

Total polyphenol and flavonoid contents and antioxidant activities of water lettuce (*Pistia stratiotes*) leave extracts

^{1,*}Sudirman, S., ¹Herpandi, ¹Safitri, E., ²Apriani, E.F. and ³Taqwa F.H.

¹Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30862, Indonesia

²Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya 30862, Indonesia

³Department of Aquaculture, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30862, Indonesia

Article history:

Received: 3 July 2021

Received in revised form: 15 August 2021

Accepted: 29 November 2021

Available Online: 26 July 2022

Keywords:

Antioxidant,
Bioactive compounds,
Polyphenol,
Water lettuce

DOI:

[https://doi.org/10.26656/fr.2017.6\(4\).484](https://doi.org/10.26656/fr.2017.6(4).484)

Abstract

Free radicals such as reactive oxygen species and reactive nitrogen species are involved in the development of various chronic diseases. Under oxidative stress conditions, the human body needs more antioxidants that can be externally obtained from foods or supplements. This study aimed to investigate the total polyphenol content and flavonoid compounds and antioxidant activities of 70% ethanol and water extracts of water lettuce (*Pistia stratiotes*). The dried water lettuce was extracted by maceration. The yield of total polyphenol content of 70% ethanol extract (238.36±28.51 mg gallic acid equivalent (GAE)/g of dry sample) was higher than water extract (70.66±29.43 mg GEA/g of dry sample). The 70% ethanol extract (209.65±7.71 mg quercetin equivalent (QE)/g of dry sample) also possesses a higher level of flavonoid content compared to water extract (140.98±1.68 mg QE/g of dry sample). As analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods, 70% ethanol extract exhibited more powerful antioxidant activities compared to water extract. The half-maximum inhibitory concentration (IC₅₀) of 70% ethanol and water extracts were about 459.08±25.38 µg/mL and 1086.44±186.53 µg/mL, respectively and the ferric reduction power was about 118.43±2.17 mM Fe²⁺ equivalent/g of dry sample and 71.76±0.45 mM Fe²⁺ equivalent/g of dry sample, respectively. Based on these results, it is understood that 70% ethanol extract is the potential source of an antioxidant agent.

1. Introduction

Free radicals such as reactive oxygen species and reactive nitrogen species are involved in the development of various chronic diseases such as cancer, diabetes, ageing disease, cardiovascular, and inflammatory diseases (Lobo *et al.*, 2010). They are characterized by unpaired electrons in their outer layer and are highly reactive to other molecules such as lipids, proteins, and DNA. Then, this reaction cause damage to some tissues in the human body (Aruoma, 1998). Source of free radicals in the human body resulting from normal metabolic processes (endogenous sources) and exogenous sources such as exposure to cigarette smoking, air pollutants, X-rays, and industrial chemicals (Phaniendra *et al.*, 2014).

Under normal conditions, the human body has the ability to reduce the harmfulness of free radicals due to the presence of endogenous antioxidants such as superoxide dismutase, catalase, and glutathione

peroxidase (Pham-Huy *et al.*, 2008). However, if the exposure of free radicals is higher than antioxidant power or under oxidative stress conditions (Pizzino *et al.*, 2017), the human body needs more antioxidants that can externally be obtained from foods and supplements. The exogenous antioxidants can be obtained from natural products or plant extracts containing bioactive active compounds such as carotenoids (lutein and β-carotene), vitamins (ascorbic acid and γ-tocopherol), and phenolic compounds (flavonoids and phenolic acids) (Roehrs *et al.*, 2011; Da Costa *et al.*, 2012).

Water lettuce (*P. stratiotes*) is a macrophyte plant that lives on the surface of tropical freshwater. Water lettuce leaves and stems contain water (92.9%), carbohydrates (2.6%), protein (1.4%), fat (0.3%), crude fibre (0.9%), and minerals (1.9%), especially phosphorus and potassium (Tulika and Mala, 2015). Vitamins, stigmaterol, and palmitic acid are also found in the leaves of this plant (Khare, 2005; Liu *et al.*, 2008).

*Corresponding author.

Email: sabrisudirman@unsri.ac.id

Recently research on these plant extracts has shown the presence of bioactive compounds such as phenols and tannins that can be extracted by using n-hexane, ethyl acetate, and methanol (Tulika and Mala, 2015; Sudirman, Herpandi, Lestari *et al.*, 2017; Sudirman, Herpandi, Nopianti *et al.*, 2017). A recent study reported that methanol fractions of this plant extract exhibited great antioxidant activity (Herpandi *et al.*, 2021). However, safer and food-grade solvents or green solvents are recommended for the extraction process such as ethanol and water, especially for human applications such as food supplements (Chemat *et al.*, 2019). Additionally, different concentrations of ethanol/water have been used to extract polyphenol compounds (Sun *et al.*, 2015), whereas 70% (v/v) ethanol has widely been used for this extraction method and exhibits the highest polyphenol content and antioxidant activity (Hwang and Nhuan, 2014; Daud *et al.*, 2017; Oosthuizen *et al.*, 2018). Therefore, this study aimed to investigate the total polyphenol content (TPC) and total flavonoid content (TFC) and antioxidant activities of 70% ethanol and water extracts of water lettuce (*Pistia stratiotes*).

2. Materials and methods

2.1 Preparation and extraction process

The water lettuce (*P. stratiotes*) was harvested from Sukaraja Village, Ogan Ilir Regency, South Sumatra, Indonesia. The fresh plant was cleaned and kept leaves for future experiments. The extraction process was conducted according to a previously reported method (Chew *et al.*, 2011; Sudirman, Herpandi, Nopianti *et al.*, 2017). Briefly, the fresh leaves were dried in an oven at 45°C for 16 hrs. After the drying process, the samples were ground into powder form. A 10 g sample was mixed either with 200 mL (1:20, w/v) of 70% ethanol (70EtOH) or distilled water (dH₂O) for the maceration process with stirring (120 rpm) at room temperature for 3 hrs. After the maceration process, the liquid phase (filtrate) was separated from residue by filtering and using filter paper (Whatman no. 42). The filtrate was kept in a collection bottle. Whereas, the residue was taken and repeated in the extraction process under the same condition as the first extraction process by adding fresh solvent. A total of five extractions were performed. The filtrates were mixed and evaporated by a rotary vacuum evaporator at 50°C and resulting in concentrated ethanol and water extracts. The concentrated extract was collected into new collection tubes and dried by using a freeze dryer (Biobase BK-FD10S, Shandong, China) to obtain final 70EtOH and dH₂O extracts. The percentage

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

of extraction yield (%) was calculated according to the following equation:

2.2 Total polyphenol content and flavonoids analysis

TPC and TFC were measured according to previous methods (Chandra *et al.*, 2014). TPC was analyzed by using Folin-Ciocalteu's phenol reagent. Briefly, 0.2 mL of extract (10 mg/mL) were mixed with the Folin-Ciocalteu's reagent (1:1, v/v). After 5 mins, 1 mL of saturated sodium carbonate (8% w/v in water) was added, followed by the addition of distilled water up to 3 mL in total. The mixture was kept in the dark at room temperature for 30 mins. After the reaction time, the mixture was centrifugated at 3,000 rpm for 30 mins and the supernatant was taken and then measured at 750 nm with a spectrophotometer. The TPC was expressed as mg gallic acid equivalent (GAE) per g of dry sample.

Whereas, the TFC was determined by the aluminium chloride colourimetric method. Briefly, the quercetin standard stock solution (5 mg/mL in methanol) was diluted to make serial concentrations. Then, 0.6 mL of standard or extract (10 mg/mL) solutions were mixed with 0.6 mL 2% aluminum chloride. The mixture was kept to react at room temperature for 60 mins. After the reaction time, the absorbance was measured at 420 nm. The TFC was expressed as mg quercetin equivalent (QE) per g of dry sample.

2.3 Antioxidant activities assay

The antioxidant activity of the extracts was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. DPPH assay was performed according to the previously described method (Chew *et al.*, 2011). Briefly, the extracts were dissolved in dH₂O to make a serial concentration (0 – 500 µg/mL). 1 mL of extract solutions were mixed with 1 mL of 0.2 mM DPPH. The mixture was incubated at 37°C for 30 mins. After incubation time, the absorbance was measured at 517

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

nm. Ascorbic acid was used as a positive control. The percentage of inhibition was calculated according to the following equation:

Whereas A_{control} is the absorbance of negative control and A_{sample} is the absorbance of sample.

The FRAP method was performed according to the previously described method (Halvorsen *et al.*, 2002). Briefly, 0.1 mL of extract solution (20 mg/mL) was reacted with 3 mL of FRAP reagent (2.5 mL buffer acetate, pH 3.6; 2.6 mL of 10 mmol/L 2,4,6-tripyridyl-

striazine [TPTZ]; 2.5 mL of 20 mmol/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were mixed before used). The mixture solution was incubated at room temperature for 30 min. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ containing Fe^{2+} was used as a calibration standard. After incubation time, the absorbance was measured at 596 nm. The FRAP value was expressed as mM Fe^{2+} equivalent/g of dry sample.

3. Results and discussion

3.1 Yield of extraction

In this present study, we successfully extract the bioactive compounds from water lettuce (*P. stratiotes*) by maceration with stirring either with 70% ethanol (70EtOH) or distilled water (dH_2O) as extraction solvents. The yields of 70EtOH and dH_2O extracts have no significant difference ($p > 0.05$) as shown in Figure 1. The yield of 70EtOH and dH_2O extracts were about $16.80 \pm 1.01\%$ and $16.45 \pm 1.06\%$, respectively. The maceration method has widely been used to extract some bioactive compounds including polyphenols. Maceration is a traditional method and simple procedure for polyphenol extraction from natural products and is suitable for thermolabile compounds (Jovanović et al., 2017; Lezoul et al., 2020). Polyphenols are thermolabile (Maghsoudlou et al., 2019; Roselló-Soto et al., 2019). Therefore, maceration is a suitable method to extract polyphenol compounds from plant materials (Sharma et

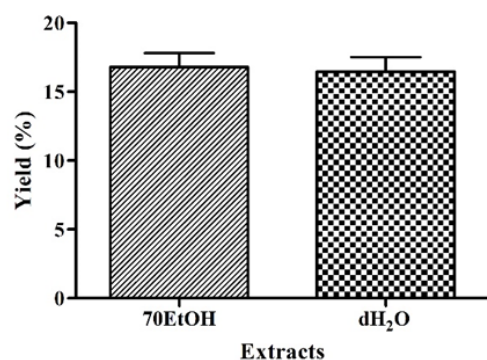


Figure 1. Extraction yield of water lettuce (*Pistia stratiotes*) extracts. 70EtOH: 70% ethanol, dH_2O : distilled water.

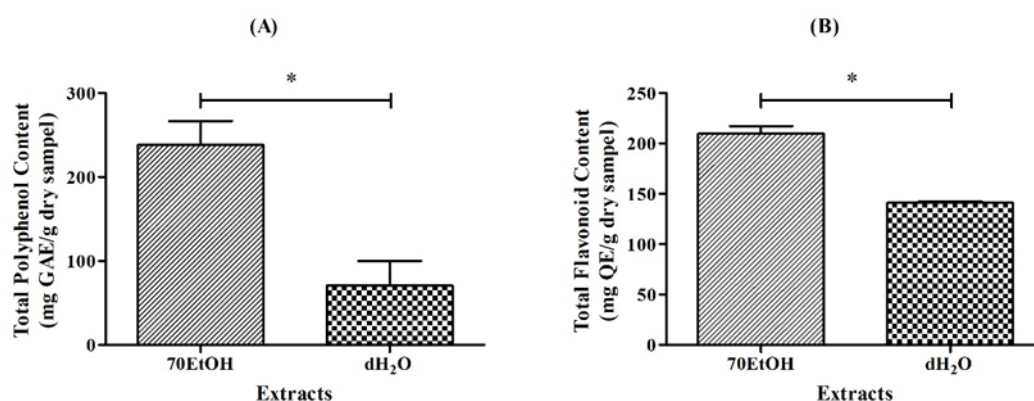


Figure 2. Total polyphenol (A) and flavonoid (B) contents of water lettuce (*Pistia stratiotes*) extracts. Statistically significance at $*p < 0.05$ versus 70% ethanol extract. 70EtOH: 70% ethanol, dH_2O : distilled water.

al., 2015; Jovanović et al., 2017). A previous study reported that the extraction yield of some leaves of the Pepino (*Solanum muricatum*) plant by using a maceration method with 70% ethanol and water as solvents were about 17.09% and 18.63%, respectively (Lezoul et al., 2020). Additionally, Sun et al. (2015) also reported that the mixture of ethanol-water solvent shows a high extraction yield from *Apis mellifera* compared to water solvent.

3.2 Total phenolic and flavonoids contents

The TPC of 70EtOH extract was significantly ($p < 0.05$) higher than dH_2O extract as shown in Figure 2A. The TPC 70EtOH extract was about 238.36 ± 28.51 mg GAE/g of dry sample. Similar to the TPC, the 70EtOH extract also contains significantly higher flavonoid content ($p < 0.05$, 209.65 ± 7.71 mg QE/g of dry sample) when compared to dH_2O extract as shown in Figure 2B. A previous study also reported that 70% ethanol is the best solvent for polyphenol extraction as indicated by the high yield in the extract (Hwang and Nhuan, 2014; Daud et al., 2017; Oosthuizen et al., 2018). The mixture of ethanol-water is suited to penetrate plant matrix including hydrophobic areas (Vongsak et al., 2013). Additionally, 70% ethanol is a polar solvent although slightly less polar than water (Kim and Lee, 2003). Previous studies reported that 70% ethanol extracts also showed a high level of total polyphenolic and flavonoid contents compared to water extract in some medical plants (Haq et al., 2019; Lezoul et al., 2020).

3.3 Antioxidant activities of water lettuce extracts

The antioxidant activity of the extracts was determined by DPPH and FRAP methods as shown in Table 1. The 70EtOH extract possessed more effective antioxidant activity as indicated by low half-maximal inhibitory concentration (IC_{50}) and this value was significantly different ($p < 0.05$) when compared to dH_2O extract. As a positive control, ascorbic acid showed

Table 1. The antioxidant activity by DPPH assay of 70% ethanol and distilled water extracts of water lettuce (*Pistia stratiotes*)

Antioxidant assay	70EtOH extract (µg/mL)	dH ₂ O extract (µg/mL)	Ascorbic acid (µg/mL)
DPPH (IC ₅₀)	459.08±25.38	1086.44±186.53*	10.85±0.52*

Statistically significance at * $p < 0.05$ versus ethanol extract. DPPH: 2,2-diphenyl-1-picrylhydrazyl, 70EtOH: 70% ethanol, dH₂O: distilled water.

lower IC₅₀ when compared to these extracts. The antioxidant activity of 70EtOH extract was also significantly ($p < 0.05$) higher than the antioxidant potential of dH₂O extract according to the FRAP assay (Table 2). A previous study also reported that 70% ethanol exhibited high antioxidant activity in the DPPH method compared to water extract (Hwang and Nhuan, 2014; Haq et al., 2019). Sun et al. (2015) also reported that ethanol extracts show strong antioxidant activity compared to water extracts.

Table 2. The antioxidant activity by FRAP assay of 70% ethanol and distilled water extracts of water lettuce (*Pistia stratiotes*)

Antioxidant assay	70EtOH extract (mM Fe ²⁺ eq/g of dry sample)	dH ₂ O extract (mM Fe ²⁺ eq/g of dry sample)
FRAP	118.43±2.17	71.76±0.45*

Statistically significance at * $p < 0.05$ versus ethanol extract. FRAP: Ferric reducing antioxidant power, 70EtOH: 70% ethanol, dH₂O: distilled water.

The majority of plant compounds such as polyphenol compounds act as antioxidants by hydrogen atom transfer (HAT) or single electron transfer (SET) mechanism to neutralize the free radicals and resulted in the reduction of the harmfulness of free radicals (Lee et al., 2015). The hydrogen donating mechanism of these compounds is involved in the prevention of free radical production (Ajila et al., 2007). Polyphenol antioxidants can provide a hydrogen atom to free radical substrate and generate a non-radical substrate (Zeb, 2020). Flavonoids can be acts as chelating metal ions and prevent their participation in free radical production (Cano et al., 2008; Lee et al., 2015).

4. Conclusion

The bioactive compounds from water lettuce (*P. stratiotes*) have been successfully extracted by the maceration method at room temperature with 70% ethanol and distilled water solvents. The 70% ethanol extract possessed a higher yield of total phenolic and flavonoid compounds and showed great antioxidant activities. Therefore, 70% ethanol extract can be used as a source material for developing a food supplement, especially as an antioxidant agent.

Acknowledgments

This research was funded by DIPA of the Public Service Agency of Universitas Sriwijaya 2020, in accordance with the Rector's Decree Number: 0685/UN9/SK.BUK.KP/2020 (July 15th, 2020).

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