# Isolation, Purification and Antioxidant Activity Analysis of Polysaccharides from the New Cultivar of Acanthopanax senticosus 'Zijia No. 1' (Post-print)

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## Abstract

'Zijia No. 1' is a new cultivar of Acanthopanax senticosus bred by our research group, featuring gray-purple tender stems and leaves with a sweet taste. This study aimed to isolate and purify polysaccharides from the tender stems and leaves of 'Zijia No. 1', determine the monosaccharide composition and molecular weight of each fraction, and evaluate their antioxidant activities. Using tender stems and leaves of 'Zijia No. 1' as raw material, crude polysaccharides (A. polysaccharides, ASPS) were obtained through water extraction and alcohol precipitation, followed by purification and decolorization via macroporous resin adsorption. Polysaccharide fractions were isolated and purified by DEAE-Cellulose 52 ion-exchange chromatography and Sephadex G-100 gel chromatography. The monosaccharide composition and molecular weight of each homogeneous fraction were determined by ion chromatography and gel permeation chromatography with refractive index and multi-angle laser light scattering detection. The in vitro antioxidant activities were analyzed by measuring the scavenging capacities of homogeneous fractions against hydroxyl radicals ( OH), superoxide anion radicals (O2- ), and 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH). The results showed that four polysaccharide fractions were obtained from ASPS through isolation and purification: acidic polysaccharides ASPA-1-1, ASPA-2-1, ASPA-3-1, and neutral polysaccharide ASPN-1, with molecular weights of 8.10, 26.15, 0.91, and 0.89 kDa, respectively. These were heteropolysaccharides mainly composed of arabinose, rhamnose, galactose, glucose, xylose, mannose, ribose, galacturonic acid, and glucuronic acid in different proportions. ASPA-1-1, ASPA-2-1, ASPA-3-1, and ASPN-1 all exhibited significant antioxidant activities, among which ASPA-2-1 showed higher scavenging abilities against OH and DPPH than ASPA-1-1, ASPA-3-1, and ASPN-1, while ASPA-3-1 demonstrated the strongest

scavenging capacity against O2- . Therefore, the polysaccharides isolated and purified from 'Zijia No. 1' possess good antioxidant activities, providing a scientific basis for their further in-depth research, development, and utilization.

## Full Text

# Isolation, Purification, and Antioxidant Activity Analysis of Polysaccharides from a New Variety of *Acanthopanax senticosus* 'Zijia 1'

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## Abstract

'Zijia 1' is a new variety of *Acanthopanax senticosus* bred by our research team, characterized by gray-purple tender stems and leaves with a sweet taste. This study aimed to isolate and purify polysaccharides from the tender stems and leaves of 'Zijia 1', determine the monosaccharide composition and molecular weight of each fraction, and evaluate their antioxidant activities. Using tender stems and leaves of 'Zijia 1' as raw material, crude polysaccharides (ASPS) were obtained through water extraction and ethanol precipitation, followed by purification and decolorization using macroporous resin adsorption. The polysaccharide fractions were then separated and purified by DEAE-Cellulose 52 ion-exchange chromatography and Sephadex G-100 gel filtration chromatography. Ion chromatography and gel permeation chromatography coupled with refractive index and multi-angle laser light scattering detection were employed to determine the monosaccharide composition and molecular weight of each homogeneous component. The *in vitro* antioxidant activity was assessed by measuring the scavenging capacity against hydroxyl radicals ($\cdot$OH), superoxide anion radicals ($O_2^{-}\cdot$), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The results showed that four polysaccharide fractions were isolated and purified from ASPS: acidic polysaccharides ASPA-1-1, ASPA-2-1, and ASPA-3-1, and neutral polysaccharide ASPN-1, with molecular weights of 8.10, 26.15, 0.91, and 0.89 kDa, respectively. These were heteropolysaccharides primarily composed of arabinose, rhamnose, galactose, glucose, xylose, mannose, ribose, galacturonic acid, and glucuronic acid in varying proportions. All four fractions exhibited significant antioxidant activity, with ASPA-2-1 showing higher scavenging capacity against $\cdot$OH and DPPH radicals than ASPA-1-1, ASPA-3-1, and ASPN-1, while ASPA-3-1 demonstrated the strongest $O_2^{-}\cdot$ scavenging ability. Therefore, the polysaccharides isolated from 'Zijia 1' possess notable antioxidant activity, providing a scientific basis for further in-depth research and development of this plant variety.

**Keywords:** Zijia 1; tender stems and leaves; polysaccharides; isolation and purification; monosaccharide composition; molecular weight; antioxidant activity

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'Zijia 1' originates from wild *Acanthopanax senticosus* collected in Mojiang County at the southern foothills of the Ailao Mountains in Yunnan Province. This perennial evergreen plant was bred by our research team (Registration No.: Yunlin Yuanzhi Xindeng 20220011). Its tender stems and leaves exhibit a gray-purple color and sweet taste, distinguishing them from common *A. senticosus* varieties that have green stems and leaves with a bitter flavor. Rich in protein, crude fiber, and vitamins, 'Zijia 1' serves as a woody vegetable with high potassium, calcium, and magnesium content but low sodium levels (Guan et al., 2018). Its roots are used medicinally and are considered warm in nature, possessing functions such as tonifying qi and spleen, nourishing kidney and calming spirit, and strengthening the body's resistance (Wang, 2018). With high medicinal value, this variety shows considerable promise for medical and healthcare applications.

Polysaccharides are complex, highly polar compounds formed by multiple monosaccharides linked through glycosidic bonds (Ullah et al., 2019). Natural polysaccharides have become a research focus worldwide due to their favorable bioactivities, non-toxicity, biodegradability, and biocompatibility (Zhou et al., 2021). Polysaccharides from *A. senticosus* have been confirmed to possess antioxidant properties (Ji & Wang, 2022), inhibit cancer cell proliferation (Peng et al., 2020), and treat immune liver injury (Zhang et al., 2019). While most studies have focused on the roots, stems, and leaves of common *A. senticosus*, the antioxidant properties of different polysaccharide fractions from 'Zijia 1' have not been reported. Polysaccharides extracted from 'Zijia 1' contain substantial impurities such as proteins and pigments, making their separation and purification a current research priority. Therefore, comprehensive comparison and evaluation of different polysaccharide fractions from 'Zijia 1' and their antioxidant activities hold significant scientific importance.

This study investigated polysaccharides from the tender stems and leaves of 'Zijia 1' using modern chromatographic separation techniques, spectroscopic methods, and pharmacological approaches to address three key questions: (1) the molecular weight of polysaccharides from 'Zijia 1' tender stems and leaves; (2) the monosaccharide composition of these polysaccharides; and (3) the *in vitro* antioxidant activity of the polysaccharides, thereby establishing a foundation for further research and utilization of 'Zijia 1'.

## 1. Materials and Methods

### 1.1 Materials and Reagents

Tender stems and leaves (the top 5–7 cm young shoots) of 'Zijia 1', a new *A. senticosus* variety (Registration No.: Yunlin Yuanzhi Xindeng 20220011) bred

by our team, were collected from Mojiang County, Yunnan Province (101°41 E, 23°25 N, altitude: 1,282 m). The samples were dried at 70 °C to constant weight, crushed, and stored in self-sealing bags under ambient dry conditions away from light. AB-8 macroporous adsorption resin was purchased from Beijing Solarbio Science & Technology Co., Ltd. Glass chromatography columns were obtained from Sichuan Shubo (Group) Co., Ltd. DEAE-Cellulose 52, Sephadex G-100, and dialysis bags were acquired from Shanghai Yuanye Bio-Technology Co., Ltd. Monosaccharide standards (HPLC $\geq$ 98%), sodium hydroxide (chromatographic grade), and sodium acetate were from Sigma-Aldrich (USA). Trifluoroacetic acid (chromatographic grade), sodium nitrate, methanol, sodium chloride, phenol, concentrated sulfuric acid, pyrogallol, hydrochloric acid, vitamin C (Vc), salicylic acid, Tris, and ferrous sulfate were all analytical grade reagents from domestic sources.

### 1.2 Experimental Instruments

The following instruments were used: DZF-6050 vacuum drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd.), ALPHA 1-2 LD plus vacuum freeze dryer (Martin Christ, Germany), UV-6100S UV-Vis spectrophotometer (Shanghai Yuanxi Instrument Co., Ltd.), XH-T vortex mixer (Xinbao Instrument), Reacti-thermo nitrogen evaporator (Thermo Fisher Scientific), ICS5000 ion chromatography system (Thermo Fisher Scientific), OPTILAB T-rEX refractive index detector (Wyatt Technology), and DAWN HELEOS-II multi-angle laser light scattering detector (Wyatt Technology).

### 1.3 Experimental Methods

**1.3.1 Extraction of Polysaccharides** Polysaccharides were extracted using the hot water extraction method (Tan et al., 2022). Briefly, 300 g of sample was soaked in 5.0 L of ultrapure water for 2.0 h, then boiled and extracted. The filtrate was collected and concentrated to one-fifth of its original volume, centrifuged at 3,500 $r \cdot min^{-1}$ for 5 min, and the supernatant was passed through an AB-8 macroporous adsorption resin column (10 cm × 30 cm) for purification and decolorization. Absolute ethanol was added to achieve a final concentration of 95%, and the mixture was precipitated at 4 °C for 12 h. The precipitate was collected and vacuum freeze-dried at -30 °C to obtain crude polysaccharides (ASPS).

**1.3.2 Determination of Polysaccharide Content** The phenol-sulfuric acid method was used to determine polysaccharide content (Yu et al., 2019). Anhydrous glucose standard (10 mg) was dissolved in 100 mL ultrapure water. Aliquots of 0, 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 mL were transferred to test tubes, diluted to 2 mL with ultrapure water, and mixed with 1 mL of 6% phenol and 5 mL of concentrated sulfuric acid. After shaking, the mixture was left for 5 min, reacted in boiling water for 15 min, cooled to room temperature, and the absorbance at 490 nm was measured. A standard curve was plotted with con-

centration ( g · $mL^{-1}$) as the x-axis and absorbance as the y-axis, yielding the regression equation y = 0.0141x + 0.0056 ($R^2$ = 0.9995), showing good linearity for anhydrous glucose in the range of 15–90 g · $mL^{-1}$.

For sample measurement, 10 mg of ASPS was dissolved in ultrapure water and diluted to 5 mL. Two milliliters of this solution were transferred to a test tube, treated with the same reagents as above, and the absorbance at 490 nm was measured. Polysaccharide content was calculated using the following formula:

$$\text{Content } (\%) = \frac{m \times V_T \times N}{V_S \times 10^6 \times m_0} \times 100$$

where $m$ is the polysaccharide amount ( g) obtained from the standard curve; $V\_T$ is the total sample volume (mL); $V\_S$ is the sample volume used for measurement (mL); $N$ is the dilution factor (1 in this experiment); $10^6$ is the conversion factor (1 g = $10^6$ g); and $m\_0$ is the sample mass (g).

### 1.3.3 Separation and Purification of Polysaccharides (1) Linear gradient elution analysis of polysaccharides on ion-exchange column

Ten milligrams of ASPS were dissolved in 10 mL ultrapure water, centrifuged at 4 °C and 3,500 r · $min^{-1}$ for 10 min, and the supernatant was loaded onto a DEAE-Cellulose 52 column (2.5 cm × 45 cm). Elution was performed sequentially with ultrapure water and 0.1–1.0 mol · $L^{-1}$ NaCl solution at a flow rate of 5 s · $d^{-1}$. Five milliliters of eluate were collected per tube, and 1 mL from each tube was analyzed by the phenol-sulfuric acid method at 490 nm. An elution curve was plotted with tube number as the x-axis and absorbance as the y-axis.

### (2) Preparative ion-exchange chromatography of polysaccharides

Ten grams of ASPS were dissolved in 200 mL ultrapure water and sequentially eluted with 4.0 L ultrapure water, 0.3 mol · $L^{-1}$ NaCl, 0.6 mol · $L^{-1}$ NaCl, and 0.8 mol · $L^{-1}$ NaCl solution at a flow rate of 5 s · $d^{-1}$. The eluates were collected, concentrated under reduced pressure, desalted and decolorized using dialysis bags with a molecular weight cutoff of 3.5 × $10^3$ Da, and freeze-dried for further use.

### (3) Preparative gel filtration chromatography of polysaccharides

Appropriate amounts of the above polysaccharide fractions were dissolved in ultrapure water, centrifuged at 10,000 rpm for 5 min, and the supernatant was loaded onto a Sephadex G-100 column (1.5 cm × 100 cm). Elution was performed with ultrapure water at a flow rate of 10 s · $d^{-1}$, collecting 10 mL per tube. One milliliter from each tube was analyzed by the phenol-sulfuric acid method at 490 nm. An elution curve was plotted with tube number as the x-axis and absorbance as the y-axis. Elution peaks were pooled, concentrated, and freeze-dried to obtain purified homogeneous polysaccharides.

**1.3.4 Molecular Weight Determination** The molecular weight of polysaccharides was determined by gel permeation chromatography coupled with refractive index and multi-angle laser light scattering detection (Hu et al., 2020). Briefly, polysaccharide samples were dissolved in 0.1 mol · $L^{-1}$ $NaNO_3$ solution at a concentration of 1.0 mg · $mL^{-1}$, filtered through a 0.45 m membrane, and transferred to sample vials for analysis. Data were collected and processed using Astra6 software. The system consisted of an Optilab T-rEX refractive index detector (Wyatt Technology, CA, USA) and a DAWN HELEOS-II multi-angle laser light scattering detector (Wyatt Technology, CA, USA). Chromatographic separation was achieved using OHpak SB-805 HQ (300 mm × 8 mm), OHpak SB-804 HQ (300 mm × 8 mm), and OHpak SB-803 HQ (300 mm × 8 mm) gel exclusion columns. The mobile phase was 0.1 mol · $L^{-1}$ $NaNO_3$, injection volume was 100 L, flow rate was 0.4 mL · $min^{-1}$, elution was isocratic for 100 min, and column temperature was maintained at 45 °C.

**1.3.5 Monosaccharide Composition Analysis** Monosaccharide composition was determined using a Thermo ICS 5000 ion chromatography system equipped with an electrochemical detector (Guo et al., 2021). Standards included fucose, arabinose, rhamnose, galactose, glucose, xylose, mannose, fructose, ribose, galacturonic acid, guluronic acid, glucuronic acid, and mannuronic acid. Polysaccharide samples (5.0 mg) were hydrolyzed in 1 mL of 2 mol · $L^{-1}$ trifluoroacetic acid (TFA) at 121 °C for 2.0 h. The hydrolysate was evaporated to dryness under nitrogen, washed with methanol, and evaporated again. This methanol wash-evaporation cycle was repeated 2–3 times to completely remove TFA. The residue was dissolved in an appropriate amount of sterile water and transferred to chromatography vials for analysis.

Chromatographic conditions: Dionex™ CarboPac™ PA20 (150 mm × 3.0 mm, 10 m) anion-exchange column; injection volume: 5 L; mobile phase A: 0.1 mol · $L^{-1}$ NaOH; mobile phase B: 0.1 mol · $L^{-1}$ NaOH + 0.2 mol · $L^{-1}$ NaAc; flow rate: 0.5 mL · $min^{-1}$; column temperature: 30 °C.

**1.3.6 Antioxidant Activity Assays (1) Hydroxyl radical (·OH) scavenging assay**

The salicylic acid method was used to measure ·OH scavenging capacity based on the Fenton reaction, where ·OH reacts with salicylic acid to form a colored product at 510 nm. The presence of antioxidants reduces colored product formation, allowing assessment of ·OH scavenging ability through absorbance measurements (Ge et al., 2021, with slight modifications). Polysaccharide powder was dissolved in ultrapure water to prepare solutions at various concentrations (0.1, 0.3, 0.5, 0.7, 0.9, 1.0 mg · $mL^{-1}$). Two milliliters of each solution were mixed with 1 mL of 9 mmol · $L^{-1}$ ferrous sulfate and 1 mL of 8.8 mmol · $L^{-1}$ hydrogen peroxide (3%, V/V). After shaking, 1 mL of 9 mmol · $L^{-1}$ salicylic acid-ethanol solution was added. The mixture was reacted at 37 °C for 1.0 h, and absorbance was measured at 510 nm. Ultrapure water and Vc solution served as blank and

positive controls, respectively. Experiments were performed in triplicate. The $\cdot$OH scavenging activity was calculated as:

$$\text{Scavenging rate } (\%) = \frac{A_1 - (A_2 - A_3)}{A_1} \times 100$$

where $A_1$ is the absorbance of the blank (ultrapure water instead of sample), $A_2$ is the absorbance with sample solution, and $A_3$ is the absorbance without hydrogen peroxide solution.

**(2) Superoxide anion radical ($O_2^- \cdot$) scavenging assay**

The pyrogallol autoxidation method was used to measure $O_2^- \cdot$scavenging capacity. Pyrogallol rapidly autoxidizes under alkaline conditions to generate $O_2^- \cdot$, and antioxidants inhibit this autoxidation process, causing a color change from green to yellow. Antioxidant activity was assessed by measuring absorbance at 325 nm (Yang et al., 2018, with slight modifications). Polysaccharide powder was dissolved in ultrapure water to prepare solutions at concentrations of 0.1, 0.3, 0.5, 0.7, 0.9, and 1.0 mg $\cdot$ mL$^{-1}$. Two milliliters of each solution were mixed with 4.5 mL of 50 mmol $\cdot$ L$^{-1}$ Tris-HCl buffer (pH 8.2) and reacted at 25 °C for 20 min. Then, 0.5 mL of 25 mmol $\cdot$ L$^{-1}$ pyrogallol solution was added. After 5 min, the reaction was terminated by adding 1 mL of 10 mmol $\cdot$ L$^{-1}$ HCl, and absorbance was measured at 325 nm. Vc served as the positive control, and 10 mmol $\cdot$ L$^{-1}$ HCl as the blank control. Experiments were performed in triplicate. The $O_2^- \cdot$ scavenging activity was calculated as:

$$\text{Scavenging rate } (\%) = \frac{A_1 - (A_2 - A_3)}{A_1} \times 100$$

where $A_1$ is the absorbance of the blank (ultrapure water instead of sample), $A_2$ is the absorbance with sample solution, and $A_3$ is the absorbance where pyrogallol solution was replaced with ultrapure water.

**(3) DPPH radical scavenging assay**

The DPPH method was used to evaluate *in vitro* antioxidant activity. In the presence of antioxidants, the DPPH solution changes from deep purple to light yellow, with decreased absorbance at 517 nm. The absorbance change is quantitatively related to electron acceptance, enabling analysis of antioxidant capacity (Wang et al., 2019, with slight modifications). Polysaccharide powder was dissolved in ultrapure water to prepare solutions at concentrations of 0.1, 0.3, 0.5, 0.7, 0.9, and 1.0 mg $\cdot$ mL$^{-1}$. Two milliliters of each solution were mixed with 2 mL of 2 mmol $\cdot$ L$^{-1}$ DPPH-anhydrous ethanol solution and reacted in the dark at 25 °C for 30 min. Absorbance was measured at 517 nm. Vc solution served as the positive control, and ultrapure water as the blank control. Experiments were performed in triplicate. The DPPH scavenging activity was calculated as:

$$\text{Scavenging rate } (\%) = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100$$

where $A_0$ is the absorbance of the blank (ultrapure water instead of sample), $A_1$ is the absorbance with sample solution, and $A_2$ is the absorbance where DPPH-anhydrous ethanol solution was replaced with ultrapure water.

### 1.4 Data Processing

Data were plotted using Excel 2010 and analyzed by one-way ANOVA using SPSS 21.0.

## 2. Results

### 2.1 Extraction and Content Determination of Polysaccharides

Three hundred grams of 'Zijia 1' sample was extracted by hot water boiling and filtration, followed by ethanol precipitation and purification/decolorization with AB-8 macroporous adsorption resin. After concentration and drying, 59.19 g of crude polysaccharide powder was obtained, with an extraction yield of 19.73% and a polysaccharide content of $(8.41 \pm 0.09)\%$.

### 2.2 Separation of Polysaccharides by DEAE-Cellulose 52 Preparative Column

As shown in [Figure 1: see original paper], linear gradient elution of ASPS on the DEAE-cellulose column yielded four major peaks, corresponding to ultrapure water, 0.3 $\text{mol} \cdot \text{L}^{-1}$ NaCl, 0.6 $\text{mol} \cdot \text{L}^{-1}$ NaCl, and 0.8 $\text{mol} \cdot \text{L}^{-1}$ NaCl solutions. Therefore, ASPS was sequentially separated using 4 L of each eluent. After concentration, dialysis, and freeze-drying, four fractions were obtained and designated as ASPA-1, ASPA-2, ASPA-3, and ASPN, with yields of 7.41%, 4.87%, 4.07%, and 3.29%, respectively.

### 2.3 Gel Filtration Chromatography of Polysaccharides on Sephadex G-100

The four fractions were further purified by Sephadex G-100 gel chromatography. As shown in [Figure 2: see original paper], each fraction eluted as a single peak, confirming them as homogeneous components with a defined molecular weight range. The elution peaks were collected to obtain different polysaccharide fractions designated as ASPA-1-1, ASPA-2-1, ASPA-3-1, and ASPN-1, with yields of 1.49%, 2.13%, 6.24%, and 2.43%, respectively.

### 2.4 Molecular Weight Determination

The molecular weights of the polysaccharides were determined by gel permeation chromatography coupled with multi-angle laser light scattering and refractive

index detectors. The results are presented in . The polydispersity indices of ASPA-1-1, ASPA-2-1, and ASPN-1 were close to 1.0, indicating monodisperse molecular weight distributions and high purity (Hu et al., 2017). ASPA-3-1 showed the highest polydispersity index, possibly due to non-uniform molecular chain lengths resulting in a broad molecular weight distribution (Liu et al., 2019). The root-mean-square radius reflects molecular looseness, with smaller radii indicating smaller molecular sizes (Jiang et al., 2019). However, the radii of gyration did not correlate consistently with weight-average molecular weights across fractions, possibly because polysaccharide molecules shrank in the salt solution, reducing their apparent size (Hu et al., 2020).

### 2.5 Monosaccharide Composition Analysis

Monosaccharide types were identified by comparing retention times with standard compounds. As shown in [Figure 3: see original paper], the standard chromatogram exhibited symmetrical peaks, indicating good separation of individual monosaccharides. Quantification by internal standardization revealed significant differences in monosaccharide types and ratios among fractions. As shown in [Figure 4: see original paper] and , ASPA-1-1 contained the highest galacturonic acid content, while ASPA-3-1 had the highest glucuronic acid content; neither contained ribose. ASPA-2-1 showed the highest ribose content, followed by galacturonic acid. ASPN-1 contained the highest glucose proportion with minimal uronic acids and lacked arabinose and ribose. None of the four polysaccharides contained guluronic acid, mannuronic acid, or fructose.

### 2.6 Antioxidant Activity Assays

**2.6.1 Hydroxyl Radical ($\cdot$OH) Scavenging Capacity** As shown in [Figure 5: see original paper], the $\cdot$OH scavenging effects of different polysaccharide fractions exhibited a dose-dependent relationship, though all scavenging rates were lower than the positive control Vc. At a concentration of 1.0 mg $\cdot$ mL$^{-1}$, the $\cdot$OH scavenging rates of ASPA-1-1, ASPA-2-1, ASPA-3-1, and ASPN-1 were 48.94%, 96.07%, 48.22%, and 67.81%, respectively. The $IC_{50}$ values for $\cdot$OH scavenging capacity were ranked as: ASPA-2-1 ($0.17 \pm 0.008$ mg $\cdot$ mL$^{-1}$) > ASPN-1 ($0.36 \pm 0.033$ mg $\cdot$ mL$^{-1}$) > ASPA-1-1 ($1.35 \pm 0.336$ mg $\cdot$ mL$^{-1}$) > ASPA-3-1 ($2.01 \pm 0.050$ mg $\cdot$ mL$^{-1}$), with lower $IC_{50}$ values indicating stronger radical scavenging ability. ASPA-2-1 demonstrated the strongest $\cdot$OH scavenging capacity, which may be related to its high uronic acid content and large molecular weight. Polysaccharides with high uronic acid content and large molecular weight can chelate $Fe^{2+}$ in the reaction solution, thereby reducing hydroxyl radical formation (Fan et al., 2019).

**2.6.2 Superoxide Anion Radical ($O_2^{-}\cdot$) Scavenging Capacity** As shown in [Figure 6: see original paper], the $O_2^{-}\cdot$ scavenging capacity of different polysaccharide fractions increased with concentration, showing a clear dose-dependent relationship, though all scavenging rates were lower than Vc.

At 1.0 mg·mL$^{-1}$, the $O_2^-$·scavenging rates of ASPA-1-1, ASPA-2-1, ASPA-3-1, and ASPN-1 were 34.30%, 46.84%, 48.31%, and 16.58%, respectively. The $IC_{50}$ values for $O_2^-$·scavenging capacity were ranked as: ASPA-3-1 (1.29 ± 0.037 mg·mL$^{-1}$) > ASPA-2-1 (1.49 ± 0.045 mg·mL$^{-1}$) > ASPA-1-1 (5.03 ± 0.253 mg·mL$^{-1}$) > ASPN-1 (7.56 ± 0.160 mg·mL$^{-1}$), with lower $IC_{50}$ values indicating stronger radical scavenging ability (Gong et al., 2021). ASPA-2-1 and ASPA-3-1 showed the strongest $O_2^-$·scavenging capacities, with high contents of glucuronic acid and galacturonic acid. As acidic polysaccharides, their activity may be related to the dissociation energy of O-H bonds. More electron-withdrawing groups such as carboxyl and aldehyde groups attached to the polysaccharide backbone weaken O-H bond dissociation energy, enhancing $O_2^-$·scavenging capacity and terminating radical chain reactions, thereby exhibiting significant antioxidant activity (Yuan et al., 2016).

**2.6.3 DPPH Radical Scavenging Capacity** As shown in [Figure 7: see original paper], all polysaccharide fractions showed consistent DPPH scavenging capacity trends, increasing gradually with concentration, though remaining lower than Vc. At 1.0 mg·mL$^{-1}$, the DPPH scavenging rates of ASPA-1-1, ASPA-2-1, ASPA-3-1, and ASPN-1 were 89.17%, 89.02%, 89.07%, and 76.18%, respectively. The $IC_{50}$ values for DPPH scavenging capacity were ranked as: ASPA-2-1 (0.01 ± 0.003 mg·mL$^{-1}$) > ASPA-1-1 (0.14 ± 0.002 mg·mL$^{-1}$) > ASPA-3-1 (0.15 ± 0.017 mg·mL$^{-1}$) > ASPN-1 (0.50 ± 0.002 mg·mL$^{-1}$). Molecular weight showed a positive correlation with radical scavenging capacity. ASPA-2-1 exhibited the strongest DPPH scavenging ability, possibly because uronic acids can activate hydrogen atoms on anomeric carbon atoms, which combine with radicals to form stable DPPH-H structures, thereby terminating radical reactions (Li et al., 2022; Wei et al., 2021).

## 3. Discussion and Conclusion

In this study, crude polysaccharides (ASPS) were extracted from the new *A. senticosus* variety 'Zijia 1' by water extraction and ethanol precipitation. ASPS was further separated and purified by DEAE-Cellulose 52 ion-exchange chromatography combined with Sephadex G-100 gel filtration to obtain four polysaccharide fractions: ASPA-1-1, ASPA-2-1, ASPA-3-1, and ASPN-1. The neutral polysaccharide ASPN-1 contained the highest proportion of glucose with minimal uronic acids, while the acidic polysaccharides ASPA-1-1, ASPA-2-1, and ASPA-3-1 contained high proportions of galacturonic acid and glucuronic acid. This is similar to the high galacturonic acid content reported by Bai et al. (2015) for polysaccharides from *A. senticosus* roots and stems. However, 'Zijia 1' showed higher glucuronic acid content, whereas in common *A. senticosus* this was below 1.10%. The molecular weights of ASPA-1-1 (8.10 kDa) and ASPA-3-1 (0.91 kDa) were lower than the acidic polysaccharides (10.0–48.0 kDa) isolated by Bai et al. (2015) from *A. senticosus*, possibly due to differences in variety, origin, extraction method, and analytical technique.

Scavenging assays for $\cdot OH$, $O_2^- \cdot$, and DPPH radicals are common methods for evaluating *in vitro* antioxidant capacity of bioactive compounds. The four polysaccharide fractions obtained in this study showed significant scavenging effects against all three radicals, with antioxidant activity demonstrating a dose-dependent relationship—antioxidant capacity increased with polysaccharide concentration. ASPA-2-1, with the largest molecular weight (26.15 kDa) and high galacturonic acid content (22.47%), exhibited the strongest scavenging capacity against $\cdot OH$ and DPPH radicals, with $IC_{50}$ values of ($0.17 \pm 0.008$) $mg \cdot mL^{-1}$ and ($0.01 \pm 0.003$) $mg \cdot mL^{-1}$, respectively. This aligns with findings by Shen et al. (2020) that *A. senticosus* fruit polysaccharides showed highest antioxidant activity at molecular weights of 10–50 kDa, and with Hu et al. (2022) who reported that WOJP-A from *Osmunda japonica* with high galacturonic acid content showed stronger $\cdot OH$ scavenging capacity than WOJP-N. The mechanism may involve high uronic acid content and large molecular weight enabling polysaccharides to block electron transfer to radicals, thereby terminating radical chain reactions. Thus, polysaccharide antioxidant activity is related to molecular weight, uronic acid content, and monosaccharide composition (Xie et al., 2016). Literature reports indicate that polysaccharides with high uronic acid content possess strong antioxidant capacity (Yan et al., 2019). ASPA-3-1 showed the strongest $O_2^- \cdot$ scavenging capacity with an $IC_{50}$ value of ($1.29 \pm 0.037$) $mg \cdot mL^{-1}$, rich in glucuronic acid (16.31%) and galacturonic acid (32.70%). This may be because uronic acids can activate hydrogen atoms on anomeric carbons to donate hydrogen radicals, converting radicals into stable compounds and blocking oxidative chain reactions, thereby conferring strong antioxidant activity (Monirsadat et al., 2020; Hepef et al., 2016).

This study advanced understanding of 'Zijia 1' polysaccharides by determining their monosaccharide composition and molecular weight through combined separation and purification techniques with multiple chromatographic methods. These findings lay the foundation for exploring the chemical composition and antioxidant mechanisms of 'Zijia 1' and provide a scientific basis for further development and utilization of this plant. However, the structural units and linkage patterns of different 'Zijia 1' polysaccharide fractions remain unclear. Future studies should employ advanced techniques to elucidate the fine structures of these polysaccharides and verify their *in vivo* antioxidant effects and molecular mechanisms through animal experiments.

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*Note: Figure translations are in progress. See original paper for figures.*