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Structure and immunostimulating activity of polysaccharides derived from the roots and leaves of dandelion

Qinggang Qiao[1], Xianzhang Song[2], Cheng Zhang[2], Chengxi Jiang[3*] and Runshen Jiang[1*]

Abstract

Two polysaccharides were obtained from dandelion roots (DPR) and dandelion leaves (DPL) via water extraction and ethanol precipitation. Both DPR and DPL were an acid heteropolysaccharide, with a molecular weight of 5.89×10^4 and 28.2×10^4 Da, respectively. Monosaccharide composition results showed that both DPR and DPL contained Man, Glc, Rha, GalA, Glc, Gal, and Ara with a molar ratios of 2.98:1.00:3.67:6.41:2.98:16.70:2.48 and 1.23:1.00:1.06:5.74:2.88:8.59:1.84, respectively. Methylation and NMR analysis showed DPR and DPL, with triple-helix conformations, were mainly composed of 4-**α**-Gal*p* and 4-**α**-GalA*p*, terminated with t-**α**-Ara*f*, t-**α**-Rha*p*, t-**α**-Glc*p* and t-**α**-Man*p*. DPL showed an immunoprotective effect in cyclophosphamide (Cy)-induced black-bone silky chickens by improving chicken growth performance, increasing the spleen, thymus, and bursa of Fabricius indices, and promoting blood lymphocyte proliferation, the secretion of cytokines (IL-2, IL-6, and INF-γ) and serum immunoglobulin (IgA, IgG, and IgM) levels in a dose-dependent manner. Moreover, the oxidative stress damage in immunosuppressed chickens was significantly reformed after DPL treatment. These findings provide useful information on the potential for application of dandelion polysaccharides as natural nutrients to enhance chicken immune and antioxidant functions.

Keywords Dandelion roots, Dandelion leaves, Polysaccharides, Immunostimulation, Black-bone silky chicken

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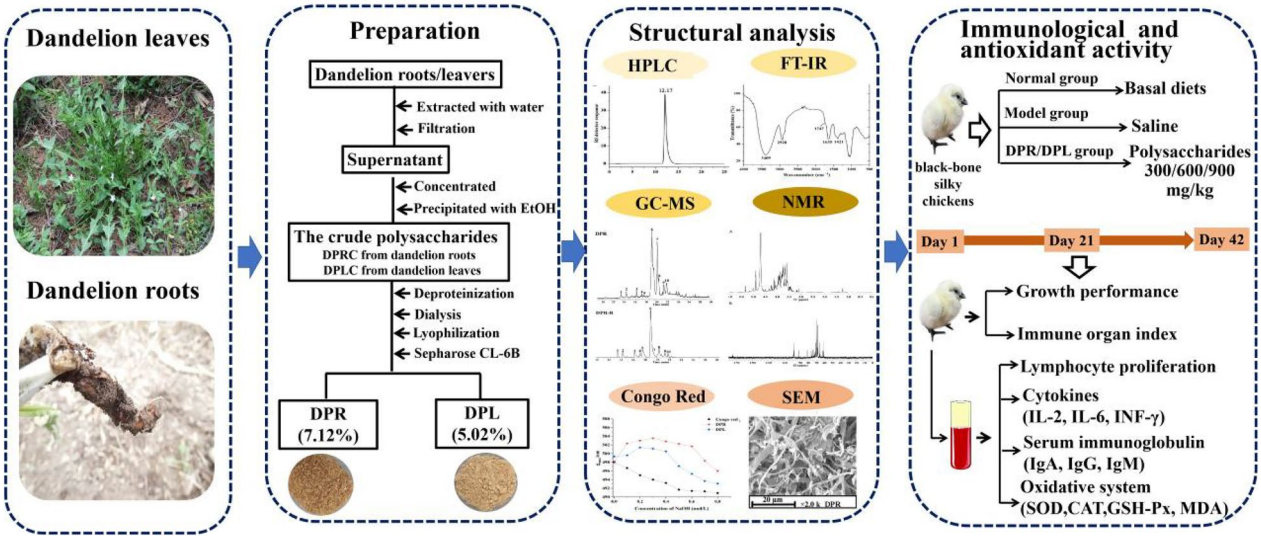
Graphical Abstract



Introduction

The use of antibiotics as feed additives in animal husbandry has recently been banned worldwide. This development has led to extensive research into substitutes for dietary antibiotic supplementation in the field of animal breeding. Plant polysaccharides are natural active ingredients with no side effects that have been used as immunopotentiators to prevent and treat bacterial, viral, and parasitic diseases in chickens [1]. Furthermore, polysaccharides are widely used as feed additives, to improve livestock and poultry immune function by promoting immune organ growth, antibody generation, lymphocyte activation, and animal growth, as well as decreasing death rates [2]. For example, Wang et al. found that *Paulownia fortunei* flowers polysaccharide (PFFPS) exerted immunological effects on spleen lymphocytes and the quantity of leukocytes in Cy-induced immunosuppressed chickens in vivo [3]. Furthermore, Su et al. reported that dietary supplementation with Yu-Ping-Feng polysaccharides improved disease resistance in *Litopenaeus vannamei*, by improving immune responses [4]. In addition, sulfate-based alginate polysaccharide supplementation benefits immune responses, growth, and disease resistance against *Vibrio harveyi* in juvenile hybrid groupers, and is considered a potential immunomodulatory enhancer [5]. Therefore, there is evidence supporting the use of plant polysaccharides as promising natural feed additives, with potential to replace antibiotics in this context.

Dandelion (*Taraxacum mongolicum Hand.-Mazz.*) is a traditional Chinese herb distributed in the northern hemisphere [6, 7] Previous studies have revealed that the plant contains a diverse range of active ingredients including flavonoids, polyphenols, terpenoids and polysaccharides [8], which exhibit multiple pharmacological activities, including anti-inflammatory, anti-infective, and antioxidant activities [8, 9]. Furthermore, dandelion is well known for its use in making functional foods and potential feed additive in animals [6–8]. Polysaccharides, represent a high proportion of dandelion active phytoconstituents, and are reported to exhibit diverse biological activities, such as anti-inflammation [10], antioxidant [11], and antitumor activities [12]. Furthermore, several studies have revealed that dandelion polysaccharides are potential immunopotentiators. For example, Ren et al. isolated a dandelion polysaccharide and demonstrated that it inhibits tumor growth in BALB/c mice by promoting lymphocyte transformation, improving spleen germinal center reaction and modulating T cell activation [13]. Furthermore, Sib et al. found that dandelion polysaccharide (DP) markedly improved the growth performance, antioxidant capacity and immunity of *Cyprinus carpio* [7].

Black-bone silky chickens (*Gallus gallus domesticus Brisson*) are a special breed of chickens with excessive melanin deposition, and a long history in China, with high medicinal and nutritional value. Cy is an alkylating agent that causes irreversible damage to B and NK lymphocytes, thereby inhibiting humoral and cellular

immunity [14]. Moreover, Cy inhibits immunoglobulin formation and reduces dietary intake, slows weight gain, and reduces poultry growth performance [15–17]. Hence, Cy is often used to establish immunosuppressed animal models, including mice, rats, pigs, and chickens.

Notably, the polysaccharides in different parts of medical plants have physicochemical properties and structural features. Moreover, there is a close correspondence between the structure of polysaccharides and their activity [18, 19]. In this study, we prepared dandelion polysaccharides from dandelion roots and dandelion leaves with the aim of investigating their immunomodulatory effects on an immunosuppressed black-bone silky chicken model generated by Cy treatment. Our results provide new evidence for the immune enhancement effects of dandelion polysaccharides in chickens and will promote the development and utilization of dandelion polysaccharides as green feed additives.

Materials and methods

Materials and reagents

Dandelion roots and leaves used in this study were purchased from Bozhou City, Anhui Province, China. Sepharose CL-6B was obtained from GE Healthcare (Pittsburgh, USA). Total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC) and malondialdehyde (MDA) kits and ELISA kits, including interleukin-2 (IL-2), interleukin-6 (IL-6), Interferon-γ (INF-γ) and immunoglobulins (IgA, IgG, and IgM) kits, were provided by Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Cy was obtained from Shanghai Yuanye Biotechnology, Ltd. (Shanghai, China). All other chemicals and reagents were of analytical or HPLC grade.

General methods

The chemical compositions, including the total carbohydrate, uronic acid, and protein contents, of polysaccharides from dandelion roots and leaves, were determined using the phenol–sulfuric acid [20], m-hydroxydiphenyl colorimetric [21], and Bradford [22] methods, respectively. Samples (500 mg) were placed on crucible tongs and calcinated at 150 ℃ for 15 h to a constant weight. Ash content was weighed after burning and ash content calculated.

Preparation of polysaccharides from dandelion roots and leaves

Air-dried dandelion roots and leaves (1000 g) were crushed and extracted three times with 16 L of boiling water for 3 h. The extract was filtered, concentrated, and centrifuged (5000×*g*, 5 min). The supernatant was precipitated with four volumes of 85% ethanol to obtain the two crude polysaccharides: DPRC (the polysaccharide from dandelion roots) and DPLC (the polysaccharide from dandelion leaves) [16]. The crude polysaccharides were further treated with Sevag reagent (butyl alcohol:chloroform = 1:4, v/v) to obtain two deproteinized polysaccharide, which were dissolved in distilled water (10 mg/mL), fractionated on the Sepharose CL-6B gel column (3.0×100 cm) and eluted using 0.15 M NaCl. Appropriate fractions were collected using a total sugar content assay. Two major fractions, DPR and DPL were obtained. Production of polysaccharides from dandelion roots and leaves is illustrated in Fig. 1.

Purity, homogeneity, and molecular weight assay

The sample (5 mg) was dissolved in distilled water (1 mL) and analyzed using a UV–VIS Spectrophotometer (Shimadzu UV-2700, Japan). Wavelengths ranging from 200 to 800 nm were recorded.

Samples homogeneity and molecular weights were measured using a high-performance gel permeation chromatogram (HPGPC) on a Dionex™ UltiMate™ 3000 high-performance liquid chromatography (HPLC) system (Thermo Fisher Scientific, United States), combined with a RID-10A detector and TSK-G3000 PWXL columns (7.8 mm×30.0 cm), according to a previously reported protocol [23]. Sample aliquots (20 μL) were injected into the column and eluted with 0.2 M NaCl at a flow rate of 0.5 mL/min; column temperature was set at 40 °C.

Monosaccharide composition

The pre-column derivatization and HPLC analysis was used to determine the monosaccharide composition of samples with a combination of 1-phenyl-3-methyl-5-pyrazolone (PMP). Briefly, 2 mg samples were hydrolyzed with TFA (2 M) at 120 °C (4 h) to obtain the hydrolysis product, then it stirred with NaOH (0.3 M) and PMP methanol solution (0.5 M). The resultant mixture was analyzed using an Agilent RRLC 1200 SL system (Agilent Technologies, DE, Wilmington, USA) after stored at 70 °C (0.5 h). It was combined with a DIKMA Inertsil ODS-3 column (4.6×150 mm, 5 μm, Dikma, Japan) and a UV–vis DAD detector. The mobile phase was PBS (0.1 mol/L, pH 7.0) and acetonitrile (83:17, v/v). Injection volume was 10 μL and column temperature was 25 °C.

Fourier transform-infrared (FT-IR) analysis

FT-IR spectra were analyzed using a Bruker Vertex 7.0 FT-IR Spectrometer (Germany) in the range 400–4000 cm^{-1}. Vibration absorption peaks near 1736 and

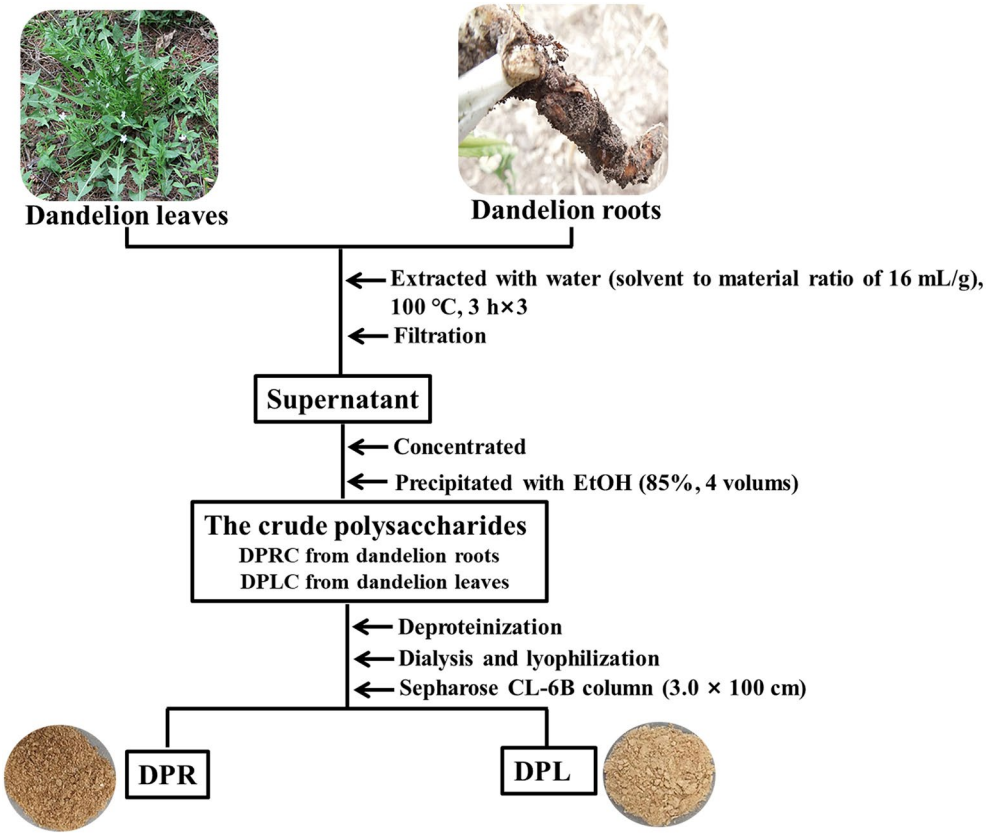


Fig. 1 Process for the extraction and purification of polysaccharides obtained from dandelion roots and leaves

1610 cm^{-1} represent peaks characteristic of C=O stretching vibration in methylated and free $-COO^-$ groups of Gal*p*A, respectively. The ratio of the area of the band at 1736 cm^{-1} to the sum of the areas of the bands at 1740 and 1630 cm^{-1} reflects the degree of methylesterification (DM) of an acid polysaccharide [24].

Reduction of uronic acid

The carboxyl group in DPL and DPR was reduced according to the method of Tong [25]. The sample (40 mg) was dissolved with distilled water (40 mL) and mixed with 400 mg C.M.C (N-cyclohexyl-3-(2-methylhexyl) carbodiimide p-toluenesulfonate). The mixture was adjusted by HCL to maintain a pH of 4.8 for 3 h, then 30 mL of KBH_4 (2 M) was added within 2 h. At the same time, the pH of the resulting solution was maintained neutral (pH = 7) with 4 M HCL Then the reaction solution was dialyzed, concentrated, lyophilized to obtain the reduced polysaccharides DPL-R and DPR-R.

Methylation and GC–MS analysis

The methylation, reduction and acetylation of polysaccharides were conducted according to method of Pettolino et al. with some modification [26]. Briefly, the polysaccharide (10 mg) was mixed with NaOH–DMSO for 16 h and methylated with iodomethane for three times, the methylated product was hydrolyzed with TFA (2 mol/L) at 120 ℃ for 2 h and reduced by $NaBD_4$ at 25 ℃ for 24 h. Then the resulting product was mixed with 1 mL pyridine/acetic anhydride (1:1, v/v) to prepare the partially methylated alditol acetates (PMAA) at 100 ℃ for 2 h. The PMAAs were analyzed by Thermo Finnigan Trace MS (Finnigan, USA) and the analytical column was HP-5MS column (30 m × 0.25 mm, i.d., 0.25 μm).

NMR spectroscopy analysis

Sample (30 mg) was dissolved in 0.5 mL D_2O (99.9%) for ^1H and ^{13}C NMR analysis using a Bruker-600 FT-NMR spectrometer (Bruker, Rheinstetten, Germany).

Congo red analysis

Samples (10 mg) were dissolved in 4 mL distilled water. Polysaccharide solution (2 mg/mL, 2 mL) was blended with Congo red solution (80 μM, 2 mL). Then, NaOH solution (1 mL, 0–0.8 mol/L) was added into the mixed solution, allowed to incubate for 30 min, and the absorption value at 490–500 nm recorded. A graph was

generated with NaOH concentration as the abscissa and maximum absorbance value (λ) as the ordinate [23].

Scanning electron microscopy (SEM)

Surface morphological analyses of samples were performed by inspection using an F50 field emission scanning electron microscope (SU-8010, Hitachi, Japan). Samples were sputtered with gold powder under vacuum conditions.

Evaluation of immunoenhancement activity mediated by DPR and DPL in Cy-induced immunosuppressed black-bone silky chickens

Experimental animals

One-day-old male black-bone silky chickens ($n=800$) were purchased from a commercial hatchery (Jiangshan, Zhejiang, China), housed in environmentally friendly metal cages (length × width × height, 70 × 70 × 40 cm; 10 per cage), and permitted to drink and eat freely. During the first week of feeding, the henhouse temperature was maintained at 35 °C. Thereafter, it was gradually decreased by 3 °C every week until it reached 25 °C, and then remained constant until the end of the experiment. The black-bone silky chickens used in the experiment were prepared in accordance with the guidelines approved by the Animal Care and Use Committee of Anhui Agricultural University. Growth status was recorded daily.

Experimental design

One-day-old black-bone silky chickens (Jiangshan) were randomly divided into eight diet groups, with five replicates per group, and 20 chickens per replicate. In each group: the control group (fed basal diet); the model group (fed sterile saline instead of polysaccharides); the DPR groups (fed basal diets supplemented with 300, 600, and 900 mg DPR/kg); and the DPL groups (fed basal diets supplemented with 300, 600, and 900 mg DPL/kg). Except for the control group, all other groups were intraperitoneally injected with 80 mg/kg Cy continuously for 3 d [14]. The experiment lasted for 42 d. The schematic diagram of the experiment design is shown in Fig. 2.

Growth performance

During the experiment, it is necessary to monitor the activities, feed, and water intake of black-bone chickens daily for each cage, and the mortality rate of chickens was recorded. The body weights of the chickens need to be recorded on days 21 and 42, and ADG, ADFI, and feed conversion ratio (FCR) need to be calculated.

Immune organ index detection

On day 42, the spleen, thymus, and bursa of Fabricius were surgically excised and weighed from four black-bone silky chickens for each replicate. The immune organ index (mg/g) was calculated as the ratio of organ weight (mg) to the body weight (g).

Serum biochemical parameter assays

On day 42, it took a vacuum tube without anticoagulants to collect blood from the brachial vein after fasting for 12 h. The blood placed at 25 °C for 2 h and centrifuged (3000 × *g*, 15 min). The obtained serum was stored at − 80 °C for further analysis. The TP, ALB, and MDA levels, ALT, AST, SOD, CAT, and GSH-Px activities, and the T-AOC ability were investigated in accordance with the manufacturer's instructions.

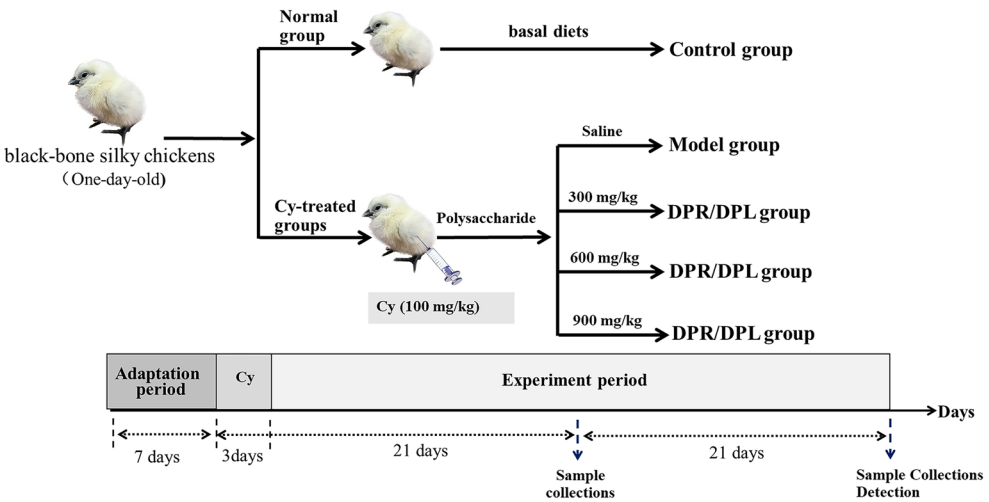


Fig. 2 The schematic diagram of the animal experiment design

Determination of cytokine and immunoglobulin

The serum was prepared, and the levels of IL-2, IL-6, INF-γ and immunoglobulins (IgA, IgG, and IgM) were determined using ELISA kits according to the manufacture's instructions.

Peripheral blood lymphocyte proliferation assay

On day 42, the fresh blood samples (1 mL/chicken) were collected from the heart of four black-bone silky chickens for each group, and the lymphocyte was prepared according to method reported previously [24]. The viability of lymphocyte was determined by trypan blue dye exclusion. 100 μL of the lymphocyte (2.5×10^6/mL) were seeded into 96-well plates and then incubated with or without 20 μL ConA (400 μg/mL) or LPS (200 μg/mL). The lymphocyte transformation was measured by CCK8 assay. The results were shown as stimulation index (SI), which was calculated using the following formula:

$$\mathrm{SI} = \text{OD value of mitogen} - \text{stimulated cells} / \text{OD value of non} - \text{stimulated cells.}$$

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) in SPSS 19.0 (SPSS Inc, Chicago, USA) and represented as mean ± S.D. Duncan's multiple-range test was used for multiple comparisons. $p<0.05$ indicates a significant difference.

Results

Preparation and characterization of polysaccharides

Two crude polysaccharides were extracted from dandelion roots and leaves using boiling water and precipitated with ethanol, with yields of 11.8% and 8.53% (relative to the dry weight of the plant). The total sugar contents evaluated in DPRC and DPLC were 78.7% and 63.2%, respectively (Table 1). The protein contents of the two polysaccharides were determined to be DPRC (10.9%) > DPRL (7.8%). The content of uronic acid was relatively lower in DPRC (11.9%) than DPLC (30.4%). Monosaccharide composition analysis showed that DPRC and DPLC mainly composed of mannose (Man), glucuronic acid (GlcA), rhamnose (Rha), galacturonic acid (GalA), glucose (Glc), galactose (Gal), and arabinose (Ara) with molar ratio of 1.36:1.00:0.87:1.61:1.36:4.80:1.32 and 1.07:1.00:1.61:3.10:1.43:4.25:1.51, respectively.

The crude polysaccharides were then deproteinated using the Sevag method, and further fractionated via Sepharose CL-6B into two major fractions, with *Mw* 58.9 kDa (DPR; yield, 59.8%) and 28.2 kDa (DPL; yield, 64.4%) (Fig. 3A–D). The total sugar content values of DPR and DPL were 95.2% and 94.1%, with uronic acid contents of 14.7% and 24.5%, respectively. Samples showed negative Bradford test results. Furthermore, no absorption at 260 and 280 nm was observed on UV spectra analysis (Fig. 3E, F), suggesting that the samples were free from protein and nucleic acid. These results demonstrate that we obtained high-purity polysaccharides from dandelion roots and leaves, and that the influence of impurities, such as proteins, was removed. Monosaccharide analysis showed that DPR was mainly composed of Gal (43.93%), Rha (9.66%), GalA (16.87%), GlcA (2.63%), Glc (7.84%), and Man (7.85%), while DPL primarily consisted of Gal (37.20%), GalA (24.86%), Glc (12.45%), Ara (7.97%), Man (5.34%) and with minor Rha (4.61%), GlcA (4.33%) and Xyl (3.24%) (Table 1).

Table 1 Yields, *Mw*, and chemical composition analysis of polysaccharides from dandelion roots and leaves

	DPRC	DPLC	DPR	DPL
Yield (w%)	11.8[a]	8.5[a]	59.8[b]	64.4[b]
Total sugar (w%)	78.7	63.2	95.2	94.1
Protein content (w%)	10.9	7.8	–	–
Ash content (w%)	11.8	18.5		
Uronic acid (w%)	11.9	30.4	14.7	24.5
Mw (kDa)	–	–	58.9	28.2
DM (%)	–	–	2.2	9.7
Monosaccharide composition (mol%)				
Man	10.87	7.56	7.85	5.34
GlcA	7.99	7.08	2.63	4.33
Rha	6.94	11.4	9.66	4.61
GalA	12.87	21.96	16.87	24.86
Glc	10.83	10.1	7.84	12.45
Gal	38.33	30.06	43.93	37.20
Xyl	Tr	1.14	2.62	3.24
Ara	10.57	10.7	6.51	7.97
Fuc	Tr	–	1.99	–

[a] Yield in relation to the dry weight of the plant

[b] Yield in relation to DPRC or DPLC

Tr: lower than 1%

FT-IR spectroscopy

FT-IR spectra are presented in Fig. 4. Obviously, the strong absorption peak approximately 3400 cm^{-1} was derived from the stretching vibration of O–H and the peak at approximately 2932 cm^{-1} was due to the stretching vibration of C–H groups ($-CH_3$, $-CH_2$, and –CH). Three characteristic absorption peaks at approximately 1746, 1630, and 1420 cm^{-1} suggested the existence of uronic acid [27]. The peaks at approximately

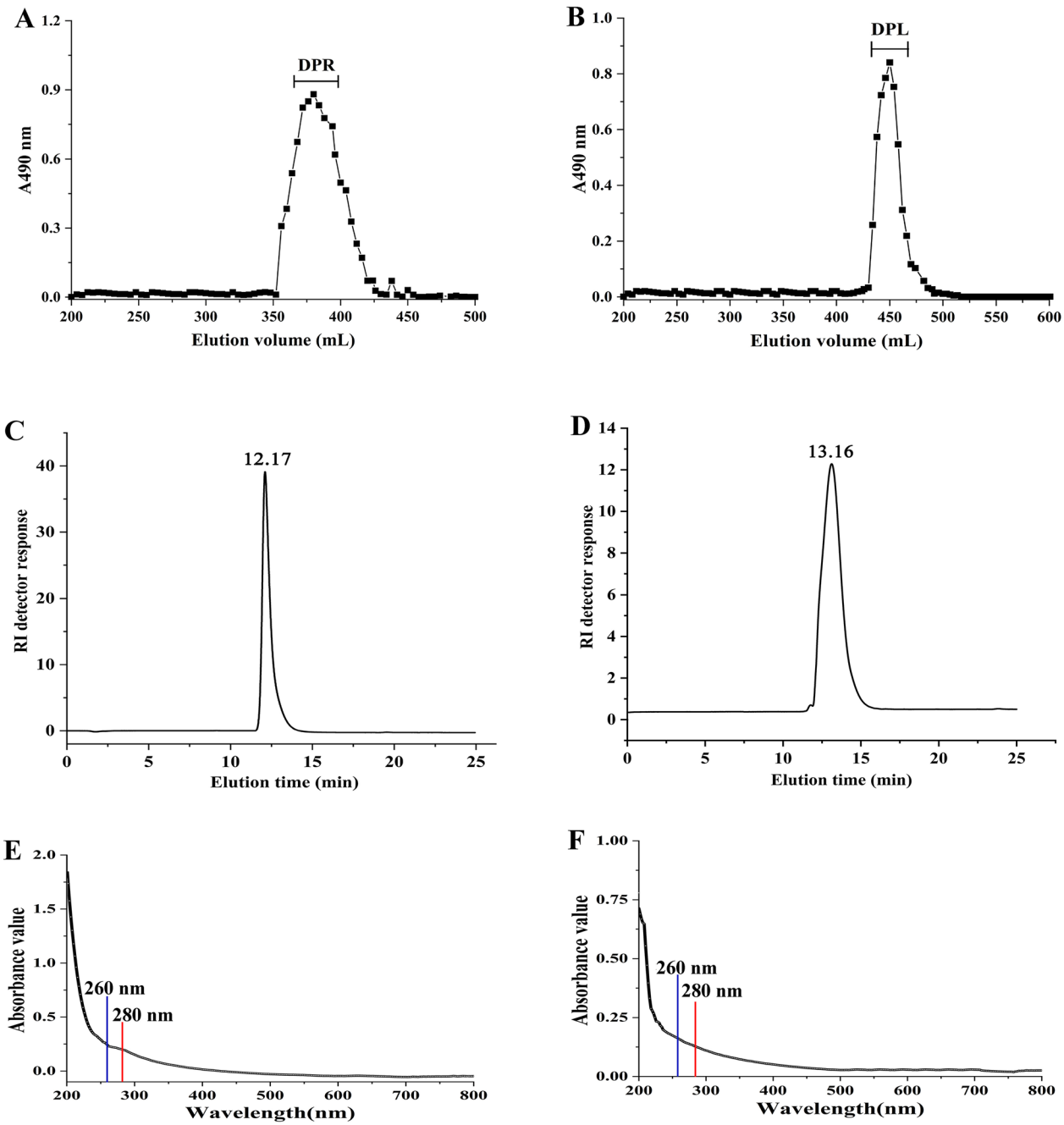


Fig. 3 Analysis of polysaccharides obtained from dandelion roots and leaves. **A**, **B** Elution profiles of DPR (**A**) and DPL (**B**) on Sepharose CL-6B column; elution profiles of DPR (**C**) and DPL (**D**) on HPGPC; UV spectra of DPR (**E**) and DPL (**F**)

1746 and 1630 cm^{-1} were assigned to the C=O stretching vibrations of methylated and free –COO– in Gal*p*A, respectively [28]. The characteristic signals at 825 and 898 cm^{-1} represented the presence of α- and β-glycosidic bonds, respectively [29]. The DM values of DPR and DPL were 2.2% and 9.7%, respectively.

Methylation analysis

Methylation is an important method for structural analysis of polysaccharides. The glycoside bond information was obtained based on the relative retention time and the main ion fragments (m/z). As shown in Table 2, DPL mainly contains eight residues, i.e., t-Ara*f*, t-Rha*p*, t-Glc*p*, t-Man*p*, 4-Gal*p*, 6-Gal*p*, 4,6-Gal*p* and 3,6-Gal*p*,

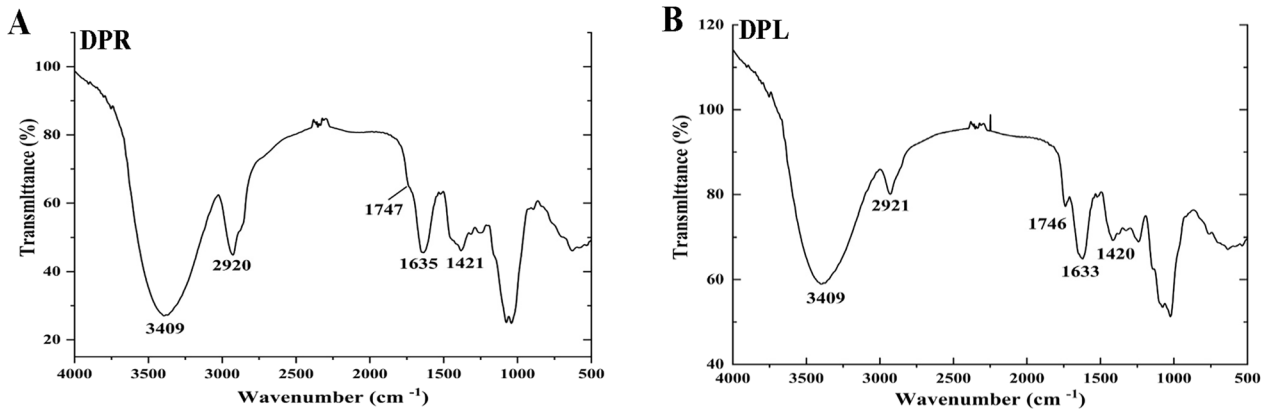


Fig. 4 FT-IR spectra of DPR (**A**) and DPL (**B**)

Table 2 Methylation analysis of polysaccharides from dandelion leaves and roots

PMAAs	Linkage pattern	Relative amount (%)				Major mass fragments (m/z)
		DPL	DPL-R	DPR	DPR-R	
2,3,5-Me_3-Ara[a]	t-Ara*f*	3.94	1.46	4.67	5.56	71,87,101,117,129,161
2,3,4-Me_3-Rha	t-Rha*p*	2.89	2.64	5.53	4.17	87,101,129,143,189,203
3,4-Me_2-Rha	2-Rha*p*	–	–	3.08	7.55	87,89,99,129,131,189
2,3,4,6-Me_4-Man	t-Man*p*	7.69	7.51	2.42	3.07	71,87,101,117,129,145,161,205
2,3,4,6-Me_4-Glc	t-Glc*p*	8.63	15.16	2.10	7.15	71,87,101,117,129,145,161,205
2,4,6-Me_3-Gal	4-Gal*p*	35.66	57.01	43.45	56.97	87,99,101,113,117,233
2,3,4-Me_3-Gal	6-Gal*p*	21.00	10.20	17.46	0.61	87,99,101,113,117,233
2,6-Me_2-Galc	3,4-Glc*p*	–	–	5.35	6.51	87,117,129
2,6-Me_2-Gal	4,6-Gal*p*	12.49	2.80	7.32	4.72	71,85,101,117,129,159,201, 261
2,4-Me_2- Man	3,6-Man*p*	7.71	3.22	8.64	3.93	87,99,101,117,129,139,159,189,201

with molar ratios of 1.36:1.00:2.66:2.99:12.34:7.27:4.32:2.67. Compared with DPL, the molar percentage content of 4-Gal*p* in DPL-R increased by about 35%, indicating that the existence of 4-GalA*p* in DPL. Similarly, the content of t-Glc*p* in DPL-R increased by 7%, indicating that it contained t-GlcA*p*. DPR mainly contains ten residues. The content of 4-Gal*p* is more than 40%. Glc exists in DPR in the forms of (1→)- and (1→4)-linkage, with approximately 7% of Glc located at the non-reducing end. t-Rha*p*, 2-Rha*p*, t-Man*p* and 3,6-Man*p* were also detected in DPR and DPR-R. Similar to DPL, DPR contains 4-GalA*p* and t-GlcA*p* as its acid sugar residues. The above results indicate that both DPR and DPL are acidic heteropolysaccharides with different branching degree. Both of them contain 4-Gal*p* and 4-GalA*p* as the main sugar residues, but the proportion of each residue is different, indicating that the two polysaccharides have different structures.

NMR analysis

NMR is an effective method for analyzing the structure of polysaccharides. The structural features of DPL and DPR were further elucidated by using ^{1}H and ^{13}C NMR spectra. The signals distributed in the range of 4.48–5.35 ppm in the ^{1}H spectra were assigned to anomeric protons, suggesting the presence of α- and β-configuration in DPL and DPR [26]. Signals at 1.16 ppm and 1.24 ppm were attributed to H-6 of α-Rha*p* (Fig. 5A and C). In the ^{13}C NMR spectra (Fig. 5B and D), the signal around 170 and 174 ppm were assigned to esterified and non-esterified carboxyl groups of uronic acid [25]. The anomeric carbon signals of DPR were distributed at 92.29 ppm ~ 103.86 ppm ppm (Fig. 5B), further indicating it contains both α- and β-sugar residues [26]. In the anomeric resign, the signals at 103.86 ppm, 99.29 ppm, 98.26 ppm, 98.01 ppm, 96.3 ppm and 92.34 ppm were assigned, respectively, to C-1 of β-4,6-Gal*p*, α-4-GalA*p*, α-4-Gal*p*, α-t-Man*p*, α-3,6-Man*p*

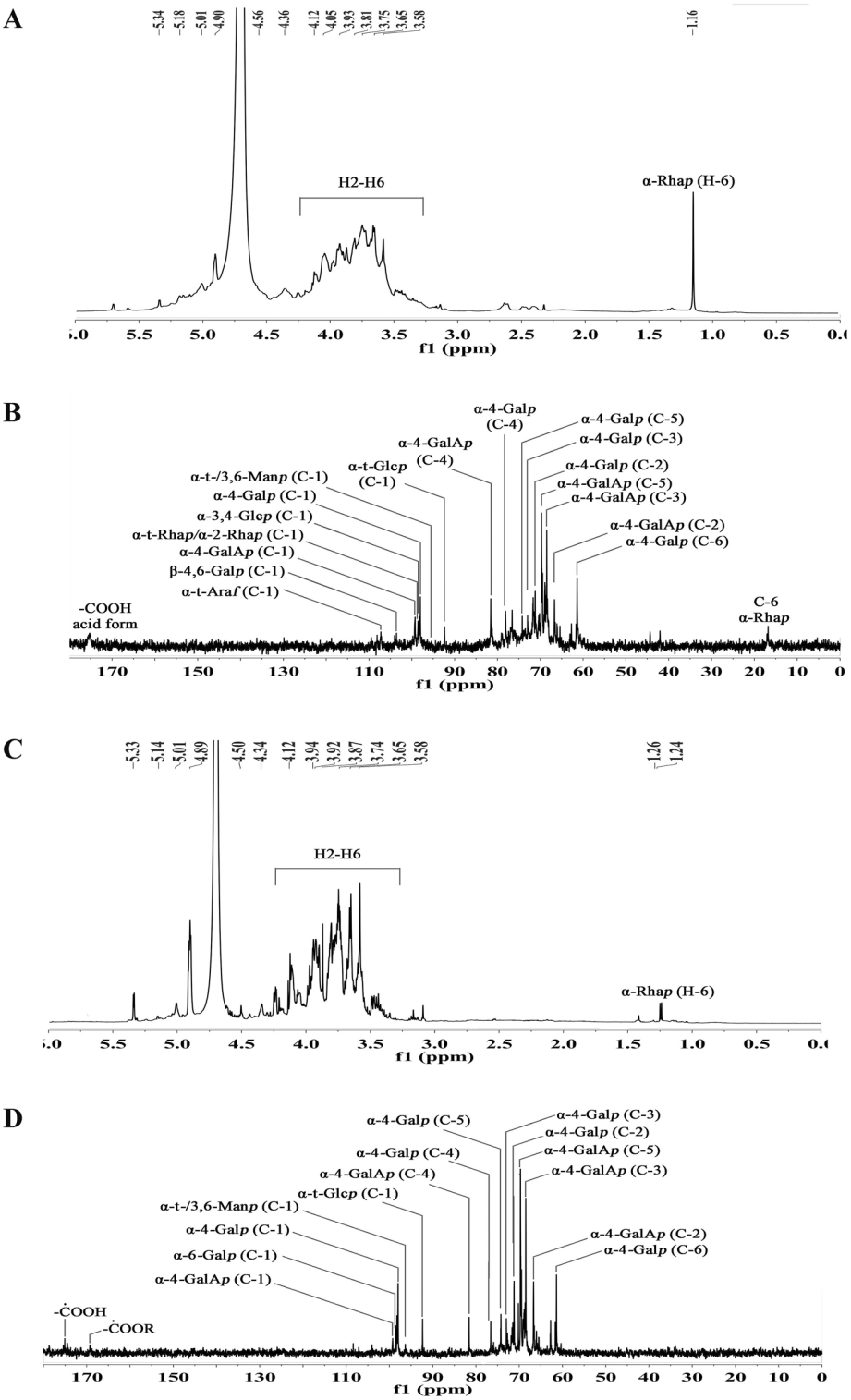


Fig. 5 NMR spectra of DPR and DPL (**A**); ^{1}H-spectrum of DPL (**B**); ^{13}C-spectrum of DPL (**C**); ^{1}H-spectrum of DPR (**D**); ^{13}C-spectrum of DPR

and α-t-Glc*p*. The chemical shift of C2-C6 of α-4-Gal*p* appeared at 71.01 ppm, 72.91 ppm, 76.85 ppm, 74.23 ppm, and 61.38 ppm. According to the ^{13}C NMR spectrum (Fig. 5D), DPL contained signals at 107.11 ppm, 103.57 ppm, 99.23 ppm, 98.55 ppm, 98.23 ppm, 98.00 ppm, 96.23 ppm, and 92.59 ppm which were attributed, respectively, to the anomic carbon signal α-t-Ara*f*, β-4,6-Gal*p*, α-4-GalA*p*, α-t-Man*p*, α-3,6-Man*p*, α-4-Gal*p*, α-t-Glc*p*. The signals at 66.68 ppm, 68.65 ppm, 81.56 ppm, and 69.45 ppm were attributed to C2-C6 of α-4-GalA*p*.

Based on the analysis of methylation, FT-IR, ^{1}H, and ^{13}C NMR, the results showed that were both acidic heteropolysaccharides, but the types and proportions of glycosidic bonds in them differed and the structure of DPR was more complex.

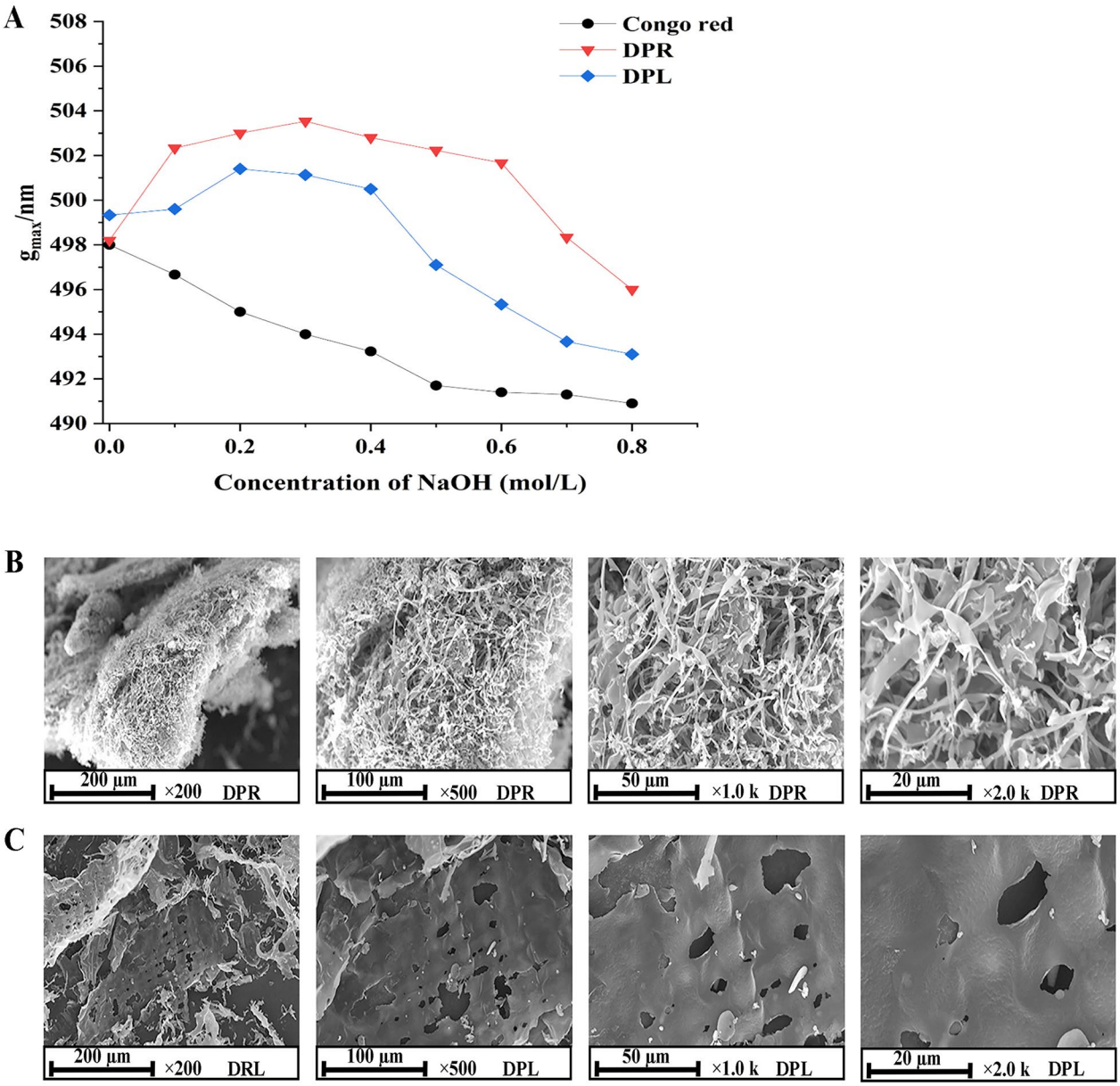


Fig. 6 Conformation analysis of DPR and DPL: Congo red analysis of DPR and DPL (**A**); SEM images of DPR (**B**) and DPL (**C**) images of different magnifications

Conformation analysis

Congo red ($C_{32}H_{22}N_6O_6S_2Na_2$) is an acid dye that can combine with polysaccharides in a helical configuration [23]. The polysaccharide–Congo red complex is stable in low-concentration NaOH solution, and its maximum absorbance demonstrates a redshift compared with that of the polysaccharide solution. Hence, Congo red can be used to investigate the presence of helical structures in polysaccharides. The maximum wavelengths of DPR and DPL in 0–0.5 M NaOH solutions are shown in Fig. 6A. The results showed an increase in the maximum wavelength of the polysaccharide and Congo red mixed solution with increasing sodium hydroxide concentration, indicating that DPR and DPL can react with Congo red to form stable complexes. The maximum wavelengths of the complexes first increased and then decreased, indicating that DPR and DPL contain triple-helix structures.

The morphological characteristics of DPR and DPL were investigated by SEM. As shown in Fig. 6B, C, the two polysaccharides presented different morphological characteristics. DPR appeared as irregular fibrous filaments, which were irregularly intertwined and formed tight clusters under 200× time magnification. At 5000× magnification, wide ribbon fibers of larger diameter were observed in DPL, accompanied by some fragments, while DPL exhibited an irregular sheet-like, uneven surface with irregular pores.

The immune enhancement activities of DPR and DPL on Cy-induced immunosuppressed chickens

Growth performance

The effects of DPR and DPL on the growth performance of immunosuppressed chickens were measured. As shown in Table 3, during the experimental period (Days 1 to 42), a significant decrease in ADG and ADFI ($p < 0.05$), and a significant increase in FCR ($p < 0.05$), were observed in the model group (Cy-treated chickens) compared with the control group. From Days 1 to 21 and Days 22 to 42, DPL (300, 600, and 900 mg/kg) supplementation dose-dependently increased the ADG and ADFI and decreased the FCR of Cy-induced chickens. During the whole experimental period, DPL at 900 mg/kg exhibited the optimal effect; ADG and ADFI increased by 14.32 and 8.52%, respectively, and FCR decreased by 9.52% compared with that of the model group.

From Days 1 to 21, the growth performance of the chickens in the DPR groups (300, 600, and 900 mg/kg) showed no significant differences compared to those of the model group ($p > 0.05$). From Days 22 to 42, the ADG and ADFI in the DPL-treated groups (900 mg/kg) significantly increased, and the FCR significantly decreased compared to that in the model group ($p < 0.05$). Additionally, the chickens received diets with DPL (900 mg/kg) possessed significantly higher ADG and ADFI and lower FCR compared with that of the model group throughout the experiment ($p < 0.05$).

Table 3 Effects of DPL and DPR supplementation on growth performance in chickens (mean ± SD, $n = 20$)

Items	Groups							
	Model	Control	DPL 300 mg/kg	DPL 600 mg/kg	DPL 900 mg/kg	DPR 300 mg/kg	DPR 600 mg/kg	DPR 900 mg/kg
IBW (g)	32.62 ± 3.51	32.41 ± 4.22	32.92 ± 2.92	31.69 ± 3.77	32.04 ± 4.2	31.85 ± 2.99	31.94 ± 3.73	32.02 ± 4.71
FBW (g)	501.54 ± 37.02	543.47 ± 24.31	517.61 ± 23.11	528.82 ± 22.23	534.49 ± 38.89	505.83 ± 41.26	516.72 ± 39.43	518.50 ± 37.67
Days 1–21								
ADG(g)	10.51 ± 1.15^{c}	12.68 ± 1.88^{a}	10.55 ± 2.03^{c}	11.11 ± 1.94^{c}	12.58 ± 1.85^{c}	10.52 ± 2.11^{c}	10.91 ± 1. 95^{bc}	11.25 ± 2.07^{ab}
ADFI(g)	18.42 ± 2.18^{d}	23.45 ± 2.83^{a}	21.94 ± 2.13^{d}	22.44 ± 2.72^{cd}	22.88 ± 3.41^{cd}	22.33 ± 2.44^{c}	22.80 ± 3.62^{b}	22.83 ± 3.73^{b}
FCR	2.13 ± 0.27^{a}	1.85 ± 0.19^{c}	2.08 ± 0.23^{a}	2.02 ± 0.24^{a}	1.91 ± 0.25^{b}	2.12 ± 0.34^{a}	2.09 ± 0.25^{a}	2.03 ± 0.34^{a}
Days 22–42								
ADG(g)	16.42 ± 2.08^{d}	21.86 ± 2.85^{a}	16.94 ± 2.13^{cd}	17.24 ± 2.72^{bc}	18.22 ± 2.47^{b}	16.76 ± 1.98^{cd}	16.96 ± 2.28^{cd}	17.23 ± 2.21^{b}
ADFI(g)	40.72 ± 4.22^{d}	42.62 ± 3.98^{a}	40.82 ± 3.88^{d}	40.94 ± 4.19^{c}	41.36 ± 4.72^{c}	40.84 ± 3.58^{d}	40.87 ± 5.17^{bc}	41.17 ± 4.47^{b}
FCR	2.48 ± 0.55^{a}	1.95 ± 0.74^{c}	2.41 ± 0.54^{a}	2.34 ± 0.66^{a}	2.27 ± 0.55^{b}	2.45 ± 0.52^{a}	2.41 ± 0.61^{a}	2.39 ± 0.48^{a}
Days 1–42								
ADG(g)	13.47 ± 1.57^{c}	17.27 ± 2.24^{a}	13.75 ± 1.62^{c}	14.18 ± 1.44^{c}	15.40 ± 2.17^{b}	13.64 ± 1.99^{c}	13.94 ± 1.95^{c}	14.24 ± 2.02^{c}
ADFI(g)	29.57 ± 2.55^{c}	33.04 ± 3.41^{a}	31.38 ± 3.25^{bc}	31.69 ± 2.92^{bc}	32.09 ± 4.11^{b}	31.56 ± 3.98^{bc}	31.83 ± 3.49^{bc}	32.00 ± 4.08^{b}
FCR	2.31 ± 0.31^{a}	1.90 ± 0.21^{c}	2.25 ± 0.32^{a}	2.18 ± 0.28^{a}	2.09 ± 0.31^{a}	2.29 ± 0.27^{a}	2.25 ± 0.29^{a}	2.21 ± 0.41^{a}

IBW, initial body weight (g); FBW, final body weight (g); ADG: daily weight gain (g); ADFI: daily feed intake (g); FCR: feed conversion ratio

$^{a–d}$ Means in a row without the same superscripts differ significantly ($p < 0.05$)

Table 4 Effects of DPL and DPR supplementation on immune organ index in chickens (mean ± SD, $n = 20$)

Organs	Groups							
	Control	Model	DPL-300	DPL-600	DPL-900	DPR-300	DPR-600	DPR-900
Thymus index	3.17 ± 0.49^{a}	2.62 ± 0.47^{b}	2.74 ± 0.52^{b}	2.81 ± 0.41^{b}	2.91 ± 0.37^{a}	2.67 ± 0.36^{bc}	2.86 ± 0.42^{b}	2.84 ± 0.49^{b}
Spleen index	2.25 ± 0.55^{a}	1.45 ± 0.51^{c}	1.74 ± 0.46^{b}	1.89 ± 0.61^{b}	1.99 ± 0.47^{a}	1.52 ± 0.39^{bc}	1.65 ± 0.46^{b}	1.77 ± 0.51^{b}
Bursa of Fabricius index	3.53 ± 0.36^{a}	2.52 ± 0.39^{d}	2.61 ± 0.37^{c}	2.84 ± 0.41^{bc}	3.09 ± 0.52^{b}	2.37 ± 0.31^{d}	2.58 ± 0.38^{cd}	2.74 ± 0.41^{c}

DPL/DPR-300, DPL/DPR 300 mg/kg; DPL/DPR-600, DPL/DPR 600 mg/kg; DPL/DPR-900, DPL/DPR 900 mg/kg; control: the normal group; model: Cy-treated group

[a–d] Means in a row without the same superscripts differ significantly ($p < 0.05$)

Table 5 The effect of DPL and DPR on lymphocyte proliferation

Groups	Dose (mg/kg)	Lymphocyte	
		T cells (A450 nm)	B cells (A450 nm)
Control	–	1.29 ± 0.12^{a}	0.96 ± 0.09^{a}
Model	–	0.74 ± 0.08^{d}	0.54 ± 0.07^{d}
DPL	300	0.85 ± 0.08^{c}	0.69 ± 0.06^{c}
	600	0.98 ± 0.09^{bc}	0.78 ± 0.07^{bc}
	900	1.12 ± 0.09^{b}	0.86 ± 0.05^{b}
DPR	300	0.78 ± 0.07^{d}	0.59 ± 0.06^{d}
	600	0.79 ± 0.05^{d}	0.62 ± 0.07^{d}
	900	0.91 ± 0.09^{bcd}	0.67 ± 0.06^{c}

[a–d] Means in a row without the same superscripts differ significantly ($p < 0.05$)

Effects of dandelion polysaccharides on immune organ index

DPR and DPL improved the index of immune organs in immunosuppressed chickens. As shown in Table 4, the thymus, spleen, and bursa of Fabricius indices in Cy-induced chickens significantly decreased compared with those of the control group ($p < 0.05$), indicating that the immunosuppressed chicken model was successfully established. In the DPL-treated groups, the relative thymus, spleen and bursa of Fabricius index of immunosuppressed chickens increased in a dose-dependent manner compared with those of the model group. Compared with the model group, significant increases in the immune organ ($p < 0.05$) were observed in chickens fed diets containing 900 mg/kg DPL. In addition, the immunological enhancement of DPL was higher than that of DPR at the same concentrations, suggesting that the contribution of DPL to the immunostimulatory activity of dandelion polysaccharides was higher than that of DPR.

Effect of dandelion polysaccharides on lymphocyte proliferation

DPR and DPL were measured for ConA- and LPS-induced mitogen activity of lymphocytes and the results are shown in Table 5. DPL and DPR were able to stimulate T and B lymphocyte proliferation in a dose-dependent manner in the tested dose range. DPL significantly enhanced both T and B cell proliferation at 300, 600 and 900 mg/kg compared with the model group ($p < 0.05$). Compared with DPL, DPR exhibited a significant improvement on lymphocyte proliferation at a high concentration (900 mg/kg). Moreover, the action of DPL was stronger than that of DPR at doses of 600 and 900 mg/kg.

Effect of dandelion polysaccharides on serum biochemical parameter levels

As shown in Fig. 7A–D, the levels of AST and ALT were obviously increased in Cy-treated chickens, while the levels of ALB and TP were significantly decreased ($p < 0.05$). Supplementation in feed with DPL at 900 mg/kg markedly and dose-dependently reversed the abnormal serum indicators by elevating the concentrations of TP, and reducing the AST level. However, no evident differences were found in the levels of TP, ALB, AST, and ALT between the DPR supplementation groups and the model group.

Antioxidant activity assay

The effects of dandelion polysaccharides to the activity of antioxidant enzymes in chickens were measured. As shown in Fig. 8A–E, the activities of SOD, CAT, GSH-Px and T-AOC in the serum of the model group were significantly lower than those of the control group ($p < 0.05$). DPL supplementation significantly improved the activities of antioxidant enzymes and the T-AOC of immunosuppressed chickens in a dose-dependent manner ($p < 0.05$). DPL at 900 mg/kg showed the best antioxidant activities, and the enzymatic activities of SOD, CAT, and GSH-Px in chickens increased by 60.06, 28.66, and 21.70%, respectively, compared with those of the model group. The serum level of MDA decreased by 25.76%. In contrast, DPR supplementation at the tested dosages reduced the MDA levels; however, no significant difference was observed compared with the model group, suggesting that DPL showed stronger effect in alleviating the oxidative damage in immunosuppressed chickens.

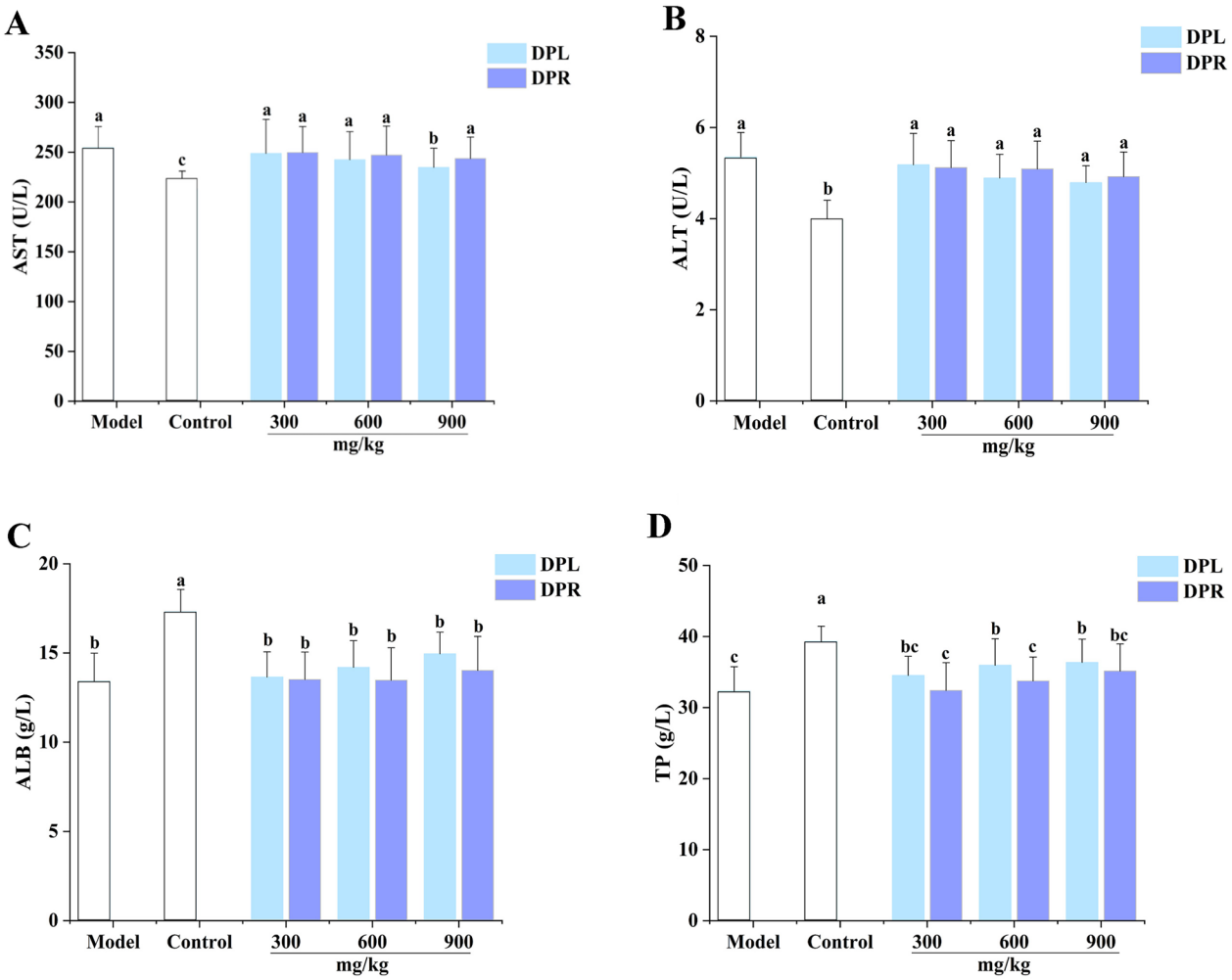


Fig. 7 Effects of DPR and DPL on serum biochemical parameter of Cy-induced immunosuppressed chickens: AST (**A**), ALT (**B**), ALB (**C**), and TP (**D**). Data ('a–d') in bars represent significant differences between groups, with different letters indicating significant differences between groups ($p < 0.05$) and the same letter indicating no significant differences between groups ($p \geq 0.05$). Model, chickens treated with Cy; control, normal group; 300, chickens treated with 300 mg/kg dandelion polysaccharides; 600, chickens treated with 600 mg/kg dandelion polysaccharides; 900, chickens treated with 900 mg/kg dandelion polysaccharides

Cytokine level assay

Serum IL-2, IL-6 and INF-γ levels are presented in Fig. 9A–C. The Cy-treated group had lower levels of serum IL-2, IL-6 and INF-γ relative to the control group ($p < 0.05$). In the DPL and DPR groups, the levels of IL-2, IL-6 and INF-γ in the serum were improved in immunosuppressed chickens compared with those of the model group. DPL supplementation (600 and 900 mg/kg) significantly increased the levels of IL-6 and INF-γ ($p < 0.05$) in the serum; in contrast, there were no significant differences between the DPR-supplemented groups and the Cy-treated group ($p > 0.05$).

Concentration of immunoglobulins

In the Cy-treated group, the levels of IgA, IgG, and IgM in the serum were decreased 24.81%, 44.13%, and 33.07%, respectively, compared to the control group (Fig. 9D–F). IgA, IgG and IgM levels in the dandelion polysaccharide-treated groups increased dose-dependently. IgA and IgG levels in chickens treated with 900 mg/kg DPL were significantly increased by 23.71% and 25.69%, respectively, compared with those of the model group. Consistent with previous results, the effect of DPL was better than that of DPR.

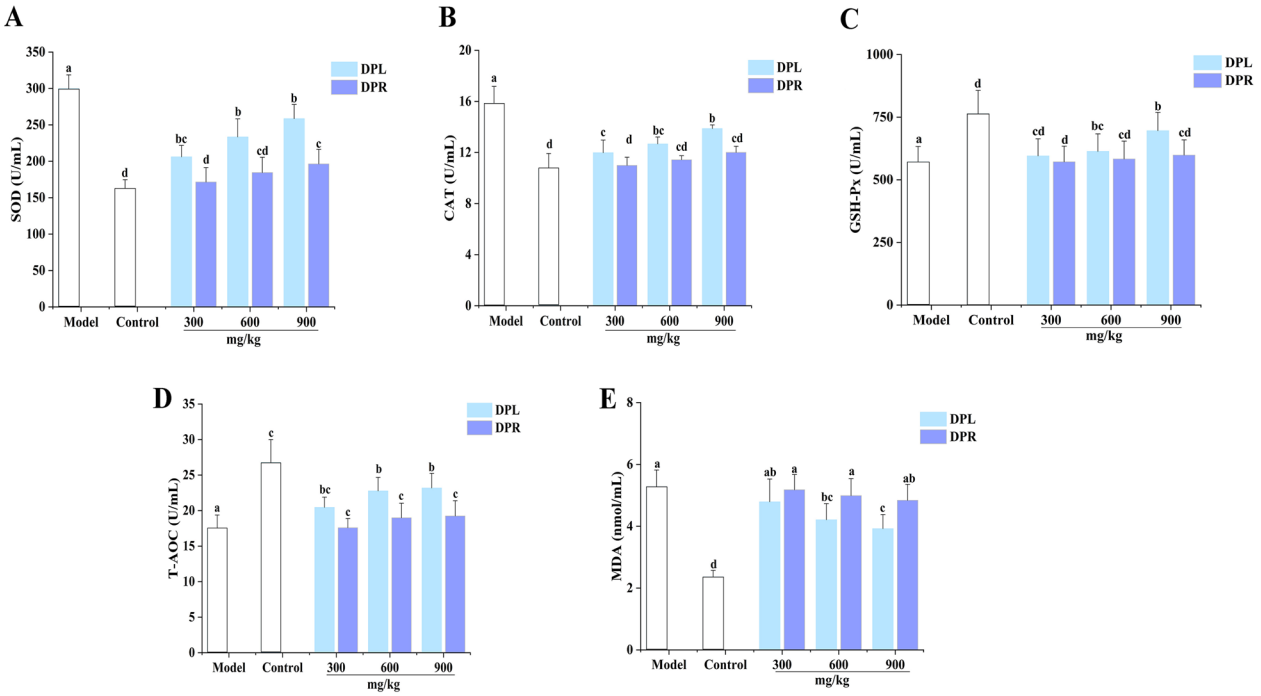


Fig. 8 Effects of DPR and DPL on the antioxidant capacity of Cy-induced immunosuppressed chickens:SOD (**A**), CAT (**B**), GSH-Px (**C**), T-AOC (**D**), and MDA (**E**). Data ('a–d') in bars represent significant differences between groups, with different letters indicating significant differences between groups ($p < 0.05$) and the same letter indicating no significant differences between groups ($p \geq 0.05$). Model, chickens treated with Cy; control, normal group; 300, chickens treated with 300 mg/kg dandelion polysaccharides; 600, chickens treated with 600 mg/kg dandelion polysaccharides; 900, chickens treated with 900 mg/kg dandelion polysaccharides

Discussion

Immunosuppression can cause concurrent or increased incidence rates of, secondary infections and reduce the protective effect of vaccines on poultry, leading to decreased chicken production capacity and even death [30]. In this study, chickens injected with Cy exhibited decreased excitability, appetite, and weight loss. Furthermore, poor growth performance, low ADG and ADFI, and high FCR were observed in Cy-treated chickens. In addition, immune organ indices, globulin concentrations, and antioxidant enzyme activity in the serum decreased, relative to those in the control group. The above results indicate that the immune function of black-bone silky chickens was dramatically affected by Cy, and demonstrate the successful establishment of an immunosuppression model.

We obtained two acidic heteropolysaccharides (DPR and DPL), with different structural features, from dandelion roots and leaves, using water extraction, ethanol precipitation and gel chromatography. To improve their purity, Sevag reagent was used to remove proteins during preparation of the polysaccharides. The effects of DPR and DPL on the immune and antioxidant systems of black-bone silky chickens were investigated, and the results showed that DPR and DPL had positive effects on growth performance, particularly at 900 mg/kg, compared with the model group. Within the range of 300 to 900 mg/kg, supplementation with DPR and DPL improved ADG and ADFI in immunosuppressed chickens, and decreased FCR during the overall experimental period. The thymus, spleen, and bursa of Fabricius are the main immune organs in chickens, and are typically used to evaluate immunological status in these birds. Lymphocyte proliferation is the key event in activation process of both cellular and humoral immune responses. Lymphocyte proliferation induced by ConA or LPS can be used to evaluate T or B lymphocyte activity. In this work, both DPR and DPL enhanced T and B cell proliferation. However, DPL exhibited better proliferation effects of T and B cells than DPR did, indicating that it is a possible potential immunopotentiator for use in livestock and poultry production. Until now, various polysaccharides, with different structural characteristics, were isolated from dandelion roots or leaves. Cai et al. obtained two polysaccharides DRP-2b (31.8 kDa) and DRP-3a (6.72 kDa) from dandelion, and demonstrated that DRP-2b is mainly composed of 5-Ara*f*, 6-Glc*p* and 6-Gal *p*composition; in contrast, DRP-3a has 6-Glc*p* and 2,6-Glc*p* as the major sugar residues [31]. The results of Li et al. indicate the existence of rhamnogalacturonan I (RG I), homogalacturonan

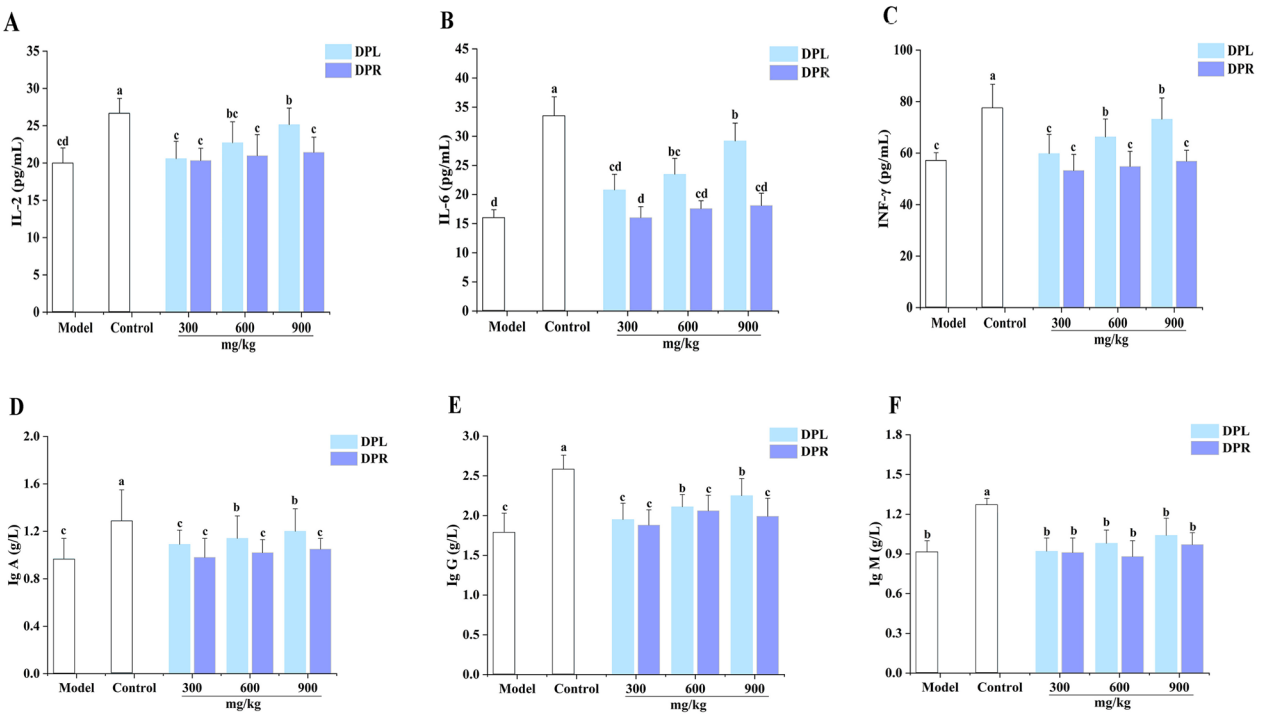


Fig. 9 Effects of DPR and DPL on the IL-2 (**A**), IL-6 (**B**), IFN-γ (**C**), IgA (**D**), IgG (**E**), and IgM (**F**) levels of Cy-induced immunosuppressed chickens. Data ('a–d') in bars represent significant differences between groups, with different letters indicating significant differences between groups ($p < 0.05$) and the same letter indicating no significant differences between groups ($p \geq 0.05$). Model, chickens treated with Cy; control, normal group; 300, chickens treated with 300 mg/kg dandelion polysaccharides; 600, chickens treated with 600 mg/kg dandelion polysaccharides; 900, chickens treated with 900 mg/kg dandelion polysaccharides

(HG), arabinogalactan, and glucomannan in dandelion leaves [32]. The extraction and purification method has important impacts on structure and activity of polysaccharides. In this article, we used the same extraction and purification methods to prepare polysaccharides from dandelion roots and leaves, and compared their immunomodulatory activities. We obtained two polysaccharides, DPR (5.89×10^4) and DPL (28.2×10^4 Da) from dandelion roots and leaves. To improve their purity, Sevag reagent was used to remove proteins during preparation of the polysaccharides. According to the GC–MS and NMR analysis, DPR and DPL were acidic heteropolysaccharides, with complex structural features. Both of them were mainly composed of 4-Gal*p* and 4-GalA*p*. However, the linkage types and proportions of sugar residues are different, which might be the reason resulting in their different immunoenhancement.

Serum globulin concentration is also a vital indicator of the humoral immunological status of an organism [15]. Our results showed that DPL improved immune organ indices and serum concentrations of IL-2, IL-6 and INF-γ, IgM, IgA, and IgG, compared with those of the model group, indicating that it can antagonize Cy-induced immunosuppression reactions. Serum immunoglobulin levels are important markers of humoral immune function [33]. Immunoglobulins, including IgG, IgA, IgM, IgD, and IgE, are related to immune responses and regulation, and several diseases are closely associated with changes in the concentrations of IgA, IgG, and IgM in serum [34]. In this study, the immune organ index, and the serum concentrations of IL-2, IL-6, INF-γ, IgM, IgA, and IgG in the model group decreased compared with those of the control group. Moreover, dandelion polysaccharides antagonized Cy-induced immunosuppression reactions. DPL at 600 and 900 mg/kg significantly increased serum IgA and IgG levels in black-bone silky chickens. However, the enhancing effects of DPR and DPL on immune function in immunosuppressed chickens differ. DPR treatment did not significantly improve the abnormalities of the above indicators, even at 900 mg/kg. Based on these results, at the same dose, both polysaccharides improved the above indicators, showing a trend of DPL > DPR, indicating that DPL has stronger effects in resisting Cy-induced immune suppression.

Polysaccharides are biomacromolecules with complex structures, and their biological activities may be influenced by several factors, including monosaccharide composition, *Mw*, category of glycosidic bond and chain

conformation [35]. Based on our results, DPL which had lower *Mw*, higher GalA content than DPL, showed lower immunoenhancement effects. Interestingly, polysaccharides are rich in hydroxyl groups, which can form numerous intermolecular and intramolecular hydrogen bonds, leading to the formation of different conformations. Triplex conformation is an important characteristic of immunoactive polysaccharides [36], and our conformation analysis showed that DPR and DPL possessed triplex chain conformations, but with different morphological structures. Therefore, we speculate that the difference in immunoenhancement between the dandelion polysaccharides may result from the synergistic effects of multiple factors.

Cyclophosphamide weakens the immune system and causes endocrine dyscrasia in poultry, leading to metabolic disorders and increasing the concentration of free radicals [37]. Excessive free radicals cause oxidative stress and reduce the growth performance of animals. Based on our results, compared to the control group, serum MDA in the Cy-treated group increased, and the activities of SOD, GSH-Px, and T-AOC in serum decreased significantly, suggesting that Cy administration weakened the antioxidant capacity of black-bone silky chickens. In addition, the levels of serum ALT and AST in the model group were significantly higher than those in the control group, which may have been due to the consumption of microsomal cytochrome P450 by Cy activation in the liver, leading to an increase in ROS and lipid peroxidation, ultimately leading to liver cell damage. Recently, the antioxidant activities of plant polysaccharides have received increasing attention. Studies have shown that plant polysaccharides can restore Cy-induced oxidative damage [14]. In our results, compared with the model group, supplementation with DPR or DPL markedly reduced MDA levels, improved the activity of SOD, CAT, and GSH-Px, and improved T-AOC, demonstrating the protective effect of dandelion polysaccharides on the antioxidant system of immunosuppressed black-bone silky chickens. Interestingly, the antioxidant ability in immunosuppressed chickens was better in the DPL groups than in the DPR groups, indicating that DPL plays a stronger role in resisting oxidative injury induced by Cy. Zhang et al. also noted that dandelion leaf polysaccharides, which are rich in GalA, showed stronger antioxidant activity than polysaccharides from dandelion roots [11, 38]. Meanwhile, significant decreases in ALT and AST levels and increases in TP and ALB levels were observed in serum from DPL-treated black-bone silky chickens, indicating that DPL supplementation can improve physiological protein synthesis capacity.

Most plant polysaccharides are considered potential prebiotics because of their complex structures. They can be easily utilized by gut microbiota to produce short-chain fatty acids (SCFAs) and other beneficial metabolites, thereby affecting animal health [39]. Various polysaccharides can improve the structure and abundance of gut microbiota in chickens, including *Bifidobacterium* and *Lactobacillus*, which enhance the activity of digestive enzymes and improve protein absorption and utilization. Furthermore, such polysaccharides can increase intestinal acidity and promote chemical barrier formation by promoting SCFA production and reducing the physiological generation of harmful nitrogen [40]. A similar phenomenon was also observed in this study, where dandelion polysaccharides improved serum biochemical indicators related to metabolism, and DPL exhibited superior effects to DPR; however, further research is needed to determine whether this effect is related to intestinal microbiota regulation.

Based on the above analysis, we conclude that DPL has stronger effects on immune enhancement and antioxidant stress in immunosuppressed black-bone silky chickens, possibly due to differences in its structural features relative to DPR.

Conclusions

DPR and DPL obtained by hot water extraction from dandelion roots and leaves and purified via proteinization and gel chromatography. DPR (58.9 kDa) had a higher *Mw* than DPL (28.2 kDa). It contained Gal as its major sugar, whereas DPL was mainly composed of Gal and GalA. They were mainly composed of 4-α-Gal*p* and 4-α-GalA*p* as the backbone structure. Moreover, both dandelion polysaccharides contained triple-helical structures, but had different morphological characteristics. Our results also indicate that the addition of the dandelion polysaccharides, particularly DPL improved growth performance by alleviating the damage of immune function and antioxidant system in Cy-induced black-bone silky chickens. Notably, the contribution of DPL to immunoenhancement and antioxidant activities of black-bone silky chickens is higher than that of DPR. Thus, polysaccharides from dandelion leaves can be used as natural feed additives to protect chickens against immune deficiency and oxidative stress. However, the molecular mechanism of DPL on immunosuppressive chickens is still unclear. In addition, the structural features of polysaccharides are closely related to their biological activities. However, it is still unclear whether the structural characteristics of DPL have an impact on its immune enhancement effect. Therefore, the immunoenhancing mechanism and structure–activity relationship of DPR will be further studied in the future work.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00568-y.

Additional file 1. Fig. S1. Total ion flow diagram of GC–MS analysis for DPR and DPR-R. **Fig. S2**. Total ion flow diagram of GC–MS analysis for DPL and DPL-R.

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Author contributions
QG. Q. methodology, investigation and writing-original draft. XZ. S., C. Z. validation and visualization. CX. J., RS. J. writing—review and editing, project funding acquisition. All authors contributed to the article and agreed to the published version of the manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The animal study was reviewed and approved by the Animal Care and Use Committee of Anhui Agricultural University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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