm, 5 ppm, 10 ppm, 15 ppm, and 20 ppm at a wavelength of 300 nm created a calibration curve for the quercetin standard solution which can be seen in table 4.

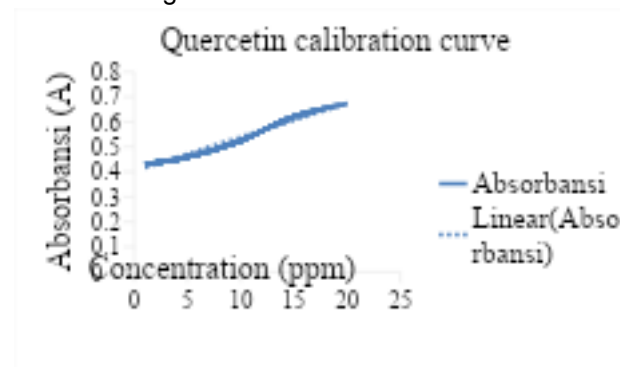
Table 4. Results of measuring the absorbance of a standard quercetin solution with a wavelength of 300 nm

No	Concentration (ppm)	Absorbance Value (A)
1	1	0,429
2	5	0,459
3	10	0,525
4	15	0,621
5	20	0,673

Source (Research data)

Based on the absorbance value data from the concentration series of 1 ppm, 5 ppm, 10 ppm,

15 ppm, and 20 ppm, a calibration curve for a standard quercetin solution was created which can be seen in Figure 2.



Gambar 2. Calibration curve of quercetin solution

The results of measuring the absorbance and total flavonoid content of tarantan leaves (*Tournefortia pubescens* Hook.f.) at a wavelength of 300 nm can be seen in table 5.

Table 5. Results of absorbance measurements and total flavonoid content of tarantan leaves (*Tournefortia pubescens* Hook.f.)

Berat ekstrak (mg)	Absorbansi (A)	Konsentrasi senyawa dalam Sampel (µg/mL)	Kadar flavonoid (mg/g QE)
25	0,483	5,904	5,904

Source (Research data, 2023)

Determination of total flavonoid content of tarantan leaf extract (*Tournefortia pubescens* Hook.f.) using a standard curve of quercetin solution was obtained from the relationship between absorbance and quercetin concentration (ppm). The linear regression equation obtained is $y = 0.0136x + 0.4027$ with a correlation coefficient value of $R^2 = 0.984$. This means that the linear equation can be used to calculate flavonoid levels because the correlation coefficient value is close to 1.

Flavonoid analysis uses a UV-Vis Spectrophotometer instrument because flavonoids contain conjugated aromatic systems which can show strong absorption bands in the UV-Vis area (Trihadi, et al., 2015). This analysis also uses a blank, namely 96% ethanol, which functions as a matrix identifier apart from the sample as an impurity. So research on tarantan leaf extract (*Tournefortia pubescens* Hook.f.) obtained a total flavonoid content of 5.904 mg/g QE or 0.0005904%.

d. Antioxidant test

Maximum Wavelength Optimization

Based on research results (Maesaroh, et al., 2018) entitled Comparison of Test Methods Antioxidant activity of DPPH, FRAP and FIC against ascorbic acid, gallic acid and quercetin. It was found that

Available online at <https://conference.lppm.unila.ac.id/index.php/icsiger>
DOI: <https://doi.org/10.23960/icsiger>

the DPPH activity test method was found to be the most effective and efficient compared to the FRAP and FIC methods. The optimum wavelength was obtained by measuring the absorbance of 100 ppm DPPH in 1 ml to which 5 mL of methanol was added. The absorbance measurement was measured at a wavelength of 512-525 nm. The optimum wavelength obtained in this study was 510 nm after being measured using UV-Vis spectrophotometry. The optimum DPPH wavelength curve is shown in figure 1.

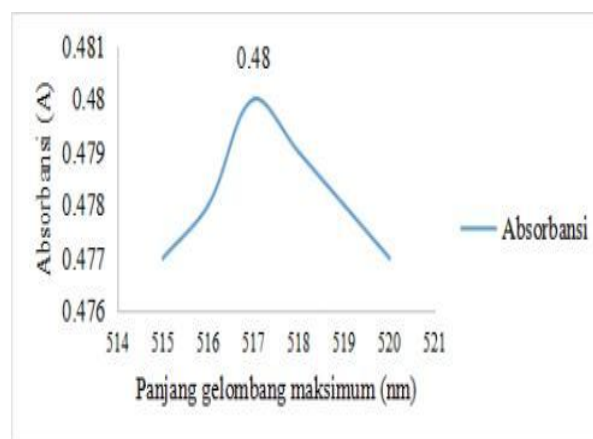


Figure 3. Results of maximum DPPH wavelength

The test solution that had been made with a concentration series of 10, 20, 30, 40 and 50 ppm had its absorbance measured at a wavelength of 510 nm. Then the test solution that had been made with a concentration series of 10, 20, 30, 40 and 50 ppm had its absorbance measured at a wavelength of 510 nm.

Table 6. Results of absorbance measurements for each sample concentration

Sample Concentration (ppm)	Absorbance
0	0,397
10	0,383
20	0,316
30	0,286
40	0,294
50	0,290

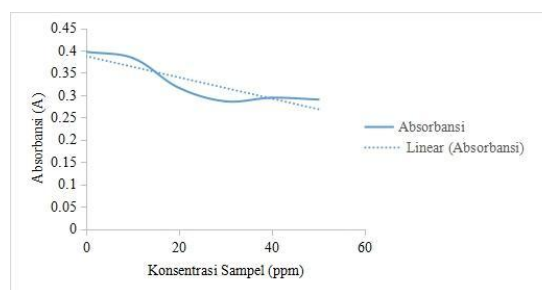


Figure 4. Relationship curve between sample concentration and absorbance

Table 7. % inhibition value for each sample concentration

Sample Concentration (ppm)	Inhibition (%)
0	0
20	3,526
20	20,403
30	27,959
40	25,944
50	26,952

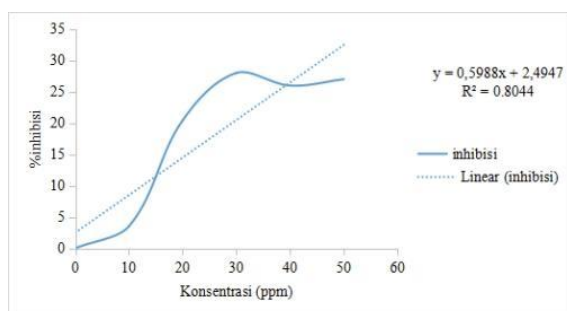


Figure 5. Relationship curve between sample concentration and % inhibition

Data from the absorbance results of each sample are used to find the % inhibition.

Sample Concentration (ppm)	Inhibition (%)
0	0
20	3,526
20	20,403
30	27,959
40	25,944
50	26,952

Based on the data obtained, the higher the sample concentration, the greater the percent inhibition. This happens because the active substance from the leaf extract of the Tarantan Plant (*Tournefortia pubescens* Hook.f.) is increasing in oxidizing free radicals (Indrawati, et al., 2022) (Kamoda, et al., 2021) also suggests that the inhibitory power against DPPH is getting higher along with the higher sample concentration..

d. Data analysis

IC50 Value Calculation

$$y = ax + b$$

Linear regression equation:

$$y = ax + b$$

$$50 = ax + b$$

$$50 = 0,5988x + 2,4947$$

$$x = \frac{50 - 2,4947}{0,5988}$$

$$x = 79,334 \mu\text{g/ml}$$

The % inhibition value is then made into a linear regression curve so that the line equation $y = ax + b$ is obtained to calculate the IC50 value. The curve of the relationship between

concentration and % inhibition can be seen in Figure 3. Determining the IC50 value is used to determine the amount of extract that can inhibit free radicals by 50% (Souhoka, et al., 2019) (Anwar & Triyasmono, 2016), stating that the smaller the IC50 value, the stronger the antioxidant activity. From the calculation results, the IC50 value of Tarantan plant leaf extract (*Tournefortia pubescens* Hook.f.) was 79.334 $\mu\text{g/mL}$. This value indicates that Tarantan leaf extract (*Tournefortia pubescens* Hook.f.) is a very strong antioxidant. This is in accordance with the antioxidant value criteria proposed by (Salim, 2018), where IC50 with a value of less than 50 is classified as very strong, IC50 with a value of 50-100 is classified as strong, IC50 with a value of 100-150 is classified as moderate and IC50 with a value of 151-200 is classified as weak..

4. CONCLUSION

Based on the research results, it is known that:

- The leaves of the Tarantan Plant (*Tournefortia pubescens* Hook.f.) contain flavonoids, alkaloids, terpenoids, steroids and phenolics
- The total flavonoid compound content of tarantan leaf extract (*Tournefortia pubescens* Hook.f.) was 5.904 mg/g QE or 0.0005904%.
- The toxicity value from the antioxidant test for the leaves of the Tarantan Plant (*Tournefortia pubescens* Hook.f.) has an IC50 value of 79.334 $\mu\text{g/mL}$. This shows that the leaves of the Tarantan Plant (*Tournefortia pubescens* Hook.f.) are a strong antioxidant.

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