

## Ethanol solvent and pH effect on antioxidant activity of purple sweet potato (*Ipomoea batatas* L.)

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### ABSTRACT

Air pollution induces the production of reactive oxygen species (ROS), which can cause tissue damage when excessive. Antioxidants help counteract this damage, and purple sweet potatoes, rich in anthocyanins, are a promising natural antioxidant source. This study aimed to determine the effect of solvent acidity variations on the antioxidant activity of purple sweet potato (PSP) tuber extract and identify the optimal pH condition. Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay, ethanol solvents at different pH levels (non-acidified ethanol, pH 2.5, pH 2, and pH 1.5) were tested. The results showed that higher acidity enhanced antioxidant activity, with the strongest activity observed at pH 1.5, yielding an IC<sub>50</sub> value of 9.74±0.23 ppm. Although less potent than Vitamin C (IC<sub>50</sub> 1.22±0.04 ppm), the extract demonstrated significant potential as a natural antioxidant source. Further studies on anthocyanin content are recommended to better understand its contribution to antioxidant activity.

**Keywords:** acidity, extraction, purple sweet potato, anthocyanin, antioxidant

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## INTRODUCTION

Air pollution is currently the world's greatest environmental health issues and has led to an increase in the incidence of disease and is responsible for 7 million deaths each year (Barbier et al., 2023). Indonesia is ranked 14th as the country with the worst air quality within the world, and ranked first in Southeast Asia for four consecutive years from 2020 to 2023 (IQAir, 2024). The body's physiological response to pollutants is the production of free radicals such as ROS where immoderate ROS production can cause damage to frame tissues (Nurzaman et al., 2024). Therefore, a pharmacological approach is needed for the prevention and overcoming of the effects of air pollution through the use of antioxidant compounds (Miller, 2022).

PSP is one of the plants known to contain antioxidants (Arifuddin, 2018; Guclu et al., 2023). The main bioactive contents in PSP tubers are phenolic content and anthocyanins (Pazos et al., 2022; Przybył et al., 2022; Sun et al., 2019) and the content of anthocyanins is the most influential component in terms of antioxidant activity (Azman et al., 2022).

In the isolation of phenolic compounds, including anthocyanins, extraction is the most important step (Azman et al., 2020). The use of acidified organic solvents can be an option in the anthocyanin extraction (Azman et al., 2022; Ferreira et al., 2020; Muangrat et al., 2017). In a study by (Pratiwi & Priyani, 2019), it was found that the pH variation of the solvent during the extraction of PSP tubers affected the anthocyanin content, with the more acidic or lower the pH of the solvent, the higher the anthocyanin content. However, research on antioxidant activity has not been carried out, so the authors are interested in conducting research on the effect of solvent at different pH variations in the extraction of PSP tubers on antioxidant activity.

## MATERIALS AND METHOD

### Tools and Materials

The substances used on this look at were PSP tubers harvested after 3 months of planting from Batubeulah village, Cisaruni, Padakembang subdistrict, Tasikmalaya Regency, West Java.

### Methods

#### Preparation of purple sweet potato tuber extract

Fresh purple sweet potatoes were harvested after 3 months of planting, then plant determination was carried out at SITH Bandung Institute of Technology No. 1891/ITI.C11.2/TA.00/2024. Fresh samples were then sorted, washed, chopped, dried and pulverized until simplicia powder was obtained. Extraction was done by maceration method for 3x24 hours with solvent change every 24 hours. The maceration process was carried out with 4 variations of acidity (96% ethanol solvent without acidification, 96% ethanol solvent at pH 2.5, 96% ethanol solvent at pH 2, and 96% ethanol solvent at pH 1.5) with powder to solvent ratio of 1:10. Solvent acidification was carried out by adding HCl. The resulting macerate was then evaporated on a rotary evaporator followed by concentrated in a water bath to give a thick extract.

#### Antioxidant activity assay

Instruction of DPPH stock solution: Weighed as a great deal as 50 mg of DPPH powder, then dissolved with methanol p.a. in a 50 mL volumetric flask and homogenized to gain a DPPH solution concentration of 1000 ppm (Moilati et al., 2020).

Preparation of blank solution: Diluted 1000 ppm DPPH stock solution to 50 ppm by using taking 5 mL of inventory solution then dissolved with methanol p.a. in a 100 mL volumetric flask. the solution changed into allowed to stand in a darkish room for 30 minutes after which measured the absorbance at a maximum wavelength with a UV-Vis spectrophotometer (Suharyani et al., 2022).

Determination of Maximum Wavelength ( $\lambda$ ): Pipetted as much as 3 mL of 50 ppm DPPH solution then measured the absorbance at a wavelength of 400-800 using a UV-Vis Spectrophotometer.

Determination of Incubation Time of Vitamin C and PSP Tuber Extract: 1 mL of 10 ppm vitamin C solution and 10 ppm extract solution were pipetted, then 2 mL of 50 ppm DPPH solution was added to each. Absorbance was observed at the maximum wavelength obtained from minute 0 to minute 30 with an interval of every 5 minutes. The operating time was determined when a stable absorbance was obtained (Hidayati et al., 2023; Larasati et al., 2023).

Preparation of vitamin C solution and PSP Tuber Extract solution: A inventory vitamin C solution with a awareness of 1000 ppm become prepared through weighing 50 mg of vitamin C and dissolving it in 50 mL of methanol p.a. in a volumetric flask. Furthermore, series solutions with concentrations of 4, 6, 8, 10 and 12 ppm were prepared in triplicates to ensure reproducibility (Nofita et al., 2021). The manner started via weighing 100 mg of PSP tuber extract and dissolving it with 20 mL of methanol p.a. to prepare a inventory solution with a attention of 5000 ppm. a sequence of solutions with concentrations of 2, 4, 6, 8, and 10 ppm became then organized, every in triplicate, to enhance accuracy (Safari et al., 2020). 1 mL of each series solution was taken and blended with 2 mL of DPPH solution, homogenized, and stored in a dark room for the duration of the operating time. The absorbance changed into then measured on the maximum wavelength.

### Data Analysis

The antioxidant activity was determined by regression analysis. Percent inhibition was determined using the formula:

$$\text{inhibisi} = \frac{\text{control abs} - \text{sample abs}}{\text{control abs}} \times 100\% \dots\dots\dots(1)$$

The percent inhibition value at each concentration was then plotted on a linear curve against sample concentration. The IC50 value was then calculated from the linear equation on the curve.

## RESULT AND DISCUSSION

The first step in processing simplisia is plant determination as the initial stage of identify plant identity and ensuring the type and name of the plant used as a sample (Sawiji et al., 2020). The determination results, recorded with the number 133/U/FIK-UP/03/2024, confirm that the identity of the sample is appropriate, namely PSP with the Latin name *Ipomoea batatas* L. Fresh PSP samples weighing 12 kg were harvested. The samples were washed thoroughly to remove impurities, followed by sorting to separate good and undamaged samples, resulting in 11.6 kg of selected material. The sorted samples were then dried and pulverized to produce 3.4 kg of *simplisia*.

The extraction turned into achieved through maceration, as maceration is the simplest method of extraction (Bitwell et al., 2023) and does not cause any damage to the active compounds in the sample, as it does not involve any heating process at all (Wiraningtyas et al., 2020), since anthocyanin compounds have properties that are not resistant to heating at high temperatures (Tan et al., 2022). HCl was chosen as the acidifier because several studies have shown that HCl is the most stable and effective acidifier for denaturing cells and dissolving anthocyanin compounds (Damayanti et al., 2020; Pratiwi & Priyani, 2019; Rismiarti, 2022; Ursu et al., 2023). The extraction was made into 4 variations based on the acidity level, namely at pH 2.5, pH 2 and pH 1.5 and extraction without acidification.

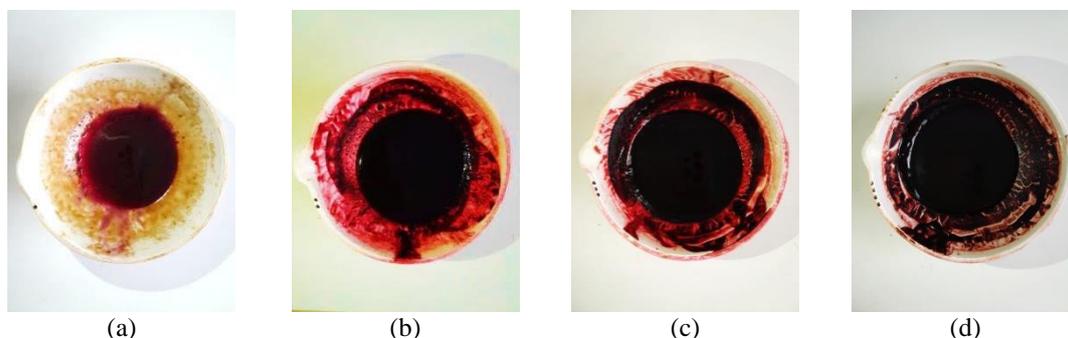
**Table I. Purple sweet potato tubers extraction yield**

Solvent	Simplisia (g)	Thick extract (g)	Yield (%)
Non-acidified	300	15.49	5.16
pH 2.5	300	29.60	9.87
pH 2	300	30.78	10.26
pH 1.5	300	31.82	10.61

*Ethanol solvent and ... (Nurzaman et al.,)*

The results of the extraction of PSP tubers show that the more acidic or the lower pH value of the solvent during extraction, the higher the yield. This indicates that the degree of acidity significantly influences the efficiency of anthocyanin extraction (Azman et al., 2022; Damayanti et al., 2020; Ferreira et al., 2020; Pratiwi & Priyani, 2019). Acidic conditions likely enhance the stability and solubility of anthocyanins, as these compounds are more stable in low-pH environments. This finding aligns with previous studies that highlight the importance of acidic solvents in maximizing anthocyanin extraction (Safari et al., 2020). Therefore, optimizing the pH of the solvent is a critical factor in obtaining high-quality extracts with greater anthocyanin content.

In addition to having an effect on the amount of yield, the acidity of the solvent also has an effect on the color of the extract (Damayanti et al., 2020; Rismiarti, 2022; Rundubelo et al., 2019). The results of the research show that the more acidic the solvent is, the more red the color of the extract will be, this is consistent with the literature where the lower pH state causes more anthocyanin pigments to be in the form of flavilium or oxonium cations that have a red color (Damayanti et al., 2020), this is an indication of the higher content of anthocyanin compounds (Ferreira et al., 2020; Rismiarti, 2022; Widyastutik et al., 2022).



**Figure 1. Color of the extract produced (a) Non-acidified extract (b) pH 2.5 extract (c) pH 2 extract (d) pH 1.5 extract**

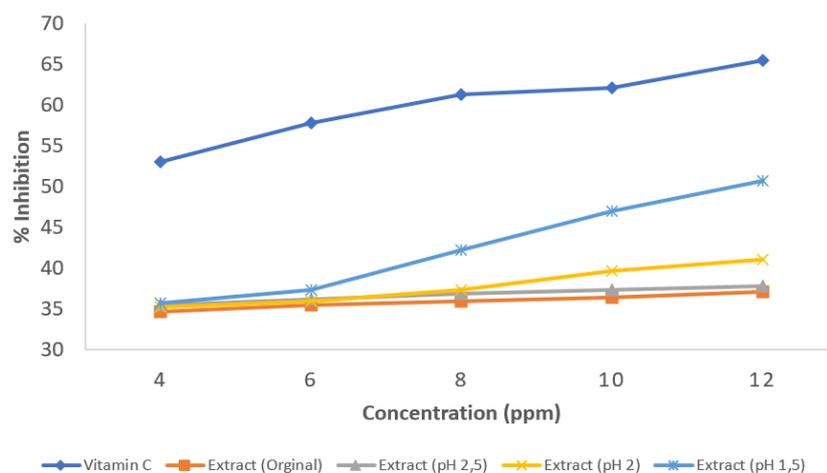
The addition of  $H^+$  atoms from acidic solutions creates flavilium cations, these cations make anthocyanins very stable, and conversely, if the anthocyanins lose  $H^+$  atoms, it will cause the anthocyanins to hydrolyze, which will cause instability and form colorless to blue carbinol or chalcone structures (Ayun et al., 2022).

The extraction was made in 4 variations based on the acidity level, namely at pH 2.5, pH 2 and pH 1.5 and extraction without acidification. The results of the extraction of PSP tubers show that the more acidic the solvent during the extraction, the more yield will be produced. Solvent without acidification, solvent at pH 2.5, pH 2 and pH 1.5 yields are 5.16%, 9.87%, 10.26% and 10.61% respectively. The outcomes of the antioxidant activity assay confirmed that the more acidic the solvent used in the extraction, the greater the antioxidant activity. The extract without acidification has the least antioxidant activity with  $IC_{50}$  value is 56.00 ppm, acidified extract at pH 2.5, pH 2 and pH 1.5 showed stronger antioxidant activity with  $IC_{50}$  value werw 51.26 ppm, 21.70 ppm and 9.74 ppm, respectively.

The antioxidant activity check in this examine used the DPPH (*2,2-diphenyl-2-picrylhydrazil*) scavenging assay because this method has advantages including a rapid process, simpler, easier, lower cost, and does not use many chemical reagents (Gulcin, 2020; Ngibad et al., 2023; Tamunu et al., 2022). The first step is to determine the maximum wavelength of DPPH which aims to find the wavelength with the highest absorbance to achieve maximum sensitivity and minimize errors (Agustiarini & Wijaya, 2022). The results of the maximum wavelength test are obtained at 516 nm, the results are in accordance with the theoretical maximum wavelength of DPPH, which is 515-519 nm

(Sari et al., 2023). The purpose of determining the incubation time is to determine the time range for a stable reaction (Suharyani et al., 2022) and to determine the incubation time (Wulandari et al., 2020). Absorbance appears stable at 25 to 30 minutes for vitamin C, while the extract test shows a stable absorbance at 20 to 25 minutes for all extract variations.

Absorbance measurements were taken after the incubation process. The consequences display decreasing absorbance values because the concentration of the sample solution increases. This is in accordance with the Lambert-Beer Law, which states that there's a linear relationship among absorbance and sample concentration (Indrawati et al., 2022). After obtaining the absorbance value at each concentration, the percentage of inhibition was calculated. Percent inhibition describes the ability of the sample at a given concentration to reduce the DPPH radical reaction (Rosaini et al., 2019).



**Figure 2. Linearity curve of the sample Vitamin C, Non-acidified extract, pH 2.5 extract, pH 2 extract and pH 1.5 extract**

The determination of  $IC_{50}$  is obtained by substituting a linear equation where the y-coefficient of the linear data is 50 and the x-coefficient is the concentration used to reduce the free radical activity of DPPH (Indrawati et al., 2022; Sinala & Dewi, 2019). Figure 2 shows the linear regression curves of vitamin C and PSP tuber extract samples of all variations.  $IC_{50}$  is the value that indicates the sample concentration (ppm) that can reduce the DPPH oxidative reaction by 50% (Gulcin & Alwasel, 2023; Wulandari et al., 2020).

**Table 2. Results of antioxidant activity assay**

Sample	$IC_{50}$ (ppm)	Category*
Vitamin C	1.22±0.04 <sup>a</sup>	Very strong
Non-acidified extract	56.00±0.27	Strong
pH 2.5 extract	51.28±0.22 <sup>a</sup>	Strong
pH 2 extract	21.70±0.08 <sup>a</sup>	Very strong
pH 1.5 extract	9.74±0.23 <sup>a</sup>	Very strong

Description :  $IC_{50}$  categories \*(Haerani et al., 2019), (Wilujeung & Anggarani, 2021): Very strong (less than 50 ppm); Strong (range of 50-100 ppm); Moderate (range of 100-150 ppm); Weak (range of 150-200 ppm); Very weak (more than 200 ppm), a=Significant compared to non-acidified extract ( $p < 0.05$ ).

PSP tuber extract without acidification obtained the highest  $IC_{50}$  value of 56.00 ppm, which means that the extract without acidification has the lowest antioxidant activity in comparison to the extract

variants with acidification. The IC<sub>50</sub> value is higher than Safari's research (2020), which was 41.1 ppm (Safari et al., 2020) and lower than Arifuddin's research (2018) which was 65.35 ppm (Arifuddin, 2018). PSP tuber extract obtained using solvent extraction at pH 1.5 demonstrates very strong antioxidant activity, with an IC<sub>50</sub> value of 9.74 ppm. This result confirms that increased acidity of the solvent enhances the activity. The high activity at pH 1.5 can be attributed to the stability and solubility of anthocyanin compounds in highly acidic environments. Moreover, acidic conditions prevent the degradation of anthocyanins throughout the extraction procedure, ensuring the preservation of these potent antioxidant compounds. This finding emphasizes the important role of pH optimization in maximizing the functional properties of natural extracts.

The results of this research are in line with the literature that the acidity of the extraction has an effect on the antioxidant activity, where the more acidic the solvent is, the more the antioxidant activity will increase (Azman et al., 2022) caused by the acidification of the solvent will attract a large number of anthocyanin compounds in the sample (Arifuddin, 2018; Azman et al., 2020; Damayanti et al., 2020; Pratiwi & Priyani, 2019).

## CONCLUSION

The antioxidant assay demonstrated that the extract without pH adjustment showed lower antioxidant activity, with an IC<sub>50</sub> value of 56.00 ppm (strong category), compared to the extract at pH 1.5, which had significantly stronger activity with an IC<sub>50</sub> value of 9.74 ppm (very strong category). Variations in the pH of the solvent can affect the antioxidant activity where the more acidic the solvent will increase the antioxidant activity of the PSP tuber extract produced.

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