Hue University Journal of Science: Natural Science
Vol. 132, No. 1D, 63–71, 2023

pISSN 1859-1388
eISSN 2615-9678

Polysaccharide extraction from *Myxopyrum smilacifolium* trunk and its antioxidant capacity

Le Trung Hieu[1]*, Le Lam Son[1], Nguyen Dang Giang Chau[1], Nguyen Minh Nhung[2], Tran Thanh Minh[1], Ho Xuan Anh Vu[1], Le Thuy Trang[1], Nguyen Viet Thang[3], Nguyen Quang Man[4], Tran Thi Van Thi[1]

[1] Faculty of Chemistry, Hue University of Sciences, Hue University, Hue, Vietnam
[2] Center for Measurement, Testing and Scientific information, Department of Science and Technology Thua Thien Hue province, Hue, Vietnam
[3] Faculty of Biology, Hue University of Sciences, Hue University, Hue, Vietnam
[4] University of Medicine and Pharmacy, Hue University, Hue, Vietnam

* Correspondence to Le Trung Hieu <letrunghieu.chem@gmail.com>
(*Received: 23 November 2022; Revised: 13 January 2023; Accepted: 15 January 2023*)

Abstract. As a traditional medicinal plant in Vietnam, *Myxopyrum smilacifolium* has been used for a long history to treat cough, nervous disorders, numbness, rheumatism, and cephalalgia. Nevertheless, reports on the antioxidant activity and extraction of *M. smilacifolium* polysaccharides are still very rare. This study was designed to extract a high yield of polysaccharides from the *M. smilacifolium* trunk and characterize it. As a result, the maximum yield of the polysaccharides of 5.13 ± 0.05% was obtained with an extraction time of three hours, extraction temperature of 100 ^{o}C, the ratio of water to sample of 1:50 as extraction solution, extraction number of times 3, and the ratio of ethanol to extract volume 5:1 (v/v). Polysaccharides was characterized by Fourier transform infrared spectroscopy (FT-IR) and high-performance gel-permeation chromatography (HPGPC). The average molecular weight of extracted polysaccharides was around 3.78 × 10^5 Da. *In vitro* assays dan impressive antioxidant activities of the extracted polysaccharides, containing 0.2423 ± 0.0028 mg GA/g or 0.2142 ± 0.0007 μmol AS/g. The IC_{50} values of polysaccharides in the DPPH and ABTS methods were 0.89 mg/mL and 3.85 mg/mL, respectively. These findings exhibited the potential for application or further research and development of polysaccharides from *Myxopyrum smilacifolium*.

Keywords: *Myxopyrum smilacifolium*; polysaccharides extraction; antioxidant activities

1 Introduction

Polysaccharides (PS) have been appealing great attention over the past decades [1, 2]. They play a wide variety of important roles in nature, and have been found to be extremely valuable sources of food and cosmetic ingredients and in nutraceutical and pharmacological applications [3-5]. The polysaccharide extraction process makes their structural diversity and potential biological functions offer unique properties [6-8]. For this reason, the extraction of polysaccharides has been concerned as a hot-spot- research topic in the field of biomedicine. In addition, polysaccharides were biological macromolecules that exhibit a variety of physicochemical and pharmacological properties that are important processes for their application or further research and development.

Myxopyrum smilacifolium belongs to the *Myxopyrum* genus of the Oleaceae family growing as part of native vegetation in Southeast Asian countries such as Vietnam, Laos, Cambodia, Indonesia Thailand, and India [9-11]. The plant was used in traditional medicine to treat cough,

rheumatism, nerve complaints, asthma, fever, neuropathy, and asthma treatments [11, 12]. Studies confirmed that the extracts from *M. smilacifolium* exert antimicrobial, cytotoxic, anti-inflammatory, and antioxidant activities. Nevertheless, the reason for inducing the bioactivities of *M. smilacifolium* has been ambiguous. Given that polysaccharides could be the primary component of *M. smilacifolium* extracts, causing the antioxidant activity. In this context, the extraction and antioxidant activity of polysaccharides can be considered the imperative piece of information explaining their outstanding bioactivities [9-12]. However, to our knowledge, there have been polysaccharide extraction and antioxidant activity of polysaccharides from *M. smilacifolium* trunk are rarely reported.

Motivated by those challenges, this study aimed to provide design experiments (extraction parameters: extraction time, extraction temperature, the ratio of water to sample, extraction number times, and the ratio of ethanol to extract volume) to appropriate the yield of polysaccharides from the *M. smilacifolium* trunk. Characterization of polysaccharides was indicated in the Fourier transform infrared spectroscopy and high-performance gel-permeation chromatography. Besides, *in vitro* antioxidant activities of polysaccharides such as total antioxidant capacity, ABTS radical scavenging activity, and DPPH radical scavenging activity, were evaluated.

2 Experimental section

2.1 Material samples and chemicals

The *Myxopyrum smilacifolium* trunks were collected from a natural source on June 10th, 2021 in Thua Thien Hue, Vietnam, and were taxonomically identified by Nguyen Viet Thang (Department of Biology, College of Sciences; Hue University). A voucher specimen was deposited at the Department of Biology, College of Sciences; Hue University.

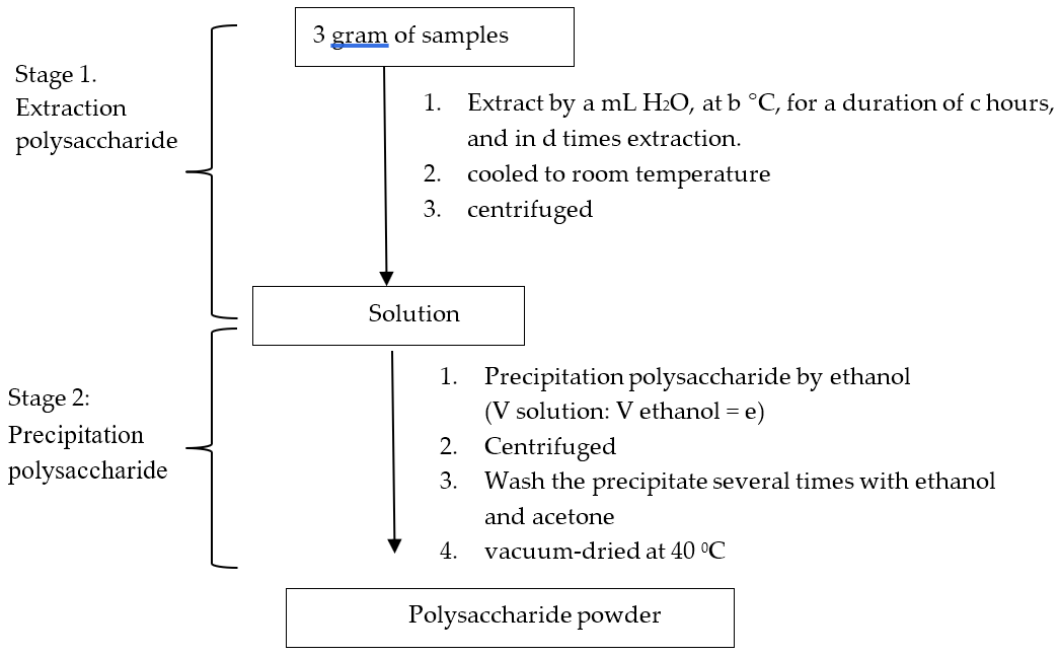
2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), $AlCl_3$, $NaNO_2$, and NaOH were purchased from Shandong Chemical Co. (China). $(CH_3)_2SO$, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and ascorbic acid were purchased from Sigma-Aldrich (USA). The ethanol used in all experiments was food grade and purchased from local suppliers.

2.2 Extraction procedure of polysaccharides (PS)

The process of extraction of polysaccharides is carried out through two main steps: extraction of polysaccharide from *Myxopyrum smilacifolium* and precipitation of polysaccharide by ethanol. In the investigations on the single-factor experiments, the variables considered are extraction parameters: extraction time, extraction temperature, the ratio of water to raw material, extraction number times, and ratio of ethanol to extract volume.

The powder samples (3 grams) were in a 250 mL flask, and the ratio of water to sample (1:30 ÷ 1:70 (g/mL)) and appropriate extraction temperature (60 °C ÷ 100 °C), and extraction number times (2 ÷ 5), and extraction time (2h ÷ 6h). The mixture was cooled to room temperature using cold water and was then centrifuged. The solution was concentrated in a rotary evaporator under reduced pressure to receive the extract solution (50 mL). The ratio of ethanol 96° to extract volume (from 2:1 to 5:1, v/v) to precipitate completely polysaccharide. The resulting precipitate was collected by centrifugation and then washed sequentially with cold ethanol and acetone. Finally, the product was vacuum-dried at 40 ^{0}C to yield a crude water-soluble PS powder [13].

pISSN 1859-1388
eISSN 2615-9678



Scheme 1. Diagram depicting the extraction of *Myxopyrum smilacifolium* trunk polysaccharide

2.3 Qualitative and quantitative analysis of polysaccharides

Polysaccharides were examined by phenol-sulphuric acid – colorimetric method using D-Glucose as standard at a wavelength of 490 nm [14]. Data were calculated as percentages in the dry weight of the sample.

2.4 Structural characterizations of polysaccharide

The FT-IR spectra of polysaccharides were recorded in an IRPrestige-21 infrared spectrophotometer (Shimadzu, Japan). Samples were dried at 55 °C in a vacuum drying oven for 48 h prior to tableting with KBr powder. Spectra were scanned in the 4000–400 cm^{-1} region.

The average molecular weight of polysaccharides was determined by gel permeation chromatography (GPC – Agilent 1100 Series coupled to MS detector, microTOF-QII Bruker, USA, an Ultrahydrogen 500 column (7.8 mm x 300 mm, 10 μm)) as described by Wang et al. with some modifications [15]. The purified polysaccharides were dissolved in $NaNO_3$ 0.1M and injected in the system by maintaining the same flow rate (1 mL/min) and column temperature (40 °C). The molecular mass was estimated by the standard curve which was calibrated using the pullulans (from 50 kDa to 800 kDa).

2.5 Antioxidant activities

Total antioxidant capacity (TAC)

The total antioxidant capacity of the samples was determined according to the method described by Nair et al [16]. The total antioxidant capacity is displayed as the number of equivalents of gallic acid (GA) [17] or ascorbic acid [18] (the standard curve equation for gallic acid and ascorbic acid is Abs = 2.172 × CGA + 0.1056; R = 0.9995 and Abs = 4.209 × CAS – 0.0463; R = 0.9993).

DPPH radical-scavenging activity

The DPPH free radical scavenging activity is used to perform the screening effects of antioxidation of the testing substances. Two mL of sample (in

dimethyl sulfoxide (DMSO) with different concentrations (from 0.4 mg/mL to 2 mg/mL)) were added to 1 mL of 100 μM DPPH (in methanol) for 30 min at room temperature. The absorbance is measured by the UV–Vis method at 517 nm. The antioxidative activity was shown by color reduction. Ascorbic acid was used as a reference substance. DMSO was used as a control. The radical scavenging activity was evaluated using the IC_{50} value [13].

ABTH·+ radical-scavenging activity

The scavenging capacity on the ABTS radical of polysaccharide was determined according to the method described by Roberta Re et al with several modifications [19]. An amount of 0.1 mL sample with different concentrations (from 1 mg/mL to 5 mg/mL) were mixed with 3.9 mL of ABTS+· solution. The absorbance was then measured at 734 nm. Ascorbic acid was used as a positive control. The radical scavenging activity was evaluated using the IC_{50} value.

2.6 Statistical analysis

Unless otherwise stated, all the experiments were performed three times (n = 3). The results are presented as means value ± Standard deviation (SD) or Standard Error of the Mean (SEM). The statistical analysis system software involved the use of both Origin 8.0 and Microsoft Excel (2010).

3 Results and discussion

3.1 The extraction of polysaccharides from *Myxopyrum smilacifolium* trunk

Table 1 showed extraction yields of polysaccharides under investigation conditions. The extraction yields ranged from 3.26 ± 0.05% to 5.10 ± 0.03% with the increasing temperature from 60 ^{0}C to 100 ^{0}C. The highest yield of polysaccharide was 5.10 ± 0.03% obtained at 100 ^{0}C. As the temperature increased, the diffusion of polysaccharides from the cells into the extraction solvent increased [20].

As the solvent volume increased, the polysaccharide yield increased rapidly in the sample: solvent ratio range of 1:30 to 1:50 mg/mL. However, with a further increase in solvent volume, the extracted polysaccharide yield did not increase further. The sample: water ratio of 1:50 mg/mL was selected for polysaccharide extraction.

The results indicated that extraction time was proportional to the yield of the PS when extraction time was between 2 and 3 h. The yield of the PS was 5.10 ± 0.03 % when the extraction time was 3 h. After this point, the yield of the polysaccharides started to decrease with increasing the extraction time. In our opinion that increasing the extraction time may enhance the yield of the polysaccharides. However, excessively lengthening extraction time will also induce a change in polysaccharides structure (maybe, the long extraction time induced the degradation of polysaccharides), as a result of which the extraction yield of polysaccharides instead decreased.

The effect of extraction number of times on the yield of the polysaccharides was investigated. The results indicated that the extraction number of times was proportional to the yield of the polysaccharides when the extraction number of times was between 2 and 4. The yield of the polysaccharides was 5.11 ± 0.02% when the extraction number of times was 4. However, the yield increased slowly when the extraction number of times increased from 3 to 4 times. To avoid the wasting consumption of solvents and bulky handling in the subsequent processes, the extraction number of times 3 was chosen.

After step 1, the effect of the ratio of ethanol to extract volume was studied. Data in Table 1

pISSN 1859-1388
eISSN 2615-9678

revealed that the larger ratio of EtOH 96°: extract volume, the higher percentage of pure polysaccharide. The highest yield of polysaccharides was 5.13 ± 0.05% obtained with a ratio of ethanol 96° to extract volume 5:1.

Table 1. Extraction yield of polysaccharides from *Myxopyrum smilacifolium* trunk under investigation conditions

The effect of extraction temperature on the yield of the polysaccharides Other extraction conditions were as follows: ratio of sample: water volume 1:50, extraction time 3 h, extraction number times of 3, and ratio of ethanol 96° to extract volume 4:1.					
extraction temperature (^{0}C)	60	70	80	90	100
Percentage of pure PS (%)	3.26 ± 0.05	4.10 ± 0.04	4.50 ± 0.04	4.94 ± 0.05	5.10 ± 0.03
The effect of sample–water ratio on the yield of the polysaccharides Other conditions were as follows: extraction temperature 100 ^{0}C, extraction time 3 h, extraction number times of 3, and ratio of ethanol 96° to extract volume 4:1.					
sample: water (g/mL)	1:30	1:40	1:50	1:60	
Percentage of pure PS (%)	4.42 ± 0.04	4.66 ± 0.05	5.10 ± 0.03	5.09 ± 0.04	
The effect of extraction time on the yield of the polysaccharides Other conditions were as follows: extraction temperature 100 ^{0}C, extraction number times of 3, ratio of ethanol 96^0 to extract volume 4:1, and the ratio of sample: water volume 1:50.					
Extraction time (h)	2	3	4	5	
Percentage of pure PS (%)	4.63 ± 0.09	5.10 ± 0.03	5.07 ± 0.07	4.97 ± 0.02	
The effect of extraction number of times on the yield of the polysaccharides Other conditions were as follows: extraction temperature 100 ^{0}C, extraction time 3 h, the ratio of ethanol 96° to extract volume 4:1, and the ratio of sample: water volume 1:50					
Extraction number times	2	3	4		
Percentage of pure PS (%)	4.66 ± 0.07	5.10 ± 0.03	5.11 ± 0.02		
The effect of the ratio of ethanol to extract volume on the yield of the polysaccharides Other conditions were as follows: extraction temperature 100 ^{0}C, extraction time 3 h, the ratio of ethanol 96°to extract volume 4:1, and extraction number times of 3.					
EtOH 96^0: extract (v/v)	2:1	3:1	4:1	5:1	
Percentage of pure PS (%)	4.44 ± 0.05	4.78 ± 0.05	5.10 ± 0.03	5.13 ± 0.05	

3.2 Characterization of polysaccharide extracted from *Myxopyrum smilacifolium* trunk

Figure 1 shows a chromatogram of polysaccharide obtained by gel permeation high-performance liquid chromatography. The polysaccharide from *Myxopyrum smilacifolium* trunk had a molecular weight of 3.78 × 10^5 Da. The ratio Mw/Mn, which represents the polydispersity index and provides a rough indication of the breadth of the distribution, was approximately 14.10. This value implied that the isolated polysaccharide contained a very large molecular weight distribution. Therefore, it can be stated that the polysaccharide extracted from *Myxopyrum smilacifolium* trunk is a heterogeneous polysaccharide.

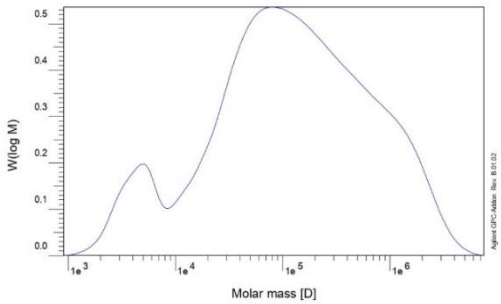


Fig. 1. Molecular mass chromatogram of polysaccharide from *Myxopyrum smilacifolium* trunk obtained by GPHPLC.

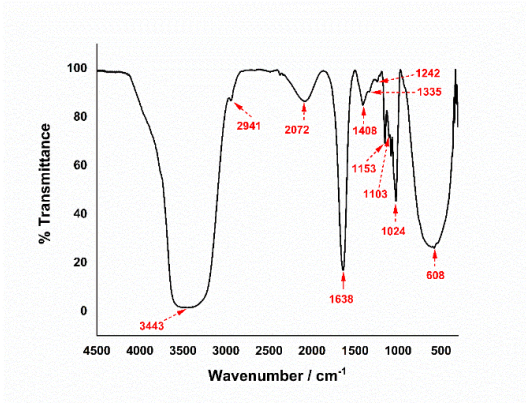


Fig. 2. Infrared spectra of polysaccharide from *Myxopyrum smilacifolium* trunk.

The FT-IR was conducted to investigate the characteristic bonding of the sample polysachraide from *Myxopyrum smilacifolium* trunk, as shown in Figure 2. The intensity of bands around 3443 cm^{-1} was due to the hydroxyl stretching vibration of the polysaccharide. The bands in the region of 2941 cm^{-1} were due to C-H stretching vibration, and the bands in the region of 1638 cm^{-1} were due to associated water. The strong absorption bands at 1408 cm^{-1} were due to C–O stretching vibrations [21]. The characteristic peak centering at 1153 cm^{-1} implies a glucopyranoside, whereas the peaks located at 1024 cm^{-1} could be indicated α-configurations and the presence of fructose residues, respectively [22]. To this end, it can be said that the extracted polysaccharide from *M. smilacifolium* trunk possesses the typical absorption groups of polysaccharides, glucopyranoside, and fructose in the structure.

3.3 *In vitro* Antioxidant activity assay of polysaccharides

One of the most commonly used methods to evaluate antioxidant activity in chemical and biological samples is the total antioxidant capacity (TAC) method. The TAC method is based on the redox potential of metal ions. In this study, we investigated the total antioxidant content of the sample based on the redox potential of Mo(VI)/Mo(V). TAC was expressed as the number of equivalents of gallic acid or ascorbic acid. The study revealed that the antioxidant capacity of polysaccharides was observed at the concentration of 1.5 mg/mL where the total antioxidant capacity of polysaccharides from *M. smilacifolium* trunk showed contained 0.2423 ± 0.0028 mg GA/g or 0.2142 ± 0.0007 µmol AS/g. The total antioxidant capacity of polysaccharides from *M. smilacifolium* trunk is higher than that of *Ophiocordyceps sobolifera* (from 0.1460 ± 0.0011 mg GA/g or 0.1222 ± 0.0003 µmol AS/g) [13]. This result suggests that this plant is a potent antioxidant.

On the other hand, free radical scavenging is a mechanism inhibiting lipid oxidation effectively, commonly used to estimate antioxidant activity. The ABTS radical scavenging activity and DPPH radical scavenging activity are splendid methods for determining the activity of substances according to their capacity to donate a hydrogen atom or electron, which is measured as the capacity of samples to degrade the colour by reacting directly with ABTS radical and DPPH radical. The electron-withdrawing group of polysaccharides and the specific structures activate the hydrogen atoms on sugar residues. The antioxidant activity of the polysaccharide from *M. smilacifolium* trunk was assessed by the

pISSN 1859-1388
eISSN 2615-9678

DPPH and ABTS methods, and the outcomes are displayed in Figure 3.

Figure 3 shows the free radical scavenging capacity of the polysaccharide increases with concentration. The DPPH radical scavenging activity at the concentration of 2.0 mg/mL of polysaccharides was over 75%, and the percentage of the ABTS radical scavenging activity rose remarkably and reached a peak at 5 mg/ mL concentration with over 60% inhibition, however, the activity of the polysaccharide was lower than that of ascorbic acid at the same concentration.

The IC_{50} values of polysaccharides in the DPPH and ABTS methods are found to be 0.89 mg/mL and 3.85 mg/mL, respectively, which is found to be considerably higher than that of the reported polysaccharide, as depicted in Table 2 (without PS from *Bletilla striata* in ABTS assay). Overall, the results suggest that polysaccharide from *M. smilacifolium* trunk is a promising source of natural antioxidants and can be used as an additive in food, pharmaceutical, and cosmetic preparations.

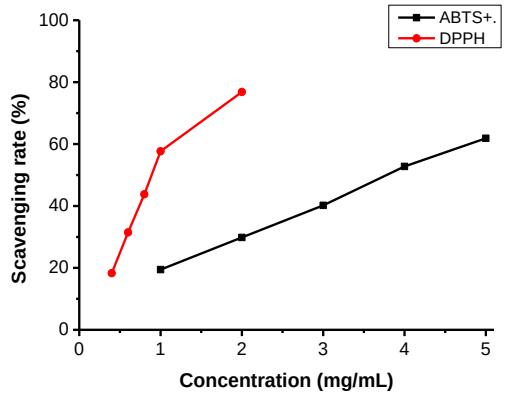


Fig. 3. In *vitro* antioxidant activity of polysaccharide from *Myxopyrum smilacifolium* trunk

Table 2. IC_{50} values obtained from DPPH and ABTS radical scavenging activity of reported polyscaccharide

No.	Sample	**IC_{50} ABTS (mg/mL)**	**IC_{50} DPPH (mg/mL)**	Ref
1	*Myxopyrum smilacifolium* trunk	3.85	0.89	This study
2	*Ophiocordyceps sobolifera*	4.83	0.97	[13]
3	Rice Brans	4.3	5.2	[23]
4	*Medemia argun* fruit	13.61	5.17	[24]
5	*Caulerpa lentillifera*	10.31	74.03	[25]
6	Wheat straw	6.4	2.84	[26]
7	*Lupinus angustifolius*	From 5.34 to 8.45	-	[27]
8	*Ganoderma tsugae*	-	From 2.84 to 12.85	[28]
9	*Physalis alkekengi*	6.053	1.284	[29]
10	*Bletilla striata*	From 3.157 to 5	> 2	[30]

4 Conclusion

Generally, an increased yield of crude polysaccharides was observed when the extraction time, extraction temperature, ratio of water to raw material, and extraction number were raised. The results from this study indicated the appropriate yield of polysaccharides from the *Myxopyrum smilacifolium* trunk. were as follows: an extraction time of 3 hrs, extraction temperature of 100 °C, the ratio of water to sample 1:50, extraction number times of 3, and the ratio of ethanol to extract volume 5:1. Under these conditions, a crude polysaccharide yield of around 5.13 ± 0.05% of dry weight. Fourier transform infrared spectroscopy indicated that polysaccharide from *M. smilacifolium* trunk possesses the typical absorption groups of polysaccharides, glucopyranoside, and fructose in the structure. The average molecular weight of extracted polysaccharides was around 3.78×10^5 Da. Impressively, the achieved polysaccharide from *Myxopyrum smilacifolium* trunk exhibits significant *in vitro* antioxidant activity. The total antioxidant capacity of polysaccharides from *Myxopyrum smilacifolium* trunk showed contained 0.2423 ± 0.0028 mg GA/g or 0.2142 ± 0.0007 μmol AS/g. The IC_{50} values of polysaccharides in the DPPH and ABTS methods were 0.89 mg/mL and 3.85 mg/mL, respectively. Thus, polysaccharides from *Myxopyrum smilacifolium* trunk may be developed as a potential natural antioxidant activity agent and functional food.

Funding: The authors acknowledge the support of Hue University under the Core Research Group Program, Grant No. NCM.DHH.2020.05. This research was funded by the Vietnamese Ministry of Education and Training under project number B2022-DHH-12 (L.T.H)

Conflicts of Interest: The authors declare no conflict of interest

References

1. Barclay TG, Day CM, Petrovsky N, Garg S. Review of polysaccharide particle-based functional drug delivery. Carbohydrate Polymers. 2019;221:94-112.
2. Chaiklahan R, Chirasuwan N, Triratana P, Loha V, Tia S, Bunnag B. Polysaccharide extraction from Spirulina sp. and its antioxidant capacity. International Journal of Biological Macromolecules. 2013;58:73-78.
3. Tzianabos AO. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. Clinical Microbiology Reviews 2000;13(4):523-533.
4. Liu Y, Sun Y, Huang G. Preparation and antioxidant activities of important traditional plant polysaccharides. International Journal of Biological Macromolecules 2018;111:780-786.
5. Chen L, Huang G. The antiviral activity of polysaccharides and their derivatives. International Journal of Biological Macromolecules. 2018;115:77-82.
6. Khatua S, Acharya K. Alkaline extractive crude polysaccharide from Russula senecis possesses antioxidant potential and stimulates innate immunity response. Journal of Pharmacy and Pharmacology. 2017;69(12):1817-1828.
7. Hambali E, Wibawa Sakti SC, Fahmi MZ, Wahyudianto FE, Nuryono, Yessi P, et al. In Effect of Extraction Time and Na_2CO_3 Concentration on The Characteristics of Alginate Extracted from *Sargassum sp*. IOP Conference Series: Earth and Environmental Science. 2018;209(1):012033.
8. Tang W, Liu D, Yin JY, Nie SP. Consecutive and progressive purification of food-derived natural polysaccharide: Based on material, extraction process and crude polysaccharide. Trends in Food Science & Technology. 2020;99:76-87.
9. Praveen R, Nair AS. Preliminary phytochemical screening of root extracts of Myxopyrum smilacifolium Blume. Asian Journal of Biomedical and Pharmaceutical Sciences. 2014; 4(38):41-45.
10. Siju EN, Samu J, Minil M, Rajalakshmi GR. Antioxidant activity of myxopyrum smilacifolium blume. Asian Journal of Pharmaceutical and Clinical Research. 2015;8:119-21
11. Vuong NQ, Chien VV, Hue NT, Hang PT, Hieu TV, Nhiem NX, et al. Chemical constituents from the

pISSN 1859-1388
eISSN 2615-9678

roots of Myxopyrum smilacifolium. Vietnam Journal of Science and Technology. 2021;59(4):498-506.

12. Peeriga R, Bonnth CK. Pharmacognostical Investigation and Preliminary Phytochemical Screening of Leaves of *Myxopyrum Smilacifolium* B. Pharmacognosy Journal. 2016;8(2).
13. Le TH, Thi TTV, Tran VK, Vu HXA, Tran TM, Chau NDG, et al. Structural Characterization of Mannoglucan Isolated from *Ophiocordyceps sobolifera* and Its Antioxidant Activities. ACS omega. 2022;7(11):9397-9405.
14. Dubois M, Gilles KA, Hamilton JK, Rebers P, Smith F. Colorimetric method for determination of sugars and related substances. Analytical chemistry. 1956;28(3):350-356.
15. Wang K, Li W, Rui X, Chen X, Jiang M, Dong M. Structural characterization and bioactivity of released exopolysaccharides from *Lactobacillus plantarum* 70810. International journal of biological macromolecules. 2014;67:71-78.
16. Nair VD, Panneerselvam R, Gopi R. Studies on methanolic extract of Rauvolfia species from Southern Western Ghats of India–*In vitro* antioxidant properties, characterisation of nutrients and phytochemicals. Industrial Crops and Products. 2012;39:17-25.
17. Megala J, Geetha A. Free radical-scavenging and H+, K+-ATPase inhibition activities of Pithecellobium dulce. Food chemistry. 2010;121(4):1120-1128.
18. Jayaprakasha G, Selvi T, Sakariah K. Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts. Food research international. 2003;36(2): 117-122.
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 1999;26(9-10):1231-1237.
20. Zhu C, Zhai X, Li L, Wu X, Li B. Response surface optimization of ultrasound-assisted polysaccharides extraction from pomegranate peel. Food Chemistry. 2015;177:139-146.
21. Tran TVT, Ho XAV, Le LS, Le TH, Nguyen THC, Nguyen CC, et al. Chemical structure of a novel heteroglycan polysaccharide isolated from the biomass of Ophiocordyceps Sobolifera. Journal of Molecular Structure. 2021;1232:129986.
22. Kacurakova M, Capek P, Sasinkova V, Wellner N, Ebringerova A. FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohydrate polymers. 2000;43(2):195-203.
23. Surin S, Seesuriyachan P, Thakeow P, You S, Phimolsiripol Y. Antioxidant and antimicrobial properties of polysaccharides from rice brans. Chiang Mai J Sci. 2018;45 (3):1372-1382.
24. Mohammed JK, Mahdi AA, Ahmed MI, Ma M, Wang H. Preparation, deproteinization, characterization, and antioxidant activity of polysaccharide from Medemia argun fruit. International journal of biological macromolecules 2020;155:919-926.
25. Chaiklahan R, Srinorasing T, Chirasuwan N, Tamtin M, Bunnag B. The potential of polysaccharide extracts from *Caulerpa lentillifera* waste. International Journal of Biological Macromolecules. 2020;161:1021-1028.
26. Xu X, Wu P, Wang T, Yan L, Lin M, et al. Synergistic effects of surfactant-assisted biodegradation of wheat straw and production of polysaccharides by Inonotus obliquus under submerged fermentation. Bioresource technology. 2019;278:43-50.
27. Thambiraj SR, Phillips M, Koyyalamudi SR, Reddy N. Antioxidant activities and characterisation of polysaccharides isolated from the seeds of Lupinus angustifolius. Industrial Crops and Products. 2015;74:950-956.
28. Tseng YH, Yang JH, Mau JL, Antioxidant properties of polysaccharides from Ganoderma tsugae. Food chemistry. 2008;107(2):732-738.
29. Liu X, Bian J, Li D, Liu C, Xu S, Zhang G, et al. Structural features, antioxidant and acetylcholinesterase inhibitory activities of polysaccharides from stem of *Physalis alkekengi* L. Industrial Crops and Products. 2019;129:654-661.
30. Chen H, Zeng J, Wang B, Cheng Z, Xu J, Gao W, et al. Structural characterization and antioxidant activities of *Bletilla striata* polysaccharide extracted by different methods. Carbohydrate Polymers. 2021;266:118149.