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Original Research Article

Antihyperlipidemic Activity of Purified Polyphenol Extracted from Water Lettuce (*Pistia stratiotes*) Leaf: An I*n Vitro* Analysis

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ABSTRACT

Obesity is a metabolic disorder disease and excessive fat accumulation. Synthetic antiobesity (antihyperlipidemic) drugs are considered to cause some side effects. Therefore, the investigation of novel antiobesity agents, with less adverse effects, is a major challenge in the future research, such as from plant extracts. Thus, this research aimed to investigate the antihyperlipidemic of purified polyphenol from water lettuce (*Pistia stratiotes*) extract. The research was conducted using crude and purified extracts of water lettuce. The purification process was performed by solid-phase extraction by using a HyperSep Retain PEP cartridge. The qualitative analysis of bioactive compounds (flavonoid, alkaloid, terpenoid, tannin) and quantitative analysis of lipase inhibition were conducted in the laboratory. The results showed that flavonoid, alkaloid, and tannin were increased after the purification process, whereas triterpenoid was reduced. The stretching of a polymeric hydroxyl group (O–H) and H-bonded stretching at 3382.36 cm^{-1} (crude extract) and 3399.25 cm^{-1} (purified extract) indicate present of phenol in the extracts. The inhibition of lipase enzyme also increased from 37.50% (crude extract) to 72.50% (purified extract). Therefore, purified polyphenol compound from water lettuce (*Pistia stratiotes*) extract is the potential agent as antihyperlipidemic agent.

Keywords: Antihyperlipidemic, *Pistia stratiotes*, polyphenol, purification

Introduction

Overweight or obesity is defined as a condition of abnormal or excessive fat storage in adipose tissue and can cause health problems in the human body.[1] Hyperlipidemia also which is a condition where the number of triglycerides continues to increase beyond normal limits in adipose tissue.[2, 3] Hyperlipidemia can be reduced by the use of pancreatic lipase enzyme inhibitors that play a role in triglyceride hydrolysis and digestion.[4] A commercial drug such as orlistat has been used for hyperlipidemia treatment. However, several clinical studies have found that taking this drug can make patients more susceptible to adverse health reactions and gastrointestinal diseases.[5, 6] Therefore, the investigation of novel antiobesity agents, with less adverse effects, is a major challenge in the future research, such as from natural resources including plant extracts.

Natural sources of lipase inhibitors generally contain bioactive compounds including polyphenols, flavonoids, alkaloids, and other active compounds.[7] Various studies reported that polyphenol compounds successfully inhibit pancreatic lipase.[8-10] Polyphenol compounds can be extracted from some aquatic and terrestrial plants.[11, 12]

Water lettuce (*Pistia stratiotes*, Family Araceae) is an aquatic plant that contains some bioactive compounds, such as polyphenols, flavonoids, and tannins.[13,14]

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A recent study reported that crude and purified polyphenol extracts from this plant show different effect on inhibition of HMG-CoA reductase activity.[15] According to these conditions, we hypothesize that crude and purified polyphenol compounds from water lettuce also show different effect on pancreatic lipase inhibition. However, there is yet no study reporting the effect of crude and purified polyphenol compounds from water lettuce on pancreatic lipase inhibition. Thus, this study aimed to investigate the activity of crude and purified polyphenol extracts from water lettuce (*Pistia stratiotes*) on pancreatic lipase inhibition.

Materials and Methods

Sample preparation and extraction

The fresh water lettuce (*Pistia stratiotes*) was collected was collected in September 2021 from Sukaraja Village, South Indralaya, South Sumatra, Indonesia (3.233844° S, 104.674735° E). The sample collected was authenticated at the Microbiology and Biotechnology Laboratory of Fisheries Product Technology, Universitas Sriwijaya (FPT0015092022). The sample was prepared according to the previous study.[15] Briefly, the leaf was rinsed with distilled water and oven-dried at 45ºC until constant weight is reached. After the drying process, the sample was milled to a size of 40 mesh using a grinding machine (Microphyte disintegrator B-One DM-120M) and kept for the extraction process.

The polyphenol compound was extracted by the following the previous studies.[14,15] Briefly, 20 mg of sample and 200 mL of 70% ethanol (Ethanol absolute, CAS No. 64-17-5, Merck) were mixed in the Erlenmeyer flask, then stirred using a magnetic stirrer. The extraction was performed at room temperature for 3 hours, then the filtrate and residue were separated with a Whatman filter paper (No. 42). The filtrate was kept in a new collection tube and the residue was extracted by a fresh solvent under the same condition as the first extraction, which was carried out five repetitions. The filtrate-mixed was evaporated using a vacuum rotary evaporator (Biobase RE-301) at 40ºC to obtain the concentrated extract. Half of the concentrated extract was dried

using a freeze dryer (Biobase BK-FD10S) to obtain polyphenol crude extract in powder form. Whereas, the purified extract was obtained by solid-phase extraction by using a HyperSep Retain PEP cartridge.

Purification process and quantitative phytochemical test

The purification of polyphenol compound was performed by solid-phase extraction (SPE) by using a HyperSep Retain PEP cartridge (Part. No. 60107-212, Thermofisher Scientific) as described by previous method.[15, 16] Briefly, 2 mL of dH_2O and then 2 mL of methanol were rinsed for cartridge preconditioned. Then, 2 mL of crude extract was loaded into the cartridge. The sample was eluted by using 2 mL of n-hexane and then 2 mL of 1 N H_2SO_4. The cartridge was washed with absolute methanol to obtain purified polyphenol extract in an aqueous form. Then, it was dried with a freeze dryer to obtain the powder form of purified extract. Whereas, the qualitative of phytochemical compounds (flavonoids, tannin, alkaloids, and terpenoids) were analyzed according to the previous methods.[17, 18]

FT-IR analysis of polyphenol compounds

The functional group of polyphenol compounds was detected by using n Fourier transform infrared (InfraRed Bruker Tensor 37) equipped with an infrared source, potassium bromide beam splitter according previous method.[19]

Lipase inhibition activity assay

The *in vitro* analysis of antihyperlipidemic was used a commercial lipase activity assay kit (MAK046-IKT, Merck) and the analysis procedure was according to manufacturer protocol and the previous method.[20] The extract was prepared according to Table 1.

After complete condition according to Table 1 at microtubes, the mixture was incubated at 37ºC for 1 h. The absorbance was measured at 570 nm by using a spectrophotometer (Genesys 150 ThermoScientific). The percentage of the inhibition was calculated according to the formula:

$$\text{Inhibition (\%)} = \frac{Abs.\ lipase - Abs.\ sample}{Abs.\ lipase} \times 100\%$$

Whereas: *Abs lipase*, Absorbance at 570 nm without treatment, *Abs sample*, absorbance at 570 nm with sample/treatment.

Statistical Analysis

The qualitative phytochemical was used descriptive analysis. The yield and lipase inhibition were analyzed by independent t-test ($p<0.05$) using SPSS (v.22.0; IBM Corp., Armonk, NY, USA). All graphics were produced using the GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA, USA).

Table 1: The lipase inhibition activity assay reaction

Sample	*Reagent* (µL)					Reaction conditions
	Lipase Assay Buffer	Peroxide Substrate	Enzyme Mix	Lipase Substrate	Lipase Positive Control	
Crude extract	465	10	10	15	50	Crude extract (50 µL)
Purified extract	465	10	10	15	50	Purified extract (50 µL)
Lipase activity	465	10	10	15	50	Lipase Assay Buffer (50 µL)
Lipase inhibition (Pravastatin)	465	10	10	15	50	Pravastatin (50 µL)
Blank	465	10	10	-	-	-

Results and Discussion

Extraction yield

The extraction yield of crude and purified extract was shown in Figure 1. Figure 1 showed that the extraction yield of the purified extract was significantly ($p<0.05$) reduced after purification process. This condition due to some unwanted components such as lipid and organic compounds have been removed by the purification process. The lipid was eluded by *n*-hexane, whereas organic compounds by low concentration of sulfuric acid [16]. A previously study also reported that the polyphenol crude extract of *Quercus crassifolia* has high yield when compared to purified extract.[21] Additionally, purified polyphenol extract of *Elaeagnus angustifolia* showed low yield extract when compared to crude extract.[22] Also, crude extract of *Anacardium occidentale* leaves showed higher extraction yield than purified extract.[23]

Qualitative phytochemical compounds

The qualitative phytochemical compounds before and after purification were shown in Table 2. A solid-phase extraction (SPE) purification method was used due to its simplicity, economic, and rapid.[24] Table 2 showed that the presence of polyphenol compounds, such as flavonoids and tannins were increased after purification process. Alkaloids also were increased, whereas terpenoids were reduced after the purification process. In the present study, the non-polyphenol compounds such lipid and polar compound non-polyphenol such as sugar and organic acids have been partially removed in the purification by *n*-hexane and low concentration of sulfuric acid, respectively.[16] A previous study reported that alkaloids are soluble in organic solvent such as ethanol.[25] Whereas terpenoids or terpenes can be removed from the substance by a non-polar organic solvent such as *n*-hexane.[26] A quantitative study from the previous study also reported that total polyphenol and flavonoids of water lettuce (*Pistia stratiotes*) leaf also were increased after the purification.[15]

FT-IR Spectra of polyphenol compounds

The absorption spectra of polyphenol compounds of crude and purified extracts were shown in Figure 2. According to Figure 2, stretching of a polymeric hydroxyl group (O–H) and H-bonded stretching at 3382.36 cm^{-1} (crude extract, intensity: 0.0291) and 3399.25 cm^{-1} (purified extract, intensity: 0.363). The region of 3400 to 3200 cm^{-1} indicates asymmetric and symmetric stretching of the polymeric hydroxyl (O–H) group, H-bonded stretching, which is characteristic of polyphenolic compounds.[27]

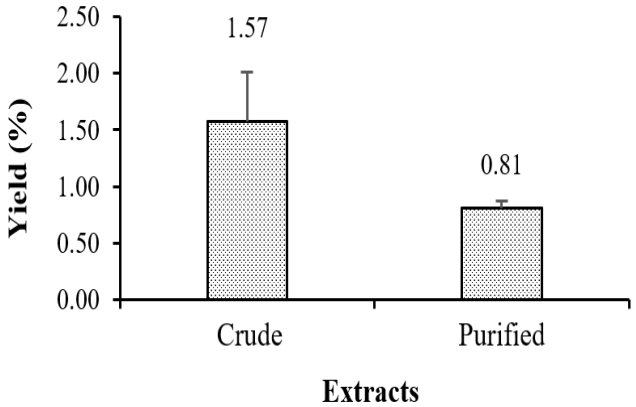

Figure 1: Yield of crude and purified extracts of water lettuce (*Pistia stratiotes*) leaf. Data represent the mean ± SD (n=3). Significantly difference at *$p<0.05$ vs crude extract.

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Table 2: The qualitative phytochemical compounds of leaf extract of water lettuce (*Pistia stratiotes*)

Phytochemical compounds	Extracts	
	Crude	Purified
Flavonoids	+	++
Tannins	+	++
Alkaloids	+	++
Terpenoids	++	+

Note: "+", indicated the presence of the metabolite; "++", indicated more presence than "+"

Figure 2 also showed C-O stretching vibration absorption bands at 1625.18 cm^{-1} (crude extract, intensity: 0.178) as well as 1719.55 cm^{-1}, 1624.41 cm^{-1} and 1173.66 cm^{-1} (purified extract, intensity: 1.017, 0.826, and 0.640, respectively). The C-O stretching vibration absorption bands at wavenumbers 1760 to 1600 cm^{-1} and 1230 to 1140 cm^{-1} also indicate the presence of phenol compounds.[28] Additionally, a previous study also detected C-O stretching at ~ 1200 cm^{-1}. This stretching is due to the C–O of pyran, typical of flavonoid C-rings.[27] In the present study, the OH stretching and C-O stretching were observed stronger at purified when compared to crude extract

Inhibition of lipase activities

The lipase inhibition activities of the crude and purified extracts were shown in Figure 3. According to Figure 3, the inhibition activity of the purified extract was significantly (p<0.05) increased when compared to the crude extract. This condition was caused by a high concentration of polyphenols compound of purified extract. A previous study reported that polyphenol compounds from several plants have been reported for their potential lipase-inhibitory activities.[8,29] A previous study also reported that the quality or bioactivity of crude extract was increased after purification.[30]

Conclusion

The purified extract of water lettuce showed high flavonoid and tannin contents when compared to the crude extract. Thus, the lipase inhibitory activity of the purified extract was also higher when compared to the crude extract. Therefore, the purified extract of polyphenol compounds from water lettuce (*Pistia stratiotes*) has the potential to be developed as an alternative anti-hyperlipidemia or antiobesity agent.

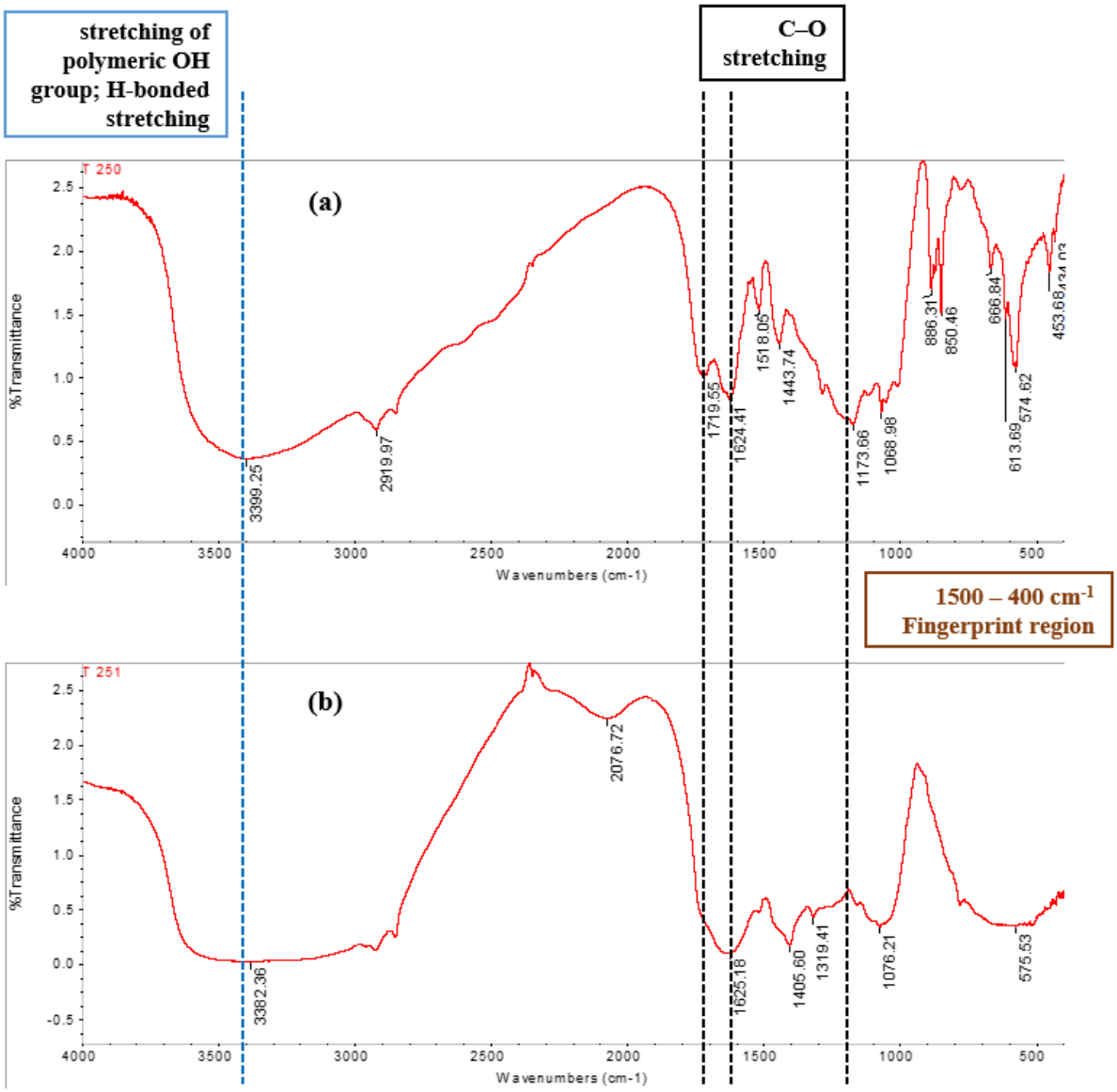

Figure 2: Infrared absorption spectra of (a) purified and (b) crude polyphenols of water lettuce (*Pistia stratiotes*).

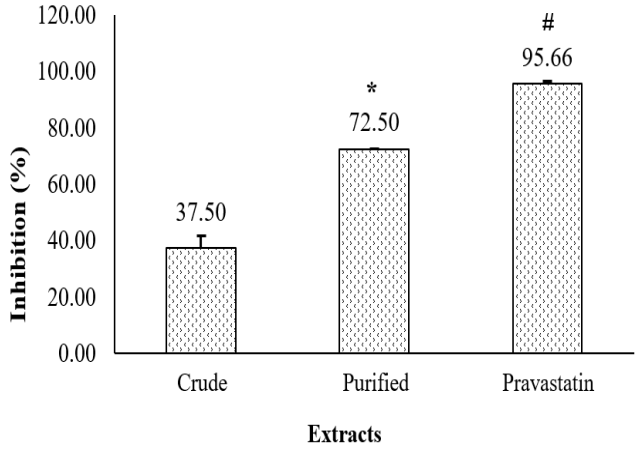

Figure 3: The inhibition of lipase activities by crude and purified extracts of water lettuce (*Pistia stratiotes*). Data are presented as mean ± SD (*n*=3). (*) and (#) indicate significant difference at $p<0.05$ vs crude extract.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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