

Antioxidant activity of the fractions from water lettuce (*Pistia stratiotes*) extract

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Abstract

Free radicals including reactive oxygen species are continuously increasing in the human body. This condition causes the unbalance between free radicals and antioxidants in the human body. An antioxidant is a compound with the ability to reduce the harmfulness of the free radical. This study aimed to determine the antioxidant activity of fractions and analyzed the functional groups of water lettuce (*Pistia stratiotes*) methanol extract. The separation process was performed by using thin-layer chromatography (TLC) and column chromatography. The separated fractions were measured for their antioxidant activity by using the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The functional groups of each fraction were determined by using Fourier-transform infrared (FT-IR) spectroscopy. The separation of water lettuce extract by using column chromatography produced seven fractions with different colors and confirmed by using TLC. The antioxidant activity showed the highest activity in the third fraction with a half-maximal inhibitory concentration (IC₅₀) value of 131.66 ppm. The fifth fraction with the IC₅₀ was about 184.62 ppm. Whereas, the first, second, fourth, sixth, and seventh fractions were relatively weak with the IC₅₀ more than 200 ppm. The FT-IR spectrum also showed that the intensity hydroxyl group in the third fraction higher than the seventh fraction.

1. Introduction

Reactive oxygen species (ROS) and free radicals, such as anion superoxide (O₂⁻), hydroxyl radical (•OH), and hydrogen peroxide (H₂O₂) are continuous increases in the human body (Phaniendra *et al.*, 2014). The high level of free radicals compared to antioxidants leading to oxidative stress conditions. This condition is involved in some chronic and low-inflammation diseases, such as insulin resistance in type-2 diabetes, rheumatoid arthritis, cardiovascular diseases, and aging disease (Khansari *et al.*, 2009). Therefore, the body needs exogenous antioxidants through functional food products, fruits, vegetables, and food supplements (Bouayed and Bohn, 2010).

An antioxidant is a compound with the ability to reduce the harmfulness of the free radicals (Lobo *et al.*, 2010). According to its source, the antioxidant can be divided into two groups include endogenous (primary) antioxidants, such as superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPx), whereas the second group is exogenous (secondary) antioxidants. This type of antioxidant can get from the

diet by eating antioxidant-rich foods or food supplements (Bouayed and Bohn, 2010). Water lettuce is one of the aquatic plants which possess antioxidant properties. This plant also contains some bioactive compounds, such as polyphenols, flavonoids, and saponin (Sudirman, Herpandi, Lestari *et al.*, 2017; Sudirman, Herpandi, Nopianti *et al.*, 2017). According to the previous study, water lettuce (*Pistia stratiotes*) methanol extract showed high antioxidant activity when compared to *n*-hexane and ethyl acetate extracts. This extract is composed of polyphenols and flavonoids (Sudirman, Herpandi, Lestari *et al.*, 2017). A previous study reported polyphenols reduced the harmfulness of the free radical by transferring the hydrogen (H) atom from their hydroxyl (OH) groups (Foti, 2007).

However, in the previous study, the authors used a crude extract of the water lettuce. Therefore, in the present study, we tried to separate the methanol crude extract by using thin-layer chromatography (TLC) and column chromatography. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014). A previous study reported that different

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fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti et al., 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao et al., 2019). According to these conditions, we hypothesized that the antioxidant activity of the water lettuce methanol extract will be increased after the separation process. Therefore, this study aimed to investigate the antioxidant activity of methanol fraction from water lettuce (*Pistia stratiotes*) after separation and confirm their hydroxyl group by Fourier-transform infrared.

2. Materials and methods

2.1 Water lettuce extraction

The fresh form of water lettuce (*Pistia stratiotes*) was collected at swampy waters in Palembang city, South Sumatera, Indonesia, and cleaned to remove unwanted materials. Then, it was reduced the size, dried, and extract by the following previous method (Sudirman, Herpandi, Nopianti et al., 2017). Briefly, 20 g of dried sample was extracted by using 200 mL of methanol (1:10, v/v) at room temperature for 24 hrs. The solution was filtrated by using a filter paper, solvent removed by using a rotary vacuum evaporator, and dried by using an oven dryer. The sample was kept for future analysis. The percent of extraction yield was calculated from the weight of dried extract divided by the weight of the dried sample and multiplied by 100%.

2.2 Separation process

The thin-layer chromatography (TLC) and column chromatography methods have been widely used to separate fractions from plant extracts. The silica TLC plate and silica gel were purchased from Merck KGaA (Darmstadt, Germany). The TLC method in this research followed the previous study (Kagan and Flythe, 2014). Briefly, the plant extract was dissolved in methanol to have a sample concentration. The TLC plate was cut in a piece (2×10 cm) and leaving a 1 cm border on the upper- and bottom sides of the plate. The sample was loaded by using a microliter syringe on the plate band and allow to dry. The plate with the sample was then developed in the cover chamber which containing mixed solvents or eluent (methanol-ethyl acetate-acetone 1:1:1, v/v/v, for this study). After the developing step, the plate was dried and observed bands under visible or UV light (254 nm and 365 nm) then marked bands with a pencil. The mixed solvent was used to further separation by using silica gel column chromatography. Whereas, the column chromatography was performed by the following previous method (Venkatesh et al., 2017). The mixed solvent was loaded into the packed silica column. The seven different separated fractions (confirmed by TLC)

were collected, dried, and kept for future analysis.

2.3. Antioxidant activity assay

The antioxidant activity assay was performed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described by the previous method (Molyneux, 2004). The DPPH powder was purchased from Merck KGaA (Darmstadt, Germany). Briefly, each of the dried fractions was dissolved in a methanol solvent to make a serial concentration (50 ppm, 100 ppm, 150 ppm, and 200 ppm). Whereas, Vitamin C (containing ascorbic acid) was used as a positive control. A hundred sixty (160) μ L of dried-fraction was added into a well containing 40 μ L of 0.76 mM of DPPH (10 mg DPPH in 10 mL of methanol). The solution was incubated at 37°C for 30 mins. after incubation time, the absorbance was immediately measured at 517 nm by using spectrophotometry. The percent of inhibition (%) was calculated from absorbance of blank minus absorbance of the sample divided by absorbance of blank and multiplied by 100%.

2.4. Functional group analysis

The functional group was analyzed by using a Fourier transform-infrared (FT-IR) spectroscopy according to the previous methods (Ouhaddouch et al., 2019). Briefly, the sample was placed into the infrared beam (sample holder), then measured in the spectral range between 500 cm^{-1} and 4000 cm^{-1} . Whereas, KBr-pressed disk was used in the FT-IR spectroscopy (Spectrum One Perkin Elmer, Massachusetts, USA).

3. Results and discussion

This study demonstrated the antioxidant activity of the fractions from the Water lettuce (*Pistia stratiotes*) methanol extract. The previous studies reported that the methanol extract is composed of polyphenols, such as flavonoids and tannin (Sudirman, Herpandi, Lestari et al., 2017; Sudirman, Herpandi, Nopianti et al., 2017). These studies also reported that methanol extract showed the highest antioxidant activity compared to *n*-hexane and ethyl acetate extracts. In addition, methanol extract also shows a high yield extract (Benhammou et al., 2009). Therefore, this present study only separated the methanol extract from water lettuce to some fractions. The yield of water lettuce methanol extract was about 16.16%. A previous study also reported that methanol is the best solvent for the extraction of leaves of *Acalypha wilkesiana* and *Atriplex halimus* were about 14.67% and 24.00%, respectively (Benhammou et al., 2009; Anokwuru et al., 2016). Methanol has been widely used for bioactive extraction from the plant. Methanol is also

more efficient in the extraction of polyphenols especially the low molecular weight (MW) of polyphenols (Do *et al.*, 2014).

The fractions from methanol extract were separated by using thin-layer chromatography (TLC) and column chromatography methods. Based on the TLC results, we found that the best-mixed solvent is composed of methanol, ethyl acetate, and acetone with the ratio of 1:1:1, as shown in Figure 1A. According to this result, this eluent was used for the mobile phase in column chromatography. After separation by column chromatography, the result showed that there are seven fractions of the methanol extracts as shown in Figure 1B. The TLC and column chromatography methods have been widely used to separate plant extract into several fractions (Kagan and Flythe, 2014).

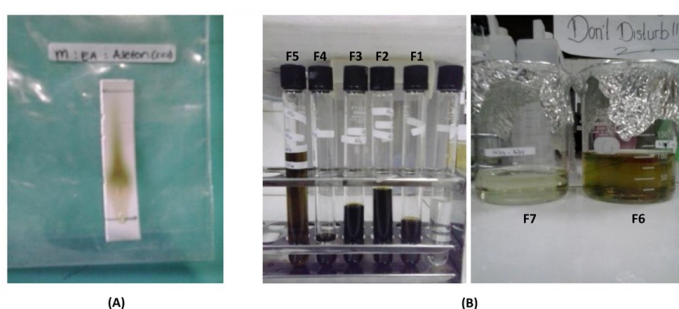


Figure 1. Methanol Extract Separation: (A) Thin-Layer Chromatography and (B) the Separated-fractions after Separation Process by using Column Chromatography. F1 – F7 are the sample fractions.

The antioxidant activity of the fraction was evaluated by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The DPPH method was widely used to measure

the antioxidant activity of the extract due to its speed, simplicity, and low cost (Alam *et al.*, 2013). As shown in Table 1, third (F3) and fifth (F5) fractions possessed highly antioxidant activities with the half-maximum inhibitory concentration (IC₅₀) was about 131.66 ppm and 184.62 ppm, respectively. Whereas, other fractions showed weak antioxidant activities with the IC₅₀ more than 200 ppm (Molyneux, 2004). A previous study reported that IC₅₀ of crude methanol extract of water lettuce was about 147.60 ppm (Sudirman, Herpandi, Lestari *et al.*, 2017). The different fractions in *Garcinia hombroniana* methanol extracts also show different antioxidant activities (Triadisti *et al.*, 2018). In addition, separated-fraction also enhanced the antioxidant activity of the extract (Zhao *et al.*, 2019).

According to the antioxidant activity assay, the highest activity (third, F3) fraction was continued for analysis of its functional group by using Fourier transform-infrared (FT-IR) spectroscopy then compared to the seventh fraction (F7) as a representative from the fraction of weak antioxidant activity as shown in Figure 2 and Table 2. A previous study reported that different bounds of the molecule also show different vibrational frequencies of FTIR spectroscopy, such as C–C, C=C, C–O, C=O, O–H, and N–H bonds (Altemimi *et al.*, 2017). The functional group of each wavenumber in Table 2 was evaluated according to the previous reference (Ouhaddouch *et al.*, 2019). Figure 2 showed that the O-H bond in the F3 fraction (3432.90 cm⁻¹) more than the F7 fraction (3366.06 cm⁻¹). The number of OH groups bound to the aromatic ring is positively correlative with

Table 1. The antioxidant activity of the fractions.

Sample	F1	F2	F3	F4	F5	F6	F7	VC
IC ₅₀ (ppm)	642.97	203.9	131.66	459.61	184.62	>2000	>2000	4.14

F: sample fraction, IC₅₀: Half-maximum inhibitory concentration, VC: vitamin C as a positive control.

Table 2. The functional groups of third fraction and seventh fraction.

Sample	Wavenumber (cm ⁻¹)	Wavenumber reference (cm ⁻¹)	Functional groups
Third fraction (F3)	3432.9	3200-3500	O-H
	2926.65	2800-3000	C-H
	2854.28	1560-1640	N-H
	1636.94	1400-1500	C-H
	1459.52	1020-1250	C-N, C-O, C-C
	1076.63		
Seventh fraction (F7)	3366.06	3200-3500	O-H
	2962.64		
	2935.11	2800-3000	C-H
	2875.55		
	1659.39	1560-1640	N-H
	1633.23		
	1466.62	1400-1500	C-H
1434.52			
1065.38	1020-1250	C-N, C-O, C-C	
1019.96			

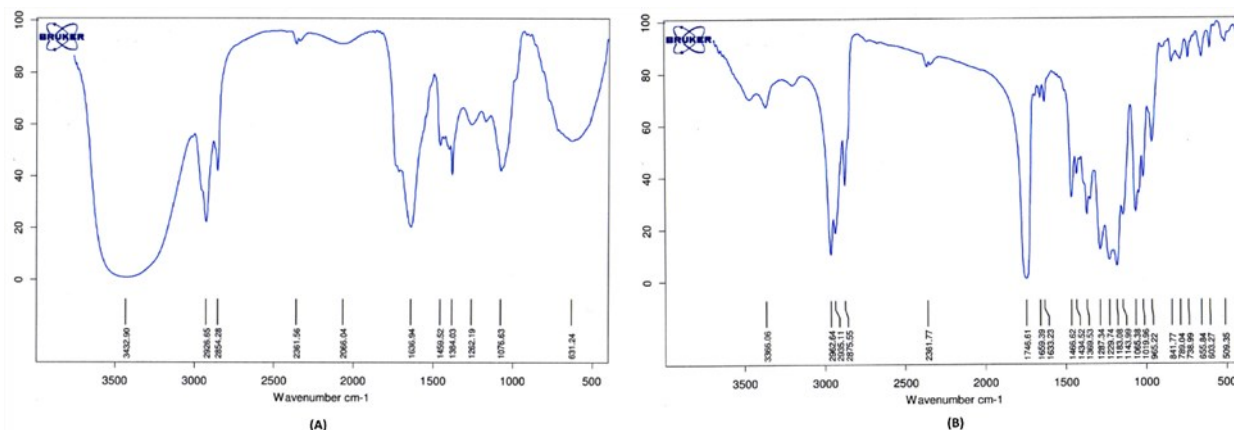


Figure 2. The Fourier Transform-infrared (FT-IR) Spectrums of the (A) Third Fraction, F3 and (B) Seventh Fraction, F7.

the antioxidant activity of polyphenols (Zielinska-Blizniewska *et al.*, 2019).

4. Conclusion

Overall, the bioactive compound from water lettuce (*Pistia stratiotes*) was successfully extracted by methanol solvent. The methanol extract showed seven fractions after separation by thin-layer chromatography and column chromatography. Whereas, the third fraction possessed high antioxidant activity. The Fourier transform-infrared confirmed that the hydroxyl group of the polyphenols play an important role in their antioxidant activity.

Conflict of interest

The authors declare no conflict of interest.

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