



# Determination of Total Flavonoid Level and Antioxidant Activity of Ethyl Acetate Fraction of Mangkokan Leaf Extract (*Nothopanax scutellarium* [Burm.f] Merr.)

Sri Lestari Ramadhani Nasution\*, Chrismis Novalinda Ginting, I Nyoman Ehrich Lister

Department of Public Health, Faculty of Medicine, Prima Indonesia University, Medan, Indonesia

## Abstract

**BACKGROUND:** Mangkokan leaves (*Nothopanax scutellarium* [Burm.f] Merr.) have several properties, containing several chemicals that have bioactivity. It contains flavonoids and is a natural source of antioxidants.

**AIM:** This study aims to determine the total flavonoid content and the potency of the ethyl acetate fraction in the leaves of the Mangkokan leaf as an antioxidant in counteracting free radicals.

**METHODS:** Identification of flavonoids was carried out with a reagent between magnesium powder and concentrated HCl, the orange color formed indicated the presence of flavonoids. Determination of the total flavonoid content of the ethyl acetate fraction of the Mangkokan leaf extract was carried out colorimetrically using a UV-Vis spectrophotometer. The antioxidant activity test of the ethyl acetate fraction of kukukan leaf extract was carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.

**RESULTS:** The results showed that the total flavonoid content of the ethyl acetate fraction of the Mangkokan leaf extract was 25.402749 mgGAE/g extract and the free radical inhibition test with the DPPH method showed that the ethyl acetate fraction of the Mangkokan leaf extract had antioxidant activity with an IC50 value of 103.36 ppm.

**CONCLUSION:** The ethyl acetate fraction of the Mangkokan leaf extract has activity as an antioxidant with a moderate category of antioxidant power so that it has the potential to be developed in the treatment of diseases caused by free radicals.

Edited by: Sasho Stoleski

Citation: Nasution SL, Ginting CN, Lister IN. Determination of Total Flavonoid Level and Antioxidant Activity of Ethyl Acetate Fraction of Mangkokan Leaf Extract (*Nothopanax scutellarium* [Burm.f] Merr.). Open Access Maced J Med Sci. 2022 Jan 26; 10(A):1001-1005. https://doi.org/10.3889/oamjms.2022.8131

Keywords: Antioxidants; DPPH; Ethyl Acetate; Flavonoids; Mangkokan

\*Correspondence: Sri Lestari Ramadhani Nasution, Faculty of Medicine, Prima Indonesia University, Medan, Indonesia. E-mail: srilestariinst@gmail.com

Received: 30-Nov-2021

Revised: 13-Jan-2022

Accepted: 16-Jan-2022

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

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## Introduction

The damage to cells and tissues that is at the root of most diseases is caused by highly active and harmful chemical species called free radicals (oxidants). Free radicals play an important role in the occurrence of diabetes mellitus, atherosclerosis, coronary heart disease, stroke, cancer, kidney failure, and the human aging process [1].

Indonesia is one of the countries that still relies on plants as traditional medicine. One of the plants from the Araliaceae family that has been scientifically proven related to its activity against a disease, namely, the Mangkokan leaf (*Nothopanax scutellarium* [Burm.f] Merr.). such as calcium, oxalate, peroxidase, amygdalin, phosphorus, iron, fat, protein, Vitamins A, B1, C, saponins, tannins, flavonoids, alkaloids, and terpenoids [2], [3], [4]. Meanwhile, it has been scientifically proven that the leaves of the Mangkokan have anti-fungal activity [6], antioxidant [3], antibacterial [5], and wound healing. This is because the mangrove leaves contain flavonoid compounds and other compounds that can provide pharmacological activity [2].

The types of flavonoids contained in the mangrove leaves are flavonols (quercetin, kaempferol

and myricetin) and flavones (luteolin and apigenin) which are thought to have antioxidant activity. Flavonoids are very useful and also act as antioxidants and provide protection from free radicals that can cause various degenerative diseases [6], [7].

Based on the background above, the researchers wanted to know how much total flavonoid content was carried out by colorimetry using a UV-Vis spectrophotometer and antioxidant activity of the ethyl acetate fraction of kukukan leaf extract with the DPPH method. So that the potential is a raw material for drugs for the prevention and treatment of various diseases and can be developed to the fullest.

## Research Methods

### Sample preparation

Fresh Mangkokan leaves were taken from the yard of the Medan city house as much as 10 kg. The leaves taken are dark green leaves that are still fresh. The leaves were washed and then drained and

weighed. The wet weight was obtained then the leaves were dried in a drying cabinet at a temperature of  $\pm 40^{\circ}\text{C}$  until the leaves were dry enough. The dried simplicia was blended into powder then the powder was weighed and the dry weight was obtained and then put into a closed container and stored at room temperature.

### ***Making ethanol extract of the leaves of the Mangkukan***

The leaves of the Mangkukan which became powder were macerated by means of 10 parts of simplicia into a vessel, then poured with 75 parts of the liquid filter, closed and left for 5 days, stirring frequently. After 5 days, the dregs were squeezed out and washed with a liquid filter until 100 parts were obtained. The juice was transferred to a closed vessel, left in a cool place for 2 days. Then, all the macerate was collected and evaporated with a vacuum rotary evaporator until extra viscous was obtained.

### ***Making fractions of Mangkukan leaf ethanol extract***

A total of 20 g of ethanol extract was dissolved in 96% ethanol until dissolved then added 40 mL of distilled water, put into a separating funnel, then added 100 mL of n-hexane, then shaken, and allowed to stand until there are two separate layers ( $\pm 30$  min). The n-hexane layer (top layer) was taken by flowing it and fractionation was carried out until the n-hexane layer gave a negative result with LB reagent. The collected n-hexane layer was concentrated with a rotary evaporator to obtain the n-hexane fraction. Then, 100 mL of ethylacetate is added to the residue, then shaken, allowed to stand until there are two separate layers ( $\pm 30$  min), the ethylacetate layer (upper layer) is taken by flowing, and fractionation is carried out until the ethylacetate layer gives a negative result with  $\text{FeCl}_3$  reagent. The ethylacetate layer collected was concentrated with a rotary evaporator to obtain the ethylacetate fraction. The water layer (remaining) is taken and concentrated with a rotary evaporator so that the water fraction is obtained.

### ***Qualitative test of flavonoid compounds***

The extract was pipetted 1 mL and put into a test tube. The extract was added with magnesium powder and 2–4 drops of concentrated HCl, then the mixture was shaken. The orange color formed indicates the presence of flavonoids [8].

### ***Determination of total flavonoid level***

#### ***Preparation of quercetin standard mother liquor***

Weighed 10 mg of quercetin dissolved with methanol to obtain a volume of 100 mL to obtain a solution of quercetin with a concentration of 100 ppm.

### ***Determination of quercetin maximum wavelength***

Pipette 2 mL of 100 ppm quercetin standard mother liquor, add 0.1 mL of  $\text{AlCl}_3$  and 0.1 mL of  $\text{CH}_3\text{COONa}$  and 2.8 mL of distilled water, then incubate for 40 min. The maximum wavelength was measured using a UV-Vis spectrophotometer in the range of 400–800 nm.

#### ***Creation of a quercetin calibration curve***

Pipettes from the standard solution of quercetin as much as 0.6 mL each; 1 mL; 1.45 mL; 1.95; and 2.3 mL and put into each 10 mL volumetric flask to obtain a solution with a concentration of 6 ppm; 10 ppm; 14.5 ppm; 19.5 ppm; and 23 ppm. Pipette 2 mL of each concentration and add 0.1 mL of  $\text{AlCl}_3$  and 0.1 mL of  $\text{CH}_3\text{COONa}$  and 2.8 mL of distilled water, then incubate for 40 min. The absorbance of each concentration was measured by UV-Vis spectrophotometry at a maximum wavelength of 438 nm. The quercetin calibration curve and the linear regression line equation  $y = ax + b$  are obtained.

#### ***Determination of total flavonoid extract level***

Weighed as much as 10 mg of thick extract, dissolved with 10 mL of methanol solvent to obtain a concentration of 1000 ppm. 2 mL of the solution was pipetted, added with 0.1 mL of  $\text{AlCl}_3$  and 0.1 mL of  $\text{CH}_3\text{COONa}$  and 2.8 mL of distilled water, then incubated for 40 min. The absorbance was measured by UV-Vis spectrophotometry at a maximum wavelength of 438 nm.

### ***Antioxidant activity test***

#### ***Preparation of DPPH standard mother liquor***

A total of 10 mg of DPPH was weighed, then put into a 50 mL volumetric flask, dissolved in methanol to the mark line (200 g/mL).

#### ***Preparation of blank solution***

DPPH solution (concentration 200 g/mL) was pipetted as much as 1 mL, then put into a 5 mL volumetric flask, then filled with methanol to the mark line (concentration 40 g/mL) (Molyneux, 2004).

#### ***Determination of maximum wavelength***

The maximum wavelength was determined using a DPPH solution with a concentration of 40 g/mL and the absorption was measured at a wavelength of 400–800 nm.

#### ***Preparation of sample master solution***

A total of 10 mg of extract was weighed and then each was dissolved in a 10 mL volumetric flask with

methanol, then the volume was filled with methanol to the mark line (concentration 1000 g/mL). The mother liquor sample was pipetted 0.5 mL; 1 mL; 1.5 mL; 2 mL; and 2.5 mL to obtain concentrations of 100 g/mL, 200 g/mL, 300 g/mL, 400 g/mL, and 500 g/mL, into a 5 mL volumetric flask then into each volumetric flask plus 1 mL of standard mother liquor DPPH 200 g/mL, then the volume was filled with methanol to the mark line and homogenized, allowed to stand for 30 min and then the absorption was measured using a UV-Visible spectrophotometer at the maximum absorption wavelength obtained, namely, 515.

*Determination of operating time*

A 500 ppm sample solution was made, 2 mL of DPPH solution was added and the absorbance was measured in a period of 0–60 min at a wavelength of 516 nm. f.

*Measurement of antioxidant activity*

Extract sample solutions of 100, 150, 200, 250, 300, 400, and 500 ppm were made in a 5 mL volumetric flask. In each solution, 1 mL of sample solution was pipetted and then 1 mL of DPPH solution was added, shaken until homogeneous and incubated at operating time range at room temperature and the absorbance was measured at the maximum wavelength obtained.

*Data analysis*

Percent inhibition is calculated by the following formula

$$\% \text{ inhibition} = \frac{A \text{ blank} - A \text{ sample}}{A \text{ blank}} \times 100$$

The relationship between sample concentration and % inhibition was made by linear regression equation and IC<sub>50</sub> was calculated by entering the percent inhibition value of 50 in the equation.

**Results and Discussion**

The results of the extraction of dried simplicia from the mangrove leaves obtained as much as 20 g of thick extract. The determination of the total flavonoid content was carried out using the colorimetric method with UV-Vis spectrophotometric instruments [9].

In this method, AlCl<sub>3</sub> and sodium acetate are used as reagents. The reaction between AlCl<sub>3</sub> with flavonoid group compounds forms a complex between paired hydroxyl groups and ketones or with neighboring hydroxyl groups. In addition, sodium acetate also serves to detect the 7-hydroxyl group.

**Table 1: Calculation of the regression equation of the quercetin absorption curve**

X	Y	XY	X <sup>2</sup>	Y <sup>2</sup>
0	0	0,0000	0,0000	0
6	0.393	2,358	36,0000	0.154449
10	0.429	4,29	100,0000	0.184041
14.5	0.514	7,453	210,2500	0.264196
19	0.729	13,851	361,00000	0.531441
23.5	0.816	19,176	552,2500	0.665856
X = 73	Y = 2.881	XY = 47,1280	X <sup>2</sup> = 1259.5000	Y <sup>2</sup> = 1.799983
$\bar{X} = 12.16667$	$\bar{Y} = 0.4802$			

Determination of total flavonoid content using linear regression (Table 1) equation of the standard quercetin curve with the average value of flavonoid content is 25.402749 mgEQ/g (Table 2). This result is different from research conducted by [10] which obtained flavonoid levels total 96% ethanol extract was 0.3525%. Differences in the growing environment and harvest age in this study and previous studies can be a factor that affects the presence and amount of chemical content in the mangrove leaf plant [9], [11].

**Table 2: Results of determination of total flavonoid levels in the ethyl acetate fraction of Mangkokan leaf extract**

Sample weight (g)	Sample volume (ml)	FP	Absorbance	Average absorbance	Concentration (µg/ml)	Total Flavonoid Content (mgGAE/g extract)
0.0113	10	1	0.986 1.044 1.024	1.018	28,70511	25,402749

$$a = \frac{(\sum XY) - (\sum X)(\sum Y) / n}{(\sum X^2) - (\sum X)^2 / n}$$

$$a = \frac{(47,1280) - (73)(2,8817) / 6}{(1259,5000) - (73)^2 / 6}$$

$$a = 0.03252$$

$$b = \bar{Y} - a\bar{X}$$

$$b = 0.4802 - (0.03252) (12.16667)$$

$$b = 0.0845$$

Hence, the regression equation is  $Y = 0.03252X + 0.0845$

Correlation coefficient (r)

$$r = \frac{\sum XY - (\sum X)(\sum Y) / n}{\sqrt{[(\sum X^2) - (\sum X)^2 / n][(\sum Y^2) - (\sum Y)^2 / n]}}$$

$$r = \frac{47,1280 - (73)(2,8817) / 6}{\sqrt{[(1259,5000) - (73)^2 / 6][(1,799983) - (2,881)^2 / 6]}}$$

$$r = 0.9709$$

Calculation of total phenol levels in the ethyl acetate fraction of Mangkokan leaf extract is

$$\text{Total levels of flavonoids} = \frac{x \text{FP concentration} (\infty \text{g / ml}) \times \text{Vol. sample (L)}}{\text{Berat Sample (g)}}$$

Calculation formula:

$$\text{Sample Volume} = 10 \text{ mL} = 0.010 \text{ L}$$

Sample weight = 0.0113 g  
 Dilution Factor = 1  
 Average Absorbance = 1.018  
 Regression equation:  $Y = 0.03252X + 0.0845$

$$X = \frac{1,018 - 0,0845}{0,03252} = 25.402749 \text{ g / ml}$$

$$\text{Total Flavonoid Level} = x \left| \frac{25.402749 (\text{mg / ml}) \times 0.010 \text{L}}{0.0113 \text{g}} \right.$$

Total Flavonoid Content = 25,402749 mgGAE/g extract

Based on the measurement results of the total flavonoid content of the ethyl acetate fraction of the Mangkukan leaf extract, it was 25.402749 mgGAE/g extract.

The DPPH method is a simple and inexpensive method to measure antioxidant capacity and is widely used to measure free radical inhibitory activity in natural ingredients and plant extracts [13], [14]. The principle of the DPPH method used in this study is the measurement of the absorbance of the DPPH radical which has decreased due to the presence of antioxidant compounds using a visible spectrophotometer at operating time and the maximum absorption wavelength that has been previously determined [4].

The results of the antioxidant activity test of the ethyl acetate fraction of the Mangkukan leaf extract using the DPPH method, the results are shown in Table 3. Based on the calculation of the linear equation obtained, it can be determined that the  $IC_{50}$  value of the ethyl acetate fraction of the Mangkukan leaf extract is 103.36 ppm.  $IC_{50}$  is a number that indicates the effective concentration that can inhibit the activity of an antioxidant by 50% [6].

**Table 3: Antioxidant activity and  $IC_{50}$  ethyl acetate fraction of the extract of the Mangkukan leaf extract**

Test solution concentration (ppm)	Sample absorbance average	% Inhibition	$IC_{50}$ (ppm)
50	0.839	45.60	103.36
100	0.654	57.58	
150	0.401	73.99	
200	0.351	77.23	
250	0.222	85.60	

From the results above, the  $IC_{50}$  of the ethyl acetate fraction of Mangkukan leaf extract is 103.36, it can be concluded that the antioxidant power of the ethyl acetate fraction of Mangkukan leaf extract is moderate.

In Figure 1, it can be seen that the results obtained from the concentration of the test solutions of 50, 100, 150, 200, and 250 gave different results and the  $IC_{50}$  value of the ethyl acetate fraction of Mangkukan leaf extract was 103.36 with an  $R^2$  value of 0.8489.

The antioxidant activity of the ethyl acetate fraction of the extract of the Mangkukan leaf is possible due to the presence of chemical compounds, especially flavonoids, saponins, and hexadecanoic acid. Flavonoids are the largest phenolic group of compounds

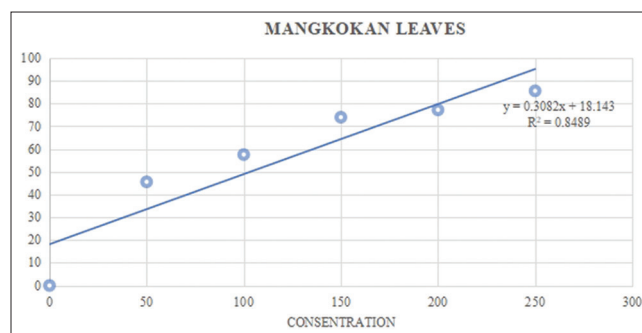


Figure 1: Curve of antioxidant activity sample and  $IC_{50}$  Ethyl acetate fraction of the Mangkukan leaf extract

found in nature and are a source of potent antioxidants because they have an ideal chemical structure that can counteract free radicals. Antioxidants play an important role in protection against oxidative cell damage that can lead to several disease conditions, such as Alzheimer's disease, cancer, heart disease, and are also associated with chronic inflammation [5].

## Conclusion

Based on the results of the research that has been carried out, the ethyl acetate fraction of the Mangkukan leaf extract contains flavonoid compounds with a total flavonoid content of 25.402749 mgGAE/g extract. This information is useful for the development of the ethyl acetate fraction of kukukan leaf extract in tracking its bioactivity based on its flavonoid content. Based on the results of data analysis, it was found that the ethyl acetate fraction of Mangkukan leaf extract has antioxidant activity potential with secondary metabolite compounds including tannins, alkaloids, terpenoids, saponins, and flavonoids. The results of the analysis of antioxidant activity which were analyzed using the DPPH method showed that the  $IC_{50}$  value of the ethanol extract of the Mangkukan leaf was 103,36 ppm.

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