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The ability of *Abelmoschus manihot* L. leaf extract in scavenging of free radical DPPH and total flavonoid determination

S Sudewi[1], W A Lolo[1], M Warongan[1], Y Rifai[2], H Rante[2]

[1] Department of Pharmacy FMIPA, Universitas Sam Ratulangi Manado, Indonesia

[2] Faculty of Pharmacy, Universitas Hasanudin Makassar, Indonesia

Email: ssudewi@gmail.com

Abstract. *Abelmoschus manihot* L. has reported to have flavonoids content. This study aims were to determine the ability of *A. manihot* extract in counteracting free radical DPPH and determine the content of total flavonoids. *A. manihot* leaf was taken from 2 regions in North Sulawesi, namely Tomohon and Kotamobagu. The maceration was carried out to extract the active compound in a 96% ethanol solvent. Free radical scavenging analysis was carried out by DPPH and determination of its total flavonoid in the extract was measured using spectrophotometri method. The results showed that *A. manihot* extract from Tomohon and Kotamobagu could counteract free radical of DPPH with value of free radical activity of 88.151 and 88.801 %, respectively. *A. manihot* leaf from Kotamobagu has higher total flavonoids content 61.763 mg/g compare to Tomohon 46.679 mg/g which presented as quercetin. *A. manihot* has antioxidant activity.

Keywords: *Abelmoschus manihot* L. leaf extract, free radical DPPH, total flavonoids

1. Introduction

In North Sulawesi, *Abelmoschus manihot* L. or known by the name of the Gedi is one of the plants of the common Malvaceae tribe planted. Easily grown stem cuttings on loose soil. The society recognizes two types of *A. manihot* ie red Gedi and green Gedi. Green leaves can be utilized by the Manado people as foodstuffs known as Manado Porridge (Tinutuan), typical of Manado, whereas for traditional red Gedi can cure some diseases [1].

The method which could be used to measure antioxidant capacity is the DPPH method (1,1-diphenyl-2-picrylhydrazyl). DPPH is widely used to test an antioxidant activity as a scavenger of free radicals of food and herbal extract. DPPH can also be used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used in both solid and liquid samples and could be applied to the whole antioxidant capacity present in the sample.

It has been extensively studied about the benefits of *A. manihot* including antioxidant activity [2], analgesics [3] as well as formulations [4]. Previous study reported antioxidant activity of the *A. Manihot* leaves which found from the areas of Palu, Makassar and Gorontalo [5]. The identification of chemical compounds reported the presence of flavonoid in *A. manihot* leaves extracted with ethanol solvent [6]. The level of flavonoid content of A. Manihot leaves extracted with 96% ethanol was 41.56% [5]. Flavonoid compounds have various important functions for health, among others, in reducing the risk of cardiovascular disease, blood pressure, atherosclerosis, and as an antioxidant [7].
This study aims to determine the ability of *A. manihot* extract in counteracting free radical compounds DPPH and determine the total content of flavonoids taken from 2 regions in North Sulawesi, namely Tomohon and Kotamobagu.

2. Materials and Methods

2.1 Plant material

A. manihot leaf samples were taken from two locations in North Sulawesi: Tomohon and Kotamobagu. The sample is washed with clean running water. The next process is cut into small pieces and dried. After drying, the sample was powdered and then made stage of extract making.

2.2. Extraction of A. manihot leaves

A. manihot leaf powder was macerated using a 96% ethanol solvent of 500 mL. Remacerated was carried out with 300 mL of 96% ethanol. The macerate was then filtered. The macerat was evaporated with a rotary evaporator until a viscous extract was obtained. Further dried in the oven to obtain a dried extract.

2.3 Free radical scavenger Activity with DPPH Method

This test was conducted to determine the presence or absence of antioxidant activity as a free radical scavenger [8]. A total of 1 mL of *A. manihot* extract with concentration of 150 µg / mL was added with 2 mL of DPPH solution in 0.08 mM methanol. The mixture is then divorted and left for 30 minutes at room temperature in the dark. The absorbance was measured at a wavelength of 517 nm and methanol was used as a blank. The percentage of DPPH free radical scavenger activity is calculated according to the equation:

$$\text{Free radical scavenger activity (\%)} = \frac{\text{Abs.control} - \text{Abs.Sample}}{\text{Abs.control}} \text{ x } 100\% \quad (1)$$

2.4 Determination of total flavonoids

A sample of 0.1 mL of *A. manihot* leaf extract was put into a test tube, added 3 ml of Aluminum chloride solution then mix by vortex for 1 min. The mixture was incubated for 30 minutes. The absorbance of the solution was determined using a Spectrophotometer at a wavelength of 415 nm. The presence of flavonoids is shown by the formation of colors [9].

2.5 Characterization of functional group by FTIR

A small quantity *A. manihot* extract were mixed with KBr. The functional group were analysed by Fourier Transform Infra Red Spectrophotometer (Shimadzu) in region of 4000-400 cm^{-1}.

3. Results and Discussion

3.1 Extraction

Extraction is done to draw chemical components contained in a material. Maceration is choosen because this method is done by soaking the sample powder without heating so thermal decomposition can be avoided. The viscous extract obtained was dark green with the percentage of rendement in the Table 1.

Table 1. Rendemen of *A. manihot* extract

Name	Rendemen (%)
Tomohon	2,366
Kotamobagu	5,796

3.2 Free radical scavenging activity

The antioxidant activity in this research was evaluated using DPPH method. The DPPH was chosen because this method is a simple, easy, fast and sensitive method and requires only a small sample to know the antioxidant activity of natural compounds [10]. Before testing of the extract, determination of the maximum wavelength and operating time was carried out. The results of the determination of the maximum wavelength in this study was 517 nm and the operating time was 30 minutes for the incubation time. The results of free radical scavenging activity testing can be seen in the Table 2.

Table 2. Free Radical scavenger activity of *A. manihot* leaf of Tomohon

Concentration (µg/mL)	Absorbance			% Free radical inhibitory
	I	II	Average absorbance	
50	0.233	0.164	0.1985	81.552
100	0.206	0.142	0.174	83.829
150	0.189	0.103	0.146	86.431
200	0.173	0.109	0.141	86.896
250	0.157	0.098	0.1275	88.151

Table 3. Radical scavenging activity of *A. manihot* leaf from Kotamobagu

Consentration (µg/mL)	Absorbance			% free radical inhibitory
	I	II	Average absorbance	
50	0.238	0.253	0.2455	77.184
100	0.217	0.164	0.1905	82.296
150	0.186	0.158	0.172	84.015
200	0.162	0.171	0.1665	84.526
250	0.103	0.138	0.1205	88.801

The existence of antioxidant activity could be caused by the compound contained in plants release the H atom and then bind to the radical DPPH form a new compound which more stable was diphenyl picrilhidrazine [11].

3.3 Total flavonoids

Determination of total flavonoid from *A. manihot* leaf extract was done according to previous [9]. Before the total measurement of flavonoids in the sample, the maximum wavelength was determined first. Maximum wavelength measurement results were obtained at a wavelength of 417 nm. The operating time stability was determined and obtained at 30 minutes. The total flavonoid measurements were determined at a wavelength of 417 nm and 30 min incubation time [12].The principle of total measurement of flavonoids based on color formation with $AlCl_3$ reagents. Flavonoid has a keto group on C-4 and has a hydroxy group on C-3 or C-5 atoms which can form a color complex with $AlCl_3$

[13]. The addition of acetic acid was to maintain the complex of C-4 keto and 3 or 5 -OH remains stable. The result of determination of quercetin standard curve can be seen the Figure 1.

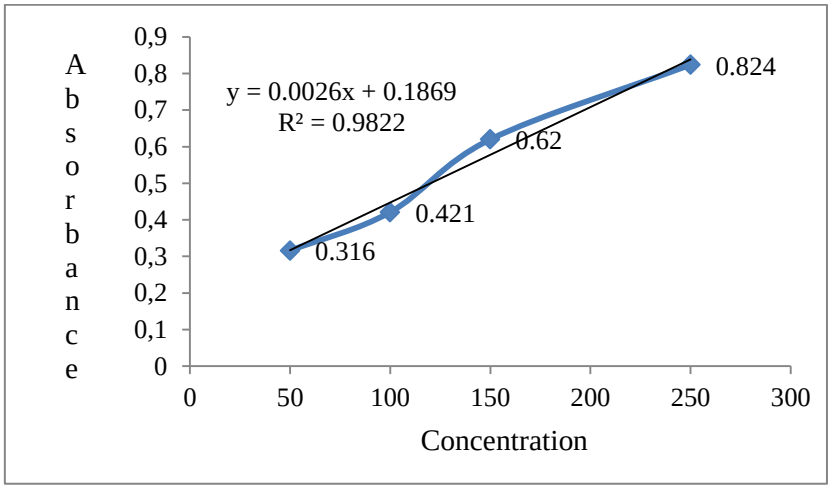

Figure 1 . Standard curve of quercetine for total flavonoid

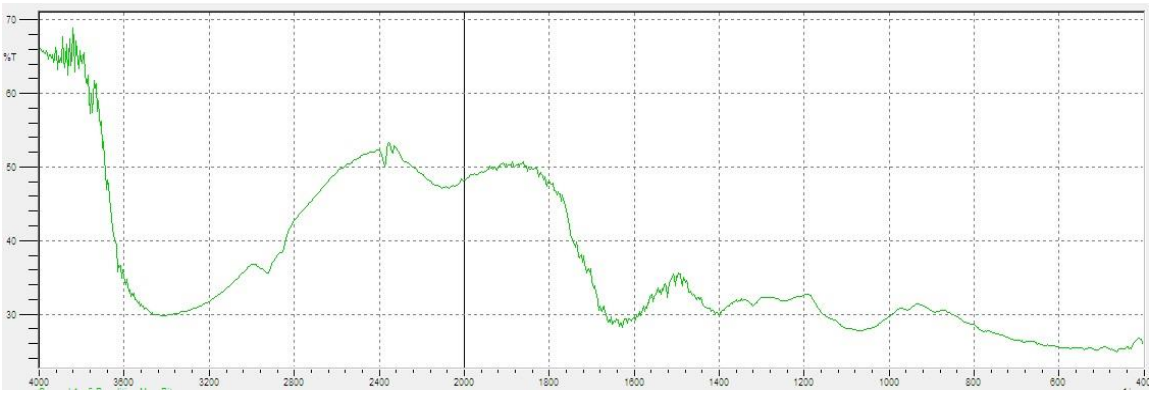

Figure 2 . FTIR spectra of *A. manihot* from Kotamobagu

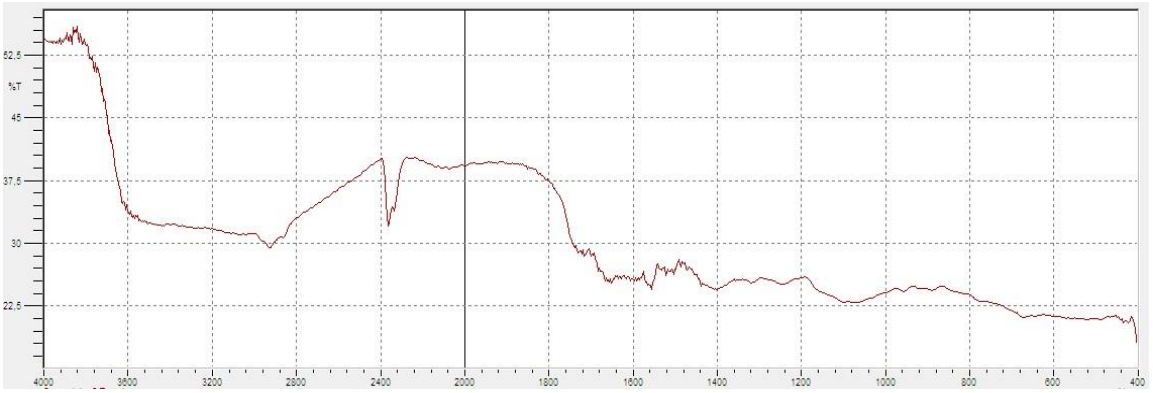

Figure 3 . FTIR spectra of *A. manihot* fromTomohon

The *A. manihot* leaves from Tomohon and Kotamobagu towns are estimated to have functional groups -OH as shown in the Figure 2 and figure 3. The absorption in wave numbers 3000-3300 cm^{-1} is typical hydroxyl group [14].

4. Conclusions

A. manihot leaf extract from Tomohon and Kotamobagu is able to scavenge of DPPH free radical.

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