In Vitro Antioxidant Activity, Flavonoid and Phenolic Contents of 70% Methanol Extract from Purple and Yellow Passion Fruit Peel

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Article Info	ABSTRACT		
Article history:	Peel of purple and yellow passion fruit has the potential to be an		
Received Jan 15 th , 2023 Revised Oct 13 th , 2023 Accepted Dec 4 th , 2023	antioxidant. The flavonoid and total phenol compound groups are two examples of natural antioxidant compounds that can potentially be antioxidants. Many studies have used 70% methanol solvent to extract flavonoids and total phenolics from plants. The aim of this study is to analyze the potential of 70% methanol extract from the peel of purple and yellow passion fruit as an in		
Corresponding Author: Khoirul Ngibad, Department of Medical Laboratory Technology Universitas Maarif Hasyim Latif Email: <u>khoirul ngibad@dosen.umaha.ac.id</u>	vitro antioxidant using the DPPH method, total flavonoid, and phenolic levels. The passion fruit peel powder was macerated with 70% methanol for four days. The total flavonoid contents were determined using quercetin, and total phenols were determined using gallic acid. The 2.2-diphenyl-1-picrylhydrazyl (DPPH) was used for in vitro antioxidant activity by determining the percent inhibition of DPPH radicals. The flavonoid content in the 70% methanol extract of purple and yellow passion fruit peel is 1.2824 and 1.9775 mg QE/g extract, respectively. In contrast, the total phenolic content in 70% methanol extract of purple and yellow passion fruit peel is 15.8 and 3.6 mg GAE/g extract, respectively. The 70% methanol extract of purple passion fruit peel at a concentration of 80 mg/L resulted in a more significant percent inhibition of DPPH free radicals (72.80%) than yellow passion fruit (64.91%). In conclusion, the results suggest that 70% methanol extract from purple and yellow passion fruit peel can decrease free		
	radicals and be a natural antioxidant. <i>Keyword:</i> Antioxidant activity, Flavonoid, Methanol extract,		
	Passion fruit peel, Total phenol		

1. INTRODUCTION

Passion fruit plants cultivated in Indonesia have three varieties, purple, yellow, and red. The part of the passion fruit plant that is widely used is the fruit's flesh. The peels of passion fruit have not been widely utilized (Aisyah & Ngibad, 2022). The main component of passion fruit peel that contains a lot of fiber and pectin is albedo (pith) which can potentially be a functional food ingredient (Coelho et al., 2017). Some studies report that passion fruit peel in purple and yellow has the potential to be an antioxidant.

The flavonoid and total phenol compound groups are two examples of natural antioxidant compounds that have been the center of attention lately. The plant's largest secondary metabolite

compound is a class of phenolic compounds belonging to aromatic alcohols because their hydroxyl group is permanently attached to the benzene ring (Nofita, Ngibad, & Rodli, 2022). Flavonoids are the most abundant phenolic compounds in all plants that can potentially be antiviral, antibacterial, anti-inflammatory, anticancer, antiallergic, and antioxidant (Aisyah & Ngibad, 2022). The potential of an extract of natural ingredients as an antioxidant is usually always related to flavonoids and total phenols contained in extracts, fractions, or pure/single compounds [(Sabarudin et al., 2021)(Zhang et al., 2021)(Maigoda, Judiono, Purkon, Haerussana, & Mulyo, 2022).

The part of the purple passion fruit peel extracted using ethanol solvent has been widely researched about its antioxidant potential [8], [(Nazliniwaty, Harun, Putra, & Nerdy, 2020)](Xiong et al., 2019)[10](Musika et al., 2021). In addition, other reports suggest that the leaves and stem of P. edulis have the potential for antitumor, antimicrobial, and antioxidant activities (Taïwe & Kuete, 2017). The part of the yellow passion fruit seed also has a total polyphenol content that correlates with an antioxidant capacity (de Santana et al., 2017). In addition, studies in vitro exhibit promising anti-inflammatory and antioxidant potential of yellow passion fruit peel extract (Baseggio et al., 2022).

The solvent used in the extraction process determines the type and quantity of flavonoids in an extract of natural materials. Methanol solvents have been widely used in the maceration process of natural materials that produce optimal total phenol levels and show good antioxidant activity results [(Saravana Kumar & Felicia, 2015)(Mohamed, Youssef, & El-Shahir, 2022). In the methanol extract of the leaves of Goniothalamus wightii, there is a high content of phenols and flavonoids compared to other extracts (Palani et al., 2020). In addition, many studies have used methanol concentrations of 70%. A 70% methanol solvent can extract flavonoids and total phenolics from plants (Hazra, Biswas, & Mandal, 2008). In addition, total flavonoids are more maximally extracted in 70% methanol solvents than in 70% acetone solvent and 70%.t (El Mansouri, Palma Lovillo, El Farissi, Oufdou, & Brigui, 2020). In addition, 70% methanol extract from the roots of Hemidesmus indicus also has the potential to be a natural source of antioxidants (S. Mandal, Hazra, Sarkar, Biswas, & Mandal, 2009).

This study aims to analyze the potential of 70% methanol extract from passion fruit peel as an antioxidant using the DPPH method. Furthermore, the 70% methanol extract was tested for flavonoid levels and total phenolic substances. This report is the first research about the antioxidant potential and total flavonoid and phenolic levels of passion fruit peel grown in Indonesia extracted using a 70% methanol solvent.

2. RESEARCH METHOD

2.1 Materials

Passiflora edulis was obtained from the Cigugur District, Kuningan Regency, West Java, Indonesia. Plant determination was carried out in the Biological Services Unit of the Science and Technology Faculty, UNAIR, Indonesia. The chemicals used include methanol (Merck), n-hexane (Merck), DPPH (Sigma-Aldrich), gallic acid (Merck), Folin-Ciocalteu reagent (Merck), quercetin (Sigma-Aldrich), demineralized water, sodium carbonate (Merck), and AlCl₃ (Merck). All reagents used were analytical grade.

2.2 Sample Preparation

The fresh purple and yellow P. edulis fruit peel were weighed and washed using clean water. Next, the samples were air-dried at room temperature. Then, the samples were blended and scrambled until the powder was formed with 60 mesh.

2.3 Extraction

One hundred grams of passion fruit peel powder were each macerated with 700 mL of 70% methanol solvent for 24 hours. Next, filtration is carried out to obtain filtrate and pulp. The filtrate was stored while the pulp was re-macerated using 700 mL of the new 70% methanol solvent. The maceration process is stopped when the pulp is already pale in color. Then, the filtrate is collected

to be concentrated with a vacuum rotary evaporator. The yield of each extract is calculated using the following equation:

% Yield =
$$\frac{\text{extract weight (g)}}{\text{sample weight(g)}} \times 100\%$$
 (1)

2.4 Antioxidant Assay

Each test sample solution and standard antioxidant was pipetted as much as 5 mL, mixed with 1 mL of 6 ×10⁻⁵ M DPPH in the test tube, and then homogenized with vortex for 1 minute. The incubation process was carried out for 30 minutes. The absorbance measurements were conducted at 515 nm using Genesys 10S UV-Vis Double Beam Spectrophotometry to obtain the sample's absorbance value. Next, the blank solution containing methanol in the DPPH solution was prepared and measured at the same wavelength to obtain the control absorbance value. The experiment was done with three repetitions. First, antioxidant activity was calculated using equation (2). Next, a graph of the relationship between the concentration of the sample (x-axis) and the percent inhibition of DPPH radicals (y-axis) was made. Finally, the calculation of IC₅₀ values was based on the formula of linear regression equations.

$$\% Inhibition of DPPH radicals = \frac{absorbance of control - absorbance of sample}{absorbance of control} \times 100\%$$
(2)

2.5 Determination of Total Flavonoid Content

Quercetin at various concentrations (5, 20, 40, 60, and 80 mg/L) was used as the standard solution to produce calibration curves. First, each methanol and n-hexane extract (25 mg) was dissolved with 25 mL methanol. Then, as much as 2.5 mL was pipetted and added with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate solution, and 2.8 mL of demineralized water. The incubation process was conducted for 22 minutes. The absorbance measurements were carried out at 444 nm. The total flavonoid level from each extract was revealed as mg of quercetin equivalent in extract weight (mg QE/g extract).

2.6 Determination of Total Phenolic Content

Gallic acid at various concentrations (10, 20, 30, 40, and 50 mg/L) was used as the standard solution to produce calibration curves. First, 250 mg of each extract was dissolved with 250 mL of methanol solvent. Then, as much as 1 mL was pipetted and inserted into a volumetric flask. The solution was added with 0.4 mL of Folin-Ciocalteu reagent and let for 8 minutes. Then, the solution was added with 4 mL of 7% Na₂CO₃ and demineralized water to the limit of the mark. After 2 hours, the absorbance measurements were conducted at 765 nm. Finally, the total phenolic level was revealed as the equivalent gram of gallic acid in extract weight (mg GAE/g extract).

3. RESULTS AND ANALYSIS

3.1. Extraction

In this study, the purple and yellow passion fruit peel powder were extracted using the maceration method. The advantages of the maceration method over other extraction methods are simplicity in extraction procedures and equipment and low operational costs. Further, active compounds that are not heat resistant will not be damaged because the maceration method does not require heating (Nofita et al., 2022). The solvent used to extract purple and yellow passion fruit peel powder is 70% methanol. The maceration process is carried out as much as possible to maximize the withdrawal of all active compounds so that the extracts obtained become more (Babota et al., 2022)

(Chuo et al., 2020). The results of the yield of passion fruit peel extract are shown in **Table 1**. The percentage of 70% methanol extract of purple and yellow passion fruit peel is 14.94 and 14.40%, respectively. The results showed that the yield of 70% methanol extract of purple and yellow passion fruit peel was almost the same.

The report on the results of the second amendment of this extract shows that the 70% methanol solvent can maximize the maceration process of purple and yellow passion fruit peel powder. Other studies have also shown that methanol solvents are more efficient than acetone solvents (Benchikh & Louailèche, 2014). This 70% methanol solvent maximizes the withdrawal of flavonoid and total phenol phytochemical compounds from purple and yellow passion fruit peels. Some natural ingredients studied for total flavonoid and phenolic levels and antioxidant potential using a 70% methanol solvent are *A. reptans* L. (Göger, Köse, Demirci, & Göger, 2021), *Populus alba* (Kuchukhidze, Jokhadze, Murtazashvili, & Mshvildadze, 2011), and leaves of *C. crista* (N. Mandal, Mandal, Hazra, Sarkar, & Biswas, 2011).

Table 1. The extract yield of passion fruit peel				
Types of Extracts	Weight extract (g)	Sample weight (g)	% Yield	
Extract of purple passion fruit peel	100	14.94	14.94	
Extract of yellow passion fruit peel	100	14.40	14.40	

3.2. Total Flavonoid and Phenolic Content

Extract of purple and yellow passion fruit peel with yield obtained from the previous procedure further determined the total flavonoid and phenol levels. Determination of flavonoid levels is carried out using a standard solution of quercetin. The quercetin is a class of flavonoid compounds that effectively inhibit free radicals. Quercetin is equivalent to mg QE/g of dry matter and is a standard for determining flavonoid levels (Saddiqe, Naeem, Hellio, Patel, & Abbas, 2020) (M. Sulaiman, Mohammed, & Manan, 2015). The method of measuring flavonoids used is the colorimeter method. The reagent AlCl₃ will cause the formation of a stable acid complex and cause the color of the test solution to become more yellow, which can be measured absorbance at 444 nm (Aisyah & Ngibad, 2022). On the other hand, the total phenolic level is determined by using a standard solution of gallic acid. The advantage of this standard solution over others is that gallic acid can increase the effectiveness in forming molybdenum-tungsten complex compounds with Folin-Ciocalteu reagents so that the test solution becomes yellow. Not enough there, the reaction was continued with adding a solution of Na₂CO₃ which caused the test solution to change color from yellow to blue so that its absorption could be measured at 765 nm (Nofita et al., 2022).

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Total flavonoid levels (mg QE/g extract)		Total phenolic le	Total phenolic levels (mg GAE/g extract)	
Extract of purple	Extract of yellow	Extract of purple	Extract of yellow passion	
passion peel	passion peel	passion peel	peel	
1.2824	1.9775	15.8	3.6	

Table 2. Total flavonoid and phenol content of passion fruit peel extract

The results of measuring total flavonoid and phenol levels in 70% methanol extract of passion fruit peel are shown in **Table 2**. The flavonoid content in the 70% methanol extract of purple passion fruit peel (1.2824 mg QE/g extract) is smaller than the peel of the yellow passion fruit (1.9775 mg QE/g extract). In contrast, the total phenolic content in 70% methanol extract of purple passion fruit peel (15.8 mg GAE/g extract) is greater than that of yellow passion fruit peel (3.6 mg GAE/g extract). These results showed that 70% methanol extract purple passion fruit peel contains more flavonoid compound group while 70% methanol extract yellow passion fruit peel contains a more phenolic compound group. Thus, this study emphasizes that there are different flavonoids and total phenols in the type of passion fruit.

Several other studies have shown that a 70% methanol solvent can extract flavonoids and total phenolics from plants. The total flavonoid and phenol levels in the 70% methanol extract from the bark of Spondias pinnata were 350.5 ± 0.004 mg/ml and 91.47 ± 0.004 mg/ml, respectively (Hazra et al., 2008). A 70% methanol solvent has also been used to extract flavonoids and total phenols from the fruits of T. chebula, T. belerica, and E. officinalis. The flavonoid levels in the fruits of Terminalia chebula, Terminalia belerica, and Emblica officinalis are 219.30 ± 0.01 138.30 \pm 0.01 176.00 \pm 0.01 mg/ml, respectively, while the total phenol levels in the fruits of T. chebula, T. belerica, and E. officinalis are 127.60 ± 0.001 , 133.00 ± 0.003 , 215.60 ± 0.004 mg/ml (Hazra, Sarkar, Biswas, & Mandal, 2010). Other reports also indicate that the total flavonoids in the 70% methanol extract of Hordeum vulgare L. are more significant than the 70% acetone extract and 70% ethanol extract (El Mansouri et al., 2020). Furthermore, H. canum extracted using a 70% methanol solvent had a higher phenolic content (284.13 \pm 0.30 mg GAE/g extract) than aqueous extract (244.55 \pm 0.35 mg GAE/g extract) (Baldemir, Gökşen, Ildız, Karatoprak, G. Ş., & Koşar, 2017).

Flavonoids are one of the essential polyphenols classes with hydroxyl groups in different positions and are soluble in water (Kumar et al., 2021). The flavonoids group are natural compounds with several pharmacological characteristics, such as antioxidant, anticarcinogenic, antibacterial, and antiviral (Jo, Kim, Kim, Shin, & Kim, 2019). Flavonoids act as antioxidants by inhibiting ROS radicals (Tavsan & Kayali, 2019) (Kim & Je, 2017). In addition, total flavonoids can also reduce blood viscosity, increase serum lipids, and prevent cerebrovascular diseases of the heart (Wang, He, Zhang, & Li, 2021). On the other hand, phenolics are the largest phytochemicals group with great potential for antioxidant activity [(Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018) (C. T. Sulaiman & Balachandran, 2012). Phenolics exhibit bioactivity as anticancer, anti-inflammatory, antibacterial, antidiabetic, anti-analgesic, antiallergic, and anti-Alzheimer's (Nurhasnawati et al., 2019).

3.3. Antioxidant Assay

The potency of 70% methanol extract of passion fruit peel containing flavonoids and total phenols as antioxidants were determined using the DPPH method. Results of inhibition of DPPH free radicals from 70% methanol extract of purple and yellow passion peel at extract concentrations of 5, 20, 40, 60, and 80 mg/L can be seen in **Table 3**. The concentration of the extract that produces the most considerable percent inhibition of DPPH free radicals is 70% methanol extract. 70% methanol extract at a concentration of 80 mg/L resulted in more significant percent inhibition of DPPH free radicals (72.80 %) than yellow passion fruit (64.91 %). Similarly, 70% methanol extract at a concentration of 5 mg/L produces a more significant percent inhibition of DPPH free radicals (55.55%) than yellow passion fruit (52.04%). On the other hand, ascorbic acid provides the most significant percentage of DPPH free radical propagation at a concentration of 80 mg/L, which is 94.12%. The large extract concentration causes a more considerable percent radical inhibition of DPPH for the whole type of testing. In in vitro antioxidant tests, the advantages of the DPPH method compared to other methods are simple, easy, and few samples or reagents. The reaction mechanism in antioxidant tests using the DPPH method is that antioxidant compounds give hydrogen atoms to DPPH free radicals (Ngibad & Lestari, 2020).

The concentration of test solution (mg/L)	Inhibition percentage (%)		
	Ascorbic acid	Extract of purple	Extract of yellow
		passion peel	passion peel
5	49.58	55.55	52.04
20	59.66	59.06	57.30
40	71.43	62.86	59.94

Table 3. Results of the antioxidant assay of extract of passion fruit peel

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The concentration of — test solution (mg/L)				
	Ascorbic acid	Extract of purple	Extract of yellow	
		passion peel	passion peel	
60	81.51	68.12	62.28	
80	94.12	72.80	64.91	

The results can inform scientifically that methanol extract of 70% purple passion peel has better in vitro antioxidant potential than yellow. This fact is supported by determining the phenolic content of 70% methanol extracts of purple passion fruit peel larger than the yellow. In vitro, antioxidant activity in this report can be attributed to extracts' total flavonoid and phenol content. Hydroxyl and carbonyl groups of phenolic compounds in 70% methanol extract of purple and yellow passion peels can inhibit DPPH free radicals and produce phenol radicals (Zhao et al., 2020) (Urmi et al., 2013). The 70% methanol solvent used in the *C. cajan* leaf extraction process has antioxidant and free radical antidote properties (Sarkar, Hazra, Mandal, Biswas, & Mandal, 2009). In addition, 70% methanol extract from the roots of *Hemidesmus indicus* also has the potential to be a potential source of natural antioxidants (S. Mandal et al., 2009). *Gymnema sylvestre* leaves extracted using a 70% methanol solvent are also potent antioxidants that can inhibit free radicals (Sarkar, Hazra, Biswas, & Mandal, 2009).

4. CONCLUSION

The percentage of 70% methanol extract of purple and yellow passion fruit peel is 14.94 and 14.40%, respectively. The flavonoid level in the 70% methanol extract of purple passion fruit peel (1.2824 mg QE/g extract) is smaller than the peel of the yellow passion fruit (1.9775 mg QE/g extract). In contrast, the total phenolic content in 70% methanol extract of purple passion fruit peel (15.8 mg GAE/g extract) is greater than that of yellow passion fruit peel (3.6 mg GAE/g extract). The concentration of the extract that produces the most considerable percent inhibition of DPPH free radicals is 70% methanol extract of purple passion fruit peel. 70% methanol extract of purple passion fruit peel at a concentration of 80 mg/L resulted in a more significant percent inhibition of DPPH free radicals (72.80 %) than yellow passion fruit (64.91 %). Similarly, 70% methanol extract of purple passion fruit peel at a concentration of 5 mg/L produces a more significant percent inhibition of DPPH free radicals (55.55 %) than yellow passion fruit (52.04 %).

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