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Secondary metabolic analysis of three shallot cultivars on coastal peatlands in Meranti Islands District, Riau Province, Indonesia

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Abstract. *Sutriana S, Samanhudi, Purwanto E, Jumin HB. 2025. Secondary metabolic analysis of three shallot cultivars on coastal peatlands in Meranti Islands District, Riau Province, Indonesia. Biodiversitas 26: 2106-2116.* Shallot (*Allium ascalonicum*) is a strategic horticultural vegetable commodity with great potential to be developed due to the various secondary metabolite content, such as flavonoid, phenol, anthocyanin, and antioxidant activity, which are crucial for health. This study aimed to identify and determine the secondary metabolism values of three shallot cultivars in coastal peatlands, namely Bima Brebes, Trisula, and SS Sakato. Shallot bulbs were extracted by maceration, followed by phytochemical content filtration. The total phenol, anthocyanin, as well as flavonoid levels were assessed using High-Performance Liquid Chromatography (HPLC), while antioxidant activity was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results indicated that SS Sakato cultivar had the highest extract yield of 12.17%. Bima Brebes and Trisula cultivars contained flavonoid, phenol, terpenoid, and steroid phytochemical, while SS Sakato had flavonoid, phenol, and terpenoid. Moreover, Bima Brebes produced the highest total phenol and flavonoid and SS Sakato had the highest anthocyanin. The standard retention time of quercetin from three shallot cultivars ranged from 9.167 to 9.227 minutes, with an average of 9.192 minutes. Bima Brebes was also found to have the best antioxidant activity as indicated by Inhibition Concentration50 (IC_{50}) value.

Keywords: Coastal peatlands, DPPH, HPLC, secondary metabolites, shallot cultivars

INTRODUCTION

Meranti Islands District has coastal peatlands formed in coastal areas and influenced by the ebb and flow of seawater. The soil is characterized by low soil fertility, pH, nutrient availability, base saturation, as well as high organic acid content, toxicity, and nutrient deficiencies (Hapsari et al. 2022). These conditions make the soil less suitable for cultivating plants, specifically vegetables such as shallot (*Allium ascalonicum* L.), which is an essential part of daily diet. The development of shallot cultivation in Meranti Islands District must be carried out to increase production with effective and efficient agricultural technology. This will ensure adequate production of quality shallot to meet the needs of the community and not depend on the Provinces of West Sumatra, North Sumatra, and Java as suppliers.

According to the Directorate of Horticultural Seeds (2018), many shallot cultivars have been developed and released in Indonesia through the Decree of the Minister of Agriculture, including Bima Brebes, Trisula, and SS Sakato. Bima Brebes cultivar originates from the local area of Brebes, Central Java, with flowering age of 50 days, and a harvest age of 60 days after planting. Other properties include plant height of 25-44 cm, number of shoots ranging from 7-12 bulbs per clump, number of leaves between 14-50 strands, the weight loss of tubers (wet-dry) is 21.5%, the tuber yield is 9.9 tons/ha of dry tubers and suitable for lowlands (Agricultural Instrument Standardization Agency 2024).

Trisula cultivar originates from the Vegetable Research Institute and is characterized by flowering age 24-35 days after planting, harvest age 50-55 days after planting, 39.92 cm plant height. Other properties include 28-39 strands per clump, diameter 1-2.5 cm, tuber weight per clump 39-93.3 g, number of tubers 5-8 per clump, tuber yield per ha 6.5-23.21 tons/ha. In addition, Trisula cultivar adapts well in lowlands with a height of 6-85 m ASL (Agricultural Instrument Standardization Agency 2024).

SS Sakato cultivar from the local area of Alahan Panjang, Solok District, West Sumatra Province, has a plant height of 24-44 cm, and 22-46 strands per clump. The age of flowering is 48-55 days after planting, and the harvest age ranges from 85-95 days after planting. In addition, the diameter is 0.8-2.7 cm, the weight per tuber is 2.4-6.8 g, and the weight of tubers per clump is 70-280 g. The number of tubers per clump is 9-25, and the tuber yield per ha is 17.52-28 tons. SS Sakato cultivar is suitable for the highlands in Solok District (Ministry of Agriculture Republic of Indonesia 2017).

Each plant cultivar possesses distinct morphological, physiological, and biochemical responses that affect metabolite content and enzyme activity during secondary metabolite formation (Kusumiyati et al. 2021, 2022; Munawar

et al. 2022). Cultivation on coastal peatlands affects the value or proportion of secondary metabolites due to environmental (soil water content, soil pH and temperature), genetics, and physiological (plant age and plant conditions) factors (Böttcher et al. 2018).

Flavonoid is secondary metabolite produced by plants, which is included in the large group of polyphenol. This compound is present in all plant parts, like leaves, roots, fruits, as well as seeds. Generally, flavonoid can scavenge free radicals and inhibit lipid oxidation (Treml and Smejkal 2016). Quercetin comprises a class of flavonoids that exhibit various biological functions due to their ability to neutralize free radicals and reactive oxygen species, such as superoxide anions as well as hydroxyl radicals. Quercetin levels contained in shallot bulb extract can be analyzed using HPLC (Ahmed 2024).

Anthocyanin is a hydrophilic pigment belonging to flavonoid subclass and known as anthocyanidin glycosides. This compound gives fruits, flowers, and various other plant parts a red or blue coloration (Khoo et al. 2017; Saha et al. 2020). In addition, anthocyanin is a powerful free radical scavenger and has been shown to protect against oxidative DNA damage, inhibit enzyme activity, prevent oxidative degradation of lipids, and enhance membrane stability (Mojzer et al. 2016).

In this study, the antioxidant activity of shallot bulb extract was evaluated using the DPPH method. Antioxidant substances generally interact with DPPH radicals by donating hydrogen atoms, leading to a color change in DPPH from purple to yellow. In this context, the Inhibition Concentration50 (IC_{50}) refers to the concentration of a substance that effectively reduces DPPH absorbance by 50%. A lower IC_{50} value indicates greater antioxidant strength (Bouhenni et al. 2021).

Based on the discussion, the objective of this study was to specify and determine the secondary metabolic value of three shallot cultivars cultivated on coastal peatlands through extract yield, phytochemical screening, HPLC chromatography, and antioxidant activity using DPPH method. The results are expected to inform the public that the three shallot cultivars cultivated on coastal peatlands have different secondary metabolic content values or proportions. These differences suggest significant potential for cultivar development to increase production with effective and efficient agricultural technology and improve the quality of shallot in Meranti Islands District, Riau Province.

MATERIALS AND METHODS

Study period and location

This study was carried out at the Vahana Scientific Laboratory, Padang, West Sumatra, Indonesia, from July to August 2024. The materials used include the extraction of three shallot cultivars (Bima Brebes, Trisula, SS Sakato) (Figures 1-2), 96% ethanol, methanol, equates, Hydrochloric Acid (HCl), chloroform, gallic acid CAS G7384, methanol PA CAS 1.06009.2500, folic ciocalteu CAS 1.09001.100 10%, Magnesium (Mg), Sodium Carbonate (Na_2CO_3) CAS 1.06392.1000 7%, ethanol PA, potassium chloride citric acid (KCl+ $C_6H_8O_7$), Natrum citrate ($Na_3C_6H_5O_7$), Iron (III) Chloride ($FeCl_3$), and Sulfuric Acid (H_2SO_4). Meanwhile, the tools used include a rotary evaporator, test tube, micropipette, analytical balance type FS-AR210 (INT-CAL), watch glass, spatula, dropper, 50 mm funnel, 10 mL and 100 mL measuring flask, ultrasonic bath Ultrasonic 40 KHz, Whatman filter paper No. 42, and Agilent Technologies carry 8454 UV-Vis Spectrophotometer.

Preparation and extraction

The materials used for extraction were the bulbs of three shallot cultivars cultivated on coastal peatlands in Alah Air Village, Tebing Tinggi District, Meranti Islands District, Riau Province, during the period from February to May 2024. The location was on the coordinates: 00°59'42.28230"N, 102°40'52.71890"E, with an altitude of 5.5 m ASL. Shallot bulbs used for this secondary metabolic test have been air-dried for 6 weeks after harvest.

Maceration extraction was carried out by weighing 1 kg of shallot bulbs and dissolving in 3000 mL of 96% ethanol (ratio 1:3). The mixture was then stirred and macerated for 3 × 24 hours with filtration every 24 hours. The solution was evaporated with a rotary evaporator at 40°C and a pressure of 0.08 MPa until the solvent was completely evaporated. The thick/concentrated extract obtained can then be used for further analysis.

Phytochemical screening of shallot bulb extract

Shallot bulb extract was analyzed to determine the content of flavonoid, phenol, alkaloid, terpenoid, steroid, and saponin. Phytochemical screening was qualitative, with (+) present and (-) not present signs.

A B C

Figure 1. Bulbs of three shallot cultivars. A. Bima Brebes; B. Trisula; C. SS Sakato

A B C

Figure 2. Shallot plants 40 days after planting. A. Bima Brebes; B. Trisula; C. SS Sakato

Flavonoid

Shallot extract weighing 0.1 g was combined with 5 mL methanol and it was shaken. The solution was taken and placed on a plate then 0.1 g Mg powder was added with one drop of HCl p.a. A red or yellow color appearance indicates the existence of flavonoid (+) (Shaikh and Patil 2020).

Phenol

About 0.1 g of shallot extract was added to a test tube, followed by the addition of 5 mL of distilled water, and the mixture was shaken well. The solution was then put onto a drop plate, as well as a single drop of $FeCl_3$ was introduced. The appearance of a blackish-blue or green hue signifies the phenols' presence (+) (Shaikh and Patil 2020).

In the phenol test, there are two chemical reactions, first, the Folin-Ciocalteu reaction comprises the addition of Folin-ciocalteu reagent to a sample containing phenol, generating a blue complex. Second, the oxidizing reaction entails the addition of reagents such as $FeCl_3$ or HCl to samples containing phenol.

Alkaloid

The alkaloid test was performed using the Mayer and Wagner methods. A 0.1 g of shallot extract was put in a test tube, followed by the addition of 3 mL of chloroform, and the mixture was shaken. Furthermore, approximately 3 mL of chloroform was put into a separate test tube, along with 3 mL of 0.05 N ammonia, and 10 drops of 2 N H_2SO_4 were included. This solution was allowed to separate, and the upper acid layer was collected. To this, a few drops of Mayer's solution were put on (tube I). The appearance of a white precipitate signifies the alkaloids presence (+). Next, from the acid part (top), a few drops of Wagner's solution were put on (tube II). The formation of a brown precipitate also signifies the presence of alkaloids (+) (Setyawati et al. 2020; Shaikh and Patil 2020).

Terpenoid and steroid

Shallot extract weighing 0.1 g was put into two test tubes, added with 5 mL of chloroform p.a, and then shaken. Two drops of chloroform solution were placed on the drop plate and left to stand until the solution was dry or the chloroform evaporated. Another three drops of acetic anhydride p.a was added along with + 1 drop of H_2SO_4 p.a. The formation of a purple-red color indicates the presence of Terpenoid (+), while green-blue suggests steroid (+) (Dhayalan et al. 2018).

Two chemical reactions were carried out in the terpenoid/steroid test, first, the Liebermann-Burchard reaction involves the addition of Liebermann-Burchard reagent to samples containing terpenoid/steroid. Second, the Salkowski reaction entails the addition of Salkowski reagent to samples containing terpenoid/steroid.

Saponin

About 0.1 g of shallot extract was placed into a test tube, then 10 mL of distilled water was added and the mixture was shaken for 10 seconds until foam formed, after which 2 N HCl was added. The formation of foam that does not disappear or is stable when added with HCl indicates the presence of saponin (+) (Sobuj et al. 2024).

Quantitative phytochemical contents

Total phenol contents

Shallot extract was weighed up to 1 g, dissolved with methanol p.a to 10 mL, sonicated for 30 minutes, and filtered. Furthermore, the extract was thinned to a final volume of 100 mL, and then 0.25 mL of this solution was combined with 1.25 mL of 10% Folin-Ciocâlteu reagent. After allowing the mixture to sit for 3 minutes, 1 mL of a 7% Na_2CO_3 solution was added, and the mixture was incubated at 40°C for 60 minutes to facilitate color development. The absorbance was subsequently calculated at 765 nm using an Agilent Technologies Cary 8454 UV-Vis spectrophotometer (Dahech et al. 2013). The total phenol content was recorded in mg/g utilizing the following equation from the calibration curve: $y = 0.0098x - 0.0841$, with $R^2 = 0.9995$, where y represents absorbance and x denotes concentration.

Total flavonoid contents

Shallot extract was weighed up to 0.25 g dissolved with 2 mL of methanol p.a and sonicated for 30 minutes. This was followed by dilution according to the required concentration with methanol p.a as a solvent. The sample was filtered with a 0.22 μm PTFE syringe to a 1.5 mL vial as well as injected into HPLC system.

Anthocyanin contents

To determine anthocyanin content, 1 g of shallot extract was weighed and liquefied in a mixture of 70% ethanol and 1 N HCl at an 85:15 ratio, reaching a total volume of 10 mL in a volumetric flask. A volume of 1.5 mL of the extract solution was pipetted as well as combined with 0.75 mL of HCl-KCl buffer at pH 1. Another 1.5 mL of the extract solution was also pippeted and then added with 0.75 mL pH 4.5 citrate buffer. The absorbance readings were taken at wavelengths of 519 and 700 nm utilizing an Agilent Technologies Cary 8454 UV-Vis spectrophotometer, and the anthocyanin content was reported in mg/L (Vieira et al. 2019).

For pH 1 buffer solution, 16.58 mL of 0.2 M HCl was pipetted and dissolved with distilled water to 1000 mL. Subsequently, 0.2 M KCl solution was weighed up to 14.9 g and dissolved with distilled water to 1000 mL. About 97 mL of 0.2 M HCl solution was taken, added with 50 mL of 0.2 KCl solution, and diluted to 200 mL. For pH 4.5 citrate buffer solution, 0.1 M $C_6H_8O_7$ solution was used, 21.01 g of monohydrate nitric acid was weighed and dissolved with distilled water to 1000 mL. About 0.1 M $Na_3C_6H_5O_7$ solution was weighed g and dissolved with distilled water to 1000 mL, then 26.75 mL of 0.1 M $C_6H_8O_7$ solution was taken, added with 23.25 mL of 0.1 M $Na_3C_6H_5O_7$ solution and diluted to 100 mL.

Flavonoid-quercetin by HPLC assay

Flavonoid content analysis was carried out using HPLC Agilent LC 1220 Infinity II. A standard solution was weighed up to 1.7 mg and dissolved with 2 mL of methanol

p.a, sonicated for 30 minutes, then the Standard (depending on the concentration) was diluted with methanol p.a as a solvent. The solution was passed through a 0.22 µm PTFE syringe filter into a 1.5 mL vial and then injected into the HPLC system. The instrument measurement conditions are as follows:

Column : C18, 250 mm × 4.6 mm
Mobile phase : A (Acetonitrile), B (water)
Flow rate : 1 mL/min
Pump : Gradient
Injection volume : 5 µL
Column temp. : 30°C
Detector : DAD, 374 nm

Antioxidant activity by DPPH assay

The shallot extract antioxidant activity was tested employing DPPH method (Dahech et al. 2013). Shallot extract was weighed up to 1 g, dissolved in methanol p.a up to 10 mL, and sonicated for 30 minutes, then filtered. Various concentrations were made with methanol p.a as a solvent (2 mL extract + 1 mL DPPH CAS A-2095 200 µM, incubated for half hour at Room Temperature (RT). The absorbance was calculated at 515 nm utilizing an Agilent Technologies Cary 8454 UV-Vis spectrophotometer. To make DPPH 200 µM, DPPH powder was weighed up to 0.0158 g and dissolved with methanol pa 100 mL, then 20 mL of DPPH was taken, and 20 mL of methanol pa was added (Dahech et al. 2013).

The DPPH color reduction percentage was measured using the equation:

$$\% \text{ Inhibition} = 1 - \frac{\text{Sample absorbance}}{Absorbance\ of\ blank\ sample} x\ 100\%$$

The absorbance of the sample refers to the absorbance measurement obtained for that specific sample, while absorbance of blank sample was the absorbance value for the control.

The IC_{50} value indicates the amount of a sample needed to reduce 50% of DPPH free radicals. This value was determined through linear regression analysis of the graphs, where the horizontal axis displayed the concentrations of the examined plant extracts along with the mean percentage of their scavenging ability from three different cultivars. To calculate the IC_{50} value, Y was set to 50, and the corresponding X value representing the concentration needed to get 50% inhibition, was derived from the regression equation.

Data analysis

Analysis of Variance (ANOVA) in the R software was used for statistical analysis, and significant differences between means were identified using Tukey test at $p < 0.05$. The test was administered three times, and the mean ± SD was used to express the results.

RESULTS AND DISCUSSION

Extract yield

Based on the data in Figure 3, there were differences in the yield of three shallot cultivars extracted using the maceration method. SS Sakato shallot cultivar produced a more significant total extract yield than Bima Brebes and Trisula due to the larger number of chemical compounds contained in the bulbs. The higher the yield value, the greater the value of the extract produced. One of the requirements for a thick extract yield is that the value must not be less than 10% (Ministry of Health of the Republic of Indonesia 2017). Among the three cultivars, SS Sakato and Bima Brebes met the thick extract yield of more than 10%, while Trisula did not.

Phytochemical screening of shallot bulb extract

The content of flavonoid, phenol, alkaloid, terpenoid, steroid, and saponin in red onion bulb extract is shown in Table 1 and Figure 4. The table shows that Bima Brebes and Trisula cultivars contain flavonoid, phenol, terpenoid, and steroid compounds, while SS Sakato cultivar contains flavonoid, phenol, and terpenoid. A red or yellow color change indicates a positive flavonoid test, a blue-black or green color change suggests a positive phenol test and a purple-red color change corresponds to a positive terpenoid test. Additionally, a green-blue color change indicates a positive steroid test. Alkaloid and saponin tests showed negative results due to the absence of color in the sample when given a reagent. The absence of steroid compounds in SS Sakato is presumably due to the genetic, environmental, cultivation, and biochemical factors combination that lead to low activity.

Quantitative phytochemical contents

Quantitative phytochemical content, namely total phenol content, total flavonoid content, as well as anthocyanin is shown in Table 2.

Table 1. Phytochemical screening on three cultivars of shallot

Cultivars	Flavonoid	Phenol	Alkaloid	Terpenoid	Steroid	Saponin
Bima Brebes	+	+	-	+	+	-
	+	+	-	+	+	-
Trisula	+	+	-	+	+	-
	+	+	-	+	+	-
SS Sakato	+	+	-	+	-	-
	+	+	-	+	-	-

Note: +: Present; -: Not present

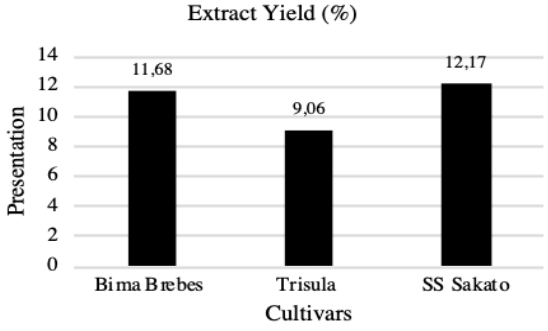

Figure 3. Average extract yield of three shallot cultivars

Table 2 illustrates that the average total phenol compounds in Trisula cultivar were more significant, namely 3.51 ± 0.04 mg GAE/g compared to Bima Brebes at 3.26 ± 0.03 mg GAE/g, and SS Sakato at 3.19 ± 0.02 mg GAE/g. The highest average flavonoid content in Trisula cultivar was 24.03 ± 0.31 mEQ/g, compared to Bima Brebes at 15.52 ± 0.23 mEQ/g and SS Sakato at 14.94 ± 0.28 mEQ/g. The highest average anthocyanin content was in Bima Brebes cultivar at 1.62 ± 0.01 mg/100 g, compared to Trisula at 1.20 ± 0.09 mg/100 g along with SS Sakato at 0.46 ± 0.07 mg/100 g.

Flavonoid-quercetin by HPLC assay

Observation of retention time (tR) is a qualitative analysis parameter that measures the time required for a solution to pass through the column ensuring detection by the detector. Retention time can be measured through a chromatogram from minute 0 until a peak appears. The advantage of using chromatography as a qualitative method is that the sample required for analysis is relatively small, and the duration is short (Chalif and Alauhdin 2024).

The data in Figure 5 indicates that the calibration curve produced the equation of the line y = 12.3x + 1.772, r = 0.9988. The requirement for linearity is the correlation coefficient value, which is good when the r value is ≥0.9988. The results of HPLC analysis carried out on the red shallot bulb extract samples are presented in Table 3 below.

Antioxidant activity by DPPH assay

Antioxidant compounds in plants are phenolics, alkaloids, saponins, and tannins (Suryanti et al. 2016, 2021). Both phenolics and alkaloids are major antioxidants in natural products. Phenolic compounds have radical elimination capabilities due to the irability to donate hydrogen and form stable radical intermediates (Abu-Lafi et al. 2020). The quantity of hydroxyl groups in a phenolic molecule and its chemical structure determine its antioxidant activity.

DPPH radical-scavenging activity was used to assess the antioxidant activity of three cultivars of shallot. The ability of a compound to donate an electron or a hydrogen atom to DPPH to neutralize free radical compounds is utilized in antioxidant assays with DPPH (Dubey et al. 2020). An antioxidant's 50% inhibition concentration (IC_{50}) is needed to scavenge 50% of free radicals DPPH. High antioxidant activity substances will have a low IC_{50} value. The IC_{50} values for DPPH of three shallot cultivars are presented in Table 4.

Table 2. Total phenol, flavonoid, and anthocyanin results of three shallot cultivars

Cultivars	Total phenol (mgGAE/g)	Average	Flavonoid (mEQ/g)	Average	Anthocyanin (mg/100g)	Average
Bima Brebes	3.28	3.26 ± 0.03	15.68	15.52 ± 0.23	1.62	1.62 ± 0.01
	3.24		15.36		1.63	
Trisula	3.54	3.51 ± 0.04	24.25	24.03 ± 0.31	1.13	1.20 ± 0.09
	3.48		23.81		1.27	
SS Sakato	3.20	3.19 ± 0.02	14.74	14.94 ± 0.28	0.51	0.46 ± 0.07
	3.18		15.14		0.41	

Table 3. Results of HPLC analysis on the red shallot bulb extract samples

Sample (cultivars)	Replication	Extract weight (g)	Sample volume (mL)	Sample concentration (mg/L)	FP	RT (min)	Area (y)	Injection concentration (mg/L)	Quercetin levels (mg/g)	Content average quercetin (mg/g)	SD	KV (%)
Bima	1	0.2491	2	124550	100	9.175	242.0371	19.53	15.68	15.52 ± 0.23	0.23	1.46
Brebes	2	0.2445	2	122250	100	9.227	232.708	18.78	15.36			
Trisula	1	0.2483	2	124150	100	9.196	372.0444	30.10	24.25	24.03 ± 0.31	0.31	1.29
	2	0.2511	2	125550	100	9.212	369.5045	29.90	23.81			
SS Sakato	1	0.2529	2	126450	100	9.175	230.9811	18.63	14.74	14.94 ± 0.28	0.28	1.89
	2	0.2699	2	134950	100	9.167	253.0579	20.43	15.14			

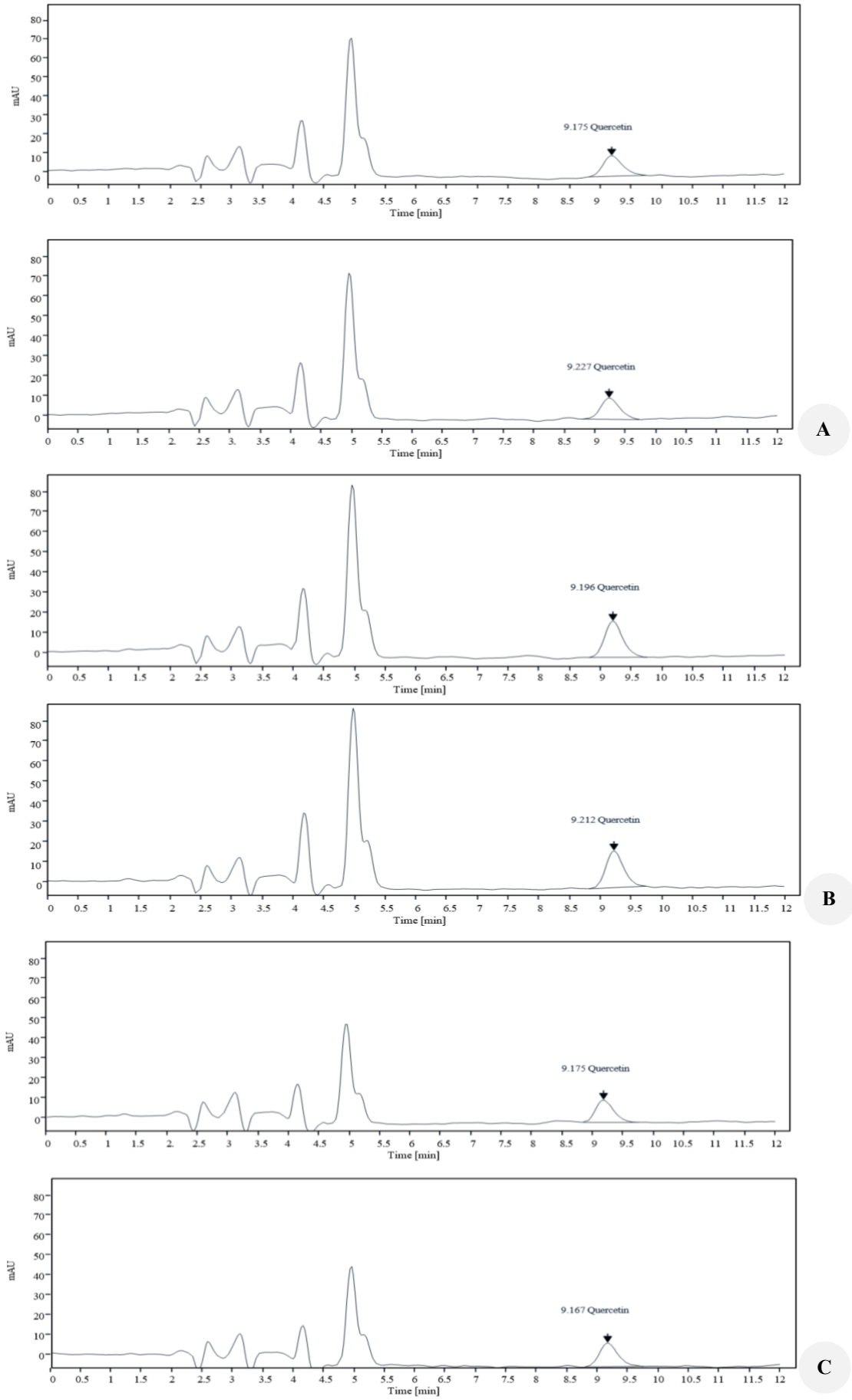

Figure 4. HPLC chromatogram results of shallot cultivars: A. Bima Brebes; B. Trisula; C. SS Sakato

Table 4. Results of antioxidant activities of three cultivars of shallot by DPPH Assay

Cultivars	Replication	Concentration (ppm)	% inhibition	Equality	IC_{50} value (ppm)	Average	SD
Bima Brebes	1	1000	25.10	Y = 0.0139x + 11.164	2794.38	2790.13 ± 6.00 a	6.00
		2000	38.81				
		3000	52.89				
		4000	67.01				
		5000	80.49				
	2	1000	24.86	Y = 0.014x + 10.964	2785.89		
		2000	39.02				
		3000	53.05				
		4000	67.31				
		5000	80.77				
Trisula	1	500	13.22	Y = 0.0141x + 6.8145	3070.46	3061.76 ± 12.30 b	12.30
		1000	21.49				
		2000	35.00				
		3000	49.26				
		4000	62.78				
	2	500	13.24	Y = 0.0141x + 6.8782	3053.06		
		1000	21.60				
		2000	35.41				
		3000	49.33				
		4000	63.11				
SS Sakato	1	1000	13.17	Y = 0.0136x - 0.0506	3687.90		
		2000	27.17				
		3000	40.47				
		4000	55.25				
		5000	80.84			3690.31 ± 3.41 c	3.41
	2	1000	13.11	Y = 0.0136x - 0.3182	3692.72		
		2000	26.89				
		3000	40.85				
		4000	54.33				
		5000	81.25				

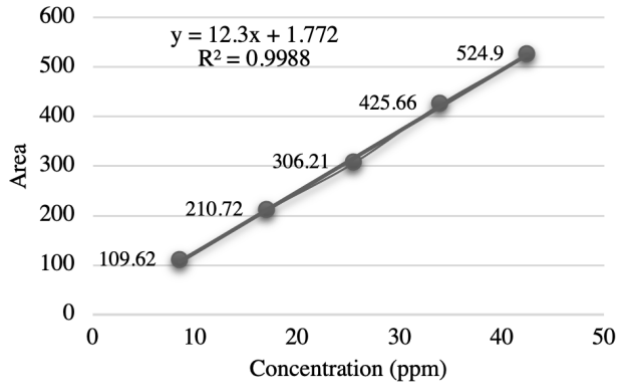

Figure 5. Quercetin calibration curve

Discussion

The size of the extract yield obtained was affected by (i) chemical factors can be categorized into internal and external factors. Internal factors include the type of active compounds present in the material, their qualitative and quantitative composition, as well as the overall average content of these compounds. External factors encompass aspects such as the method of extraction, the effectiveness of the extraction process, the dimensions of the extraction tool, and the size of the sample material (a smaller surface area of the sample allows for wider contact as well as enhances interaction with the solvent). Additionally, external factors involve the solvent utilized in the extraction, the presence of heavy metals, as well as the temperature and time involved in the process (Prasedya et al. 2021; Gil-Martín et al. 2022). (ii) Biological factors include plant species, growing location, harvest time, storage of plant material, age of the plant, and part of the plant used for extracting.

Three shallot cultivars have distinct advantages, for example, based on the description of quality shallot seeds, Bima Brebes cultivar originates from Brebes, Central Java, and well adapted to lowlands. Meanwhile, Trisula originates from the Vegetable Research Institute (Balitsa) and well adapted to lowlands. SS Sakato is from Alahan Panjang local area, Solok District, West Sumatra, and suitable for highlands (Directorate of Horticultural Seeds 2018). These three shallot cultivars were then cultivated on coastal peatlands in Meranti Islands District, Riau Province. The results obtained both in quality and quantity of bulbs, have not reached national production due to the low fertility and pH levels in peat soil. This shortage affects the percentage of extract yield, coupled with the long storage time after harvest for 7 weeks.

In general, phytochemicals are non-nutritional chemicals found in plant parts and have a bioactive capacity that acts as an inhibitor of the growth of disease pathogens and antioxidant capacity by protecting cells against oxidative stress as well as damage attributed to free radicals (Sharifi-Rad et al. 2020).

Trisula cultivar has a higher flavonoid content than Bima Brebes and SS Sakato due to a combination of genetic, environmental, and cultivation factors. Variations in gene expression levels likely contribute to differences in flavonoid accumulation. Trisula cultivar has been able to adapt better to coastal peat environments, showing resilience against drought stress or heavy rainfall. The application of cultivation and fertilization methods further contributes to the enhanced flavonoid production. Furthermore, the F3H enzyme also contributes to the flavonoid biosynthesis pathway and regulates the accumulation of flavonols and antioxidants. High flavonoid content can affect the quality and benefits of shallot plant.

Bima Brebes cultivar was found to contain a higher anthocyanin content than Trisula and SS Sakato. This is presumably due to the more intense red color of the bulbs, which affects the anthocyanin content. Anthocyanin is a pigment responsible for the red color in shallot. In addition, genetics, gene expression, environmental factors, cultivation techniques, and fertilization also affect the color and quality of shallot bulbs. Although environmental factors, cultivation techniques, and fertilization for these three cultivars were the same, Bima Brebes is able to produce higher anthocyanin than other cultivars.

Changes in total phenol, flavonoid, and anthocyanin content in shallot can be attributed to variations between cultivars. High flavonoid levels indicate that plants are better able to manage environmental stress (Salem et al. 2011). In this regard, the influencing factors include plant varieties, climate, growing area, cultivation process, harvest period, storage, light, and temperature (Mattioli et al. 2020). Different varieties or cultivars have distinct genetic diversity to impact the synthesis along with compilation of phenol, flavonoid, and anthocyanin. Furthermore, environmental conditions including temperature, humidity, light intensity, and interactions between plants and microorganisms can affect the synthesis and accumulation of phenol, flavonoid, and anthocyanin. Physiological factors of shallot plants, such as age, health, and enzyme activity related to synthesizing phenol, flavonoid, and anthocyanin, may differ between cultivars. Different colors and shapes of shallot and water content are also affected.

The composition of coastal peat soil in Meranti Islands District has a relatively low pH and low nitrogen, phosphorus, and potassium levels. The texture of coastal peat is soft and muddy, with high water content and relatively low density, affecting the total phenol, flavonoid, and anthocyanin of shallot bulbs. Therefore, before cultivating plants, a more specific peat soil analysis should be carried out to understand the condition of the land used and the type and dose of fertilizer to increase the fertility of the soil and cultivated plants. The level of stress in plants potentially affects the content of total phenol, flavonoid, and anthocyanin through several mechanisms, namely increasing secondary metabolic synthesis, changing gene expression (activating genes related to stress and inhibiting genes related to growth), and varying physiological conditions (changing water content and changing temperature).

Yeloojeh et al. (2020) noted that the total flavonoid content depends on the species, plant part, and drought level. A study conducted by Olsovska et al. (2024) for 2 years stated that fertilization has a differential influence on the flavonoid content of several shallot varieties. The Kamal variety exhibited the highest flavonoid content at 0.286 mg g^{-1} FW when fertilized with Nitrogen and Sulfur, followed closely by the Robin variety, which reached 0.299 mg g^{-1} FW with Nitrogen, Sulfur, and Iron fertilization. Nonetheless, the overall highest flavonoid content was recorded for the Nitrogen-fertilized Robin variety at 0.353 mg g^{-1} FW. The yellow Mundo variety showed no significant changes in flavonoid levels due to fertilization. In contrast, the Pueblo variety consistently had the lowest flavonoid content across all fertilization treatments, ranging from 0.104 to 0.116 mg g^{-1} FW. Meanwhile, in Nitrogen and Sulfur fertilization, the white Pueblo variety displayed a 10% increase in flavonoid levels than the control group (O). Implementing optimal fertilization methods is vital for ensuring food security, promoting environmental sustainability, and enhancing human health. A balanced approach to fertilization, particularly the combined effects of Nitrogen and Sulfur, boosts shallot yield as well as nutritional value, aiding in shallot farming long-term sustainability.

Kusumiyati et al. (2024) reported that the total flavonoid of three shallot cultivars, namely Sumenep (95.07 ± 0.30), Bima Brebes (221.78 ± 0.76), and Trisula (334.73 ± 1.09) were relatively low. Metrani et al. (2020) examined two shallot cultivars namely Honeysuckle and Sweet Italian which produced total flavonoid of 444.37 ± 75.21 and 345.34 ± 58.09, with anthocyanin of 103.40, 37 ± 22.06, and 86.47 ± 18.43, respectively.

Phenol and flavonoid compounds are responsible and closely linked to antioxidant activity. Dudonné et al. (2009) examined 30 plant extracts, which showed a significant link between the concentration of phenol compounds as well as antioxidant activity using a method based on free radical capture and iron-reducing capacity. Phenol compounds inhibit mutagenesis and carcinogenesis in the human body, consuming up to 1 gram per day of fruit and vegetable foods (Sun et al. 2011).

Anthocyanin is a flavonoid compound responsible for shallot purple or red color (Metrani et al. 2020). Shallot is a good source of anthocyanin, with the highest concentration often found in dry outer skin, ranging from 109-219 mg/100 g (Zhang et al. 2016). About 25 anthocyanins have been identified in shallot, including cyanidin 3-O-glucose, the main component in epidermal cells. The outer layer of half a shallot bulb contains anthocyanin, while the inner layer is minimal (Collings 2019). The intensity and stability of anthocyanin can vary based on various factors, including the amount and structure, pH levels, temperature, light intensity, as well as the presence of other pigments, metal ions, enzymes, oxygen, sulfur dioxide, ascorbic acid, and sugar metabolites (Enaru et al. 2021).

HPLC chromatogram results have an abscissa (x-axis), the retention time in minutes, while the ordinate (y-axis) is the detector response in mAU units. The chromatogram results in Figure 2 show that in Bima Brebes, Trisula, and SS Sakato, the standard retention time of quercetin was 9.175 minutes and 9.227 minutes, 9.196 minutes and 9.212 minutes, as well as 9.175 minutes and 9.167 minutes,

respectively. The standard retention time of quercetin of three shallot cultivars ranged from 9.167 to 9.227 minutes, with an average of 9.192 minutes. The standard retention time of quercetin ranges from 9.148 to 9.218. Therefore, it can be concluded that the three cultivars of shallot, such as Bima Brebes, Trisula, and SS Sakato, contain quercetin.

Table 3 shows that based on the quantitative analysis, the average quercetin content in Bima Brebes, Trisula, and SS Sakato cultivars was 15.52 mg/g, 24.03 mg/g, and 14.94 mg/g, respectively. Moreover, the high levels in Trisula cultivar can be attributed to internal as well as external factors. Internal factors are related to the cultivar genetics, as well as the quality of shallot bulbs comprising the size, shape, and color. Meanwhile, the external factors are temperature, humidity, sunlight, nutrients, drought, pest attacks, and plant diseases.

The results obtained were greater than Major et al. (2022), who explained that the three species produced quercetin levels as follows of *Allium* × *cornutum* of 8.12 ± 0.84 mg/100 g DW1, *Allium* × *proliferum* of 6.73 ± 0.36 mg/100 g DW1 and *A. cepa* aggregatum of 26.63 ± 3.26 mg/100 g DW1. Furthermore, Sukasih et al (2017) examined 10 varieties of shallots cultivated in Indonesia, producing an average of 199.68-1766.4 ppm (0.19968-1,766.4 mg/g). Bima Brebes variety produced a quercetin content of 443.74 ppm (0.44374 mg/g).

Antioxidant activity test results of three shallot cultivars indicated by IC_{50} value are shown in Table 4. The best IC_{50} value was found in Bima Brebes at 2790.13 ± 6.00, followed by Trisula at 3061.76 ± 12.30, and SS Sakato at 3690.31 ± 3.41. These three shallot cultivars produced IC_{50} values classified as very weak antioxidants >200 ppm. IC_{50} value describes the concentration at which an antioxidant can inhibit 50% of radicals. The percentage of inhibition showed an increase with rising concentration, starting from 500, 1000, 2000, 3000, 4000, to 5000 ppm. This implies that antioxidant activity is based on the extract concentration used. The evaluation of IC_{50} value aims to determine the concentration of the fraction in reducing DPPH activity, indicated by a decrease in radical absorbance of 50%. A lower IC_{50} value indicates a more potent antioxidant ability to inhibit radicals, while a higher IC_{50} value signifies a weaker antioxidant activity (Sariwati et al. 2024).

The concentration for the three shallot varieties was very high because, at a concentration of 500-5000 ppm, only 50% inhibition could be produced. These results suggest that the three shallot cultivars contain slight antioxidant activity presumably due to several factors including the type of peat soil used, water content, and use of fertilizers. At the beginning of planting, there was stress due to the dry season until the plants were 21 days old. Subsequently, at harvest time, shallot bulbs were air-dried for 6 weeks. A secondary metabolic test was carried out, where at 6 weeks, the percentage of bulb weight loss had decreased by 30-35%. This explanation was also reinforced by Zhang et al. (2016) who stated that phytochemical production in plants was influenced by genetic and environmental factors (microclimate, location, planting season, soil type, and nutrients), plant maturity, storage, as well as post-harvest processing. Kurnia et al (2021) observed the antioxidant compounds in *Allium sativum* leaves, showing IC_{50} of 7.21 ± 0.39 mg/mL (7210 ppm) using DPPH test.

DPPH is a stable organic free radical with an absorption band at 517 nm. It loses absorption when accepting electrons or free radical species, causing the visual fading of the purple color to yellow. Antioxidants reportedly capture free radicals from the oxidation chain and form stable free radicals that do not trigger or cause further oxidation inhibition (Kedare and Singh 2011). DPPH radicals are widely used to evaluate antioxidants free radical scavenging capacity. The results showed that the methanol extract of Dayak shallot and ascorbic acid effectively reduced stable DPPH radicals to yellow diphenyl ficrylhydrazyl, suggesting the extract is active in scavenging DPPH radicals. The radical scavenging activity correlated highly with total flavonoid content (R = 0.9468). A lower correlation was observed between this activity and total phenol content (R = 0.8908). The polarity of the solvent and the effect on the extraction of phenol and flavonoid were mainly responsible for the differences between DPPH radical scavenging capacities of the extracts. The capacity of phenol and flavonoid compounds to scavenge free radicals may be due to the presence of hydroxyl groups in the structure and the ability to donate electrons that transfer hydrogen to radicals and produce phenoxide radicals to stabilize the product (Khalili et al. 2022).

In conclusion, shallot cultivar containing the highest extract yield was SS Sakato at 12.17%, followed by Bima Brebes at 11.68% and Trisula at 9.06%. All three cultivars contained flavonoid, phenol, terpenoid, and steroid, except SS Sakato, which did not contain steroid. Trisula cultivar produced the highest total phenol and flavonoid, while Bima Brebes had the highest anthocyanin. The highest quercetin content was in Trisula at 24.03 mg/g, followed by Bima Brebes at 15.52 mg/g, and SS Sakato at 14.94 mg/g. In contrast, the highest IC_{50} value was produced by Bima Brebes at 2790.13 ± 6.00, followed by Trisula cultivar at 3061.76 ± 12.30, and SS Sakato at 3690.31 ± 3.41.

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