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DETERMINATION OF FLAVONOID LEVELS OF Macaranga gigantea AND ITS ACTIVITY AS ANTIOXIDANTS

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Abstract: One of the ways to prevent generative diseases is by using natural antioxidant compounds. The flavonoid compounds in the flower parts of M. gigantea have a role in antioxidants. Thus the study aimed to test the antioxidant activity and flavonoid levels of M. gigantea flowers. The antioxidant test was carried out by UV-vis spectrophotometry using 2,2-diphenyl-1-picrylhydrazyl and flavonoid assay using 10% aluminum chloride and potassium acetate, both tests using quercetin as a comparison. The results of this test obtained IC50 antioxidants 145,725 ± 3.858 ppm, and flavonoids were 1,860 ± 0.007% w/w eq quercetin. The conclusion from the experiments that have been carried out is that *M. gigantea* flowers have moderate antioxidant abilities.

Keywords: *Antioxidant, Flavonoid, M. gigantea, DPPH, $AlCl_3$*

INTRODUCTION

Antioxidant compounds as a barrier to the existence of free radicals are of concern to various health workers. Unpaired electrons in unstable molecules are the forerunners of free radicals. Antioxidants are electron donor compounds that could stabilize radical free by donating a hydrogen atom capable of hindering radical free. The degenerative disease begins with oxidative stress caused by free radicals. This degenerative disease is atherosclerosis, stroke, diabetes mellitus, cancer, and aging early [1]

One of the plants as an antioxidant is Ambaratan (*Macaranga gigantea*). By empiric, *M. gigantea* is used by the Orang Rimba in Jambi, the Tunjung Dayak Tribe, and the Ngaju Dayak Tribe for drug fever, diarrhea, dysentery, medicine wounds, and medicine sprue [2]. This plant contains phytochemicals that are dominated by flavonoids [3]. Flavonoids include in nutritious polyphenyl _ for treating cancer, antidiabetic, antimicrobial, hypoallergenic, anti-inflammatory, and antioxidant [4]. So, Thing could strengthen the reason to use the plant *M. gigantea* as an ingredient in raw herbal medicine or traditional for society. Based on describing the so destination from this test for knowing the ability antioxidant flower *M. gigantea* and set flower flavonoid content by spectrophotometry.

RESEARCH METHODS

Tools and materials

The tools used that is equipment glass laboratory, TLC chamber, paper filter, UV lamp (Local), macerator balance sheet analytical (Ohauss, Pioneer), oven (Finco Inc OV 50), tweezers, capillary tube, propipet, rack tube reaction, spoon horn, spatel, UV -Vis spectrophotometry (Spectronic Genesys 10uv), vial, vortex (Lab Companion). Materials used that is flower *M. gigantea*, aquadest, $AlCl_3$ 10%, ammonia pa, potassium acetate 1 M, ethanol 96% technical, ethanol, chloroform, and methanol standard pa, DPPH reagent (Sigma), silica gel plate F254 (Merck) and standard quercetin (Sigma).

Procedure

1. Collection ingredient raw

The raw material flower *M. gigantea*, which will be used was obtained from Subdistrict Haruai Regency Tabalong, South Kalimantan, in January 2021.

2. Determination *M. gigantea* plant

Determination plant *M. gigantea* was conducted at the Regional Research and Development Agency of South Kalimantan-UPT. Banua Botanical Gardens , Banjarbaru .

3. Making powder simplicia flower *M. gigantea*

Flower sample *M. gigantea* separated from the impurity, then washed clean with running water. Next sample was dried with a warming up ray sun. Samples have been dry blended and sifted using size 14 mesh.

4. Making extract flower *M. gigantea*

100 g of simplicia powder was put into the macerator, and 96% ethanol 1:24 (w/v) was added. The extraction process using the maceration method was carried out for three days with a replacement bullet each day once in a while stirred. The maceration results were separated using filter paper. Then evaporated in a water *bath* with a temperature of 50 ^{o}C to obtain a thick extract.

5. Qualitative analysis of antioxidants with TLC

Extract thick as much as 10 mg and comparison quercetin dissolved with 50 mL methanol and ready for spotting using a TLC plate.

The mobile phase used in the study is chloroform: methanol with a ratio of 9.5:0.5. Next observed in a UV lamp. Sample for antioxidants sprayed with DPPH while the sample for flavonoids to be evaporated with the ammoniac [5], [6]

6. Test antioxidant quantitative with DPPH

Test this started with the preparation of 0.4 mM DPPH in 25 mL methanol. Determination lamda max implemented with scanning Visible wavelength [7].

a. Determination IC_{50} value solution comparison quercetin

Quercetin concentrations were 2, 3, 4, 5, and 6 ppm, with an initial stock of 1000 ppm. The sample was later added to solution DPPH 1 mL and incubated for 22 minutes, and the sample was next measured for absorbance and IC_{50} calculation.

b. Determination of IC_{50} value extract flower *M. gigantea*

The concentration of M. *gigantea* flowers used is 110, 130, 150, 170, and 190 ppm. Sample be read vortex more first and done filtering. Obtained liquid _, then 1 mL of DPPH was added and incubated for 22 minutes, and the sample next measured absorbance and IC_{50} calculation.

Determination of total flower flavonoids *M. gigantea*

a. Determination curve raw

Scanning Wavelength _ is carried out at 400-500 nm. Quercetin concentrations were used _ to make curve raw is 5,10,20,40,80,160 ppm read at max lambda obtained from scanning long wave with operating time of 38 minutes[8]

b. Determination of total flower flavonoids *M. gigantea*

Extract sample flower weighed 10 mg dissolved with 10 mL ethanol, then Added $AlCl_3$ and potassium acetate as well as aquadest 2.8 mL, next be read absorbance like curve raw [9]

7. Analysis data

Data analysis of antioxidants and flavonoids was determined using Microsoft Excel with linear regression equation formula [10]. Then ability data antioxidants determined using the following formula:

$$\%\ \text{inhibition} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \text{X}100\%$$

RESULTS AND DISCUSSION

Samples *of M. gigantea* based on results determination of SK number 050/713-LIT/KRB including family Euphorbiaceae with species *Macaranga gigantea*. Samples of flowers that have been collected and selected are dried. Drying is conducted with light sun and covered cloth black for light sun no about simplicity and circulation of the air good, so that maximize the drying process and content substance active no broken. Then sort dry and pollinated for the expanded surface to enlarge contact direct Among simplicia with solvent to optimize the extraction process. Extraction flower *M. gigantea* with maceration use ethanol 96%. Using ethanol-caused eluent, this could be an interesting compound actively contained in sample flowers. The maceration method is suitable for easy antioxidants and flavonoids damaged at a temperature tall [12]. The result extraction is thick with the color chocolate, and the yield obtained is 2.31%.

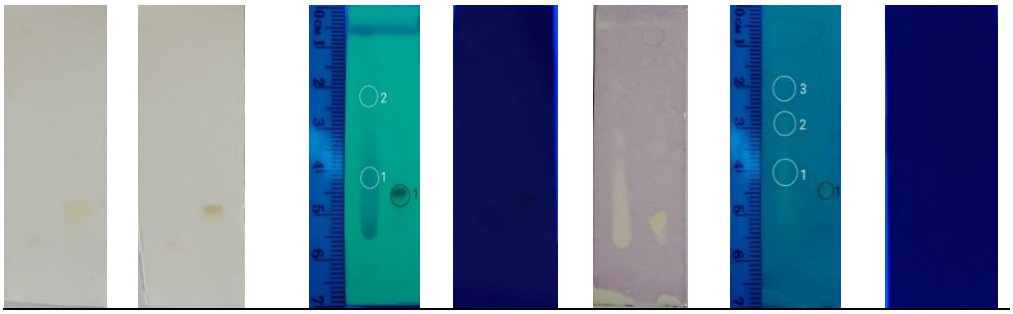
Qualitative analysis TLC aims to study the beginning content of a chemical sample on a chromatogram [13]. The result there is spotting colored expected yellow _ as flavonoid compounds. The results of flavonoid Rf (table 1) are Rf_1 : 0.28; Rf_2 :0.66; and Rf of quercetin 0.22. While the antioxidant Rf, namely Rf_1:0.30; Rf_2:0.54; Rf_3:0.68, and Rf quercetin 0.22. Rf value between flavonoids, antioxidants, and quercetin have almost the same, a difference of 0.08, concluding that stain 1 contains flavonoids and antioxidants. The result said the same with compound comparison if the difference stain distance is 0.2 [14].

Determination of antioxidants *M. gigantea* flower extract using DPPH reagent. The quantitative antioxidant activity is determined by the value of $IC_{50,}$ which is the concentration value of the extract capable of scavenging 50% free radicals. The principle of this method is the interaction between N atoms which suppress their antioxidant activity by AH molecules. Then a bond will form between free radicals and DPPH with donor hydrogen from antidote free radicals to form 1,1-diphenyl-2 picrylhydrazyl. As a result of this interaction, there will be a change in the color of the solution from purple to yellow [15]. The purpose of scanning lambda is to obtain lambda DPPH with the maximum absorbance in a certain absorption area, where absorption light will be transmitted to a sample [16]. The Results obtained were 516 nm, and according to devotional research, that maximum lamda is 516 [7].

Determination IC value $_{50}$ solution comparison quercetin and extract ethanol flower *M. gigantea* conducted with make five concentration then read at length 516 nm wave. Election quercetin is a comparison because quercetin is flavonol from a large group of flavonoids found in various plant types and has a very strong antioxidant ability [17]. IC50 Determination result quercetin and extract ethanol *M. gigantea* in table 2.

Table 1. Thin layer chromatographic analysis of extract ethanol flower *M. gigantea*

Visible	Flavonoids			Antioxidant		
	Visible	UV 254 nm	UV 366 nm	Visible	UV 254 nm	UV 366 nm



Description: sample (circle white) and comparison quercetin (circle black)

Table 2. IC_{50} Results solution comparison Quercetin and Extract skin stem *M. gigantea*

Sample	Concentration (ppm)	Percent inhibition			Average	IC_{50}±SD	% RSD
		R1	R2	R3			
Quercetin	2	22.849	21.662	15.964	20.158	5.210 ± 0.097	1.855
	3	28.131	26.944	27.478	27.517		
	4	40.950	41.009	40.772	40.910		
	5	49.496	45.875	46.706	47.359		
	6	57.626	56.439	57.685	57.250		
Extract M. gigantea flower	110	38.164	42.326	43.515	41.335	145.725 ± 3.858	2.648
	130	46.785	47.231	46.785	46.934		
	150	50.650	50.650	50.204	50.501		
	170	55.258	55.406	54.960	55.208		
	190	59.420	59.420	64.325	61.055		

Regression results in quercetin that is y = 8.9703x + 3.2166 with R-value ^{2} of 0.9858. While the results of *M. gigantea* flower extract are y = 0.2386x + 15.221, and the value of r is 0.9941. The obtained r value and RSD value following condition acceptance, where the value of r is 0.98. The RSD value requirement for a concentration of 0.1% is ≤3.7%. The IC_{50} results for quercetin were 5.210 ± 0.097 ppm (Table 2) with a very strong category and an IC_{50} yield of *M. gigantea* flower extract of 145.725 ppm with a moderate category [18].

Determination of flavonoids aims to determine the total flavonoid content of the ethanol extract of *M. gigantea* flowers by colorimetric method using AlCl3 reagent. The principle of $AlCl_3$ is to form a keto group complex in C-4 and a hydroxyl group in C-3 or C-5 from flavones and flavonols, thus forming a color complex and Wavelength undergoing a shift towards visible marked by a yellow color in the solution [8]. Quercetin serves as a standard because it has such a cluster [17].

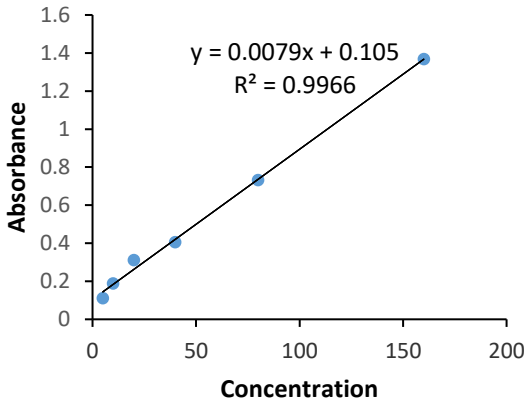


Figure 1. Standard curve flavonoid levels

The Lambda maximum earned is 427 nm and corresponds to research by Muhtadi (2014). Determination curve raw quercetin made of 6 series concentration. Results obtained, namely y = 0.0079x + 0.105 and the value of r ^{2} of 0.9966 (Figure 1), the value of r ^{2} shows a strong relationship between concentration with absorbance obtained [19-20].

Next, the determination rate of total flower flavonoids *M. gigantea*. The absorbance result sample entered in curve linear regression raw quercetin, so that will obtained total flavonoid content of the sample. The results of the determination of flavonoid levels are in Table 3.

Table 3. Determination results total extract flavonoid content skin stem *M. gigantea*

Sample	Absorbance sample	Absorbance ± SD	RSD (%)	content (%w/w EK)	content (%w/w EK) ± SD	RSD (%)	Total flavonoids (mg/g)
1	0.257	0.252		1,924	1.860		
2	0.248	±	1.818	1,810	±	3.117	25.2
3	0.251	0.004		1,848	0.058		

The determination result flower flavonoid content of *M. gigantea* obtained is 1.860 ± 0.058% w/w equivalent quercetin. So in 10 mg extract ethanol flower *M. gigantea* contains a total flavonoid of 1.860%. Whereas the value of % RSD is 3.117%, the score for this fulfills requirements is below 5% [21] (Table 3). Total flavonoids play a role in the ability of antioxidants, where the more many groups hydroxy from the flavonoid group, the more activity antioxidants will be good and effective. So that there is a correlation Between activity antioxidants and total flavonoids; namely, the effectiveness of activity antioxidants will increase along with the height of total flavonoid content.

CONCLUSIONS

Extract M. gigantea flower has the ability antioxidant in category currently with IC50 value is 145.725 ± 3.858 and the level of total flavonoid obtained from the interest rate of *M. gigantea* is 1.860 ± 0.007% w/w equivalent quercetin.

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