Effect of extraction method and solvent ratio on antioxidant activity of Dayak onion extract

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Abstract. Dayak onion has antioxidant compounds obtained through the extraction process. The extraction and isolation methods affect the amounts and the quality of extract obtained. Multiple solvents can be used sequentially in the extraction to obtain an optimum amount of compounds in the desired yield. The amount of extract obtained can be related to its antioxidant activity. This study aims to determine the effect extraction method and ratio of solvent to the antioxidant activity of Dayak onion extract. The method used in the research was Factorial Random Block Design (FRBD) with two factors. The factor I was the extraction method (maceration, reflux, and soxhletation). Factor II was the ratio of solvent (ethanol and hexane with ratio of 70:30, 60:40, and 50:50). The observation data analysed with ANOVA, then followed by DMRT with a confidence interval of 99%. The best treatment of the extraction method was maceration with the ratio of solvent ethanol:hexane was 70:30. The results were 3.76% dried extract yield, moisture content of 14.01%, total phenolic content of 8.62 mg GAE/g, total flavonoid of 28.20 mg QE/g, and antioxidant activity IC\textsubscript{50} of 17.33 ppm.

1. Introduction
Dayak onion (Eleutherine palmifolia L. Merr) is an indigenous plant from Central Kalimantan, Indonesia. It contains several active compounds such as alkaloids, flavonoids, glucosides, and saponins [1], triterpenoids, and quinones [2]. Dayak onion has health benefits and contains antioxidants to prevent free radicals [3]. The bioactive compounds in Dayak onion are obtainable through the extraction process [3]. The effectiveness of the extraction process influenced by several factors, such as extraction method and type of solvent used.

Method selection and type of solvent causes the bounding of bioactive compounds during the extraction process. Commonly used extraction method are maceration [4], reflux, soxhletation [5], and ultrasonic [3]. Moreover, commonly used solvents are ethanol. Ethanol solvents well used for extracting polar compounds, so that the extraction result of nonpolar compounds that is antioxidant less than the maximum [6]. Hence, one of the nonpolar solvents is hexane. Based on the theory, antioxidant compounds are extracted not only from polar compounds such as phenols and flavonoids but also nonpolar compounds such as anthraquinone.

The purpose of this study was to compare yields, level of bioactive compounds, and antioxidant activity of Dayak onion extract obtained by several extraction methods using multiple ethanol and hexane solvents simultaneously.
2. Materials and Method

2.1. Materials
The material used in this study is Dayak onion obtained from Batu City with a harvested at the age of six months, length of 5 cm, and a diameter of 3 cm. Chemicals used were 96% ethanol solvent, ethanol p.a. Hexane, n-Hexane p.a., 5% NaNO₂ solution, standard quercetin solution, gallic acid powder, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in 0.2 mM ethanol, 1 M NaOH solution, Follin-ciocalteau reagent, solution 7% Na₂CO₃, and 10% AlCl₃ solution.

2.2. Experimental design
The experimental design in this study was factorial randomized group design (FRGD), using two factors. The first factor was the extraction method with three levels (maceration, reflux, and soxhletation); the second factor was the ratio of ethanol:hexane solvents with three levels of 70:30, 60:40, and 50:50 (v/v).

2.2.1. Preparation of Dayak onion [3]
Dayak onion is sorted by size with 5 cm length and 3 cm diameter. The Dayak onion was sorted, peeled, and washed with running water and drained. Dayak onions were then sliced with a thickness of 1-2 mm and dried with a vacuum dryer at 45°C for 7 hours. Dried Dayak onions were milled using a grinder for 2-3 minutes, then sieved using a 60-mesh sieve.

2.2.2. Extraction of Dayak onion
In preliminary research, Dayak onion extracted using the maceration method with a single solvent of 96% ethanol resulted in ethanolic extract. This ethanolic extract only used for characterisation. In primary research, dayak onions powder extracted following the treatment condition. Three extraction methods used in this research were maceration [5], reflux [7], and soxhletation [8]. After the extraction, the concentrated extract was filtered using filter paper. The extract left in a separating funnel for 30 minutes, then the ethanol and hexane fraction was separated. The ethanolic and hexane fraction of extract was evaporated separately using a rotary vacuum evaporator with temperature is 42°C speed 30 rpm for 2.5 hours and the dried extract obtained. Then, analysis of the yield, water content, total phenolic, total flavonoid, and antioxidant activity IC₅₀ conducted.

2.2.3. Physicochemical analysis of Dayak onion
Physical analysis conducted in this study include water content on a dry basis (% db) and wet basis (% wb) [9], yield (% db), total phenol for gallic acid equivalent (GAE) [9], antioxidant activity for the half-maximal inhibitory concentration (IC₅₀) [9], total flavonoids for quercetin equivalent (QE) [10], and best treatment test [11]. Data were analysed using the analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) with a confidence interval of 99% (α = 1%).

3. Results and Discussion

3.1. Dayak onion
Dayak onion extract obtained by maceration using a single solvent of 96% ethanol. This extraction method and ethanolic extract of dayak onion used only for characterisation in preliminary research. The yields and characteristics of the dried extract given in Table 1. The yield produced in this study was lower than the previous experiment [5], possibly due to the material characteristics and proportion ratio between materials and solvents [13]. Short drying time in this study might affect dried extract yields, water content, IC₅₀, total phenol, and flavonoids.

3.2. The yield of Dayak onion extracts
The yield of Dayak onion extract ranged from 3.14-4.52% db. ANOVA analysis showed that the extraction method and the ratio of ethanol:hexane solvents had a very significant effect on yields (α=0.01), but there was no interaction between two factors. The average yields of Dayak onion from
several extraction methods and the ratio of double solvent ethanol:hexane showed in Figure 1. It shows that the soxhletation method produced the highest yields at all different ethanol:hexane solvent ratio. It is due to the use of heat in soxhletation, which makes it easier for solvents to diffuse into the materials with similar polarity. The study indicates that an increase in the ethanol ratio used resulted in increasing the extract yields. The highest yields obtained from ethanol:hexane solvent ratio of 70:30 show that it is likely the compounds contain in Dayak onion are polar [3].

Table 1. Dried extract yield and the characteristics of Dayak onion

| Analysis                      | Experimental Result $^{a1}$ | Reference research [5] |
|-------------------------------|------------------------------|------------------------|
| Yield (%)                     | 4.76 ± 0.23 (4.31 ± 0.21) $^{b1}$ | 5.30$^{c}$           |
| Water content (% db)          | 10.46 ± 0.29                 | 9.89$^{d}$            |
| Total phenol (mgGAE/g)        | 10.44 ± 0.97 (wb)            | 9.72$^{d}$            |
| Total flavonoids (mgQE/g)     | 29.33 ± 1.12 (wb)            | 7.67$^{d}$            |
| IC$_{50}$ (ppm)$^{e}$         | 25.72 ± 0.75                 | 105.48$^{d}$          |

Notes: a) standard deviation of triplicate measurements; b) number in parentheses is wet weight; c) yield; d) recalculated to dry weight [10]; e) DPPH solvent 2 ml 0.2 Mm

Figure 1. The yield of Dayak onion extracts from the three extraction methods

3.3. Water content

Many researches usually employed a dried powder of plants in bioactive compounds extraction to eliminate the interference of water at the same time. Thus, in this study, the water content of dried Dayak onion was measured to ensure it was well-dried and to minimise the interference of water in the extraction process. The water content of dried Dayak onion obtained was in the range of 14.01-18.93 %db or 12.28-15.91 %wb. ANOVA analysis shows that the extraction method and the ratio of double solvents had interactions and had a very significant effect on the confidence interval of 99% ($\alpha=0.01$). Water content data, as shown in Table 2, indicates that increasing water content was parallel to an increase in the ratio of hexane solvent used. It is possibly due to the use of multiple solvents in the treatment may enhance the effectiveness of the extraction process. Thus, more extracted compounds were obtained because the water bounding by polar compounds on Dayak onions was carried over to the extract.

3.4. Total phenol

The average total phenol of dried Dayak onion extract ranged from 5.15-8.62 mg GAE/g (db), as can be seen in Table 3. ANOVA analysis shows that extraction methods and solvent combinations gave a very significant effect on total phenol ($\alpha=0.01$), and there were interactions between the two factors.
Table 3 shows that total phenol was influenced by the extraction method, rather than the double solvent used. The data indicates that, at the same double solvent ratio, using soxhletation method resulted in the lowest total phenol. This was possibly due to the use of heat in the soxhletation process can damage the phenol compounds. Furthermore, the use of many solvents can inhibit the effectiveness of ethanol during the extraction process. It is because ethanol solvents have two functional groups, namely polar hydroxyl (-OH) and nonpolar alkyl groups (-R), which bind polar and nonpolar compounds [14–16].

Table 2. The water content of Dayak onion extract obtained from various extraction methods and double ethanol: hexane solvent ratio

| Extraction Method                        | Solvent Ratio Ethanol:Hexane (%) | Water Content (% db) | Water Content (% wb) |
|-----------------------------------------|----------------------------------|----------------------|----------------------|
| Maceration (3×24 hours, solvents replaced every 24 hours) | 70:30                            | 14.01 ± 1.33a        | 12.28 ± 1.02a        |
|                                          | 60:40                            | 16.81 ± 0.80b        | 14.39 ± 0.59b        |
|                                          | 50:50                            | 17.44 ± 1.40b        | 14.84 ± 1.01b        |
| Reflux (40⁰C, 5 hours)                   | 70:30                            | 16.84 ± 0.47b        | 14.41 ± 0.34b        |
|                                          | 60:40                            | 17.07 ± 1.21b        | 14.58 ± 0.87b        |
|                                          | 50:50                            | 18.31 ± 1.26b        | 15.47 ± 0.90b        |
| Soxhletation (40⁰C, 5 hours)             | 70:30                            | 17.73 ± 0.75b        | 15.05 ± 0.54b        |
|                                          | 60:40                            | 16.52 ± 1.27a        | 14.17 ± 0.93b        |
|                                          | 50:50                            | 18.93 ± 1.34b        | 15.91 ± 0.94b        |
| DMRT 1%                                  |                                  | 1.67-1.79            | 2.29-2.39            |
| Comparative treatment maceration, 24 hours, ethanol 96% | 1:5                             | 10.46 ± 0.29         | 9.47 ± 0.23          |

Notes: different notation in the same column are significantly different at α=0.01; ± indicates the standard deviation of triplicate measurements

Table 3. Total phenol obtained from several extraction methods and double solvent ratio ethanol: hexane

| Extraction Method                        | Solvent Ratio Ethanol: Hexane (%) | Total phenol (mg GAE/g db) |
|-----------------------------------------|----------------------------------|----------------------------|
| Maceration (3×24 hours, solvents replaced every 24 hours) | 70:30                            | 8.62 ± 0.55c               |
|                                          | 60:40                            | 6.87 ± 0.46c               |
|                                          | 50:50                            | 6.59 ± 0.50ab              |
|                                          | 70:30                            | 7.86 ± 0.33bc              |
| Reflux (40⁰C, 5 hours)                   | 60:40                            | 6.55 ± 0.29b               |
|                                          | 50:50                            | 5.33 ± 0.26ab              |
|                                          | 70:30                            | 5.67 ± 0.62b               |
| Soxhletation (40⁰C, 5 hours)             | 60:40                            | 6.13 ± 0.52ab              |
|                                          | 50:50                            | 5.15 ± 0.46a               |
| DMRT 1%                                  |                                  | 1.15-1.26                  |
| Comparative treatment maceration, 24 hours, ethanol 96% | 1:5                             | 10.44 ± 0.97               |

Notes: different notation in the same column are significantly different at α=0.01; ± indicates the standard deviation of triplicate measurements
3.5. **Total flavonoids**

Total flavonoids of Dayak onion extracted ranged from 23.43-28.20 mg QE/g (db). ANOVA analysis shows that the method and ratio of double solvents gave a significantly different to total flavonoids ($\alpha=0.01$), but no interactions between the two factors observed. Figure 2 shows that, in all extraction methods, the higher the ethanol solvent, the higher the total flavonoids. Again, using the soxhletation method has impact on reducing the total flavonoids. Several studies have reported that the heat in the soxhletation process can contribute to defect the flavonoids compounds [15,17,18]. Furthermore, previous study by Sharma et al. [15] shown that heating process decreases total flavonoids by 15-78%. The use of dual solvents may also influence the total flavonoids. It assumed that ethanol has higher polarity than hexane. Moreover, the higher ethanol ratio in the extraction process has increased the total flavonoids [19, 20].

![Figure 2](image)

**Figure 2.** Total flavonoids of Dayak onion in various extraction method and solvent ratio ethanol:hexane

| Extraction Method | Solvent Ratio Ethanol: Hexane (%) | Average IC$_{50}$ (ppm) |
|-------------------|----------------------------------|-------------------------|
| Maceration (3×24 hours, solvents replaced every 24 hours) | 70:30 | 17.33 ± 1.67a |
| | 60:40 | 19.88 ± 0.31a |
| | 50:50 | 44.83 ± 2.98cd |
| Reflux (40 °C, 5 hours) | 70:30 | 36.19 ± 1.34b |
| | 60:40 | 45.07 ± 0.90cd |
| | 50:50 | 50.28 ± 1.05d |
| Soxhletation (40 °C, 5 hours) | 70:30 | 41.69 ± 2.90c |
| | 60:40 | 49.12 ± 2.55d |
| | 50:50 | 51.52 ± 1.67d |

**Table 4.** IC$_{50}$ obtained from several extraction methods and double solvent ratio ethanol: hexane

Duncan Multiple Range Test (DMRT) 1%

| Comparative treatment maceration, 24 hours, ethanol 96% | 1:5 | 25.72 ± 0.75 |

Notes: different notation in the same column are significantly different at $\alpha=0.01$; ± indicates the standard deviation of triplicate measurements
3.6. IC$_{50}$

IC means inhibition concentration and IC$_{50}$ defines as the half concentration of inhibition, indicating the concentration of antioxidant that inhibits free radical (DPPH). The IC$_{50}$ value is inversely proportional to the free radical scavenging activity of the antioxidant property of the sample [24]. It means that the sample requires less amount in scavenging the free radical if the IC$_{50}$ value is lower or vice versa. The scavenging activity of free radicals in the sample is due to presence of molecules known as antioxidants. The average IC$_{50}$ of Dayak onion extract was in the range of 17.33-51.52 ppm.

ANOVA analysis shows that there was an interaction between the extraction methods to double solvents, as can be seen in Table 4. The highest antioxidant activity (i.e. the lowest IC$_{50}$ value) obtained from the maceration method with a solvent ratio of 70:30 (ethanol:hexane). Moreover, a higher concentration of ethanol gave lower IC$_{50}$ value. It was possibly because polar compounds have more antioxidant activity than non-polar compounds [8]. However, using bot reflux and soxhletation methods have the lowest antioxidant activity. It was possibly due to the heat use caused an electron excitation, which has negative influence on damaging the antioxidant. Several studies have found that the excited electrons during the electron excitation produce heat and could damage the antioxidant compounds as electron donors [21,22]. The presence of multiple solvents inhibits the action of ethanol solvents in binding antioxidant compounds. Ethanol is usually used to extract active compounds, in which the smaller the ratio of ethanol indicating, the less amount of ethanol used to extract the antioxidant compounds [23].

4. Conclusions

The findings confirm that the extraction method and the solvent ratio (ethanol:hexane) affects yields, total phenol content, total flavonoid content, and antioxidant activity (IC$_{50}$) of the dried Dayak onion extract. The maceration method with ethanol:hexane solvent ratio of 70:30 gave best result compared to other treatments. A high ethanol solvents ratio found in lowering the IC$_{50}$ value. The extraction method using heating (reflux and soxhletation) was found to have damage impact on antioxidant activity. This study also demonstrated that polar compounds (dissolved in ethanol) have more antioxidant activity than non-polar compounds (dissolved in hexane).

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