Journal of Food and Nutrition Sciences
2017; 5(3): 73-78
http://www.sciencepublishinggroup.com/j/jfns
doi: 10.11648/j.jfns.20170503.13
ISSN: 2330-7285 (Print); ISSN: 2330-7293 (Online)

Isolation, Purification and Structural Characterization of a Polysaccharide from *Rheum Palmatum* L.

Jinhua Li, Sihao Zheng, Weiguang Ren, Labin Wu, Linfang Huang*

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

Email address:

lfhuang@implad.ac.cn (Linfang Huang)

*Corresponding author

To cite this article:

Jinhua Li, Sihao Zheng, Weiguang Ren, Labin Wu, Linfang Huang. Isolation, Purification and Structural Characterization of a Polysaccharide from *Rheum Palmatum* L. *Journal of Food and Nutrition Sciences.* Vol. 5, No. 3, 2017, pp. 73-78.
doi: 10.11648/j.jfns.20170503.13

Received: February 14, 2017; **Accepted**: March 22, 2017; **Published**: April 14, 2017

Abstract: Rhubarb is a widely used Traditional Chinese Medicine in treating various diseases for thousands of years, and polysaccharide is one of the bioactive constituents in this herb. However, study of the polysaccharide is very limited, especially structural characterization. In the present study, a polysaccharide from *Rheum palmatum* L. (PR) was isolated and puried. Based on UV, IR, GC, GC-MS, 1H NMR and 13C NMR analyses, the structure of PR was characterized. Its molecular weight was measured to be 1.21×10^4 Da. The results of PR structural analysis showed that four 1→4 galactose were linked with one 1→ 3, 6 galactose and one 1→6 glucose, and each repeating unit had a branched chain consisting of a α-1→ xylose, which was linked with the C-6 of galactose backbone chain.

Keywords: *Rheum Palmatum* L., Polysaccharide, Isolation, Purification, Structural Characterization

1. Introduction

Rhubarb (Da-Huang in Chinese medicine) is one of the most important herbal medicines that has been widely used for thousands of years in many Asian countries [1, 2]. The dried roots and rhizomes of *Rheum palmatum*, *R. tanguticum* and *R. offcinale* are officially recorded by the Chinese Pharmacopoeia and European community monograph [1]. According to the principles of traditional Chinese medicine, rhubarb is frequently used in clinical treatment for its wide range of therapeutic activities, such as analgesic, choleretic, purgative, antibacterial, antiplatelet, anti-cancer, anti-inflammatory, antioxidant, liver-protective activities [3-5]. These pharmacological effects are attributed to components isolated from rhubarb, including anthraquinone derivatives, anthocyanin, polyphenols, polysaccharide and stilbenes [2, 6, 7]. To the best of our knowledge, secondary metabolites in the rhubarb have been widely studied by numerous researchers [6, 8]. However, there are few reports on the structure and conformation of polysaccharides.

Polysaccharides are macromolecular carbohydrates formed from long chains of monosaccharides and their main functions include energy storage, structural support, antigens, and so on [9] Recently, extensive attention has been paid to polysaccharides due to their crucial physiological and biological functions, such as anti-coagulant, cardiac protection, immunomodulatory and antitumor [10, 11]. According to previous reports, the biological activities of polysaccharides were closely related to their chain conformation and structure including chemical compositions, molecular weight, backbone and degree of branching [12]. In this regard, the elucidation of structure and conformation is necessary to better understand biological functions of polysaccharides. Herein. A polysaccharide from *R. Palmatum* (RP) was isolated and purified, *and* its structural characteristics were analyzed comprehensively by a variety of analytical techniques such as UV, IR, GC, GC-MS and NMR.

2. Materials and Methods

2.1. Plant Materials

R. palmatum was collected in November 2012 from Gansu Province, China. It was identified by Prof Linfang Huang. The specimen was deposited at the Herbarium of Medicinal Plant

Development, Chinese Academy of Medical Sciences. Standards glucose (Glc), galactose (Gal) and xylose (Xyl) with >98% purity were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Extraction of Crude Polysaccharide

Crude powder of *R. palmatum* was weighed for 300g accurately, and added 1L petroleum ether-absolute ethyl alcohol (1:1 for volume) to reflux degreasing for 2 h. Volatile petroleum ether was removed from the residue, and added 1L water to reflux extracted 3 times for each time with 2 h. The extracting solutions were combined and concentrated to 100 mL. The concentration was added ethanol to ethanol containing of 80%, and put at 4°C for 12 h, and centrifuged to obtain the precipitate. The precipitate was washed with diethyl ether and acetone several times respectively, and dried in low temperature to obtain the crude RP. The obtained crude polysaccharide was dissolved by water and added 3% papain, and put at 60°C for 3 h. The solution was removed proteins by Sevag method.

2.3. Purification of Polysaccharide

The crude polysaccharide was re-dissolved with 5 mL water, and purified by AB-8 (4 cm × 80 cm) macroporous resin column, which eluted by water with flow rate of 2ml/min, collected and concentrated eluant. The eluant was further purified by SephadexG-100 gel filtration chromatography (4 cm × 80 cm), eluted by water with flow rate of 2mL/min. The eluant was collected by tubes respectively (5 mL per tube), and measured the absorbance by phenol-sulfuric acid method at 490 nm. Combining the eluant, concentrating and freeze-dried were performed to obtain RP.

2.4. Measurement of Molecular Weights

The Polysaccharide was dissolved into the concentration of 2.0 mg/mL by water, and then filtrated by millipore filter and measured on a GPC using a Waters 1525 instrument. The purity and relative molecular weight of RP were determined on HPLC by using Waters series (Waters Ultrahydrogel TM 1000 column; ELSD detector; the temperature of column was 30°C; the mobile phase was 0.05% mixed solution of phosphoric acid-water; isocratic elution; injection volume was 20 μL; flow rate was 0.4 mL/min). Dextran T-12.6, T-60, T-110, T-300, T-2000 and glucose were as standard [13].

2.5. $NaIO_4$ Oxidation

The polysaccharide (25.0 mg) was dissolved in 25mL 15 mM $NaIO_4$ solution and reacted in the dark condition. The solution was sampled every 6 h and followed by spectroscopy at 223 nm. Ethylene glycol was added to terminate the reaction when the consumption of IO_4^- was stable. Consumption of IO_4^- was calculated according to the standard curve that was made with $NaIO_4$ solution at 223 nm. The content of formic acid was determined by titration using 0.01 mol/L NaOH solution.

2.6. Monosaccharide Composition Analysis

50.0 mg polysaccharide was dissolved in 4.0 mL 2 mol/L trifluoroacetic acid (TFA) in a 10 mL test tube. The test tube was heated in water-bath at 100°C for 3 h to hydrolyze the polysaccharide to monosaccharides. The hydrolyzed solution was cooled to room temperature, and centrifuged for 8 min in 6000 r/min. The supernatant was collected and evaporated to dry under reduced pressure. The dried RP was dissolved in 600 μL hydroxylamine hydrochloride solution and then mixed by supersonic method for 1 min. The mixed solution was used to carry on the water bath for at 90°C 30 min, and cooled to room temperature. Then, 600 μL acetic anhydride was added to the cooled solution to carry on water bath at 90°C for 30 min again, cooled to room temperature. The solution was mixed with 500 μL chloroform, and next washed three times by 500 μL deionized water. The organic portion was collected and removed the excess water with anhydrous Na_2SO_4. Finally, the resultant solution was evaporated to dryness and re-dissolved in 500 μL chloroform for GC analysis. D-Glucose, D-galactose and D-xylose were also derivatized as standards. The analysis was performed using an Agilent 6890N chromatograph (USA) equipped with a capillary column of DB-5 (30 m × 0.25 mm × 0.25μm); The GC was run using the following conditions: injection temperature, 270°C; detector, flame-ionization detector; the temperature of detector, 270°C; the carrier gas, N2 at a flow rate 30 mL/min; sample injection was as the splitless method; the temperature program was that initial temperature was 150°C for 3 min, and as the rate of 3°C/min to 170°C, and holding for 5 min, and then as the rate of 5°C/min to 210°C, and holding for 5 min, and then as the rate of 10°C /min to 270°C, and holding for 5 min.

2.7. Methylation Analysis

Methylation analysis was carried out based on the previous method [14]. 10 mg RP which was sufficiently dried by P_2O_5 was depolymerized the methylated products and hydrolyzed by trifluoroacetic acid, and then acetylated to generate alditol acetate correspondingly. For speculating the branch structure, RP was further analyzed by partial acid hydrolysis method. RP was hydrolyzed by 0.05, 0.2 and 0.5 mol/L trifluoroacetic acid respectively, and evaporated to dry under reduced pressure. RP was transferred to a dialysis bag after removed the trifluoroacetic acid, and dialyzed by ultrapure water for 48 h to obtain the high molecular weight retentate RP-a and low molecular weight permeate RP-b respectively. The solution was dried and treated with methylation process, and then analyzed by GC-MS. GC/MS analyses were conducted using a Shimadzu GC/MS QP2000 system under the same GC conditions. An electron impact quadropolar system was used in the detection.

2.8. Infrared Spectra

2 mg polysaccharide was processed by pressed-disk technique for Infrared spectra analysis A Fourier transform infrared spectropho-tometer (PerkinElmer Spectrum 400

FT-IR, Massachusetts, USA) in the range of 4000–500 cm^{-1} were applied [15].

2.9. NMR Spectra

12 mg RP was dissolved in 400 μL D_2O for the analysis of NMR. The spectrogram of ^1H NMR and ^{13}C NMR were obtained with the following condition. ^1H NMR analysis, the frequency, 600 MHz; the temperature, 25°C. 13C NMR analysis, the frequency, 125 MHz; the temperature, 30°C. TMS was used as the internal standard.

3. Results and Discussion

3.1. Isolation and Purification of RP

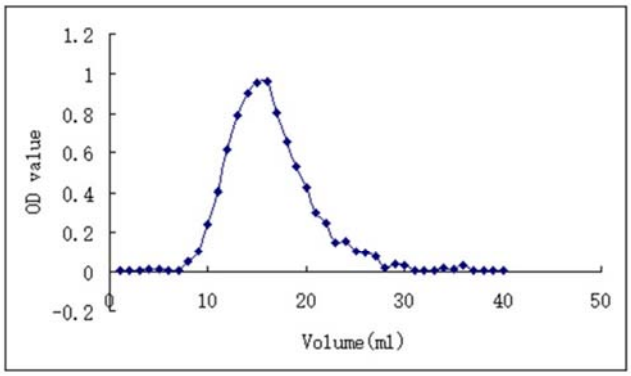

Figure 1. *Size-exclusion chromatography of RP on a Sephadex G-100 column eluted with water.*

Crude polysaccharide (31.25 g) was extracted from crude powder of *R. palmatum* by water-extraction and alcohol-sedimentation method, and then crude polysaccharide was further purified by AB-8 macroporous resin and Sephadex-G 100 column chromatography. As a result, 1.05 g pure polysaccharide (RP) was obtained. The absorbance was measured at 490 nm by phenol-sulfuric acid method. The elution curves were shown in Figure 1.

3.2. Molecular Weight Determination

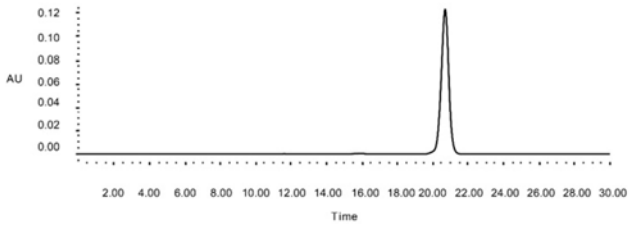

Figure 2. *GPC chromatogram of RP on an Ultrahydrogel 1000 column (7.8 mm×300 mm).*

As showed in Figure 2, GPC chromatogram showed that the chromatographic peak of RP was single symmetrical peak of a homogeneous component. Based on the standard curve which was calculated by dextran (Y=12.171-0.4476X, r=0.9996) and the retention time of samples, the relative molecular weight of RP was determined to be 1.21×10^4 Da. The UV scanning in 260nm and 280nm wavelength shown that RP had no absorption, and the ninhydrin reaction was negative, which indicated that RP did not contain nucleic acid and protein.

3.3. $NaIO_4$ Oxidation Reaction

The glycosidic linkage locations of RP were preliminarily determined by consumption of NaIO4 and production of formic acid. The results showed that 1 mol of sugar residues consumed 0.64 mol of NaIO4 and produced 0.08 mol of formic acid. Meanwhile, the consumption of $NaIO_4$ was two times higher than the production of formic acid, which indicated that RP contained linkages which could produce formic acid such as (1→6, 1→) linkage, but also contained some amount of linkage types which only could consume NaIO4 such as (1→4) linkage or some amount of linkage types which could not be oxidized by $NaIO_4$ such as (1→3, 6) linkage.

3.4. Monosaccharide Composition Analysis

In order to obtain more information about the monosaccharide residues of RP, the product of $NaIO_4$ oxidation was degraded by Smith degradation method and then analyzed by GC. The chromatograms of standard monosaccharides and degradation products were shown in Figure 3. The results indicated that RP was mainly consist of galactose as well as a small amount of glucose and xylose, whose molar ratio was 5.1: 1: 1.

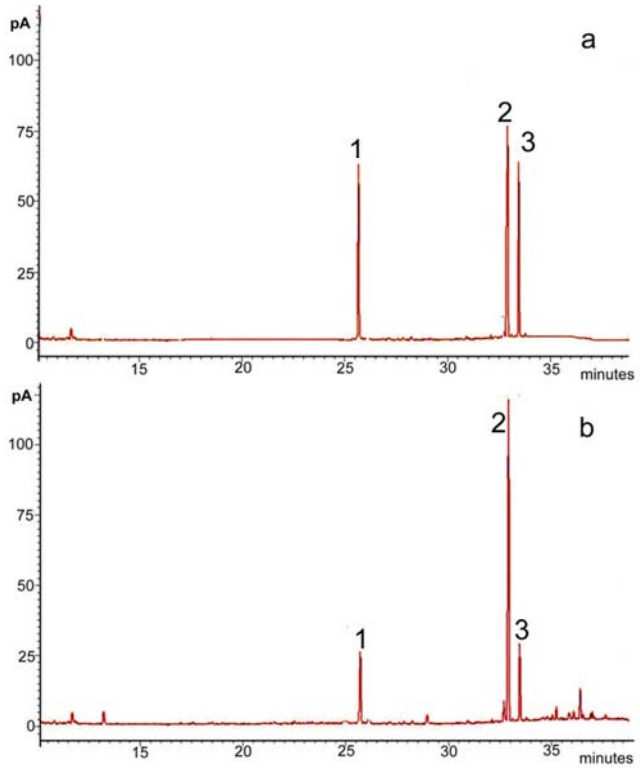

Figure 3. *GC analysis of acetylated standard monosaccharide (a) and acetylated RP (b), 1. Xylose 2. Galactose 3. Glucose.*

3.5. Methylation Reaction

To further determine the linkage types between

monosaccharide residues, RP was permethylated, hydrolyzed and converted into partially methylated alditol acetates for GC-MS analysis. The results were shown in Table 1, the backbone structure of the sugar chain was mainly consist of 1→4 linked galactose (Gal), 1→ terminal xylosel (Xyl) and 1→3, 6 linked galactose (Gal), small amounts of 1→6 linked glucose (Gal), 1→3 linked glucose (Glc) and galactose (Gal). In Mass spectrum, m/z 43 was the base peak of methylated acid sugar esters (PMAA), and was formed due to the loss of acetyl ion (CH3CO+). The m/z 145 and 259 fragments were the characteristic peaks of hexose mass spectrum, while the erythritol and glycerol were generated.

Table 1. *Methylation analysis of RP.*

Methylated alditol	Mainion ion fragment (m / z)	Type of linkage	Molar ratio
2,4,6--tri-O-methyl galactitol	43 45 87 101 117 129 161 259	→3) Gal→1	3.0
2,3,6--tri-O-methyl galactitol	43 45 87 99 101 113 117 161 233	→4) Gal→1	41.0
2,4--tri-O-methyl galactitol	43 73 85 117, 129, 189, 233.	→3, 6) G alp→1	15.8
2,4,6-tri-O-methyl glucitol	43 45 87 101 117 129 161	→3) Glc→1	3.6
2,3,4-tri-O-methyl glucitol	43 87 99 101 117 129 189	→6) Glc→1	10.4
2,3--tri-O-methyl Xyl	43 71 87 101 117 129 143 161	Xyl→1	26.2

The further research on branch structure of RP was shown in Table 2. After partial acid hydrolysis, the macromolecular fragment RP-a was the RP backbone structural components which consisted of galactose and glucose. Thus, the backbone structure of RP was composed of galactose and glucose alternately. The analysis by GC-MS for RP-b was shown to be 1→ terminal xylose. Because the RP-b was the lower molecular weight part with partial acid hydrolysis, so it was hydrolyzed as a branched chain of RP.

Table 2. *Methylation analysis of RP-a and RP-b.*

Group	Methylated alditol	Mainion ion fragment (m / z)	Type of linkage	Molar ratio
RP-a	2,4,6--tri-O-methyl galactitol	43 45 87 101 117 129 161	→3) Gal→1	4.3
	2,3,6--tri-O-methyl galactitol	43 45 87 99 101 113 117 161 233	→4) Gal→1	58.4
	2,4--tri-O-methyl galactitol	43 73 85 117 133 147 159	→3，6) G al→1	22.5
	2,3,4-tri-O-methyl glucitol	43 87 99 101 117 129 189	→6) Glc→1	14.8
RP-b	2,3--tri-O-methyl Xyl	43 71 87 101 117 129 143 161	Xyl→1	100

3.6. Infrared Spectroscopy Analysis

To characterize polysaccharide fractions, the characteristic absorption of RP was performed in the range of 4000-400 cm^{-1} by IR. The result was shown in Figure 4. It is indicated that distinct polysaccharide characteristic bands appeared at 3435, 2951, 1648, 1617, 1413 and 1101 cm^{-1}. The wide and strong absorption band at 3435 cm^{-1} was the O-H stretching vibration of sugar; resulting from intermolecular and intramolecular hydrogen bonding [16]. A weak peak at 2951 cm^{-1} was caused by C-H asymmetric stretching vibration; The peak at 1617 cm-1 was caused by the H-O-H bending of water adsorbed on the polysaccharide sample; The peak near 1413 cm-1 was caused by the vicinity of deformation vibration of CH_2; the peak near 1101 cm^{-1} 1was attributed to the stretching vibration of C-O-C and the deformation vibration of O-H in C-O-H bond [17]. 955 cm^{-1} was caused by the symmetric stretching vibration of the furan ring; 779 cm^{-1} was caused by the stretching vibration of the pyran ring. Hence, monosaccharide residues in RP was appeared in form of furan ring and pyran ring. The result is consistent with monosaccharide composition analysis.

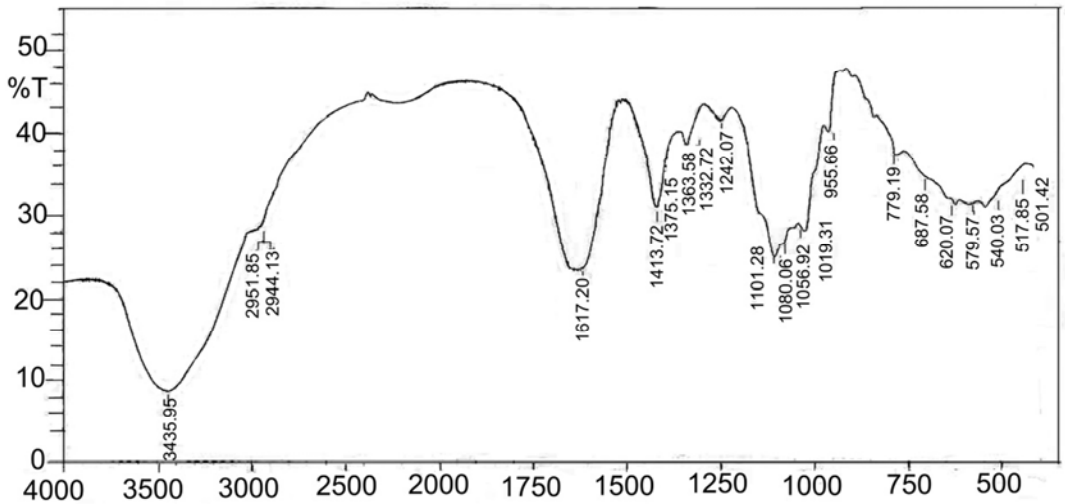

Figure 4. *The FT-IR spectrum of RP.*

3.7. NMR Analysis

The structural feature of RP was also elucidated by NMR spectral analysis. The spectrums were shown in Figure 5. The chemical shifts at δ 4.30-5.90 ppm in the ^{1}HNMR spectrum could be assigned to the typical signals of anomeric protons. In the spectrum, three main signals at δ 5.47, 5.14 and 4.47 in the anomeric proton region indicated that RP was mainly composed of three types of monosaccharides. Among them, one signal at δ 4.47 was assigned to the configuration of β-glycosidic bond and others were assigned to the configuration of α-glycosidic bond. The strong signal at around 4.8 ppm was attributed to the chemical shifts of D_2O.

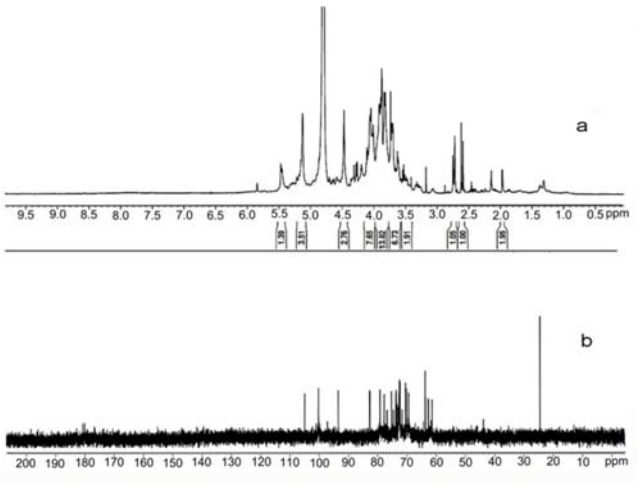

Figure 5. *The ^{1}H NMR spectrum (a) and ^{13}C NMR spectrum (b) of RP.*

Correspondingly the ^{13}C NMR spectrum showed three carbon signals of δ 104.966, 100.244 and 93.454 in anomeric region, which were assigned to β-xylose, α-galactose and α-glucose, respectively. This is consistent with those obtained in GC and GC-MS analysis. In the non-anomeric carbon region, a large number of signals accumulated in the range of δ 61~77. The absorption peak in δ 82.6 indicated that the substitution occurred on C3 of glucose; the appears of signal δ79.19 indicated that substitution occurred on C4 of galactose residues; the strong absorption peak of signal δ 82.6 indicated that the substitution occurred on C3 of glucose residue; the presence of signal δ 63.6 indicated that sugar chain contained fructofuranosyl units; the strong absorption peak at δ 62.6 indicated that C6 was not substituted in most glucose.

The combined results from the analysis of NaIO4 oxidation, Smith degradation, IR, GC-MS and NMR indicated that the backbone chain of RP was composed of galactose linked by 1→4 and 1→3,6 glycosidic bonds as well as glucose linked by 1→6 glycosidic bonds, and the branch chains were attached to backbone chain by xylose linked by 1→ terminal glycosidic bonds. Namely, four 1→4 galactose were linked with one 1→ 3, 6 galactose and one 1→6 glucose, and each repeating unit had a branched chain consisting of a α- 1→ xylose, which was linked with the C-6 of galactose backbone chain.

4. Conclusion

In conclusion, a polysaccharide from *R. Palmatum* (RP) was obtained in this study. The relative molecular weight of RP was 1.21×10^4. Through structural characterization analysis, it is demonstrated that the backbone chain of RP is composed of glucose linked by α-(1→4) galactose, α-(1→3,6) galactose and α-(1→6) glucose, the branch chains are attached to backbone chain by β-1→terminal xylose. RP was built up of β-xylose, α-galactose and α-glucose residues in the molar ratio of 1:5.1:1. This study provided useful information for further study of the polysaccharide from *R. palmatum*.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 81274013 and 81473315), Key National Natural Science Foundation of China (No. 81130069). All the author did not have any potential conflict of interest.

References

[1] Sun, M.; Li, L.; Wang, M.; van Wijk, E.; He, M.; van Wijk, R.; Koval, S.; Hankemeier, T.; van der Greef, J.; Wei, S., Effects of growth altitude on chemical constituents and delayed luminescence properties in medicinal rhubarb. *Journal of photochemistry and photobiology. B, Biology* 2016; 162: 24-33.

[2] Ren;, G.; Li;, L.; Hu;, H.; Li;, Y.; Liu;, C.; Wei, S., Influence of the Environmental Factors on the Accumulation of the Bioactive Ingredients in Chinese Rhubarb Products. 2016;11(5): e0154649.

[3] Sun, W.; Zhang, X.; Zhang, Z.; Zhu, R., Data fusion of near-infrared and mid-infrared spectra for identification of rhubarb. *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy* 2017; 171: 72-79.

[4] Zhu, T.; Liu, X.; Wang, X.; Cao, G.; Qin, K.; Pei, K.; Zhu, H.; Cai, H.; Niu, M.; Cai, B., Profiling and analysis of multiple compounds in rhubarb decoction after processing by wine steaming using UHPLC-Q-TOF-MS coupled with multiple statistical strategies. *Journal of separation science* 2016; 39 (15): 3081-90.

[5] Lin, T. J.; Lin, C. F.; Chiu, C. H.; Lee, M. C.; Horng, J. T., Inhibition of endosomal fusion activity of influenza virus by Rheum tanguticum (da-huang). *Scientific reports* 2016; 6: 27768.

[6] Liu, Y.; Li, L.; Xiao, Y. Q.; Yao, J. Q.; Li, P. Y.; Yu, D. R.; Ma, Y. L., Global metabolite profiling and diagnostic ion filtering strategy by LC-QTOF MS for rapid identification of raw and processed pieces of *Rheum palmatum* L. *Food chemistry* 2016; 192: 531-40.

[7] Takeoka, G. R.; Dao, L.; Harden, L.; Pantoja, A.; Kuhl, J. C., Antioxidant activity, phenolic and anthocyanin contents of various rhubarb (Rheumspp.) varieties. *International Journal of Food Science & Technology* 2013; 48 (1): 172-178.

[8] Zheng, Q. X.; Hai-Feng, W. U.; Jian, G.; Nan, H. J.; Chen, S. L.; Yang, J. S.; Xu-Dong, X. U., Review of Rhubarbs: Chemistry and Pharmacology. *Chinese Herbal Medicines* 2013; 5 (1): 9-32.

[9] Tang, J.; Nie, J.; Li, D.; Zhu, W.; Zhang, S.; Ma, F.; Sun, Q.; Song, J.; Zheng, Y.; Chen, P., Characterization and antioxidant activities of degraded polysaccharides from Poria cocos sclerotium. *Carbohydrate polymers* 2014; 105: 121-6.

[10] Zhang, X.; Liu, L.; Lin, C., Isolation, structural characterization and antioxidant activity of a neutral polysaccharide from Sisal waste. *Food Hydrocolloids* 2014; 39: 10-18.

[11] Hu, H.; Liang, H.; Wu, Y., Isolation, purification and structural characterization of polysaccharide from Acanthopanax brachypus. *Carbohydrate polymers* 2015; 127: 94-100.

[12] Ye, H.; Zhou, C.; Li, W.; Hu, B.; Wang, X.; Zeng, X., Structural elucidation of polysaccharide fractions from brown seaweed Sargassum pallidum. *Carbohydrate polymers* 2013; 97 (2): 659-64.

[13] Wen, L.; Lin, L.; You, L.; Yang, B.; Jiang, G.; Zhao, M., Ultrasound-assited extraction and structural identification of polysaccharides from Isodon lophanthoides var. gerardianus (Bentham) H. Hara. *Carbohydrate polymers* 2011; 85 (3): 541-547.

[14] Anumula, K. R.; Taylor, P. B., A comprehensive procedure for preparation of partially methylated alditol acetates from glycoprotein carbohydrates. *Analytical biochemistry* 1992; 203 (1): 101.

[15] Wang, X. T.; Zhu, Z. Y.; Zhao, L.; Sun, H. Q.; Meng, M.; Zhang, J. Y.; Zhang, Y. M., Structural characterization and inhibition on alpha-d-glucosidase activity of non-starch polysaccharides from Fagopyrum tartaricum. *Carbohydrate polymers* 2016; 153: 679-85.

[16] Nep, E. I.; Carnachan, S. M.; Ngwuluka, N. C.; Kontogiorgos, V.; Morris, G. A.; Sims, I. M.; Smith, A. M., Structural characterisation and rheological properties of a polysaccharide from sesame leaves (Sesamum radiatum Schumach. & Thonn.). *Carbohydrate polymers* 2016; 152: 541-7.

[17] He, P. F.; He, L.; Zhang, A. Q.; Wang, X. L.; Qu, L.; Sun, P. L., Structure and chain conformation of a neutral polysaccharide from sclerotia of Polyporus umbellatus. *Carbohydrate polymers* 2017; 155: 61-67.