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DETERMINATION OF TOTAL FLAVONOID LEVELS AND ANTIOXIDANT ACTIVITY FROM ETHANOL EXTRACTS GEDI BARK *(ABELMOSCHUS MANIHOT* L. MEDIK*)* WITH DPPH METHOD (2, 2-DIPHENYL-1-PICRYLHYDRAZYL)

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ABSTRACT

Gedi plant (Abelmoschus manihot L. Medik) is a plant from the Malvaceae tribe that grows in tropical temperatures and is widely used as a vegetable, traditional medicine, and as a complement to Manado porridge in North Sulawesi. In treating diseases such as kidney disease, lowering blood cholesterol and ulcers, the people of North Sulawesi traditionally use a decoction of gedi leaves without the addition of salt to treat them (Mamahit and Soekamto, 2010). Flavonoids are one of the largest natural phenol group compounds and are found in all green plants so they are found in every plant extract (Markham KR, 1988). Flavonoids are known to have properties as an antidote to free radicals or are antioxidants, inhibitors of hydrolysis enzymes, oxidative and work as anti-inflammatory (Pourmourad et al., 2006). This study aimed to determine the total flavonoid content and antioxidant activity of the ethanolic extract of the gedi stem bark using the DPPH method (2,2-diphenyl-1-picrylhydrazil). The study was started by collecting material from the Manoko Lembang Plantation, West Bandung Regency, then determining the moisture content and drying loss. Extraction was carried out by maceration method with 96% ethanol solvent and the yield of 17.6% was obtained. Furthermore, phytochemical screening, determination of total flavonoid levels and measurement of antioxidant activity with quercetin as a comparison. The results of the determination of total flavonoids were 1.33 mg QE/gr, while the results of antioxidant testing of the ethanol extract of gedi stem bark with UV-Vis spectrophotometry measured at a wavelength of 516 nm obtained an IC50 value of 65.71 g/mL indicating strong antioxidant activity.

KEYWORDS: Gedi bark, total flavonoids, antioxidants, DPPH.

INTRODUCTION

Gedi plants (Figure 1) are plants that contain chemical compounds, namely alkaloids, flavonoids, polyphenols, saponins, tannins and steroids that have the potential as antioxidants. Antioxidants are compound molecules that are able to remove, clean and resist the formation or combine the effects of reactive oxygen species (ROS) or free radicals (Dewi, 2016). In a chemical sense, antioxidants are compounds that donate electrons, while in a biological sense, antioxidants are compounds that can reduce free radical activity by preventing cell oxidation. In general, antioxidants are compounds that can change radical compounds into a stable form by donating one or more electrons so that the chain reaction can stop (Rochmah, 2017).

Free radicals are molecules that have unpaired electrons in their outer orbits so they are reactive and very easy to bond with other elements. The presence of excess free radicals can trigger damage to DNA, lipids, proteins and carbohydrates, causing various diseases such as diabetes mellitus, cancer and arteriosclerosis (Chen et al., 2007), besides this condition also causes body cells to degenerate, metabolic processes are disturbed. and decreased immune response, triggering various degenerative diseases. The levels of free radicals in the body can be seen from the activity of antioxidant enzymes and levels of malondialdehyde (Zakaria et al., 2000). Antioxidants are needed that can help protect the body from the effects of free radicals and reduce their negative effects (Winarsi, 2007).

The antioxidant test method used in this study is the DPPH free radical scavenging method (2,2-diphenyl-1-picrylhydrazyl). This method requires a small sample, is simple, easy, fast, and sensitive to evaluate the antioxidant activity of natural compounds (Hanani et al., 2005).

UV-Vis spectrophotometry is a method used to test the amount of light absorbed at each wavelength in the ultraviolet and visible regions. In this instrument a light

ray is split partly of light directed through a transparent cell containing a solvent (Molyneux, 2004). The basic principle is electromagnetic radiation in the ultraviolet region and visible light through compounds that have double bonds, part of the radiation by compounds. The amount of radiation absorbed depends on the wavelength of the radiation and the structure of the compound. The absorption of radiation rays is caused by the reduction in energy from the radiation beam when electrons in low energy orbitals are excited to higher energy orbitals (Silalahi Jansen, 2006).

EXPERIMENT

Materials

Erlenmeyer, analytical balance, brown bottle, knife, spatel, watch glass, porcelain cup,volumetric flask, funnel, beaker, measuring cup, drop pipette, volume pipette, oven, evaporator, bath water, test tube, moisture balance, UV –Vis spectrophotometer Ethanol 96%, $AlCl_3$, quercetin, HCL, acetic acid, DPPH, vitamin C, aluminum foil, filter paper, distilled water, H_2SO_4, Dragendroff's reagent, Mayer's reagent, Mg, NaCl 10%, $FeCl_3$.

Preparation of sample

Gedi bark was colected from the Manoko Lembang plantation, West Bandung Regency. First of all, the bark of the gedi stem is washed clean of dirt and then rinsed with running water, then peeled and cut into small pieces, in the wind and then stored in a dry place. Avoid direct sunlight, After drying, grind until smooth, then sieved using a 40 mesh.

Extraction

Five hundred gram of gadi bark powder was extracted using 96% ethanol solvent by maceration method for 3 x 24 hours protected from light while stirring occasionally, then filtered using filter paper to obtain the filtrate. Then evaporated using a Rotary Vacuum Evaporator at a temperature of 70^{o}C, obtained a thick extract and then evaporated using a water bath at a temperature of 50^{o}C to obtain a concentrated ethanol extract of gadi barks as much as 88 g with a yield of 17.6%.

Phytochemical Screening

Phytochemical screening is a preliminary stage that can provide an overview of the content of certain compounds in natural materials to be studied (Fajriyah dkk, 2018). Screening for alkaloids, flavonoids, tannins and saponins was carried out.

Total Flavonoid Content (TFC)

Total flavonoid content with quercetin as a comparison, 15 mg of extract, dissolved in 10 mL of ethanol, in order to obtain a concentration of 1500 ppm. From this solution, 1 mL of pipette was added, 1 mL of 2% AlCl3 solution and 1 mL of 120 mM potassium acetate were added. Samples were incubated for one hour at room temperature. The absorbance was determined using UV-Vis spectrophotometry at a maximum wavelength of 435,2 nm. (Stankovic, 2011). The total flavonoid content was figured as g quercetin equivalent per 100 g extract.

DPPH scavenging activity

Preparation of DPPH solution by mixing 4 mg of DPPH with 96% ethanol in a 100 mL volumetric flask to obtain a concentration of 100 ppm. Then the absorption was measured at a wavelength of 400-600 nm using UV-Vis spectrophotometry.

10 mg of ethanol extract of gedi barks was dissolved with ethanol in a 10 mL volumetric flask and then centrifuged. Various concentrations of 50 ppm, 60 ppm, 70 ppm, 80 ppm and 90 ppm were made, homogenized and incubated for 30 minutes in a dark place at 37°C. The absorbance was measured at a wavelength of 516 nm using UV-Vis spectrophotometry. The IC_{50} value is calculated using the linear regression equation formula.

RESULTS AND DISCUSSION

Plant determination was done in Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Universitas Padjadjaran Jatinangor and stated that the plants used were gadi bark (*Abelmoschus manihot* L. Medik) (Figure 1)

Characterization of simplicia exhibited that water content 2% and loss drying 11%.

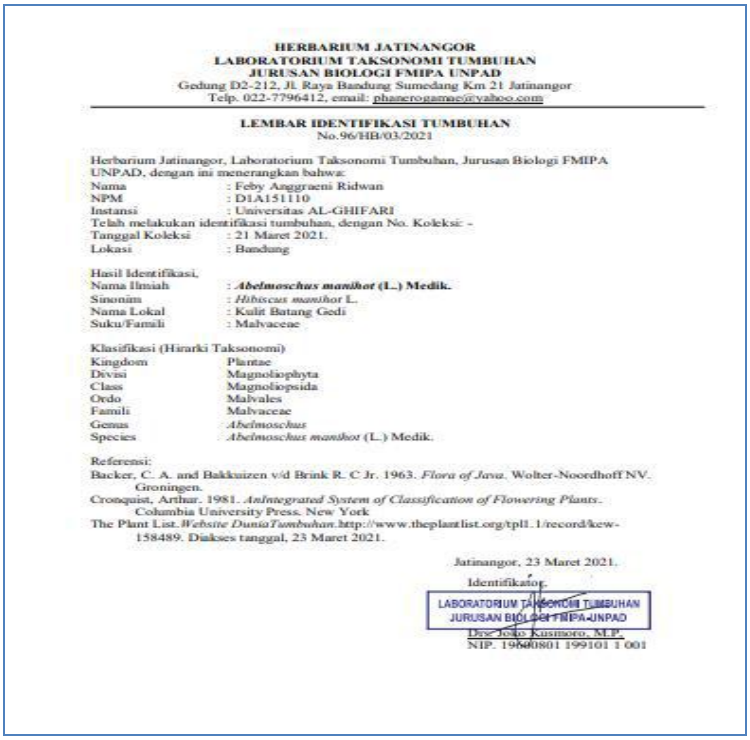
Results pumpkin seed extraction 88 g with a yield of 17, 6%.

The chemical screening was performed in extracts to find out the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins in extracts. The results of the phytochemical screening can be seen in Table 1.

The total flavonoid test results were calculated in mg QE/100g and the total flavonoid content of the ethanol extract of gedi barks was 1,33 mg QE/100g.

Measurement of antioxidants using the DPPH method was characterized by a purple to yellow color change after being incubated for 30 minutes. The DPPH method was chosen because it is simple, easy, fast and sensitive and requires a small sample to determine the antioxidant activity of natural compounds (Pratimasari, 2009). Quercetin was used as a comparison because it has very strong antioxidant properties (Karinda et al., 2013). The IC50 calculation results for quersetin is 1,205 µg/mL exhibit very strong antioxidant activity and ethanol extract of gedi barks is 65,71 µg/mL, exhibit strong antioxidant activity.

The standard linear regression curve of inhibition of antioxidant activity of quercetin and ethanol extract of gedi barks against DPPH can be seen in Figures 3 and 4.



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LEMBAR IDENTIFIKASI TUMBUHAN
No.96/HB/03/2021

Herbarium Jatinangor, Laboratorium Taksonomi Tumbuhan, Jurusan Biologi FMIPA UNPAD, dengan ini menerangkan bahwa:
Nama : Feby Anggraeni Ridwan
NPM : D1A151110
Instansi : Universitas AL-GHIFARI
Telah melakukan identifikasi tumbuhan, dengan No. Koleksi: -
Tanggal Koleksi : 21 Maret 2021.
Lokasi : Bandung

Hasil Identifikasi,
Nama Ilmiah : ***Abelmoschus manihot* (L.) Medik.**
Sinonim : *Hibiscus manihot* L.
Nama Lokal : Kulit Batang Gedi
Suku/Famili : Malvaceae

Klasifikasi (Hirarki Taksonomi)
Kingdom Plantae
Divisi Magnoliophyta
Class Magnoliopsida
Ordo Malvales
Famili Malvaceae
Genus *Abelmoschus*
Species *Abelmoschus manihot* (L.) Medik.

Referensi:
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Cronquist, Arthur. 1981. *An Integrated System of Classification of Flowering Plants*. Columbia University Press. New York
The Plant List. *Website DuniaTumbuhan*.http://www.theplantlist.org/tpl1.1/record/kew-158489. Diakses tanggal, 23 Maret 2021.

Jatinangor, 23 Maret 2021.
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Figure 1: Result of determination.

Figure 2: Gedi Plant.

Table 1: Chemical screening of extract.

Secondary metabolites	Solvent	Extract
Alkaloid	Dragendroff	+
	Mayer	+
Flavonoid	Magnesium	+
Tannin	FeCl3	+
Saponin	HCl	+

(+) = detected

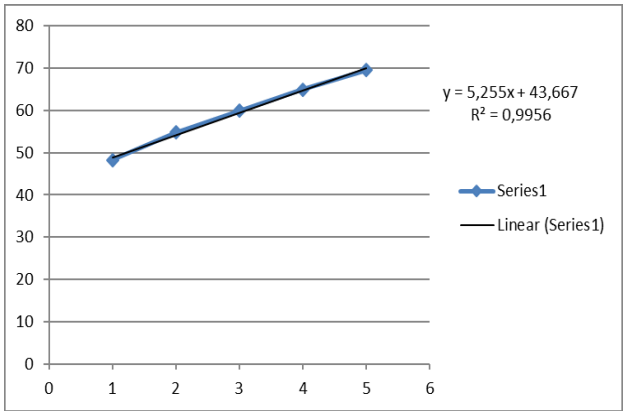

Figure 3. Standard Curve of Linear Regression % inhibition of Antioxidant Activity of Quercetin with DPPH.

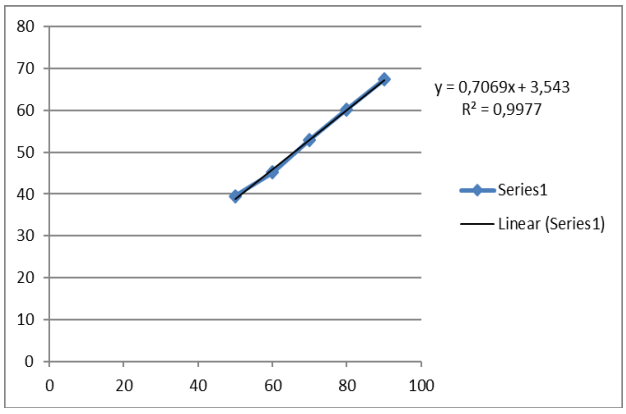

Figure 4: Standard Curve of Linear Regression % Antioxidant Activity Ethanol Extract Gedi bark with DPPH.

CONCLUSION

The total flavonoid content of the ethanol extract gedi barks (*Abelmoschus manihot* L. Medik) was 1,33 mg QE/100g and IC_{50} antioxidant activity 65,71 µg/mL, indicating a strong antioxidant activity using the DPPH method.

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