Mucolytic Activity Test of Shallot Extract (Allium Ascalonicum L) by in Vitro

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Abstract. This paper aims to explain the results of research on the mucolytic activity of shallot extract is proportional to 0.2% N-Acetylcysteine. Shallot (Allium ascalonicum L.) is efficacious for treating cough. This research was conducted by examining the mucolytic activity of shallot extract made with various dose concentration 5%, 10%, 15%, 20%, and 25%. The mucolytic activity test was performed in vitro based on the decrease in the viscosity of the egg whites by using the Brookfield viscometer. The results showed that shallot extract with dose concentration of 5%, 10%, 15%, 20%, 25% had mucolytic activity by decreasing the viscosity of egg white solution. The effective concentration almost equal to 0.2% N-Acetylcysteine is at 25% concentration.

1. Introduction
Herbal medicine is the oldest and still the most widely used system of medicine in the world today. It is medicine made exclusively from plants. It is used in all societies and is common to all cultures. There are many different "types" of herbal medicine that spring from different cultures around the world. All these have the use of medicinal plants in common, but they vary in the plants they use, the way they prepare and use medicines from these plants, and the philosophy of their treatment approaches. Different cultures may also use the same plants but differ in how it is used, or the part they use [1].

One of the herbal remedies is shallot (Allium ascalonicum L.). The shallot plant is one of the annual crops included in the Liliaceae family [2]. Shallots consist of a cluster of small to medium-sized bulbs with a reddish brown to orange brown outer skin. The shallot is commonly used as a condiment in Southeast Asian countries, including Indonesia. They are usually used in combination with other spice blends and condiments [3].

Shallot (Allium ascalonicum L.) contains essential oils consisting of dialylsulfide, propantiol-S-oxide, S-Alyl-L-Cysteine-sulphoxide or Aliine, Allisin, phenolic acid, fumaric acid, kaphryl acid, floroglusin, phosphorus, phytosterol, flavonol, flavonoid, pectin, saponin, sterols, cycloallin and propyl-methyl disulfide, prostaglandin A-1, diphenylamine and cycloalkine, methylaliin, dihydroaliin, kaemferol and foroglusinol [4]. Benefits of shallots are: oils that evaporate in the shallot water is very useful to kill most of the microbes [5], nutrient content in shallot can be used by the body to provide energy, can reduce the possibility of trombofilia or hypercoagulation, and can cure cough [6].

Cough is a natural protection and defense action to remove mucus, foreign substances and infections from the larynx, trachea and large bronchi. Cough is also the most efficient mechanism to clean the upper respiratory tract with natural mechanisms [7]. Mucolytic are a working mechanism that helps
lower the viscosity of sputum, particularly from the lower respiratory tract. So changing the physical nature of chemistry of mucus causes the mucus viscosity to decrease and it will be easier to cough [8]. One of the mucolytic drugs is N-Acetylcysteine, which is a cysteine derivative of an amino acid that is able to shorten long chains of sputum mucoprotein, to become more fluid and easier to remove by cough [7].

2. Methods

2.1. Collection and identification of plant materials
The sample plant materials used is shallot (Allium ascalonicum L.) obtained from the market. shallots were determined at Herbarium Sekolah Ilmu Teknologi Hayati (SITH) Institut Teknologi Bandung. To determine the class of secondary metabolite content present in a simplicia, phytochemical screening was performed. To identify the flavonoid content, performed with some of the samples in the test tube are mixed with Magnesium powder and 2N Chloride Acid. The mixture is heated and then filtered. In the filtrate added amyl alcohol, then shaken strongly. The presence of flavonoid compounds characterized by the occurrence of yellow-red color changes, which can be drawn by Amyl alcohol. Furthermore, alkaloid identification was done by reagent mayer and reagent Dragendorff.

Identification of saponin content is also done. Saponin is a strong surface-active compound that causes foam when shaken in water and at low concentrations often cause red blood cell hemolysis, and work as an antimicrobial agent. Saponin solubility is soluble in water, but not soluble in ether. Saponin stimulates the secretion of the bronchioles [9]. Saponins can increase the activity of the ciliary epithelium, an event that generates a cough, and release phlegm.

Tannin identification was performed by taking 500mg of simplicia samples with 10ml distilled water, filtered and then filtrate diluted with distilled water. Taken 2ml of solution and then added iron (III) chloride 1%, blue or greenish black color indicates the existence of tannin.

Another material used is albumen from duck eggs. Albumen is used because it shares the same physical physicochemical properties with the human mucus. Composition of human airways mucus 95% water and 5% glycoprotein [10]. Albumen (egg white) is composed of most of the water and organic matter of protein. Albumen consists of 4 fractions namely, chalaziferous layer, inner thin layer, gel-like layer firm, and thin layer outer. Albumin is composed of mostly water and organic protein materials. Eggs have very useful physicochemical properties in food processing include the properties of foam, emulsion, coagulation, rheology and color [10]. Based on the physicochemical properties that egg white is selected as a medium for mucolytic activity test.

2.2. Extraction of the plant materials
The preparation of the extract was carried out by reflux method. Fresh Allium ascalonicum L. bulbs were washed with freshly prepared sterile distilled water. The outer covering of the bulbs was peeled off and the fleshy part of the shallots rewashed separately with the distilled water. A part of 100g of each shallot bulbs were cut into small parts. Further refluxed by adding ethanol 96% 500 ml for 1 hour at 40°C. All the reflux results (filtrates) are concentrated with a rotary vaporator at 40 °C, to obtain the viscous extract the extract is concentrated again in the vapor plate on top of the water bath, then the rendement is calculated.

2.3. Preparation of phosphate buffer solution
Preparation of phosphate buffer pH 7 solution by mixing 50 ml of 0.2M dihydrogen phosphate potassium with 29.1 ml of 2N sodium hydroxide and diluted with carbon-free water sufficiently so that 200 ml was then tested with pH meter. Separate the egg white duck with its egg yolk, then the egg white is stirred slowly until completely homogeneous, then in the container in a glass of chemistry. The obtained egg white was mixed with a phosphate buffer solution of pH 7 (20: 80) stirred, to obtain a homogeneous solution of egg white.
2.4. Observation of in vitro mucolytic activity

Testing of mucolytic effect, buffer phosphate buffer pH 7 is prepared, then make an egg white solution in buffer (20: 80). Test solution preparations in 5 concentrations, i.e. 5%, 10%, 15%, 20%, 25% were tested for their mucolytic power. The test was performed by calculating the flow time of the egg white solution which was added test solution with Brookfield viscometer tool with Rpm range 12, 30 and 60 using spindle no 2. Negative control solution was egg white, and positive control was N-Acetylcysteine. 20 ml test solution and 80 ml egg white solution placed in a 100-ml glass beaker were then measured by adjusting the appropriate spindle and Rpm in the viscometer apparatus used at room temperature and the readable scale showed the viscosity of the sample examined with cP (centipoise).

3. Results and discussion

3.1. Plan determination

Determination is intended to verify the identity of a plant. The results of shallot determination conducted at Herbarium School of Biological Sciences (SITH) Bandung Institute of Technology showed that the plants used in this study is true plant of Shallot (Allium ascalonicum L).

3.2. The results of phytochemical screening

Based on the results of phytochemical screening on simplicia and shallot extract (Allium ascalonicum L.) (shown in Table 1), it can be seen that the simplicia and extract of shallot have secondary metabolites Alkaloids, Flavonoids, Saponins, Tannin, Polyphenols. There is no difference between simplicia and extract, because there is no change after extraction.

| Content     | Simplicia | Extract |
|-------------|-----------|---------|
| Alkaloid    | +         | +       |
| Flavonoid   | +         | +       |
| Tannin      | +         | +       |
| Polyphenol  | +         | +       |
| Saponin     | +         | +       |

3.3. Mucolytic test result of shallot extract on albumen from duck eggs

To determine the effect of shallot extract on the viscosity of egg white solution, the researcher processed the result of viscosity measurement data using Statistical Analysis of Variance Single Factor. The measurement results are shown in Table 2.

In a study with a range of 12 Rpm performed some test treatment to determine the viscosity, the first of which was control which consisted only of 20% egg white mixture with phosphate buffer (20:80). In the control test obtained a viscosity of 27.50 cPois. In the positive control treatment visible viscosity decrease of N-Acetylcysteine by 12.50 cPois after some test. While at concentration 5% obtained viscosity equal to 24.17 cPois, at dose 1.143 gram obtained viscosity equal to 21.67 cPois, at dose 1.714 gram obtained viscosity equal to 18.33 cPois, at dose 2.286 gram obtained viscosity equal to 15.83 cPois, at dose 2.857 grams obtained viscosity of 11.67 cPois. This shows a large viscosity at a dose of 2.286 grams and 2.857 grams decreased viscosity, whereas at a dose of 0.571 grams did not change significantly, this is because at a dose of 0.571 grams of extract used has not been able to reduce viscosity because the compound is efficacious mucolytic at a dose of 0.571 grams has not been able to break the disulfide bonds in egg white solution to the maximum.
Table 2. Viscosity Measurement Results

| Testing group | 12 Rpm | 30 Rpm | 60 Rpm |
|---------------|--------|--------|--------|
| X ± SEM       | X ± SEM| X ± SEM|
| K (-)         | 27.50 ± 0.64 | 25.33 ± 1.46 | 15.67 ± 1.81 |
| K (+)         | 12.50 ± 0.29  | 11.83 ± 0.68  | 10.83 ± 1.25  |
| Dose 1        | 24.17 ± 0.56  | 20.00 ± 1.27  | 14.50 ± 1.67  |
| Dose 2        | 21.67 ± 0.50  | 19.33 ± 1.12  | 13.83 ± 1.60  |
| Dose 3        | 18.33 ± 0.42  | 16.00 ± 0.92  | 12.67 ± 1.46  |
| Dose 4        | 15.83 ± 0.37  | 13.33 ± 0.77  | 11.50 ± 1.33  |
| Dose 5        | 11.67 ± 0.27  | 10.83 ± 0.63  | 9.33 ± 1.08   |

Description:
- X ± SEM: Mean Viscosity Value of Egg White Solution ± SEM (Standard Error Mean)
- Negative Control (Comparison of 20% egg white solution with phosphate pH 7 (20:80))
- Positive Control (Acetylcysteine white solution Eggs 20% in phosphate pH 7 (20:80))
- Dose 1 (shallot extract 5% plus 20% egg white solution in phosphate pH 7)
- Dose 2 (shallot extract 10% plus 20% egg white solution in phosphate pH 7)
- Dose 3 (shallot extract 15% plus 20% egg white solution in phosphate pH 7)
- Dose 4 (shallot extract 20% plus 20% egg white solution in phosphate pH 7)
- Dose 5 (shallot extract 25% plus 20% egg white solution in phosphate pH 7)

In the viscosity test of the egg white solution of the range of 30 Rpm also performed some test treatments, the first of which was control which consisted only of 20% egg white mixture with phosphate buffer (20:80). In the negative control test obtained viscosity of 25.33 cPois, and on the positive control there was a decrease in viscosity of 11.83 cPois after several tests. This is in accordance with the literature that the work of N-Acetylcysteine is as mucolytic. N-Acetylcysteine has a sulfhydryl (SH) group in its molecule and is powerless to open a disulfide (S-S) group of sputum mucoproteins [10]. The result is a sputum depolymerization that causes its viscosity to fall. While at viscosity test at dose 0.571 gram equal to 22.00 cPois, at dose 1.143 gram obtained viscosity equal to 19.33 cPois, at dose 1.714 gram obtained by viscosity equal to 16.00 cPois, at dose 2.286 gram obtained viscosity equal to 13.33 cPois, at dose of 2.857 gram obtained viscosity of 10.83 cPois. This shows a significant decrease in viscosity at doses of 2.286 grams and 2.857 grams.

In the viscosity test the egg white solution of the range of 60 rpm also performed some test treatments, the first of which was control which consisted only of 20% egg white mixture with phosphate buffer (20:80). In the negative control test obtained viscosity of 15.67 cPois. On the positive control test, the viscosity decrease was 10.83 cPois. In the dose 0.571 grams viscosity test at 14.50 cPois, at dose 1.143 gram obtained viscosity equal to 13.83 cPois, at dose 1.714 gram obtained viscosity equal to 12.67 cPois, at dose 2.286 gram obtained viscosity equal to 11.50 cPois, at dose 2.857 obtained viscosity equal to 9.33 cPois. From these data it shows a large viscosity at a dose of 2.286 grams and 2.857 grams of viscosity decreasing near the viscosity of positive control.

Based on the results of viscosity measurement results obtained analysis of variance as shown in table 3.

Table 3. Analysis of Variance (ANOVA)

| Viscosity of Egg White Solution | SS   | DF  | MS   | F    | F Crit | P-value |
|--------------------------------|------|-----|------|------|--------|---------|
| Factor                         | 369,2116 | 6   | 61,53526 | 4.462136 | 2.85   | 0.00099 |
| Error                          | 193,0675 | 14  | 13,79054 |        |        |         |
| Total                          | 544,812 | 20  | 17,478 |        |        |         |
It can be concluded that the giving of extract has an effect on the decrease of viscosity of egg white solution. Due to the influence, it is necessary to do further test, the researcher do follow-up test with Duncan DMRT (Duncan Multiple Range Test) test. Test results can be seen in table 4.

From the results of the duncant test, it can be seen that Dose 5, Dose 4, and Dose 3 did not differ significantly with positive control. This means the use of doses of 2.857 g, 2.286 g and 1.714 g has an effect similar to the use of N-Acetylcysteine in the market. Nevertheless, the use of extract - dose 5 (2.857g) is the most closely approximated positive control.

| Table 4. Ducant Test Results |
|-----------------------------|
| Ducant test | 1 | 2 | 3 |
| D5          | 10,610 |   |   |
| k (+)       | 11,720 |   |   |
| D4          | 13,553 |   |   |
| D3          | 15,667 |   |   |
| D2          | 18,277 |   |   |
| D1          | 20,223 |   |   |
| k (-)       | 22,833 |   |   |

4. Conclusion
Based on the research that has been done can be concluded several things:

- Extract of shallot (Allium ascalonicum L.) gives mucolytic effect that is decrease the viscosity of egg white solution.
- Effective concentration of red onion extract at dose 2,286 gram and 2,857 grams almost equal to N-Acetylcysteine 0,2%.

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