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PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT OF WATER AND ETHANOL EXTRACT OF *Eleutherine americana* (L.) Merr

Ika Ayu Mentari[1*], Reviana Amalia Dinata[1], Indah Hairunisa[1,2], Paula Mariana Kustiawan[1]

[1]Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur (UMKT), Samarinda, Indonesia

[2]Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malayasia (UTHM), 84600 Muar, Johor, Malaysia

*Coresponding author: iam856@umkt.ac.id

Abstract: Free radicals are reactive and inconsistent molecules that lack a partner for a portion of their electrons. Oxidative stress is the consequence of free radicals in the body surpassing their capacity. Oxidative stress has the potential to cause damage to a variety of cell components, including carbohydrates, proteins, DNA, and fat. Degenerative disease will result if oxidational stress is not adequately addressed. Antioxidant compounds can be employed to combat free radicals. It is believed that dayak onions (*Eleutherine americana* (L.) Merr) contain flavonoids and phenolic substances that have the potential to act as antioxidants. The objective of the investigation is to ascertain the flavonoids, total phenolic levels, and content of secondary metabolites in the water extract and ethanol extract of Dayak onions collected from Tenggarong seberang (L3), East Kalimantan. The Folin-Ciocalteu method is employed to ascertain total phenolic levels, the colorimetric method is employed to ascertain total flavonoid levels, and the tube method is employed to conduct phytochemical screening tests. The results obtained from the total phenolic content of dayak onions water extract (0.45±0.07 mg GAE/g) and dayak onions ethanol extract (1.21±02.02 mg GAE/g), as well as the total level of flavonoids in dayak onions aquatic extracts (1.17±0.66 mg QE/g) and dayak onions etanolic extraction (1.33±0.41 mg QE/g), suggest that the ethanol extract of dayak onions contains higher total levels of phenolics and flavanoids.

Keywords: Dayak onions, *Eleutherine americana* (l.) Merr, Free radicals, Total phenolic content, Total flavonoid content

1. Introduction

Free radicals are molecules that possess unpaired electrons in their outer orbit. They are prone to react with other molecules, resulting in the theft of electrons and the development of instability (Pratama *et al.,* 2020). Reactive oxygen species (ROS) are substances that contain reactive oxygen, which enables the formation of new substances when combined with other molecules, including hydrogen peroxide, superoxide, and hydroxyl radicals. The reaction between free radicals and oxygen can result in the formation of more reactive ROS, which are detrimental to cells. If the body's intrinsic defense mechanism, which involves the production of ROS or free radicals, exceeds the antioxidants, oxidative stress may result (Phaniendra *et al.,* 2015; Hikmah *et al.,* 2021).

Oxidative stress is the accumulation of free radicals in the body that surpasses the body's capacity to neutralize them. Various components of cells, including carbohydrates, proteins, DNA, and fats, can be damaged by oxidative stress. Degenerative diseases, such as diabetes, cancer, and heart disease, may result from this (Arief and Widodo, 2018). Free radicals are generated by vehicle exhaust fumes, air pollution, ultraviolet rays, and a variety of chemicals that have been burned (known as carbonate materials). The human body can overcome free radicals with the assistance of antioxidants. Antioxidants are substances that have the capacity to supply hydrogen atoms to free radicals, halt chain reactions, and convert them into a consistent form that does not impede the body's metabolism. Antioxidants are present in a variety of medicinal plants, grains, fruits, and vegetables (Kamoda *et al.*, 2021). Dayak onion is recognized for its antioxidant properties.

Dayak onion is a member of the *Iridaceae* family and is a common plant in Kalimantan. Dayak onion is frequently employed as a medication to treat stroke, breast cancer, colon cancer, ulcers, and cholesterol reduction (Mokoginta *et al.*, 2020). These onion bulbs contain a variety of compounds, such as flavonoids, phenolics, polyphenols, saponins, glycosides, alkaloids, tannins, steroids, quinones, and triterpenoids (Sàadah *et al.*, 2017). Dayak onions are also a source of phenolics and flavonoids that function as antioxidants. Phenolic compounds are secondary metabolites that are produced by plants and are distinguished by aromatic rings that contain hydroxyl groups. This substance prevents heart disease, acts as an anti-inflammatory, antimicrobial antioxidant, and anti-cancer agent. This compound has the potential to diminish ROS due to its antioxidant properties (Mahardani *et al*., 2021). Flavonoid compounds are secondary metabolites that are classified as phenol substances due to the substitution of the hydroxyl (-OH) group in their benzene structure. Flavonoids execute three mechanisms for managing free radicals: they inhibit the formation of ROS, degrade ROS, and regulate antioxidants. Flavonoids, as secondary antioxidants, decompose the oxidation reaction of the free radical chain and absorb it (Ningsih *et al.*, 2023).

Based on the background above, this study aims to determine secondary metabolites, total phenolic content and flavonoid content in water and ethanol extracts of dayak onions taken from the Tenggarong Seberang (L3) area, East Kalimantan. In this study, extraction activities were carried out using the maceration and infusion methods, and are expected to provide the latest information on the total phenolic content and flavonoid content in dayak onion extracts using the infusion method.

2. Materials and Methods

2.1 Materials

The materials used are water and ethanol extract of Dayak onion, Ethanol 96% (Merck), NaOH 10% (Merck), Ammonia 25%(Merck), Chloroform, HCl 10% (Merck), n-hexane (Merck), $FeCl_3$ 1%, Dragendroff reagent, Libermann Burchard reagent (Nitra kimia), Gallic acid, Sodium Carbonate 10%, Folin-Ciocalteu reagent (Merck), quercetin (Merck), Aluminum chloride 2%, Methanol (Smartlab), and distilled water. The tools used are containers for maceration, scissors, knives, infusion pots, cloth filters, measuring glasses (Pyrex), beakers (Pyrex), analytical scales (Smith), test tubes, measuring flasks (Pyrex),

microtubes, micropipettes (Dragonlab), water baths (Faithful), UV-Vis spectrophotometers (T60 UV-VIS PG).

2.2 Sample Preparation

Dayak onion plants (*Eleutherine americana* (L.) Merr.) were taken in the L3 area of Tenggarong Seberang, East Kalimantan. Fifteen (15) kg samples of fresh Dayak onions were collected, sorted, washed with running water, drained, cut into pieces and dried in the sun for 3 days until the sample is dry. After the sample is dry, the sample is then stored in a dry container and stored at a cold temperature.

2.3 Extraction

The Dayak onion extraction process is carried out using the infusion method and maceration method. In the infusion method, the dried Dayak onion sample is weighed, the dried Dayak onion sample is weighed according to the required dose then heated with distilled water and boiled at 90° for 15 minutes, calculated from the boiling point of the bottom of the pan, after 15 minutes the sample is filtered using cloth filter (Muthia and Astuti, 2018).

In the maceration method, the dried simplicia is extracted with 96% ethanol solvent with a ratio of 1:3 between sample and solvent for 3 days. The liquid extract was then concentrated using a rotary evaporator at a temperature of 50ºC, then concentrated again using a water bath until it reached a constant weight (Muthia *et al*., 2023).

2.4 Phytochemical Sscreening

Phytochemical screening was carried out to observe secondary metabolite compounds in Dayak onion bulbs.

a. Flavonoid test

A total of two mL each of ethanolic and water extract Dayak onions was added with two drops of 10% NaOH. A yellow, red, brown or green color change indicates the presence of flavonoids (Hidayah *et al.,* 2016).

b. Alkaloid test

A total of two mL of extract was added with 25% ammonia and chloroform. Then extracted with 10% HCl. Next, Dragendroff's reagent was added. The presence of a red precipitate indicates the presence of alkaloids (Hidayah *et al.,* 2016).

c. Triterpenoid test

A total of two mL of extract was added with two mL of n-hexane, shaken. The n-hexane layer was added with Liebermann-Burchard reagent. A change in color to red indicates the presence of triterpenoids (Hidayah *et al.,* 2016).

d. Steroid test

A total of two mL of extract was added with two mL of n-hexane, shaken. The n-hexane layer was added with Liebermann-Burchard reagent. A change in color to greenish blue indicates the presence of steroids (Hidayah *et al.,* 2016).

e. Tannin test

A total of two mL of extract was combined with two mL of 1% $FeCl_3$, and the mixture was agitated. The presence of tannins is indicated by a shift in the color of the solution to a blackish brown hue (Hidayah *et al.*, 2016).

f. Saponin test

Two mL of extract was combined with two mL of distilled water, and the mixture was vigorously shaken vertically. Subsequently, HCl was introduced. The presence of saponin is indicated by the presence of foam that remains stable in solution after the addition of HCl (Hidayah *et al.,* 2016).

g. Phenolic test

The extract is added to a 2% $FeCl_3$ which results in the formation of a green, purple, blue or black color which indicates the presence of phenol.

2.5 Determination of Total Phenolic Content

Starting with making a standard solution of gallic acid with a concentration of 10 mg/ml and making a 10% Na_2CO_3 solution. The standard curve for gallic acid was made with a concentration series of 1 ug/ml, 2 ug/ml, 4 ug/ml, 8 ug/ml, and 16 ug/ml. The gallic acid solution was pipetted, and 0.5 mL of Folin-Ciocalteu reagent was added. The mixture was shaken, and the mixture was incubated for 8 minutes. Add 3 mL of sodium carbonate solution and 10 mL of distilled water. In dark conditions, incubate gallic acid standards for 2 hours. Read the Gallic acid standard at a wavelength of 767 nm. The total phenolic content was determined by pipetting 100 µL of the sample into a 10 ml volumetric flask, adding 0.5 mL of Folin-Ciocalteu reagent, shaking, and incubating for 8 minutes. Add 3 mL of sodium carbonate solution and 10 mL of distilled water. Place the sample in a dark environment for a period of two hours (Hairunisa *et al*., 2021). Read the gallic acid standard at a wavelength of 767 nm and repeat 3 times. The total phenolic content can be calculated using the following formula:

$$\text{Total Phenolics} = \frac{C \times V \times fp}{g}$$

Description :

C= phenolic concentration which is the x value and has been converted in mg/mL units

V = is the sample volume in mL

Fp = is the dilution factor, and g is the sample weight in grams (Anggarani *et al.,* 2021).

2.6 Determination of Total Flavonoid Content

Starting with making a standard quercetin solution with a concentration of 5 mg/mL and making an $AlCl_3$ solution with a concentration of 2%. Preparation of a quercetin standard curve with a concentration series of 5 ug/mL, 10 ug/mL, 20 ug/mL, and 40 ug/mL. The quercetin solution was pipetted then put into a 10 ml volumetric flask and methanol was added to the mark. Take 750 µL of solution, put it in a microtube. Add 750 µL of $AlCl_3$ solution, shake and store in a dark room for 1 hour. The sample was read at a wavelength of 420 nm, with a methanol blank. And determining the total flavonoid content was carried out by pipetting 150 µL of the sample and placing it in a 10 ml volumetric flask, adding methanol to the limit mark, leaving it until a precipitate appeared. Take 750 µL of sample, put it in a

microtube. Add 750 µL of $AlCl_3$ solution, shake and store in a dark room for 1 hour. The sample was read at a wavelength of 420 nm, with a methanol blank (Hairunisa *et al.*, 2021).

3. Results and Discussion

3.1 Extraction of water and ethanolic extract dayak onion bulbs

The methods used in the extraction process are the Infusion and Maceration methods. The infusion method is used to extract active substances from Dayak onions, such as flavonoids, saponins, alkaloids, and tannins, which are polar secondary metabolites that are easily soluble in water (Dienina *et al.*, 2015). Meanwhile, the maceration method was chosen because it allows to maintain natural materials that are not heat resistant and allows many compounds to be extracted (Makalunsenge *et al.*, 2022). Dayak onion ethanolic extract uses 96% ethanol as a solution because of its universal properties that can be used to extract stems, leaves, and roots. Ethanol has 2 parts in the form of a polar -OH group and CH_2CH_3, which is nonpolar, so it is expected that many secondary metabolite compounds will be attracted to ethanol. Table 1 shows the results of the Dayak onion water and ethanolic extract yield.

Table 1. The results of the extraction of Dayak onion samples using ethanol and water solvents

Extract	Sample weight (g)	Extract weight (g)	% Yield (%)
Water	90	23	25,55
Ethanol	100	13	13,00

The ethanolic extract of Dayak onion produced a yield of 13%, while the water extract of Dayak onion produced a yield of 25.55%. Consequently, the attracted substance was declared to be of good quality and accounted for 13% and 25.55% of the raw material used, respectively. Farmakope Herbal Indonesia (2017) stipulates that the extract yield must not be less than 10% in order to meet the yield requirement.

3.2 Phytochemical screening test

This phytochemical screening test is carried out using a qualitative tube method, namely, a chemical reaction occurs which causes the test solvent to change in harmony with the positive component. The results of the phytochemical screening carried out on water and ethanol extract Dayak onion can be seen in Table 2.

Table 2. Phytochemical screening results from water and ethanol extract of Bawang dayak

Compound classes	Water extract	Ethanol extract
Flavonoid	+	+
Alkaloid	+	+
Triterpenoid/ Steroid	-	-

Tanin	+	+
Saponin	+	-
Phenolics	+	+

The Dayak onion water extract sample contained alkaloids, flavonoids, saponins, tannins, and phenolics, as indicated by the phytochemical screening. The Dayak onion ethanol extract did not contain saponin. The Dayak onion water extract contains saponins, which are polar compounds that are soluble in water due to the hydrophilic sugar groups they contain. Foam can be generated by saponins due to the fact that the polar groups of saponins face outward and the nonpolar groups face inward during micelle formation. Consequently, the foam appears as foam. In contrast, ethanol is less effective in dissolving saponins due to its lower polarity, particularly at high concentrations. Saponins are more easily extracted with water than with ethanol due to this distinction (Rukmini *et al.*, 2020). Previous research conducted by Toar *et al.*, (2023) on ethanol extract of Dayak onion with a 96% ethanol solvent was macerated for five days, and re-macerated twice for three days each, resulting in the secondary metabolite saponin. Several environmental factors, such as the location where the sample was taken, altitude, soil type, climate and temperature, pH, water activity and light intensity, can affect the content of secondary metabolite compounds in a plant (Kusuma *et al.*, 2018).

3.3 Total phenolic content and total flavonoid content

Analysis of total phenolic content in this study used the Folin-Ciocalteu method. To determine the phenolic compounds in the sample, gallic acid was used as a standard solvent because it is a pure phenol that has strong antioxidant properties to identify phenolic compounds in the sample. To determine the total flavonoid content using the colorimetric method with UV-Vis spectrophotometry, and the reagent used is $AlCl_3$. The principle of aluminum chloride ($AlCl_3$) is based on the formation of a colored complex between aluminum chloride and flavonoids with certain hydroxyl groups. When $AlCl_3$ is added to a sample solvent containing flavonoids, a chemical reaction occurs that produces a yellow flavonoid-AlC_3 complex (Prima *et al.*, 2020).The results of determining the total phenolic and flavonoid content in water extracts and ethanol extracts of Dayak onions are shown in Table 3.

Table 3. Results of total phenolic content water and ethanol extract of Dayak onion

Sample	**Replication**	**Determination**	
		Total phenolic content (mg GAE/g)	**Total flavonoid content (mg QE/g)**
Water Extract	1	0.463628	1.93754
	2	0.374219	0.785437
	3	0.519184	0.795146
	x±SD	**0.45 ± 0.07**	**1.17 ± 0.66**

Ethanolic Extract	1	1.246615	0.888997
	2	1.202344	1.714239
	3	1.210156	1.40356
	x±SD	**1.21 ± 0.02**	**1.33 ± 0.41**

The results of the determination of higher total phenolic and flavonoid levels are in the ethanol extract of Dayak onion. This study produced different total phenolic and flavonoid levels from previous studies. In the study (Toar et al., 2023) the total flavonoid content was 1.2% in the ethanol extract of Dayak onion (*Eleutherine americana* (L.) Merr.) with 96% ethanol as a solvent and this sample was taken in the Passi Village area, North Sulawesi. In the study (Fitriansyah *et al.,* 2021) the total phenolic and flavonoid content of the ethanol extract of Dayak onion (*Eleutherine americana* (L.) Merr.) was 3.721 g GAE/100g and 0.378 g QE/100g using 70% ethanol as a solvent and the sample was taken in Lembang, Bandung. And in the study (Laila *et al.,* 2022) the total phenolic and flavonoid content of methanol extract of dayak onion (*Eleutherine americana* (L.) Merr.) was 14.49 mg GAE/g dry matter and 5.41 mg QE/g dry matter using methanol as a solvent and samples were taken in Bogor. In the study (Supriningrum *et al.,* 2017) the total flavonoid content was 2.41% in the ethanol extract of dayak onion (*Eleutherine americana* (L.) Merr.) with 70% ethanol as a solvent and the dayak onion sample was purchased from a trader on Jalan Kadri Oening, Samarinda.

The results are different from previous studies because the solvents used are different, and the extraction methods are different. And these results can show different total phenolic and flavonoid levels. In previous studies using methanol and 70% ethanol solvents, and in this study using 96% ethanol. Where when compared the results of methanol extraction are higher, then 70% ethanol extract and 96% ethanol. In line with the theory that, based on the principle that each compound will dissolve in a solution through harmonious polarity properties, the effectiveness of the compound extraction process by solvents is highly dependent on how soluble the compound is in the solvent (Wahyuningtyas *et al.,* 2017; Putri *et al.,* 2023). Compared to ethanol solvents, methanol solutions have a high dielectric constant. In addition, ethanol at different concentrations, namely 70% ethanol and 96% ethanol, affects the level of solution polarity. The higher the concentration, the lower the level of solution polarity (Listiawati *et al.,* 2022).

The presence of plant growth environmental factors and temperature will also affect the compound content of plants. The low total flavonoid & phenolic content of Dayak onion water extract using the infusion method is because phenolic compounds are thermosensitive, they allow hydrolysis and reduction of phenol levels at high temperatures (Komala and Husni, 2021) and flavonoid substances have conjugated aromatics that are easily damaged at high temperatures. This can cause damage to phenolic and flavonoid compounds in Dayak onion infusion extract (Hasanah *et al.,* 2023).

4. Conclusion

The results of phytochemical screening showed the presence of alkaloids, flavonoids, saponins, tannins, and phenolics in the Dayak onion infusion sample, and there were no saponins in the Dayak onion ethanolic extract. The total phenolic content of the Dayak onion

water extract was 0.45 ± 0.07 mg GAE / g and the Dayak onion ethanol extract was 1.21 ± 0.02 mg GAE / g, and the total flavonoid content of the Dayak onion water extract was 1.17 ± 0.66 mg QE / g and the Dayak onion ethanolic extract was 1.33 ± 0.41 mg QE / g.

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Conflict of Interest

All Authors declare no conflict of interest and agree with the content of the manuscript.

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