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Structural Characteristics and the Antioxidant and Hypoglycemic Activities of a Polysaccharide from *Lonicera caerulea* L. Pomace

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Abstract: In this study, a novel polysaccharide, LPP, was obtained from *Lonicera caerulea* L. pomace by ultrasonic-assisted heating and was purified by Sephadex G-100. The structural characteristics of LPP showed that the molecular weight (Mw) was 8.53×10^4 Da; that it was mainly composed of galacturonic acid, followed by galactose; that it possessed the characteristic functional groups of polysaccharides; and that it had an absence of O-glycosidic bonds and crystalline and triple helix structures. Furthermore, LPP exhibited a favorable thermodynamic stability and antioxidant, hypoglycemic, and hypolipidemic activities in a dose-dependent manner in vitro, demonstrating that LPP can be used as an agent to regulate glycolipid metabolism. Additionally, the relationship between its bio-activities is discussed in this paper. The results revealed that the RP, •OH, and NO_2^- radicals had synergistic promoting effects, and polysaccharides with a strong antioxidant ability may have excellent hypoglycemic and hypolipidemic effects. Collectively, these results suggest that LPP has a strong bio-activity, and that *Lonicera caerulea* L. pomace can be used as a potential polysaccharide source.

Keywords: blue honeysuckle; polysaccharide; structural characterizations; glycolipid metabolism; correlation analysis

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1. Introduction

The prevalence of metabolic syndromes, including hyperglycemia, hypertension, hyperlipidemia, insulin resistance, and obesity, is expected to increase to 53% by 2035 [1]. According to statistics, one-third of the world's population is facing the crisis of being overweight or obese, and the highest incidence of adult and teenage obesity in the world is in Egypt, with about 35%, and the USA, with about 13% [2]. At present, Orlistat, which reduces intestinal fat absorption by inhibiting pancreatic lipase, and Sibutramine, which inhibits appetite, are common drugs on the market for obesity treatment, but these drugs have specific side effects [3]. Therefore, seeking a new method for intervening and preventing obesity has become a research hotspot. Polysaccharides have received much attention in recent years due to their safety, efficacy, and absence of side effects.

Lonicera caerulea L. (blue honeysuckle) is a perennial berry plant that belongs to the Caprifoliaceae family, with strong frost resistance (−46 °C) and a wide pH tolerance (5–8). It is mainly distributed throughout China, Japan, South Korea, and regions with a low temperature in North America [4,5]. In China, *L. caerulea* L. is mainly distributed in the Daxing'anling, Xiaoxing'anling, and Changbai Mountains, and the main varieties are the wild type and the artificially cultivated "Beilei" [6].

In recent years, with the development of science and technology, various active ingredients that can promote human health have been extracted from *L. caerulea* L. Recently, our team demonstrated the bio-activities of polyphenols isolated from *L. caerulea* L. However,

after extracting the polyphenols, the residue still contained many active components, such as polysaccharides. Results about extracted polysaccharides from *L. caerulea* L. pomace have not been reported, whereas extracted polysaccharides from other pomaces and waste products have been reported. For example, Canalejo, Guadalupe, Martínez-Lapuente, Ayestaían, and Pérez-Magariño [7] and Andreani, Karboune, and Liu [8] investigated the polysaccharides from white grape pomace and cranberry pomace, respectively. Gu et al. [9] extracted the acidic polysaccharides from *Annona squamosa* residue, which were found to inhibit the activity of α-amylase and α-glucosidase. Wu et al. [10] obtained two polysaccharides with hypoglycemic and immunomodulatory activities from *Rosa setate* × *Rosa rugosa* waste. These results demonstrate that the extraction of polysaccharides from *L. caerulea* L. pomace is feasible and could also improve the utilization of resources.

As a complex macromolecule, polysaccharides have many bio-activities, such as antioxidant, hypoglycemic, hypolipidemic, anti-tumor, and immunity-supporting properties [11–13]. Moreover, studies have shown that the properties of polysaccharides are closely related to the structure. Wang et al. [14] extracted three natural polysaccharides from *Flos Magnoliae* and analyzed their structure and function. The results showed that the polysaccharide FMP-2, with the highest yield and average molecular weight (Mw) of the three, exhibited excellent thermal stability and effective antioxidant activities, and could be used as a potential stabilizer and natural antioxidant. In addition, Fu et al. [15] identified the structural features and properties of polysaccharides extracted from the blue honeysuckle. They discovered that the Mw of the acidic heteropolysaccharide HEP-2 was 3.01×10^6 Da, and that it contained the presence of α- and β-pyranose rings and six sugar residues. Meanwhile, they demonstrated the hypoglycemic function of HEP-2 in vitro. Subsequently, this team studied the hypoglycemic activity of the degraded polysaccharides of the blue honeysuckle. The results showed that the polysaccharide PD70 had a more linear structure, a lower Mw, and longer side chains than the polysaccharide PD50, and it also possessed stronger α-amylase and α-glucosidase inhibition activity [16]. Therefore, analyzing the structure of *L. caerulea* L. pomace polysaccharides to evaluate their bio-activities is necessary.

In general, the accumulation of free radicals, which are the by-products of cellular metabolism, causes many diseases, such as diabetes, cancer, and obesity [17]. Therefore, exploring the correlation between free radical scavenging activities and the hypoglycemic and bile-acid-binding abilities of polysaccharides is necessary. In the current study, a novel polysaccharide, LPP, was extracted from the pomace of *Lonicera caerulea* L. after extracting the polyphenols through the co-fermentation of probiotics. The partial structures and physicochemical properties of LPP were characterized and reported for the first time in this report. Furthermore, the thermal stability, antioxidant activities, inhibition of α-amylase and α-glucosidase, and bile-acid-binding capacities of LPP were evaluated in vitro. The correlations between the bio-activities of the polysaccharide are also discussed in this paper, which provides a theoretical basis for the comprehensive utilization of *Lonicera caerulea* L. pomace.

2. Materials and Methods

2.1. Materials and Reagents

The results of our team's previous studies demonstrated that the microbial fermentation of *L. caerulea* L. had an effect on its polysaccharide structure and bioactivities [18]. Meanwhile, our team found that after extracting the polyphenols, the residue still contained many active components, such as polysaccharides. Therefore, in this study, the residual pomace (after the extraction of the polyphenols) obtained from *L. caerulea* L. by the co-fermentation of *Saccharomyces cerevisiae* W5 and *Lactobacillus rhamnosus* 6224 was used as the raw material, which was provided by the Key Laboratory of Microbiology, Heilongjiang University (Heilongjiang, China). The recovered pomace was freeze-dried, thoroughly ground and mixed, and then sealed and stored at room temperature, away from light, until use. The reagents used in the experiment are listed in Table S1.

2.2. Procedure for the Extraction and Purification of Polysaccharides

The *L. caerulea* L. pomace was mixed with deionized water (1:20, g/mL), and then ultrasonic extraction was carried out at 50 °C for 90 min. The purification was performed according to the methods in our previous report, with some modifications [19]. Briefly, the water extracts were centrifuged (12,000 rpm, 10 min) to gather the supernatant, which was mixed with 3 volumes of 95% ethanol overnight at 4 °C. Then, the liquid was centrifuged (12,000 rpm, 10 min). Subsequently, 80% (*w*/*v*) trichloroacetic acid (TCA) was added to the supernatant to achieve a final concentration of 4% (*w*/*v*), and the mixture was kept at 4 °C for 10 h, then centrifuged to remove protein. Afterward, the supernatant was precipitated by mixing it with 3 volumes of 95% ethanol overnight at 4 °C and centrifuging at 12,000 rpm at 4 °C for 10 min. After being dialyzed (with the Mw cut off of 8–12 kDa) at 4 °C for 2 d, the crude polysaccharide was obtained, then loaded onto a Sephadex G-100 gel-filtration chromatography column (1.6 cm × 50 cm) at a flow rate of 0.2 mL/min, followed by an elution step with deionized water. The polysaccharide fractions were collected and concentrated at 50 °C using a rotary vacuum evaporator. Finally, lyophilization was performed to obtain the pure LPP polysaccharide.

2.3. Characterization of Polysaccharides

2.3.1. Chemical Composition Analysis

The content of C, H, S, and N in LPP was measured using an elemental analyzer (Vario EL cube, Elementar, Hanau, Germany). The total sugars, uronic acid, and sulfuric groups in the LPP were measured using the phenol-sulfuric acid method, the sulfuric acid-carbazole method, and the barium chloride-gelatin method, respectively [20–22]. The protein content in LPP was assayed by the BCA Protein Assay Kit. The extraction yield (%) of LPP was calculated using the following equation: extraction yield (%, *w*/*w*) = (weight of dried LPP (g)/weight of raw materials (g)) × 100% [23].

2.3.2. Determination of Molecular Weight (Mw)

The Mw of LPP was determined by high-performance liquid chromatography (HPLC) (LC-20Ai, Shimadzu, Shimane-ken, Japan) equipped with a TSK-Gel G4000 PWXL column (7.8 mm × 300 mm, TOSOH, Yamaguchi-ken, Japan) [12,16]. Briefly, different molecular dextran standards and LPP (2.0 mg) were dissolved in 0.2 mol/L of NaCl solution (400 μL) at room temperature, and an ultrasonic treatment was performed to avoid the formation of aggregates. After centrifugation at 8000 rpm for 5 min, 20 μL of supernatant was collected and filtered through a 0.22-micron water filtration membrane, injected into the column, and eluted with NaCl (0.2 mol/L) at a flow rate of 0.6 mL/min at 40 °C.

2.3.3. Monosaccharide Composition Analysis

The monosaccharide composition of LPP was measured using HPLC (LC-20AT, Shimadzu, Shimane-ken, Japan) equipped with a COSMOSIL 5C18-PAQ column (4.6 ID × 250 mm, COSMOSIL, Kyoto, Japan), as described by a previous report with slight modifications [10,17]. Firstly, LPP was methanolyzed with 1 mL of an anhydrous hydrochloric acid–methanol solution at 80 °C for 16 h and then with 2 mol/L of trifluoroacetic acid (TFA) at 120 °C for 1 h. Secondly, 0.3 mol/L of NaOH (500 μL) and 0.5 mol/L of 1-phenyl-3-methyl-5-pyrazolone (PMP) (500 μL) were added to the solution. The solution was then mixed well and maintained at 70 °C for 30 min. Subsequently, 0.3 mol/L of HCl (100 μL) was added to neutralize the reaction product, and the product was extracted with 700 μL of dichloromethane. Finally, the water phase was collected and passed through a 0.22 μm membrane. The sample was eluted with a mixture of acetonitrile (19.2%, *v*/*v*) and monopotassium phosphate buffer (80.8%, *v*/*v*, 0.1 mol/L, pH 7.0) at 35 °C with a 1 mL/min flow rate. The injection volume of the sample was 10 μL and the UV detection wavelength was 245 nm. Mannose (Man), glucuronic acid (GlcA), rhamnose (Rha), galacturonic acid (GalA), glucose (Glc), galactose (Gal), xylose (Xyl), arabinose (Ara), and fucose (Fuc) were used as standards.

2.3.4. X-ray Diffraction (XRD)

XRD (D8 Advance, Bruker, Karlsruhe, Germany) was used to determine the degree of LPP crystallinity [11,15]. The LPP samples were exposed to the X-ray beam from the X-ray generator, running at 40 kV and 150 mA, and the patterns were collected in the 2θ range of 5–80° with a step size of 0.02°.

2.3.5. Ultraviolet-Visible (UV-Vis) Spectra and Fourier-Transform Infrared (FT-IR) Spectra Analyses

LPP was dissolved in ultrapure water, and the UV spectra of LPP were recorded in the wavelength range from 200 to 400 nm using a UV-vis spectrophotometer (Lambda 950, PerkinElmer, Waltham, MA, USA) [9]. The FT-IR of LPP was determined using the KBr disk method on an FT-IR spectrometer (Spectrum One, PerkinElmer, Waltham, MA, USA) at the wavelength of 400–4000 cm^{-1} [10,12].

2.3.6. Congo Red Experiment

The Congo red experiment was used to determine the conformation of LPP according to a previous report, with slight modifications [13,16,24]. Briefly, 2.0 mL of LPP solution (1.0 mg/mL) was mixed with 2.0 mL of Congo red (80 μmol/L), and a NaOH solution (1 mol/L) was added to the mixture until the final concentration of NaOH reached 0–0.5 mol/L. The maximum absorption wavelength (λmax) was measured using a microplate reader (Spectra Max iD3, Molecular Devices, Silicon Valley, CA, USA) at 40–800 nm.

2.3.7. β-Elimination Reaction

The β-elimination reaction can be used to explore the type of glycosidic presence in polysaccharides. The type of glycosidic presence in LPP was investigated using the methods of Zhang et al. [25]. Briefly, 10 mg of LPP was incubated in 5.0 mL of a $NaBH_4$-NaOH (1 mol/L) solution at 45 °C for 24 h and scanned from 190 to 400 nm. The same concentration of polysaccharides without alkaline hydrolysis was used as the control.

2.3.8. Field Emission Scanning Electronic Microscope (FESEM) Observation

After thinly sputtering with a layer of Au, the morphological features of LPP were observed by an FESEM (S-4800, Hitachi, Tokyo, Japan) and studied at a 5 kV accelerating voltage under high vacuum conditions with the image at 1000× and 2000× magnification [23].

2.3.9. Atomic Force Microscopy (AFM) Determination

An LPP aqueous solution (10 μg/mL) was dispersed on the surface of an amica plate and dried at room temperature. Images of the LPP were acquired via AFM (NTEGRA Spectra, NT-MDT, Moscow, Russia) under tapping mode [15].

2.3.10. Nuclear Magnetic Resonance (NMR) Spectroscopy

The structural information of LPP was studied using NMR spectroscopy (AVANCE NEO 600 MHz, Bruker, Karlsruhe, Switzerland). Briefly, LPP (30.0 mg) was frozen and thawed with D_2O (0.55 mL, 99.9%) 3 times and then collected in an NMR tube. The 1D-^1H and ^{13}C NMR experiments were performed [8,10,26]. MestReNova software was used to analyze the data.

2.4. General Functional Properties

2.4.1. Thermodynamic Stability

The thermal properties of LPP were evaluated by performing a thermogravimetric analysis (TGA) using a thermogravimetric analyzer (TGA-7, PerkinElmer, MA, USA). The purified LPP sample (3 mg) was placed in an aluminum pan and heated at a rate

of 10 °C/min over a temperature range from 30 to 800 °C under a nitrogen atmosphere (99.9999%) [19].

2.4.2. Water-Holding Capacity (WHC) and Water Solubility Capacity (WSC)

Lyophilized LPP (45 mg) was suspended in deionized water (0.5 mL). The water-holding capacity (WHC) and water solubility capacity (WSC) were measured according to the methods of Jeddou et al. [27] and Ahmed, Wang, Anjum, Ahmad, and Khan [28], respectively.

2.5. Antioxidant Activities In Vitro

The antioxidant activities of LPP in vitro were evaluated by assaying the radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) ($ABTS^+$), a hydroxyl radical (•OH), nitroso (NO_2^-), and the reducing power (RP). Prior to all assays, the samples were dissolved in deionized water to a final concentration of 0.2, 0.4, 0.5, 0.6, 0.8, or 1.0 mg/mL. Deionized water was used as the blank control and vitamin C (Vc) was used as the positive control. The absorbance was measured with a microplate reader at room temperature. Details of the operating conditions and methods have been described previously [26,27,29,30].

2.6. Hypoglycemic Activities In Vitro

The hypoglycemic activities of the LPP in vitro were evaluated by detecting the α-amylase and α-glucosidase inhibitory capacities. Prior to the two experiments, LPP and acarbose were dissolved in phosphate buffer (pH 6.9) to a final concentration of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, or 2.0 mg/mL. Detailed experimental methods were obtained from previous reports [9,10,31]. Acarbose and deionized water were used as positive and blank controls, respectively. The absorbance was detected by a microplate reader at 540 nm and 405 nm under room temperature.

2.7. Bile Acid Binding In Vitro

Bile acid binding in vitro was analyzed using methods described previously [32,33]. The bile acid stock solution and sample, including taurochenocholic acid (TA, 1 mg/mL), glycocholic acid (GA, 1 mg/mL), and LPP (0.05–2 mg/mL), were prepared with a phosphate buffer solution (pH = 6.9). The absorbance was measured at 387 nm using a microplate reader, and the phosphate buffer without the bile acid was used as the blank. The bile-acid-binding capacity was estimated from the calibration curves obtained for taurochenocholic acid ($y = 2.6355x + 0.0808$, $R^2 = 0.997$) and glycocholic acid ($y = 2.865x + 0.1116$, $R^2 = 0.9906$).

2.8. Statistical Analyses

All data were statistically analyzed with SPSS (version 25.0). TBtools (version 1.087) was used for a heatmap analysis of the bio-activities. A correlation analysis of the bio-activities was performed using Origin 2021 (OriginLab Corp., Greenbelt, MD, USA). Statistical significance in the research was set as $p < 0.05$. Data were reported as means ± standard deviations (n = 3) and analyzed by ANOVA.

3. Results and Discussion

3.1. Extraction and Purification of Polysaccharides

A single fraction was eluted and then collected, concentrated, and freeze-dried for further analysis and designated as LPP. Furthermore, the extraction yield of purified LPP was 7.43 ± 0.07%, which was similar to previous reports. Fu et al. [15] showed that the yield of polysaccharides extracted from blue honeysuckle berry homogenate was 8.31 ± 0.23%. Therefore, it can be inferred that the extraction of polysaccharides from *Lonicera caerulea* L. pomace is feasible.

3.2. Characterization of the Polysaccharide

3.2.1. Chemical Composition Analysis

The total sugar, uronic acid, sulfate groups, and protein content of LPP were 72.37 ± 1.05%, 27.23% ± 0.48%, 0.15 ± 0.01%, and 0.25% ± 0.05%, respectively. The results of the elemental analysis showed that LPP is composed of carbon (36.47 ± 0.86%), hydrogen (6.20 ± 0.16%), nitrogen (0.95 ± 0.02%), and sulfur (0.18 ± 0.01%), indicating that LPP has proteins and sulfate groups, which is consistent with the results of the protein and sulfate group content determination.

3.2.2. Molecular Weight and Monosaccharide Composition

The HPLC system was applied to measure the Mw of LPP. The average Mw of LPP was calculated as 8.53×10^4 Da, which was lower than the polysaccharide HEP-2 (3.01×10^6 Da) obtained by Fu et al. [15]. A reason for this may be that LPP was extracted from *Lonicera caerulea* L. pomace after microbial fermentation in this experiment, which may have reduced the Mw of the polysaccharides. In addition, Ma et al. [16] verified that the blue honeysuckle polysaccharides with a lower Mw possessed stronger anti-glycation and hypoglycemic activity.

The chromatographic analysis of the monosaccharide standards and LPP are shown in Figure 1A. Compared to the monosaccharide standards, LPP was composed of Man, GlcA, Rha, GalA, Glc, Gal, Xyl, Ara, and Fuc in percentages of 5.36, 2.53, 6.2, 40.38, 7.21, 20.56, 1.97, 14.25, and 1.55, respectively, which indicates that LPP is an acidic heteropolysaccharide. The values for the homogalacturonan (HG) and rhamnogalacturonan (RG-I) regions were calculated using the formulas HG = GalA − Rha and RG-I = (GalA − HG) + Rha + Ara + Gal, and the results were 34.18% and 47.21%, respectively, indicating that the linear portion was less than the side chain portion in LPP [14,34]. Furthermore, the linearity of LPP was calculated by the formula GalA / (Gal + Rha + Xyl + Fuc + Ara + GlcA), and the result was 0.86 [14].

3.2.3. X-ray Diffraction

The crystalline structure of LPP was examined using XRD, with patterns recorded from 5° to 80° (Figure 1B). A broad peak spanning the 2θ values from 10° to 30° and distinctive diffraction peaks at 20.42° indicated that the LPP formed an amorphous body, which was consistent with the result of the Congo red experiment. Moreover, Jeddou et al. [27], Ma et al. [35], Zhao et al. [24], and Abuduwaili et al. [11] also obtained similar results.

3.2.4. Ultraviolet-Visible Spectra and Fourier-Transform Infrared Spectra

The UV-vis spectra of LPP are presented in Figure 1C. The maximum absorption peak of LPP was from 200 to 210 nm, and there were no significant absorption bands detected at 260 and 280 nm, which indicated no or little nucleic acids and proteins in LPP. This was in line with the results of the chemical composition.

FT-IR spectroscopy was performed to identify the functional groups of LPP. As shown in Figure 1D, the typical intense absorption band at 3421 cm^{-1} was attributed to the intermolecular O-H stretching vibration of hydroxyl [10,30]. The weak band at 2928 cm^{-1} was assigned to the C-H stretching vibration of alkyl. These two bands both belong to the characteristic absorption bands of polysaccharides [26,27]. The relatively weaker absorption bands at 1617 cm^{-1} and 1412 cm^{-1} could be associated with the C=O asymmetric and C-O stretching vibrations of carboxyl, which implies the existence of uronic acids [32,33]. In addition, the characteristic bands of ester's C=O stretch vibration and carboxyl vibrations were observed at 1743 cm^{-1} and 1640 cm^{-1}, respectively [15]. The absorptions at 1101 cm^{-1}, 1020 cm^{-1}, and 1049 cm^{-1} were assigned to the asymmetric stretching vibration of C-O-H side groups or C-O-C glycosidic bond vibrations [36]. The absorption band at 924 cm^{-1} was attributed to the asymmetric vibration expansion of a pyran ring, suggesting that LPP might have a pyranose ring structure [37]. Meanwhile, the weak bands at 830 cm^{-1} and 896 cm^{-1} confirmed that both α- and β-glycosidic bonds simultaneously

existed in LPP, which was further proven by the NMR analysis [23,31]. Consequently, the LPP had the α-and β-configuration of a pyranose saccharide.

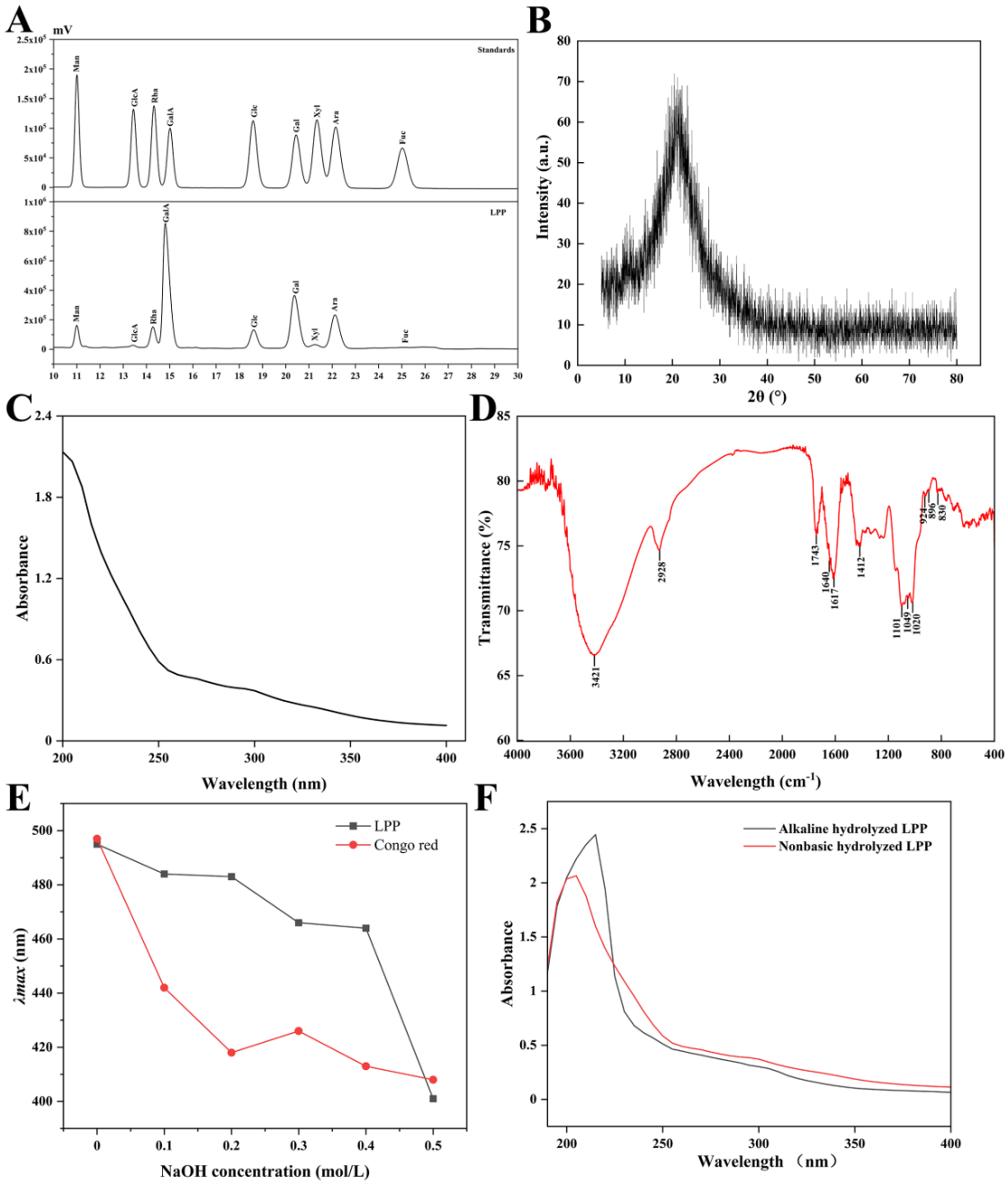

Figure 1. (**A**) Monosaccharide composition, (**B**) XRD, (**C**) UV-vis spectrum, (**D**) FT-IR spectroscopy, (**E**) Congo red experiment, and (**F**) β-elimination reaction for the *Lonicera caerulea* L. pomace polysaccharide LPP.

3.2.5. Congo Red Experiment

The Congo red experiment was implemented to study whether a triple helix structure exists in LPP due to the fact that the biological functions of polysaccharides are closely related to their spatial structure. As shown in Figure 1E, when the concentration of the NaOH solution increased, the λmax of the Congo red-LPP and the control group decreased gradually with no observed redshifts, demonstrating that the conformation of LPP in the solution was not a triple helix, but a random coil conformation. It was clear that the λmax of the Congo red-LPP complex showed a similar blue shift tendency with increasing

concentrations of the NaOH solution (0–0.5 mol/L). Therefore, LPP had no triple helical conformation in solution. This is consistent with previous research showing that heteropolysaccharides do not easily form triple helix structures [10,15,16,36]. However, the polysaccharides from ginger and *Annona squamosa* residue extracted by Gu et al. [9] and Yang et al. [13] were found to have a triple helical structure.

3.2.6. β-Elimination Reaction

The β-elimination reaction is often used in the structural analysis of glycoproteins to distinguish the glycopeptide chain from linking properties. Under alkaline conditions, the O-glycoside bond is easily broken, and the characteristic absorption peak appears at 240 nm. The results of the β-elimination assay for LPP, as shown in Figure 1F, showed that no characteristic absorption peak was detected at 240 nm, suggesting that the O-glycoside bond was nonexistent in LPP; it may instead possess a N-glycosidic bond, or neither, which is different from most results [34].

3.2.7. Morphological Properties

FESEM and AFM were used to identify the microstructures and morphological features of the LPP (Figure 2). As shown by the FESEM images (Figure 2A,B), the LPP exhibited a multi-layer flake-like connected network structure with a relatively smoother surface, similar to that of pectic polysaccharides from carrot pulp [38]. Moreover, the fresh bitter gourd polysaccharide BPS-W was obtained using water as an extraction solution with a similar surface structure, and the extraction method of Yan et al. [23] was similar to this study. Meanwhile, Yan et al. [23] found that the polysaccharide BPS-W exhibited higher antioxidant, α-amylase inhibitory, and α-glucosidase inhibitory activities in vitro than polysaccharides extracted from other extracting solutions (NaOH, $(NH_4)_2SO_4$, or citric acid). These results indicate that using an ultrasonic-assisted heating extraction method with water as the extracting solution will protect the structure and bio-activities of polysaccharides.

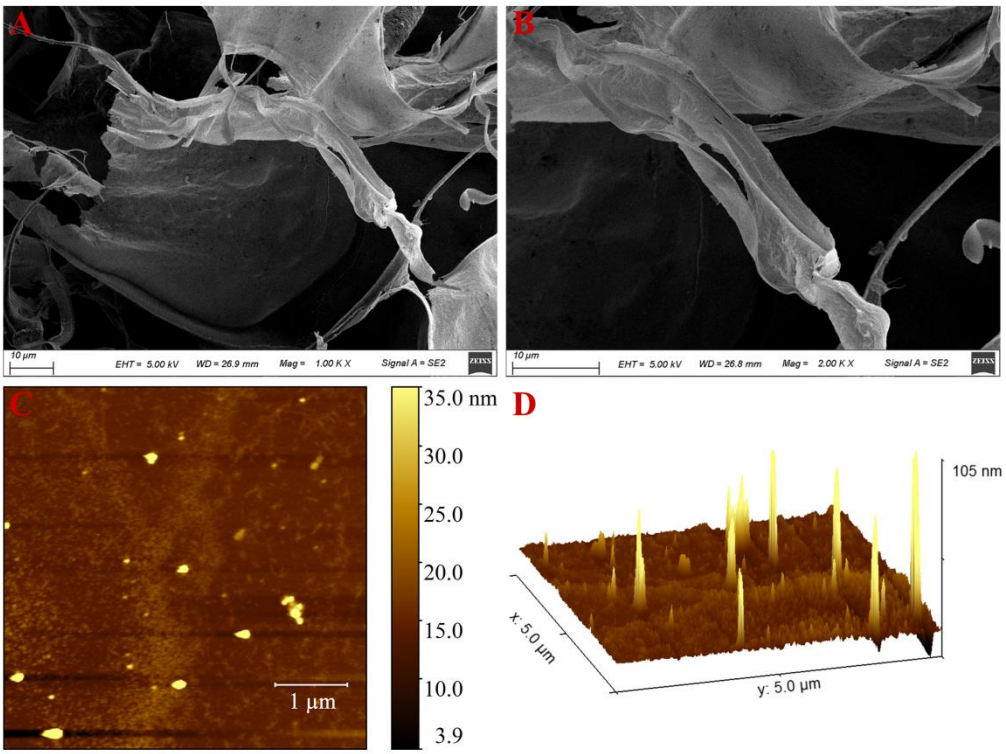

Figure 2. (**A**) 1000 × SEM images of LPP, (**B**) 2000 × SEM images of LPP, (**C**) planar AFM images of LPP, and (**D**) three-dimensional AFM images of LPP.

AFM has been established as a valuable technique for analyzing the three-dimensional morphology of polysaccharides in water. As shown in Figure 2C,D, the surface of LPP was rough with some pointed or thorny ridges. Furthermore, the maximum height of the lumps was 92.65 nm, and the average roughness was 1.38 nm.

3.2.8. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is widely used to analyze the structures of complex polysaccharides, such as *α*- or *β*-configurations, glycosidic linkages, and the linkage sequence of sugar residues [8,15,16,26,29,34,38]. The ^{1}H and ^{13}C NMR spectra of LPP are presented in Figure 3. The anomeric hydrogen at 4.4–5.5 ppm and the anomeric carbon at 95–110 represented both *α*-glycosidical and *β*-glycosidical configurations in LPP [10]. In the ^{1}H spectra (Figure 3A), there were nine major anomeric proton signals in the anomeric region from 4.4 to 5.5 ppm, meaning that LPP contains heteropolysaccharides with nine residues, which is consistent with the monosaccharide composition results [36]. The signals at 4.95 ppm, 5.14 ppm, and 5.09 ppm were assigned to the H-1 of *α*-1,4-linked GalA*p*, *α*-Ara*f*, and *α*-1,5-linked Ara*f*, respectively [10]. Meanwhile, methyl proton signals of the Rha and acetyl groups were observed around 1.25 and 2.17 ppm, respectively [38].

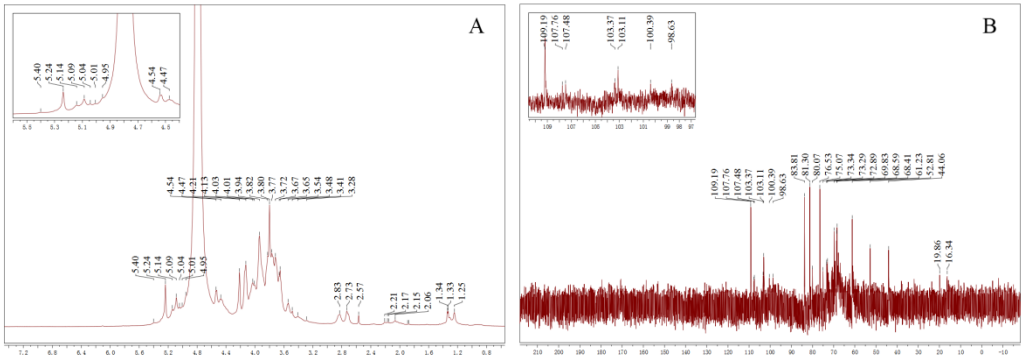

Figure 3. NMR spectra of LPP. (**A**) ^{1}H NMR spectrum and (**B**) ^{13}C NMR spectrum.

In the ^{13}C spectra (Figure 3B), the methyl group signals of Rha and the acetyl groups of GalA were observed at 16.34 and 19.86 ppm, respectively [26]. The carbon signals at δ 109.19 ppm and δ 107.48 ppm could be attributed to the C-1 of *α*-Ara*f* and 1,5-*α*-Ara*f*. The signals at 98.63 ppm could be assigned to the C-1 of the *α*-GalA*p* residues. Moreover, the carbon signals at 16.34 ppm and 19.86 ppm could be attributed to the presence of rhamnosyl residues and acetyl groups [8,10].

By combining the results of the FT-IR spectra, monosaccharide composition, and NMR analysis, LPP was found to be a polysaccharide consisting of *α*-1,4-linked GalA*p*, *α*-Ara*f*, and *α*-1,5-linked Ara*f* residues.

3.3. General Functional Properties

The thermodynamic stability analysis for LPP is presented in Figure 4A. The TGA curve depicted the weight loss in three stages; in the initial stage, 10.87% of the weight was lost between 30 °C and 103.03 °C, which might be associated with the loss of adsorbed water and structural water, indicating the existence of a certain number of carboxyl groups in LPP [37]. No significant weight changes were observed when the temperature was elevated from 103.03 °C to 197.90 °C, suggesting that LPP is stable below 197.90 °C. Subsequently, a significant loss of 58.29% of the weight occurred from 197.90 °C to 568.23 °C, due to the cleavage of the chemical structure and chemical bonds through depolymerization. The third stage occurred between 568.23 °C and 800 °C, accompanied by the gradual cracking of more stable structures that were charred to produce ash. Ultimately, the weight loss gradually reached equilibrium, with only 15.26% of the residue remaining. As shown in the DTG curve (Figure 4A), the maximum decomposition rate of LPP was observed at

231.69 °C, which indicates that LPP has a high thermal stability. The WHC and WSC of LPP were 3160.82 ± 192.26% and 57.33 ± 1.15%, respectively. Furthermore, the results of Jeddou et al. [27] and Olawuyi and Lee [26] show that better thermodynamic stability, WHC, and WSC can improve the bio-activities of polysaccharides.

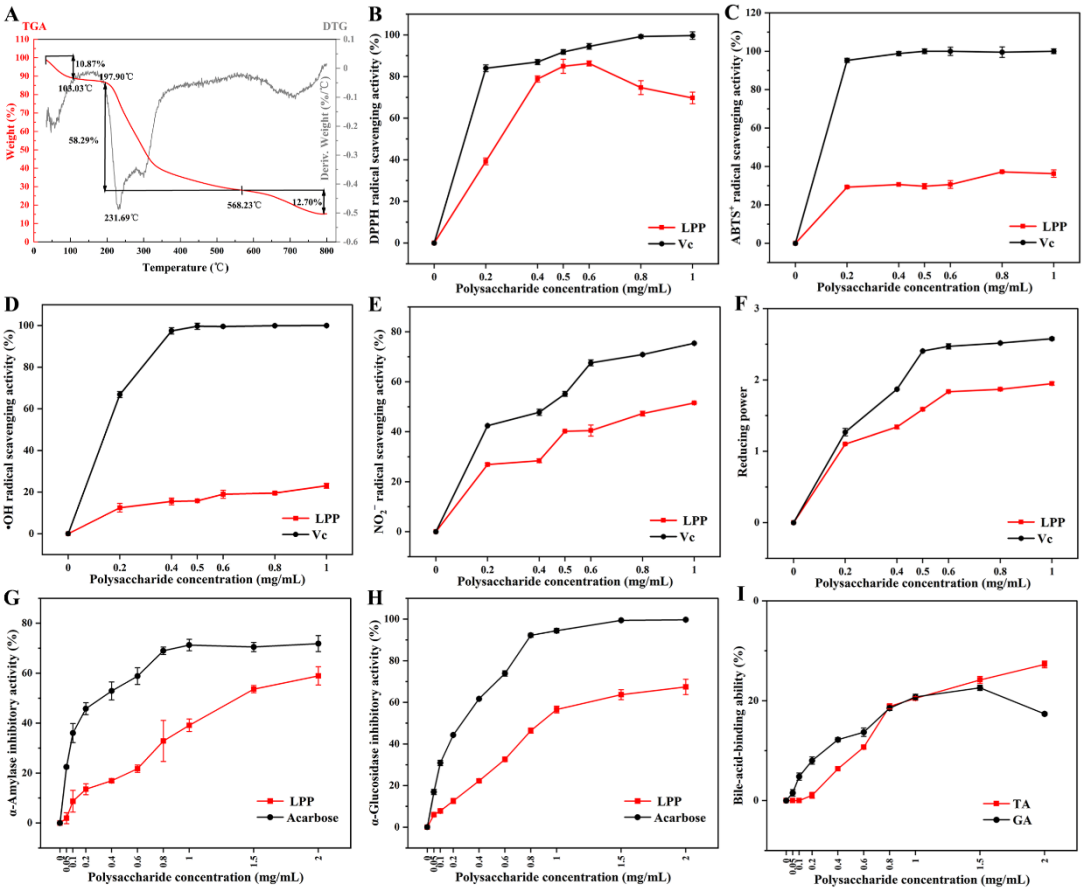

Figure 4. The properties of LPP. (**A**) Thermal properties, (**B**) DPPH radical scavenging activity in vitro, (**C**) $ABTS^+$ radical scavenging activity in vitro, (**D**) •OH radical scavenging activity in vitro, (**E**) NO_2^- radical scavenging activity in vitro, (**F**) reducing power in vitro, (**G**) α-amylase inhibitory activity, (**H**) α-glucosidase inhibitory activity, and (**I**) bile-acid-binding ability.

3.4. Antioxidant Activities In Vitro

The radical scavenging activities of LPP were determined, calculated, and compared to Vc. As shown in Figure 4B–F, the scavenging activity of LPP on DPPH radicals, $ABTS^+$ radicals, •OH radicals, NO_2^- radicals, and RP were increased in a dose-dependent manner (0–1 mg/mL). At 0.2 mg/mL, the LPP showed scavenging abilities of 39.24 ± 1.63%, 29.25 ± 0.68%, 12.49 ± 2.08%, and 26.90 ± 0.56% on DPPH radicals, $ABTS^+$ radicals, •OH radicals, and NO_2^- radicals, respectively. The maximum scavenging abilities were 96.37 ± 1.22% (0.6 mg/mL), 37.19 ± 0.39% (0.8 mg/mL), 23.03 ± 1.21% (1.0 mg/mL), and 51.53 ± 0.25% (1.0 mg/mL), respectively, which were much weaker than those of Vc, at 94.48 ± 1.37%, 99.52 ± 2.70%, 99.98 ± 0.39%, and 75.43 ± 0.12%, respectively. Compared to the results of previous studies, the scavenging abilities for DPPH and $ABTS^+$ radicals in this study were higher than those of the polysaccharide BPS-W (85.1%, 4.0 mg/mL) obtained from fresh bitter gourd and those of the polysaccharide OLP (approximately 18%, 1.0 mg/mL) obtained from okra leaves, respectively [23,29]. Meanwhile, the scavenging ability for NO_2^- radicals was superior to that of P-EPS (34.5%, 5.0 mg/mL) extracted from *Leuconostoc lactis*. L2 [28]. On the contrary, the scavenging ability for •OH radicals in this research were weaker than those of the polysaccharides BPS-I (69.12%, 0.3 mg/mL) and SeTPS-1 (59.43%, 5 mg/mL) from bitter gourd and selenium-enriched

tea, respectively [33,35]. Compared to the polysaccharides extracted from other small berries, such as *Pinus koraiensis* (about 20%, 1 mg/mL), LPP (69.70 ± 2.80%, 1 mg/mL) showed a better DPPH radical scavenging ability. The $ABTS^+$ radical scavenging abilities of LPP (36.28 ± 1.96%, 1 mg/mL) are also far better than those of *Pinus koraiensis* (about 4%, 1 mg/mL) [25]. These results indicate that LPP exhibited a remarkably high effect for DPPH radical, $ABTS^+$ radical, and NO_2^- radical scavenging. The reason for these phenomena may be due to the presence of a small number of polyphenols in LPP, which in turn contributes to the enhancement of the antioxidants of LPP [19,26]. Furthermore, the RP of LPP was 1.948 ± 0.022 (1 mg/mL), which was slightly lower than that of Vc (2.577 ± 0.019). Jeddou et al. [27] reported that a lower Mw could increase the RP of the polysaccharides by exposing more reduced and non-reduced ends.

3.5. Hypoglycemic Activities In Vitro

Carbohydrates, such as starch and glycogen, are firstly hydrolyzed to oligosaccharides by α-amylase, followed by degradation into monosaccharides by α-glucosidase, in turn raising blood glucose [39]. Thus, α-glucosidase and α-amylase inhibition tests were performed as simple and effective strategies for evaluating the hypoglycemic activity of LPP in vitro, with acarbose as a positive control. As illustrated in Figure 4G,H, LPP and acarbose showed similar trends for inhibiting α-amylase and α-glucosidase in a dose-dependent manner with increasing concentrations of 0.05–2 mg/mL. At the concentration of 2.0 mg/mL, the maximum inhibitory rate of LPP on α-glucosidase and α-glucosidase (58.94 ± 3.65%, 67.42 ± 3.69%) was lower than that of acarbose (71.84 ± 3.20%, 99.68 ± 0.31%). However, studies have shown that acarbose has certain side effects and dependence. Therefore, LPP may be an alternative because of the multiple therapeutic effects and minimal side effects [15,40]. Moreover, LPP exhibited better α-glucosidase inhibitory activity than the α-amylase inhibitory activity, which might be due to the fact that acarbose is an inhibitor of α-glucosidase. Similar results have been reported by Gu et al. [9] and Yan et al. [23]. In addition, the inhibitory activity on α-amylase and α-glucosidase of the polysaccharide HEP-2 (about 45% and 52%, Mw = 3.01×10^6 Da) from blue honeysuckle berries was lower than that of LPP (58.94 ± 3.65% and 67.42 ± 3.69%, Mw = 8.53×10^4 Da) in concentrations of 2 mg/mL [15]. It is speculated that lower Mw can improve the hypoglycemic bio-activities of polysaccharides, which is consistent with the results of Ma et al. [16] and Wu et al. [10]. However, Wang, Yu, Chen, Wang, and Yan [31] and Yan, Wu, Qiao, Cai, and Ma [33] found that the polysaccharides OPS and BPS, obtained from fresh okra and bitter gourd, had stronger inhibitory activities on α-amylase than α-glucosidase. Further validation of the hypoglycemic activity of LPP should be performed in vivo.

3.6. Bile Acid Binding In Vitro

The bile-acid-binding ability is usually used to evaluate the effects of polysaccharides in the hypolipidemic aspect in vitro. As shown in Figure 4I, the TA and GA maximum binding capacities of LPP were approximately 27.27 ± 0.68% (2.0 mg/mL) and 22.58 ± 0.52% (1.5 mg/mL), respectively. The binding ability of LPP to TA was positively correlated with the LPP concentration, ranging from 0.05 mg/mL to 2.0 mg/mL, whereas the binding rate of LPP and GA increased at first, and then decreased at 1.5 mg/mL. The results of Yan et al. [23] showed that the polysaccharide BPS-A has a stronger bile-acid-binding capacity due to the lower Mw, a higher content of Gal, a looser surface morphology, and its multiple-branching construction. In addition, the early research results of Yan, Wu, Qiao, Cai, and Ma [33] showed that the bile-acid-binding abilities of the polysaccharide BPS-I were correlated with the content of uronic acid, neutral sugars, and polyphenols, indicating that the chemical composition is related to the bile-acid-binding ability. Qin et al. [32] showed that the bile-acid-binding ability of Liupao tea polysaccharides after fermentation was better than without fermentation due to the lower GalA content in the fermented samples. Considering all the above, the binding capacity of bile acids is affected by many factors, which should be further explored in subsequent experiments.

3.7. Correlation Analysis of Bio-Activities

At present, the research on polysaccharide bio-activities has focused on a single property of antioxidant and hypoglycemic activities, and few scholars have discussed the correlation between activities. However, polysaccharides have various bio-activities, and whether these activities are synergistically promoted or mutually offset has not been reported. Therefore, the correlation among the nine kinds of bio-activities of LPP is discussed in this section.

In the present study, the DPPH radical scavenging ability combined with the $ABTS^+$, •OH, NO_2^-, and RP radical scavenging abilities were used to evaluate and compare the antioxidant activities of LPP in vitro. As a stable radical, the DPPH radical can significantly clear away through radical proton scavenger via donating hydrogen to form a stable molecule [26]. The •OH radical is considered the most harmful reactive oxygen radical, and can easily cross cell membranes to react with various macromolecules such as carbohydrates, lipids, and amino acids, and then destroy cell membranes. It may not only cause cell damage, but also accelerate cell aging, which will facilitate the occurrence and development of diseases related to oxidative stress [41]. NO_2^- is easily converted to nitrite by bacteria, which can become pathogenic in acidic environments, thus significantly increasing the risk of human disease. RA is usually associated with reductants that act as antioxidants by damaging free radical chains to produce hydrogen atoms [27]. Hence, it can be seen that the oxidation of organisms is not caused by the existence of one reactive oxygen radical, but by the interaction of multiple radicals. Therefore, to study the antioxidant activity of LPP, it is necessary to comprehensively analyze the scavenging ability of LPP for various reactive oxygen radicals. As shown in Figure 5, the correlation between the DPPH and $ABTS^+$ radical scavenging effect by LPP was not significant for other reactive oxygen radicals ($p > 0.05$), while RP had a significant positive correlation with the •OH, and NO_2^- radical scavenging abilities ($p < 0.01$), indicating that the scavenging abilities of LPP for the DPPH and $ABTS^+$ radicals were not affected by the scavenging ability of other radicals, while RP, •OH, and NO_2^- radicals had a synergistic promoting effect.

Modern pharmacological research has illustrated that polysaccharides showing strong antioxidant capacities may play a positive role in the prevention and treatment of diabetes [39]. As indicated in Figure 5, the inhibitory activity of LPP on α-amylase was positively correlated with the radical scavenging abilities of •OH and NO_2^- ($p < 0.01$), the correlation between $ABTS^+$ and the RP radical scavenging abilities were second ($p < 0.05$), and there was no significant correlation with the radical scavenging ability of DPPH ($p > 0.05$). The inhibitory rate of LPP on α-glucosidase was not significantly correlated with the scavenging rate of the DPPH radical ($p > 0.05$), but it was significantly positively correlated with the scavenging abilities of other reactive oxygen radicals. Meanwhile, the inhibitory rate of LPP on α-amylase and α-glucosidase was significantly positively correlated ($p < 0.01$). Furthermore, previous reports have suggested that increased reactive oxygen radicals in the body may increase the risk of diabetes. Collectively, there was a significant positive correlation between the hypoglycemic ability of LPP and the antioxidant activities, which indicated that polysaccharides with a strong antioxidant ability might have an excellent hypoglycemic effect. This conclusion was consistent with the results of Yan et al. [23].

Obesity is often accompanied by hyperglycemia and hyperlipidemia, which shows a certain correlation between blood glucose and hyperlipidemia. As shown in Figure 5, the binding ability of LPP to TG and GA was significantly positively correlated with the ability of LPP to inhibit α-amylase and α-glucosidase ($p < 0.01$), indicating that LPP can not only reduce blood glucose, but also significantly reduce blood lipids. Meanwhile, the binding capacities of LPP to TG and GA also showed a significant positive correlation with the antioxidant activities of LPP, except for the DPPH and $ABTS^+$ radical scavenging abilities ($p < 0.01$). These results demonstrate that LPP was positively correlated with antioxidant, hypoglycemic, and hypolipidemic activities. The three were closely related, which means that eliminating reactive oxygen radicals in vivo is expected to reduce hy-

perglycemia and hyperlipemia effectively [32,33]. Although several studies have obtained similar conclusions, the mechanistic details of this process remain unclear and require further validation.

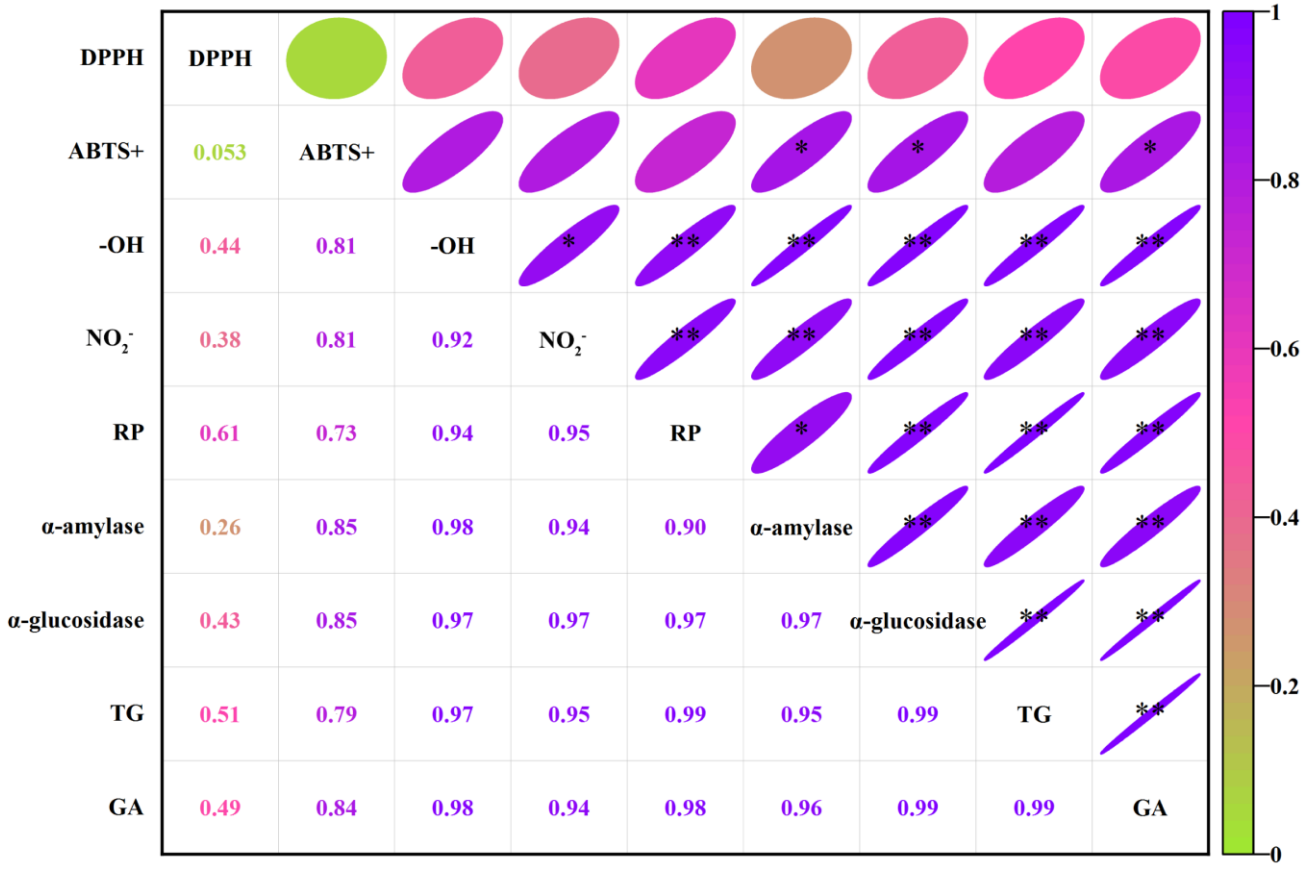

Figure 5. Correlation analysis of bio-activities based on the Pearson correlation (* $p < 0.05$, ** $p < 0.01$).

4. Conclusions

In this study, a polysaccharide called LPP was extracted and purified from *Lonicera caerulea* L. pomace. The results showed that LPP is composed of Man, GlcA, Rha, GalA, Glc, Gal, Xyl, Ara, and Fuc in a molar ratio of 5.36:2.53:6.2:40.38:7.21:20.56:1.97:14.25:1.55. LPP consists of α-1,4-linked GalA*p*, α-Ara*f*, and α-1,5-linked Ara*f* residues and has a Mw of 8.53 × 10^4 Da. LPP possesses a multi-layer flake-like connected network structure with a relatively smooth surface. Furthermore, LPP has a high thermal stability and the maximum scavenging abilities of 39.24 ± 1.63%, 29.25 ± 0.68%, 12.49 ± 2.08%, and 26.90 ± 0.56% for DPPH radicals, $ABTS^+$ radicals, •OH radicals, and NO_2^- radicals, which were 96.37 ± 1.22% (0.6 mg/mL), 37.19 ± 0.39% (0.8 mg/mL), 23.03 ± 1.21% (1.0 mg/mL), and 51.53 ± 0.25% (1.0 mg/mL), respectively. The maximum RP, the inhibitory effect on α-glucosidase and α-glucosidase, and the TA and GA binding capacities were 1.948 ± 0.022 (1 mg/mL), 58.94 ± 3.65% (2.0 mg/mL), 67.42 ± 3.69% (2.0 mg/mL), 27.27 ± 0.68% (2.0 mg/mL), and 22.58 ± 0.52% (1.5 mg/mL), respectively. In addition, this study is the first to reveal the correlation between bio-activities, which will be helpful for developing more properties of LPP in functional food. Meanwhile, the extracted polysaccharides from *Lonicera caerulea* L. pomace also increased the waste utilization of resources.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8090422/s1, Table S1: The reagents used in the experiment.

Author Contributions: Each of the authors contributed to the following: conceptualization, F.P. and J.G.; Writing—Original Draft, F.P.; Writing—Review & Editing, F.P. and J.G.; methodology, Y.L., X.C. and X.W.; Software, Y.L., Y.R. and X.C.; project administration, J.G. and F.P.; funding acquisition, J.G. and F.P. All authors have approved the final version of the manuscript.

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