Extraction of Flavonoid, Phenolic, and Saponin in Shallot Skin (*Allium Cepa var. Aggregatum*) Extract for Antibacterial Application

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ABSTRACT

Shallot skin (Allium cepa var. aggregatum) contains secondary metabolites of flavonoid, phenolic, and saponin that can be antibacterial. This research was conducted by extracting the shallot skin using maceration and ultrasound-assisted methods with 3, 5, and 7 days for maceration extraction and 10, 20, and 30 minutes for ultrasonication-assisted extraction (UAE). The extract was then tested for phytochemical screening and analysis of its flavonoid, phenolic, and saponin levels. Three extracts with the best secondary metabolite compounds were then tested for the inhibitory power of the extracts against Escherichia coli through the diffusion method. This research contribution is to show that shallot skin extract contained flavonoid and phenolic compounds but not saponin. Based on the analysis of the levels of these compounds using UV-Vis spectrophotometry, three extracts with the highest flavonoid and phenolic content were obtained from maceration extraction for seven days, the UAE method for 20 minutes, and the UAE extraction method for 30 minutes. The extract using the UAE method has relatively higher flavonoid and phenolic content (6.93 and 2.59 ppm, respectively) than the extract using the maceration method (5.48 and 2.46 ppm, respectively), which is also considered more efficient in terms of time. A preliminary antibacterial activity test showed antibacterial activity in the shallot skin.

1. Introduction

In February 2022, the coronavirus outbreak reached 300,000 active cases in Indonesia [1]. This outbreak increased people's awareness about personal hygiene and health, thus causing the sales of antibacterial products such as hand sanitizers and disinfectants to increase rapidly. Generally, these products are alcohol-based, causing the skin to dry out. For this reason, the development of nonalcohol-based antibacterial products was carried out. Products with natural ingredients have a better perception in consumers' eyes regarding mildness to the skin, so it is better to focus more on developing products made from natural ingredients.

Shallots (*Allium cepa L. var aggregatum*) are typical dishes in Indonesia and many others. Mostly, the shallot skin is discarded. Shallot skin contains chemical compounds with antibacterial activity, such as phenolic, flavonoid, and saponin [2], [3]. The antibacterial activity of ethanol extract of shallot skin showed inhibitory activity against the growth of several bacteria, such as *Staphylococcus Aureus* and *Escherichia coli* [4]. This result shows that the shallot skin can be developed as an antibacterial agent.

Escherichia coli (*E. coli*) is one of the most common bacteria that cause infection in daily life [5]. This bacteria is widely found on human hands due to the unhygienic habit of not washing hands or due to recontamination [6]. Therefore, in this study, *E. coli* bacteria were used to test the antibacterial activity of the shallot skin. Testing it against *E. coli* does not mean it can kill coronavirus. Nevertheless, it can reduce the chance of getting infected by coronavirus due to lower immunity caused by bacteria infections.

In order to produce a good quality antibacterial agent, a simple yet reliable extraction method is required. A simple extraction method that has been carried out for shallot skin extraction is maceration, but this extraction takes 4-5 days in total [7], [8]. In another study, heat can increase the yield of secondary metabolites extracted [9]. However, the high heat in microwave-assisted extraction is feared to damage the compound. Another study shows that ultrasonication-assisted extraction (UAE) results in a better secondary metabolite content and a much shorter extraction time (20 minutes) [10].

The contribution of this study is to extract shallot skin using maceration and UAE methods to obtain the best secondary metabolite results with a feasible extraction time. The extracts were then tested for their antibacterial activity for future application in manufacturing antibacterial products.

2. Research Methodology

2.1. Materials

Shallots were obtained from the local market. Folin ciocalteu's phenol reagent, synthesis grade (purity ≥98.0%) of gallic acid, and pro analysis grade (purity 99.9%) of aluminum chloride, sodium acetate, sodium hydroxide, ethanol, ferric chloride, and sodium carbonate were obtained from Merck. Quercetin with a purity of ≥95.0% was obtained from Sigma. The technical grade of alcohol (70 and 96%) and aquadest were obtained from local chemical stores.

2.2. Procedures

The flow diagram of research procedure is shown in Fig. 1.

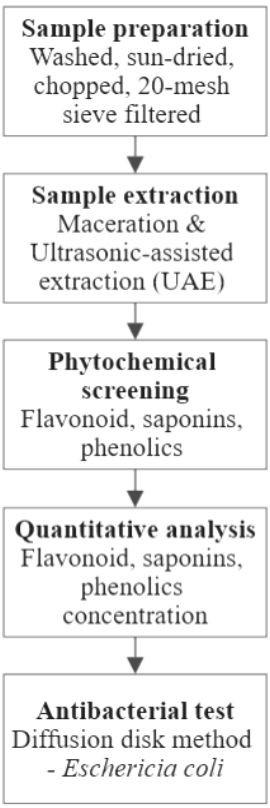

Fig. 1. Flow diagram of research procedure.

2.2.1. Simplicia Preparation

Fresh shallot skins (*Allium cepa var. aggregatum*) with no defect or disease were selected, washed in running water, and sundried for two days. The skin was then chopped and mashed using a blender to increase its surface area and sieved through a 20-mesh sieve.

2.2.2. Sample Extraction

The procedure was modified from the study by Rahayu et al. for maceration extraction [11]. A 10 g of simplicia was soaked in 150 mL of 96% ethanol for three days at room temperature and stirred manually three times a day. The simplicia was then filtered through a Whatman 1004-110 filter paper. The extract was then put into a rotary evaporator at a temperature of 70°C until a thick extract was obtained. The procedure was repeated at 5 and 7 days extraction time.

For ultrasonic-assisted extraction, a 10 g shallot skin sample was mixed with 150 mL of 96% ethanol and then extracted using sonication for 10 minutes at 38°C and 48 kHz. The extract was then evaporated using a rotary evaporator. Evaporation was carried out at 70°C until a thick extract was obtained. The procedure was repeated at 20 and 30 minutes of extraction time.

2.2.3. Phytochemical Screening

The phytochemical screening was done on the extract to detect the presence of flavonoid, phenolic, and saponin compounds. 1 ml of the extract was added to two drops of 10% NaOH in a test tube and shaken vigorously. The extract is positive for flavonoid if a yellow color appears [12]. 2 g of extract was put in a test tube, then distilled water was added until the sample was submerged and then boiled for 2-3 minutes, cooled, and then shaken vigorously. The presence of stable foam indicates the presence of saponin [12]. 1 ml of extract was added with 2-3 drops of 10% $FeCl_3$. If the solution was blue, green, purple, red, or black, it was positive for phenolic content [11].

2.2.4. Quantitative Extract Analysis

The content of the extract was analyzed quantitatively to determine the levels of secondary metabolites using a UV-Vis spectrophotometer at its maximum wavelength of 435 nm for flavonoids [9] and 750 nm for phenolic [13]. Analysis of flavonoids refers to previous research. First, a standard solution of 100 ppm quercetin was made by adding as much as 0.05 mg in a 50 mL volumetric flask. Add 15 mL of 70% ethanol, 1 mL of 10% $AlCl_3$, and 1 mL of 1 M CH_3COONa. Then, dilute with distilled water to the limit of the volumetric flask. After that, stir by shaking until homogeneous and let stand for 30 minutes at room temperature. Then, measure the absorbance using a UV-Vis spectrophotometer at a wavelength of 435 nm [9].

The next step was to create standard quercetin curves. Quercetin standard solutions were prepared at concentrations of 2, 4, 6, 8, and 10 ppm from a 100 ppm solution. The standard solution of 100 ppm was dripped using a pipette into a 50 mL volumetric flask. The volumes used were 1, 2, 3, 4, and 5 mL, then added 15 mL of 70% ethanol, 1 mL of 10% $AlCl_3$, and 1 mL of 1 M CH_3COONa and diluted with distilled water to the volume of the volumetric flask. After that, it was shaken until homogeneous and incubated at room temperature for the optimal time. The absorbance of the solution was measured at 435 nm using UV-Vis spectrophotometry.

Subsequently, the extracts produced in the maceration and UAE methods were measured at 50 mg each, then dissolved in 50 mL of 70% ethanol. 10 ml of the solution was dripped using a 10 mL pipette into a 50 mL volumetric flask. Then, 15 mL of 70% ethanol, 1 mL of 10% $AlCl_3$, and 1 mL of 1 M CH_3COONa, and diluted with distilled water to the limit of the flask. Next, 10 ml of the mixed solution was added into a 50 mL volumetric flask and diluted with water to the limit of the flask. After that, the solution was shaken until homogeneous and incubated at room temperature for the optimal time. Absorbance was measured using UV-Vis spectrophotometry at 435 nm.

The first step in analyzing phenolic content was making a 100 ppm gallic acid standard solution by diluting gallic acid in pro-analysis grade ethanol (purity 99.99%). This standard solution was then diluted to 1, 2, 3, and 4 ppm. A total of 0.4 ml of Folin Ciocalteau reagent was added and allowed to stand for 8 minutes. Na_2CO_3 was then added and shaken until homogeneous and added with distilled water until the total volume was 10 ml. After that, let stand for 2 hours at room temperature. Then the absorbance was measured at a wavelength of 750 nm [13]. Next, 10 mg extract was dissolved in 10 mL pro-analysis grade ethanol until homogeneous. A total of 1 ml of the solution was pipetted, and then Folin Ciocalteau reagent was added, shaken, and allowed to stand for 8 minutes. 4 mL of

Na_2CO_3 was added and shaken again until homogeneous. Distilled water was added until a total volume of 10 ml, then let stand at room temperature for 2 hours. The absorbance was then measured using UV-Vis spectrophotometry at 750 nm.

2.2.5. Antibacterial Test

The antibacterial activity was measured using the diffusion disk method [14]. The bacteria used were *E.* coli. This test was conducted on three extracts with the highest levels of secondary metabolites and was carried out by a third party at the Biology Education Science Laboratory, Universitas Sultan Ageng Tirtayasa.

3. Results and Discussion

3.1. Phytochemical Screening

Table 1 shows positive results for flavonoids and phenolic compounds but negative for saponins. The same results were obtained in shallot leaf extract from the same island [15]. On the contrary, as in [3] found different results where flavonoids, phenolic, and saponin compounds exist in shallot skin. Reference [2] also found that saponin exists in shallot roots from other islands in Indonesia.

Just as humans differ, plants of the same species can have differences, such as the secondary metabolite content. The latitude and altitude of the plant's location dictate many factors essential for the plant, such as light intensity, humidity, temperature, and soil fertility. Reference [16] examined the response of plant secondary metabolites in adapting to environmental changes, which can lead to differences in the content of secondary metabolites. Reference [17] studied differences in shallot morphology and chemical compounds in several Asian countries owing to their adaptability to local conditions.

Table 1. Phytochemical Screening of Extracts

Secondary metabolites	Reagent	Indicator	Result
Flavonoid	NaOH 10%	Yellowish, reddish, or brownish	+
Saponin	Aquadest	Formation of foam	-
Phenolic	$FeCl_3$	Dark blue	+

Notes: + positive; - negative

3.2. Extract Metabolites Analysis

Based on the results of phytochemical screening, shallot skin extract contains antibacterial compounds in the form of flavonoid and phenolic. The quantitative analysis of these secondary metabolites is shown in Fig. 2.

In Fig.2.a, there was an increase in flavonoid and phenolic levels along with the extraction time. A longer extraction time allows more contact between the compounds and the solvent. Thus, a mass transfer occurs due to concentration differences until equilibrium is reached. This phenomena is in accordance with the research conducted as in [18], where extended extraction time generates more concentrated extract.

In Fig. 2.b, the longer the extraction time, the more flavonoids and phenolic compounds are extracted. Cavitation due to ultrasound produces waves that can disrupt cells and break up particles [19]. The ultrasonic waves break the cell walls and release the components into the solvent. At some point, longer extraction time causes the compounds to decrease. Sonication transfers energy to the solvent and generates heat through friction. It is widely known that the extraction rate of most compounds will increase with increasing temperature [20]. However, some active compounds, such as flavonoids and phenolics, are heat-sensitive. When these materials are in contact with heat, the compound is susceptible to damage and decomposition. Temperatures exceeding 40℃ can cause a decrease in antioxidant levels [21]. A similar result was obtained as in [22], [23], [21] that a longer sonication time can cause reduced secondary metabolite concentration due to decomposition.

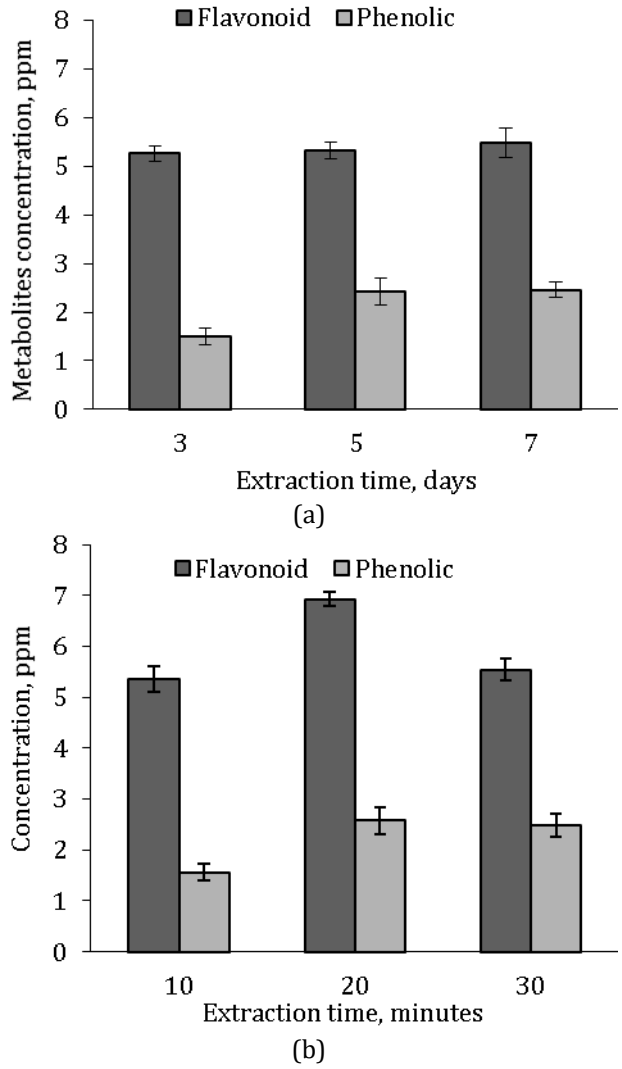

Fig. 2. Effect of extraction time on flavonoid and phenolic contents (a) maceration extraction, (b) ultrasonic-assisted extraction.

It can be seen from Fig. 2. that the phenolic and flavonoid content obtained using the UAE (2.59 ppm phenolic and 6.93 ppm flavonoids) is higher than maceration extraction (2.46 ppm phenolic and 5.48 ppm flavonoids). It is more favourable to use the UAE method because the time required is relatively short compared to maceration, with better results. Rather than depending on the diffusion of the molecules to the solvent, ultrasonication energy accelerates the diffusion by generating a current which aids the mass transfer. Another study explained that UAE could increase particle swelling and hydration so that the cell wall pores widen and facilitate a mass transfer, thereby shortening extraction time [24]. Reference [22], [25] also obtained similar results where ultrasound-assisted extraction has better extract quality than maceration extraction. Based on the data, three extracts with the highest levels of flavonoids and phenolic were macerations seven days, 20 minutes UAE, and 30 minutes UAE.

3.3. Antibacterial Test

Antibacterial tests were carried out for the top three extracts with the best concentrations of flavonoids and phenolics. Based on Fig. 3., it can be seen that each variation of the extract has an inhibition zone against *E. coli* bacteria. The more antibacterial substances in the extract, the greater the inhibition against *Escherichia coli*. The commonly proposed mechanism is the interaction between antibacterial substances and the cell wall, which damages the cell wall. Damaged cell walls reduce their resistance to unfavorable conditions [26]. Reference [27] stated that the mechanism of flavonoids in inhibiting bacterial growth is through preventing nucleic acid synthesis, inhibiting the function of the cytoplasmic membrane, and energy metabolism. According as in [28], the activity of

flavonoids and phenols as antibacterials is due to the formation of complexes with bacterial proteins that can deactivate bacteria enzymes.

All the tested samples had weak antibacterial activity (inhibition zone ≤12 mm) [29]. This result is in line with the research as in [30], where the *Azadirachta indica* extract did not show an inhibition zone on the gram-negative bacteria *Escherichia coli* but had an impact on the gram-positive bacteria *Staphylococcus aureus*. Gram-negative bacteria can change cell walls, so antibiotic compounds cannot attach or produce enzymes to deactivate antibiotics. Although bacteria have no brain, they can process information. Just as bacteria search for food sources, they can move away from antibacterial compounds as a way to survive.

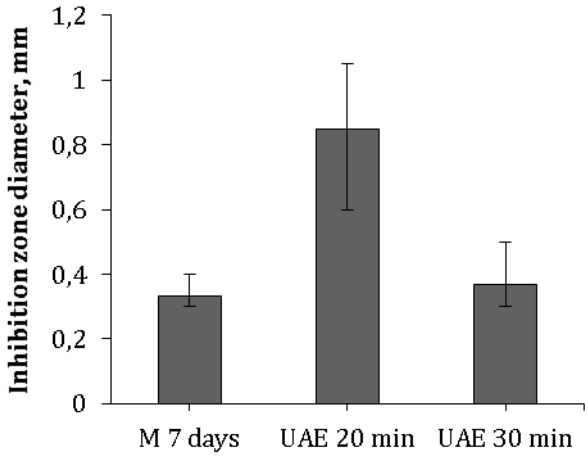

Fig. 3. Disk diffusion test results for various extracts

*M = maceration extraction
*UAE = ultrasonic-assisted extraction

4. Conclusion

The shallot skin extract is positive for flavonoids and phenolic compounds but negative for saponins. The longer the extraction time, the more flavonoids and phenolic compounds are extracted. The quality of extract from ultrasound-assisted extraction (2.59 ppm phenolic and 6.93 ppm flavonoid) can compete with the results of maceration extraction (2.46 ppm phenolic and 5.48 ppm flavonoid) in a shorter extraction time. The resulting extract showed antibacterial activity, and the extract with more secondary metabolites had a bigger inhibition zone.

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