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Research Article

The Comparison of Antioxidant Activity of Ethanol Extract of Fruit, Seeds and Leaves of Yellow Pumpkin (*Cucurbita moschata* D.) using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Method

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ABSTRACT

Yellow pumpkin (*Cucurbita moschata* D.) has various compounds that can be used as antioxidants, such as tocopherol, carotene, and phenolics. This research aims to determine which parts of the plant have potential as antioxidants by comparing the antioxidant activity of fruit, leaves, and seeds. Antioxidant activity measurements were carried out using the DPPH method by comparing the absorbance of the control and the absorbance of the sample to determine the percentage of antioxidant activity and IC_{50} value of each sample. From the measurement results, the IC_{50} values for pumpkin leaves, seeds, and fruit were 182.85 ppm, 57.04 ppm, and 90.88 ppm, respectively. Based on these results, leaves have the highest antioxidant activity compared to seeds and fruit. The results of this study show that pumpkin fruit and seeds have lower antioxidant activity than leaves. The results of this study show that pumpkin leaves had lower antioxidant activity than seeds and flesh.

Keywords: *Cucurbita moschata* D.; ethanol extract; antioxidant activity; DPPH.

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INTRODUCTION

We are always surrounded by free radical compounds in our daily lives. Endogenous free radicals can arise from phagocytosis during respiration, enzymatic oxidation, autoxidation, electron transport in mitochondria, and oxidation of transition metal ions, among other processes. Exogenous free radicals, on the other hand, originate from sources outside of the body, such as UV light and environmental factors like radiation, pollution, cigarette smoke, foods (especially those cooked at high temperature, which can form free radicals), beverages such as alcohol, sugary drinks, or energy drinks, ozone, and pesticides (Haider et al. 2020).

According to Nakamura and Lipton (2020), molecules or molecular fragments with one or more unpaired electrons in atomic or molecular orbitals are classified as free radicals. Usually, these single electrons are adequate (Nakamura and Lipton 2020; Sies 2020). Oxidants are these unpaired electrons that, by attaching to electrons in cell molecules, transform free radicals into extremely reactive substances against bodily cells (Nakamura and Lipton 2020). Excessive oxidation of nucleic acids, proteins, lipids, and cell DNA might start degenerative diseases like cancer, cataracts, coronary heart disease, and cognitive impairments (Sharifi-Rad et al. 2020; Sies 2020; Leyane, Jere, and Houreld 2022). Making lifestyle changes, such as increasing one's intake of nutrient-dense foods, is a therapeutic approach to the prevention and treatment of degenerative and chronic illnesses. Consuming antioxidants such as polyphenols, which include antioxidant vitamins C, E, A, and carotene; antioxidant minerals Se, Cu, Fe, Mn, and Zn; and flavonoids (flavones, flavonols, flavins, catechins, and proanthocyanidins) and non-flavonoids (phenolic acids, stilbene, and lignin) can enhance the activity of endogenous antioxidants that can reverse the harmful effects of oxidative stress (Jomova et al. 2023; Zujko and Witkowska 2023). Compounds known as antioxidants have the ability to prevent or postpone the oxidation of others (Moldoveanu and Oden 2021).

The human body has defense mechanisms against external and internal oxidants. Internal defenses such as glutathione, peroxidase, catalase, and histidine peptide enzymes, although they frequently fall short when oxidants are present because of external factors (Morris et al. 2022). As a result, extra antioxidant substances are needed by humans to combat free radicals. Antioxidant chemicals include synthetic antioxidants like BHT (Butylated Hydroxy Toluene), TBHQ, and BHA (Butylated Hydroxy Anisole) as well as natural antioxidants found in fruits and vegetables (Atta, Mohamed, and Abdelgawad 2017). But using synthetic antioxidants can have negative effects and even be dangerous for people (Atta, Mohamed, and Abdelgawad 2017; Fadhil, Suryati, and Jayanegara 2023). The previous study of Fadhil et al. (2023), provided a comparison of the effects of synthetic and natural antioxidants, claiming that adding natural antioxidants produces comparable results and can be utilized as an alternative to synthetic antioxidants. Pumpkin (*Cucurbita moschata* D.) is one fruit that has natural antioxidant components (Dash and Ghosh 2017; Pratama, Supriningrum, and Supomo 2024; Xu et al. 2024). Various plant components, such as leaves, seeds, and fruits, possess distinct phytochemicals that affect antioxidant capacity. Many antioxidant substances are found in *C. moschata* D., including vitamin A, vitamin E, phenolic compounds (Pratama, Supriningrum, and Supomo 2024), lutein (Xu et al. 2024), β-carotene, α-carotene, and lycopene (Dash and Ghosh 2017). Consequently, comparative research on the antioxidant activity of the fruit, leaves, and seeds of *Cucurbita moschata* D. is essential to ascertain which portion exhibits the highest antioxidant efficacy.

MATERIALS AND METHODS

Materials

The primary components utilized are pumpkin fruit, seeds, and leaves sourced from the Matesih region of Karanganyar Regency. Other materials used are distilled water, 96% ethanol solution, potassium iodide, iodine, ferric chloride ($FeCl_3$), Lead acetate [$Pb(C_2H_3O_2)2$], perchloric acid ($HClO_4$), vitamin C (Sigma Aldrich®), pro analysis ethanol solution, pro analysis methanol, DPPH powder (Sigma Aldrich®, USA). All the solvents and chemical used in this study were in good analytical grade.

Methods

Preparation of Extract

All components, specifically fruit, seeds, and leaves of pumpkin, are desiccated until they transform into simplicia, which are thereafter extracted utilizing the maceration technique with a 96% ethanol solvent. Soaking is carried out for three days, and stirring is repeated. The resulting macerate is filtered using filter paper, and then remaceration is carried out for two days (Asjur et al. 2023). The macerate obtained is then evaporated to obtain a thick extract using a rotary evaporator.

Screening of Phytochemical

According to many scientific research, bioactive components found naturally in various portions of *Cucurbita moschata* D. confirm the presence of various phytochemicals, including phenol, flavonoids, alkaloids, terpenoids, and steroids. The aforementioned components have been qualitatively estimated using standard approaches (Muhammed et al., 2020; Neelamma et al., 2021; González et al., 2023).

Determination of Antioxidant activity of Vitamin C

To prepare test solutions with the specified concentrations, measure 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, and 2.5 mL of vitamin C stock solution and transfer each into a 10 mL volumetric flask. The volume of the solution was increased with methanol pro analysis up to the limit mark. 1 mL of each solution was taken, and 3 mL of DPPH solution was added. The solution was incubated for 30 minutes in the dark and then measured for absorption at a maximum wavelength of 515 nm (Kar, Dutta, and Yasmin 2023).

Determination of Antioxidant activity of Samples

To prepare test solutions with the specified concentrations, measure 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, and 2.5 mL of sample extract and transfer each into a 10 mL volumetric flask. The volume of the solution was increased with methanol pro analysis up to the limit mark. 1 mL of each solution was taken, and 3 mL of DPPH solution was added. The solution was incubated for 30 minutes in the dark and then measured for absorption at a maximum wavelength of 515 nm (Kar, Dutta, and Yasmin 2023).

Data Analysis

The following formula was used to calculate the percentage of antioxidant activity (Baliyan et al. 2022) :

$$\%of\ antioxidant\ activity = \frac{Ac - As}{Ac}\ x\ 100\%$$

Where:
Ac = Absorbance of Control
As = Absorbance of Sample

RESULTS

Extraction Process

Leaves, seeds, and fruits obtained were dried by oven (Hereaus). After the drying process for 4 x 24 days, we weighed the simplicial, and the results are in Table 1.

Table 1. Result of Extraction

Result	Leaves	Seeds	Fruit
Weight of simplicia (g)	300	300	200
Weight of extract (g)	40,83	45,19	63,45
Yield of extract (%)	13,61	15,06	31,72

Screening of Phytochemical

A variety of phytochemicals, including alkaloids, flavonoids, phenolic compounds, steroids, and terpenoids, were detected in the sample upon phytochemical screening, as shown in Table 2.

Table 2. Result of Phytochemical Screening

Phytochemicals	Name of Test	Pumpkin Leaves (Muhammed et al. 2020)	Pumpkin Seeds (Neelamma et al. 2021)	Pumpkin Fruit (González, Botella, and Quintas 2023)
Alkaloids	Wagner's test	+	+	+
Phenols	Ferric cyanide test	+	+	+
Flavonoid	Lead acetate Test	+	+	+
Steroids and Terpenoids	Salkowski reaction Test	+	+	+

Determination of Antioxidant Activity of Vitamin C

A strong antioxidant, vitamin C has a number of uses in the pharmaceutical and cosmetic industries (Caritá et al. 2020). Vitamin C in this study used as control of antioxidant activity assay.

Table 3. Antioxidant Activity of Vitamin C

Sample	Concentration (ppm)	Absorbance	Antioxidant activity (%)
Vitamin C	5	0,274	15,17
	10	0,244	24,45
	15	0,232	31,26
	20	0,216	33,12
	25	0,187	42,10
	IC_{50}		**31,61±10,06**

Determination of Antioxidant Activity of Sample Extract

In order to demonstrate the sample extract as antioxidant agent, they were determined for their antioxidant activity by using DPPH method.

Table 4. Antioxidant Activity of Sample Extract

Sample Extract	Concentration (ppm)					IC_{50} (ppm)
	Antioxidant activity (%)					
	120	140	160	180	200	
Leaves	31,85± 1,71	38,22 ± 0,8	44,21± 0,47	47,80 ±0,87	54,21 ±1,52	182,85±8,6
Seeds	8,74±0,67	12,40±0,79	22,371±0,176	27,42±0,49	33,93±1,79	57,04±10,4
Flesh	6,54±0,94	7,898±1,11	11,82±1,20	17,33±2,01	27,39±1,72	90,88 ±8,48

Based on the data in Table 4, it can be concluded that the effectiveness of the seed extract of *Cucurbita moschata* D. has the strongest antioxidant activity compared to fruit and leaf extract.

DISCUSSION

According to the extract yield results shown in Table 1., the yield obtained in the extraction process of pumpkin leaves, seeds and flesh was 13.61%, 15.06%, and 31.72%, respectively. The yield of thick extract from the three parts of the pumpkin plant meets the requirements, more than 8.3% (Kementerian Kesehatan Republik Indonesia 2022). This indicates that the extraction process is progressing smoothly (Hasan et al. 2023).

Phenols, flavonoids, alkaloids, steroids, and terpenoids can all be detected using a variety of qualitative phytochemical screening techniques. Qualitative phytochemical screening revealed notable levels of flavonoid and phenolic contents (Table 2). All the qualitative test results showed the presence of phytochemicals. These phytochemicals are known to possess antioxidant effect (Abdillah 2018; Dash and Ghosh 2017; González, Botella, and Quintas 2023; Hossain et al. 2023)

Absorbance measurements at each concentration were carried out in three replications. The values listed in the table are the average values of the three absorbances, which then become a reference in calculating the percent inhibition. Based on the information in Table 2, the IC_{50} of the comparison solution (vitamin C) was 31.61 ± 10.06 ppm. From these results, it is known that vitamin C is a very strong antioxidant. The IC_{50} value is the value at which a concentration can reduce 50% of DPPH (González et al., 2023). Vitamin C plays multiple essential roles in the human body, most of which stem from its ability to donate electrons, making it a physiologically essential reducing agent (Jakubek et al. 2023).

One of the straightforward and widely used techniques for estimating antioxidant activity is DPPH radical scavenging activity. The result from the IC_{50} value of the dried leaf, seeds, and flesh ethanol extract of *Cucurbita moschata* D. has demonstrated 182.85 ± 8.6, 57.04 ± 10.4, and 90.88 ± 8.48 ppm, respectively. The result shows that the most potent part is pumpkin seeds, which have IC_{50} value of 57.04 ppm. This is consistent with the result of a previous study that determined the total phenolic content in pumpkin seeds and leaves by Kar et al. (2023). Kar et al. (2023), found out that pumpkin seeds had a higher total phenolic content compared to pumpkin leaves. The physiological perspective of humans indicates that phenolic compounds are important for defensive mechanisms such as anti-aging, anti-inflammatory, antioxidant, and anti-proliferative properties (Muhammed et al. 2020).

In this study, the presence of terpenoids was also tested, and the result was positive. Beta carotene and terpenoids are also plays a role in antioxidant activity

(Bufka et al. 2024). The antioxidant properties of beta carotene can reduce oxidative stress, prevent oxidative DNA damage, and reduce the risk of reactive oxygen species (Bohn 2019; Bufka et al. 2024).

Another study had mentioned that micronutrients can be found in pumpkin flesh, whereas substantial levels of proteins, minerals, phytosterols, and vital fatty acids can be found in pumpkin seeds. Moreover, bioactive substances, particularly carotenoids, polyphenols, amino acids, vitamins, and minerals, are abundant in pumpkin fruit. It is a great way to get trace elements, including magnesium, phosphate, and potassium (González, Botella, and Quintas 2023).

CONCLUSIONS

Phenols, flavonoids, and terpenoids play a role in promoting long-term health and lowering the risk of degenerative and chronic illnesses. Those compounds are presented in *Cucurbita moschata* D., or pumpkin. This study involves the antioxidant activity of three parts of pumpkin (leaves, seeds, and fruit). Based on the results of this study, it is known that the pumpkin seeds have the potential to be an antioxidant agent, followed by the fruit and leaves.

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