Research Article

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Content and composition analysis of polysaccharides from *Blaps rynchopetera* and its macrophage phagocytic activity

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Abstract: *Blaps rynchopetera* Fairmaire has a long history of use as a folk medicine in China for treating fever, cough, gastritis, boils, and tumors. In the present study, the content analyses, monosaccharide composition analyses, and the macrophage phagocytic activity of *rynchopetera* polysaccharides (RPS) were reported. *B. rhynchoptera* is rich in polysaccharides (content value 3.97%). Through PMP (1-phenyl-3-methyl-5-pyrazolone) pre-column derivatization and high performance liquid chromatography (HPLC) testing, the results showed that RPS consist of 8 known monosaccharides, including D-mannose (Man), Rhamnose (Rha), D-glucuronic acid (GlcUA), D-galacturonic acid (GalUA), D-glucose (Glc), D-galactose (Gal), Arabinose (Ara), and Fucose (Fuc), with the total content of 171.70 mg g^{-1} and Glc has the highest content of 45.40 mg g^{-1}. The phagocytic ability of mouse peritoneal macrophages was investigated after RPS stimulating alone and combined with lipopolysaccharide (LPS). RPS played an important role in the engulfment of mouse peritoneal macrophages and can significantly enhance the phagocytic ability of macrophages. However, no synergistic effects were observed when RPS combined with LPS.

Keywords: *Blaps rynchopetera*, polysaccharides, content determination, monosaccharide composition, macrophage phagocytic activity

1 Introduction

Polysaccharide exists in almost all organisms, with diverse biological activities such as antitumor, antioxidant, antidiabetic, radiation protecting, antiviral, hypolipidemic, immunomodulatory activities, antiaging, and so on [1–4]. Based on a large number of basic research and application development, polysaccharide products, in addition to various forms of functional food [5], health care products [6], and auxiliary drugs [7], such as heparin, chondroitin sulfate, hyaluronic acid, chitin, and amylum, have been widely used in clinical practice as drugs or medical materials. However, polysaccharides in different organism may have different composition, structure, and activity, which would worth more attention and investigation.

B. rynchopetera, also called 'smelly fart bug', is widely used for fever, cough, gastritis, boils, and even tumors in Yunnan province for a long time [8,9]. This medical insect belongs to the family Tenebrionidae (*Coleoptera*), which consists of 12 subfamilies, more than 1,500 genera, and about 25,000 species with a global distribution, and 9 subfamilies, more than 280 genera, and nearly 1,300 species in China [10]. In the early stage, we carried out a series research on material basis and biological activities [8,9,11–14]. *B. rynchopetera* was rich in phenolics, especially catechol substances [8,12], and phenolics had strong *in vitro* and *in vivo* antioxidant effects [15]. In this paper,

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the preparation, content determination, monosaccharides composition, and macrophage phagocytic activity of polysaccharides from *B. rynchopetera* (RPS) were reported.

2 Materials and methods

2.1 Reagents and materials

TU-1901 UV-Vis Spectrophotometer (Beijing Puyi General Instrument Co., Ltd.); Agilent 1100 High Performance Liquid Chromatography, including G1313AALS Automatic sampler, G1315A/B DAD Detector, and 1100 Chromatography Workstation (Agilent, USA); Type 5510 CO_2 incubator (US NUAIRE company); CKX41 inverted microscope (Olympus Co., Ltd.).

All the chemical reagents were AR grade. All the monosaccharide standards, including mannose (Man), rhamnose (Rha), glucuronic acid (GlcUA), galacturonic acid (GalUA), glucose (Glc), galactose (Gal), xylose (Xyl), arabinose (Ara), and fucose (Fuc), were purchased from China Institute for the Control of Pharmaceutical and Biological Products or Guizhou Dida Biotechnology Co. Lipopolysaccharide (LPS) (Sigma, Lot number: 026M4021V).

B. rynchopetera materials were purchased from the Farmers' Market in Dali City, Yunnan Province, China. The original medicinal material was identified as *B. rynchopetera* Fairmaire by Professor Ren Guodong of Hebei University and the specimen was kept in the Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical R&D.

2.2 Extraction and purification of RPS

The adults of *B. rynchopetera* were collected, dried, and crushed. Insect powder (1.0 Kg) was defatted with petroleum ether, then reflux extracted twice with 80% ethanol and filtered. The filter residue was reflux extracted with distilled water twice (1 h each time). Extracts solution was combined and concentrated to a certain volume and cooled, then oscillated with Sevage reagent (chloroform: *n*-butanol = 5:1) and centrifuged at 4,000 rpm, the upper aqueous solution was separated, repeating three times until it was cleared of protein layer. After that, 95% ethanol was added to the aqueous solution and the alcohol content was adjusted to 80%, placed at 4°C for 24 h, then filtered and the precipitate was collected. The precipitate was washed several times with anhydrous ethanol, acetone, and ether successively, dried at 60°C, then the gray-white refined polysaccharide, RPS was obtained.

2.3 Identification of RPS

Taking 1.0 mg of the above purified RPS into a plug tube, dissolving with 2.0 mL distilled water, and mixing with 2.0 mL α-naphthol solution (1 mg mL^{-1}), 1 mL of concentrated H_2SO_4 was slowly added along the tube wall. The result showed that there was a purplish red ring at the junction of RPS solution and sulphuric acid.

2.4 Content determination of polysaccharide

Polysaccharide content in *B. rynchopetera* was determined with phenol-sulfuric acid method using glucose as the standard.

B. rynchopetera (0.5 g) was accurately weighed and placed in a round bottom flask, then defatted with petroleum ether, ultrasonic extracted with 80% ethanol for 30 min, filtered and washed once with hot 80% ethanol and dried. The filtered residue was ultrasonic extracted with distilled water for about 30 min, repeated 4 times, combined with filtrates to a volumetric flask to constant volume as 200.0 mL, shook well, and the sample solution for content determination was obtained.

In a plug test tube, 2.0 mL test solution and 1.0 mL prepared phenol reagent were added and mixed, then 5.0 mL concentrated sulfuric acid was added quickly and blended, stayed at room temperature for 5 min, heated in a 60°C water bath for 10 min, then cooled to room temperature. The absorbance value of colored solution was determined at 490 nm in triplicates. The procedure was repeated with 2.0 mL distilled water used as blank.

2.5 Monosaccharide composition and content analysis of RPS

PMP pre-column derivatization HPLC method was used to analyze the monosaccharide composition and content of RPS; 9 different standard monosaccharides, including mannose, rhamnose, glucuronic acid, galacturonic acid,

glucose, galactose, arabinose, fucose, and xylose were used as control.

Nine different standard monosaccharide substances were precisely weighed respectively and resolved together to obtain the mixed standard monosaccharide solution.

The purified RPS was hydrolyzed before content and composition analyses by sealed heating [16]: RPS (10.0 mg) was accurately weighed in an ampoule bottle, mixed with 1.0 mL of 4 mol L^{-1} trifluoroacetic acid (TFA), then filled with nitrogen and sealed. It was hydrolyzed at 110°C for 2 h and cooled to room temperature. The hydrolyzed solution was vacuum distilled to solvent-free, diluted with pure water, fixed volume to 5.0 mL, and then centrifuged. The supernatant was the hydrolyzed monosaccharide solution for further derivatization.

Derivatization of mixed standard monosaccharide substance and hydrolyzed RPS were as follows [16]: 200 μL sample solution was precisely measured into the test tube, 200 μL of 0.3 mol L^{-1} NaOH solution was added, shook well, 200 μL 0.5 mol L^{-1} PMP methanol solution was added, vortex mixing for 30 s, then reacted 70 min in a constant temperature water bath at 70°C, cooled to room temperature, and then neutralized using 200 μL of 0.3 mol L^{-1} hydrochloric acid. After that, 2 mL of chloroform was added, vortex mixing for 3 min, centrifugated for 5 min, the organic layer was discarded, the supernatant was extracted repeatedly 3 times, and the water phase was filtered through 0.45 μm filter membrane before HPLC analysis.

The HPLC analysis of the derivatized monosaccharide was carried out using the following chromatographic conditions [17]: Phenomenex Gemini C18, 110 Å column (4.6 × 250 mm, particular size 5 μm); mobile phase: acetonitrile-0.05 mol L^{-1} phosphate buffer (17: 83, pH 6.7); column temperature: 30°C; flow rate: 0.8 mg mL^{-1}; detection wavelength: 250 nm; and injection volume: 10 μL.

2.6 Phagocytes assay

Purified peritoneal macrophages were obtained as described [18]. The macrophages were added into 96-well culture plates and divided into a cell control group (cell and medium only), an LPS model group with final concentration of 10 μg mL^{-1}, RPS groups (500, 250, 125, 62.5, 31.25 μg mL^{-1}), and combined groups with both LPS and RPS treatment, 3 times in parallel. After cultured, the supernatant was discarded and neutral red solutions were added and incubated. Then the supernatant was removed, and the cells in 96-well plates were washed with PBS buffer. After cell lysate-acidic ethanol solution was added and the plate was placed at 4°C overnight, the absorbance was measured at 540 nm.

2.7 Statistical analysis

Statistical significance was analyzed by one-way ANOVA using SPSS 19.0. After the homogeneity test of the variance, the experimental data of the variance was statistically analyzed by the pairwise LSD method and the rank sum test. p value <0.05 was regarded as statistically significant.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 The maximum absorption wavelength for polysaccharide content determination

Standard glucose solution (1.0 mg mL^{-1}) and polysaccharide extract solution were colored and scanned at 400–600 nm to determine the absorbance value (A); distilled water with same operation was used as blank. The results showed that both solutions had maximum absorption at 490 nm (Figure 1).

3.2 Standard curve for polysaccharide content determination

A series of glucose standard solution was prepared, and the absorbance values were measured at 490 nm in triplicates. Drawing relational graph with the absorbance values as Y axis and the concentration as X axis (Figure 2), the standard curve equation was obtained as $Y = 14.482X + 0.0221$ ($R^2 = 0.9991$), with a linear range from 0.005 to 0.05 mg mL^{-1} (Figure 2).

3.3 Conversion factor of RPS

Before RPS content determination, the conversion factor was tested [17]. The absorbance values of the colored RPS

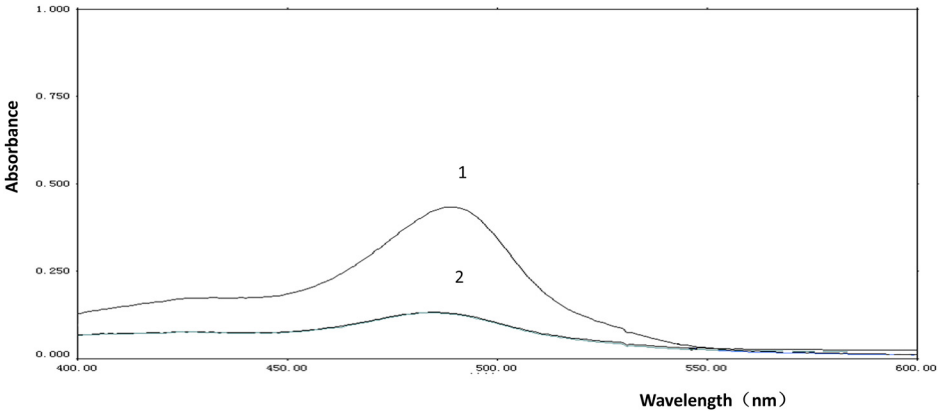

Figure 1: UV absorption curve of *B. rynchopetera* extract and glucose standard solution. (1) Glucose standard solution, (2) sample solution of *B. rynchopetera* extract.

solution were determined at 490 nm, then A value was substituted into the above equation to calculate the glucose content, and the conversion factor was calculated according to the following formula: $f = W/(C \times D)$, in which "W" is the amount of RPS (mg), "C" is the calculated concentration (mg mL^{-1}), and "D" is the dilution multiple. The RPS conversion factor "f" was determined and calculated as 5.05 (n = 3).

3.4 Methodological investigation of polysaccharide content determination

The absorbance value of colored glucose standard solution was determined at 490 nm (6 times in parallel) to evaluate the precision, the RSD = 1.6%, indicating the method has a good precision.

The absorbance values of glucose standard, RPS, and polysaccharide extract solution were measured at the points of been colored for 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 min to test the stability, respectively. The data showed good sample stability with RSD of 1.41, 1.30, and 1.18% for glucose, RPS, and polysaccharide extract solution, respectively.

Five samples with same amount were taken, extracted, and colored as above and the absorbance values were tested for reproducibility. The absorbance values were 0.495, 0.483, 0.457, 0.476, and 0.473, with RSD 3.79%, indicating a receivable reproducibility for the method.

Taking 6 powder samples of *B. rynchopetera*, approximately 200 mg each, adding about 5.0 mg of glucose standard, then extracting the polysaccharide and testing the absorbance values with the same method, the recovery rates were inspected. Calculated according to the standard curve equation, the results met the requirement with the average recovery rate as 97.6% and the RSD = 2.26% (Table 1).

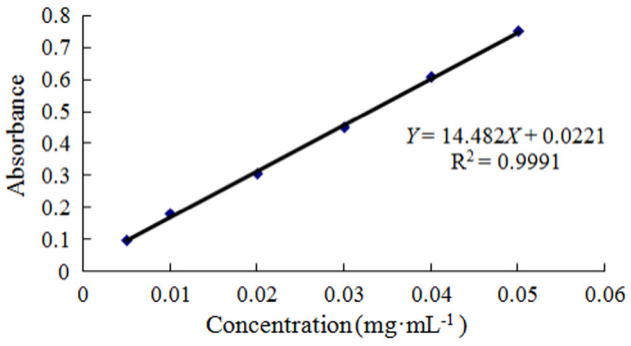

Figure 2: Standard curve of glucose.

3.5 Polysaccharide content in *B. rynchopetera*

Three polysaccharide extract solutions of *B. rynchopetera* were prepared and colored and the absorbance values tested; the polysaccharide content was calculated through the standard curve equation of item 3.2 and the following formula:

Polysaccharide content (mg g^{-1}) = $C \times D \times f/W$, in which "W" is the amount of sample (g), "C" is the calculated concentration (mg mL^{-1}), "D" is the dilution multiple of solution, and "f" is conversion factor obtained from item 3.3. The content of polysaccharide in *B. rynchopetera* was determinate as 39.7 mg g^{-1} (Table 2).

Table 1: Recovery experiment result for polysaccharide determination of *B. rynchopetera* extract

No.	Sample weight (g)	Amount of RPS (mg)	Adding standard matter amount (mg)	Measured amount (mg)	Recovery rate (%)	Average value (%)	RSD (%)
1	0.2040	7.874	4.91	12.55	95.2		
2	0.2036	7.859	5.25	12.86	95.3		
3	0.2037	7.863	5.21	13.11	100.7	97.6	2.26
4	0.2039	7.871	5.23	12.93	96.7		
5	0.2043	7.886	5.17	12.97	98.3		
6	0.2031	7.840	5.42	13.21	99.1		

Table 2: Result for polysaccharide determination of *B. rynchopetera* extract

Sample weight (g)	Average absorbance value (*A*)	Content (mg g^{-1})	Average content (mg g^{-1})	RSD (%)
0.2011	0.485	39.7		
0.2052	0.502	40.3	39.7	1.69
0.1990	0.472	39.0		

3.6 Monosaccharide composition analysis of RPS

The HPLC spectra of 9 standard monosaccharide derivatives were showed (Figure 3a); 9 peaks were separated well, except galactose, xylose, and arabinose (peaks 7, 8, 9) not been baseline separated. The hydrolyzed RPS derivatives spectra showed that there were 8 known monosaccharides, mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, arabinose, and fucose, but no xylose was detected (Figure 3b).

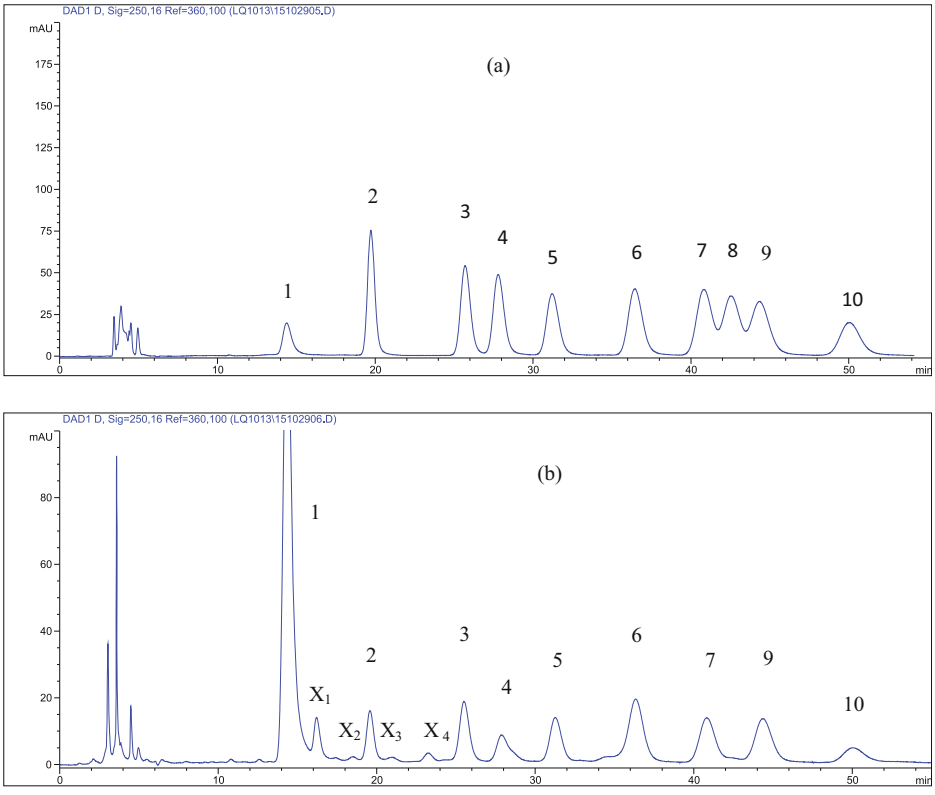

Figure 3: HPLC spectra of mixed standard monosaccharide derivatives (a) and hydrolyzed monosaccharide derivatives of RPS (b) (1. PMP reagent; 2. Man; 3. Rha; 4. GlcUA; 5. GalUA; 6. Glc; 7. Gal; 8. Xyl; 9. Ara; 10. Fuc; X = Unknown).

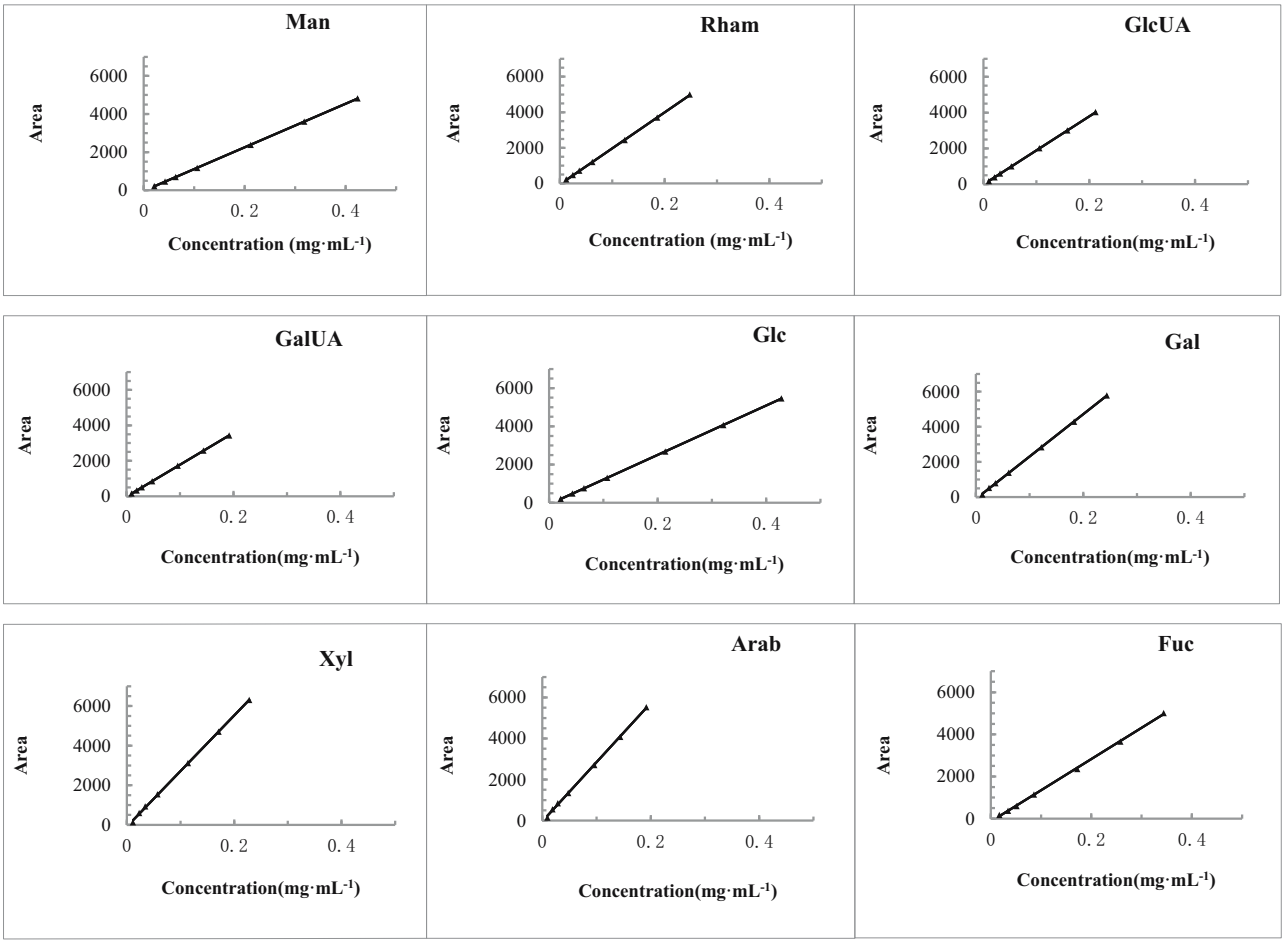

Figure 4: Standard curves of 9 monosaccharides.

3.7 Content determination of monosaccharide in RPS

Series solutions of 9 mixed monosaccharide standards with different concentration were prepared and derived, then processed for HPLC analyses with the item 2.5 testing chromatographic conditions. Through SPSS analysis, the standard curves and the equations between concentration (X) and peak area (Y) for 9 monosaccharides were obtained (Figure 4 and Table 3).

Methodological study on determination of monosaccharide content in RPS indicated that the method was accurate, stable, and reliable. After derivatization, the peak area of the mixed monosaccharides standard substance

Table 3: Standard curve equation of 9 monosaccharides

Monosaccharide	Linear equation	Correlation coefficient (R^2)	Linear range (μg mL^{-1})
Man	$Y = 11433X - 32.634$	1	0.0212–0.424
Rha	$Y = 20204X - 48.595$	0.9999	0.0124–0.248
GlcUA	$Y = 19040X - 20.109$	1	0.0106–0.212
GalUA	$Y = 17955X - 15.488$	1	0.0096–0.192
Glc	$Y = 12913X - 74.918$	1	0.0214–0.535
Gal	$Y = 24061X - 96.033$	0.9998	0.0122–0.244
Xyl	$Y = 28047X - 86.409$	0.9995	0.0114–0.228
Ara	$Y = 28910X - 47.975$	0.9994	0.0096–0.192
Fuc	$Y = 14835X - 142.93$	0.9994	0.0112–0.224

was tested, 6 times in parallel to evaluate the precision; the RSD of each monosaccharide was less than 2.0% (Table 4). The RPS solution was hydrolyzed, derivatized, and analyzed with above HPLC conditions at 0, 2, 4, 6, 8, and 12 h to evaluate the stability. The testing data showed good sample stability with RSD not more than 3% (Table 4). Six samples of RPS were accurately weighed, hydrolyzed, derivatized, and analyzed in order to test the reproducibility. And the calculated average content of monosaccharide with RSD was not more than 3%, indicating a receivable reproducibility for the method (Table 4).

Taking 6 samples of the hydrolyzed RPS and adding the mixed solution of monosaccharides, derivatization and HPLC determination were carried out; the results met the requirement with the average recovery rate and the RSD, respectively (Table 5).

At last, sample of RPS was hydrolyzed, derivatized, and determined using same HPLC conditions, three times each. The content of each monosaccharide in RPS was calculated by substituting the average peak area into the above equation and the results are shown in Table 6. The total content of monosaccharide was 171.70 mg g^{-1}; 8 monosaccharides content varied from 11.47 to 45.40 mg g^{-1} and glucose was the richest in RPS.

3.8 Effect of RPS on phagocytosis of macrophages

The phagocytic capability of mouse peritoneal macrophages was investigated by RPS stimulation alone and co-stimulation with LPS [19] for evaluating the immunological activity. Experimental result indicated that RPS could significantly enhance the phagocytic ability of macrophages. The effect was a little better than that of the positive drug LPS, but did not show a good dose-dependent relationship (Figure 5a), and there was no tendency of synergistic enhancement when co-stimulatied with LPS (Figure 5b).

Table 5: Recovery rates of 8 monosaccharides content of RPS

Monosaccharide	Average recovery rate (%)	RSD (%)
Man	97.5	1.58
Rha	97.0	2.32
GlcUA	96.5	1.38
GalUA	98.9	2.60
Glc	100.8	2.03
Gal	98.0	2.13
Ara	101.7	1.78
Fuc	98.3	1.95

4 Discussion

In this report, the RPS were prepared by water extraction, alcohol precipitation, and Sevage method getting rid of protein, and the content of polysaccharides was 39.7 mg g^{-1} determined by phenol-sulfuric acid method. Sun [20] optimized the extraction methods of three kinds of insect polysaccharides; the contents of dilute alkali extraction, protease hydrolysis extraction and water extraction were 5.95, 6.0, and 3.8%, respectively, which were basically consistent with our studies.

HPLC monosaccharide analysis results showed that RPS was rich in monosaccharide types, containing not only all the testing samples except xylose, but also other unknown ones, such as peaks X_1 to X_4 in Figure 3b between t_R 16 to 25 min. Meanwhile, there were other

Table 4: Methodology investigation result of monosaccharide content determination in RPS

Monosaccharide	Precision RSD (%)	Stability RSD (%)	Reproducibility RSD (%)
Man	0.81	0.66	1.90
Rha	1.37	0.19	0.69
GlcUA	1.37	1.50	1.19
GalUA	0.21	1.89	1.72
Glc	0.92	1.94	0.94
Gal	1.17	2.35	1.38
Ara	0.37	1.14	2.78
Xyl	—	—	—
Fuc	1.38	2.03	2.31

Table 6: Content determination result of monosaccharides in RPS (n = 3)

Monosaccharide	Retention time (min)	Average peak area	Content (mg g^{-1})
Rha	25.7	750.3	19.77
GlcUA	27.8	416.8	11.47
GalUA	31.2	650.6	18.55
Glc	36.4	1092.5	45.40
Gal	40.8	840.9	19.47
Xyl	42.6	—	—
Ara	44.3	895.7	16.32
Fuc	50.0	381.5	17.67
Total content			171.70

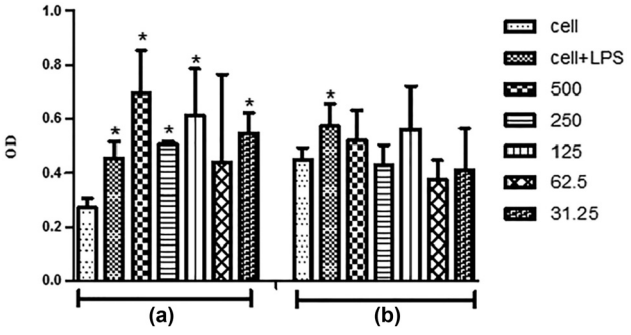

Figure 5: Effect of RPS on phagocytosis of the macrophages ($n = 3$; *, $p < 0.05$). (a) Stimulation with RPS alone, (b) co-stimulation with RPS and LPS.

non-sugar macromolecules in RPS; the total content of monosaccharides was 171.70 mg g^{-1}, and even the richest monosaccharide, glucose, had a content of 45.40 mg g^{-1}.

Immunomodulation is an important activity for polysaccharides [4]. Sun [20] reported that polysaccharide from *B. rynchopetera* had a wide range of effects on body-specific, nonspecific immunity, cellular immunity, and humoral immunity. In this study, the immunological activity of RPS was verified and our results show that RPS had significant effect to enhance the phagocytic ability of mouse macrophages.

B. rynchopetera has been used in the treatment of tumor in many areas of Yunnan Province, China. Immune regulation played an important role for tumor therapy and other medical application. Further study of biological activity, the identification of possible substance(s) of the non-sugar macromolecules in RPS, and the mechanism of RPS would be necessary to explore its medical potentials.

5 Conclusions

In this study, the content of polysaccharide was 39.7 mg g^{-1} in *B. rynchopetera* determined by phenol-sulfuric acid method. There were at least 8 familiar monosaccharides in RPS, which had the total content of 171.70 mg g^{-1}, with absence of xylose. RPS showed effective immunological activity for the enhancement of mouse macrophages phagocytosis. RPS is a complex institution composed of macromolecules with potential activity for disease treatment and worth for more investigation.

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Author contributions: Huai Xiao and Yu Zhao conceived and designed the experiments; Ying Wang, Qing Luo, Qian Lu, Wan-Xin Yu, Jin-Hu Chen, Yu-Jia Wang, and Mu-Ling Wang performed the experiments; Qian Lu and Yuan Hu analyzed the data; Ying Wang, Yin-He Yang, and Huai Xiao wrote the manuscript; and Yu Zhao polished it. Huai Xiao and Yu Zhao acquired funding for the research. All authors read and approved the final manuscript.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: All the data of this manuscript are available from the authors.

References

[1] Chen S, Ding R, Zhou Y, Zhang X, Zhu R, Gao XD. Immunomodulatory effects of polysaccharide from marine fungus *Phoma herbarum* YS4108 on T cells and dendritic cells. Mediat Inflamm. 2014;2014:738631.

[2] Huang SQ, Ning ZX. Extraction of polysaccharide from *Ganoderma lucidum* and its immune enhancement activity. Int J Biol Macromol. 2010;47(3):336–41.

[3] Qiu SL, Chen J, Qin T, Hu YL, Wang DY, Fan Q, et al. Effects of selenylation modification on immune-enhancing activity of garlic polysaccharide. PLoS One. 2014;9(1):e86377.

[4] Zeng P, Li J, Chen Y, Zhang L. The structures and biological functions of polysaccharides from traditional Chinese herbs. Progress in molecular biology and translational science. 2019;163:423–44.

[5] Bernaerts TMM, Gheysen L, Kyomugasho C, Kermani ZJ, Vandionant S, Foubert I, et al. Comparison of microalgal biomasses as functional food ingredients: focus on the composition of cell wall related polysaccharides. Algal Res. 2018;32:150–61.

[6] Zhang YL, Huang XX, Cai MQ. Advances of functions of antioxidation and relieving physical fatigue of *Lycium barbarum* polysaccharide. Journal of Shanghai Jiaotong University. 2015;35(6):911–4 and 925.

[7] García-González CA, Jin M, Gerth J, Alvarez-Lorenzo C, Smirnova I. Polysaccharide-based aerogel microspheres for oral drug delivery. Carbohydr Polym. 2014;117:797–806.

[8] Xiao H, Yin TP, Dong JW, Wu XM, Luo Q, Luo JR, et al. Five new phenolic compounds with antioxidant activities from the medicinal insect *Blaps rynchopetera*. Molecules. 2017;22(8):1301.

[9] Xiao H, Dong JW, Zhou DJ, Wu XM, Luo JR, Zhang CG, et al. Cytotoxicity of the defensive secretion from the medicinal insect *Blaps rynchopetera*. Molecules. 2018;23(1):10.
[10] Liu XL, Ren GD. 16S rDNA sequences and relationships of nine darkling beetles species. J Hebei Univ. 2004;24:399–405.
[11] Shi GR, Zhuang XL, Guo MX, Wu XM, Xiao H. Study on the *in vitro* antineoplastic activity of the extract from *Blaps rynchopetera* fairmaire. J Anhui Agric Sci. 2012;40(6):3387–8.
[12] Shan H, Luo Q, Xiao H. The polyphenols content analysis of *Blaps rynchopetera* fairmaire. Lishizhen Med Mater Med Res. 2016;27(3):532–4.
[13] Cui WB, Qian JF, Luo JR, Xiao H. Antioxidant activity of solvent extracts of *Blaps rynchopetera* fairmaire *in vitro*. Lishizhen Med Mater Med Res. 2013;24(7):1566–7.
[14] Luo Q, Cai L, Wu XM, Guo NN, Luo JR, Xiao H. Cyclodipeptides from medicinal insect *Blaps rynchopetera*. Chin Herb Med. 2015;46(16):2381–4.
[15] Hu XF, Ding ZB, Chen Y, Luo JF, He JM, Yin TP. Comparative study on the antioxidant activities of ten common flower teas from China. Open Chem. 2019;17:841–8.
[16] Wan Q, Wu XH, Fan HJ, Luo A, Chen Z, Huang XW, et al. Derivative chromatographic analysis of monosaccharides in hydrolyzed polysaccharide from *Cassia obtusifolia L*. and their composition characteristic. J Instrum Anal. 2014;33(11):1231–6.
[17] Shang XH, Qu CL, Zhang JP, Liu XY, Jiao BH, Zheng H, et al. Study on the extraction technology of the sea urchin shell polysaccharide and monosaccharide composition by pre-column derivatization HPLC. Lishizhen Med Mater Med Res. 2015;26(4):830–2.
[18] Tang M, Yu WX, Wu TQ, Ma XY, Liu H, Zhang CG, et al. Effects of periplaneta Americana extract Ento-A on immune function in immunosuppressed mice. Chin Pharmacol Bull. 2018;34(1):72–6.
[19] Amura CR, Kamei T, Ito N, Soares MJ, Morrison DC. Differential regulation of lipopolysaccharide (LPS) activation pathways in mouse macrophages by LPS-binding proteins. J Immunol. 1998;161(5):2552–60.
[20] Sun L. Isolation, purification and immunological activity of three insect polysaccharides. Beijing: Chinese Academy of Forestry; 2007.