Effect of Extraction Time on Tannin Antioxidant Level and Flavonoid on Pandan Wangi Leaf (*Pandanus amaryllifolius* Roxb) Using Hydrothermal Extractor

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Abstract. Pandanus leaf (*Pandanus amaryllifolius*) is one of the plants that have chemical content in the form of tannins, saponins, polyphenols (phenolics), alkaloids and flavonoids that can be used as antioxidants. The research that is done is a total tannin and alkaloid test on pandan leaves. The purpose of this research was to determine the effect of extraction time on the amount of tannin and alkaloids in pandan leaves. In the tannin and alkaloids test, the extraction of pandan leaves was first used hydrothermal extract with 60°C temperature in 60 minutes using aquades solvent with variation of extraction time 20, 30, 40, 50 and 60 minutes. Tannin content of 0.2%; 0.8%; 2.5%; 6.2%; 7.1% and flavonoid levels of 2.05%; 2.36%; 4.44%; 0.78%; 0.34%. From the results of the research showed that the longer the extraction time, the more tannin content produced. While flavonoids have optimum time at 40 minute extraction time. Optimal tannin content obtained by using aquades solvent is the time of extraction for 60 minutes, while the optimum flavonoid content at the time of extraction 40 minutes.

Keywords: Extraction; Pandanus Leaf; Tannin; Flavonoid; Hydrothermal

1. Introduction

Pandan leaves are plants that are commonly found in Indonesia, tropical plants. Pandanus leaves, often known as pine screw[1].

This plant is often used as a natural deodorizer and dye in the manufacture of food. Pandan leaves also have many benefits, especially tannin and flavonoids compounds contained there in. The composition contained within the Pandan leaf include alkaloids, flavonoids, saponins, tannins and polyphenols each of which has its own function. For the total content of each compound in 1 gram of pandan leaf, for total flavonoid content: 478,7629 mg / g (9.941%), total phenolic content of 99.4086 mg / g (47.87%), the total saponin and tannin content contained in the leaves has a total saponin content of 2.13% and a total tannin content of 7.04% as contained in the leaves of inggu[2][3].

In practice these compounds can be obtained in various ways, one of them by using a hydrothermal extractor equipped with a stirrer with temperature and time control. The purpose of this research is as follows: (1) to know the effect of time of extraction of tannin and flavonoid compound from pandan leaves to amount of tannin and flavonoid compound, (2) knowing the optimal extraction time resulting in the largest weight of tannins and flavonoids.

Pandan wangi is a plant that can be used as a dye and natural fragrance in food. The pandanus leaves belong to the tropical plants commonly used for natural fragrance[4]. The systematics of pandan leaf taxonomy can be seen in Table 1 as follows:
Table 1. Systematic taxonomy of pandan wangi leaves[5]

| Kingdom      | Plantae                                 |
|--------------|-----------------------------------------|
| Division     | Magnoliophyta                           |
| Class        | Liliopsida                              |
| Order        | Pandanales                              |
| Family       | Pandanaceae                             |
| Genus        | Pandanus                                |
| Species      | Pandanusamaryllifolius                   |

Tannin is a very complex organic compound and can be found in a variety of plants including pandan leaves. Tannin is amorphous and has the power to raise the skin of animals. The tannin structure can not yet be determined precisely, but is defined as a natural compound with BM (molecular weight) between 500 and 3000, and has a phenolic hydroxyl group (1-2 per 100 molecular weight units) and can form stable crosslinks with proteins and bipolimers[6].

Flavonoids a group the largest phenol compounds found in nature, is one of the bioactive components in plants [7]. Flavonoids have a carbon base framework consisting of 15 carbon atoms, in which two benzene rings (C₆) are bonded to a pop chain (C₃) to form a C₆-C₃-C₆ array. This compound is a dye of red, purple, blue, and some of the yellow dye found in plants.

Extraction is the process of separating one or more components from a homogeneous mixture using a solvent as a separating agent. Separation occurs on the basis of different solubility capabilities of the components in the mixture. Extraction includes the process of separation through the basis of diffusion operations. The diffusion process occurs because of the movement of the solute, in the direction of the phase diluent to the solvent phase as a result of the potential difference between the two phases that are in contact so that at some point the system is in equilibrium[8].

In general, based on the materials and methods, extraction can be divided into two types, namely liquid solid extraction and liquid-liquid extraction. Here's the explanation:

1. Liquid Solid Extraction (Leaching)

Liquid solid extraction (leaching) is the process of separating a solute present in a solid by contacting the solid with a solvent so that the solids and liquids are mixed and then the solute is separated from the solid as it dissolves in the solvent. In liquid solid extraction there are two phases of overflow phase and underflow phase[9].

2. Liquid-Liquid Extraction

Liquid-liquid extraction is an extraction used when separation of the mixture by distillation is not possible (eg due to the formation of the azeotrope or due to its heat sensitivity) or uneconomical. Like liquid-liquid extraction, liquid-liquid extraction always consists of at least two stages is the intensive mixing of extraction materials with the solvent and separation of the two liquid phases as perfectly as possible.

2. Materials and Methods

2.1 Pandanus

Pandan obtained from the plantation residents of Gunungpati, Semarang. Pandanus used is fresh pandanus.

2.2 Aquades

Aquades are used as much as 5 liters per each test. Aquades obtained from Chemical Engineering Operations Laboratory, Vocational School, Diponegoro University.
2.3 The First Treatment

Pandan fresh washed and cut as small as 1 kg for one test. After that blend until smooth.

2.4 Second Treatment

The pandanus extraction uses aquades, regulating the temperature of the extractor 60°C. Controls the extraction time for 20, 30, 40, 50 and 60 minutes.

2.5 Testing Content

Tests for tannin and flavonoids were performed at the Vocational School’s Analytical Chemistry Laboratory, Diponegoro University. Test the tannin content using oven. Extraction filtrate was taken as much as 20mL and then heated after it was filtered then tannins obtained then in the oven. Testing of flavonoids using methanol and water, then heated above crucibles until mongering and then weighed.

3. Results and Discussion

3.1. Tannin levels

Final Research Tests test of pandan leaf extract content has been conducted in the Laboratory of Chemical Engineering Operations, Vocational School, Diponegoro University. The sample used was pandan leaf extract (PandanusamarilifoliusRoxb) with aquades solvent which had previously been extracted using hydrothermal extractor in 60 minutes with temperature of 60°C. The results of the observed levels of tannins obtained after the study can be seen in Table 2.

Based on result of research of pandan leaf extract (PandanusAmaryllifoliusRoxb) that have been done got tannin level as in Figure 1. The result obtained showed that the longer time of extraction, tannin level obtained more and more. This is because the longer time extraction used, the more easily solvent to attract the chemicals contained in the pandanus leaves that can penetrate the walls of pandan leaves.

![Figure 1. Graph of Effect of Extraction Time on Tannin Level](image-url)
Table 2. Data Analysis of Tannin Level After Extraction

| Sample | Sample Volume (mL) | Weight of Grate + Filter paper + residue (gram) | Weight of Grate + Filter paper (gram) | Tannin Levels (gram) | Tannin Levels (%) |
|--------|-------------------|-----------------------------------------------|--------------------------------------|---------------------|------------------|
| 1      | 20                | 50,45                                         | 50,43                                | 0,02                | 0,2%             |
| 2      | 20                | 51,64                                         | 51,56                                | 0,08                | 0,8%             |
| 3      | 20                | 44,21                                         | 43,96                                | 0,25                | 2,5%             |
| 4      | 20                | 45,01                                         | 44,39                                | 0,62                | 6,2%             |
| 5      | 20                | 45,02                                         | 44,31                                | 0,71                | 7,1%             |

From the result of the research, the tannin level has been obtained with the tannin level that is in the sample 1 with the extraction time of 20 minutes for 0,02 gram, sample 2 with 30 minute extraction time 0,08 gram, sample 3 with 40 minute extraction time 0,25 gram, sample 4 with time of extraction 50 minutes equal to 0,62 gram and sample 5 with time extraction for 60 minutes equal to 0,71 gram. This shows that the length of time of extraction has an effect on the amount of tannin obtained.

3.2. Test of Flavonoid Levels

The material used in this research is pandan leaves as much as 1 kg in 5 liter aquades. The variables used are temperature and time of extraction. Temperature used is 60°C while the extraction time is 20, 30, 40, 50 and 60 minutes.

In Table 3 can be seen flavonoid levels at most 4.44%, that is, at the extraction time of 40 minutes. While the least flavonoid levels at the time of extraction time of 20 minutes for 0.34%. The extraction temperature is too low and the short extraction time will result in a low yield [10].

From Figure 2 the chart shows a fluctuating graph. Levels of flavonoids most commonly obtained at the time of extraction 40 minutes. Increased temperature and extraction time should be noted, excessively high extraction temperatures and long extraction times and exceeding versa if the extraction temperature is too low will cause not all active compounds to be extracted from the material and the low yield of the active compound obtained.

![Figure 2. Graph of Effect of Extraction Time on Flavonoid Levels](image_url)
### Table 3. Flavonoid Level Observation Table

| Sample | Sample Volume (mL) | Extraction Time (minute) | Temperature (°C) | Flavonoid Massa (gram) | Flavonoid levels |
|--------|-------------------|--------------------------|-----------------|-----------------------|-----------------|
| 1.     | 50                | 60                       | 60              | 2.98                  | 2.05 %          |
| 2.     | 50                | 50                       | 60              | 3.21                  | 2.36 %          |
| 3.     | 50                | 40                       | 60              | 4.73                  | 4.44 %          |
| 4.     | 50                | 30                       | 60              | 2.05                  | 0.78 %          |
| 5.     | 50                | 20                       | 60              | 1.73                  | 0.34            |

### 4. Conclusions

From the results showed that the longer the extraction time used, the more tannin content produced. While flavonoid have optimum time at extraction time 40 minute that is equal to 7,1%. Optimal tannin content obtained by using aquades solvent is the time of extraction for 60 minutes, while the optimum flavonoid content at the time of extraction 40 minutes amounted to 4.44%.

Increased temperature and extraction time need to be considered, the extraction temperature that is too high and the long extraction time and the optimum time limit can cause the loss of compounds in the solution due to evaporation, if the extraction temperature is too low it will cause not all active compounds to be extracted from the material and produce the low active compound obtained.

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