Research Article

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Bioactive polysaccharides in different plant parts of *Aconitum carmichaelii*

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Abstract

BACKGROUND: *Aconitum carmichaelii* is an industrially cultivated medicinal plant in China and its lateral and mother roots are used in traditional Chinese medicine due to the presence of alkaloids. However, the rootlets and aerial parts are discarded after collection of the roots, and the non-toxic polysaccharides in this plant have attracted less attention than the alkaloids and poisonous features. In this study, five neutral and 14 acidic polysaccharide fractions were isolated systematically from different plant parts of *A. carmichaelii*, and their structural features and bioactivity were studied and compared.

RESULTS: The neutral fraction isolated from the rootlets differed from those isolated from the lateral and mother roots. It consisted of less starch and more possible mannans, galactans, and/or xyloglucans, being similar to those of the aerial parts. Pectic polysaccharides containing homogalacturonan and branched type-I rhamnogalacturonan (RG-I) were present in all plant parts of *A. carmichaelii*. However, more arabinogalactan (AG)-II side chains in the RG-I backbone were present in the aerial parts of the plants, while more amounts of arabinans were found in the roots. Various immunomodulatory effects were observed, determined by complement fixation activity and anti-inflammatory effects on the intestinal epithelial cells of all polysaccharide fractions.

CONCLUSION: This study highlighted the diversity of polysaccharides present in *A. carmichaelii*, especially in the unutilized plant parts, and showed their potential medicinal value.

Supporting information may be found in the online version of this article.

Keywords: *Aconitum carmichaelii*; polysaccharide diversity; complement fixation; anti-inflammatory activity

INTRODUCTION

Aconitum carmichaelii Debeaux (Ranunculaceae) is mainly distributed in Sichuan, Shaanxi, and Yunnan provinces in China. It is a medicinal plant, and its lateral roots (Aconiti Lateralis Radix Praeparata, 'Fuzi') and mother roots (Aconiti Radix, 'Chuanwu') are used independently in traditional Chinese medicine (TCM).[1-3] 'Fuzi' is generally applied as a cardiotonic, while 'Chuanwu' is used more frequently for treating rheumatism, joint pain, and abdominal colic, and as an anesthetic.[3] 'Fuzi' or 'Chuanwu' with the highest quality is collected and produced in Jiangyou, Sichuan province, where *A. carmichaelii* has been grown for over 1000 years. More than 300 ha are cultivated with *A. carmichaelii* in Jiangyou, and around 9–12 tons of fresh roots can be produced per hectare annually, which is higher than the amount of traditional cereal crops such as wheat and rice.[4,5] Further, *A. carmichaelii* is an industrial traded herb. Around 2000 ton of roots is exported commercially to other Asian countries annually, including Japan and Korea.[5] As an industrial herbal plant, the rootlets and the aerial parts (including 60–150 cm high stems, and 6–11 cm long and 9–15 cm wide pentagonal leaves) of *A. carmichaelii* are normally discarded when the roots are collected, creating a huge amount of waste material.

The alkaloids in both 'Fuzi' and 'Chuanwu' have been reported to be the main contributors to the cardiovascular modulatory effects, as well as exhibiting anti-inflammatory, analgesic, anti-tumor, and immunomodulatory effects.[1,6,7] However, these alkaloids also cause toxicity or side effects on ion channels, neuron systems, and other organs,[8] and variation in the content and types of alkaloids in 'Fuzi' and 'Chuanwu' might be a reason for their different clinical applications[9,10] and toxicity.[11] Polysaccharides, as another promising bioactive constituent present in both 'Fuzi' and 'Chuanwu', have been shown to exhibit immunomodulatory, cardiovascular protective and anti-tumor activity.[3] The

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varying contents of polysaccharides in different root parts could be a factor in their independent applications in TCM.[12] However, a comprehensive investigation including primary structural information and biological activity of polysaccharides from different root parts of *A. carmichaelii* is still absent.[3]

On the other hand, the unutilized plant parts discarded during the collection of the roots of *A. carmichaelii* could be recycled as possible resources of valuable products such as phytochemicals, as has been done with other traditional medicinal herb residues.[13,14] Previous studies have reported on the content of polysaccharides[12,15,16] and total alkaloids[11,17] in the rootlets of *A. carmichaelii*. In the current study, different monosaccharide compositions of polysaccharides from the rootlets have been determined compared to the lateral and mother roots.[15] Polysaccharides,[12,16] alkaloids,[16-21] and flavonoids[22,23] have been reported from the aerial parts. It was suggested that they would have similar analgesic and anti-inflammatory activity to that of traditionally used roots.[19] However, systematic and detailed investigations of the chemical and biological properties of polysaccharides in these plant parts of *A. carmichaelii* are still limited.

This study aims to describe the polysaccharide diversity in the different plant parts of *A. carmichaelii*, and their immunomodulatory and anti-inflammatory effects *in vitro*, to discover future applications of the whole plant for sustainable medicinal purposes.

MATERIALS AND METHODS

Materials

The whole plant of *A. carmichaelii* Debeaux was collected from Jiangyou City, Sichuan Province, China in 2019 (located at 31° 50′ 24.0″ N/ 104° 47′ 24.0″ E, 517 m a.s.l.). The aerial and different root parts were separated immediately after collection and then dried in a drying oven at 40 °C with flowing air.

Isolation and purification of polysaccharides from different parts of *A. carmichaelii*

Polysaccharides from different parts of *A. carmichaelii* were isolated and purified following methods used and described previously.[24] Working flow is shown in Fig. 1. Briefly, 50 g of dried materials was pre-extracted with 96% ethanol, and further extracted with boiling water. The water extracts of each material were filtered and concentrated to 200 mL individually, and 800 mL of ethanol (final concentration of 80% ethanol) was added to precipitate polysaccharides at 4 °C for 24 h. These precipitants were further dialyzed with cut-off 3500 Da and lyophilized, yielding crude polysaccharides from different plant materials, named ALR (polysaccharide from *A. carmichaelii* lateral roots), AMR (polysaccharide from *A. carmichaelii* mother roots), ARL (polysaccharide from *A. carmichaelii* rootlets), AAP (polysaccharide from *A. carmichaelii* aerial parts), and AS (polysaccharide from *A. carmichaelii* stems). The polysaccharides from *A. carmichaelii* leaves have been isolated previously in the same way.[24]

Briefly, the crude polysaccharides mentioned above were applied to an ion exchange chromatography column (IEC, 5 × 40 cm) packed with matrix ANX Sepharose 4 Fast Flow (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The neutral and the acidic polysaccharide fractions were obtained from the eluates of the distilled water and 0–1.5 mol L^{-1} gradient NaCl solution, respectively. Size exclusion chromatography (SEC) connected with Hiload 16/60 Superdex 200 prep grade column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was used to purify acidic fractions, as described in a previous publication.[24]

Determination of chemical and monosaccharide compositions and glycosidic linkage types

The total content of phenolics and proteins was determined quantitatively by Folin–Ciocâlteu[25] and Bio-Rad protein assays,[26] respectively. The presence of starch was determined by adding two drops of an aqueous iodine-potassium-iodide solution (2% iodine in 2% aqueous KI solution) to each sample (1 mg mL^{-1}).[27]

Monosaccharide compositions were determined following previously published methods[28] with a few modifications.[29] Briefly, samples were subject to methanolysis with 3 mol L^{-1} hydrochloric acid in water-free methanol for 24 h at 80 °C, followed by trimethylsilylation (TMS) and quantification by capillary gas chromatography (GC) on a Trace 1300 GC (Thermo Scientific, Milan, Italy). Monosaccharide standards (Sigma-Aldrich, St. Louis, MO, USA) including arabinose (Ara), rhamnose (Rha), fucose (Fuc), xylose (Xyl), mannose (Man), galactose (Gal), Glc, glucuronic acid (GlcA), and galacturonic acid (GalA) were processed in the same way.

Determination of glycosidic linkage types was performed by carboxyl reduction, methylation, hydrolysis, reduction, and acetylation. The partially methylated alditol acetate (PMMA) fragments were detected and quantified using gas chromatography–mass spectrometry (GC–MS) QP2010 (Shimadzu, Kyoto, Japan) with a Restek Rxi-5 MS column (30 m; 0.25 mm i.d.; 0.25 mm film), as described previously.[24,30-32] The estimation of relative amounts of each linkage type was related to the total mole percentage of their monosaccharide compositions determined by methanolysis.

Determination of molecular weight

The homogeneity and the weight-average molecular weight (*Mw*) of samples (2 mg mL^{-1}, 0.5 µL) were determined by SEC on a Superose 6 10/300 column (Amersham Biosciences, Piscataway, USA) combined with the Äkta FPLC system. Dextran polymers with a different *Mw* were used to establish the calibration curve as described before.[24]

Complement fixation assay

The complement fixation assay is based on the inhibition of hemolysis of antibody-sensitized sheep red blood cells by human sera, as described by Michaelsen *et al.*[33] (Method A). BP-II, a highly active pectic polysaccharide from the aerial parts of *Biophytum petersianum* Klotzsch,[34] was used as a positive control. All samples were dissolved in a veronal buffer at a dose range from 0.48 to 500 µg mL^{-1} in twofold dilution, by which the 50% inhibition of lysis (ICH_{50}) was calculated according to the dose–response manner. In this study, a value of the $ICH_{50BP\text{-}II}/ICH_{50sample}$ was determined for a comprehensive comparison of all polysaccharide samples as a numeric expression of the degree of activity of each sample relative to BP-II.[35] The higher the value is, the better complement fixation activity of the tested sample.

Anti-inflammatory effects on porcine jejunum epithelial cells

The porcine jejunum epithelial cells (IPEC-J2) were obtained from the Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences (Shanghai, China), and were cultured as described in a previous study.[36] The cytotoxic effects of all tested samples were evaluated using Cell Counting Kit-8 (CCK-8) kit after co-cultivation with all samples (10 µg mL^{-1}) for 24 h.[37]

The IPEC-J2 cells were seeded into six-well plates (5×10^5 cells/well) and stimulated with 20 µg mL^{-1} lipopolysaccharides (LPS, Sigma-Aldrich) (purity ≥ 99%) for 12 h to induce inflammation. Cells were further incubated together with samples (20 µg mL^{-1} in medium) for another 12 h, while those treated with medium

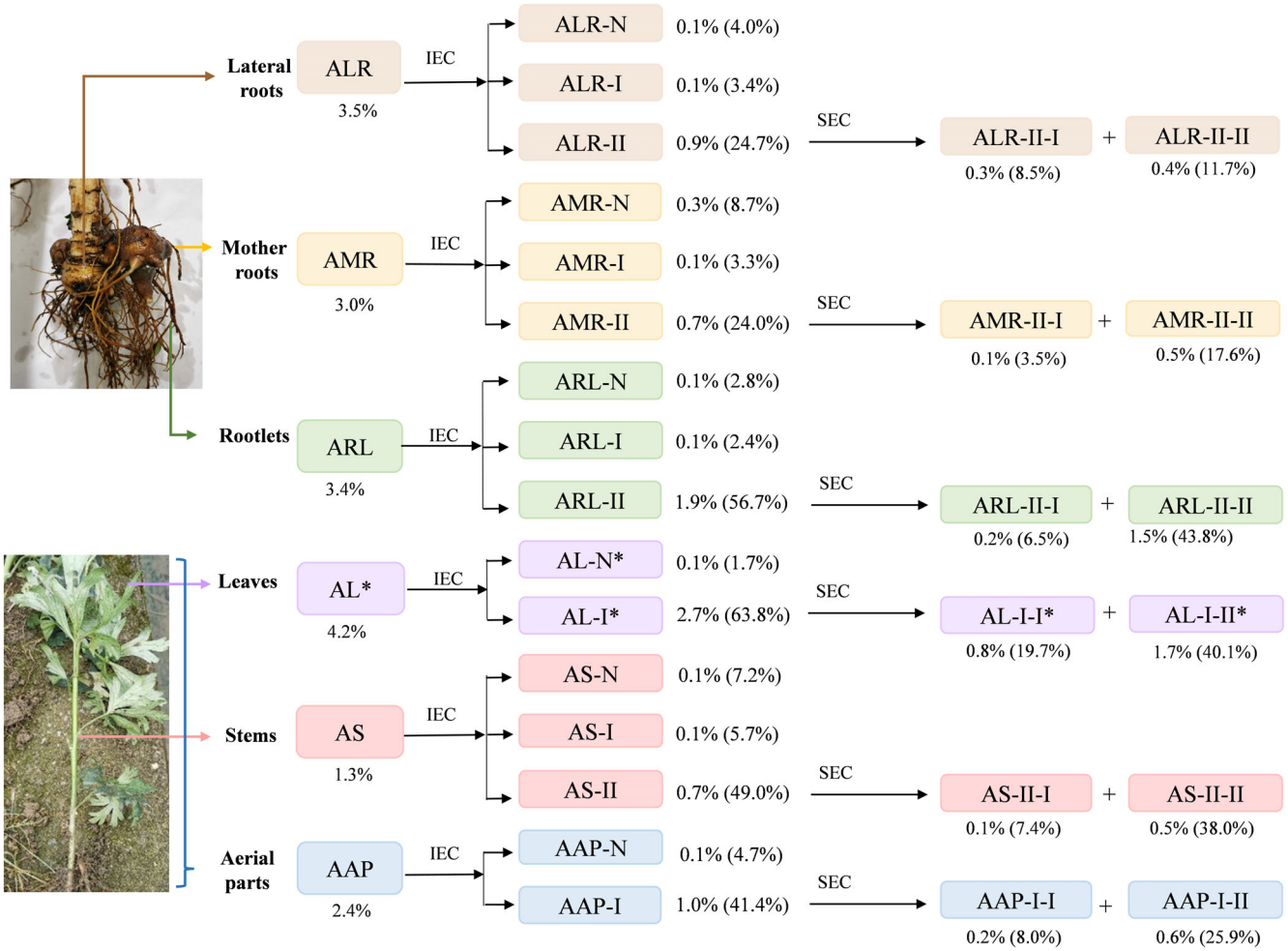

Figure 1. Overview of the workflow and yields of polysaccharides from different plant parts of *A. carmichaelii*. IEC, ion exchange chromatography; SEC, size exclusion chromatography; yields (%), mass percent related to dried plant materials (crude polysaccharides); *, data from our previous publication.[24] AAP, polysaccharide from *A. carmichaelii* aerial parts; ALR, polysaccharide from *A. carmichaelii* lateral roots; AMR, polysaccharide from *A. carmichaelii* mother roots; ARL, polysaccharide from *A. carmichaelii* rootlets; AS, polysaccharide from *A. carmichaelii* stems.

only were set as the LPS control group. After co-cultivation, all cells were collected, and the quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was performed to determine the gene expressions of pro-inflammatory cytokines including interleukin Iβ (IL-1β), tumor necrosis factor α (TNF-α), and IL-6, as previously described.[37] Primers of all genes are shown in Supporting Information, Table S1.

Statistical analysis

All experimental data are expressed as means ± SDs and analyzed using one-way ANOVA and the Fisher's least significant difference (LSD) or Duncan test (IBM SPSS Statistics version 24, IBM Corp., Armonk, NY, USA). Clustering analysis based on the Spearman test was performed using Genescloud tools (https://www.genescloud.cn).

RESULTS AND DISCUSSION

Isolation, chemical composition and *Mws* of polysaccharides from different plant parts of *A. carmichaelii*

Crude polysaccharides were obtained from different plant parts of *A. carmichaelii* after water extraction, ethanol precipitation, and dialysis. The yields of the crude and isolated polysaccharide fractions are shown in Fig. 1, and elution profiles are shown in Fig. 2.

The monosaccharide composition of neutral and purified acidic polysaccharide fractions from different plant parts were determined, and are shown in Table 1. Their chemical similarities were analyzed by hierarchical clustering, as presented in Fig. 3. The neutral fractions from the lateral and mother roots, ALR-N and AMR-N, consisted mainly of Glc (≥ 85 mol%). The one isolated from the rootlets, ARL-N, was different from ALR-N and AMR-N, containing 10 mol% more of Ara, 20 mol% more of Man, and 40 mol% less of Glc, and was similar to the neutral fractions from stems (AS-N) and aerial parts (AAP-N) (Fig. 3). The neutral fraction from leaves, AL-N, which had been reported to contain high amounts of Xyl,[24] was distinct from all neutral fractions (Fig. 3). All neutral fractions were shown to contain starch, and also had similar *Mws* (Table 1). The monosaccharide composition of the minor acidic fractions from the lateral and mother roots, ALR-I and AMR-I, were also similar, containing mainly of Glc and Ara. However, ARL-I, isolated from the rootlets, consisted of more Xyl and Man, and less Glc, which was similar to AS-I from stems. The monosaccharide composition of both neutral and minor acidic fractions might indicate the presence of similar functional carbohydrates in the rootlets and stems of *A. carmichaelii*. The patterns

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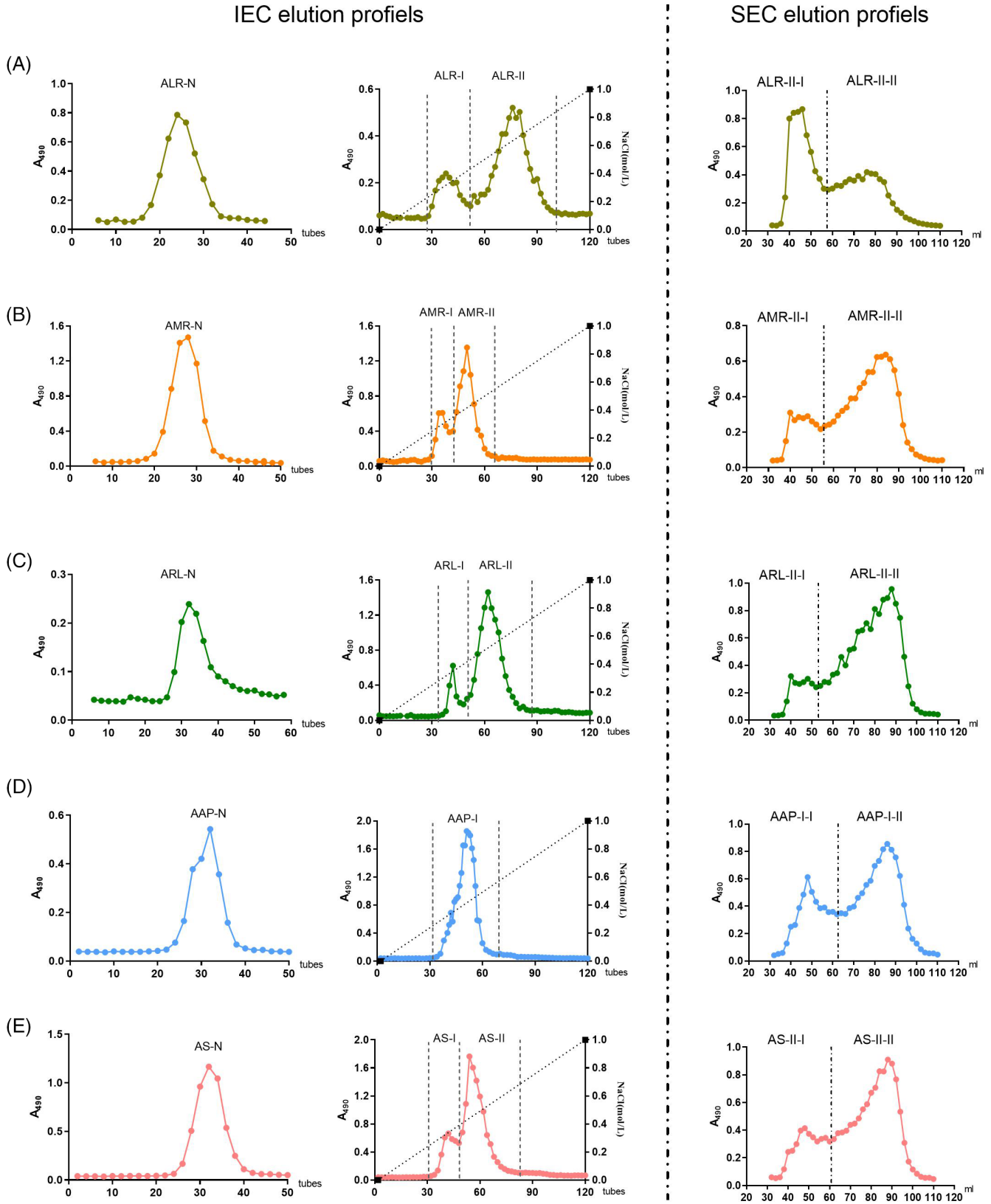

Figure 2. Ion exchange chromatography (IEC) and size exclusion chromatography (SEC) elution profiles of polysaccharides from the lateral roots (a), mother roots (b), rootlets (c), aerial parts (d) and stems (e) of *A. carmichaelii*. AAP, polysaccharide from *A. carmichaelii* aerial parts; ALR, polysaccharide from *A. carmichaelii* lateral roots; AMR, polysaccharide from *A. carmichaelii* mother roots; ARL, polysaccharide from *A. carmichaelii* rootlets; AS, polysaccharide from *A. carmichaelii* stems.

Table 1. Chemical composition and average *Mw* of polysaccharide fractions from different plant parts of *A. carmichaelii*

	Ara	Rha	Fuc	Xyl	Man	Gal	Glc	GlcA	GalA	Protein (mg/g)	Starch	*Mw*/kD
ALR-N	12.4	Tr.	Tr.	Tr.	0.8	0.7	85.1	n.d.	Tr.	15	+	12.9
ALR-I	30.4	Tr.	Tr.	0.8	0.8	4.6	60.6	Tr.	2.0	10	+	41.6
ALR-II-I	50.0	7.0	0.8	2.6	Tr.	8.9	1.6	0.8	28.1	12	–	>475
ALR-II-II	6.4	3.4	1.1	4.6	Tr.	2.9	4.9	0.6	75.8	6	–	105.9
AMR-N	8.6	Tr.	Tr.	Tr.	2.5	1.1	87	n.d.	Tr.	11	+	10.2
AMR-I	18.3	0.9	Tr.	1.5	1.7	8.1	61.5	Tr.	7.5	9	+	26.0
AMR-II-I	33.9	9.6	0.9	4.2	Tr.	14.0	3.9	0.7	32.3	16	–	>475
AMR-II-II	4.2	3.9	0.9	6.3	Tr.	2.3	3.4	Tr.	78.4	10	–	52.5
ARL-N	20.3	Tr.	0.8	3.0	23.4	8.7	40.9	n.d.	2.7	16	+	12.9
ARL-I	31.8	0.5	1.0	6.7	12.5	13.8	27.1	Tr.	6.4	2	+	16.3
ARL-II-I	41.8	8.8	0.7	5.4	0.5	9.7	2.8	0.6	29.7	18	–	>475
ARL-II-II	4.4	3.6	0.9	8.1	Tr.	2.1	4.0	Tr.	76.5	15	–	52.5
AAP-N	14.0	Tr.	0.8	3.0	23.4	4.8	52.2	n.d.	1.7	18	+	12.9
AAP-I-I	28.3	8.9	0.8	4.8	0.5	19.9	2.7	1.5	32.5	17	–	270
AAP-I-II	6.0	5.0	1.1	5.3	0.7	3.6	4.2	0.8	73.3	12	–	41.6
AS-N	19.1	Tr.	Tr.	1.2	22.1	6.3	50.0	n.d.	0.9	18	+	10.2
AS-I	42.4	0.8	Tr.	8.2	6.7	10.7	27.2	Tr.	3.2	8	+	12.9
AS-II-I	29.6	14.6	0.8	4.2	0.6	14.9	2.3	1.4	31.6	14	–	213.7
AS-II-II	5.2	5.8	1.2	4.6	0.5	3.5	3.5	1.1	74.7	14	–	41.6

Abbreviations: ALR, the lateral roots; AMR, the mother roots; ARL, the rootlets, AAP, the aerial parts, AS, the stems; —N, neutral fractions; —I, —I-I/II, —II-I/II, acidic fractions. The content of monosaccharide composition (mol%) was related to the total content of Ara, Rha, Fuc, Xyl, Man, Gal, Glc, GlcA and GalA; Tr., traces: less than 0.5 mol%, n.d., not detected.

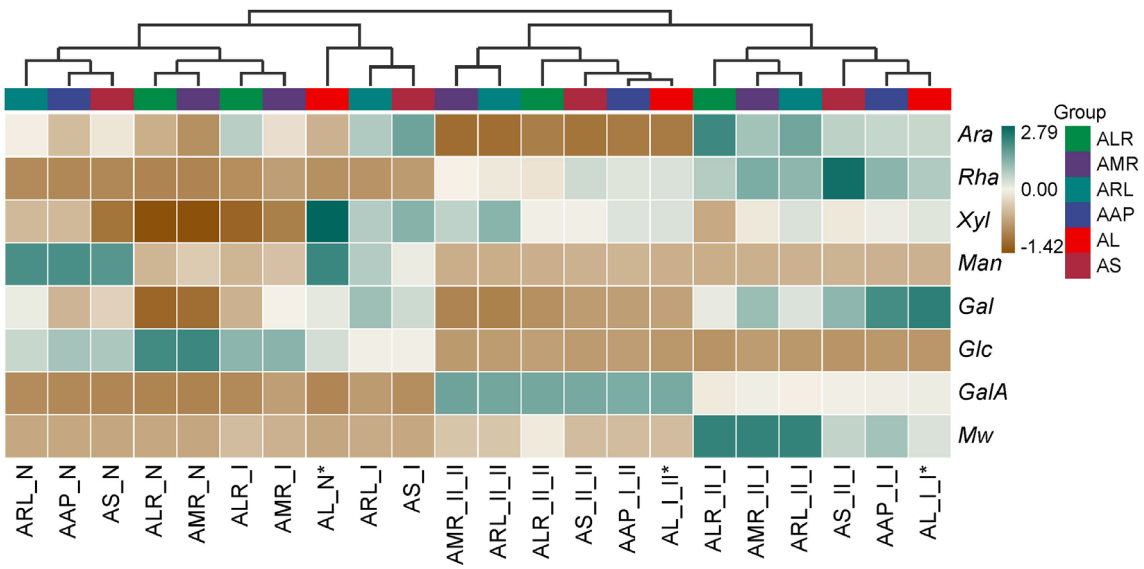

Figure 3. Heatmap with dendrogram of monosaccharide compositions and *Mw* of polysaccharides from different plant parts of *A. carmichaelii*. Hierarchical clustering was analyzed based on Euclidean distance, and the value represents the row Z scores; *, data of AL-N, AL-I-I and AL-I-II in our previous publication.[24] AAP, polysaccharide from *A. carmichaelii* aerial parts; ALR, polysaccharide from *A. carmichaelii* lateral roots; AMR, polysaccharide from *A. carmichaelii* mother roots; ARL, polysaccharide from *A. carmichaelii* rootlets; AS, polysaccharide from *A. carmichaelii* stems.

of monosaccharide composition in the rest of the acidic fractions, including those reported earlier from leaves,[24] were shown to be similar. The first SEC fractions (ALR-II-I, AMR-II-I, ARL-II-I, AAP-I-I, AS-II-I and AL-I-I) were mainly composed of Ara, GalA, and Gal, while the second ones were mainly composed of GalA (ALR-II-II, AMR-II-II, ARL-II-II, AAP-I-II, AS-II-II and AL-I-II, Fig. 3). In addition to the polysaccharide yields, the close clustering of AL-I-I/AL-I-II with AAP-I-I/AAP-I-II also suggested that the leaves contained a major part of the polysaccharides present in the aerial parts of *A. carmichaelii*. The *Mws* of most acidic polysaccharide fractions that were obtained by the same process were similar (Table 1 and Fig. 3). However, it was noticeable that the *Mw* of ALR-II-I, AMR-II-I, and ARL-II-I in the root parts were higher than those of AS-II-I, AL-I-I and AAP-I-I in the aerial parts. The protein content in all fractions was below 20 mg g^{-1} (Table 1) and no phenolics were detected.

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Table 2. Glycosidic linkage types of neutral polysaccharide fractions isolated from different plant parts of *A. carmichaelii*

Linkage types	ALR-N	AMR-N	ARL-N	AAP-N	AS-N
T-Ara*f*	2.9	1.8	6.6	2.8	3.6
1,5-Ara*f*	3.5	3.5	5.5	6.5	8.9
1,3,5-Ara*f*	6.0	3.2	7.9	4.1	6.2
T-Fuc*p*	n.d.	n.d.	0.8	0.8	Tr.
T-Xyl*p*	Tr.	Tr.	2.0	1.7	0.7
1,2-Xyl*p*	n.d.	n.d.	1.0	1.3	0.5
T-Man*p*	n.d.	n.d.	1.0	1.4	1.3
1,2-Man*p*	0.8	1.1	Tr.	n.d.	Tr.
1,4-Man*p*	n.d.	n.d.	19.6	20.9	20.6
1,4,6-Man*p*	n.d.	1.4	2.3	1.1	n.d.
T-Gal*p*	n.d.	Tr.	2.9	2.2	4.7
1,3-Gal*p*	n.d.	n.d.	0.6	0.8	1.0
1,4-Gal*p*	0.6	1.0	4.6	n.d.	n.d.
T-Glc*p*	4.4	5.7	1.2	2.0	2.0
1,3-Glc*p*	Tr.	Tr.	1.0	3.9	1.0
1,4-Glc*p*	78.8	78.5	35.0	40.5	45.0
1,4,6-Glc*p*	1.6	2.4	3.4	5.3	2.0
1,4-Gal*p*A	n.d.	Tr.	2.3	n.d.	n.d.

Abbreviations: Tr., trace, relative amount less than 0.5%; n.d., not detected.

To date, there has been limited information in the literature on the differences of polysaccharides from different plant parts of *A. carmichaelii*. Lv *et al.*[15] only compared the relative amounts of monosaccharides in the polysaccharide fraction from different root parts while only polysaccharide yields in the aerial parts and roots exist.[16] Information on the linkage types still remains unknown.

Glycosidic linkage types of polysaccharides from different plant parts

The glycosidic linkages of all polysaccharide fractions were determined and are shown in Tables 2 and 3. The main linkages of all polysaccharide fractions were further analyzed by hierarchical clustering, as presented in Fig. 4. Gas chromatography chromatograms of PMMA fragments and MS spectra of corresponding fragments are presented in Supporting Information, Figs S1 and S2 and Supporting Information, Table S2.

As mentioned above, ALR-N and AMR-N were similarly composed of Glc, which was thought to be derived from starch. This was confirmed by linkage analysis, because 1,4-linked Glc*p* was detected in both ALR-N and AMR-N (Tables 1 and 2), and these two fractions are clustered in the same main subgroup (Fig. 4(a)). ARL-N contained higher amounts of Ara*f* linkages than ALR-N and AMR-N, higher amounts of 1,4-linked Man*p*, T- and 1,4-linked Gal*p*, and the presence of 1,4-linked Gal*p*A and T- and 1,2-linked Xyl*p* (Table 2 and Fig. 4(a)). These linkage types were also found in the neutral fractions from the aerial parts. In the roots of *A. carmichaelii*, ALR-N and AMR-N were similar and mainly composed of starch, whereas ARL-N was distinct from them, as a mixture of starch, arabinans (1,5- and 1,3,5-linked Ara*f*), galactans (1,4-linked Gal*p*),[38] as well as possible hemicellulose, such as mannans (1,4- and 1,4,6-linked Man*p*, 1,4-linked Glc*p*) or xyloglucans (T-Xyl*p*, 1,2-Xyl*p*, 1,4- and 1,4,6-linked Glc*p*),[39,40] similar to those from the aerial parts. AL-N was clustered into an independent group due to the higher amounts of xyloglucans[24] (Fig. 4(a)). There was no obvious difference between the neutral fractions from the entire aerial parts compared to the stems of *A. carmichaelii*, except for the relative amount of starch represented by 1,4-linked Glc*p* (Table 2).

Regarding the acidic fractions eluted at a low concentration of NaCl, ALR-I, AMR-I, ARL-I, and AS-I were similar to the neutral polysaccharides from the respective plant parts (Fig. 4(a)), as a large amount of T- and 1,4-linked Glc*p*, most probably from starch, was detected in all of them. The presence of starch in these fractions could be due to the physical boundary between starch and an RG-I backbone with a few Gal units, and the starch could not be removed by the water elution in IEC.[35] ARL-I and AS-I differed from ALR-I and AMR-I by having relatively higher amounts of Ara*f* (T-, 1,5- and 1,3,5-linked) residues, Gal*p* (1, 3, 6-linked) residues, 1,4-linked Xyl*p*, and 1,4-linked Man*p* (Table 3). AMR-I also contained detectable levels of 1,4-Glc*p*A. The rest of the acidic polysaccharide fractions probably contain pectic polysaccharides due to the presence of 1,4-linked Gal*p*A.[38,41] The highest *Mw* fractions from the different root parts obtained from SEC (ALR-II-I, AMR-II-I, ARL-II-I) were clustered together with the one from stems (AS-II-I) (Fig. 4). The highest *Mw* fraction from the aerial parts, AAP-I-I and the reported AL-I-I,[24] were similar, with a lower amount of 1,3,5-linked Ara*f*, typical linkages of arabinans, and higher amounts of 1,6-, 1,4-, 1,3,6-, and 1,3,4,6-linked Gal*p*, typical linkages of (1 → 4)-*β*-arabinogalactan (type I, AG-I) and (1 → 3,6)-*β*-arabinogalactan (type II, AG-II) (Table 3 and Fig. 4). The content of AG-II in AL-I-I and AAP-I-I is higher than that of AG-I or arabinans (Fig. 4(b)), and almost equal amounts of AG-I and AG-II moieties (4–8 mol%) were present in AMR-II-I and AS-II-. However, ALR-II-I and ARL-II-I contained more arabinans (30.6 and 24.9 mol% respectively), followed by AG-II side chains (around 5 mol%) and minor amounts of AG-I (1 mol%) (Fig. 4(b)). The fractions with lower *Mw* and high percentage of GalA (more than 70 mol%) were assembled into the same subgroup, which could consist of homogalacturonan (HG) regions in pectic polysaccharides possibly substituted with neutral monomers, such as T-Xyl*p*[42] or T-Rha*p*, as reported in AL-I-II.[24] T-Fuc*p* could be the terminal sugar of AG side chains.[41]

Table 3. Glycosidic linkage types of acidic polysaccharide fractions isolated from different plant parts of *A. carmichaelii*

Linkage types	ALR-I	AMR-I	ARL-I	AS-I	AAP-I-I	AAP-I-II
T-Ara*f*	11.7	8.6	12.4	15.1	17.8	4.3
1,3-Ara*f*	Tr.	Tr.	Tr.	0.6	1.0	n.d.
1,5-Ara*f*	7.4	5.1	7.5	15.6	5.3	1.0
1,3,5-Ara*f*	10.9	4.2	10.7	9.2	3.9	0.5
1,2,5-Ara*f*	Tr.	Tr.	0.7	1.7	n.d.	Tr.
T-Rha*p*	n.d.	0.6	n.d.	Tr.	1.5	3.0
1,2-Rha*p*	n.d.	n.d.	n.d.	n.d.	4.9	1.5
1,2,4-Rha*p*	n.d.	Tr.	n.d.	0.5	2.5	Tr.
T-Fuc*p*	Tr.	n.d.	1.0	Tr.	0.8	1.1
T-Xyl*p*	Tr.	Tr.	2.9	0.6	3.0	3.4
1,2-Xyl*p*	n.d.	n.d.	1.3	n.d.	n.d.	Tr.
1,4-Xyl*p*	Tr.	1.2	2.5	7.6	1.8	1.6
T-Man*p*	n.d.	n.d.	0.6	Tr.	n.d.	n.d.
1,2-Man*p*	0.8	n.d.	Tr.	n.d.	n.d.	n.d.
1,4-Man*p*	n.d.	n.d.	10.8	6.0	n.d.	n.d.
1,4,6-Man*p*	n.d.	n.d.	0.9	Tr.	n.d.	n.d.
T-Gal*p*	Tr.	0.5	1.9	0.9	1.5	0.8
1,3-Gal*p*	0.5	1.1	2.5	1.4	2.3	Tr.
1,4-Gal*p*	0.7	1.8	n.d.	n.d.	2.1	0.6
1,6-Gal*p*	Tr.	0.6	1.1	1.2	1.4	Tr.
1,3,6-Gal*p*	2.2	2.9	5.8	4.7	6.4	0.6
1,3,4-Gal*p*	n.d.	n.d.	2.1	Tr.	1.0	n.d.
1,4,6-Gal*p*	n.d.	Tr.	Tr.	Tr.	1.1	Tr.
1,3,4,6-Gal*p*	0.5	1.0	2.1	1.7	4.1	0.6
T-Glc*p*	3.1	3.3	0.9	1.0	n.d.	0.6
1,3-Glc*p*	Tr.	Tr.	0.9	0.6	Tr.	n.d.
1,4-Glc*p*	55.3	56.0	21.8	24.5	2.3	3.5
1,4,6-Glc*p*	1.9	2.2	3.3	1.0	n.d.	Tr.
T-Glc*p*A	Tr.	Tr.	n.d.	Tr.	Tr.	n.d.
1,4-Glc*p*A	Tr.	1.3	n.d.	Tr.	1.2	n.d.
T-Gal*p*A	n.d.	n.d.	1.8	n.d.	Tr.	3.7
1,4-Gal*p*A	2	7.5	4.6	3.2	28.0	63.1
1,3,4-Gal*p*A	n.d.	n.d.	n.d.	n.d.	4.0	6.5

Linkage types	ALR-II-I	ALR-II-II	AMR-II-I	AMR-II-II	ARL-II-I	ARL-II-II	AS-II-I	AS-II-II
T-Ara*f*	19.3	4.5	13.1	2.9	16.7	2.9	11.1	3.4
1,3-Ara*f*	Tr.	n.d.	Tr.	n.d.	Tr.	n.d.	0.9	n.d.
1,5-Ara*f*	8.1	0.7	8.6	0.7	8.7	0.7	10.0	1.1
1,3,5-Ara*f*	22.3	1.1	11.7	Tr.	15.9	0.7	7.4	0.6
1,2,5-Ara*f*	n.d.	Tr.	n.d.	Tr.	n.d.	Tr.	n.d.	0.1
T-Rha*p*	0.4	0.7	1.0	2.9	Tr.	2.6	0.9	3.1
1,2-Rha*p*	4.9	2.0	5.9	0.7	5.8	0.6	10.3	2.0
1,2,4-Rha*p*	1.8	Tr.	2.4	Tr.	2.6	Tr.	3.4	Tr.
T-Fuc*p*	0.8	1.1	0.9	0.9	0.7	0.9	0.9	1.2
T-Xyl*p*	2.6	4.3	3.4	6.3	4.7	7.2	2.3	3.1
1,2-Xyl*p*	n.d.	Tr.	n.d.	n.d.	n.d.	0.9	n.d.	Tr.
1,4-Xyl*p*	n.d.	n.d.	0.8	n.d.	0.7	n.d.	1.9	1.2
T-Man*p*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1,2-Man*p*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1,4-Man*p*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1,4,6-Man*p*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
T-Gal*p*	1.6	n.d.	2.1	0.7	2.1	n.d.	1.8	1.4
1,3-Gal*p*	1.5	Tr.	1.7	n.d.	1.6	Tr.	2.3	Tr.
1,4-Gal*p*	1.4	0.5	4.9	0.2	1.2	n.d.	3.0	0.5
1,6-Gal*p*	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	1.1	Tr.
1,3,6-Gal*p*	3.8	1.2	3.4	0.5	3.2	0.7	4.6	0.5

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Table 3. Continued

Linkage types	ALR-II-I	ALR-II-II	AMR-II-I	AMR-II-II	ARL-II-I	ARL-II-II	AS-II-I	AS-II-II
1,3,4-Gal*p*	n.d.	n.d.	n.d.	n.d.	Tr.	n.d.	0.6	n.d.
1,4,6-Gal*p*	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
1,3,4,6-Gal*p*	Tr.	Tr.	1.0	Tr.	0.7	Tr.	1.2	Tr.
T-Glc*p*	Tr.	n.d.	Tr.	n.d.	0.6	2.7	Tr.	0.6
1,3-Glc*p*	Tr.	n.d.	Tr.	n.d.	Tr.	n.d.	0.7	n.d.
1,4-Glc*p*	1.1	4.7	3.3	3.4	1.5	1.1	1.0	2.8
1,4,6-Glc*p*	n.d.	Tr.	Tr.	n.d.	Tr.	Tr.	Tr.	Tr.
T-Glc*p*A	0.6	0.6	0.7	n.d.	Tr.	n.d.	0.7	0.9
1,4-Glc*p*A	Tr.	n.d.	n.d.	n.d.	Tr.	n.d.	0.7	Tr.
T-Gal*p*A	Tr.	5.5	Tr.	5.4	Tr.	8.0	1.4	4.9
1,4-Gal*p*A	25.1	65.2	26.6	63.3	24.3	57.8	28.3	62.1
1,3,4-Gal*p*A	2.5	5.0	5.2	9.7	5.1	10.7	1.9	7.6

Abbreviations: Tr, relative amount less than 0.5 mol%; n.d., not detected.

The current study is the first to conclude that pectic polysaccharides are present in all plant parts of *A. carmichaelii*, with the majority consisting of both RG-I cores with branches of arabinogalactans and arabinan, and linear HG regions, as well as possibly other types of polysaccharides in minor amounts, such as mannans or xyloglucans in the aerial plant parts. However, the pectic polysaccharides from the aerial parts of *A. carmichaelii*, particularly those from the leaves,[24] were composed of more AG-II side chains than those from the stems and root parts, which contained more arabinans (in ALR-II-I and ARL-II-I) followed by AG-II moieties. These findings also highlighted the potential medicinal value of the rootlets and the aerial parts of *A. carmichaelii* due to the presence of pectic polysaccharides.

Complement-fixating activity of polysaccharides isolated from different plant parts of *A. carmichaelii*

Complement-fixating activity has been attributed to immunomodulatory polysaccharides such as pectic polysaccharides, β-glucans and AG-II regions.[43] In the current study, the neutral polysaccharide fractions from the different plant parts of *A. carmichaelii* showed only weak-to-moderate complement-fixation activity (Fig. 5). The neutral fraction from the lateral roots (ALR-N) exhibited weak complement-fixating activity, with an average ICH_{50} of 280.7 μg mL^{-1} (Supporting Information, Table S3), and the fractions from the mother roots and rootlets, AMR-N and ARL-N, showed moderate activity, with an average ICH_{50} of 142.5 and 138.1 μg mL^{-1}, respectively (Supporting Information, Table S3). In comparison, the neutral fractions from the aerial parts of *A. carmichaelii*, AAP-N and AS-N, exhibited stronger complement fixation activity than those of AMR-N, ALR-N, or ARL-N, with average ICH_{50} values of 34.5 and 74.4 μg mL^{-1}, respectively (Supporting Information, Table S3). As reported previously, the neutral fraction from the leaves (AL-N) showed an ICH_{50} of 18.3 μg mL^{-1},[24] and has the strongest activity among all the neutral fractions. This finding might be due to the higher amounts of xyloglucans present in AL-N in comparison with the other neutral fractions, being represented by T- and 1,2-linked Xyl*p*.

Regarding the acidic polysaccharides, ARL-II-I from the rootlets displayed the highest complement fixating ability among the acidic polysaccharides, with an average ICH_{50} of 1.1 μg mL^{-1}. However, this fraction occupied a relatively small part of ARL-II (11%), which explains the weaker fixating activity of the mother fraction ARL-II (average ICH_{50} of 7.7 μg mL^{-1}) compared with ARL-II-I and being similar to the activity of ALR-II or AMR-II. The same applied to AS-II-I (average ICH_{50} of 1.5 μg mL^{-1}), which constituted 15% of AS-II (average ICH_{50} of 12.3 μg mL^{-1}). Even though the reported ratio of $ICH_{50\ BP\text{-}II}/ICH_{50\ sample}$ of the acidic fractions from *A. carmichaelii* leaves (approximately 6.3 for AL-I and 9.0 for AL-I-I)[24] was lower than AS-II-I (approximately 42), AL-I, and AL-I-I, gave the highest yield in the aerial parts (Fig. 1), and would be a productive plant-derived substance for industrial application.

It was noticed that the first SEC fractions showed the highest complement fixation activity, and they are mainly composed of T-, 1,5- and 1,3,5-linked Ara*f*, and/or T-, 1,3- and 1,3,6-linked Gal*p* in addition to a HG linear chain. This is consistent with previous conclusions for pectic polysaccharides from various medicinal plants, that arabinans and AG-II moieties are important to attach to human complement.[43-45] Earlier studies also concluded that pectic polysaccharides with higher *Mw* had much more potent complement fixation activity,[46] which matched with our primary finding that the second SEC fractions with lower *Mw* (41.6–105 kDa) were shown to be inactive (Fig. 5). However, the influence of *Mw* seemed to be less important for the first SEC fractions with more than 200 kDa of *Mw*. For instance, ALR-II-I, with higher *Mw* than AS-II-I (Table 1), exhibited weaker activity (Fig. 5).

Complement fixation activity of pectin could be achieved through either activation or inhibition of the complement system.[45,47] This study showed that several polysaccharide fractions from different plant parts of *A. carmichaelii* possess the ability to bind human complement, even though the exact effects on the complement system has not yet been determined.

Anti-inflammatory activity of polysaccharides isolated from different plant parts of *A. carmichaelii*

Natural bioactive polysaccharides have attracted growing attention owing to their safety, accessibility, anti-inflammatory and immunomodulatory activities, and many of them have shown for the amelioration of intestinal inflammatory disease both *in vivo* and *in vitro*.[48-50] In the present *in vitro* study, an intestinal epithelial cell line IPEC-J2 was employed, and all polysaccharide fractions were shown to be non-toxic under 10 μg/mL (final concentration) after co-cultivation for 24 h (data not shown). Inflammatory reactions were observed after LPS-treatment of IPEC-J2 cells, evidenced by a significant up-regulation of mRNA

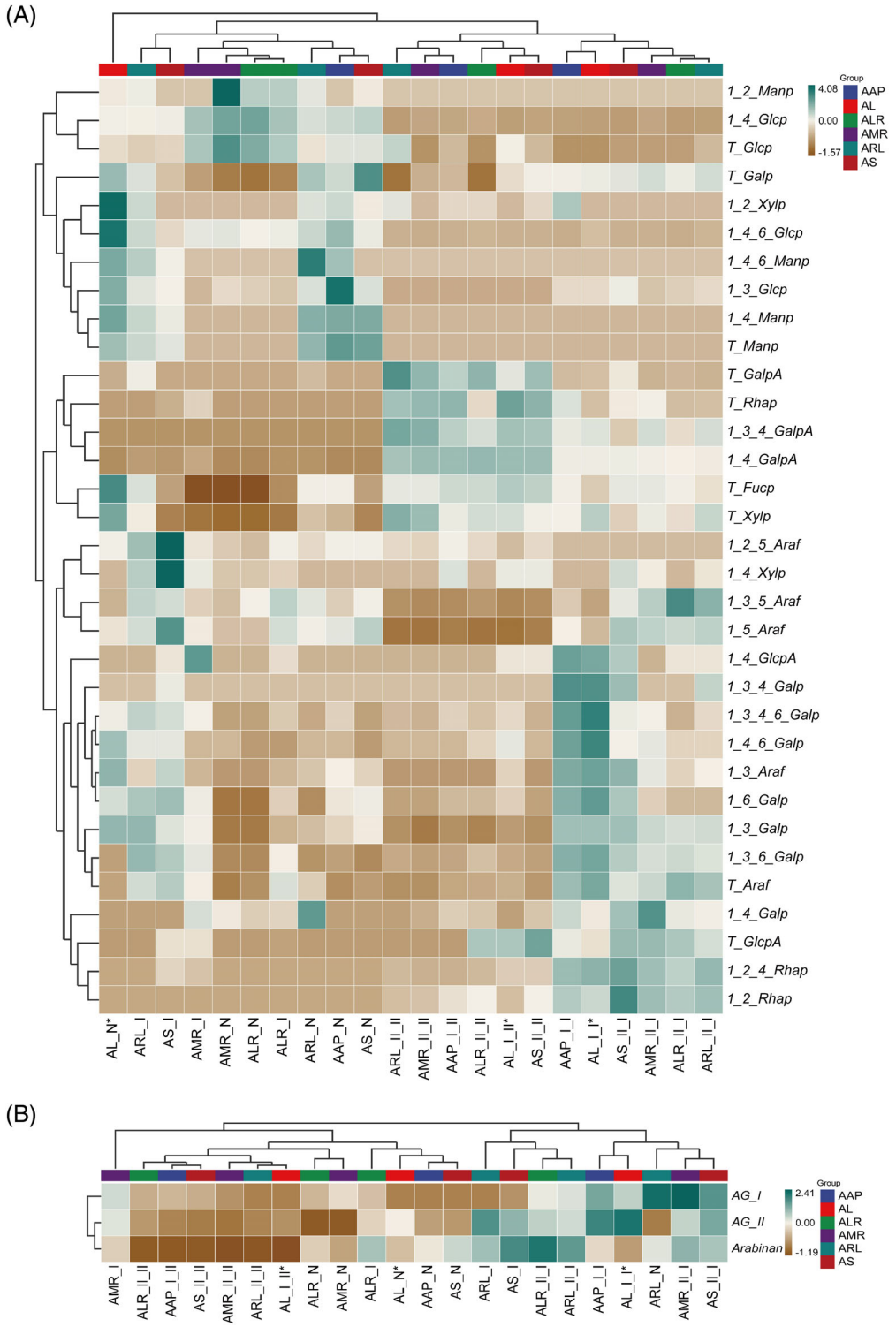

Figure 4. Heatmap with dendrogram of glycosidic linkages (A) and the relative contents of arabinan and AG regions (B) of polysaccharides from different plant parts of *A. carmichaelii*. Hierarchical clustering was analyzed based on Euclidean distance, and the value represents the row Z scores; *, data of AL-N, AL-I-I and AL-I-II in our previous publication.[24] The amount of AG-I was represented by the sum molar amount of 1,4- and 1,3,4-linked Gal*p* residues; AG-II was represented by the sum molar amount of 1,3-, 1,6, and 1,3,6-linked Gal*p* residues; arabinan was represented by the sum molar amount of 1,3-, 1,5-, 1,2,5- and 1,3,5-linked Ara*f* residues. AAP, polysaccharide from *A. carmichaelii* aerial parts; ALR, polysaccharide from *A. carmichaelii* lateral roots; AMR, polysaccharide from *A. carmichaelii* mother roots; ARL, polysaccharide from *A. carmichaelii* rootlets; AS, polysaccharide from *A. carmichaelii* stems.

transcriptions of the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 (Fig. S3). These inflammatory reactions were further down-regulated by polysaccharide fractions isolated from different plant parts of *A. carmichaelii*. The inhibition related to the mRNA transcription levels of IL-1β, TNF-α and IL-6 of LPS-treated cells was calculated for further analysis. A heatmap with clustering

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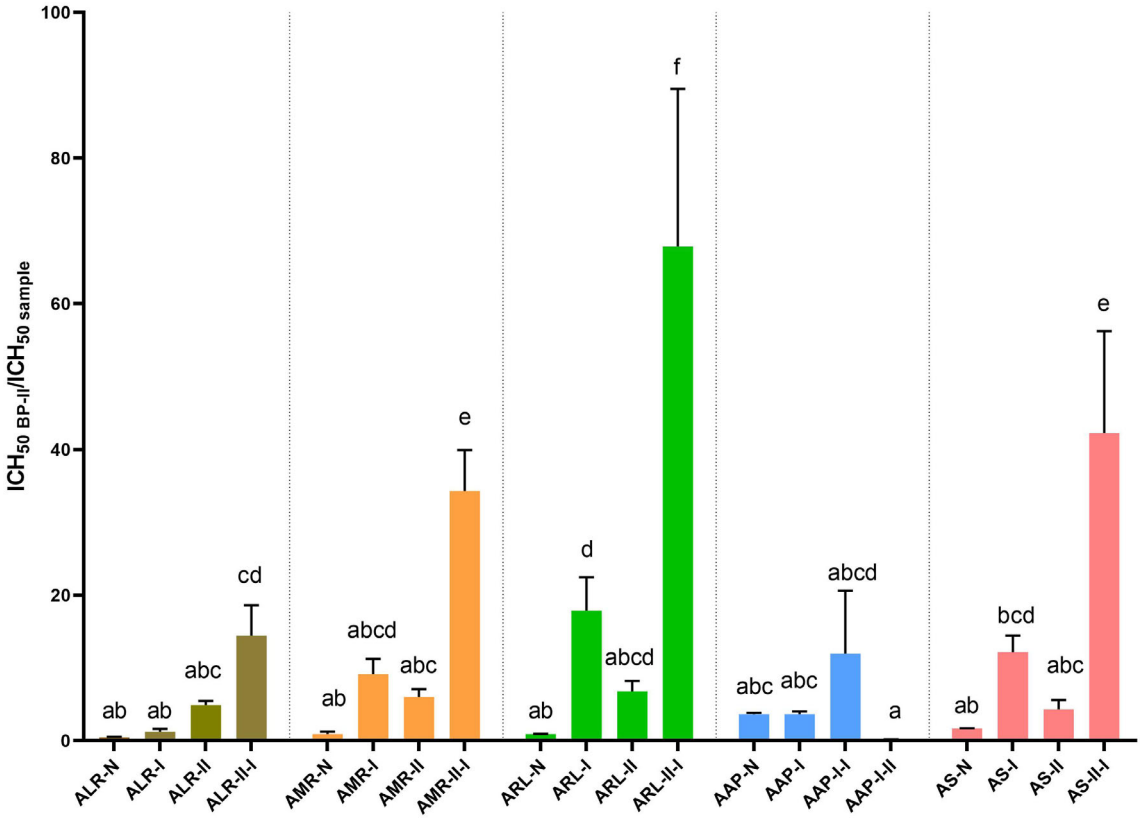

Figure 5. Complement fixating activity of polysaccharides from different plant parts of *A. carmichaelii* related to positive control (BP II, a pectic polysaccharide from *Biophytum petersianum)*. The higher value of $ICH_{50\ BPII}/ICH_{50\ Sample}$, the better complement fixating activity of the tested sample. The different lowercase letters labeled above the column indicate statistical differences among groups according to the Duncan's multiple range test at $P < 0.05$; $n = 3$. AAP, polysaccharide from *A. carmichaelii* aerial parts; ALR, polysaccharide from *A. carmichaelii* lateral roots; AMR, polysaccharide from *A. carmichaelii* mother roots; ARL, polysaccharide from *A. carmichaelii* rootlets; AS, polysaccharide from *A. carmichaelii* stems.

analysis was performed to compare the anti-inflammatory effects of different polysaccharides, as shown in Fig. 6. It is obvious that most of the acidic polysaccharide fractions showed potent anti-inflammatory effects compared to the neutral ones. However, the neutral fraction from leaves, AL-N, was an exception and has previously been reported with potent inhibitory effects[24] (Fig. 6). The acidic polysaccharides from lateral roots (ALR-I, ALR-II-I and ALR-II-II), mother roots (AMR-I and AMR-II-I), as well as the minor acidic fractions from rootlets (ARL-I) and stems (AS-I) were the most promising fractions with more than 45% inhibition related to LPS-treated cells. ALR-II-I and ALR-II-II showed more than 70% of inhibition on IL-1β and IL-6 transcriptions, higher than those of AMR-II-I and AMR-II-II. It was further shown that all polysaccharide fractions previously isolated from the leaves, AL-N, AL-I, AL-I-I, and AL-I-II,[24] were more active than those from the stems (AS-N, AS-II, AS-II-I, and AS-II-II). Most rootlets fractions, including ARL-N, ARL-II, ARL-II-I, and ARL-II-II did not show significant anti-inflammatory effects except ARL-I.

Many plant-derived polysaccharides, especially pectins, have been reported to display anti-inflammatory activity.[49,50] However, polysaccharides from *Aconitum* plants, such as glucans, and pectic polysaccharides with RG-II regions, which have displayed broad bioactivities such as anti-oxidant, anti-tumor, cardiovascular protective and immunomodulatory activity,[3,51] are rarely studied for their anti-inflammatory activity. One study reported a pectic polysaccharide containing a RG-II region isolated from *A. coreanum* with anti-inflammatory effects on LPS-stimulated macrophages.[52] The current study not only systematically screened the presence of pectins consisting of RG-I and HG regions in each plant part of *A. carmichaelii*, but also investigated their inhibition on the gene transcription of pro-inflammatory cytokines. In this study, almost half of the isolated polysaccharide fractions showed potent anti-inflammatory effects *in vitro*, being either composed of HG and branched RG-I regions with arabinan and/or AG domains (first SEC fractions), or of mainly HG regions (the second SEC fractions). The anti-inflammatory effects could be due to specific structural regions. For instance, it has previously been reported that HG domains are thought to regulate inflammation directly through electrostatic forces between non-esterified galacturonic acids and positive charges on the toll-like receptor 2 (TLR2) ectodomain.[49] The negative charge due to free carboxyl groups could also prevent the formation of hydrogen bonds and mucin–pectic aggregates, which is beneficial for the penetration of pectin through mucin layer and promotes interaction between pectin and intestinal epithelial cells.[53] However, in this study, it was hard to conclude how the polysaccharide fractions act, and which regions of pectin could be responsible for the observed anti-inflammatory effects. Other structural characteristics could be the reason of these differences, such as the degree of esterification, the degree of branching and/or two- and three-dimensional conformation.

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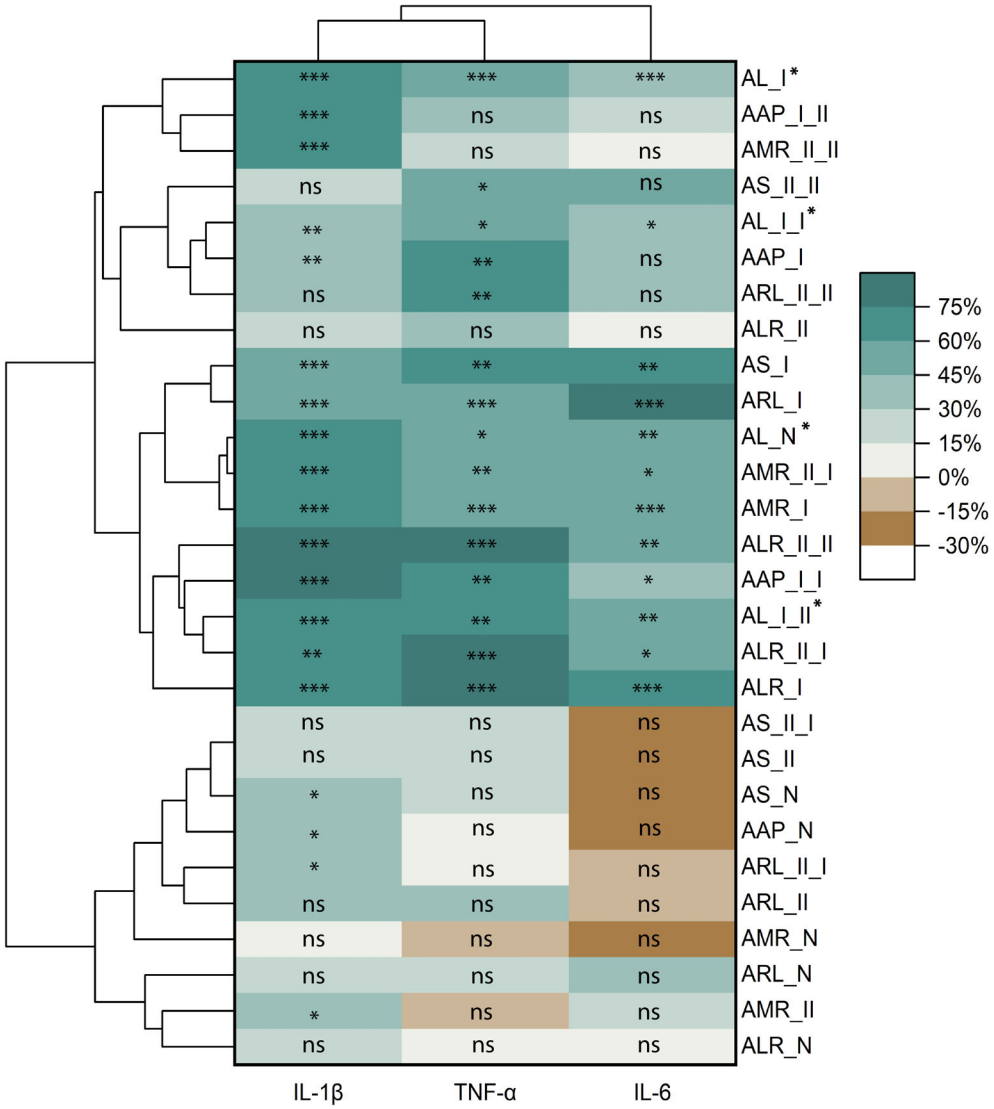

Figure 6. Inhibition on the gene transcription of inflammatory cytokines by polysaccharides from different plant parts of *A. carmichaelii*. Statistical analysis was performed based on the gene transcription levels related to those of lipopolysaccharides (LPS)-treated cells according to the Fisher's least significant difference (LSD) test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, no significance). * in sample list, data of AL-N, AL-I-I and AL-I-II in our previous publication[24]; $n = 3$. AAP, polysaccharide from *A. carmichaelii* aerial parts; ALR, polysaccharide from *A. carmichaelii* lateral roots; AMR, polysaccharide from *A. carmichaelii* mother roots; ARL, polysaccharide from *A. carmichaelii* rootlets; AS, polysaccharide from *A. carmichaelii* stems.

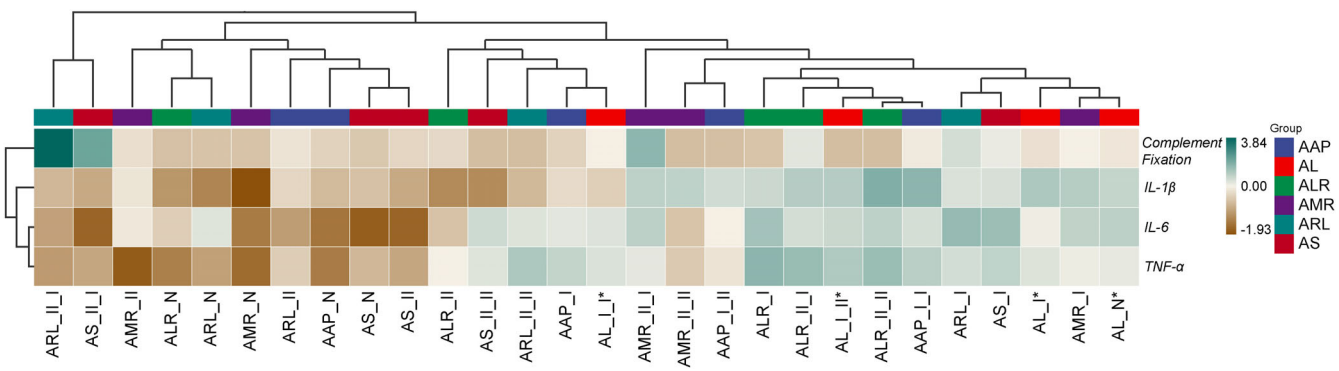

Figure 7. Clustered heatmap of anti-inflammatory and complement fixation activities of polysaccharides from different plant parts of *A. carmichaelii*. The complement fixation activity was expressed related to BP-II (*Complement Fixation*). Hierarchical clustering was analyzed based on Euclidean distance, and the value represents the row *Z* scores; *, data of AL-N, AL-I-I and AL-I-II in our previous publication.[24] AAP, polysaccharide from *A. carmichaelii* aerial parts; ALR, polysaccharide from *A. carmichaelii* lateral roots; AMR, polysaccharide from *A. carmichaelii* mother roots; ARL, polysaccharide from *A. carmichaelii* rootlets; AS, polysaccharide from *A. carmichaelii* stems.

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Combining the results of the anti-inflammatory and complement fixating activities of all polysaccharide fractions, it was obvious that the isolated polysaccharide fractions exhibited various effects in the two bioassays (Fig. 7). For instance, ARL-II-I and AS-II-I displayed potent complement fixation activity but weak anti-inflammatory effects, being clustered in a distinct group from the rest of polysaccharide fractions. Further, it was clear that most neutral fractions were shown with weak activity, except for AL-N, which showed potent activity in both bioassays and was similar to the activities of AMR-I, ARL-I, and AS-I. The purified acidic polysaccharides from aerial parts and leaves (AAP-I-I, AAP-I-II, AL-I-I, and AL-I-II) showed similar activity to those from the lateral and mother roots (ALR-II-I, ALR-II-II, AMR-II-I and AMR-II-II) but different from the polysaccharides isolated from stems and rootlets. The results on bioactivities in this study demonstrate that the polysaccharide fractions isolated from unutilized plant parts of *A. carmichaelii*, especially the rootlets and previously reported leaves, could exert immunomodulatory and moderate anti-inflammatory activities, similar to the traditional used roots. This supports the medical use of these plant resources.

CONCLUSION

In this study, the diversity of polysaccharides in the traditionally used roots – the lateral and mother roots – of *A. carmichaelii*, as well as in the unutilized parts, including the entire aerial parts, stems and rootlets, was reported. The neutral polysaccharide fraction from the rootlets, ARL-N, was different from those from lateral and mother roots but was similar to those from the aerial parts, possibly being a mixture of starch, arabinans, galactans, mannans and/or xyloglucans. The RG-I regions possibly branched with arabinans and AGs, and HG regions were found in each plant part of *A. carmichaelii*. However, the acidic polysaccharide fractions in the aerial parts, and predominantly from leaves, were composed of more AG-II moieties compared with the acidic fractions from the root parts. Most of the polysaccharide fractions mentioned above were shown to have immunomodulatory and anti-inflammatory activities *in vitro*. These diverse bioactive polysaccharides present in the different plant parts of *A. carmichaelii* suggest the potential medicinal value of *A. carmichaelii*, especially the unutilized plant parts, which should be collected and used in industrial production.

AUTHOR CONTRIBUTIONS

Yu-Ping Fu: Data curation, investigation, methodology, visualization, roles, writing – original draft. Cen-Yu Li: Data curation, investigation, methodology, visualization. Yuan-Feng Zou: Funding acquisition, methodology, project administration, resources, supervision, writing – review and editing. Xi Peng: Data curation, software, methodology. Berit Smestad Paulsen: Writing – review and editing. Helle Wangensteen: Project administration, supervision, writing – review and editing. Kari Tvete Inngjerdingen: Methodology, project administration, supervision, writing – review and editing.

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CONFLICT OF INTEREST

None of the authors has any competing financial interest to declare

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Zhou G, Tang L, Zhou X, Wang T, Kou Z and Wang Z, A review on phytochemistry and pharmacological activities of the processed lateral root of *Aconitum carmichaelii* Debeaux. *J Ethnopharmacol* **160**: 173–193 (2015).
2 Chinese Pharmacopoeia Committee, *Chinese Pharmacopoeia*. Chemical Industry Press, Beijing (2020).
3 Fu Y-P, Zou Y-F, Lei F-Y, Wangensteen H and Inngjerdingen KT, *Aconitum carmichaelii* Debeaux: a systematic review on traditional use, and the chemical structures and pharmacological properties of polysaccharides and phenolic compounds in the roots. *J Ethnopharmacol* **291**:115148 (2022).
4 Yu M, Yang Y-X, Shu X-Y, Huang J and Hou D-B, *Aconitum carmichaelii* Debeaux, cultivated as a medicinal plant in western China. *Genet Resour Crop Evol* **63**:919–924 (2016).
5 Cui H, Qu Y, Huang Y, Cheng C, Liang Z and Zhou H, Investigation on medicinal plant resources of *aconitum* in Sichuan Shaanxi and Yunnan. *Mod Chin Med* **24**:1264–1271 (2022).
6 Wang FP and Chao RB, On the famous traditional chinese medicine "Fu zi": discovery, research, and development of cardioactive constituent mesaconine. *Nat Prod Bioprospect* **11**:15–20 (2020).
7 Zhang J, Mao R and Du G, Research advance on alkaloids and their pharmacological effects of radix aconite. *Herald Med* **30**:1048–1051 (2019).
8 Liu S, Li F, Li Y, Li W, Xu J and Du H, A review of traditional and current methods used to potentially reduce toxicity of *aconitum* roots in traditional Chinese medicine. *J Ethnopharmacol* **207**:237–250 (2017).
9 Wang L, Zhang X, Wang X, Zhang Y, Chen J, Tan P *et al.*, Comparative study on unprocessed Aconiti radix and unprocessed Aconiti lateralis radix by UPLC-MS^n. *Chin J Pharm Anal* **37**: 1640–1647 (2017).
10 Yang S, Sun M, Zhang D, Song X, Liu P, Li F *et al.*, Differences in cultivated and wild Fuzi and Wutou based on alkaloids and polysaccharide content. *Pract Pharm Clin Rem* **24**:836–839 (2021).
11 Chen R, Sun G, Xu H, Ji F, Lv Y, Li F *et al.*, Comparative study on toxicity of different parts of monkshood medicinal material. *Trad Chin Drug Res Clin Pharmacol* **22**:4–7 (2011).
12 Ou S, Wang S, Zheng Q, Hu P, Chen Z and Yang M, Investigation of dynamic differences of crude polysaccharides content in various tissues of *Aconitum carmichaeli*. *Chin J Exp Trad Med Formulae* **19**:7–9 (2013).
13 Huang C, Li ZX, Wu Y, Huang ZY, Hu Y and Gao J, Treatment and bioresources utilization of traditional Chinese medicinal herb residues: recent technological advances and industrial prospect. *J Environ Manage* **299**:113607 (2021).
14 Saha A and Basak BB, Scope of value addition and utilization of residual biomass from medicinal and aromatic plants. *Ind Crops Prod* **145**: 111979 (2020).
15 Lv Y, Bo H, Yang L, Li X and Li F, Comparasion of polysaccharides in parent root, daughter root and rootlet of *Aconitum carmichaeli*. *Chin J Chin Mater Medica* **36**:1154–1157 (2011).

16 Sun M, Zhang D, Yang S, Peng S, Song X, Liu P *et al*., Comparison of alkaloids and polysaccharides in different parts of wild *aconitum* in Nanyang. *World Sci Technol/Mod Trad Chin Med Mater Medica* **20**: 2231–2236 (2018).
17 Hou D, Zhao X, Wang H and Xu Y, Determination of alkaloid content in different tissues of *Aconite carmichaelii*. *J Southwest Univ Sci Technol* **24**:98–102 (2009).
18 Zhang Y, Gao S and Han F, Determination of chemical constituents in different parts of radix Aconiti lateralis Preparata by RP-HPLC. *Cent South Pharm* **11**:551–553 (2013).
19 He Y-N, Ou S-P, Xiong X, Pan Y, Pei J, Xu R-C *et al*., Stems and leaves of *Aconitum carmichaelii* Debx. as potential herbal resources for treating rheumatoid arthritis: chemical analysis, toxicity and activity evaluation. *Chin. J Nat Med* **16**:644–652 (2018).
20 Wang X, Li Y, Shi J, Qin Y, Zhang C, Zhang X *et al*., Study on the chemical constituents of the aerial part of *Aconitum carmichaeli*. *West Chin J Pharm Sci* **33**:115–118 (2018).
21 Yang L, Zhao N, Wang J, Wang X and Liu S, Chemical constituents of the aerial part of *Aconitum carmichaeli* Debx. *Anhui Med Pharm J* **15**:1068–1069 (2011).
22 Zhang L, Yang C, Luo Q, Liu J, Chen Y and Yong S, Non-diterpenoid alkaloids in aerial parts of *Aconitum carmichaelii*. *Chin Trad Herbal Drugs* **51**:588–593 (2020).
23 Duc LV, Thanh TB, Thanh HN and Tien VN, Flavonoids and other compound isolated from leaves of *Aconitum carmichaeli* Debx. Growing in Viet Nam. *J Chem Pharm Res* **7**:228–234 (2015).
24 Fu Y-P, Li C-Y, Peng X, Zou Y-F, Rise F, Paulsen BS *et al*., Polysaccharides from *Aconitum carmichaelii* leaves: structure, immunomodulatory and anti-inflammatory activities. *Carbohydr Polym* **291**:119655 (2022).
25 Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am J Enol Vitic* **16**:144–158 (1965).
26 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Chem* **72**:248–254 (1976).
27 Hunter RA, McIntyre BL and McIlroy RJ, Water-soluble carbohydrates of tropical pasture grasses and legumes. *J Sci Food Agric* **21**:400–405 (1970).
28 Chambers RE and Clamp JR, An assessment of methanolysis and other factors used in the analysis of carbohydrate-containing materials. *Biochem J* **125**:1009–1018 (1971).
29 Wold CW, Kjeldsen C, Corthay A, Rise F, Christensend BE, Duusb JØ *et al*., Structural characterization of bioactive heteropolysaccharides from the medicinal fungus *Inonotus obliquus* (chaga). *Carbohydr Polym* **185**:27–40 (2018).
30 Ciucanu I and Kerek F, A simple and rapid method for the permethylation of carbohydrates. *Carbohydr Res* **131**:209–217 (1984).
31 Pettolino FA, Walsh C, Fincher GB and Bacic A, Determining the polysaccharide composition of plant cell walls. *Nat Protoc* **7**:1590–1607 (2012).
32 Sweet DP, Shapiro RH and Albersheim P, Quantitative analysis by various g.l.c. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydr Res* **40**:217–225 (1975).
33 Michaelsen TE, Gilje A, Samuelsen AB, Høgåsen K and Paulsen BS, Interaction between human complement and a pectin type polysaccharide fraction, PMII, from the leaves of *Plantago major* L. *Scand J Immunol* **52**:483–490 (2000).
34 Grønhaug TE, Kiyohara H, Sveaass A, Diallo D, Yamada H and Paulsen BS, Beta-D-(1-4)-galactan-containing side chains in RG-I regions of pectic polysaccharides from *Biophytum petersianum* Klotzsch. Contribute to expression of immunomodulating activity against intestinal Peyer's patch cells and macrophages. *Phytochemistry* **72**:2139–2147 (2011).
35 Zou Y-F, Zhang B-Z, Inngjerdingen KT, Barsett H, Diallo D, Michaelsen TE *et al*., Complement activity of polysaccharides from three different plant parts of *Terminalia macroptera* extracted as healers do. *J Ethnopharmacol* **155**:672–678 (2014).
36 Zou Y-F, Zhang Y-Y, Paulsen BS, Rise F, Chen Z-L, Jia R-Y *et al*., New pectic polysaccharides from *Codonopsis pilosula* and *Codonopsis tangshen*: structural characterization and cellular antioxidant activities. *J Sci Food Agric* **101**:6043–6052 (2020).
37 Zou YF, Li CY, Fu YP, Jiang QX, Peng X, Li LX *et al*., The comparison of preliminary structure and intestinal anti-inflammatory and antioxidative activities of polysaccharides from different root parts of *Angelica sinensis* (Oliv.) Diels. *J Ethnopharmacol* **295**:115446 (2022).
38 Zaitseva O, Khudyakov A, Sergushkina M, Solomina O and Polezhaeva T, Pectins as a universal medicine. *Fitoterapia* **146**: 104676 (2020).
39 Hayashi T and Kaida R, Functions of xyloglucan in plant cells. *Mol Plant* **4**:17–24 (2011).
40 Fry SC, Cell wall polysaccharide composition and covalent crosslinking, in *Annual Plant Reviews, Plant Polysaccharides: Biosynthesis and Bioengineering*, ed. by Ulvskov P. Wiley-Blackwell, West Sussex, Chichester, pp. 1–42 (2011).
41 Kaczmarska A, Pieczywek PM, Cybulska J and Zdunek A, Structure and functionality of rhamnogalacturonan I in the cell wall and in solution: a review. *Carbohydr Polym* **278**:118909 (2022).
42 Patova OA, Luanda A, Paderin NM, Popov SV, Makangara JJ, Kuznetsov SP *et al*., Xylogalacturonan-enriched pectin from the fruit pulp of *Adansonia digitata*: structural characterization and antidepressant-like effect. *Carbohydr Polym* **262**:117946 (2021).
43 Ferreira SS, Passos CP, Madureira P, Vilanova M and Coimbra MA, Structure–function relationships of immunostimulatory polysaccharides: a review. *Carbohydr Polym* **132**:378–396 (2015).
44 Dénou A, Togola A, Inngjerdingen KT, Moussavi N, Rise F, Zou YF *et al*., Isolation, characterisation and complement fixation activity of acidic polysaccharides from *Argemone mexicana* used as antimalarials in Mali. *Pharm Biol* **60**:1278–1285 (2022).
45 Yamada H and Kiyohara H, Immunomodulating activity of plant polysaccharide structures, in *Comprehensive Glycoscience*, ed. by Kamerling H. Oxford, Elsevier, pp. 663–694 (2007).
46 Zou Y-F, Zhang B-Z, Barsett H, Inngjerdingen KT, Diallo D, Michaelsen TE *et al*., Complement fixing polysaccharides from *Terminalia macroptera* root bark, stem bark and leaves. *Molecules* **19**: 7440–7458 (2014).
47 Sjoberg AP, Trouw LA and Blom AM, Complement activation and inhibition: a delicate balance. *Trends Immunol* **30**:83–90 (2009).
48 Yuan D, Li C, Huang Q, Fu X and Dong H, Current advances in the anti-inflammatory effects and mechanisms of natural polysaccharides. *Crit Rev Food Sci Nutr* **1-21**:5890–5910 (2022). https://doi.org/10.1080/10408398.2022.2025535.
49 Jin M-Y, Li M-Y, Huang R-M, Wub X-Y, Sun Y-M and Xu Z-L, Structural features and anti-inflammatory properties of pectic polysaccharides: a review. *Trend Food Sci Technol* **107**:284–298 (2021).
50 Hou C, Chen L, Yang L and Ji X, An insight into anti-inflammatory effects of natural polysaccharides. *Int J Biol Macromol* **153**:248–255 (2020).
51 Yin T, Zhou H, Cai L and Ding Z, Non-alkaloidal constituents from the genus *Aconitum*: a review. *RSC Adv* **9**:10184–10194 (2019).
52 Li X, Jiang J, Shi S, Bligh SWA, Li Y, Jiang Y *et al*., A RG-II type polysaccharide purified from *Aconitum coreanum* alleviates lipopolysaccharide-induced inflammation by inhibiting the NF-kB signal pathway. *PloS One* **9**:e99697 (2014).
53 Popov SV and Ovodov YS, Polypotency of the immunomodulatory effect of pectins. *Biochemistry (Mosc)* **78**:823–835 (2013).

