ANTIOXIDANT ACTIVITY OF TANNIN-RICH EXTRACT FROM WATER LETTUCE (Pistia stratiotes) LEAF

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ANTIOXIDANT ACTIVITY OF TANNIN-RICH EXTRACT FROM WATER LETTUCE (*Pistia stratiotes*) LEAF

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Abstract: Oxidative stress is a condition in which the free-radical substance is higher than the potential antioxidant in the human body. Therefore, exogenous antioxidants are needed to resolve this problem. Water lettuce (*Pistia stratiotes*) is an aquatic floating plant that contains several bioactive compounds, including tannins and exhibits antioxidant activity. However, since organic solvent extraction results in a crude polyphenol extract, purification is needed to remove the undesirable compounds. This study was aimed at determining the total tannin content and antioxidant activity of water lettuce leaf extract before and after the purification process. The purification was done using a solid-phase extraction method, the total tannin contents were analysed using Folin-Ciocalteu's method, and the antioxidant activity was analysed using 2,2-diphenyl-1-picrylhydrazyl. The results showed that the purification process successfully removed undesirable compounds and increased the total tannin content of the extract from 8.71 ± 1.50 mg TAE/g of the dry sample to 13.84 ± 0.28 mg TAE/g of the dry sample. The antioxidant activities also increased after the purification from IC_{50} 135.14 ± 22.44 µg/mL to IC_{50} 78.45 ± 5.42 µg/mL. According to these results, the tannin from water lettuce extract can be used as a source of natural antioxidants and formulated as an ingredient in food supplements.

Keywords: Antioxidant, *Pistia stratiotes*, polyphenols, purification, tannins.

Introduction

Oxidative stress is a condition in which the free radical substance is higher than the potential antioxidant in the human body (Vona *et al*., 2021). Free radicals are normally produced in the body and are usually destroyed by the body's natural antioxidant system. However, this substance can increase more than antioxidants due to external factors such as radiation, smoking, alcohol intake, industrial residue, and antibiotic residue. Free radicals can attack and damage some important molecules such as lipids and DNA, as well as cause some diseases (Rad *et al*., 2020). Therefore, exogenous antioxidants are needed to resolve this problem. Generally, this antioxidant contains some bioactive compounds such as polyphenols, flavonoids, and tannins (Spiegel *et al*., 2023). These compounds can be extracted from some plants, including water lettuce (*Pistia stratiotes*) (Herpandi *et al*., 2021; Sudirman *et al*., 2022a).

Water lettuce (*P. stratiotes*) is an aquatic floating plant widely found in tropical areas, including Indonesia. A previous study reported that this plant extract contains several bioactive compounds such as phenolics, tannins, and flavonoids (Sudirman *et al*., 2017; Sudirman *et al*., 2022a). Tannins are polyphenol compounds widely found in terrestrial and aquatic plants (Tong *et al*., 2022). Polyphenols are categorised as secondary metabolites and exhibit excellent antioxidant activity (Das *et al*., 2020). Generally, tannins can be extracted from plants using organic solvents such as ethanol, acetone, and methanol solutions (Sirisangsawang & Phetyim, 2023). Since the extraction using organic solvent resulted in a crude polyphenol extract; therefore, purification was needed to remove the undesirable compounds such as lipids, sugars, and organic acids (Zeka *et al*., 2017).

A solid-phase extraction (SPE) method is widely used for the purification process due to its rapidity, simplicity, and economy (Dai & Mumper, 2010). Previous studies reported that purification increases the total polyphenol content of *Inga edulis* (Souza *et al.*, 2007) and the flavonoid compound (luteolin) also increases after the purification process (Zhao *et al.*, 2022). Additionally, purified polyphenol compounds exhibit stronger antioxidant activity than crude extract (Wang *et al.*, 2019). The purification process also increases the inhibition of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase and the pancreatic lipase activity of polyphenol extract from water lettuce (Sudirman *et al.*, 2022b; Sudirman *et al.*, 2023). According to these conditions, we hypothesised that purification would also increase tannin content and antioxidant activity of the ethanol extract of water lettuce leaf. Therefore, this study aims to determine the total tannin content and antioxidant activity of water lettuce leaf extract before and after the purification process.

Materials and Methods

Extraction and Purification

The young and mature leaves of water lettuce (*Pistia stratiotes*) were collected from Sukaraja Village, South Sumatra, Indonesia. The plants were transported to the laboratory to be cleaned and undesirable substances removed. They were cut into small pieces and dried in the oven at 45ºC for 24 hours. After drying, the sample was ground into powder and kept at a cold temperature for further use. The tannin extraction was done by maceration method and 70% ethanol was used as a solvent according to the previous methods (Markom *et al.*, 2007; Sirisangsawang & Phetyim, 2023). Briefly, 20 g of dried sample was extracted using 200 mL of 70% ethanol in an Erlenmeyer flask. The extraction was performed at room temperature for three hours and stirred with a magnetic stirrer (IKA C-MAG HS 7) at 100 rpm. After the extraction, the mixture was filtered through Whatman No. 4 filter paper to separate the filtrate and residue. The filtrate was transferred to a new collection tube, whereas the residue was extracted using a fresh solvent in the same condition as the first extraction and was performed five times in total. After the extraction, the filtrate was mixed in new tubes and the solvent was evaporated using a rotary vacuum evaporator at 40ºC. The extraction was fully dried using a freeze dryer (Biobase BK-FD10S, Shandong, China) to obtain powder (crude extract). The yield of extraction was calculated according to this formula:

$$\text{Yield (\%)} = \frac{\text{Extract weight (g)}}{\text{Dried sample (g)}} \text{ x } 100\% \qquad (1)$$

The purification process used the solid-phase extraction (SPE) method using HyperSep Retain PEP Cartridges (Part. No. 60107-212, Thermofisher Scientific) according to the previous method (Sudirman *et al.*, 2022b). Briefly, 2 mL of distilled water (dH_2O) and then 2 mL of absolute methanol were used to wash the cartridge in the preconditioning step. After preconditioning the cartridge, 2 mL of the crude extract (1 mg/10 mL) was pipetted into the cartridge and was eluded using 2 mL of *n*-hexane and 2 mL of a low concentration of sulfuric acid (H_2SO_4 1 N), respectively. The absolute methanol was then used to wash the cartridge to collect the purified extract, which was dried by a freeze dryer to obtain extract powder (purified extract).

Tannin Content Analysis

The total tannin content was analysed by Folin-Ciocalteu's method according to the previous methods (Vijaytha *et al.*, 2020; Rajkumar *et al.*, 2022). Briefly, 0.1 mL of sample solution (1 mg/mL) was pipetted into the reaction tube. The tube was added with 7.5 mL of distilled water (dH_2O), 0.5 mL of Folin-Ciocalteu's reagent, and 1 mL of 35% $NaCO_3$, the volume was made up to 10 mL by dH_2O. The mixture was allowed to react at room temperature for 30 minutes. After the reaction time, the absorbance

was measured immediately by using a UV-Vis spectrophotometer (Genesys 150 Thermo-Scientific) at 700 nm. Tannic acid was used as a standard for total tannin determination. Therefore, the total tannin was expressed as mg total tannin equivalent per g of dried weight of the sample (mg TAE/g DW).

Antioxidant Activity Assay

The antioxidant activity was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to the previous methods (Chew *et al*., 2011; Sudirman *et al*., 2021). Briefly, the extract was dissolved in ethanol to make a serial concentration (0-1,000 µg/mL). Then, 1 mL of sample solution at each concentration was mixed with 1 mL of 0.2 mM DPPH in a reaction tube. The mixture was then incubated at 37ºC for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The antioxidant activity was expressed as the ability of the sample to inhibit the DPPH radical according to this formula:

$$\text{Percentage (\%) of inhibition} = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \times 100\% \quad (2)$$

where:
Abs_{blank} = Absorbance at 517 nm without sample
Abs_{sample} = Absorbance at 517 nm with sample

Functional Groups Analysis by FT-IR

The functional groups of tannin-rich extract were detected using a Fourier-transform infrared (InfraRed Bruker Tensor 37) according to the previous reference (Bhateja *et al*., 2020). The FT-IR spectra of the extract were obtained by mixing the extract with potassium bromide, further, it was pressed into pellets.

Data Analysis

All the data were presented as mean ± standard deviation (SD). The data were analysed 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).

Results and Discussion

Yield of Extraction and Purification

The yield of extraction (crude extract) and purified extract of the water lettuce leaf is shown in Figure 1. The yield of the crude extract is about 14.46 ± 1.07%, whereas, after purification, the yield was significantly ($p < 0.05$) reduced to 5.91 ± 0.57%. A previous study reported that the

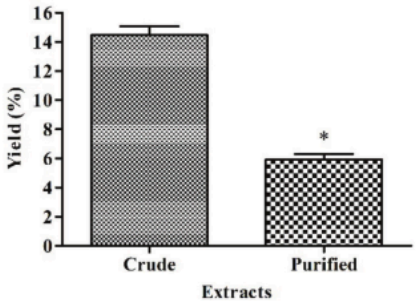

Figure 1: The yields of extraction (crude extract) and purified extract from water lettuce (*Pistia stratiotes*) leaf. The data are shown as the mean ± SD ($n = 3$). Statistical significance at $*p < 0.05$ versus crude extract

yield of crude extract was reduced from 1.57% to 0.81% after purification using the solid-phase extraction (SPE) method (Sudirman *et al*., 2023). Additionally, a previous study also reported that the purified extract of *Quercus crassifolia* was lower than the crude extract (Valencia-Avilés *et al*., 2018). This condition indicated that some compounds were successfully removed by the purification process, resulting in a lower yield of the purified extract when compared to the crude extract. During the purification process, *n*-hexane was used to remove lipoidal substances. In contrast, the low concentration of sulfuric acid was used to elude organic acids, sugar, and other non-polyphenol polar compounds (Pérez-Magariño *et al*., 2008). The

SPE method is widely used during polyphenol purification due to its rapidity, simplicity, and economy (Dai & Mumper, 2010).

Tannin Content

The total tannin contents of the crude and purified extracts are shown in Figure 2. The total tannin of the purified extract was significantly higher when compared to the crude extract, at about 13.84 ± 0.28 mg TAE/g of the dry sample and 8.71 ± 1.50 mg TAE/g of the dry sample, respectively. Tannins are a group of polyphenolic substances that are widely found in plants (Tong *et al*., 2022). A previous study reported that the total polyphenol content of *Inga edulis* increased from 496.5 mg GAE/g to 518.8 mg GAE/g (Souza *et al*., 2007). Also, the flavonoid compound (luteolin) increases after the purification process from 0.68 mg/g to 3.52 mg/g (Zhao *et al*., 2022). This condition indicated that some unwanted compounds such as sugar impurities were removed during the purification process. Similar to this present study, the purified extract contains a high concentration of tannin-polyphenol compounds due to some unwanted compounds being removed. *N*-hexane and a low concentration of sulfuric acid are used to remove undesirable compounds during SPE purification such as non-polar compounds (lipid) and non-polyphenol polar compounds (sugar and organic acids), respectively. A previous study also reported that purification using the SPE method successfully increased the total polyphenol and flavonoid contents of *P. stratiotes* ethanol extract (Sudirman *et al*., 2022b).

Antioxidant Activity

The antioxidant activity of crude and purified tannin from water lettuce leaf extract is shown in Figure 3. Both of the extracts exhibit antioxidant activity. However, the antioxidant activity of the purified extract was significantly ($p < 0.05$) higher than that of the crude extract, as indicated by the low half-maximum inhibition concentration (IC_{50}) of the purified extract. A low IC_{50} value indicated that the substances exhibit higher antioxidant activity and vice-versa (Goutzourelas *et al*., 2023). The antioxidant activity of the purified extract is about IC_{50} 78.45 ± 5.42 µg/mL and that of the crude extract is about IC_{50} 1[illegible]5.14 ± 22.44 µg/mL. In this study, we found that the purification process successfully increased the antioxidant activity of the tannin-polyphenol compound of water lettuce extract. Increased antioxidant activity in the purified extract is because this extract also contains high levels of tannin-polyphenol compounds when compared to crude extract. A previous study also reported that the purification process increased the antioxidant activity of distiller grains (Wang *et al*., 2019). The bioactivity or quality of the crude extract increased after purification (Barbosa-Pereira *et al*., 2013). This is because undesirable

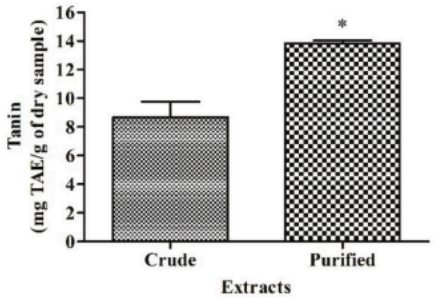

Figure 2: The tannin content of the crude and purified extracts from water lettuce (*Pistia stratiotes*) leaf. The data are shown as the mean ± SD ($n = 3$). Statistical significance at *$p < 0.05$ versus crude extract

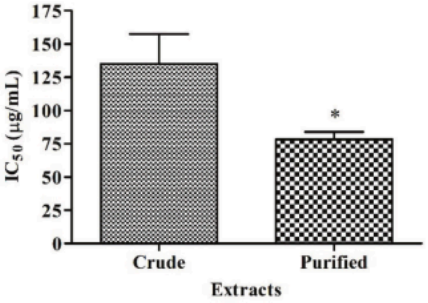

Figure 3: The antioxidant activities of the crude and purified tannin extract from water lettuce (*Pistia stratiotes*) leaf. The data are shown as the mean ± SD ($n = 3$). Statistical significance at *$p < 0.05$ versus crude extract

compounds have been removed, thereby improving the overall quality of the crude extract. The polyphenol compounds, including tannin, act as antioxidants by donating the hydrogen (H) atom to the free radicals (Matyas *et al*., 2019) and reducing the oxidation reaction by transferring electrons (Lee *et al*., 2015).

Functional Groups of the Tannin

The functional groups of the tannin-rich extract are shown in Figure 4. In this present study, the stretching of a polymeric hydroxyl (O–H) group and H-bonded stretching were detected at 3,334.12 cm^{-1}. A previous study reported that phenolic compounds, including tannin are characterised by asymmetric and symmetric stretching of the polymeric hydroxyl group and H-bonded stretching, which is detected in the region of 3,400 to 3,200 cm^{-1} (Wongsa *et al*., 2022). Whereas, 1,618.33 cm^{-1} indicates the C-O stretching vibration absorption bands and also indicates the presence of a phenolic group in the extract (1,760-1,600 cm^{-1}) (Kannan *et al*., 2011).

Conclusions

Overall, the tannin compounds were successfully extracted from water lettuce (*P. stratiotes*) using an ethanol solution. The purification using the solid-phase extraction method increases the total tannin content of this extract and also increases its antioxidant activity. Therefore, tannin from water lettuce can be used as a source of natural antioxidants and formulated as a food supplement.

Acknowledgements

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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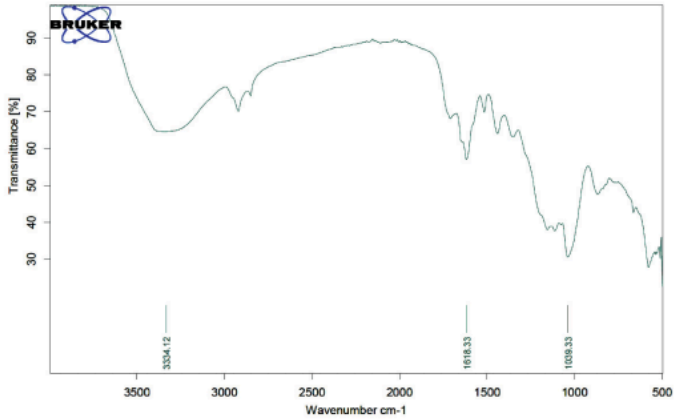

Figure 4: The FT-IR spectra of the purified tannin-rich extract from water lettuce (*Pistia stratiotes*) extract

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