

DETERMINATION OF TOTAL FLAVONOID LEVELS AND ANTIOXIDANT ACTIVITY FROM ETHANOL EXTRACTS OF PUMPKIN SEEDS (*CUCURBITA MOSCHATA* DUCH) WITH DPPH METHOD (2,2-DIPHENYL-1-PICRYLHYDRAZYL)Slamet Tuty*¹, Desi Dwi Riani² and Sri Maryam³^{1,2,3}Department of Pharmacy Al-Ghifari University Bandung Jl. Cisaranten Kulon no.140 Soekarno-Hatta, Bandung 40293.***Corresponding Author: Slamet Tuty**

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ABSTRACT

Pumpkin is a local food that is abundantly available and contains complete nutrients, such as carbohydrates, protein, fat, minerals and vitamins, a source of dietary fiber and antioxidants. The use of pumpkin seeds as a traditional medicine has been done by Native Americans. Pumpkin seeds have been used as an anthelmintic agent and supportive treatment in functional bladder disorders. The utilization of its seeds in Indonesia is limited to the production of kuaci, pumpkin seeds (*Cucurbita moschata* Duch) contain saponins, tannins, flavonoids, and phenolic compounds which are a source of antioxidants. Flavonoids are one of the largest natural phenol group compounds and are found in all green plants. This study aims to determine total flavonoid levels and measure antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method using UV-Vis Spectrophotometry. Extraction was carried out by maceration method using 96% ethanol as a solvent with a yield of 5.41%. The determination of the total flavonoid content of the ethanol extract of pumpkin seeds was determined based on the absorbance value measured at a wavelength of 437 nm using quercetin as a comparison. The results of the determination of total flavonoid levels obtained were 46.116 mg QE/gr. Antioxidant activity test using UV-Vis Spectrophotometer with free radical inhibition method DPPH to ethanol extract of pumpkin seeds at a wavelength of 517 nm. The results of the antioxidant activity test of pumpkin seed extract showed an IC₅₀ value of 8.273 g/mL. Based on the IC₅₀ value, it can be concluded that the ethanol extract of pumpkin seeds has very strong antioxidant activity.

KEYWORDS: Antioxidant, pumpkin seeds, flavonoids, DPPH.**INTRODUCTION**

Pumpkin (Figure 2) is a local food that is readily available and contains complete nutrients such as carbohydrates, protein, fat, minerals and vitamins, a source of fiber and antioxidants.

The use of pumpkin seeds as a traditional medicine has been carried out by Native Americans. Utilization of seeds in Indonesia is limited to the production of kuaci, pumpkin seeds (*Cucurbita moschata* Duch) contain saponins, tannins, flavonoids, and phenolic compounds which are known to be a source of antioxidants, which are also used as anti-inflammatory and cardioprotective. The use of pumpkin in Indonesia is that the flesh of the fruit is processed into snacks such as cakes, compotes and vegetable soup, while the seeds are not utilized optimally, only processed into kuaci (Hargono, 1999), and as an anthelmintic agent and supportive treatment in bladder disorders (Salehi et al., 2019).

Flavonoids are one of the largest natural phenol group compounds and are found in all green plants and are found in every plant extract (Markham KR, 1988). Flavonoids are known to have properties as free radical scavengers, are antioxidants, inhibitors of hydrolysis enzymes, oxidative and work as anti-inflammatory (Pourmourad et al., 2006). 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 free radicals can trigger damage to DNA, lipids, proteins and carbides, causing various diseases such as diabetes mellitus, cancer and arteriosclerosis (Chen et al., 2007), besides this condition also causes body cells to degenerate, disrupt metabolism and decrease immune response. thus triggering various degenerative diseases. Free levels 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). Antioxidants are compounds that can inhibit reactive oxygen species/reactive nitrogen species (ROS/RNS) and also free radicals (Halliwell *et al.*, 1992). Antioxidants inhibit oxidation reactions and prevent cell damage by binding to free radicals and highly reactive molecules. Consumption of antioxidants in adequate amounts is reported to reduce the incidence of degenerative diseases, improve immunological status and inhibit the onset of degenerative diseases due to aging. Therefore, optimal adequacy of antioxidant intake is necessary (Winarsi, 2007). The antioxidant test method used in this study is the free radical scavenging method of DPPH. 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 that electromagnetic radiation in the ultraviolet region and visible light through compounds that have double bonds, part of the radiation is absorbed by the compound. 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 a reduction in the energy of the radiation beam when electrons in low energy orbitals are excited to higher energy orbitals (Silalahi Jansen, 2006). This study aims to determine total flavonoid levels and test antioxidant activity using the DPPH method from the ethanolic extract of pumpkin seeds, so that it is expected to be able to provide scientific information for the public regarding the content of seeds. Pumpkin fruit so that its use is not only processed as a kuaci snack.

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%, AlCl₃, quercetin, HCL, acetic acid, DPPH, vitamin C, aluminum foil, filter paper, distilled water, H₂SO₄, Dragendroff's reagent, Mayer's reagent, Mg, NaCl 10%, FeCl₃.

Preparation of sample

Pumpkin seeds (*Cucurbita moschata* Duch) was collected from Cikole plantation in the Ciburial, West Bandung. Pumpkin seeds are washed under running water, drained and then dried in the sun. The dried pumpkin seeds were powdered using a blender, then sieved using a 60 mesh, then weighed again and obtained 300 gr powder.

Extraction

Three hundred gram of pumpkin seed 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 60°C, obtained a thick extract and then evaporated using a water bath at a temperature of 60°C to obtain a concentrated extract of pumpkin seeds as much as 16.25 g with a yield of 5.41%.

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 (Kristanti *et al.*, 2008). Screening for alkaloids, flavonoids, tannins and saponins was carried out.

Total Flavonoid Content (TFC)

Total flavonoid content with quercetin as a comparison, 200 mg of extract, dissolved in 10mL of ethanol, in order to obtain a concentration of 2000 ppm. From this solution, 1mL 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 nm. 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 40 ppm. Then the absorption was measured at a wavelength of 400-600 nm using UV-Vis Spectrophotometry. 200 mg of ethanolic extract of pumpkin seeds was dissolved with ethanol in a 10 mL volumetric flask and then centrifuged so that the concentration became 2000 ppm as stock solution. Various concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm were made, homogenized and incubated for 30 minutes in a dark place at 37°C. The absorbance was measured at a wavelength of 517 nm using UV-Vis spectrophotometry. The IC₅₀ 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 pumpkin seeds (*Cucurbita moschata* Duch) (Figure 1)

Characterization of simplicia exhibited that water content 2.4% and loss drying 5.5%. Results pumpkin seed extraction 16.25 g with a yield of 5.41%. 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 pumpkin seeds was 46.116 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 (Hanani *et al.*, 2005). Vitamin C was used as a comparison because it has very strong antioxidant properties (Haeria, 2016). The IC₅₀ calculation results for vitamin C is 1,210 g/mL and ethanol extract of pumpkin seeds is 8,273 g/mL. both exhibit very strong antioxidant activity.

The standard linear regression curve of inhibition of antioxidant activity of vitamin C and ethanol extract of pumpkin seeds with DPPH can be seen in Figures 3 and 4.

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LEMBAR IDENTIFIKASI TUMBUHAN
 No.15/HB/07/2020

Herbarium Jatinangor, Laboratorium Taksonomi Tumbuhan, Departemen Biologi FMIPA UNPAD, dengan ini menerangkan bahwa :

Nama : Desi Dwi Riani
 NPM : D1A191868
 Instansi : AL-GHIFARI
 Telah melakukan identifikasi tumbuhan, dengan No. Koleksi : -
 Tanggal Koleksi : 25 Juli 2020
 Lokasi : Jabar

Hasil Identifikasi,
 Nama Ilmiah : *Cucurbita moschata Duch*
 Sinonim : *Cucurbita moschata var. argyrosperma (C.Huber) Naudin*
 Nama Lokal : Labu kuning
 Suku/Famili : Cucurbitaceae

Klasifikasi (Hierarki Taksonomi)
 Kingdom : Plantae
 Divisi : Magnoliophyta
 Class : Magnoliopsida
 Ordo : Cucurbitales
 Famili : Cucurbitaceae
 Genus : Cucurbita
 Species : *Cucurbita moschata Duch*

Referensi:
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 Diakses tanggal, 29 Juli 2020.

Jatinangor, 29 Juli 2020
 Identifikator,

 Drs. Joko Kusumoro, M.P.
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Figure 1: Result of determination.



Figure 2: Pumpkin Seeds.

Table 1: Chemical Screening of Extract.

Secondary metabolites	Solvent	Extract
Alkaloid	Dragendroff	+
	Mayer	+
Flavonoid	Magnesium	+
Tannin	FeCl ₃	+
Saponin	HCl	+

(+) = detected

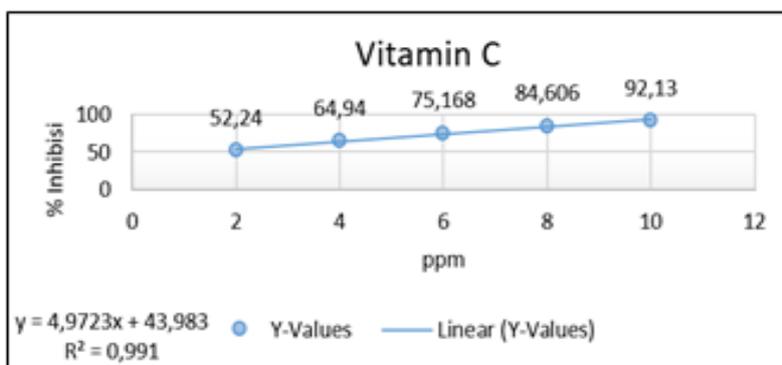


Figure 3: Standard Curve of Linear Regression % inhibition of Antioxidant Activity of Vitamin C with DPPH.

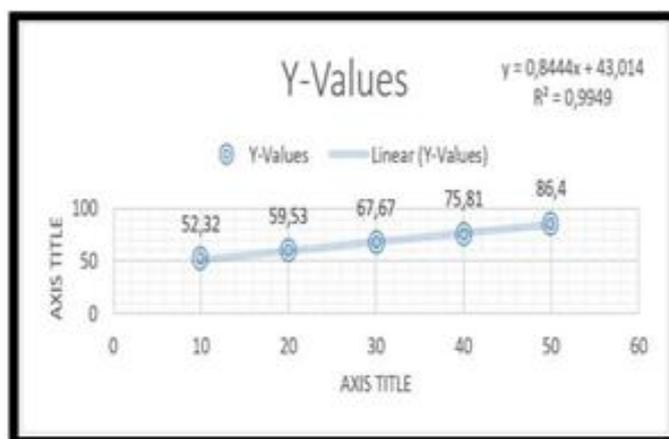


Figure 4: Standard Curve of Linear Regression % Antioxidant Activity Pumpkin Seed Extract with DPPH.

CONCLUSION

The total flavonoid content of the ethanolic extract of pumpkin seeds (*Cucurbita moschata* Duch) was 46.116 mg QE/100g and IC₅₀ antioxidant activity 8.273 g/mL, indicating a very strong antioxidant activity using the DPPH method.

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