Antioxidant Activity of Ethanolic Extract and Aqueous Fraction of Purple Sweet Potato (*Ipomoea batatas* L.) Leaves Using DPPH Method

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Abstract. Oxidative stress, triggered by an imbalance between the production of Reactive Oxygen Species (ROS) and the body's antioxidant defence mechanisms, contributes significantly to various chronic diseases. This study aimed to compare the antioxidant activity of ethanolic extract and aqueous fraction of purple sweet potato (*Ipomoea batatas* L.) leaves using the DPPH method. Extraction was performed using maceration with ethanol, followed by liquid-liquid fractionation with n-hexane, ethyl acetate, and water. Phytochemical screening confirmed the presence of flavonoids in both samples. Antioxidant activity testing revealed IC50 values of 95.57 µg/mL for the ethanolic extract and 85.69 µg/mL for the aqueous fraction, indicating strong antioxidant activity for both samples. The aqueous fraction demonstrated superior antioxidant activity compared to the ethanolic extract, suggesting that the fractionation process successfully concentrated polar antioxidant compounds. The results indicate that purple sweet potato leaves, particularly the aqueous fraction, possess significant potential as a natural source of antioxidants.

Keywords: Antioxidant, aqueous fraction, DPPH, ethanolic extract, *Ipomoea batatas*

1 Introduction

Oxidative stress, characterised by an imbalance between the production of Reactive Oxygen Species (ROS) and the body's antioxidant defence mechanisms, plays a significant role in the pathogenesis of various chronic diseases, including cancer, diabetes, and cardiovascular disorders [1]. Free radicals, such as DPPH radicals with unpaired electrons, are highly reactive species that can damage cellular components, including lipids, proteins, and DNA. The administration of exogenous antioxidants represents a crucial approach to counteract these detrimental effects, with natural antioxidants from plants being generally preferred over synthetic alternatives due to their better safety profile [2].

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Purple sweet potato (*Ipomoea batatas* L.) leaves constitute an abundant yet underutilized agricultural resource. Previous phytochemical studies have identified this plant material as a rich source of bioactive compounds, particularly flavonoids that exhibit significant antioxidant properties [3]. These compounds function as effective radical scavengers through electron donation mechanisms, neutralizing free radicals [4]. Previous research has primarily focused on evaluating the activity of crude extracts, with particular emphasis on ethanolic extracts [5]. However, the complex mixture of compounds in crude extracts often leads to suboptimal and non-specific bioactivity.

Notably, no previous studies have specifically compared the crude ethanolic extract with the aqueous fraction of purple sweet potato leaves in terms of their antioxidant activity. Fractionation serves as an effective separation technique for concentrating target compounds based on polarity [6]. Aqueous, being a polar solvent, is expected to selectively concentrate polar antioxidant compounds from the leaves, particularly flavonoid glycosides. Therefore, a systematic comparison between crude ethanolic extract and its resultant aqueous fraction is essential to evaluate fractionation efficiency and identify the most potent antioxidant fraction.

The DPPH method remains widely employed for preliminary antioxidant assessment due to its simplicity, reliability, and rapid results [7]. This study aims to analyze and compare the antioxidant activity of the crude ethanolic extract and aqueous fraction of purple sweet potato (Ipomoea batatas L.) leaves using the DPPH method with the objective of identifying promising natural antioxidant sources and validating the effectiveness of fractionation.

2 Materials and Methods

2.1. Research Design

This study employed a quasi-experimental design with analytical descriptive approach. The research involved extracting *I. batatas* L. leaves using an analytical-grade ethanol solvent with the Ultrasound-Assisted Extraction (UAE) method, followed by sequential liquid-liquid fractionation. The obtained aqueous fraction was subjected to phytochemical testing and antioxidant activity assessment using UV-Vis Spectrophotometry.

2.2. Materials

Dry simplicia of *I. batatas L*. leaves were authenticated by the UPT Laboratory of Herbal Materia Medica, Batu City. Chemicals included analytical-grade ethanol, ethyl acetate, methanol, n-hexane (Smart-Lab brand), and quercetin (Sigma-Aldrich brand), all obtained from Nurra Gemilang. Additional materials included aquadest, magnesium powder, HCl, NaOH (Smart-Lab brand), and DPPH powder (Sigma-Aldrich brand), which were obtained from the Integrated Laboratory of the Faculty of Medicine at Universitas Islam Malang.

2.3. Equipment

Laboratory equipment included glassware, analytical balance (Shimadzu Type ATX224), ultrasonic bath cleaner (Rocker), vacuum buchner (Rocker), rotary vacuum evaporator (Heidolph Hei-VAP Core Standard G1), waterbath (Memmert WNB14RACK), oven (Binder Type ED 115), UV-Vis spectrophotometer (Shimadzu Single-beam UV-1280), and analogue vortex mixer (Ohaus Vxmnal).

2.4. Fractionation Procedure

The preparation of the aqueous fraction began with the extraction of *I. batatas* L. leaf powder using the UAE method with an analytical-grade ethanol solvent (1:15, % v/v) for 15 minutes [10]. The concentrated extract was then subjected to sequential liquid-liquid fractionation using n-hexane, ethyl acetate, and water in a separation funnel. The solution was shaken constantly for 15 minutes and allowed to settle until complete phase separation occurred. The aqueous fraction was collected and concentrated using a waterbath at 40°C until a thick fraction formed [11].

2.5. Phytochemical Screening

Phytochemical screening using three different reagents consistently confirmed the presence of flavonoids in both samples. Positive results with the Wilstater reagent indicated the presence of flavone and flavonol subgroups [8], while the Bate-Smith-Metcalfe reaction suggested the presence of anthocyanidin flavonoids [9]. The 10% NaOH test confirmed the presence of phenolic hydroxyl groups in flavonoid structures. These findings are consistent with established protocols for phytochemical identification of flavonoids [10].

2.6. Antioxidant Activity Testing

Test solutions of the aqueous fraction were prepared at concentrations of 60, 80, 100, and 120 µg/mL. One millilitre of each solution was mixed with 4 mL of a 40 µg/mL DPPH reagent (prepared by dissolving 40 mg in 100 mL of analytical-grade methanol). The mixtures were homogenized and incubated in the dark for 30 minutes. Absorbance was measured at λ 515.5 nm. The same procedure was applied to quercetin as a reference standard. Antioxidant activity was expressed as IC_{50} value, determined by substituting absorbance data into the linear regression equation derived from the relationship between concentration (x) and percentage inhibition (y) [9]. The percentage inhibition for each sample was calculated using the formula:

$$\%Inhibition = \frac{A0 - A1}{A0} x\ 100$$

Where:
A0 = Blank absorbance
A1 = Sample absorbance

2.7. Data Analysis

Statistical analysis in this study employed descriptive statistics. All experiments were performed in triplicate, and the data are expressed as the mean ± standard deviation. Research data were analyzed using Microsoft Excel 2021 for Windows.

3 Results and Discussion

3.1. Phytochemical Screening Results

Phytochemical screening using three different reagents consistently confirmed the presence of flavonoids in both samples. Positive results with the Wilstater reagent indicated the presence of flavone and flavonol subgroups [8], while the Bate-Smith-Metcalfe reaction suggested the presence of anthocyanidin flavonoids [9]. The 10% NaOH test confirmed the presence of phenolic hydroxyl groups in flavonoid structures. These findings are consistent with established phytochemical protocols for flavonoid identification [10]

Table 1. Phytochemical Screening Results of *I. batatas* L. Leaf Extracts

Sample	Phytochemical Test	Reagent	Result
Ethanolic Extract	Flavonoid	Wilstater	+
		Bate-Smith Metcalfe	+
		NaOH 10%	+
Aqueous Fraction	Flavonoid	Wilstater	+
		Bate-Smith Metcalfe	+
		NaOH 10%	+

Note: (+) Detected; (-) Not detected

3.2. Antioxidant Activity

The antioxidant activity testing revealed concentration-dependent increases in radical scavenging activity for both samples. The aqueous fraction demonstrated superior antioxidant activity with an IC50 value of 85.69 µg/mL compared to the ethanolic extract (IC50 95.57 µg/mL). Both values are classified as strong antioxidant activity according to established parameters [11].

Table 2. Antioxidant Activity Test Results

Sample	C(µg/ mL)	n	%Inhibition ($\bar{x}$ ± SD)	IC50 (µg/mL)
Ethanolic Extract	60	3	28,47 ± 0,09	95,57 ± 0,003
	80	3	41,57 ± 0,17	
	100	3	53,92 ± 0,09	
	120	3	63,54 ± 0,26	
Aqueous Fraction	60	3	28,60 ± 0,09	85,69 ± 0,145
	80	3	47,47 ± 0,09	
	100	3	59,91 ± 0,18	
	120	3	77,83 ± 0,16	

Note: C: Concentration; n: Replication; SD: Standard Deviation; IC50: Inhibitory Concentration of 50%

3.3. Phytochemical Screening of Flavonoid Content in Ethanolic Extract and Aqueous Fraction of Purple Sweet Potato (Ipomoea batatas L.) Leaves

The identification of secondary metabolite compounds in ethanolic extracts and aqueous fractions of *I. batatas* L. leaves was carried out qualitatively through phytochemical testing. This study focused on the detection of flavonoid compounds, which are one of the largest phenol groups, characterized by a basic structure consisting of two C6 rings connected by a three-carbon aliphatic chain. In addition to their antioxidant activity, which was the focus of this study, flavonoids are also known to exhibit various other biological activities, including antiviral, antibacterial, anti-inflammatory, and hepatoprotective properties [1]. The phytochemical test for flavonoids in this study was conducted using three different detection methods: Wilstater reagent, Bate-Smith Metcalfe reagent, and 10% NaOH. The test results showed that both the ethanolic extract and aqueous fraction of *I. batatas* L. leaves gave positive results for the three flavonoid tests conducted, as presented in Table 1.

Testing with the Wilstater reagent (magnesium and hydrochloric acid) showed the presence of flavonoid compounds in both samples, indicated by the formation of an orange colour. The addition of magnesium powder and hydrochloric acid served to reduce the

benzopyron nucleus in the flavonoid structure, which then formed flavilium salts and produced orange or red complexes [8,12]. The Wilstater reagent is specifically used to identify flavonoid compounds of the flavone and flavonol groups. The chemical reaction that occurs between flavonoid compounds and magnesium and HCl can be observed in Figure 1 [12].

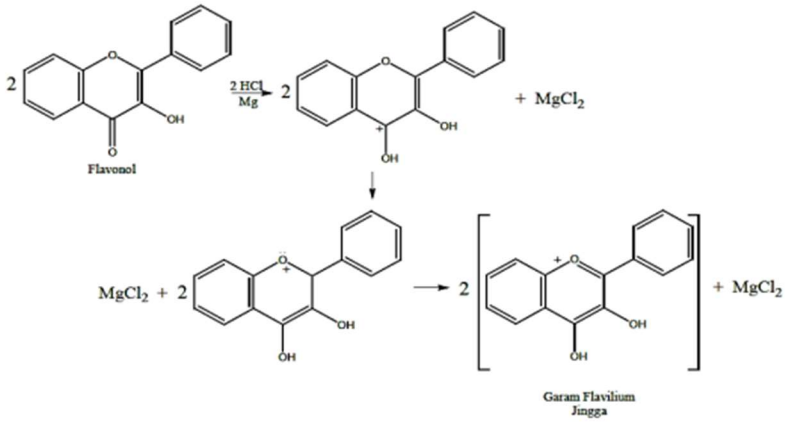

Fig. 1. Flavonoid Reaction with Magnesium and Hydrochloric Acid

Positive flavonoid results are also indicated by the Bate-Smith Metcalfe reagent, which uses concentrated HCl and heating. The concentrated HCl reagent plays a crucial role in the O-glycosyl hydrolysis process, breaking down flavonoids into their aglycones. The conversion of O-glycosyl to H+ from acid is due to its electrophilic properties [9]. Heating accelerates the hydrolysis reaction process and is indicated by the presence of a red colour that identifies anthocyanidin flavonoids.

The results of flavonoid testing with a 10% NaOH reagent yielded positive results, indicated by the formation of an orange complex in both the ethanolic extract and aqueous fraction samples from leaf I. batatas L. The 10% NaOH is a basic catalyst. When the sample is added to this reagent, it causes the decomposition and breakdown of the isoprene structure in the flavonoid compound, forming yellow to brown acetophenone molecules [10]. The flavonoid compounds identified with the 10% NaOH reagent belong to the phenol group. The reaction between flavonoids and NaOH can be observed in Figure 2 [13].

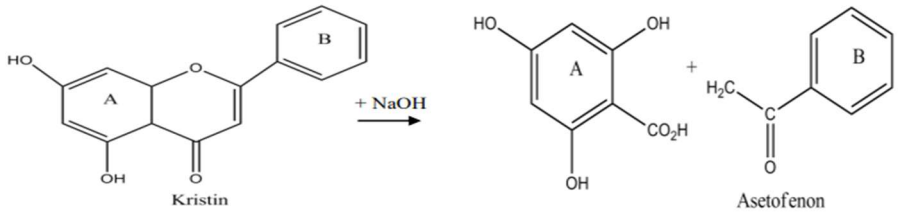

Fig. 2. Reaction of Flavonoids with NaOH

The successful detection of flavonoids using three different reagents provides important information about the flavonoid profile in ethanolic extracts and aqueous fractions of *I. batatas* L. leaves. Positive results with Wilstater reagent confirmed the presence of flavone and flavonol subgroups [8], while the Bate-Smith Metcalfe reaction indicated anthocyanidin flavonoids [9]. The diversity of these flavonoid subclasses contributes to the observed antioxidant activity, as each subclass exhibits different mechanisms and effectiveness in

counteracting free radicals through various electron donation and radical scavenging pathways [4].

3.4. Antioxidant Activity of Ethanolic Extract and Aqueous Fraction of *Ipomoea batatas* L. Leaves. DPPH Method

The antioxidant test method used in this study was the DPPH method, which is based on the principle of hydrogen atom donation or electron transfer from antioxidant compounds to radical compounds (diphenylpicrylhydrazyl), thereby converting the radical compounds into more stable non-radical compounds (diphenylpicrylhydrazine). This ability can be observed visually, namely the change in the colour of the solution from purple to yellow. A high sample concentration will result in lower absorbance, indicating that the antioxidant activity of a compound in suppressing free radicals is stronger, causing the colour of the resulting solution to fade. The reaction mechanism is illustrated in Figure 3 [14].

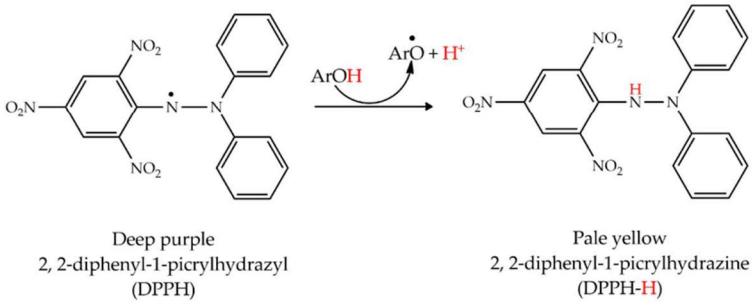

Fig. 3. DPPH Reaction Mechanism

The main parameter in determining antioxidant activity using the DPPH method is the IC_{50} value, which describes the concentration of a compound required to inhibit 50% of DPPH free radicals [11]. Based on **Table 3** [7], antioxidant activity is inversely proportional to the IC_{50} value, where a lower IC_{50} value indicates a stronger ability of the compound to suppress free radicals.

Table 3. IC_{50} Value Parameters

IC_{50} Value (µg/mL)	**Category**
< 50	Very Strong
50 - 100	Strong
101 - 150	Moderate

151 - 200	Weak

Analysis revealed that the ethanolic extract and aqueous fraction of I. *batatas* L. leaves exhibited strong antioxidant activity with IC_{50} values of 95.57 µg/mL and 85.69 µg/mL, respectively. Comparative analysis revealed that the aqueous fraction had more potent activity than the ethanol extract, with a difference in IC_{50} values of 9.88 µg/mL. The pattern of increased antioxidant activity in line with increased concentration in both samples indicated a dose-dependent response [7].

The observed antioxidant activity is strongly suspected to be related to the role of flavonoid compounds detected in both extracts. Flavonoids work through the mechanism of hydrogen atom donation to DPPH radicals, thereby converting them into a more stable and non-reactive reduced form [4]. In addition, flavonoids also act as scavengers that can directly capture Reactive Oxygen Species (ROS), inhibit the regeneration of ROS, and increase the activity of cellular antioxidant enzymes [3]. Additional mechanisms include the inhibition of xanthine oxidase and NADPH oxidase enzymes, as well as the ability to chelate metal ions [15]. The superior antioxidant activity of the aqueous fraction can be explained by the effectiveness of the fractionation process in concentrating polar antioxidant compounds, particularly flavonoid glycosides [6]. This aligns with previous findings that fractionation enhances bioactivity by selectively concentrating active compounds [7]. The observed dose-dependent response is characteristic of antioxidant compounds and has been well-documented in previous studies [12].

The antioxidant effectiveness of flavonoids is influenced by several structural factors, including the number and location of phenolic hydroxyl groups, as well as an extensive conjugation system [4]. Several studies have reported a positive correlation between total flavonoid content and antioxidant activity [15]. However, it should be noted that total antioxidant activity does not always depend solely on flavonoid content, given the contribution of other secondary metabolites such as alkaloids, saponins, tannins, anthocyanins, and other phenolic compounds [3].

The results of this study confirm the effectiveness of fractionation in enhancing the antioxidant potential by concentrating target compounds with specific polarities. Thus, it can be concluded that although both samples have potential as natural antioxidant sources, the aqueous fraction exhibits more optimal performance due to the fractionation process, which successfully concentrates polar antioxidant compounds, particularly flavonoids, that significantly contribute to DPPH radical scavenging activity.

4 Conclusion

The aqueous fraction of purple sweet potato I. *batatas* L. leaves demonstrated superior antioxidant activity (IC_{50} 85.69 µg/mL) compared to the ethanolic extract, recommending its potential as a natural antioxidant source for pharmaceutical applications while suggesting further isolation of active compounds and adoption of fractionation as a standard practice for enhancing bioactive compound recovery.

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