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Effects of extraction temperature on bioactive compounds and antioxidant activity of yellow velvetleaf (*Limnocharis flava*) and water lettuce (*Pistia stratiotes*) leaf extract

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Abstract

Oxidative stress is considered a source of metabolic disorders. It is caused by an imbalance between the body's antioxidant potential and free radical production. Therefore, the body needs exogenous antioxidants, such as the bioactive compounds of plants. The yellow velvetleaf (*Limnocharis flava*) and water lettuce (*Pistia stratiotes*) leaf showed potential antioxidant activity because of their polyphenol compounds. This study aimed to investigate the effects of extraction temperature on polyphenols and flavonoids as well as their antioxidant activity on yellow velvetleaf and water lettuce leaf. The plant was extracted by the maceration method in various processes using 70% ethanol. The results showed the total polyphenol (TPC) and flavonoid content (TFC), as well as the antioxidant content determined using the DPPH method. The highest TPC (512.5 mg GAE/g of dried sample; 273.7 mg GAE/g of the dried sample) and TFC (209.5 mg QE/g of dried sample; 641 mg QE/g of the dried sample) were observed in low-temperature extraction. Under these conditions, the highest antioxidant activity (IC_{50}) of yellow velvetleaf and water lettuce was 0.207 mg/mL and 0.227 mg/mL, respectively. A low extraction temperature is optimal for extracting these bioactive compounds, primarily from yellow velvetleaf and water lettuce leaf.

1. Introduction

Oxidative stress has been recognized as a source of metabolic disorders. It is caused by an imbalance between the antioxidant potential and free radical production in the cells and tissues. Therefore, exogenous antioxidants are widely needed to maintain these conditions. Synthetic antioxidants used in the food industry have been restricted due to safety concerns, such as DNA damage and other side effects (Lourenço *et al.*, 2019). Its alternative sources are one of the emerging fields in health care, including functional foods or dietary supplements. In addition, bioactive compounds such as vitamins and polyphenols are widely used as a source of natural antioxidants (Sinbad *et al.*, 2019; Zeb, 2020).

The aquatic plants widely spread in Indonesia include yellow velvetleaf (*Limnocharis flava*, Family Alismataceae) and water lettuce (*Pistia stratiotes*, Family Araceae), which float on the water surface. Flavonoid compounds have been observed in yellow velvetleaf extracts (Baehaki *et al.*, 2020). The successful extraction of polyphenols and flavonoid compounds from water lettuce (Herpandi *et al.*, 2021) has been reported in a previous study. Furthermore, it demands several parameters, such as extraction temperature, type of solvent, the ratio of solvent to solid, and extraction number, for its optimization (Andres *et al.*, 2020).

Polyphenols are thermolabile compounds (Li *et al.*, 2017). A previous study reported that a high temperature reduced the polyphenol content in *Thymus vulgaris* extract (Vergara-Salinas *et al.*, 2012). Additionally, it (>40°C) caused the degradation of some thermolabile bioactive compounds in *Gordonia axillaris* extract (Li *et al.*, 2017). Temperature is an essential element in the extraction process to find the optimal condition, especially in polyphenol compounds from yellow velvet and water lettuce leaf. The effects of temperature on this extraction process have not been reported. Therefore, this study investigates the optimal extraction temperature for polyphenol compounds from yellow velvetleaf and water lettuce leaf.

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2. Materials and methods

2.1 Preparation and extraction

The fresh forms of yellow velvetleaf (*Limnocharis flava*) and water lettuce (*Pistia stratiotes*) leaf were collected from a village in Ogan Ilir Regency, South Sumatra, Indonesia. They were cleaned, kept, and transported to the laboratory for further experiments. The extraction process was conducted according to the following methods (Chew *et al.*, 2011). Briefly, the fresh leaf of each plant was dried in an oven at 45°C for 12 hours, after which it was ground into powder form. Then, the bioactive compound from the plants was extracted by the maceration method (120 rpm). Furthermore, the samples were extracted with 70% ethanol at 30°C, 45°C, and 60°C. After the maceration period, the filtrate (liquid phase) and residue (solid phase) were separated using filter paper (Whatman No. 42) and then transferred into the new collection tube. The residues were collected with fresh solvent, as mentioned above, and five extractions were performed. The mixed filtrate of each plant was evaporated at 45°C (Biobase RE-301, Shandong, China) to obtain concentrated extracts. Additionally, they were freeze-dried (Biobase BK-FD10S, Shandong, China) to obtain the final powder of yellow velvetleaf (YVE) and water lettuce extract (WLE). The samples were kept at a cold temperature for further analysis. The extraction yield percentage (%) was calculated according to the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dried sample (g)}} \times 100\%$$

2.2 Total polyphenol content analysis

The total polyphenol content (TPC) was analyzed using Folin-Ciocalteu's phenol reagent, and the experiment was performed according to the method described by Chandra *et al.* (2014). Gallic acid was used as a standard in this calculation. Briefly, 0.2 mL of each extract (10 mg/mL) or standard was mixed with the Folin -Ciocalteu's reagent and kept for 5 mins. After the reaction time, 1 mL of saturated sodium carbonate (8% in water, w/v) and distilled water were added to generate 3 mL. The mixture was allowed to react in dark conditions at room temperature for 30 mins. Subsequently, it was centrifuged at 3,000 rpm for 30 mins, and the absorbance of the supernatant was measured at 750 nm using a UV-Visible spectrophotometer (Genesys 150 ThermoScientific, Massachusetts, USA). The total polyphenol content was expressed as mg gallic acid equivalent (GAE) per g of dry sample.

2.3 Total flavonoid content analysis

Total flavonoid content (TFC) was analyzed by the aluminum chloride colorimetric method according to the method described by Chandra *et al.* (2014). Quercetin was used as a standard in performing this calculation. Briefly, 0.6 mL of each extract (10 mg/mL) or standard solution was reacted with 2% aluminum chloride (1:1, v/v) at room temperature for 60 mins. After the incubation time, the absorbance was measured using a UV-Visible spectrophotometer (Genesys 150 ThermoScientific, Massachusetts, USA) at 420 nm. Finally, the total flavonoid content was expressed as mg quercetin equivalent (QE) per g of dry sample.

2.4 Antioxidant activity assay

The antioxidant activity of each extract was measured with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by Chew *et al.* (2008). Briefly, each extract was dissolved in distilled water to make a serial concentration of 0 – 500 µg/mL, while ascorbic acid was used as a positive control. Subsequently, 1 mL of extract or control was mixed with 0.2 mM DPPH solution (1:1, v/v). The mixture was incubated at 37°C in a dark condition for 30 mins. Afterwards, the absorbance was measured at 517 nm using UV-Visible spectrophotometry (Genesys 150 ThermoScientific, Massachusetts, USA). The inhibition percentage was formulated according to the following equation:

$$\text{Percentage (\%) of inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\%$$

Abs_{blank} = Absorbance at 517 nm without treatment, and Abs_{sampel} = Absorbance at 517 with sample treatment.

2.5 Statistical analysis

All data are expressed as the mean ± standard deviation (SD) and analyzed by one-way ANOVA with Duncan's post-hoc test (p<0.05) using SPSS (v.22.0; IBM Corp., Armonk, NY, USA). Furthermore, the graphics were produced using the GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, USA).

3. Results and discussion

3.1 Extraction yield

The maceration method collected bioactive compounds using solvent extraction, separating the soluble solute from a solid matrix by diffusion. This method is also cheap and simple compared to others (Zhang *et al.*, 2018). However, some parameters optimize the condition, including extraction temperature. Figure 1 shows that the temperature significantly affects the yield of extraction. It was indicated that the extraction yield in high-temperature conditions was significantly higher than the lowest. This influences the solubility and diffusion coefficient during extraction.

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Additionally, an increase in temperature is directly proportional to the penetrability of the solvent, resulting in rising extraction speed and efficiency (Lee *et al.*, 2016).

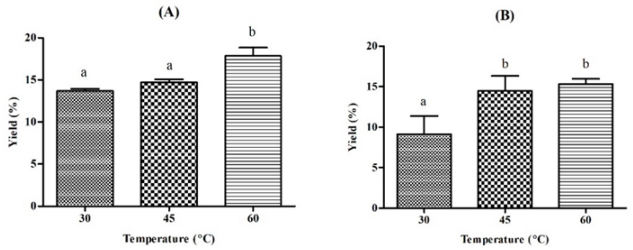

Figure 1. Effects of extraction temperature on the extraction yield of (A) yellow velvetleaf and (B) water lettuce leaf. Values are presented as mean±SD (n = 3). Bars with different notations are statistically significantly different (p<0.05) between groups determined using one-way ANOVA with Duncan's post-hoc test.

3.2 Total polyphenol and total flavonoid content

Figure 2 shows that a high extraction temperature of 60°C significantly decreased the polyphenol content (TPC; 435.7 mg GAE/g and 211.9 mg GAE/g of the dried sample, respectively) in the yellow velvetleaf and water lettuce extracts. The polyphenol of yellow velvetleaf and water lettuce at 30°C extraction temperature was 512.5 mg GAE/g and 273.7 mg GAE/g of the dried sample. Additionally, high extraction temperatures significantly reduced the TFC in the plant extract by 162.7 mg QE/g of the dried sample and 446.0 mg QE/g of the dried sample, respectively, compared to otherwise (209.5 mg QE/g of the dried sample and 641 mg QE/g of the dried sample, respectively), as shown in Figure 3. According to these results, a low temperature is the optimum condition for the extraction of polyphenols and flavonoid compounds, especially TPC and TFC, from yellow velvetleaf and water lettuce leaf.

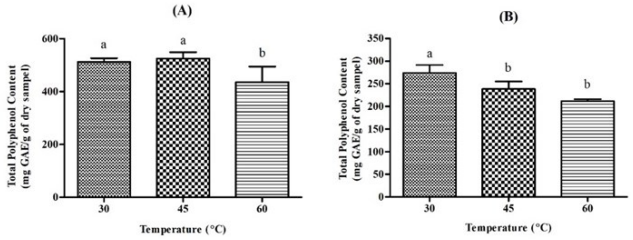

Figure 2. Effects of extraction temperature on total polyphenol content of (A) yellow velvetleaf and (B) water lettuce leaf. Values are presented as mean±SD (n = 3). Bars with different notations are statistically significantly different (p<0.05) between groups determined using one-way ANOVA with Duncan's post-hoc test.

A previous study reported that the optimum condition for TPC was 35°C (Roselló-Soto *et al.*, 2019). The highest TFC from *Momordica charanti* was due to an extraction temperature of 40°C (Tan *et al.*, 2014). Even though the high temperature increased the extraction yield, as shown in Figure 1, polyphenol remains a thermolabile compound. The polyphenol compounds tend to decompose due to a high extraction temperature. Additionally, previous literature reported that 40°C remains the optimal condition for extracting phenolic compounds from *Gordonia axillaris* (Li *et al.*, 2017).

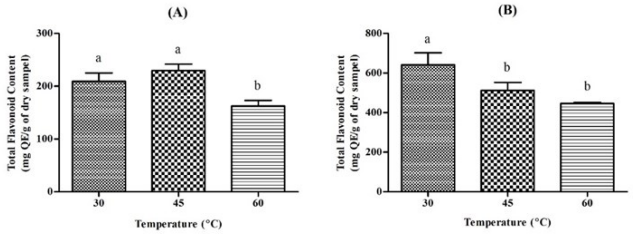

Figure 3. Effects of extraction temperature on total flavonoid content of (A) yellow velvetleaf and (B) water lettuce leaf. Values are presented as mean±SD (n = 3). Bars with different notations are statistically significantly different (p<0.05) between groups determined using one-way ANOVA with Duncan's post-hoc test.

3.3 Antioxidant activity

According to the DPPH method, Figure 4 shows that the temperature significantly affected the antioxidant activities of the plant extract. Meanwhile, the low temperature significantly had more potent antioxidant activity than the high extraction temperature, as indicated by the lower half-maximum inhibitory concentration (IC_{50} 0.207 mg/mL, and 0.227 mg/mL for yellow velvetleaf and water lettuce, respectively). This condition showed a higher level of bioactive compounds (polyphenol and flavonoid) at a low extraction temperature than at a high extraction temperature (Figures 2 and 3). A previous study reported that polyphenol compounds act as antioxidants by HAT or SET mechanisms to prevent oxidation reactions or reduce free radicals (Lee *et al.*, 2015).

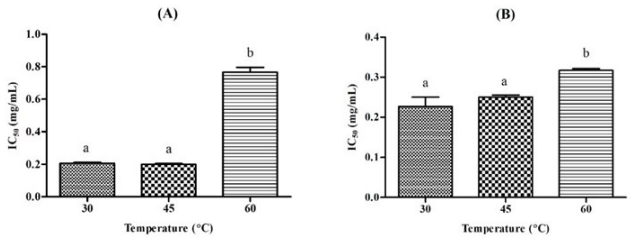

Figure 4. Effects of temperature extraction on antioxidant activity of (A) yellow velvetleaf and (B) water lettuce leaf. Values are presented as mean±SD (n = 3). Bars with different notations are statistically significantly different (p<0.05) between groups determined using one-way ANOVA with Duncan's post-hoc test.

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4. Conclusion

Polyphenol and flavonoid compounds were successfully extracted from yellow velvetleaf and water lettuce leaf at different extraction temperatures. Higher polyphenol and flavonoid contents were observed after low-temperature extraction. The same condition also raises the antioxidant potential. Therefore, the low extraction temperature is considered the optimal condition for extracting these bioactive compounds, especially from yellow velvetleaf and water lettuce leaf.

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