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ORIGINAL ARTICLE

TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF YELLOW PUMPKIN (*Cucurbita moschata* Duch) FRUIT EXTRACT

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Abstract Pumpkin (*Cucurbita moschata* Duch) is a food rich in vitamin A, vitamin C, minerals, carbohydrates, and flavonoid compounds. Flavonoids have several pharmacological activities, including as antioxidants to ward off free radicals. This study aims to determine the total flavonoid content and antioxidant activity of pumpkin fruit. The extraction of pumpkin fruit was done by the maceration method in a 70% ethanol solvent. The total flavonoid test used the colorimetric method with a 10% $AlCl_3$ reagent and quercetin as a positive control. Antioxidant activity was tested using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The results obtained for the total flavonoid content of the ethanol extract of pumpkin fruit amounted to 1.741 mgQE/g ± 0.2438 and an IC50 value of 379.44 ppm, including the category of weak antioxidant, compared with the positive control.

Keywords: Antioxidant activity • *Cucurbita moschata* • Flavonoids • DPPH • Colorimetry

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Introduction

Pumpkin (*Cucurbita moschata* Duch) belongs to the Cucurbitaceae family. Pumpkin fruit is a food rich in vitamin A, vitamin C, minerals, carbohydrates, carotenoids, and flavonoids (Adlhani, 2014). Flavonoids are found in almost all parts of plants including fruits, seeds, roots, leaves and outer bark. A number of medicinal plants containing flavonoids have been reported to have antibacterial, antiviral, anti-inflammatory, antiallergic, anticancer, and antioxidant activities (Ahmad et al., 2015; Pardede and Koketsu, 2017).

Antioxidants are compounds that at certain levels, are able to inhibit or slow down damage due to the oxidation process. Antioxidants have a very important role for the health of the human body because their function can inhibit and neutralize the occurrence of oxidation reactions involving free radicals. The inhibitory mechanism of antioxidants usually occurs during the initiation reaction in the oxidation reaction of fat or other molecules in the body by absorbing and neutralizing free radicals (Parwata, 2016). Several studies have revealed the role of oxidative stress caused by free radicals in various dangerous diseases, such as cancer, cardiovascular-related diseases, and degenerative diseases (Barhe and Tchouya, 2014). The most commonly used method to test antioxidant activity is using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals.

DPPH acts as a free radical that will react with antioxidant compounds, so that DPPH will

turn into a non-radical. This reaction causes a dark purple color to change to a pink or pale-yellow color that can be measured by a visible light spectrophotometer at a wavelength of 400–800 nm, so that the free radical suppression activity of the sample can be found (Rachmawati and Ciptati, 2011). Pujiastuti et al. (2022) have conducted research on the test of total flavonoid content using a UV-Vis spectrophotometer and the antioxidant activity test of ethanol extract (96%) of pumpkin using the ABTS (2,2-azinobis-3-ethylbenzothiazoline-6 Sulfonic Acid) method, with results of 93.017 mgQE/g of total flavonoid and an IC50 value of 69.00 ppm, including the strong antioxidant activity category. Research by Purwaningsih et al. (2018) concluded that the antioxidant activity of a 96% ethanol extract of pumpkin fruit skin using the DPPH method gave IC50 results of 64.82 ppm and included the category of strong antioxidant activity. Based on the description and literature, research was conducted on the total flavonoid content and antioxidant activity of yellow pumpkin fruit extract (*Cucurbita moschata* Duch).

Materials and Methods

Glassware (Pyrex®), macerator (Thermo®), micropipette, analytical balance, rotary evaporator, UV-Vis spectrophotometry (Shimadzu) UV-1800. Materials Used 70% ethanol, bouchardat reagent, dragendorf reagent, meyer reagent, amyl alcohol, Mg powder, $FeCl_3$ 1%, concentrated HCl, HCl 2 N, concentrated H_2SO_4, $AlCl_3$ 10%, potassium acetate 1M, quercetin, distilled water, and DPPH compound.

Simplisia Processing

Simplisia processing begins with the collection of raw materials in the form of pumpkin fruit. Furthermore, wet sorting is carried out, namely separating the pulp from the skin and seeds. The pulp samples were washed, then cut into pieces, dried in the sun and wet-sorted. Dried simplisia is mashed until it becomes simplisia powder.

Extract Preparation

Pumpkin fruit simplisia powder (300 g) was put into a glass vessel, and 70% ethanol solvent was added to as much as 1.5 L. Continuous stirring was carried out for 6 hours and allowed to stand for 18 hours. The macerate was filtered, and the pulp was re-macerated with the same type and amount of solvent. All the obtained macerates were evaporated until a thick extract was obtained (Kemenkes RI. 2017).

Phytochemical Screening

The test solutions were made by weighing 1 g of extract and adding 50 ml of distilled water. They were heated until dissolved, then filtered, and the filtrate was used for the testing of alkaloid, flavonoid, tannin, and saponin compounds. Phytochemical screening procedures follow previous research reports (Pardede et al., 2013; Supomo et al, 2016).

Determination of Total Flavonoid Content

Determination of Maximum Wavelength of Quercetin Standard Solution

Weighed 10 mg of quercetin standard solution and dissolved it in 10 mL of ethanol for 1000 ppm. A quercetin standard solution of 1000 ppm was pipetted into 1 mL and dissolved in 10 mL of ethanol for 100 ppm. Then a solution with a concentration of 6 ppm was made by pipetting 0.6 mL of 100 ppm quercetin standard solution and adding ethanol into the flask up to 10 mL. Pipetted 1 mL was put into a test tube with 0.2 mL of $AlCl_3$ 10% and 0.2 mL of 1 M potassium acetate. After that, it was incubated for 30 minutes at room temperature and measured for absorbance with UV-Vis spectrophotometry at the maximum wavelength (Aminah et al., 2017).

Determination of Quercetin Standard Curve

Quercetin standard solution of 1000 ppm, then pipetted 1 mL and dissolved in 10 mL ethanol for 100 ppm, then made several concentration of 2, 4, 6, 8, and 10 ppm. Then each concentration of quercetin standard solution was pipetted 1 mL put into a test tube and added 0.2 mL of $AlCl_3$ 10% and 0.2 mL of potassium acetate 1M. After that, it was incubated for 30 minutes at room

temperature and measured for absorbance using a UV-Vis spectrophotometer with the maximum wavelength (Aminah et al., 2017)

Determination of Total Flavonoid Content of Sample Extract

Weighed 25 mg of extract, dissolved in 25 mL of ethanol. Pipetted 1 mL was put into a test tube with 0.2 mL of $AlCl_3$ 10% and 0.2 mL of potassium acetate 1M. After that, it was incubated for 30 minutes at room temperature and measured for absorbance using a UV-Vis spectrophotometer with the maximum wavelength (Azizah et al., 2014). The determination of the total flavonoid value was calculated based on the formula:

$$\text{Total Flavonoid} = \frac{c.v.fp}{g}$$

c = sample concentration (mg)
v = volume of extract used (mL)
fp = dilution factor
g = sample weight (g)

Antioxidant Activity Test

Preparation of 40 ppm DPPH solution

4 mg of DPPH was weighed with 100 ml of 70% ethanol in a beaker. The solution was kept at room temperature, protected from light, for immediate use.

Determination of Maximum Wavelength of 40 ppm DPPH

The 40 ppm DPPH solution that has been made, pipetted 2 mL and then put into a 10 mL volumetric flask, added 70% ethanol to the limit mark, shaken until homogeneous. Then left for 30 minutes, then measured the absorption at a wavelength of 400-800 nm using a UV-Vis spectrophotometer. Preparation of 100 ppm concentration of quercetin stock solution. Weighed as much as 10 mg of quercetin then dissolved in a 10 mL volumetric flask with 70% ethanol until the limit mark so as to obtain 1000 ppm quercetin stock solution, then diluted by taking 1 mL of 1000 ppm quercetin stock solution and put into a 10 mL volumetric flask and added ethanol until the limit mark to make 100 ppm stock solution.

Preparation of Quercetin Concentration Solution

The 100 ppm quercetin stock solution was made into 1, 2, 3, 4 and 5 ppm. then put each into a 10 mL volumetric flask and added ethanol to the limit mark.

Antioxidant Activity Test of Quercetin

Take 1 mL of quercetin concentration solution and put it into a test tube. Added 2 mL of a 40 ppm DPPH solution. The mixture was homogenized and then incubated for 30 minutes at room temperature. The absorbance was measured by a UV-Vis spectrophotometer at the maximum wavelength.

Preparation of 1000 ppm Solution of Ethanol Extract of Yellow Pumpkin Fruit

Weighed 50 mg of extract, added a few drops of 70% ethanol, and stirred. Then put into a 50-mL volumetric flask with sufficient volume of ethanol to obtain a 1000 ppm ethanol extract stock solution.

Preparation of Serial Solution of Ethanol Extract of Yellow Pumpkin Fruit

A concentration solution of ethanol extract of yellow pumpkin fruit was made with concentrations of 50, 100, 150, 200, and 250 ppm, each of which was put into a 10 mL volumetric flask and filled with 70% ethanol (Aminah et al., 2017).

Antioxidant Activity Test of Ethanol Extract of Yellow Pumpkin Fruit

Take 1 mL of each sample concentration solution and put it into a test tube. Added 2 mL of a 40 ppm DPPH solution, let it stand for 30 minutes at room temperature, and then measured the absorbance with a UV-Vis spectrophotometer at a wavelength of 523 nm. The IC50 value was calculated using the linear regression equation formula obtained by plotting the extract

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concentration on the x-axis and percent (%) inhibition on the y-axis (Purwanto et al., 2017).

Results and Discussion

Processing of Pumpkin Fruit Simplicia

Samples of pumpkin fruit flesh that have been collected, washed, and cut into pieces to facilitate the drying process. Samples were dried in the sun and covered with black cloth. The purpose of drying is to obtain simplisia that is not easily damaged, so that it can be stored for a long time (Sinaga et al., 2021).

Preparation of Pumpkin Fruit Extract

Preparation of an ethanol extract of yellow pumpkin fruit by the maceration method. This method was chosen because the procedure used is more practical and does not require heating, so it can minimize the decomposition of active compounds in simplisia (Istiqomah, 2013).

Alkaloid tests were carried out using Meyer, Bouchardat and Dragendorff reagents. The identification results show that a precipitate is formed, which means it is positive for alkaloid compounds. This is in accordance with research conducted by Adlhani (2015), where pumpkin fruit flesh contains alkaloid compounds. The flavonoid test is done by adding Mg powder to the extract solution, 1 mL of concentrated HCl, and 2 mL of amyl alcohol. The addition of concentrated HCl in the flavonoid test is used to hydrolyze flavonoids into aglycones. The test results showed a yellow color formed on the amyl alcohol layer, which means positive flavonoids. The tannin test is carried out by the addition of $FeCl_3$ 1%, one of the hydroxyl groups present in tannin compounds. The addition of $FeCl_3$ 1% produces a blackish green color that shows condensed tannins (Sangi et al., 2018).

The saponin test of pumpkin fruit extract is carried out by shaking vigorously for 10 seconds. If foam forms for not less than 10 minutes, is 1–10 cm high, and does not disappear with the addition of 1 drop of HCl 2N, it shows the presence of saponins. The test results do not form foam, which means negative saponins. The steroid/triterpenoid test of the ethanol extract of pumpkin fruit showed negative results because no color change occurred. The results of the phytochemical screening test of the ethanol extract of pumpkin fruit can be seen in Table 1.

Table 1. Phytochemical Screening Results of Ethanol Extract of Yellow Pumpkin Fruit

Compound	Reagen	Result	Description
Alkaloid	Meyer	-	There is a brown to black precipitate
	Bouchardat	+	There is a brown to black precipitate
	Dragendorff	+	There is an orange to red brown precipitate
Flavonoid	HCl, Mg powder, amil alkohol	+	yellow color on amyl alcohol layer
Saponin	HCl 2N	-	No foam formed
Tanin	$FeCl_3$ 1%	-	No green-black or blue-black color formed
Steroid/Triterpenoid	H_2SO_4	-	No red or blue color formed

(+): contains secondary metabolite compounds (-): does not contain secondary metabolite compounds

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Determination of Total Flavonoid Content of Ethanol Extract of Yellow Pumpkin Fruit

Determination of flavonoid compound content of pumpkin fruit extract using a UV-Vis spectrophotometer with the colorimetric method. The compound used as a standard for determining flavonoid content is quercetin. Quercetin is the most widely distributed compound found in plants. The concentration of the quercetin standard solution was 100 ppm. The standard quercetin was made at 5 concentrations, namely 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. Then, the maximum wavelength measurement with the UV-Vis spectrophotometer was 437 nm at a concentration of 6 ppm, and the absorbance values of each concentration in order are as follows: 0.0170; 0.0353; 0.0603; 0.0884; 0.1115. The absorbance values can be seen in Table 2.

Table 2. Absorbance Value Of Quercetin Standard Solution

No	Concentration (ppm)	Absorbance
1	2	0,0170
2	4	0,0353
3	6	0,0603
4	8	0,0884
5	10	0,1115

Table 3. IC_{50} of Antioxidant Activity of Ethanol Extract of Yellow Pumpkin Fruit

Sampel	*IC50* (ppm)	Average *IC50* (ppm)
Quercetin	3,03 3,31 3,73	3,35
Ethanol extract of yellow pumpkin fruit	325,45 367,30 445,47	379,44

Antioxidant Activity Test of Ethanol Extract of Yellow Pumpkin Fruit

One of the parameters that can be used to determine the antioxidant activity of a compound is the IC50 value, which is the concentration of the sample that is able to reduce free radicals by 50%. The smaller the IC50 value, the stronger the antioxidant activity of a compound. Quercetin was used as a comparator or positive control because quercetin is an antioxidant source that can act as an inhibitor of other molecules (Zhao et al., 2018). Pumpkin fruit extract was made at concentrations of 50, 100, 150, 200, and 250 ppm. At this concentration, there is a color change from purple to yellow. The concentration solution was then measured at a wavelength of 523 nm. Three replications of measurements were carried out, and the average IC_{50} result was 379.44 ppm, which was categorized as a weak antioxidant. Research by Pujiastuti et al. (2019) concluded that the ethanol extract of pumpkin fruit has strong antioxidant activity with an IC50 value of 69.00 ppm. The difference in IC50 values is due to differences in solvent concentration and antioxidant test methods used. Pujiastuti et al. (2019) in their research, used a 96% ethanol solvent and the ABTS method.

There is a relationship between flavonoids and antioxidant activity (Mahani et al., 2022). According to Ustadi et al. (2017), the higher the flavonoid content, the greater the ability of antioxidants to donate electrons to suppress free radicals, and the smaller the IC50 value. Based on the measurement of flavonoid content in the ethanol extract of pumpkin fruit, the average value is 1.741 mgQE/g, with an average value of antioxidant activity of 379.44 ppm, including the weak antioxidant category. The average IC50 value of quercetin is 3,35 ppm, including the strong antioxidant

Conclusion

The flavonoid content of ethanol extract of pumpkin fruit was 1.741 mgEQ ± 0.2438, and the antioxidant activity of ethanol extract of pumpkin fruit obtained IC50 value of 379.44 ppm including weak antioxidant category.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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