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Research Article

Total polyphenols and antioxidant activity of yellow velvetleaf (*Limnocharis flava*) extract

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Abstract: An increase in free radicals can cause damage to cells and tissues in the body. This is caused by a lack of antioxidant ability in the body, so it requires antioxidants outside the body. One of the plants that can be used as a source of antioxidants is the yellow velvetleaf (*Limnocharis flava*), which has a class of polyphenolic compounds. The crude extract of this plant still has other components that are not included in the polyphenol compound. This study aimed to measure the total polyphenol and flavonoid contents as well as the antioxidant activity of the yellow velvetleaf plant (L. flava) before and after purification. The purification process is carried out using the solid-phase extraction method. The antioxidant activity was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The total polyphenol and flavonoid contents increase after the purification process. Therefore, the antioxidant activity of purified extract is also increased when compared to crude extract. These results show that the purification process successfully increased the levels of polyphenol compounds from the yellow velvetleaf plant and its antioxidant activity. Thus, the purified extract can be used as an alternative source of natural antioxidants and can be developed as a food supplement ingredient.

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1. INTRODUCTION

Free radicals are a group of compounds that have unpaired electrons in their outer orbitals and are naturally the result of biological processes in the body. Free radical compounds can also come from pollution, cigarette smoke, exposure to heavy and transition metals, pesticides, and industrial wastes (Phaniendra *et al*., 2015). Under normal conditions, the body's antioxidant defense system can reduce exposure to existing free radicals because it has primary antioxidants (endogenous antioxidants), such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ighodaro & Akinloye, 2019). However, the body requires additional antioxidants that come from outside when the levels of free radicals exceed the antioxidants contained in the body. This type of antioxidant can be classified as exogenous

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antioxidants and can generally be obtained through functional foods or dietary supplements (Roehrs *et al*., 2011).

Yellow velvetleaf (*Limnocharis flava*) is an aquatic plant commonly found in South Sumatra, Indonesia. This plant contains some bioactive compounds, such as polyphenols and flavonoids (Jamila *et al*., 2021). This plant extract also shows antioxidant activity by scavenging free radicals using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method (Baehaki *et al*., 2019).

Phenolic compounds are classified as secondary metabolites commonly distributed in the kingdom of plants, with immense structures and functions. Polyphenol compounds are a source as natural antioxidant (Swallah *et al*., 2020). Since polyphenol extraction with organic solvent was obtained as a crude extract composed of other non-polyphenol components, such as sugar, lipid, and organic acids, a purification process is required to remove these compounds (Zeka *et al*., 2017). A solid-phase extraction (SPE) method was used for the purification process due to its simplicity, rapidity, and economics (Dai & Mumper, 2010). A previous study reported that purified polyphenol compounds exhibit stronger antioxidant activity than crude (unpurified) extract (Wang *et al*., 2019). Additionally, the purification process of polyphenol extract by the SPE method also increases the inhibition of HMG-CoA reductase activity (Sudirman *et al*., 2022). According to these conditions, we hypothesized that the purification process also showed different effects on the antioxidant activity of the yellow velvetleaf extract. Therefore, this study aimed to investigate the effect of the purification process on the polyphenol and flavonoid contents of yellow velvetleaf (*L. flava*) extract as well as its antioxidant activity.

2. MATERIAL and METHODS

2.1. Preparation and Extraction

The fresh yellow velvetleaf (*L. flava*) was collected and washed using tap water. The whole plant was used except the root. The plant was cut into pieces and dried in the oven at 45ºC for 24 hours. After the drying process, the plant was ground to obtain dried powder. The extraction process was conducted at 30ºC for 3 hours by the maceration method with stirring at 100 rpm by a magnetic stirrer. Whereas, 70% ethanol was used as a solvent (Sudirman *et al*., 2022). Briefly, 20 g of dried sample was put into an Erlenmeyer tube, which contains 200 mL of 70% ethanol. After 3 hours of maceration, the filtrate and residue were separated by filter paper (Whatman No. 42). The filtrate was kept in the new collection tube and the residue was extracted using a fresh solvent under the same conditions as the first extraction, for a total of five extractions. After the extraction process, the filtrates were mixed in a new collection tube and the solvent was evaporated by using a rotary vacuum evaporator at 40ºC to obtain paste form, and then dried by using a freeze dryer. The yield of extraction was calculated according to this formula:

$$\text{Yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Dried sample (g)}} \text{ x } 100\%$$

2.2. Purification Process

The purification process was conducted using a solid phase extraction method (SPE) by using HyperSep Retain PEP Cartridges (Part. No. 60107-212, Thermofisher Scientific) according to the previous method (Sudirman *et al*., 2022). Briefly, the cartridge was washed with 2 mL of distilled water and 2 mL in the precondition step, respectively. Then, 2 mL of the crude extract (1 mg/10 mL) was pipetted into the cartridge and was eluded by using 2 mL of *n*-hexane and 2 mL of H_2SO_4 1 N, respectively. The cartridge was then washed with absolute methanol to collect the purified extract and it was dried by a freeze dryer to obtain extract powder.

2.3. Total Polyphenol and Flavonoid Contents Analysis

The total polyphenol content was analyzed using *Folin-Ciocalteu's* method according to the previous method (Chandra *et al*., 2014). Briefly, 0.2 mL of each extract (10 mg/mL, crude and purified extracts) was mixed with Folin-Ciocalteu's phenol reagent in a reaction tube and allowed to react for 5 minutes. After the reaction process, the solution was added with 8% sodium carbonate and a volume of up to 3 mL using distilled water. The mixture was reacted in a dark condition for 30 minutes. The supernatant was separated by centrifugation at 3,000 rpm for 30 minutes. The absorbance of the supernatant was measured by a spectrophotometer (Genesys 150 ThermoScientific, Massachusetts, USA) at 750 nm. Gallic acid was used as a standard to determine the total polyphenol compound and the total polyphenol content was calculated as mg gallic acid equivalent (GAE) per g of dried weight (mg GAE/g DW).

The total flavonoid content was analyzed by the aluminum chloride colorimetric method according to a previous study (Chandra *et al*., 2014). Briefly, 0.5 mL of extract solution (crude and purified extract) was reacted with 2% aluminum chloride (1:1) at room temperature for 60 minutes. After the reaction time, the absorbance was measured immediately by using a spectrophotometer (Genesys 150 ThermoScientific, Massachusetts, USA) at 420 nm. The quercetin was used as a standard to determine the total flavonoid compound and the total flavonoid content was calculated as mg quercetin equivalent (QE) per g of dried weight (mg QE/g DW).

2.4. Antioxidant Activity Assay

The antioxidant activity was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the previous study (Chew *et al*., 2008). Briefly, the extracts (crude and purified) were diluted at serial concentrations (0 – 1,000 µg/mL). Then, the extract solution (1 mL) was mixed with 0.2 mM DPPH solution (1:1). The mixture was incubated at 37ºC for 30 minutes in dark conditions. The absorbance was measured immediately using a spectrophotometer at 517 nm. The percentage of radical scavenging or free radical inhibition was calculated by following this formula:

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

Whereas: Abs_{blank}, the absorbance at 517 nm without sample; Abs_{sample}, the absorbance at 517 nm with sample.

2.5. Data Analysis

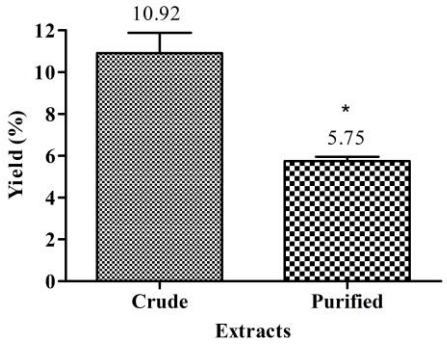
All the data were presented as mean ± standard deviation (SD). The data was analyzed using an independent t-test with SPSS 22.0 (IBM Corporation, Armonk, NY, USA). All graphics were produced using the GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA, USA).

3. RESULTS

3.1. Extraction and Purification Yields

The yield of crude extract was about 10.92±3.59% and the yield was reduced to 5.75±0.25% after the purification process as shown in Figure 1.

Figure 1. The yield of crude and purified extracts of yellow velvetleaf (*L. flava*). Data was shown as mean ± SD (*n*=3). Significant difference at *p<0.05 vs crude extract.



3.2. Total Polyphenol and Flavonoid Contents

The total polyphenol content of yellow velvetleaf after purification significantly (p<0.05) increased when compared to crude extract (494.83 mg GAE/g DW and 379.25 mg GAE/g DW, respectively), as shown in Figure 2. Whereas, Figure 3 showed that the total flavonoids also significantly (p<0.05) increased after the purification process when compared to crude extract (280.98 mg QE/g DW and 177.61 mg QE/g DW, respectively).

Figure 2. The total polyphenol content of crude and purified extracts of yellow velvetleaf (*Limnocharis flava*). Data was shown as mean ± SD (*n*=3). Significant difference at *p<0.05 vs crude extract.

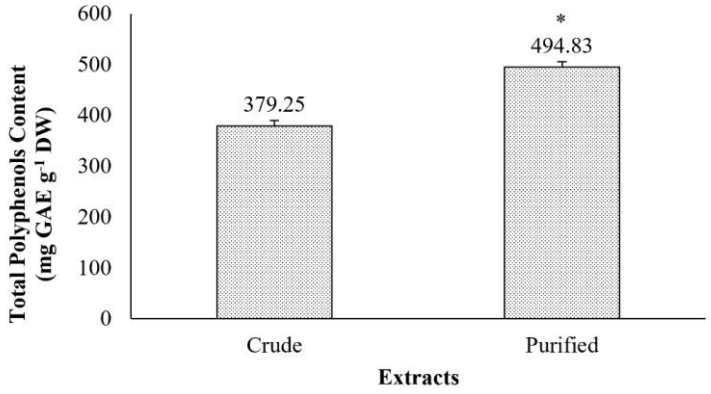
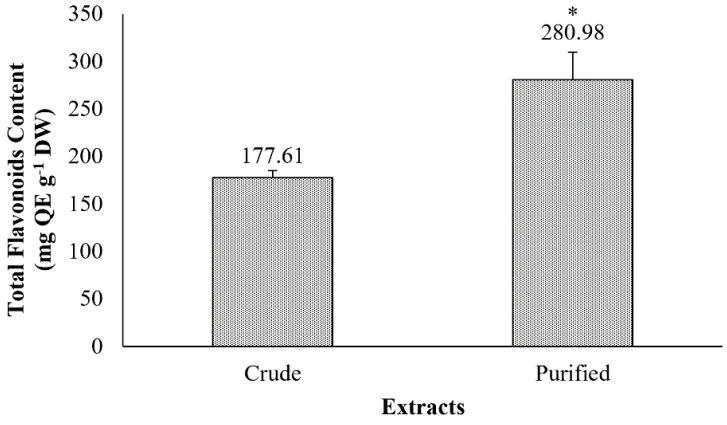


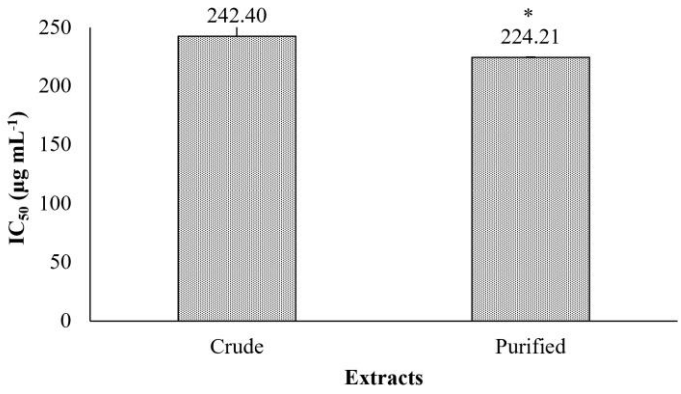
Figure 3. The total flavonoid content of crude and purified extracts of yellow velvetleaf (*Limnocharis flava*). Data was shown as mean ± SD (*n*=3). Significant difference at *p<0.05 vs crude extract.



3.3. Antioxidant Activity

The antioxidant activity of crude and purified extracts is shown in Figure 4. The antioxidant activity of purified extract significantly (p<0.05) increased when compared to crude extract (IC_{50} 224.21 µg/mL and 242.40 µg/mL, respectively).

Figure 4. The antioxidant activities of crude and purified extracts of yellow velvetleaf (*Limnocharis flava*). Data was shown as mean ± SD (n=3). Significant difference at *p<0.05 vs crude extract.



4. DISCUSSION and CONCLUSION

In this study, we successfully extracted polyphenol compounds from yellow velvetleaf (*Limnocharis flava*). The crude extract of polyphenol was purified using the solid-phase extraction method. The purified extract yield was reduced after the purification process (Figure 1). A previous study also reported that the crude extracts of *Quercus crassifolia* (Valencia-Avilés *et al*., 2018) and *Anacardium occidentale* (Nugroho *et al*., 2013) have a high yield when compared to purified extracts. This condition indicates that some unwanted compounds were successfully removed by the purification process. A solid-phase extraction method was used for the purification process due to its simplicity, rapidity, and economics. The purification process uses *n*-hexane solvent to remove nonpolar compounds such as lipoidal materials from the crude extract, whereas a low concentration of sulfuric acid was used to eliminate the non-phenolic and other organic compounds (Dai & Mumper, 2010; Sudirman *et al*., 2022).

The total polyphenol and flavonoid contents of the purified extract significantly increased compared to the crude extract after the purification process (Figure 2 and Figure 3). A previous study also reported that the total polyphenol content from water lettuce (*Pistia stratiotes*) leaf extract increased from 29.03 mg GAE/g dry sample to 65.63 mg GAE/g dry sample after the purification process. Whereas, the total flavonoid contents also increased after the purification process (crude extract, 27.58 mg QE/g dry sample; purified extract, 88.02 mg QE/g dry sample) (Sudirman *et al*., 2022). This condition indicated that the purified extract contained a concentration of polyphenols and flavonoids. The undesirable compounds were successfully removed from crude extract during the purification to obtain purified extract, such as lipoidal substances, non-phenolic compounds, and other organic compounds (Pérez-Magariño *et al*., 2008; Sudirman *et al*., 2022). A solid-phase extraction (SPE) purification method was used due to its being economic, simple, and rapid. The low concentrate of sulfuric acid was used to remove non-polyphenol polar compounds, such as organic acids and sugar as well as *n*-hexane was applied for the removal of non-polar from the crude extract (Pérez-Magariño *et al*., 2008).

The purification process successfully increases the antioxidant activity of the purified extract (Figure 4). A previous study also reported that the antioxidant activity of distiller grains increased after the purification process (Wang *et al*., 2019). There was high antioxidant activity in purified extract due to this extract also containing high-levels of polyphenol and flavonoid compounds. These compounds were categorized as secondary metabolites that are widely found

in plants and recognized as natural antioxidants (Dini & Grumetto, 2022). A previous study also reported that the high polyphenol content in the extract also shows high antioxidant activity (Yi & Wetzstein, 2011). Additionally, flavonoids (a group of polyphenols) are also reported as strong antioxidative agents (Panche *et al.*, 2016). The polyphenol compounds act as antioxidants by donating the hydrogen (H) atom to the free radicals (Matyas *et al*., 2019). Also, these compounds can reduce the oxidation reaction by transferring electrons (Lee *et al*., 2015).

Overall, in this present study, the polyphenol was successfully extracted from yellow velvetleaf (Limnocharis flava). The purification process increases the total polyphenol and flavonoid contents of the yellow velvetleaf extract. The antioxidant activity of this plant extract also increases after the purification process. Therefore, this extract potentially uses an alternative antioxidant agent and can be used as a food supplement ingredient.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Sabri Sudirman: Supervision and writing, editing and final approval. **Mey Arianti:** Methodology and formal analysis. **Gama Dian Nugroho:** Supervision and validation. **Sherly Ridhowati:** Formal analysis, and writing - original draft. **Puspa Ayu Pitayati**: Formal analysis and writing - original draft. **Miftahul Janna**: Methodology and formal analysis.

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