Extraction yield, antioxidant activity and total phenolic content of *Mimusops elengi* L. fruit

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Abstract. *Mimusops elengi* L. fruit or known as Bakul fruit is believed to have a lot of benefit due to its flavonoid group compound that can have antioxidant effects. This study aimed to determine the effect of temperature and ratio between material and solvent in the extraction process on the antioxidant activity and content of phenolic compounds in *Mimusops elengi* L. fruit extract. This research used factorial completely randomized design with 2 factors: extraction temperature (45°C, 60°C, and 75°C) and ratio of the material and solvent (1:4, 1:6, and 1:8) with 3 replications. The extraction yield, total phenolic contents and antioxidant activity of *Mimusops elengi* L. fruit extract were examined. Furthermore, the application of extraction temperature in 75°C with ratio 1:8 material to solvent (w/v) has the highest extraction yield, total phenolic contents and antioxidant activity compared to others treatments.

1. Introduction

*Mimusops elengi* L. is one of the plants that are easily found in Indonesia. This plant is widely planted in office yards, schools and on the roadside. One part of *Mimusops elengi* L. plant that can be used is the fruit. *Mimusops elengi* L. fruit or known as Bakul fruit is one of the crops rich in antioxidants so that it can be one of natural sources as free radical scavenger. Several compounds that can perform as antioxidants include polyphenolic compounds (phenolic acids and flavonoids), alkaloids, steroids or triterpenoids (saponins), and anthraquinones [1][2]. Boonyuen et al [3] stated that the *Mimusops elengi* L. fruit contains flavonoid compounds and is considered a bioactive compound. The amounts of bioactive natural products are always fairly low [4]. Perwiratami et al. [5] stated that *Mimusops elengi* L. fruit extracted using ethanol at 60°C contained a phenolic compound of 6.5 mg/g. Nowadays, it is very important to develop effective and selective methods for the extraction and isolation of those bioactive compounds.

Extraction of bioactive or phenolic compounds from *Mimusops elengi* L. fruit through a process called extraction. This process has a separation principle that aims to get the content or extract from a biosource such as a plant or fruit. Temperature is a very critical factor in the extraction process [6]. Antioxidant and phenolic compounds are increasing by raising the temperature between 45-100°C [7][8]. The ratio of material and solvent, time, temperature, and particle size can affect the extraction process [9]. Extraction is the initial phase to isolate the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing and sublimation according to the extraction principle. Solvent extraction is the most commonly used process. The extraction of...
natural products progresses through the following points: the solvent penetrates into the solid material; the solute dissolves in the solvents; the solute is diffused out of the solid material; the extracted solutes are collected [4]. In this study, a maceration extraction method was used with various temperatures. The temperatures used are 45°C, 60°C, and 75°C. The use of different extraction temperatures aims to understand the consequence of extraction temperature on antioxidant activity and phenolic compounds in *Mimusops elengi* L. fruit extraction.

Throughout the extraction process, bioactive compounds in the matrix will be dissolved by a solvent that matches its polarity. Phenolic compounds are polar compounds [10]. Current study used ethanol because ethanol is a polar solvent which is proper for extracting phenolic compounds. Furthermore, ethanol is also a type of solvent safe for food products and it can extract antioxidants quite well [11]. The ratio of materials and solvents is also part of the factors that affect the rate of extraction, in this study used ratios of 1:4, 1:6, and 1:8 between materials and solvents. Rifai et al. [12] reported that during the extraction process the yield will increase along with the increased amount of solvent. However, when the solvent ratio is increased to a certain level, on the other hand yield is still fairly low [13]. Therefore this study aimed to determine the effect of temperature and ratio between material and solvent in the extraction process on the antioxidant activity and content of phenolic compounds in *Mimusops elengi* fruit extract.

2. Materials and methods

2.1. Material

Materials used in this study are fresh *Mimusops elengi* L. fruit, ethanol 96%, Folin-Ciocalteu, sodium carbonate (Na₂CO₃), gallic acid, metanol, and 2,2’-diphenyl- 1-picrylhydrazyl (DPPH).

2.2. Fruit material [5]

Sample preparation is done by Perwiratami et al [5] with slight modification. *Mimusops elengi* L. fruit is thoroughly washed and peeled to get flesh of the fruit. Flesh of *Mimusops elengi* L. fruit is aerated in room temperature for 5 days until it completely dried. Then dried-sample is grinded and sieved to get powder of dried *Mimusops elengi* L. fruit. Powdered material was analyzed for moisture and ash content.

2.3. Preparation of extracts

The extraction was conducted according to Perwiratami et al [5] and Soehendro et al [8]. 30 g of Powdered *Mimusops elengi* L. fruit were extracted with 96% ethanol at required ratio; 1:4, 1:6, and 1:8 in a glass conical flask. The conical flask was wrapped with aluminum foil to prevent solvent loss. The mixture was extracted using a hot plate for 8 hours at the determined temperature starting from 45°C, 60°C, and 75°C. After completing the extraction process, *Mimusops elengi* L. fruit extract was filtered through Whatman No.1 filter paper in order to obtain a clear crude extract solution and condensed with rotary evaporator. All the extraction processes were done in replicate and all the analyses on each sample were done in triplicate. Subsequently, this extract was subjected to yield, DPPH radical scavenging activity and total phenol analysis.

2.4. Determination of phenolic content

Determination of total phenolic content in recent study was according to Sam et al [14]. 1 mg of gallic acid diluted in ethanol 96% and making the volume up to 100 mL. Then, 0.2, 0.4, 0.6, 0.8, and 1 mL were taken into volumetric flask and made up to 100 ml with ethanol 96%. 1 mL of Folin-Ciocaltelu 10% was mixed and incubated for 8 minutes. After incubating the mixture at room temperature for 8 min, 1 mL of 6% (w/v) sodium carbonate anhydrous (Na₂CO₃) solution was added into the mixture. The mixture was then immediately vortexed for 10 s (homogenous) and added with 5 mL of deionized water. After incubating in a dark environment at room temperature for 2 h, the absorbance at 765 nm was measured. A calibration curve for gallic acid was used for determining total phenolics in the sample fractions and the findings were expressed as µg/mL of gallic acid per gram of the extracted fraction. 0.2 ml diluted *Mimusops elengi* L. fruit extract then add 5 mL of distilled water, 1 mL of Folin-Ciocaltelu
The reagent and 1 mL of 6% \( \text{Na}_2\text{CO}_3 \) then shake and let it rest for 4-8 minutes. The absorbance at 765 nm was measured and the definitions were expressed as mg gallic acid/g fresh sample.

2.5. DPPH radical-scavenging capacity

Procedure of antioxidant activity was described by [15] with slight modification to determine the DPPH radical scavenging activity [16]. In brief, 0.2 mL of sample dissolved in distilled water and 25 mL of 0.0098 g DPPH (2.2-diphenil 1-pichylhydazyl) were mixed in ethanol. For the control, distilled water was used to replace the extract mixture. After vortex mixing, the mixture was left to rest at 25 °C in dark condition for 60 min. The absorbance was measured at 516 nm using a spectrophotometer (UV-Visible, Shimadzu Japan). The following equation was used to calculate the DPPH scanning effect as a percentage of the DPPH discoloration:

\[
\text{AA} (\%) = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100
\]  

(1)

2.6. Statistical analysis

Recent study used a completely randomized design (CRD) factorial with extraction temperature (45°C, 60°C, and 75°C) and the ratio of the material to the solvent (1:4, 1:6, and 1:8). This treatment was replicated 3 times. In examining the effect of each factor and the interaction between factors on the yield analysis parameters, antioxidant activity, and total phenol, it performed statistical analysis using two-way analysis of variance (ANOVA) with Duncan Multiple Range Test (DMRT) test which was used to determine the significant differences between the means at the 5%.

3. Results and discussion

3.1. Moisture and ash content of Powdered Mimusops elengi L. Fruit

The moisture content analysis aims to identify water contained in powder material. In this study, the moisture content ranged from 10.42-10.88% with average 10.58% and in accordance with Perwiratami et al [5]. According to Indonesian national standard (SNI 01-3709-1995), Mimusops elengi L. fruit powder is classified pretty good where the maximum quality requirement for powder has moisture content of 12%. The determination of moisture content is also related to the purity of the extract, material with high moisture content will stimulate microorganisms to grow and result in decreasing extract quality. In this study, the average of ash content from Mimusops elengi L. fruit powder was 1.43%. This result is higher than declared by Vineeta singh et al [17] by 0.7% and Manik et al [18] by 0.89%. Based on the research, the samples used for the extraction process have met the quality requirements of the ash content of the dried fruit. The ash content in dried fruit is a maximum of 3.5% [5].

3.2. Yield of Powdered Mimusops elengi L. Fruit

The yield produced in this study ranged from 13.62 to 33.21% with average 24.69 % at 75°C extraction temperature contributing the highest yield. There is an interaction between the extraction temperature and the ratio of materials and solvents which has a significant effect (P<0.05). Based on Figure 1, the yield of Mimusops elengi L. fruit extraction that contains phenolic compounds was increased correspondingly with the increasing of extraction temperature. Every single temperature experiment showed a similar trend, although 60°C was no significant difference with 75°C at the same ratio between material and solvent, 1:6 and 1:8. Increased temperature could stimulate the phenolic extraction by increasing both diffusion coefficient and solubility of phenolic compounds in extraction solvent [19][20]. The recent study was linearly with [6] [19]. Furthermore, ratio material and solvent are also contributed to yield the phenolic compound. It shows that ratio material and solvent of 1:8 presented the highest yield compared to others (Table 1). However, ratio 1:6 in 75°C extraction temperature was no significant difference (p>0.05) in yield percentage with this 1:8 ratio in 75°C as represented in Table 1.
Figure 1. Effect of temperature and ratio of material:solvent on percentage yield of powdered *Mimusops elengi* L fruit extracts. Values are presented as means ± standard deviation of 3 measurements. Note: Error bars represent the standard deviation.

Handayani et al. [14] and Delazar et al. [21] stated that higher composition of ethanol solvent demonstrated high yield value due to contact period with material as the extraction medium tend to be better. Figure 1 proved that increasing ethanol volume will exhibit more yield in recent study. This trend occurs because of polarity factor between material and solvent. Ethanol itself can be both polar and nonpolar. The process of extraction arises by the flow of solvent into the cell which causes swelling of protoplasm, and the material bounded in cell will dissolve according to its solubility. This high dissolving capacity is associated to solvent polarity and the polarity of the extracted compounds. It’s indicated that solvents only extract those phytochemicals which have similar polarity with the solvents [22]. Delazar et al. [21] stated that yield will increase along with increasing amount of solvent. By using more solvent, releasing of target compound into the solvent could run more flawlessly and solvent saturation could be also avoided. Therefore, the study would preferably selected ratio 1:8 at 75°C based on yield percentage.

3.3. Phenolic content of Powdered *Mimusops elengi* L. Fruit
Total phenol found in this study ranged from 4.64 mg/g - 7.71 mg/g with an average of 6.21 mg/g. The highest total phenol was achieved at 75°C but there was no significance difference (p>0.05) with others except for total phenol obtained from 45°C in ratio material and solvent 1:4 (Table 1). The effects of extraction temperature on phenolic contents crude extract are shown in Figure 2. phenolic compounds were increased consistently with the increasing extraction temperature, reaching maximum values at 75°C. Based on the figure 1, high temperature was found to enhance the retrieval of phenolic compounds from *Mimusops elengi* L. fruit. This result was supported by Lim and Murtijaya [23] and Silva et al. [24], who reported that higher temperature could affect a significantly higher total phenol in extracting the Indian medicinal plant and mashua, respectively. Al-Farsi and Lee [19] reported that increased temperature could promote the phenolic extraction by increasing both diffusion and solubility of phenolic compounds in extraction solvent. Additionally, intense heat from solvent was also able to discharge the cell wall phenolics and bounded phenolics by breaking down of cellular constituents [25] and consequently increased the phenolic yield in extract.
Figure 2. Effect of temperature and ratio of material:solvent on phenolic content of powdered *Mimusops elengi* L fruit. Values are presented as means ± standard deviation of 3 measurements. Note: Error bars represent the standard deviation.

### Table 1. Interaction between ratio material:solvent and temperature data on yield, phenolic content and DPPH radical scavenging capacity.

| Ratio material:solvent | Temperature (°C) | | | | |
|------------------------|------------------|--|--|--|--|
|                        | 45  | 60  | 75  | | |
| 1:4                    | 13.62<sup>a</sup> | 23.78<sup>c</sup> | 24.25<sup>c</sup> | | |
| 1:6                    | 16.94<sup>b</sup> | 28.62<sup>d</sup> | 29.98<sup>de</sup> | | |
| 1:8                    | 19.13<sup>b</sup> | 32.64<sup>c</sup> | 33.21<sup>c</sup> | | |
| 1:4                    | 4.64<sup>a</sup> | 5.87<sup>ab</sup> | 7.09<sup>ab</sup> | | |
| 1:6                    | 4.81<sup>ab</sup> | 6.50<sup>ab</sup> | 7.49<sup>ab</sup> | | |
| 1:8                    | 4.94<sup>ab</sup> | 6.86<sup>ab</sup> | 7.71<sup>b</sup> | | |
| 1:4                    | 61.09<sup>a</sup> | 71.07<sup>c</sup> | 76.53<sup>de</sup> | | |
| 1:6                    | 62.78<sup>ab</sup> | 73.36<sup>ed</sup> | 78.41<sup>c</sup> | | |
| 1:8                    | 65.58<sup>b</sup> | 73.86<sup>ed</sup> | 78.94<sup>c</sup> | | |

Values marked by different lower-case letters (a-e) are significantly different (p<0.05).

3.4. DPPH radical-scavenging capacity of Powdered *Mimusops elengi* L Fruit

Antioxidant activity observed in this study ranged from 61.09% to 78.94%, the average was 71.29%. According to Figure 3, the recovery of the antioxidant activity increased linearly with increasing extraction temperature. The highest DPPH was achieved at 75°C of extraction temperature and ratio material and ethanol concentration at 1:8. This present study indicates that *Mimusops elengi* L. fruit extract had the highest antioxidant capacity at 78.94%. However, based on Table 1, this result was no difference with ratio 1:4 and 1:6 respectively. Earlier reports stated that antioxidant activity of phenolic compounds were associated with the availability of the phenolic compound acting as hydrogen-donating radical scavengers [26][27]. Therefore, it could be projected that there was a moderate availability of phenolic compounds which can act as hydrogen-donating radical scavengers in *Mimusops elengi* L. fruit extract at treatment of 1:8 ethanol concentration with 75°C of extraction temperature.
In this circumstance, antioxidant capacity started to increase when the extraction temperature was beyond 45°C and stable up to 75°C. It was expected to decrease if extraction temperature is increased beyond 75°C. According to Narsih and Agato [28], intense heat can break down the stability of plant cell tissue in extracted material. Thus, there was an increase in the active substances released from the material. This is accordance with previous study by Soehendro et al. [8], where the material extracted at a temperature of 75°C produces antioxidant activity that tends to increase compared to the material extracted at a temperature of 60°C and 45°C. However, temperatures that are too high can also reduce antioxidant activity. Perwiratami et al. [5] reported in their research that the extract of *Mimusops elengi* L. fruit contains compounds of alkaloid group (flavonoids), which are susceptible to damage at temperatures around 90°C [29]. On the other hand, a previous study stated that the antioxidant capacities of Centella asiatica extract started to decrease when the extraction temperature exceeded 45°C [6]. Similarly, Abdul Hamid et al. [30] also revealed that the antioxidant capacity of C. asiatica extract was stable up to 50°C. According to Chan et al. [31] and Liyana Pathirana and Shahidi [32], the loss of the antioxidant capacity of plant extracts at high extraction temperatures is likely due to the breakdown of phenolic compounds that were previously mobilized at low temperatures. Therefore, phenolic compounds extracted at high temperatures have a lower antioxidant capacity than those extracted at low temperatures, a high phenol content does not necessarily mean a high antioxidant capacity, the antioxidant capacity also depends on the structure and interaction between the phenolic compounds [33]. It can be assumed that due to the solubility of the extracted material with a certain solvent and a certain temperature in the same material, different degrees of antioxidant activity can occur. Therefore, further studies should be carried out to identify phenolic compounds in *Mimusops elengi* L. fruit extracts extracted at different temperatures and solvent with regard to their antioxidant mechanism and synergistic effects.

4. Conclusions
The extraction parameter of ratio between material and ethanol concentration, and extraction temperature had significant (p < 0.05) effect on yield, the phenolic contents and of *Mimusops elengi* L. fruit extract. The optimal extraction conditions for optimized phenolic and antioxidant activity recovery
from *Mimusops elengi* L. fruit 1:8 ratio of material and ethanol at 75°C, with values of 33.21% for yield, 7.71 mg/g for total phenolic content, and 78.94% for antioxidant activity. Further studies should be carried out to identify phenolic compounds in *Mimusops elengi* L. fruit extracts extracted at different temperatures and solvent with regard to their antioxidant mechanism and synergistic effects.

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