Pharmacoscript

Muthia et al./Pharmacoscript, Volume 9, No, 1, February 2026, 1-11

P-ISSN : 2622-4941 | E-ISSN : 2685-1121

DETERMINATION OF TOTAL PHENOL AND FLAVONOID CONTENT OF 96% ETHANOL EXTRACT OF FRESH AND BLACK SUNA ONION BULB (*Allium schoenoprasum* L.)

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Received: 10/07/2025 Revised: 30/10/2025 Accepted: 05/11/2025 Published: 28/02/2026

ABSTRACT

The use of traditional medicinal plants is increasing because they are easy to find, safer, and affordable. One of the potential local plants from Central Kalimantan is the suna onion (*Allium schoenoprasum* L.), whose bulbs are used to cook spices and medicine. This study aims to determine the compound, total phenol and flavonoid content in fresh and black suna onion bulbs. Thermal fermentation was carried out for 7 days at ±70°C using an electric slow cooker. Extraction was done using the maceration method and 96% ethanol as a solvent. Qualitative tests for phenol used $FeCl_3$ and Millon reagents for flavonoids used Shinoda, Bate-Smith, and 10% NaOH reagents. The determination test used a UV-Vis spectrophotometer. For total phenols, gallic acid was used as a comparator, Folin-Ciocalteau as a reagent, with a wavelength of 760 nm. For total flavonoids, quercetin was used as a comparator, and $AlCl_3$ reagents with a wavelength of 415 nm. The qualitative test showed fresh and black suna onion bulbs contained positive phenol and flavonoid compounds. Quantitative results showed an increase in phenolic content from 0.733 ± 0.095 mg Gallic Acid Equivalent(GAE)/g (fresh) to 11.024 ± 0.165 mg GAE/g (fermented) and total flavonoid content from 15.310 ± 0.372 mg QE/g (fresh) to 30.786 ± 0.179 mg QE/g (fermented). Based on these results, it can be concluded that the fermentation process increases the levels of phenolic compounds, flavonoids, and total phenolics in the suna onion bulbs, thus potentially developing them as a traditional medicinal ingredient. This is due to chemical changes that occur during the thermal fermentation process (Maillard reaction).

Keywords: Bulbs, Flavonoids, Phenols, Fresh, Thermal.

INTRODUCTION

Kalimantan is an island rich in medicinal plants. Generally, plants containing secondary metabolites can be used as medicine (Hermawan et al., 2023). One type of plant that has the potential to be used as a source of traditional medicine is the suna onions (*Allium schoenoprasum* L.), which are commonly found in Central Kalimantan. In Indonesia, suna onions are known as kucai, lokio, bawang batak, or chives (Materia Medika Batu, 2024). Previous studies have shown that the results of examination of suna onion (*A.*

schoenoprasum L.) bulbs contain flavonoids, alkaloids, saponins, steroids, tannins (Sinta et al., 2020), and phenolic compounds (Lin et al., 2016; Sinaga, 2024).

Previous research showed that 96% ethanol extract of suna onion bulbs contained a total phenol content with an average of 112.67±5.08 mg GAE/g extract (Jovanova et al., 2019). Other studies showed that 96% ethanol extract of *A. schoenoprasum* L. bulbs contained total flavonoid levels with an average of 19,000 mg/mL QE (Sinta et al., 2020). Suna onion bulbs extracted with sterile distilled water stated that the average total flavonoid content of Suna onion bulbs was 14.583 mg QE/g (Utamy *et. al*, 2021), while with 96% ethanol, the average total flavonoid content was 17.417 mg/ml QE (Agustin et al., 2022).

The type and concentration of secondary metabolites can influence the pharmacological activity of a plant. The concentration of secondary metabolites is influenced, among other things, by the processing of the raw materials. The fermentation process in the tubers studied aims to increase biological activity and chemical content (Fitriansyah et al., 2021). The phenol and flavonoid compounds in a sample play a role in neutralizing free radicals, meaning there is a relationship between the amount of phenol and flavonoids and antioxidant activity. The higher the total phenol and flavonoid levels, the higher the potential antioxidant activity in suppressing free radicals (Mar'atirrosyidah & Estilasih, 2015; Mahani et al., 2022).

No research has been conducted on the fermentation of suna onion bulbs. However, according to the hypothesis put forward by Hegnaur, plants from the same family generally have similar compound content (Utamy et al., 2021). Based on the kinship between *Allium schoenoprasum* L.) and garlic (*Allium sativum* Linn.), which both come from the same family and same genus Allium, the basis of this study refers to the results of research by Romsiah et al. (2020) which stated that antioxidant activity in garlic bulbs increased after undergoing a fermentation process for 7 days at temperature of 70–80°C, with an IC_{50} value of fermented garlic (black garlic) of 46.61 ppm and fresh garlic of 149.49 ppm. From these results, it can be concluded that there is a possibility that the higher the antioxidant activity, the higher the total phenol and flavonoid content will also be.

Based on the results of previous studies, the author is interested in conducting this research, which aims to determine the total phenol and flavonoid content after the fermentation process, which was extracted

using the maceration method with 96% ethanol solvent.

METHODS

Tools and Materials

Tools such as an electric slow cooker (BabySafe), chopper (Phillips), micropipette (Dragon Lab), analytical balance (Scount Pro), macerator, waterbath (Memmert), test tubes, UV-Vis spectrophotometer (T60), hotplate, rotary evaporator (IKRF 10) were used. Materials such as suna onion bulbs, acetic acid, 96% ethanol, concentrated hydrochloric acid, quercetin, aluminum chloride reagent, Mg powder, amyl alcohol, natrium hydroxide, gallic acid, distilled water, folin-ciocalteau, ferric chloride, sodium carbonate, Millon reagent, ethanol p.a.

Research Path

1. Plant determination

Plant determination was carried out at the Batu Herbal Materia Medica Laboratory, East Java Provincial Health Office, and the results obtained were number 00093/3299/102.20/2024

2. Sample preparation

Suna onion bulbs as samples were obtained from Lunuk Ramba Village, Basarang District, Kapuas Regency, Central Kalimantan, approximately 2–3 months old, 2–3 cm long, approximately 1 cm wide, and white in color. The samples consisted of fresh and fermented. The samples were sorted and washed until clean. Thermal fermentation was done by wrapping 100 g of the sample in 2 layers of aluminum foil. Leave in an electric slow cooker at a warm temperature of 70 °C for 7 days (Romsiah et al., 2020).

3. Extraction

The previously prepared samples were ground using a chopper and then macerated with 96% ethanol (1:3 ratio). Maceration was carried out for 24 hours with two remacerations. Concentration was carried out using a rotary evaporator and a water bath (Romsiah et al., 2020).

4. Qualitative test of phenol and flavonoid

A test solution was prepared by dissolving 0.5 g of the extract in 25 mL of warm distilled water. The phenol test was performed in two ways: 3-5 drops of 1% $FeCl_3$ solution were added to 5 mL of the test solution. A positive reaction was indicated by the appearance of a green, blue, or black color (Dewantara et al., 2021). Millon's reagent was also added to 1 mL of the test solution. A positive reaction was indicated by the presence of a white precipitate, which, when heated, turns red (Heliawati, 2018).

For flavonoids, three tests are performed: the Shinoda test, in which 0.1 g

of magnesium powder, 2-4 drops of concentrated HCl are added to 1 mL of the test solution, then shaken, and then amyl alcohol is added. A positive result is indicated by a red, yellow, or orange color change in the amyl alcohol layer (Saepudin et al., 2024). In the Bate-Smith test, a few drops of concentrated HCl were added to 1 mL of the test solution and then heated. A red color indicates the presence of flavonoids from the anthocyanin group (Rahayu et al., 2015). In the 10% NaOH test, two drops of NaOH solution are added to 1 mL of the test solution and shaken vigorously. If a strike color change occurs from the original color to yellow, red, brown, or green, it is concluded that the sample contains flavonoids (Mailuhu et al., 2017).

5. Determination of total phenol contents

To determine the maximum wavelength (λmax) and operating time (OT), 5 mL of 10% Folin-Ciocalteau reagent was added to 0.5 mL of a 30 ppm gallic acid solution and allowed to stand for 5 minutes. Then, 4 mL of a 1 M Na_2CO_3 solution was added, mixed homogeneously, and allowed to stand at room temperature. Absorbance was measured at a wavelength of 600–800 nm. For OT, absorbance was measured over 0-60 minutes (Andriani & Murtisiwi, 2018).

To determine the standard curve for gallic acid, a series of concentrations of 30, 40, 50, 60, and 70 ppm was created (Qonitah et al., 2023). Each concentration of 0.5 mL of gallic acid solution was added with 5 mL of 10% Folin Ciocalteau reagent, and left for 5 minutes. Then, 4 mL of 1 M Na_2CO_3 was added, left for OT time, and measured at λmax (Yuliasari, 2024). On samples with a concentration of 5000 ppm, the same test procedure was carried out and replicated 3 times.

6. Determination of total flavonoid contents

The maximum wavelength (λmax) was determined in the 370-450 nm range, and operating time (OT) at 2-minute intervals for 60 minutes until a stable absorbance was obtained with the following test procedure. The 1000 ppm stock solution was diluted with 40, 60, 80, 100 and 120 ppm concentration in determining the standard curve of quercetin. Each 0.5 mL was taken and reacted with 0.5 mL of 10% $AlCl_3$ and 4 mL of 5% acetic acid. It was left for the OT time. The absorbance was measured with a UV-Vis Spectrophotometer (Hidayatullah et al., 2024). The 1000 ppm sample was tested according to the procedure above with three replications (Daipadli et al., 2024).

Data Analysis

The initial step in data analysis is the creation of a standard curve and determining a linear regression equation in the form y = bx + a from the reference absorbance values. Next, the total phenol and flavonoid levels are determined by entering the absorbance data into the standard curve equation for gallic acid and quercetin as the y value. The resulting x value represents the milligram equivalents of gallic acid per gram of extract (GAE) (Andriani & Murtisiwi, 2018). The formula for calculating total phenol and flavonoid contents (Sukma, 2022) is as follows:

$$\text{TPC / TFC} = \frac{\text{C . V x Fp}}{\text{M}}$$

Note: TPC = Total Phenolic Content (mg GAE/g extract); TFC = Total Flavonoid Content (mg QE/g extract); C = phenol/flavonoid content from the standard curve (mg/L); V = volume used (mL); Fp = Dilution Factor; M = sample weight (mg).

RESULTS AND DISCUSSION

Samples were identified to ensure the accuracy of the plant's identity, avoid sampling errors, and prevent potential contamination with other plants (Klau & Rosa, 2021). The results showed that the plant used in this study was *Allium schoenoprasum* L., a member of the Liliaceae family (Figure 1).

Figure 1. Suna onion bulbs

Two samples were used, fresh and fermented. A macroscopic examination revealed that the fresh bulbs are white, have a distinctive odor, and have a slightly firm texture. Meanwhile, the fermented samples showed changes in color, smell, and texture from day 1 to 7, resulting in a black color, a characteristically weak odor, and a soft texture (Figure 2).

Figure 2. Sample changes during fermentation

The spontaneous fermentation method was selected for the samples, observing the color change to dark brown and the emergence of a sweet and fresh flavor due to the Maillard reaction. This reaction affects color, smell, shape, and taste, and increases antioxidant potential. In addition to

physicochemical changes, the heating process can also increase the content of bioactive compounds such as phenols, flavonoids, and S-allyl cysteine (SAC) (Solichah & Herdyastuti, 2021).

Extraction was carried out using the maceration method with 96% ethanol solvent because it can dissolve various polar compounds, and its non-toxic nature makes it safe for use in the extraction of natural materials (Muthia et al., 2023). The percentage yield value for fresh samples is 12,68% and for fermented samples is 17,65%. This is due to the heated process during fermentation, which reduces the water content through evaporation, resulting in a more concentrated extract. This water evaporation increases the concentration of secondary metabolites and reduces the material's weight, directly affecting the yield obtained (Komala et al., 2022).

Qualitative test results showed positive values for fresh and fermented samples, indicating they contained phenols and flavonoids. Determining total phenol contents used the Follin-Ciocalteu reagent, which works based on the principle of redox reactions. Gallic acid, used as a comparison, reacts with the Folin-Ciocalteu reagent to form a yellow color. Na_2CO_3 solution is added to create a blue color. Na_2CO_3 reacts with the Folin-Ciocalteau reagent in an alkaline environment to dissociate protons in phenolic compounds into phenolate ions (Ahmad et al., 2015). The chemical reaction of phenol with the Folin-Ciocalteau reagent can be seen in Figure 3.

The maximum wavelength of gallic acid obtained in this study was 760 nm.

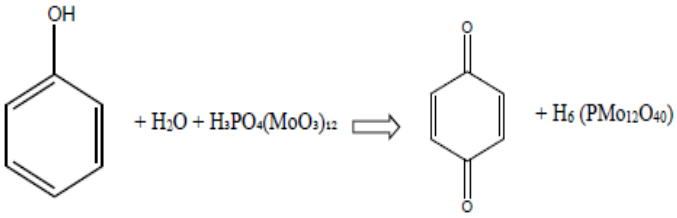

Figure 3. Chemical Reaction of Phenol with Folin-Ciocalteau

The operating time obtained in this study was stable at 53 to 60 minutes. In the Determination of the Gallic Acid Standard Curve, the linear regression equation was y = 0.0032x + 0.2296 with a correlation coefficient (r) value of 0.9989. The graph for determining the gallic acid standard curve can be seen in Figure 4. The correlation coefficient (r) value has met the acceptance criteria for linearity, which is ≥ 0.98. Therefore, it can be concluded that the linear standard curve found in gallic acid is linear and shows a strong relationship between solution concentration and absorbance. The next step is determining the total phenol content and obtaining data as in Table 1.

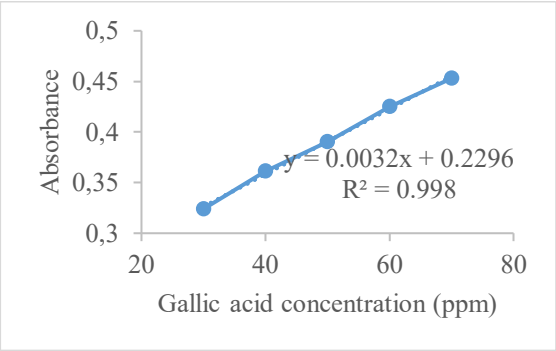

Figure 4. Gallic acid standard curve

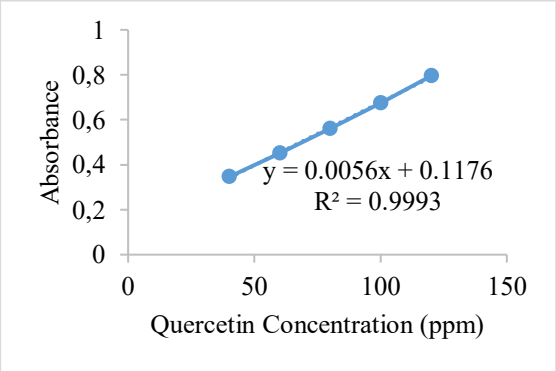

Figure 5. Quercetin standard curve

Table 1. Total phenol content determination data of samples

Sample	Absorbance	TPC (mg GAE/g extract)	Average TPC ± SD (mg GAE/g extract)
Fresh	0.240	0.650	0.733 ± 0.095
	0.243	0.837	
	0.241	0.712	
Fermented	0.404	10.090	11.024 ± 0.165
	0.405	10.962	
	0.409	11.212	

Table 2. Total flavonoid content determination data of samples

Sample	Absorbance	TFC (mg QE/g extract)	Average TFC ± SD (mg QE/g extract)
Fresh	0.205	15.607	15.310 ± 0.372
	0.201	14.893	
	0.204	15.429	
Fermented	0.291	30.964	30.786 ± 0.179
	0.289	30.607	
	0.290	30.786	

In determining total flavonoid content, 10% $AlCl_3$ plays a role in forming a complex with the flavonoid compounds in the extract, which causes a wavelength shift towards the visible region, indicated by a change in the solution color to yellow. The addition of acetic acid maintains the wavelength to remain in the visible region (Awilda et al., 2024). The comparator used was quercetin with a maximum wavelength of 415 nm and an operating time of 30-36 minutes. This study prepared a standard curve for quercetin from a stock solution of 1000 ppm with five different concentrations: 40, 60, 80, 100, and 120 ppm (Estikawati & Novena, 2019). The standard curve for quercetin can be seen in Figure 5. The reaction between quercetin and $AlCl_3$ can be seen in Figure 6.

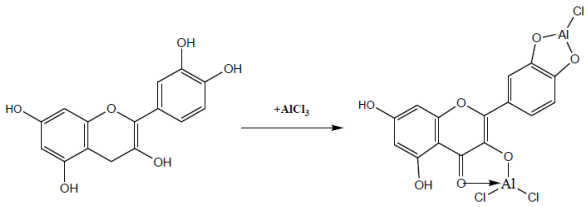

Figure 6. Chemical reaction of quercetin (Flavonoid) with $AlCl_3$

The linear regression equation for the standard curve of quercetin is y = 0.0056x + 0.1176 with an (r) value of 0.9996. The (r)

value obtained is the relationship between quercetin concentration and absorbance, and an (r) value close to 1 indicates that the linear regression equation is linear (Awilda et al., 2024). The next step is determining the total flavonoid content and obtaining data as in Table 2.

Total phenol and flavonoid content increased after fermentation. The Maillard reaction is also a significant factor in the fermentation process. This non-enzymatic browning process occurs through the interaction between the amine groups of proteins and the carbonyl groups of reducing sugars, causing the color of onions to change from brown to black (Sadewo, 2021). The Maillard reaction is known to increase the formation of bioactive compounds, including polyphenols and flavonoids, which have great potential as highly active antioxidants. This increase in phenol compound levels is due to the breakdown of chemical bonds such as esters, glycosides, and other ester bonds (Solichah & Herdyastuti, 2021).

CONCLUSIONS

The study showed that 96% of the ethanol extract of suna onion (*Allium schoenoprasum* L.) bulbs, both fresh and fermented, contained positive phenol and flavonoid compounds based on qualitative tests. The fermentation process for 7 days at 70°C increased the total phenol and flavonoid levels. The total phenol and flavonoid levels in the 96% ethanol extract of black suna onion bulbs were higher than in fresh conditions. This is due to chemical changes during the thermal fermentation process (Maillard reaction).

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