

Determination of Total Flavonoid Content of Saputangan Leaves (*Maniltoa grandiflora* (A. Gray) Scheff) and Its Ability as Antioxidant

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Abstract

Determination of phenolic content using uv-visible and measured IC_{50} as antioxidant of saputangan leaves. Methods: Screening was used $FeCl_3$, NH_3 , Alkali and $Pb(CH_3COO)_2$. The maceration process was carried out using ethanol as a solvent and soaked for more than 24 hours. The measurement of total flavonoid levels began with determining the maximum wavelength of quercetin, which was 442 nm. The linear regression equation for the resulting quercetin calibration curve was the equation $y = 0.0155x + 0.0387$ with a correlation value (R^2) of 0.9943. The linear regression of antioxidant of saputangan leaves on percentage of inhibition is $y = 0.6899x + 1.5616$ with a correlation (R^2) of 0.9895. The *Maniltoa grandiflora* (A. Gray) Scheff leaves contained flavonoid compounds which are proven based on the results of screening. Meanwhile, the weight of the extract after partitioning using ethyl acetate and n-hexane was 50 gr. Total flavonoid levels in *Maniltoa grandiflora* (A. Gray) Scheff leaves were 33.87 mg QE/g extract or 33.87%. IC_{50} value of 70.2108 ppm which was categorized as an antioxidant at the medium (medium) level. Conclusions: Total flavonoid content was 3.87 mg QE/g extract or 33.87% and categorized as antioxidant on medium level.

Keywords: total flavonoid content, screening of flavonoid, antioxidant, maceration, partition

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1 Introduction

Flavonoid compounds are included in secondary metabolites that have a C₆-C₃-C₆ carbon skeleton and are widely distributed in

higher plants. Flavonoids are mostly obtained from several plant families but are known to be widespread in the Fabaceae family. Flavonoids

are usually formed in the stems, leaves and fruits of plants [1]

Saputangan leaves plant or *Maniltoa grandiflora* (A. Gray) Scheff is a plant species belonging to the Fabaceae family and the *Maniltoa* genus. This plant is usually used as an ornamental plant that can reduce pollution by absorbing pollutants such as carbon monoxide [2]

Several flavonoid compounds have antioxidant activity and are able to inhibit bacterial activity. Antioxidants from natural ingredients can protect the body from free radical attacks and can slow the occurrence of chronic diseases caused by an increase in reactive oxygen species (ROS) such as hypertension, diabetes, heart failure, stroke, atherosclerosis, and other chronic diseases. [3]

Based on the results of screening conducted by Sinurat JP (2018), it was stated that *Maniltoa grandiflora* (A. Gray) Scheff leaves contain flavonoid, phenolic and terpenoid compounds.[4] Several studies that support the research to be conducted include: Research conducted by Elin Novia Sembiring et al (2018) which determines levels of The total flavonoid in *Caesalpinia bonduc* (L.) Roxb leaves was 31.05 mgQE/g and the IC₅₀ value (Antioxidant) of total flavonoids to DPPH was 79.802 g/ml, including in the category of strong antioxidants [5].

Based on the description above, the researchers were interested in determining the total flavonoid content *Maniltoa grandiflora* (A. Gray) Scheff leaves using a UV-Visible spectrophotometer. In addition, to test the ability of flavonoid compounds as antioxidants using the DPPH method.

2 Methods

2.1 Apparatus and Reagents

Apparatus: Maserator (Schott/ Duran), Rotary Evaporator (Büchi R-114), Separation Funnel (Duran), UV-Visible Spectrophotometer (Shimadzu), Waterbath, Incubator and Micro pipette.

Reagents: *Maniltoa grandiflora* (A. Gray) Scheff leaves powder, Ethanol, Ethyl acetate, n-Hexane, Methanol, DPPH, Quercetin, Iron (III) Chloride, Ammonia, Sodium Hydroxide, Sulfuric Acid, Lead Acetate, Aluminum Chloride.

2.2 Procedures

2.2.1 Screening of Flavonoid

a. FeCl₃ 5%

Sample powder was dissolved with ethyl acetate, filtered and then the filtrate was dripped with 5% FeCl₃. Changes in the filtrate to black indicates the presence of flavonoid compounds.

b. Ammonia

Sample powder diluted with ethanol was smeared on the surface of the TLC plate. Then the surface of the TLC was evaporated using ammonia until a yellow stain appeared which indicated the presence of flavonoid compounds

c. Base Reagent

The ethanol extract was dripped with 10% NaOH to produce a thick yellow solution. Then it will become a colorless solution when the addition of dilute H₂SO₄ proves the presence of flavonoids

d. Lead Acetate

The ethanol extract was added with a few drops of lead acetate solution to form a yellow solution indicating the presence of flavonoid compounds. [6]

2.2.2 Maceration

As much as 2000 g of *Maniltoa grandiflora* (A. Gray) Scheff leaves powder was macerated for ± 24 hours with 8 liters of ethanol solvent at room temperature. Maceration was carried out repeatedly using ethanol as a solvent until the flavonoid screening gave results. The ethanol extract obtained was concentrated using a rotary evaporator at a temperature of 60°C with a rotation of 80 rpm.

2.2.3 Partition

The sample was dissolved in distilled water, then partitioned using ethyl acetate as solvent. Part of the ethyl acetate was evaporated and then dissolved in methanol. Then partitioned using n-hexane solvent and the n-hexane fraction was evaporated in a rotary evaporator at a temperature of 70°C with a rotation of 40 rpm to obtain total flavonoids. [7]

2.2.4 Determination of Total Flavonoid Content

a. Preparation of quercetin standard solution

A total of 10 mg of quercetin was weighed and dissolved in 10 ml of ethanol as the main

solution. Then, the quercetin was diluted in concentrations of 100, 80, 60, 40 and 20 ppm as a comparison solution of quercetin. Then 0.1 ml of 10% AlCl_3 , 0.1 ml of 1M CH_3COONa and 2.8 ml of distilled water were added. The solution was incubated for 30 minutes.

b. Maximum Wavelength And Quercetin Calibration Curve

One of the concentrations of quercetin solution was determined to measure absorbance at a wavelength of 400-800 nm. The wavelength that shows the high absorption value is the maximum wavelength. Each comparison solution was measured 3 times. After obtaining the absorbance of each comparison solution, a calibration curve was made to obtain a linear regression equation.

c. Measurement of Total Flavonoid Level

A total of 25 mg of the sample was weighed and dissolved in 25 ml of ethanol to obtain a concentration of 1000 mg/ml. Then 0.5 ml of the sample was taken, then 0.1 ml of 2% AlCl_3 was added, 0.1 ml of CH_3COONa 1 M. After being incubated for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer. Total flavonoid amount was determined as mg Quercetin Equivalent per g sample.

2.2.5 Antioxidant Activity

A total of 1 ml of 0.3 mM DPPH solution added 2.5 ml of flavonoid compounds with a concentration of 20 ppm, homogenized and incubated for 30 minutes in a dark room. After that, the absorbance was measured at a maximum wavelength of 516 nm. The same working procedure was carried out to test the antioxidant flavonoid compounds with concentrations of 40, 60, 80 and 100 ppm and against the blank (methanol) [8]. The concentration of the sample solution to reduce 50% DPPH was determined by the IC_{50} value which was calculated based on the percent attenuation of various concentrations using the equation linear regression[2].

3 Result and Discussion

3.1 Screening of Flavonoid

Screening of the flavonoid compounds in the leaf extract of *Maniltoa grandiflora* (A. Gray) Scheff leaves confirmed that the leaf of Saputangan leaves contained flavonoid

compounds. Flavonoid screening was tested using several chemical reagents. The screening results are shown in table 1.

Table 1 Result of Flavonoid Screening

No	Reagent used	Result	Observations
1	FeCl_3 5%	+	Black
2	NH_3	+	Yellow
3	NaOH 10% + H_2SO_4 p	+	Brown
4	$\text{Pb}(\text{CH}_3\text{COO})_2$	+	Cream

3.2 Maceration and Partition

The macerate obtained after evaporation in a rotary evaporator was 200 g. The obtained maserate is reddish brown in color. Then the maserate was dissolved in distilled water and partitioned using ethyl acetate and then partitioned using n-hexane. The extract was obtained as much as 50 g after the partition was evaporated and dried. The maceration method was chosen in extracting the sample because this method is relatively simple but very efficient in producing maserate followed by the selection of the appropriate solvent. Partition technique is very useful in carrying out the liquid-liquid extraction process so that more specific extracts of secondary metabolites are obtained.[9]

3.3 Total Flavonoid Content

3.3.1 Maximum Wavelength

The determination of the maximum absorption wavelength of quercetin was made at a concentration of 20 ppm which was carried out at 20 minutes after adding 1 M AlCl_3 , CH_3COONa reagent, then measured using UV-Vis spectrophotometry to obtain a maximum wavelength of 442 nm.[10]

3.3.2 Quercetin Calibration Curve

The standard calibration curve of quercetin was measured at concentrations of 20, 40, 60, 80, and 100 ppm at a wavelength of 442 nm. The linear regression equation obtained in determining the quercetin calibration curve is $y = 0.0155x + 0.0387$ with a correlation value (R^2) of 0.9943.

3.3.3 Total Flavonoid Content

The total flavonoid content in the of *Maniltoa grandiflora* (A. Gray) Scheff leaves extract was 33.87 mg QE/g extract or 33.87%. Data regarding sample absorbance, quercetin concentration and flavonoid content are shown in table 2.

Table 2 Total Flavonoid Content

No	Weight of Extract	Absorbance (y)	Concentration (x)	Flavonoid Content
1	0.0253	0.5680	34.1484	33.743
2	0.0249	0.5692	34.2258	34.363
3	0.0255	0.5684	34.1742	33.504
Average content				33.87 mg QE/g extract
Percentage content				33.87%

3.4 Antioxidant Activity

Total flavonoid compounds were tested for antioxidants using the DPPH method to obtain IC₅₀ values measured on a UV-Visible spectrophotometer at a maximum wavelength of 515 nm. The blank and sample concentrations were determined to obtain the absorbance shown in table 3. While the curve and linear regression equation are shown in Figure 2. The IC₅₀ value is defined as the concentration of the test compound that can reduce free radicals by 50%. The smaller the IC₅₀ value, the higher the free radical scavenging activity. The working principle of this measurement is the presence of stable free radicals, namely DPPH mixed with antioxidant compounds that have the ability to donate hydrogen, so that free radicals can be reduced. [11]

Table 3 Absorbance vs % Inhibition

No	Concentration	Absorbance	%Inhibition
1	Blank	0.802	0.000
2	20 ppm	0.650	18.953
3	40 ppm	0.569	29.052
4	60 ppm	0.490	38.903
5	80 ppm	0.332	58.604

The linear regression equation obtained based on the concentration data on percentage of inhibition is $y = 0.6899x + 1.5616$ with R² correlation of 0.9895. The linear regression equation was used to determine the IC₅₀ of total

flavonoid compounds in reducing DPPH free radicals. So that the IC₅₀ value of the total flavonoid of saputangan leaves is 70.2108 ppm, which indicates that the total flavonoid in the of *Maniltoa grandiflora* (A. Gray) Scheff leaves extract can act as an antioxidant with medium strength.

4 Conclusion

The *Maniltoa grandiflora* (A. Gray) Scheff leaves extract contained flavonoid compounds which are proven based on the results of screening using the reagents of Iron(III) chloride, Ammonia, Sodium Hydroxide and concentrated sulfuric acid and lead acetate. The results of the measurement of total flavonoid levels in saputangan leaves were 33.87 mg QE/g extract 33.87%. Meanwhile, the results of antioxidant measurements of the methanol extract of handkerchief leaves in reducing DPPH resulted in an IC₅₀ value of 70.2108 ppm which was categorized as an antioxidant at the medium (medium) level.

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