Antioxidant Activity of Polysaccharides from Water Lettuce (Pistia stratiotes) Leaf Extract

by Herpandi Napis

Submission date: 07-May-2025 02:14PM (UTC+0700)
Submission ID: 2668890703
File name: of_Polysaccharides_from_Water_Lettuce_Pistia_stratiotes_Leaf.pdf (561.43K)
Word count: 3404
Character count: 18850

Trop J Nat Prod Res, November 2023; 7(11):5055-5058

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Short Communication

Antioxidant Activity of Polysaccharides from Water Lettuce (*Pistia stratiotes*) Leaf Extract

Sabri Sudirman[1]*, Yohana N. Sirait[1], Aatikah D. Ghaisani[1], Herpandi[1], Indah Widiastuti[1], Miftahul Janna[2]

[1]*Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya, Indralaya 30862, Ogan Ilir Regency, South Sumatra, Indonesia;*
[2]*Master Program in Agribusiness, Faculty of Agriculture, Universitas Sriwijaya, Palembang City 30139, South Sumatra, Indonesia*

ARTICLE INFO

Article history:
Received 21 July 2023
Revised 17 October 2023
Accepted 02 November 2023
Published online 01 December 2023

ABSTRACT

An increase in free radicals in the body causes oxidative stress. Therefore, our body needs exogenous antioxidants. This study aimed to determine the antioxidant activity of crude and defatted polysaccharide extracts from water lettuce (*Pistia stratiotes*) leaf. The polysaccharide was extracted by hot-water extraction and defatted with acetone. The total sugar content was determined by the Phenol-Sulfuric acid method, the antioxidant activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, and the functional group was analyzed by Fourier transform infrared (FT-IR) spectroscopy. The results showed that the total sugar of the crude polysaccharide (96.80±14.60 mg glucose eq./g dry sample) had a higher total sugar than the defatted polysaccharide (44.0±2.16 mg glucose eq./g dry sample). However, the antioxidant activity in defatted polysaccharides (IC_{50} 0.406±0.011 mg/mL) more effectively inhibits free radicals than crude extract (IC_{50} 0.484±0.028 mg/mL). The O-H, C-H, C=O, and C-O functional groups were identified in the polysaccharide extracts. The polysaccharides extract from water lettuce exhibits antioxidant activity. Therefore, the polysaccharide from water lettuce (*P. stratiotes*) leaf can be used as a source of antioxidant agents and as a food supplement.

Keywords: Antioxidant, defatted, extraction, glucose, polysaccharides. .

Introduction

A free radical is a substance without unpaired electrons in the outer orbital and can cause oxidative stress. Oxidative stress is a condition in which free radicals have higher antioxidant potential in the body.[1] Therefore, exogenous antioxidants are needed to resolve this condition through functional foods or food supplements. Generally, antioxidants contain several bioactive compounds, such as polyphenols, polysaccharides, oligosaccharides, vitamins, and minerals.[2, 3] These compounds can be extracted from plants, such as water lettuce (*Pistia stratiotes*).

Water lettuce (*P. stratiotes*) is an aquatic floating plant that is widely found in tropical areas, including South Sumatra, Indonesia. A previous study reported that polyphenol compounds were successfully extracted from the leaf of this plant.[4] Generally, polyphenol compounds are not only present in free forms in plants but are also found in conjugated forms with one or more sugar residues linked to a carbon atom of the aromatic ring or by β-glycosidic bonds to a hydroxyl group.[5] Additionally, the leaf of the plant is also a source of polysaccharide compounds. As reported by the previous studies, polysaccharides were successfully extracted from the leaves of *Nelumbo nucifera*,[6] *Eucommia ulmoides*,[7] and *Talinum triangulare*.[8] These polysaccharides showed some biological activities, including immunomodulatory effects,[8] antioxidant activity,[7] and alpha-glucoside inhibitor.[9]

*Corresponding author. E mail: sabrisudirman@unsri.ac.id
Tel: (+62) 711580934

Citation: Sudirman S, Sirait YN, Ghaisani AD, Herpandi, Widiastuti I, Janna M. Antioxidant Activity of Polysaccharides from Water Lettuce (*Pistia stratiotes*) Leaf Extract. Trop J Nat Prod Res. 2023; 7(11):5055-5058. http://www.doi.org/10.26538/tjnpr/v7i11.7

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Polysaccharides can be extracted by hot water extraction, resulting in a crude polysaccharides extract. The crude polysaccharide is composed of some non-polysaccharide compounds, such as lipids, proteins, minerals, and pigments.[10, 11] Therefore, the purification process is needed to remove some of these unwanted compounds, such as defatted lipid compounds, by using organic solvents such as methanol, n-hexane, and ethanol.[12] Acetone is also generally used during the defatted process.[13] Additionally, the defatted process is also used as a pretreatment of polysaccharides before analysis and it affected bioactivity assay.[13, 14] However, there is yet no study reporting the effects of defatting the polysaccharides in water lettuce and its antioxidant activity. Therefore, this study aimed to determine the total sugar, antioxidant activity, and functional groups of the polysaccharides extracted from water lettuce (*P. stratiotes*) before and after the defatted process with acetone.

Materials and Methods

Preparation, extraction, and defatting process

The fresh water lettuce (*Pistia stratiotes*) leaf was collected on September 2022 from Sukaraja Village, 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 leaf was then cleaned with water and cut into small pieces. The small pieces were dried in the oven at 45°C for 12 hours. After the drying process, the sample was ground to powder. The polysaccharide from the water lettuce leaf was extracted by the hot-water extraction method according to previous methods.[10] Briefly, 10 g of dry sample was added into an Erlenmeyer flask containing 400 mL of distilled water (dH_2O). The extraction was performed at 90°C and stirred at 120 rpm by using a hot magnetic stirrer for 3 hours. After extraction time, the filtrate and supernatant were separated by centrifugation at 4,300 rpm for 20 minutes. The supernatant was collected and put into the new collection tube. The polysaccharide was then precipitated by using 95% ethanol for 24 hours at freeze temperature. The polysaccharide extract was collected by

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

centrifugation at 4,300 rpm for 20 minutes and the filtrate was dried by using an oven-dryer to obtain polysaccharide powder, which was then kept at a cold temperature for further analysis. The polysaccharides were then defatted using an acetone solvent, according to the previous studies.[15, 16] Briefly, the polysaccharides were mixed with acetone (1:3, w/v) at room temperature and stirred at 100 rpm for 15 minutes. The machine was centrifuged at 4,300 rpm for 20 minutes. The supernatant was removed, and the pellet containing defatted polysaccharides was dried by using an oven dryer to obtain defatted polysaccharide powder, which was then kept at a cold temperature for further analysis.

Total sugar analysis

The total sugar of the crude and defatted polysaccharide extracts was analyzed according to the previous method.[17] Briefly, 10 mL of sample solution (5 mg/mL in 80% ethanol) was pipetted into the reaction tube and put in the water batch at 90°C for 10 minutes. After the reaction time it was cooled to room temperature. Then, 1 mL of the solution was mixed with 0.5 mL of 5% phenol (phenol in water, v/v). The mixture was added to 2.5 mL of 36 N sulfuric acid and incubated at room temperature for 20 minutes. The absorbance was immediately measured by a UV-Vis spectrophotometer at 490 nm. The glucose was used as a sugar standard. Therefore, the total sugar of the polysaccharide was expressed as mg glucose equivalent per gram of dried sample (mg glu.eq./g dry sample).

Antioxidant activity analysis

The antioxidant activity of the crude and defatted polysaccharides was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, according to the previous studies.[18] Briefly, the extract was dissolved in methanol to make a serial concentration (0 – 1000 μg/mL). Then, 1 mL of the sample solution was mixed with 1 mL of 0.2 mM DPPH solution and incubated at 37°C for 30 minutes. After the incubation time, the absorbance was immediately measured at 517 nm by using a UV-Vis spectrophotometer. The antioxidant activity was expressed as the inhibition of DPPH free radicals by the sample solution according to this formula:

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

Whereas: Abs_{blank}, absorbance at 517 nm without sample, Abs_{sample}, absorbance at 517 nm with sample.

FT-IR analysis

The functional groups, including the hydroxyl group and glycosidic linkage of the polysaccharides, were detected by using Fourier-transform infrared (FT-IR, InfraRed Bruker Tensor 37) according to the previous method.[19] The FT-IR spectra of polysaccharides were obtained by mixing polysaccharides with potassium bromide, further, they were pressed to form pellets.

Statistical analysis

All data are expressed as the mean ± standard deviation (SD) and analyzed by an independent sample t-test with a statistically significant test at $p<0.05$ using SPSS (v.22.0; IBM Corp., Armonk, NY, USA).

Results and Discussion

Extraction yield

In this study, the crude polysaccharide extract was successfully extracted by the hot-water extraction method from a water lettuce leaf. The yield of the crude polysaccharide was about 5.83±1.38%. A previous study also extracted polysaccharides by hot water extraction from *Sargassum longifolium* (temperature: 80°C; yield: 3.76%).[20] After the defatted process using acetone, the defatted polysaccharide yield was about 72.67±2.16%. This condition indicated that some compounds were successfully removed during the defatted process, including the fat of the extract. A previous study reported that the yield of the purification (defatting) process using some organic solvents was about 31.0% – 72.4%.[21] Additionally, the defatted process is used to remove the fat from polysaccharides before polysaccharide analysis by using organic solvents such as acetone and ethanol.[14, 22] Acetone also known as a dehydrating agent, is used to achieve complete lipid removal from polysaccharides.[23]

Total sugar content

The total sugar of the crude and defatted polysaccharides is shown in Figure 1. The total sugar of the polysaccharides was significantly ($p<0.05$) decreased after the defatted process, whereas the total sugar of the crude extract was about 97.55±20.65 mg glu.eq./g of dry sample. A previous study reported that polysaccharides from *Lycium chinense* ranged from 43.15 mg glucose/g to 53.29 mg glucose/g.[24] Figure 1 also showed that the defatted polysaccharide was about 44.12±3.06 mg glu.eq./g of dry sample. Acetone was widely used to remove fat from polysaccharides.[23] However, sugar compounds such as fructose and glucose were also partially dissolving in organic solvents, including acetone.[25-27] Therefore, some sugar compounds were also removed during the defatting process by acetone.

Antioxidant activity

The antioxidants of the crude and defatted polysaccharides are shown in Figure 2. The antioxidant activity of the defatted extract was significantly ($p<0.05$) higher when compared to the crude extract. The half-maximum inhibitory concentration (IC_{50}) of the crude extract was about 0.48±0.40 mg/mL, whereas the defatted extract was about 0.41±0.02 mg/mL. A previous study reported that polysaccharides were extracted from Ajwa date (*Phoenix dactylifera*) also showed antioxidant activity with the IC_{50} about 1.73 mg/mL to 3.39 mg/mL.[28] The lower IC_{50} value indicated that the polysaccharides were more potent at scavenging DPPH radicals which implies a high antioxidant activity, and vice versa.[29, 30] The ability of polysaccharides to scavenge free radicals produced by the body is referred to as their antioxidant activity.[31] Polysaccharides can act as antioxidants through hydrogen atom transfer mechanism.[32]

Functional groups of the polysaccharide

The functional group of the polysaccharide extracts from water lettuce leaf is shown in Figure 3. The O–H stretching vibration was detected at 3419.84 cm^{-1} whereas, the C–H linkage was at 2918.98 cm^{-1}. The carbonyl (C=O) stretching has appeared at 1646.49 cm^{-1} whereas, the glycosidic linkage C–O–C at 1076.12 cm^{-1}. The functional group was analyzed according to previous reference.[33] 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.[19] Additionally, the peaks at 950–1200 cm^{-1} were attributed to the heavy atoms C–C and C–O stretching vibrations in the pyran ring of the polysaccharides.[34]

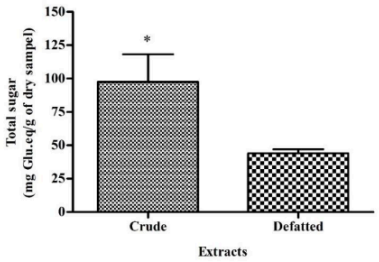

Figure 1: Total sugar contents of crude and defatted polysaccharides from water lettuce (*Pistia stratiotes*) leaf. Data are shown as the mean ± SD ($n=3$). Statistically significance at *$p<0.05$ versus defatted extract.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

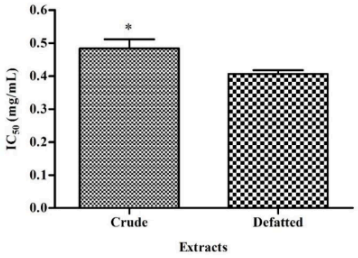

Figure 2: Antioxidant activity of crude and defatted polysaccharides from water lettuce (*Pistia stratiotes*) leaf. Data are shown as the mean ± SD (n=3). Statistically significance at *p<0.05 versus defatted extract.

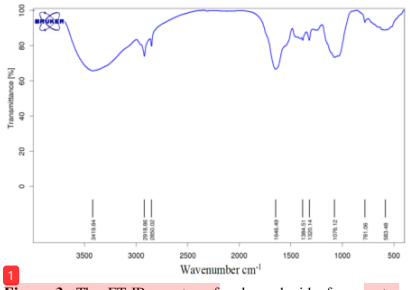

Figure 3: The FT-IR spectra of polysaccharide from water lettuce (*Pistia stratiotes*) leaf.

Conclusion

Overall, the polysaccharide from water lettuce (*Pistia stratiotes*) was successfully extracted by the hot-water extraction method. The defatting process using acetone also increases antioxidant activity. The polysaccharides from water lettuce can be used as a source of natural antioxidants. However, some sugar compounds were lost during the defatting process. Therefore, the alternative to the defatting process needs to increase the recovery of the polysaccharides.

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.

References

1. Meo SD, Venditti P. Evolution of the Knowledge of free radicals and other oxidants. Oxid Med Cell Longev. 2020; 2020:9829176.
2. Bai L, Xu D, Zhou YM, Zhang YB, Zhang H, Chen YB, Cui YL. Antioxidant activities of natural polysaccharides and their derivatives for biomedical and medicinal applications. Antioxidants. 2022; 11:2491.
3. Sudirman S, Herpandi, Safitri E, Apriani EF, Taqwa FH. Total polyphenol and flavonoid contents and antioxidant activities of water lettuce (*Pistia stratiotes*) leave extracts. Food Res. 2022; 6:205-210.
4. Sudirman S, Janna M, Herpandi, Widiastuti I. *In vitro* inhibitory HMG-CoA reductase activity of purified polyphenol compounds from water lettuce (*Pistia stratiotes*) leaf extract. Trop. J. Nat. Prod. Res. 2022; 6:1131-1134.
5. Šamec D, Karalija E, Šola I, Bok VV, Sondi BS. The role of polyphenols in abiotic stress response: the influence of molecular structure. Plants. 2021; 10:118.
6. Song YR, Han AR, Park SG, Cho CW, Rhee YK, Hong HD. Effect of enzyme-assisted extraction on the physicochemical properties and bioactive potential of lotus leaf polysaccharides. Int. J. Biol. Macromol. 2020; 153:169-179.
7. Xu J, Hou H, Hu J, Liu B. Optimized microwave extraction, characterization and antioxidant capacity of biological polysaccharides from *Eucommia ulmoides* oliver leaf. Sci. Rep. 2018; 8:6561.
8. Yeh SH, Hsu WK, Chang ZQ, Wang SH, Hsieh CW, Liou GG, Lee HB, Jiang BH, Tsou HK, Tsai MS. Purification and characterization of fractions containing polysaccharides from *Talinum triangulare* and their immunomodulatory effects. Processes. 2021; 9:709.
9. Zhang S, Li XZ. Inhibition of α-glucosidase by polysaccharides from the fruit hull of *Camellia oleifera* Abel. Carbohydr. Polym. 2015; 115:38-43.
10. Wang B, Liu Q, Huang Y, Yuan Y, Ma Q, Du M, Cai T, Cai Y. Extraction of polysaccharide from *Spirulina* and evaluation of its activities. Evid Based Complement Alternat Med. 2018; 2018:3425615.
11. Liu C, Liu Q, Sun J, Jiang B, Yan J. Extraction of water-soluble polysaccharide and the antioxidant activity from semen cassiae. J Food Drug Anal. 2014; 22:492-499.
12. Kim TK, Yong HI, Kim YB, Jung S, Kim HW, Choi YS. Effects of organic solvent on functional properties of defatted proteins extracted from *Protaetia brevitarsis* larvae. Food Chem. 2021; 336.
13. Jeong MS, Lee SD, Cho SJ. Effect of Three defatting solvents on the techno-functional properties of an edible insect (*Gryllus bimaculatus*) protein concentrate. Molecules. 2021; 26:5307.
14. Nor NANM, Abbasiliasi S, Marikkar MN, Ariff A, Amid M, Lamasudin DU, Manap MYA, Mustafa S. Defatted coconut residue crude polysaccharides as potential prebiotics: Study of their effects on proliferation and acidifying activity of probiotics *in vitro*. J. Food Sci. Technol. 2016; 54:164-173.
15. Kim TK, Lee JH, Yong HI, Kang MC, Cha JY, Chun JY, Choi YS. Effects of defatting methods on the physicochemical properties of proteins extracted from *Hermetia illucens* larvae. Foods. 2022; 11:1400.
16. Njanje I, Bagla VP, Beseni BK, Mbazima V, Lebogo KW, Mampuru L, Mokgotho MP. Defatting of acetone leaf extract of *Acacia karroo* (Hayne) enhances its hypoglycaemic potential. BMC Complement Altern. Med. 2017; 17:482.
17. Nielsen SS. *In Food Analysis Laboratory Manual*, 2017, DOI: 10.1007/978-3-319-44127-6_14, ch. Chapter 14, pp. 137-141.
18. Egharevba E, Nwani PC, Eboh U, Okoye E, Bolanle I, Oseghale I, Imieje V, Erharuyi O, Falodun A. Evaluation of the antioxidant and hypoglycaemic potentials of the leaf extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop. J. Nat. Prod. Res. 2019; 3:170-174.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

19. Bhateja PK, Kajal A, Singh R. Amelioration of diabetes mellitus by modulation of GLP-1 via targeting alpha-glucosidase using *Acacia tortilis* polysaccharide in streptozotocin-nicotinamide induced diabetes in rats. Journal of Ayurveda and Integrative Medicine. 2020; 11:405-413.
20. Shofia SI, Jayakumar K, Mukherjee A, Chandrasekaran N. Efficiency of brown seaweed (*Sargassum longifolium*) polysaccharides encapsulated in nanoemulsion and nanostructured lipid carrier against colon cancer cell lines HCT 116. RSC Adv. 2018; 8:15973-15984.
21. Amid BT, Mirhosseini H. Effect of Different purification techniques on the characteristics of heteropolysaccharide-protein biopolymer from durian (*Durio zibethinus*) seed. Molecules. 2012; 17:10875-10892.
22. Kakar MU, Karim H, Shabir G, Iqbal I, Akram M, Ahmad S, Shafi M, Gul P, Riaz S, Rehman RU, Salari H. A review on extraction, composition, structure, and biological activities of polysaccharides from different parts of *Nelumbo nucifera*. Food Sci. Nutr. 2023; 00:1-20.
23. Aldairi AF. Evaluation of various methodologies used in purification of biologically active carbohydrates derived from marine life. Biomed. J. Sci. Technol. Res. 2020; 27:20919- 20927.
24. Yang L, Liang Q, Wang S, Yuan F, Wang J, Zhang Y, He Y. Quality evaluation of thirteen geographical populations of lycium chinense using quantitative analysis of nutrients and bioactive components, J. Food Qual. 2019; 2019:1-14.
25. Danilchuk YV. Thermal effect of carbohydrate dissolution in aqueous-organic media. Health Food Biotechnol. 2021; 2:49-59.
26. Zhu H, Zhang Y, Guo X, Cheng Y, Wang L, Li X. Efficient one-pot production of 5-hydroxymethylfurfural from glucose in an acetone–water solvent. Ind. Eng. Chem. Res. 2022: 61:5661-5671.
27. Motagamwala AH, Huang K, Maravelias CT, Dumesic JA. Solvent system for effective near-term production of hydroxymethylfurfural (HMF) with potential for long-term process improvement. Energy Environ. Sci. 2019, 12, 2212-2222.
28. Dhahri M, Sioud S, Alsuhaymi S, Almulhim F, Haneef A, Saoudi A, Jaremko M, Emwas AHM. Extraction, characterization, and antioxidant activity of polysaccharides from ajwa seed and flesh, Separations. 2023; 10:103.
29. Olugbami JO,. A. Gbadegesin MA, Odunola OA. *In vitro* evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*. Afr. J. Med. Med. Sci. 2014; 43:101-109.
30. Matuszewska A, Jaszek M, Stefaniuk D, Ciszewski T, Matuszewski L. Anticancer, antioxidant, and antibacterial activities of low molecular weight bioactive subfractions isolated from cultures of wood degrading fungus *Cerrena unicolor*. Plos One. 2018; 13:e0197044
31. Huang H, Huang G. Extraction, separation, modification, structural characterization, and antioxidant activity of plant polysaccharides. Chem Biol Drug Des. 2020; 96:1209-1222.
32. Fernandes PAR, M. A. Coimbra MA. The antioxidant activity of polysaccharides: A structure-function relationship overview. Carbohydr. Polym. 2023; 314:120965.
33. Coates J. *In Interpretation of Infrared Spectra: A Practical Approach*, ed. R. Meyers, John Wiley & Sons Ltd., Chichester. 2000. pp. 10881-10882.
34. Yang K, Zhang Y, Cai M, Guan R, Neng J, Pi X, Sun P. *In vitro* prebiotic activities of oligosaccharides from the by-products in *Ganoderma lucidum* spore polysaccharide extraction. RSC Adv. 2020; 10:14794-14802.

Antioxidant Activity of Polysaccharides from Water Lettuce (Pistia stratiotes) Leaf Extract

ORIGINALITY REPORT

16%	18%	3%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	office2.jmbfs.org Internet Source	14%
2	www.kunsttherapie-berlin.de Internet Source	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		

Antioxidant Activity of Polysaccharides from Water Lettuce (Pistia stratiotes) Leaf Extract

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

PAGE 1

PAGE 2

PAGE 3

PAGE 4