Proceedings of the International Conference on Economics, Education, Technology, and the Env
Vol. 3, No. 1, July 2025
https://prosiding.utp.ac.id/index.php/ICEETE

Antioxidant Activity Test of Purple Passion Fruit Peel Extract (*Passiflora edulis Sims)* Using The DPPH Method

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ABSTRACT

Skin damage induced by free radicals, particularly from ultraviolet (UV) radiation, is a major contributor to degenerative diseases in Indonesia. Antioxidants play a vital role in mitigating this damage by neutralizing free radicals through electron donation, thereby preventing oxidative stress in cells and tissues. The peel of Passiflora edulis Sims (purple passion fruit), an underutilized agricultural byproduct, contains natural antioxidant compounds such as polyphenols, flavonoids, and vitamin C. This study evaluated the antioxidant activity of an ethanol extract derived from Passiflora edulis Sims peel using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, analyzed via UV-Vis spectrophotometry. The extraction process employed maceration with 96% ethanol, and antioxidant efficacy was quantified by measuring the IC_{50} value. Phytochemical screening revealed the presence of phenolic compounds, flavonoids, alkaloids, tannins, saponins, and steroids in the extract. The results demonstrated that the purple passion fruit peel extract exhibited an IC_{50} of 188.4 ppm, indicating weak antioxidant activity compared to the strong activity of vitamin C (IC_{50}= 4.36 ppm). These findings suggest that while the ethanol extract of Passiflora edulis Sims peel possesses measurable antioxidant properties, its activity is significantly lower than that of vitamin C. Further research is warranted to optimize extraction techniques and explore other plant components for more potent antioxidant sources.

KEYWORDS: DPPH method, antioxidant, flavonoid, purple passion fruit peel_ extract, spectrophotometer_UV Vis

INTRODUCTION

Degenerative diseases have become a serious health concern and a leading cause of mortality in Indonesia. Excessive exposure to ultraviolet (UV) radiation is a major contributor to cellular and tissue damage due to the generation of free radicals. Prolonged UV exposure can also increase the risk of long-term conditions such as cancer. Therefore, counteracting the threat of free radicals—which cause skin damage—is crucial (A. N. Sari, 2005).

Antioxidants are compounds capable of donating one or more electrons to free radicals, thereby inhibiting their harmful reactions and preventing the formation of new free radicals (Amnestiya et al., 2023). These compounds protect cells from oxidative damage by neutralizing reactive molecules. Notable antioxidant phytochemicals include polyphenols, flavonoids, vitamin C, vitamin E, and β-carotene (Amnestiya et al., 2023).

Passion fruit (*Passiflora spp.*) is widely cultivated in South America, Oceania, Africa, Asia, and Indonesia. However, its peel remains underutilized, often discarded as agricultural waste. Recent studies suggest that purple and yellow passion fruit peels exhibit significant antioxidant properties (Nazir et al., 2022).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a common method for evaluating antioxidant activity due to its simplicity, rapidity, and minimal sample requirements (T. M. Sari et al., 202). DPPH

produces a strong absorption peak at 517 nm, appearing as a dark violet solution. Free radical scavengers decolorize DPPH in proportion to the number of electrons absorbed (Lung & Destiani, 2007).

Phytochemical screening of purple passion fruit has revealed the presence of flavonoids, alkaloids, tannins, and saponins (Septiningrum et al., 2024). Further studies by Septiningrum et al. (2024) identified additional bioactive compounds, including phenolics, steroids, and saponins. Building on these findings, this study aims to assess the antioxidant activity of purple passion fruit peel ethanol extract using the DPPH method**.**

The research seeks to optimize the use of agricultural waste while providing scientific evidence for the health benefits of purple passion fruit peel.

METHODOLOGY

Research Design .

This study employs a quantitative experimental design to evaluate the antioxidant activity of purple passion fruit (*Passiflora edulis* Sims) peel using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

Population and Sample

Population and sample used .

Population: The total set of objects or subjects with specific characteristics defined for analysis (Suriani et al., 2023). In this study, the population consists of purple passion fruit peels. Sample: A subset of the population selected for observation (Suriani et al., 2023). Here, the sample is ethanolic extract of purple passion fruit peel.

Tools or Instruments Used

Tool

Maceration bottle, measuring cup (*pyrex*), *rotary evaporator* (Biobasi), stirring rod (*pyrex*), test tube (*pyrex*), dropper pipette, funnel (*pyrex*), dropper plate, measuring flask, *UV-Vis spectrophotometer* (Genesi), vial, aluminum foil, analytical balance, filter paper, cotton, evaporator cup, scale pipette, micro pipette, waterbatch, spatula, measuring flask (*pyrex*), glass beaker (*pyrex*).

Material

The materials used were passion fruit peel, 96% ethanol, chloroform, distilled water, HCl, $FeCl_3$, acetic acid, H_2SO_4, ammoniacal chloroform, 2N sulfuric acid, Wagner reagent, Dragendorf reagent, PA ethanol, vitamin C (Merck), DPPH (Merck).

Data Analysis Methods .

1. Direct Observation: The absorbance values of the DPPH solution mixed with the peel extract were measured using a UV-Vis spectrophotometer.
2. Calculation of Antioxidant Activity:
   - The percentage of radical scavenging inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A\ control - A\ sampel}{A\ control}\ x\ 100\ \%$$

     where *A*control = absorbance of DPPH without sample, and *A*sample = absorbance of DPPH with sample.
   - IC_{50} values (concentration required to inhibit 50% of DPPH radicals) were derived from the linear regression equation y=bx+a where: *x* = extract concentration (µg/mL or ppm), *y* = percentage inhibition.

RESULTS AND DISCUSSION

Plant Determination

Authentication was conducted to verify the botanical identity of the research material and prevent errors in sample collection or potential contamination with other plant species. The taxonomic identification was performed at the Biology Learning Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University**.** The results confirmed that the plant material used in this study was indeed *Passiflora edulis* Sims (purple passion fruit).

Purple Passion Fruit Peel Extraction

The extraction process employed maceration using 96% ethanol as the solvent. Ethanol was selected due to its polar properties**,** accessibility, and universal suitability for phytochemical extraction (Putri & Mahfur, 2023). The process yielded 39.09 grams of concentrated extract. The percentage yield was calculated as 10.04%**.** The extract yield obtained in this study (10.04%) showed close agreement with the findings of Azzahrah et al. (2023), who reported a 11.04% yield from purple passion fruit peel. Comparable yields have been documented for other passion fruit components: 10.01% for seed extracts (Zuriyah et al., 2024) and 11.9% for fruit pulp extracts (Sudarwati et al., 2024). The 10.04% yield observed in this study can be considered satisfactory as it exceeds the 10% threshold and aligns closely with values reported in previous literature. This consistency suggests that the extraction protocol was properly optimized and reproducible.

Table 1. Results of % Extract Yield

Sample	Weight of Simple Drugs	Extract Weight	% Yield
Purple Passion Fruit Skin	389 grams	39.09 grams	10.04%

Table 2. Organoleptic Test Results

Extract Form	Flavor	Color	Smell
Thick Liquid	Bitter	Dark brown	Passion Fruit Specialty

Table 3. Water Content Test Results

Sample	Results
Ethanol extract of purple passion fruit peel	2.64%

Phytochemical Screening Test Results

Phytochemical testing in this study included flavonoid, tannin, phenolic, saponin, terpenoid and steroid, and alkaloid tests. The results obtained were:

Table 4. Phytochemical Screening Test Results

Compound	Treatment	Results	Information
Flavonoid	2 drops of test solution in the water layer were added with 2 drops of HCl (p) and Mg metal powder.	There is a color change from light yellow to orange.	+
Tannin	2 drops of water layer added 3 drops of $FeCl_3$.	There is a color change to dark blue to blackish	+
Phenolic	2 drops of water layer added 3 drops of $FeCl_3$.	There is a color change to dark blue to black.	+
Saponin	The water layer is shaken	Foam formation occurs for 15 minutes.	+
Terpenoids and Steroids	The chloroform layer is filtered with norit, then 3 drops are taken, 2 drops of anhydrous acetic acid are added,	There is a green color change.	+ steroid

	and H_2SO_4 (p) is added.		
Alkaloid	2 drops of the chloroform layer were added with ammoniacal chloroform and 1 drop of 2N sulfuric acid, shaken and the top layer was taken and 2 drops of Wagner reagent were added.	After adding Dragendorf's reagent, a red-orange precipitate forms, and with Wagner's reagent, a brown color forms.	+

Interpretation of Results:
(+) Positive **:** Compound is present
(-) Negative **:** Compound is not detected

Phytochemical screening is a qualitative analysis method used to identify bioactive chemical compounds in plant or animal samples, either in whole or partial extracts. This test serves as a preliminary indicator for discovering novel pharmacologically active compounds, potentially leading to the development of new antibacterial, antiviral, or therapeutic agents. The phytochemical screening of purple passion fruit (*Passiflora edulis* Sims) peel extract revealed the presence of flavonoids, saponins, tannins, steroids, and alkaloids.

Results of Analysis of Antioxidant Compound

a. Maximum Wavelength Determination
The sample absorbance was measured using a UV-Vis spectrophotometer at the 517 nm wavelength**,** corresponding to the peak absorption of DPPH radicals.

b. DPPH Control Preparation
Procedure**:**
1) 1 mL of DPPH solution was pipetted into a volumetric flask.
2) The volume was adjusted to 5 mL with analytical-grade ethanol (p.a.)**.**
3) Absorbance was measured at 517 nm to establish the baseline DPPH activity.

c. Vitamin C (Ascorbic Acid) Reference Solution

Table 5. Results of vitamin C comparison solution data

Conc (ppm)	% Inhibition		
	R1	R2	R3
1	11.52	11.63	14.54
2	20.04	26.47	26.37
4	40.02	42.51	40.83
6	63.12	78.29	79.41
8	90.34	88.14	88.03

The calibration curves for vitamin C testing were constructed to determine IC_{50} values, yielding the following regression equations: replicate $y = 11.217x-2.0456$ and $R^2 = 0.9943$, replicate 2 : $y = 11.339x + 1.7905$ and $R^2 = 0.9726$ and replicate 3, $y = 11.121x + 3.1326$ and $R^2 = 0.9632$.

d. Purple Passion Fruit Peel Ethanol Extract

Table 6. Results of Data on Ethanol Extract of Purple Passion Fruit Peel

Concentration (ppm)	% Inhibition		
	R1	R2	R3
1	27.41	28.06	26.06
2	34.57	38.75	42.39
4	49.07	47.02	54.16
6	67.39	69.47	73.54
8	79.73	79.46	82.91

The calibration curves for sample testing produced these regression equations: replicate 1 $y = 0.1529x + 19.535$ and $R^2 = 0.9968$, replicate 2 : $y = 0.1479x + 21.501$ and $R^2 = 0.9794$ and replicate 3, $y = 0.1585x + 22.529$ and $R^2 = 0.9733$.

e. **Antioxidant Activity Test Measurement**

Evaluating antioxidant activity in plants is essential to verify their capacity to scavenge free radicals. This study utilized purple passion fruit (*Passiflora edulis* Sims) peel as the test material. Passion fruit contains vitamin C, which enhances immune function and serves as a natural antioxidant (Susanti & Putri, 2014). Antioxidant activity measurements were conducted using UV-Vis spectrophotometry at a wavelength of 517 nm, with the results presented in the following table:

Table 7. Results of antioxidant activity measurements

Antioxidant Activity in IC_{50} (ppm)				
Sample	**R1**	**R2**	**R3**	**Mean IC_{50} ± SD**
Ethanol extract of purple passion fruit peel	199.24	192.69	173.31	188.41 ± 11.00
Vitamin C	4.63	4.25	4.21	4.36 ± 0.19

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was conducted to evaluate the sample's effectiveness in inhibiting stable DPPH radicals through hydrogen atom transfer. Samples containing antioxidant compounds reduce DPPH to DPPH-H. This reduction is characterized by a visible color change from violet to yellow. The decrease in DPPH color intensity corresponds with a reduction in DPPH absorbance. Higher antioxidant activity in the sample results in greater fading of the DPPH color (Aryanti et al., 2021). The parameter used to assess antioxidant activity is the Inhibition Concentration (IC_{50}), which represents the concentration of an antioxidant required to neutralize 50% of DPPH radicals. A lower IC_{50} value indicates higher antioxidant potential of the compound (Lim, 2012). The test results showed that the purple passion fruit peel extract had an IC_{50} value of 188.41 ppm, while the vitamin C reference solution showed 4.36 ppm. This indicates that the purple passion fruit peel extract has relatively weak antioxidant activity compared to the positive control (vitamin C). In previous research by T.M. Sari et al. (2021), the antioxidant activity of koyal passion fruit peel extract using the DPPH method was classified as strong, with an IC_{50} value of 53.34 ppm. Another study by Fitria and Ngibad (2022) reported that a 1:1 combination of 96% ethanol extracts from purple and yellow passion fruit peels demonstrated the highest antioxidant activity (IC_{50}=12.46 mg/L), outperforming ascorbic acid (IC_{50}=24.47 mg/L). These variations in research findings may be attributed to differences in the geographical origin of samples, solvent types used, and their subsequent effects on secondary metabolite content and compound concentrations (Utomo et al., 2020).

CONCLUSION

The study demonstrated that: The ethanolic extract of purple passion fruit peel (*Passiflora edulis* Sims) exhibited weak antioxidant activity (IC_{50} = 188.41 ppm). Vitamin C (positive control) showed strong antioxidant activity (mean IC_{50} = 4.36 ppm)

ACKNOWLEDGMENT

I would like to thank all parties who have helped me, especially my supervisors 1 and 2 who have guided me during the research in Universitas Sahid Surakarta

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