

Structural characterization and *in vitro* anti-inflammatory activity test of polysaccharides isolated from the fruits of *Rosa laevigata*

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Supplementary Method

S1. HPAEC conditions

The sample extracts were analyzed by high-performance anion-exchange chromatography (HPAEC) on a CarboPac PA-20 anion-exchange column (3 by 150 mm; Dionex) using a pulsed amperometric detector (PAD; Dionex ICS 5000 system) by Sanshu Biotech. Co., LTD (Shanghai, China). Flow rate, 0.5 mL/min; injection volume, 5 μ L; solvent system A : (ddH₂O), solvent system B : (0.1M NaOH), solvent system C : (0.1M NaOH, 0.2M NaAc); gradient program, the volume ratio of solution A, B, C was 95:5:0 at 0 min, 85:5:10 at 26 min, 85:5:10 at 42 min, 60:0:40 at 42.1 min, 60:40:0 at 52 min, 95:5:0 at 52.1 min, 95:5:0 at 60 min.

S2. Methylation analysis of RLPa-2

Approximately 5 mg of the sample was dissolved in pure water and reacted with 1 mL of 1-cyclohexyl-2-morpholinoethylcarbodiimide methyl p-toluenesulfonate (CMC, 100 mg/mL) for 2 h. The product was mixed with 1 mL of imidazole (2 mol/L), and divided equally into two parts. Then, one part was added with 1 mL NaBH₄ (30 mg/mL), and the other part was added with 1 mL NaBD₄ (30 mg/mL), dialyzing and lyophilizing to acquire the reduzates, and the reduzates were methylated in DMSO/NaOH with CH₃I. After complete methylation, the permethylated products were hydrolyzed with TFA (2 mol/L) at 121°C for 1.5 h, reduced by NaBD₄ (1 mol/L) and acetylated with acetic anhydride for 2.5 h (100 °C). Finally, 500 μ L of dichloromethane was added, vortexed, mixed, centrifuged, and repeated thrice. The

aqueous phase was discarded, the lower dichloromethane phase was taken, and the product was analyzed by gas chromatography-mass spectrometry (GC-MS) (Agilent 7890A-5977B; Agilent Technologies Inc., USA) for analysis, and high purity helium (split ratio 10:1) was used as the carrier gas with an injection volume of 1 μ L. Mass spectrometry analysis was performed at the initial temperature of 140 $^{\circ}$ C for 2.0 min, and the temperature is increased to 230 $^{\circ}$ C by 3 $^{\circ}$ C/min for 3 min. The scan mode was SCAN with a range from 30 to 600 m/z.

Supplementary Data

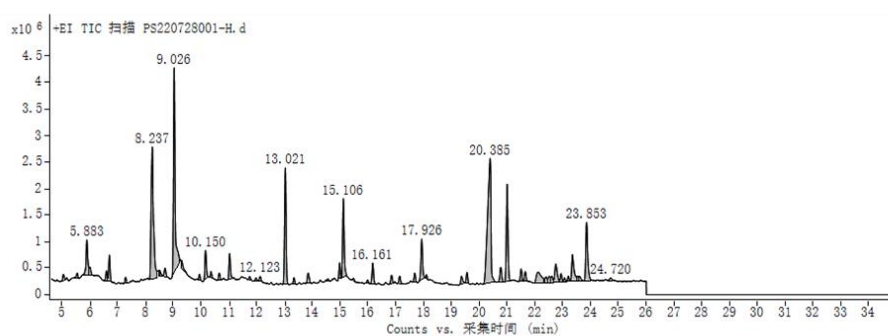
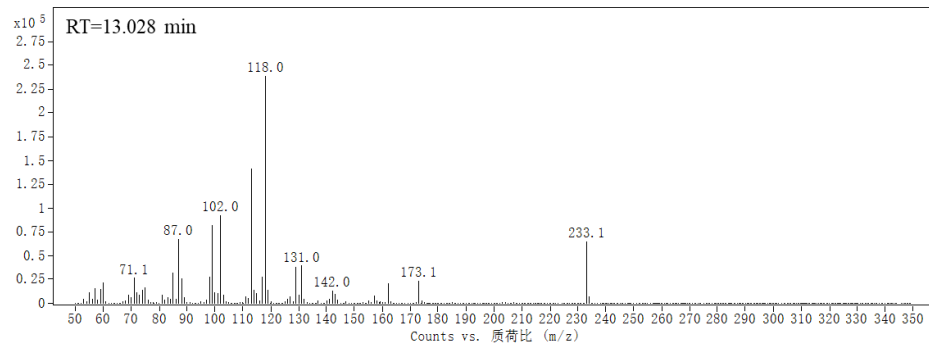
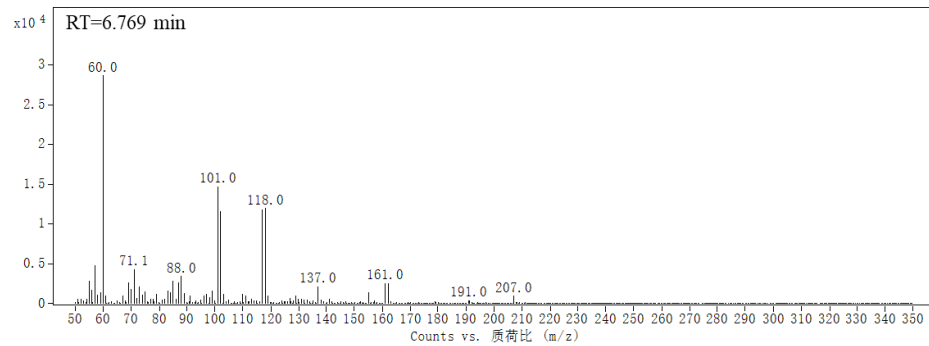
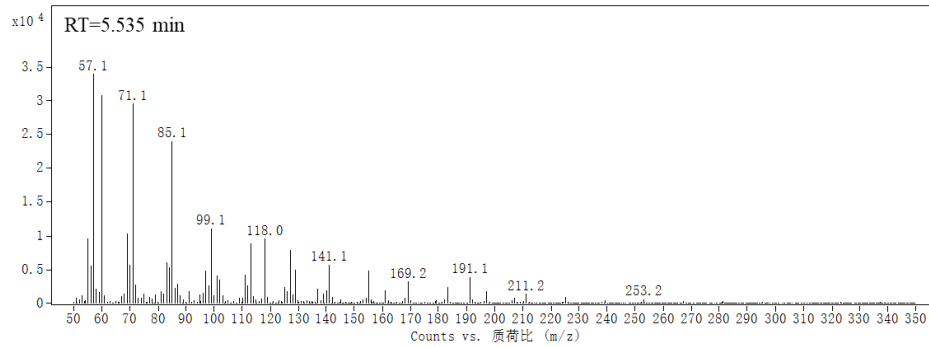
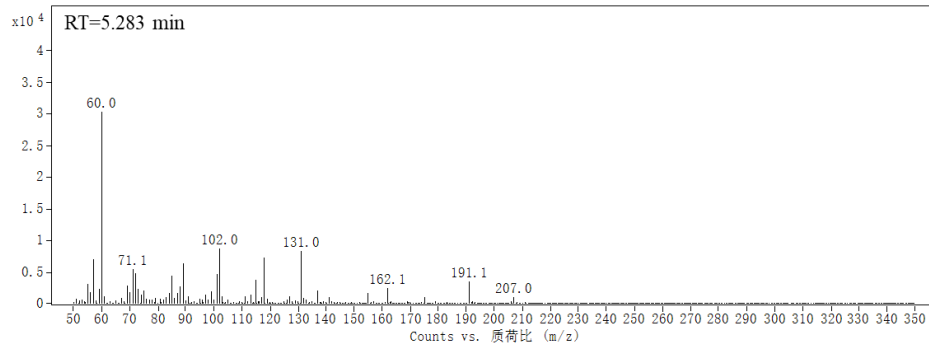


Figure S1. The total ion chromatogram of RLPa-2 polysaccharides was methylated.



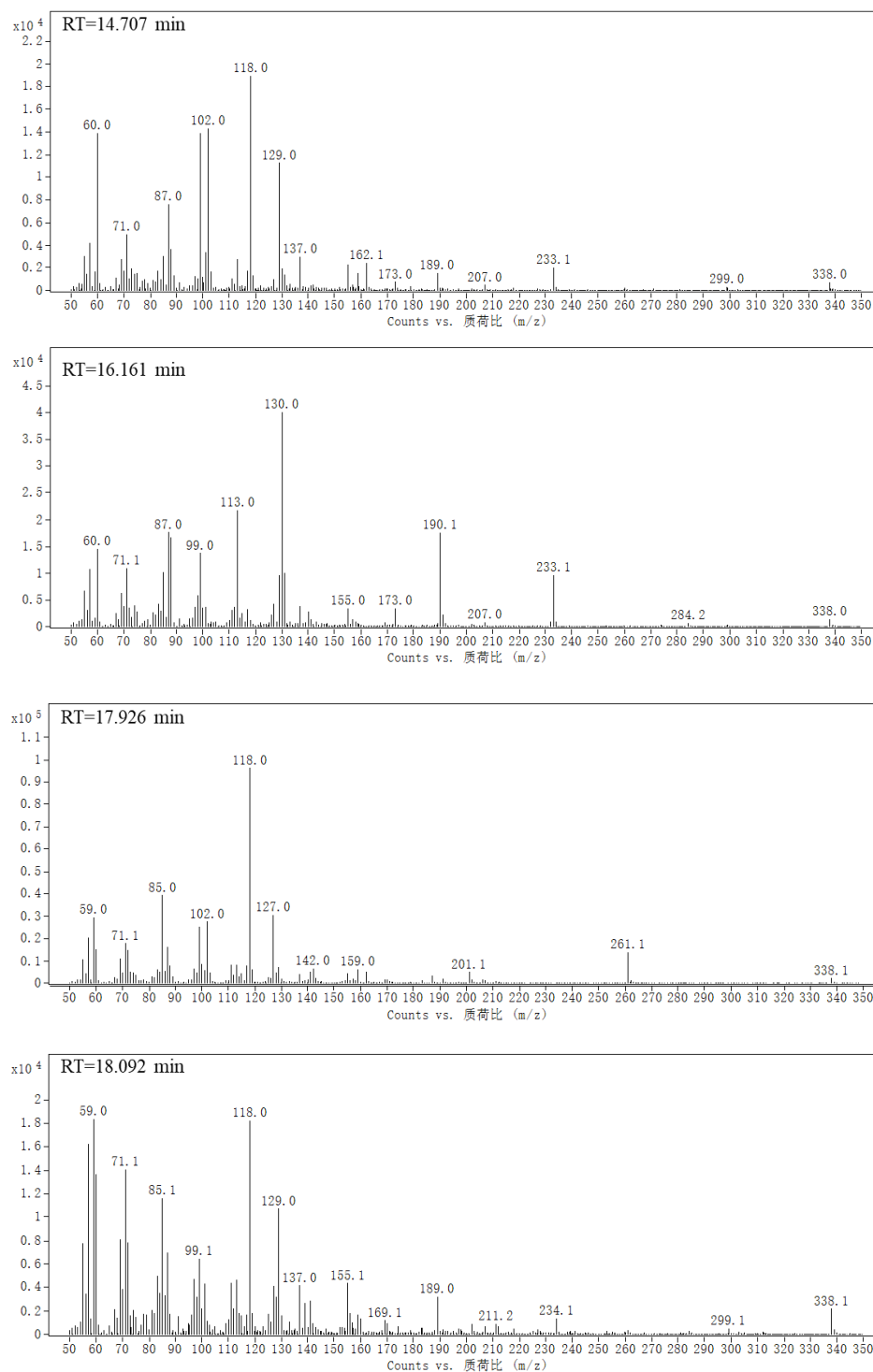


Figure S2. Tandem mass spectra of the characteristic peaks.

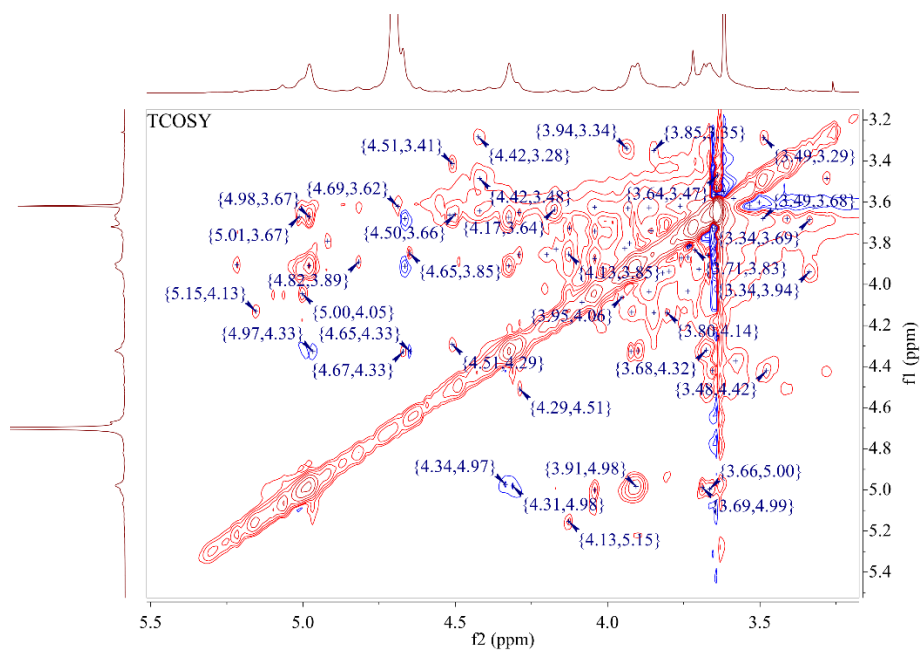
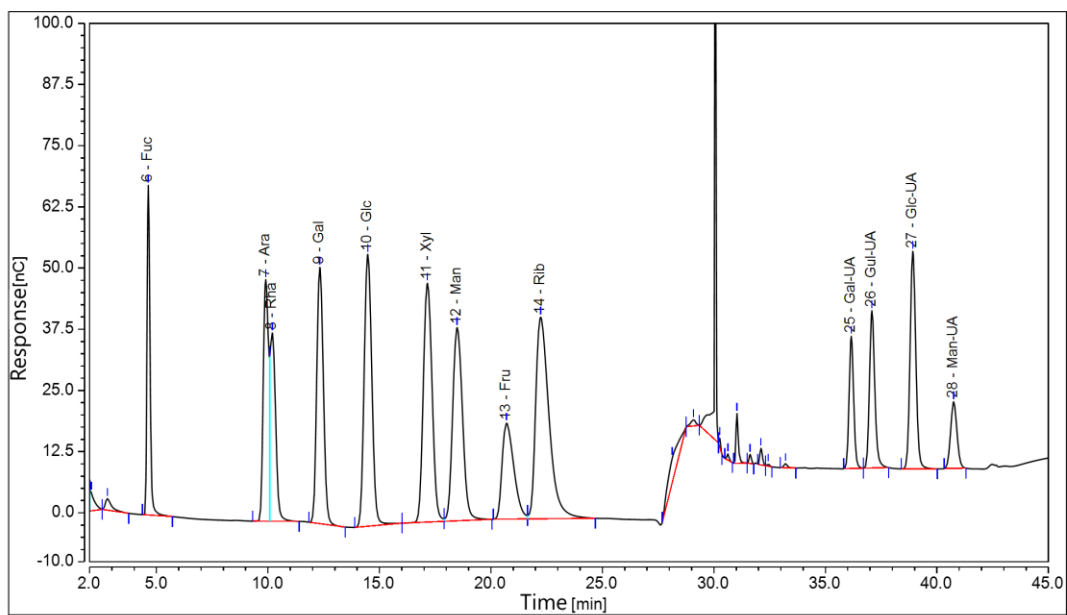


Figure S3. TOCSY spectra of RLPa-2 glycosyl residues.



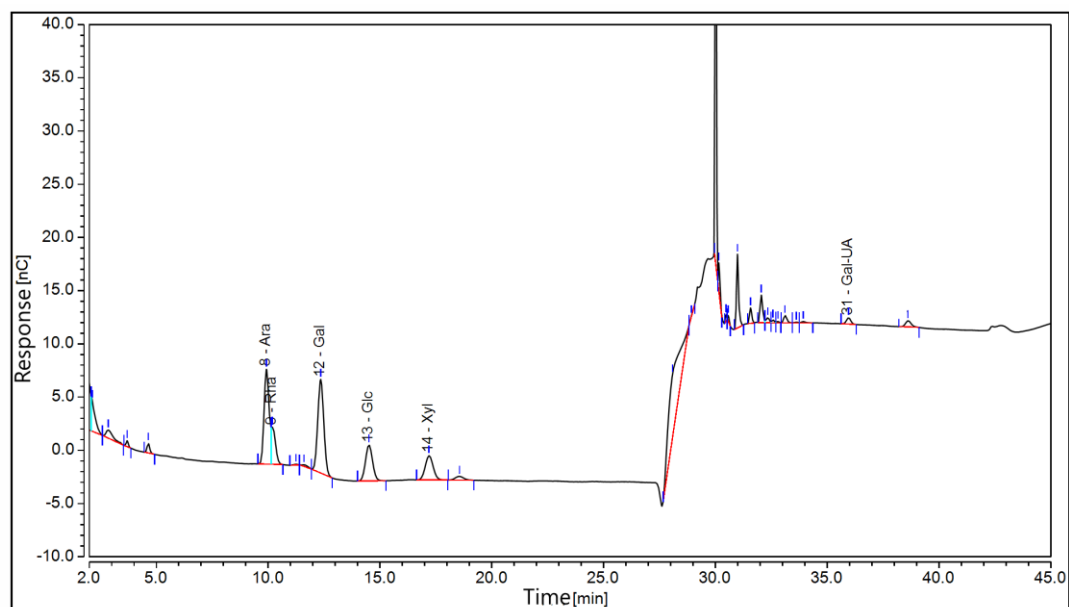


Figure S4. Chromatogram of monosaccharide standards and monosaccharide components in RLPa-2.

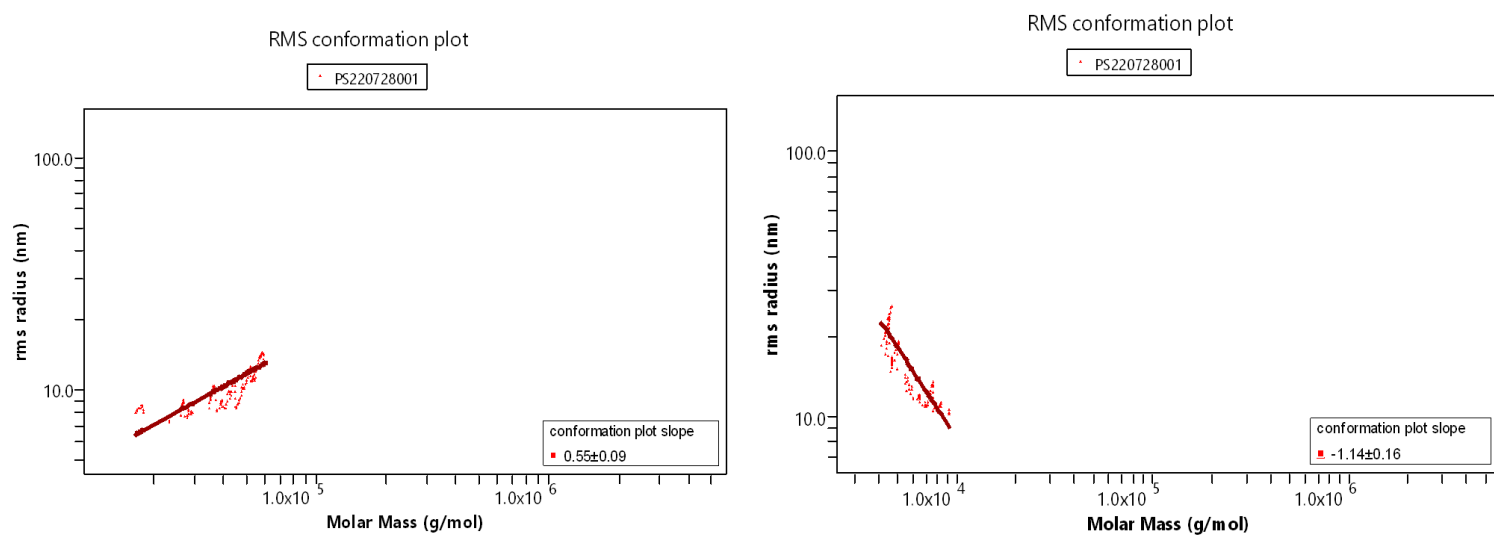


Figure S5. Molecular conformation diagram with different molar masses.

Table S1. The molecular parameters of RLPa-2 determined by SEC-MALLS-RI.

Molecular Characteristics	Parameter	Detection Results	Uncertainty
Molar mass moments	M_n	8.9 kDa	0.02709
	M_p	9.0 kDa	0.01535
	M_w	15.6 kDa	0.01387
	M_z	29.6 kDa	0.02933
	Polydispersity (M_w/M_n)	1.7	0.03043
Rms radius moments	R_n	12.3 nm	0.593
	R_w	10.6 nm	0.572
	R_z	10.3 nm	0.425