Original Article

Secondary metabolitm determination from Brebes shallot's ethanol extract and its ethyl acetate fraction "*Allium ascalonicum* L."

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ABSTRACT

Shallot is a type of onion (*Allium ascalonicum* L). Brebes is one of the most extensively consumed horticultural crop commodities, and it includes medicinal secondary metabolites. Furthermore, Brebes shallots are high in flavonoids, which are the most important phytochemical in food and provide a wide range of biological advantages for humans. The purpose of this study was to analyze the secondary metabolites and flavonoids content in shallot from Brebes, Indonesia. Maceration extraction with 70% ethanol solvent and fractionation with ethyl acetate solvent was used in this study. The results of the secondary metabolite compound test qualitatively showed that the Brebes shallot Simplicia contains volatile oil compounds, saponin, tannin, flavonoids, and terpenoids, and TLC test with toluene mobile phase: Ethyl acetate (4:0.5) obtained one spot on quercetin with an Rf value of 0.372, On the shallot extract, there was one spot with an Rf value of 0.359, and three spots with Rf values of 0.205, 0.359, and 0.449 on the shallot fraction.

Keywords: Flavonoids, Fractionation, Maceration, Shallot

Introduction

Shallot (*Allium ascalonicum* L. of Brebes) is one of the most frequently used horticultural commodities. Shallot is commonly used as a flavoring in food or as a cooking spice, and they have a variety of therapeutic effects [1]. Secondary metabolites in onion bulbs, such as tannins, saponins, essential oils, kaempferol, flavon glycosides, fluroglucin, dihydroalin, cycloalkene , methylalin, quercetin, polyphenols, sulfur, and flavonoids, can be used as traditional medicines.

Flavonoid is one of the compounds found in shallot with the highest content [2]. Flavonoids are pigments found in fruits, flowers, and leaves that contribute to the production of red, orange, blue, and purple colors. Flavonoids are a type of water-soluble polyphenol. The most important phytochemical in food is flavonoid bioactive, which have a wide range of biological benefits for humans [3].

Macerated extraction is a simple method of extracting flavonoid compounds from a sample based on the principle of immersing and stirring the sample in an appropriate solvent. When compared to other extraction methods, macerated extraction has the advantage of using more solvents [4]. This method is better because the extraction process is at room temperature to prevent flavonoid damage [5].

This study is about a qualitative test of Brebes shallot for secondary metabolites and flavonoid content, which was used as a preliminary investigation to determine the active substance content so that it could be used for more extensive research.

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Materials and Methods

Tools and materials

Tools

The tools used for this research are glassware (Pyrex), oven (Memmert), rotary evaporator (bio base), maceration set, fractionation set, TLC, and UV lamp.

Materials

The materials used for this research are the Bima variety of shallot purchased from Brebes farmers in Central Java, Indonesia with GPS latitude location: -6° 52' 59.99" S and longitude location: 109° 02' 60.00" E., ethanol, ethyl acetate, acetic acid, quercetin, aqua dest, $AlCl_3$, methanol, acetic acid, a TLC plate, filter paper, and spirtus(Denatured alcohol). HCL_2N, Mayer's reagent, Bouchardat's reagent, Wegener's reagent, Sudan III, $FeCl_3$, concentrated hydrochloric acid, magnesium.

Work procedures

Extraction

A 200-gram sample was placed in the macerator container, and then 1:10 aqua dest was added. Maceration for 4 x 24 hours was followed by stirring for 5 minutes before being evaporated with a rotary evaporator at 70° to yield a thick extract.

Phytochemical screening

The presence of secondary metabolites in shallots was determined using phytochemical screening.
Identification of compound groups:

- *Identification of alkaloid compounds*

2 grams of the sample extract were added to a test tube and 5 ml of heated HCL2N were poured into it, After cooling, divided the mixture into three tubes and added Mayer's reagent; if the alkaloids were positive, white precipitate forms. If positive, the second extract is added with Bouchardat's reagent, which results in a brown-black color change. If positive, the third extract is added with Wagner's reagent, which results in a brown precipitate.

- *Identification of saponin compounds*

If 1 gram of extract is placed in a test tube, 10 ml of hot water is added, allowed to cool, and then shaken vigorously for 10 seconds, and there is foam with a height of 1-10 cm in not less than 10 minutes, and 1 drop of HCL2N is added, the foam does not disappear, it is positive for saponins.

- *Identification of tannin compounds*

Add 1 gram of extract into a test tube, add 10 ml of hot water, then bring to a boil and add 3-4 drops of FeCl3 to the filtrate. If a blue-black color is formed, it is positive for tannin.

- *Identification of essential oil compounds*

Add the extract into the test tube, and then add Sudan III. If it is positive, the filtrate is orange.

- *Identification of flavonoid compounds*

Add the extract into a test tube, and then add 1 ml of 95% ethanol, 0.1 magnesium, and 10 drops of concentrated hydrochloric acid. If positive, a red color will be formed.

- *Identification of terpenoids and steroids*

In a test tube, 2 grams of the sample extract were combined with 2 ml of ethyl acetate and agitated. After drying, 2 drops of anhydrous acid and 1 drop of concentrated sulfuric acid were added to the ethyl acetate layer, which was put on a drip plate. If a red or yellow hue appears, it indicates the presence of terpenoids; if a green color appears, it indicates the presence of steroids.

Results and Discussion

The School of Biological Science and Technology, Bandung Institute of Technology, Indonesia, was used to determine the identity of the shallot plant from Brebes, Central Java, Indonesia. The shallot from Brebes was identified as Allium Ascalonicum L., a part of the Alliaceae family.
Using ethanol and ethyl acetate as solvents, extraction and fractionation results were obtained. In the maceration and fractionation processes, the solvent used is determined by the desired target compound. Non-polar solvents dissolve non-polar compounds, while polar solvents dissolve polar components. This is following the "like dissolves like" principle of dissolving a substance. A solvent's polarity can be determined using its chemical properties, namely the dielectric constant. The dielectric constant is a measurement of a solvent's polarity. Insolvents with a high dielectric constant, more polar compounds will dissolve [6].

Results of identification of secondary metabolic compounds

A small sample of shallot and a simple procedure wasused to conduct phytochemical screening to determine whether secondary metabolites are present. The results of qualitative compound identification based on observations can be seen in **Table 1** and **Table 2** below.

Table 1. Identification of shallot ethanol extract secondary metabolite compounds

Phytochemical test	Reactor	Color	Result	Reference
Minyak atsiri	Ethanol 95% + sudan III	orange	+	orange

Saponins	Water+ HCL	frothy	+	frothy
Tannin	Water + $FeCl_3$	black	+	blue-black
Flavonoid	Ethanol 95% + Magnesium + Concentrated HCL	orange	+	orange-purple
Terpenoids	Ethyl Acetate +acetic anhydride +concentrated sulfuric acid	yellow	+	red- yellow
Alkaloid	HCL2N + Wagner's reagent	red	-	brown
	HCL2N + Mayer's reagent	Red sediment	-	white sediment
	HCL2N + Bouchardat's reagent	red	-	brown - black

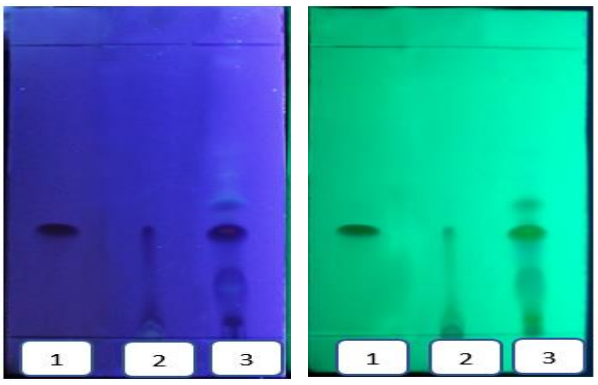
Table 2. Identification of secondary metabolite compound ethyl acetate fraction of shallot

Phytochemical Test	Reactor	Color	Result	Reference
Saponins	Water + HCL	frothy	+	frothy
Tannin	Water + $FeCl_3$	black	+	blue-black
Flavonoid	Ethanol 95% + Magnesium + Concentrated HCL	orange	+	orange-purple
Terpenoids	Ethyl Acetate +Acetic Anhydride +Concentrated Sulfuric Acid	yellow	+	red-yellow
Alkaloid	HCL2N + Wagner's reagent	red	-	brown
	HCL2N + Mayer's reagent	Red sediment	-	white sediment
	HCL2N + Bouchardat's reagent	red	-	brown-black

The test results showed that the ethanolic extract of shallot contains volatile oil compounds, saponins, tannins, flavonoids, terpenoids, and the ethyl acetate fraction of shallots contains saponins, tannins, flavonoids, and terpenoids. The results of a qualitative test were conducted on extracts and fractions thought to have the most flavonoids compounds. It can be seen by the color change that occurs from clear yellow to red-orange. The higher the content of flavonoid compounds, the darker the red color produced [7].

Thin layer chromatography

Identification of Thin Layer Chromatography compounds to prove that the extracts and fractions of shallot obtained contain flavonoids and then qualitatively tested by TLC. This method was chosen because it is easy, only requires a little sample of material, produces precise results, and takes a short time to process. The Rf value of quercetin is calculated based on the results of identification using TLC with a quercetin type flavonoid control and visible spots on the TLC plate, ethanol extract and ethyl acetate fraction of shallots is listed in **Table 3** and TLC results from UV light analysis at wavelengths of 366 nm and 254 nm in listed in **Figure 1** below.



a) b)

Figure 1. TLC results from UV light analysis at wavelengths of 366 nm (a) and 254 nm (b) on a spot

Table 3. ResultRf Calculation

No	Sample	Sample's travel distance		
		1	2	3
1	Quercetin standard		0,372	
2	Onion ethanol extract		0,359	
3	Fraction of onion ethyl acetate	0,205	0,359	0,449

TLC analysis of shallot extract and fractionation with toluene mobile phase: Ethyl acetate (4:0.5) obtained one spot on quercetin with an Rf value of 0.372, On the shallot extract, there was one spot with an Rf value of 0.359, and three spots with Rf values of 0.205, 0.359, and 0.449 on the shallot fraction. From the Rf value, it can be seen that flavonoid quercetin has more semipolar properties. When viewed from the structure of the following chalcone compounds, there are several hydroxyl groups (OH) attached to the benzene ring [8].

The TLC test was conducted on extracts and fractions that were thought to contain the highest levels of flavonoid compounds, namely the ethyl acetate fraction. This can be seen in the results of wider spots, so quantitative identification of flavonoid compounds is required.

Conclusion

Based on the findings, it can be concluded that shallot (*Allium ascolicum* L)phytochemical screening is beneficial. Chemical compounds found in Brebes include essential oils, flavonoids, saponins, tannins, and terpenoids. As a follow-up, TLC results were used to determine the flavonoid quercetin in the mobile phase. Toluene: Ethyl acetate (4:0.5) obtained one spot on quercetin with an Rf value of 0.372, On the shallot extract, there was one spot with an Rf value of 0.359, and three spots with Rf values of 0.205, 0.359, and 0.449 on the shallot fraction.

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