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INHIBITION OF ALPHA-GLUCOSIDASE BY POLYSACCHARIDES FROM WATER LETTUCE (*Pistia stratiotes*) LEAF EXTRACTS WITH DIFFERENT EXTRACTION METHODS

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder that has a significant impact on the quality of life and health. This condition is indicated by high blood glucose or hyperglycemia. Alpha-glucosidase is an important enzyme during carbohydrate digestion to obtain monosaccharides such as glucose. This study aimed to investigate the alpha-glucoside inhibitory activity of polysaccharides from water lettuce (*Pistia stratiotes*) leaf with different extraction methods. The leaf was extracted by hot water extraction (HWE) and ultrasound-assisted extraction (UAE). The total sugar, crude fiber, and alpha-glucosidase inhibitory activity were analysis by *in vitro*, then an independent-sample t-test was used to analyze the statistically different. The UAE method showed a high yield of crude extract than the HWE method. However, the total sugar of HWE is higher than UAE, whereas no different of crude fiber level. The extract from the HWE method more effectively inhibits the alpha-glucosidase activity when compared to UAE. Therefore, hot water extraction is the suitable method to extract polysaccharides from a water lettuce (*Pistia stratiotes*) leaf. This condition also indicated polysaccharides extract potential to develop as an antidiabetes agent.

Keywords: Alpha-glucosidase, extraction methods, inhibitory activity, *Pistia stratiotes*, polysaccharides

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that has a significant impact on the quality of life and health. This condition is indicated by high blood glucose or hyperglycemia that is caused either by insufficient insulin secretion (Type I DM), insulin resistance (Type II DM) or both (**ADA, 2009**). Some treatments that have been used for DM management include non-pharmacological such as physical activity or exercise (**Raveendran, 2018**) and pharmacological treatments such as consuming antidiabetic drugs (**Su *et al.*, 2015**). Acarbose is an alpha-glucoside inhibitor (AGI) that is widely used for DM treatment (**Gu *et al.*, 2015; Liu *et al.*, 2021**). Alpha-glucoside is an important enzyme during carbohydrate digestion that hydrolyzed polysaccharides, oligosaccharides, or disaccharides to monosaccharides such as glucose (**Gong *et al.*, 2020**). As an AGI, acarbose inhibits carbohydrate digestion, therefore it can delay glucose absorption in the small intestine thus having a blood-glucose-lowering effect (**van de Laar *et al.*, 2005**). However, despite the ability of antidiabetic drugs on the diabetes management, it has some adverse effects such as gastrointestinal diseases and cardiovascular risks (**He *et al.*, 2014; DiNicolantonio *et al.*, 2015**). Therefore, research to find alternative alpha-glucosidase inhibitors is an emerging field, such as using plant extract as either a food supplement or functional food.

Water lettuce (*Pistia stratiotes*) is a floating aquatic plant that is commonly found in Indonesia. A recent study reported that the leaf of this plant contains some bioactive compounds that extracted by aqueous ethanol solvent, such as polyphenols, tannins, and flavonoids (**Herpandi *et al.*, 2021**). Leaf is also containing other bioactive compounds including polysaccharides. As reported by the previous studies, polysaccharides were successfully extracted from the leaf of *Eucommia ulmoides* (**Xu *et al.*, 2018**), *Nelumbo nucifera* (**Song *et al.*, 2020**), *Talinum triangulare* (**Yeh *et al.*, 2021**). These polysaccharides showed some biological activities including antioxidant activity (**Xu *et al.*, 2018**), immunomodulatory effects (**Yeh *et al.*, 2021**), and alpha-glucoside inhibitor (**Zhang and Li, 2015**).

Various methods have been used for polysaccharides extraction such as hot-water extraction (**Zhao *et al.*, 2017**), ultrasound-assisted extraction (**Qu *et al.*, 2013**), microwave-assisted extraction (**Xu *et al.*, 2018**), and freeze-thaw methods (**Wang *et al.*, 2018**). Generally, hot water extraction is the most common method for extracting polysaccharides with the advantages of no special equipment required, low cost, simple operation, excellent quality of final product, and most suitable for preparation of acidic and neutral polysaccharides (**Dong *et al.*, 2016; Wang *et al.*, 2021**). However, all these methods show different effects on the yield, activity, and bioavailability of the polysaccharides. According to these conditions, we hypothesized that the different extraction methods also affect to polysaccharides of water lettuce. Therefore, this study aimed to investigate the effect of hot-water and ultrasound-assisted extraction on the yield, total sugar, and crude fiber contents as well as alpha-glucoside inhibitory activity of polysaccharides from water lettuce (*Pistia stratiotes*).

MATERIALS AND METHODS

Preparation and extraction

The water lettuce (*Pistia stratiotes*) was collected from Sukaraja Village, Indralaya, South Sumatra, Indonesia. The plant was transported to the Laboratory for preparation. Briefly, the leaf was cleaned with water to remove the unwanted substances then was cut to smaller pieces. After that, it was dried by oven-drying at 40ºC for 12 h. The sample then was grounded to obtain powder form and kept for extraction process.

The polysaccharides were extracted from the water lettuce leaf by hot water extraction (**Wang *et al.*, 2018**) and ultrasound-assisted extraction (**Liu *et al.*, 2014**). Briefly, 10 g of dry sample was extracted by 400 mL of distilled water (dH_2O) in an Erlenmeyer flask. The extraction with hot water was used hot plate magnetic stirrer at 90ºC for 8 h and stirring at 120 rpm. Whereas the ultrasound-assisted extraction was performed at 180 W, 40 kHz, and 50ºC for 3 h. After that, the mixture was centrifugated at 4,300 rpm for 20 min. The supernatant of each extraction method was collected into a new collection tube and then the polysaccharides precipitated by using a 95% ethanol for 24 h at freeze temperature. The polysaccharides were collected by centrifugation at 4,300 rpm for 20 min. The polysaccharides extract was dried by using a freezer dryer to obtain dried-polysaccharides extract in powder form. It was then kept at cold temperature (4ºC) for further analysis.

Total sugar, crude fiber, and FT-IR analysis

The total sugar was analyzed by the Phenol-Sulfuric acid methods (**Nielsen, 2017**). Briefly, 10 mL (5 mg/mL in 80% ethanol) of the sample was placed into water bath at 90ºC for 10 min. After that, it was cooled at room temperature then 1 mL of the sample solution was pipetted into new reaction tube and mixed with 0.5 mL of 5% phenol (phenol in water, v/v). The mixture was added by 2.5 mL of 36 N sulfuric acid then incubated at room temperature for 20 min. After incubation time, the absorbance was measured by a UV-Vis spectrophotometer at 490 nm. The glucose was used as a sugar standard then the total sugar content was expressed as mg glucose per g of dry sample. Whereas, the crude fiber was analyzed according to the AOAC Official Methods 978.10 (**AOAC, 2005**).

FT-IR analysis

The FT-IR analysis was used to detect the glycosidic linkage of polysaccharides. FT-IR spectra of water lettuce polysaccharide were obtained by mixing polysaccharide with potassium bromide, further it was pressed to form pellets. The analysis used a Fourier transform infrared (InfraRed Bruker Tensor 37) and performed according to previous methods (**Bhateja *et al.*, 2020**).

Alpha-glucoside inhibitory activity assay

The inhibitory of α-glucosidase activity was analyzed according to previous methods (**Sancheti *et al.*, 2009**). Briefly, the test sample was diluted at a serial concentration (0, 20, 40, 60, 80, and 100 ppm). Then, 50 µL of 0.1 M phosphate buffer and 25 µL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer) was added with 10 µL of the sample solution in a 96-wells microplate. The mixture was added with α-glucosidase solution (0.04 Units/mL, pH 7.0). The mixture was incubated at 37ºC for 30 min and then was terminated by the addition of 100 µL of 0.2 M Na_2CO_3 solution. After that, the absorbance was measured at 410 nm by using a microplate reader.

RESULTS AND DISCUSSION

Extraction yields

The ultrasound-assisted extraction (UAE) showed a highly significant (p<0.05) yield when compared to hot water extraction (Figure 1). The extraction yield of UAE is about 9.16±2.14% and hot-water extraction is about 4.41±0.36%. A previous study also reported that the UAE method (2.97%) showed high extraction yield when compared to hot-water extraction (1.58%) (**Chen *et al.*, 2019**). The hot-water extraction is commonly used for polysaccharides extraction due to being safe and simple, but takes a long time of extraction, whereas UAE is reducing the consumption of solvents and shorten the extraction time. The UAE method also can increase the yield of extraction than hot-water extraction (**Wang *et al.*, 2015**). This condition is due to ultrasound-assisted resulting disruption of the cell wall by generating cavitation through wave migration, whereas hot-water extraction exerted low damage on the cell wall (**Lu *et al.*, 2013**).

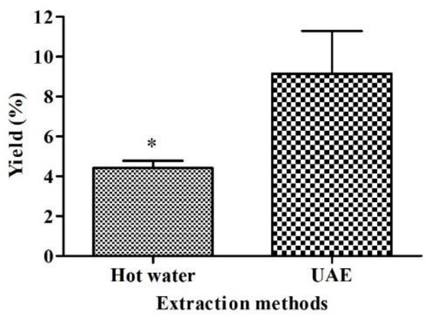

Figure 1 The extraction yield of polysaccharides extracts from water lettuce (*Pistia stratiotes*) with different extraction methods. Data shown as mean ± SD (n=3). Significantly difference at *p<0.05 vs UAE. UAE, ultrasound-assisted extraction.

Total sugar and crude fiber

Figure 2 showed that the hot water extraction (538.29±17.45 mg glucose equivalent/g of dry sample) method has a highly significant (p<0.05) of total sugar when compared to the UAE method (370.35±46.38 mg glucose equivalent/g of dry sample). However, in the crude fiber value, there is no significant difference between the hot water extraction (6.49±1.18%) and UAE (6.20±0.37%). A previous study reported that crude fiber from *Moringa oleifera* leaf was about 9.20% (**Asghar *et al.*, 2022**) and *Ficus carica* leaf from 6.53% (**Teruel-Andreu *et al.*, 2022**). The total sugar analyzes by the Phenol-Sulfuric acid method. This method is commonly used in the analysis of total sugar content during polysaccharides studies, whereas polysaccharides are composed of monosaccharides which linkage by glycosidic bonds (**Yue *et al.*, 2022**). Polysaccharides have been reported that it shows alpha-glucosidase inhibition activities (**Bisht *et al.*, 2013; Zhang and Li, 2015**). A previous study reported that total carbohydrate of hot-water extraction (73.31%) was highly when compared to ultrasound-assisted extraction (61.17%). This condition is due to some monosaccharides being easy to destroy, especially by acid, alkali, and ultrasound treatment. It also reported that the monosaccharides content also increased in hot-water extraction when compared to the UAE method (**Chen *et al.*, 2019**). Additionally, a previous study also reported that extraction methods not affected the monosaccharides composition, but affected its contents (**Wang *et al.*, 2010**). Additionally, hot water extraction is the most common method for extracting polysaccharides with the advantages of no special equipment required, low cost, simple operation, excellent quality of final product, and most suitable for preparation of acidic and neutral polysaccharides (**Dong *et al.*, 2016; Wang *et al.*, 2021**).

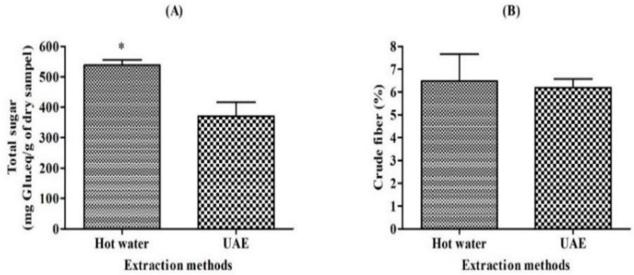

Figure 2 Total sugar content (A) and crude fiber (B) of polysaccharides extracts from water lettuce (*Pistia stratiotes*) with different extraction methods. Data shown as mean ± SD (n=3). Significantly difference at *p<0.05 vs UAE. UAE, ultrasound-assisted extraction.

FT-IR spectra of polysaccharides

Figure 3 showed that the spectra of polysaccharides of water lettuce. The spectra were obtained from 5000 cm^{-1} to 400 cm^{-1}. The O–H stretching vibration was detected at 3422.37 cm^{-1} whereas the C–H linkage was at 2918.98 cm^{-1}. The carbonyl (C=O) stretching was appeared at 1642.90 cm^{-1}, whereas the glycosidic linkage C–O–C at 1076.56 cm^{-1}. The functional group was analyzed according previous reference (**Coates, 2000**) as shown in Table 1. A previous study also detected the O–H stretching (3430.6 cm^{-1}), C–H linkage (2940.3 cm^{-1}), carbonyl stretching (1630.4 cm^{-1}), and glycosidic linkage (1030.5 cm^{-1}) from *Acacia tortilis* polysaccharides (**Bhateja *et al.*, 2020**).

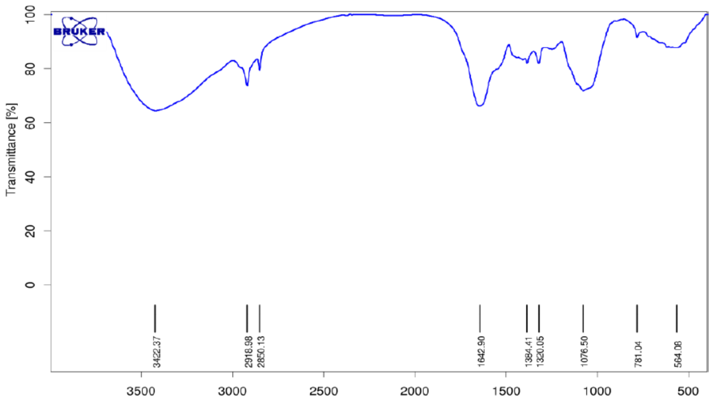

Figure 3 The FT-IR spectra of polysaccharides of water lettuce (*Pistia stratiotes*).

Table 1 The wave number of polysaccharides of water lettuce (*P. stratiotes*)

Functional groups	Wavenumber (cm^{-1})	
	P. stratiotes	Reference*
O−H	3422.37	3570-3200
C−H	2918.98	3095-2850
C=O	1642.90	1680-1620
C−O	1076.56	~1050

*According to previous method (**Coates, 2000**).

Alpha-glucosidase inhibitory activity

Figure 4 showed that hot water extraction more significantly (p<0.05) effectively inhibits the alpha-glucosidase when compared to UAE. The half-maximal inhibitory concentration (IC_{50}) of hot water extraction is about 394.84±10.92 mg/mL, whereas UAE is about 490.78±20.87 mg/mL. This condition is due to the hot-water extraction composed of high polysaccharides when compared to UAE. Therefore, its *alpha*-glucosidase inhibition activity is also higher than in UAE. A lower IC_{50} value indicated the potential inhibition activity is more effective and *vice versa* (**Matuszewska *et al.*, 2018**). However, this value lower that other plant extract, such as from polysaccharides from *Artocarpus heterophyllus* (IC_{50} 10.52 mg/mL) (**Zhang *et al.*, 2017**). The highest of alpha-glucosidase inhibition of hot-water extraction when compared to UAE was shown in polysaccharides from *Fructus mori* (**Chen *et al.*, 2019**). Previous studies reported that in-vitro analysis of polysaccharides from *Camellia oleifera* fruit (**Zhang and Li, 2015**) and *Acacia tortilis* gum exudate (**Bisht *et al.*, 2013**) inhibit alpha-glucosidase activities. According to these conditions, bioactive polysaccharides can be used as novel alternative of anti-diabetic agent. A previous study reported that polysaccharides as alpha-glucoside inhibitor has ability to prolong and increase the glucagon-like peptide-1 (GLP-1) secretion in normal and type-2 diabetes patients (**Bhateja *et al.*, 2020**).

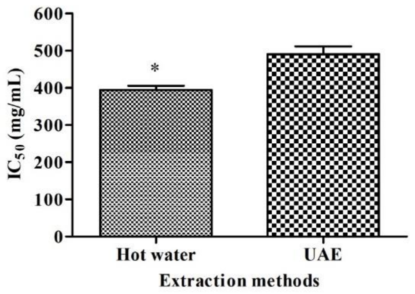

Figure 4 The alpha-glucosidase inhibitory activity of polysaccharides extracts from water lettuce (*Pistia stratiotes*) with different extraction methods. Data shown as mean ± SD (n=3). Significantly difference at *p<0.05 vs UAE. UAE, ultrasound-assisted extraction.

CONCLUSION

In this study, we successfully extracted polysaccharides from water lettuce (*Pistia stratiotes*). The hot-water extraction shows a high number of polysaccharides and inhibition of alpha-glucoside activity. Therefore, polysaccharides from water lettuce can be used as an alternative of novel antidiabetic agent.

Author's contribution: Ghaisani, Sirait, and Janna carried out the research, wrote and revised the article. Sudirman and Herpandi designed the research, supervised research progress; Sudirman and Widiastuti anchored the review, revisions and approved the article submission.

Conflict of Interest Statement: The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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