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Comparative Study on Anti-Inflammatory Effect of Polysaccharides from Vinegar-Baked *Radix Bupleuri* Using Different Methods

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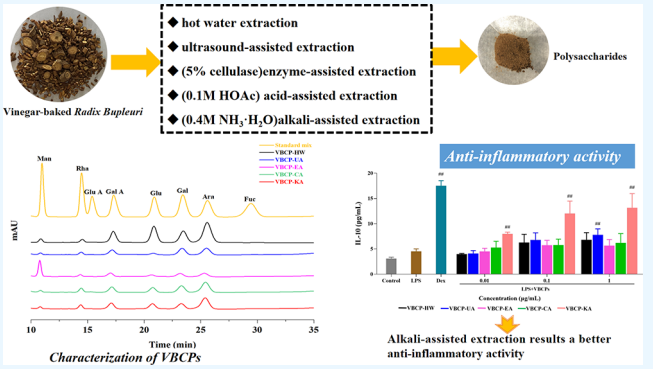
ABSTRACT: The impact of the extraction method on the physiochemical characteristics and anti-inflammatory effect of polysaccharides from vinegar-baked *Radix Bupleuri* (VBCPs) was studied. Five extraction methods were employed to obtain the VBCPs: hot water extraction (HW), ultrasound-assisted extraction (UA), enzyme-assisted extraction (EA), citric acid-assisted extraction (CA), and ammonia-assisted extraction (KA). The results showed that the extraction method affects the yield, characteristics, and anti-inflammatory effect of the polysaccharides significantly. KA produced the highest yield, Ara content, and the strongest effect of enhancing IL-10 secretion. VBCP-EA exhibited the largest molecular weight (Mw), the highest Man content, and the poorest effect on inhibiting NO, VBCP-UA possessed more Gal than other VBCPs, the lowest Mw, and a comparable effect on inhibiting NO and TNF-α with VBCP-KA and VBCP-CA. All VBCP self-assembled into nanoparticles in solutions, and VBCP-KA presented the lowest particle size. The structure−activity analysis showed that Mw and Man content are negatively correlated and Ara content is positively correlated with the NO inhibition and IL-10 secretion effects; Rha and Gal A content are positively correlated and Glu is negatively correlated with the TNF-α inhibiting effect. The above results indicated that KA is an efficient method for obtaining anti-inflammatory VBCP, which provides new insight into the extraction of VBCP.



1. INTRODUCTION

As a newly developing area of natural products research, the varied activities of polysaccharides have attracted increasing attention. Unlike small molecules, the structure and activities of polysaccharides are greatly affected by the extraction method.[1−5] However, a few reports have focused on the extraction process, and the results seem to depend on the herb and the activity which is studied.[6]

Radix Bupleuri, also called Chaihu in China, is derived from the roots of *Bupleurum chinense* DC and *Bupleurum scorzonerifolium* Willd.[7] One of the major ingredients of Radix Bupleuri is polysaccharides, which serve as the foundation for the treatment of various immune-related diseases, including hepatitis, systemic lupus erythematosus, nephritis, rheumatoid arthritis, etc.[8−12] In Chinese medicine, herbs are usually baked to reduce toxicity or strengthen activity. Radix Bupleuri is usually baked with vinegar. A previous study showed that when baked with vinegar, levels of saikosaponin A and D were decreased and saikosaponin b2 levels increased; the hepatoprotective activity is also increased.[13] Polysaccharides from vinegar-baked Radix Bupleuri (VBCP) have a much larger molecular weight (Mw) than polysaccharides from the original herb, increasing the solubility of baicalin and enhancing the anti-virus effect of oxymatrine.[14,15] However, it is unknown whether VBCP has anti-inflammatory effects, which are one of the main causes of liver diseases. Moreover, different methods may induce changes in the characteristics and activity of polysaccharides,[1−5] and which method produces the highest yield and the most powerful anti-inflammatory effect is not clear.

Therefore, in this study, the effects of different extraction techniques on the physicochemical characteristics and biological activities of VBCP were investigated and compared. Hot water extraction (HW), ultrasound-assisted extraction (UA), enzyme-assisted extraction (EA), acid-assisted extraction (CA), and alkali-assisted extraction (KA) were included. The results offer a valuable potential clue for the efficient utilization of VBCPs and benefit their development as functional foods and potential drugs.

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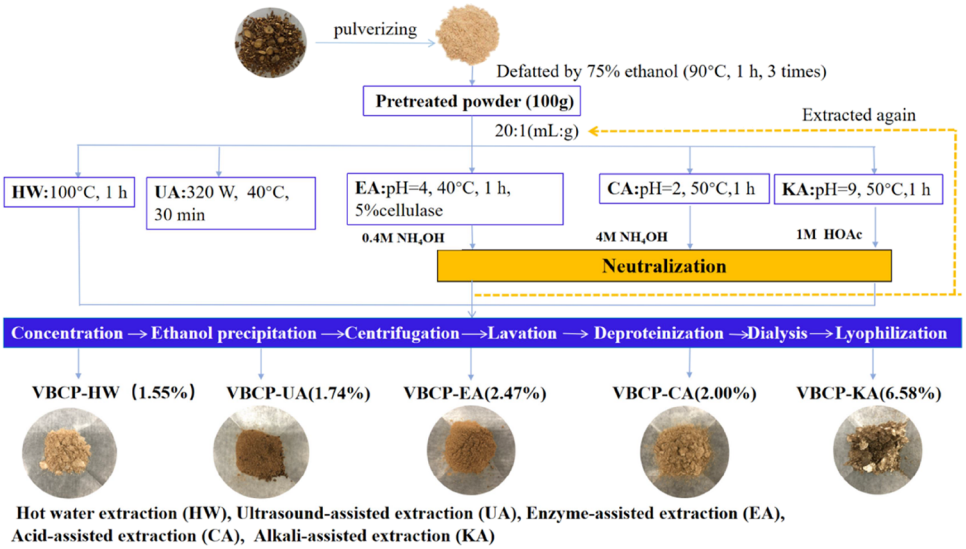

Figure 1. Processes of polysaccharide extraction from VBRB with different extraction methods.

2. EXPERIMENTAL SECTION

2.1. Materials. Vinegar-baked Radix Bupleuri (VBRB) was purchased from Kang Mei Medical Company (Guangzhou, China, No.120100341) and authenticated by Professor Danyan Zhang (Guangzhou University of Chinese Medicine). The vouchers were deposited in the herbarium of The Second Affiliated Hospital of Guangzhou University of Chinese Medicine. Cellulase (lot: J0611A) and neutral red (lot: A0425A) were obtained from Dalian Meilun Biological Technology (Dalian, China), and 1000 Da of dialysis membrane (lot: M18GS141821) was purchased from Shanghai Yuanye Bio-Technology Corporation (Shanghai, China). Dextran standards with different Mws: Dextran 60 K (lot: D620D1-9), D95K (lot: D620D120319), D220K (lot: D620D8-4), D550K (lot: D620D7-3), and Dextran 3690 K (lot: D620D10-1) were purchased from American Polymers Standards Corporation (USA). D-mannose (Man, lot: S08J12G137083), D-galactose (Gal, lot: Z22J9H64187), D-galacturonic acid (GalA, lot: S29J8I40844), D-arabinose (Ara, lot: Z23D11H135480), D-glucose (Glu, lot: Y19F11J108781), D-glucuronic acid (GluA, lot: K14M10S82777), D-fucose (Fuc, F05D9Y75981), D-ribose (SM0516GA14), and rhamnose (Rha, C11366744) were all purchased from Yuanye Biotechnology Co. Ltd. (Shanghai, China). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, lot: 7101KMP) was purchased from MP Biomedicals (France). The murine macrophage cell line RAW264.7 was obtained from the China Center for Type Culture Collection (Wuhan, China). RPMI 1640 medium (lot: 8120509), fetal bovine serum (FBS, lot: 2176398), phosphate buffer solution (lot: 8121467), and penicillin−streptomycin (lot: 23621135) were obtained from Gibco (USA). Lipopolysaccharide (LPS, lot: 0000114326) was purchased from Sigma Chemical (USA). Nitric oxide (NO, lot: 022421210729) was purchased from Beyotime Biotechnology (Nanjing, China). ELISA kits including TNF-α (lot: EGJSPRYYDL) and IL-10 (lot: CYNALNNJEX) were purchased from Elab Science Bioscience (Wuhan, China).

2.2. Extraction of Polysaccharides from VBRB by Different Methods. VBRB (1 kg) was ground into powder using a high-speed disintegrator and passed through a 65-mesh sieve. The powders were refluxed with 4 L of 75% ethanol at 90 °C (1 h) three times to remove the liposoluble ingredients. Thereafter, the residue was dried in an oven (60 °C, 12 h), and defatted VBRB was obtained.

The conditions of HW and UA were according to the optimized extraction conditions of extracting *Bupleurum chinense* polysaccharides in the literature.[16,17] The conditions of EA, CA, and KA were according to the optimized extraction conditions of other plant polysaccharides in the literature.[3,4] The extraction procedure is shown in Figure 1. In short, 100 g of defatted VBRB powder was added to 2000 mL of water or water containing enzyme, water containing 0.1 M acetic acid, or NH_4OH (w/v = 1:20), respectively. After being immersed for 0.5 h, the VBRB was extracted at a scheduled time and temperature, as shown in Figure 1. All extractions were repeated once. After extraction, the supernatants were combined and concentrated to 100 mL with a rotary evaporator at 60 °C under a 0.01 Pa vacuum. After cooling to room temperature, extracts were precipitated with 400 mL of alcohol to a final concentration of 75% (v/v) overnight at 4 °C. After that, the precipitate was washed sequentially with ethanol, acetone, and anhydrous diethyl ether to obtain crude VBCP. The crude VBCP was redissolved in hot distilled water, deproteinized by the Sevag method, and intensively dialyzed for 72 h (MWCO 1000 Da) to further remove small molecules.[14] Ultimately, the retention in the dialysis bag was collected, concentrated, and lyophilized, thus obtaining VBCPs, which were coded as VBCP-HW, VBCP-UA, VBCP-EA, and VBCP-KA. The polysaccharide yield (%) was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{weight of dried VBCP}}{\text{weight of defatted VBRB}}$$

2.3. Characterization of VBCPs. *2.3.1. Determination of Protein Levels.* The protein contents in VBCPs were measured according to Bradford's method using bovine serum albumin as the standard.

2.3.2. Ultraviolet (UV) and Fourier Transform Infrared (FT-IR) Analysis. A UV-2910 spectrophotometer (Hitachi, Ltd., Tokyo) was used to record the UV/vis spectrograms of the VBCP solutions within the limits of 200−600 nm. The IR

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Table 1. Characteristics of VBCPs[a]

sample	VBCP-HW	VBCP-UA	VBCP-EA	VBCP-CA	VBCP-KA
yield (%)	1.55 ± 0.04	1.74 ± 0.03	2.47 ± 0.38	2.00 ± 0.07	6.58 ± 0.27
proteins (%)		2.20 ± 0.14	9.13 ± 1.59		1.95 ± 1.27
diam. 50 (nm)	156.92 ± 0.89	419.79 ± 11.22	186.64 ± 4.76	192.16 ± 0.49	114.09 ± 0.69
diam. 90 (nm)	301.91 ± 2.59	826.15 ± 24.31	410.61 ± 14.21	366.33 ± 0.84	221.43 ± 0.95
zeta potential (mV)	−7.50 ± 1.49	−38.38 ± 0.26	38.39 ± 0.37	−10.57 ± 0.25	−0.40 ± 1.07
monosaccharide composition (mol%)					
mannose	2.82 ± 0.59	4.07 ± 0.11	33.95 ± 0.55	tr	2.90 ± 0.04
rhamnose	2.41 ± 0.42	7.16 ± 0.27	6.52 ± 0.18	7.19 ± 0.14	6.43 ± 0.15
galacturonic acid	12.99 ± 0.19	17.76 ± 0.06	17.03 ± 0.27	14.62 ± 0.54	15.08 ± 0.14
glucose	24.10 ± 0.26	tr	tr	16.07 ± 0.50	14.94 ± 0.17
galactose	14.37 ± 0.07	27.21 ± 0.42	15.40 ± 0.35	19.16 ± 0.51	13.03 ± 0.36
arabinose	43.30 ± 0.13	43.80 ± 0.86	27.10 ± 0.23	42.97 ± 1.24	47.63 ± 0.32

[a]Values are mean ± SD (n = 3); tr: trace (ratio < 1%).

spectra of VBCPs were recorded by FT-IR spectra (PerkinElmer, UK) in the range of 4000 to 400 cm^{-1}.

2.3.3. Molecular Weight Determination. The Mw distribution of VBCPs was determined by high-performance gel permeation chromatography (Agilent 1260, USA) with a refractive index detector according to ref 20. In short, VBCPs (2.0 mg) were dissolved in 1.0 mL of pure water and injected into a TSK-GEL G5000 PWXL gel filtration chromatographic column (7.5 × 300 mm^2, 0.5 μm, YMC Co. Ltd., Kyoto, Japan). The mobile phase was ultrapure water with a flow rate of 0.5 mL/min, and the injection volume was 10 μL. Dextran standard series (60, 95, 220, 550, and 3690 KD) were used for calibration.

2.3.4. Monosaccharide Composition Analysis. Monosaccharide composition analysis of VBCPs was performed by high-performance liquid chromatography (HPLC) analysis after 1-phyenyl-3-methyl-5-pyrazolone (PMP) derivatization as described previously with slight modifications.[18] In short, 2 mg of dried VBCPs was hydrolyzed by 2 mL of trifluoroacetic acid (TFA, 4 M) at 110 °C for 4 h under a nitrogen atmosphere. After removing the excess TFA, the hydrolyzed sample was derivatized by PMP. Then, the PMP derivatives were subjected to an Agilent 1260 Series instrument (Agilent, USA) with an analytic column (Zorbax Eclipse Plus-C18 column, 4.6 × 250 mm^2, 5 μm). The mobile phase was composed of 0.1 M phosphate buffer (pH 6.7) and acetonitrile (87:13, v/v) with a flow rate of 1.0 mL/min. The UV detection wavelength was set at 245 nm.

2.3.5. Dynamic Light Scattering (DLS) Granulometry Test. VBCPs (2.0 mg) were accurately weighed, 4 mL of ultrapure water was added (2 mg/mL), and dispersed by ultrasonic treatment for 5 min. The particle size distribution and zeta potential (AQ, Brookhaven, USA) were measured at 25 °C, and each measurement was repeated six times.

2.4. In Vitro Anti-Inflammatory Activity of Polysaccharides. *2.4.1. Cell Culture.* RAW264.7 cells were inoculated with RPMI 1640 medium containing 10% (v/v) FBS and 1% (v/v) penicillin−streptomycin in an incubator with a humidified atmosphere of 5% CO_2 at 37 °C.

2.4.2. Cell Viability Assay. The effect of different VBCPs of the proliferation of RAW264.7 was analyzed by the MTT method. The absorbance value was determined at 540 nm.

2.4.3. Assay of NO and Cytokine Production. RAW264.7 cells at a density of 3 × 10^5 cells/well were seeded in a 24-well plate. After being cultured for 24 h, LPS at a final concentration of 1 μg/mL and different concentrations of VBCPs (0.01, 0.1, and 1 μg/mL) and Dex (10 μg/mL) were added, respectively, and cultured for 24 h. Subsequently, the supernatants in the cell wells were collected. The contents of NO, TNF-α, and IL-10 in the supernatant were detected by NO kits and ELISA kits (Elabscience Bioscience, Wuhan, Hubei) according to the manufacturer's protocol.[18]

2.5. Data Analysis. All data are the averages from at least three independent experiments presented as the means ± SDs and were analyzed by SPSS 26.0 software (Version 16; SPSS Inc., Chicago, IL, USA). The differences between groups were analyzed using one-way analysis of variance and Duncan's multiple-range test, * and ** represent $p < 0.05$ and $p < 0.01$ differences, respectively. Graphical processing was performed by Origin 8.0 (Origin Lab Corporation, Northampton, MA, USA) SIMCA 14.1 software (Sartorius Stedim Biotech AS, Malmo, Sweden) was used for correlation analysis of physicochemical characteristics (Mws, contents of monosaccharide composition) and anti-inflammatory bioactivities of VBCPs.

3. RESULTS

3.1. Different Methods Affect the Yield and Purity of VBCPs. To determine the purity of the VBCPs, the protein content was determined by the bicinchoninic acid assay method. No protein was detected in VBCP-HW; however, a minor amount of protein was found in the polysaccharides extracted by other methods. Among them, EA had a relatively higher content of protein, up to 9.13%. Since the free proteins in polysaccharides were removed by the Sevag method, proteoglycans may exist.[19]

Yield affects the development cost and is the key factor for its utilization. Therefore, we detected the different methods on the yield first. As shown in Table 1, the polysaccharide yields of HW, UA, EA, CA, and KA were 1.55, 1.74, 2.47, 2.00, and 6.58%, respectively.

Herbs include two types of polysaccharides: intracellular polysaccharides, which are insoluble carbohydrates found in the cell walls, and extracellular polysaccharides, which are soluble polysaccharides found in the cytoplasm. The latter could be extracted by hot water, and the former needs to transform into soluble polysaccharides first.[20] High temperatures, prolonged extraction durations, containing assistant agents (acid, alkaline, enzyme), or ultrasonic-assisted procedures are common methods used to achieve this goal.[4,21] However, all these factors also split the extracted polysaccharides, and when the split effect is too powerful, dissolved

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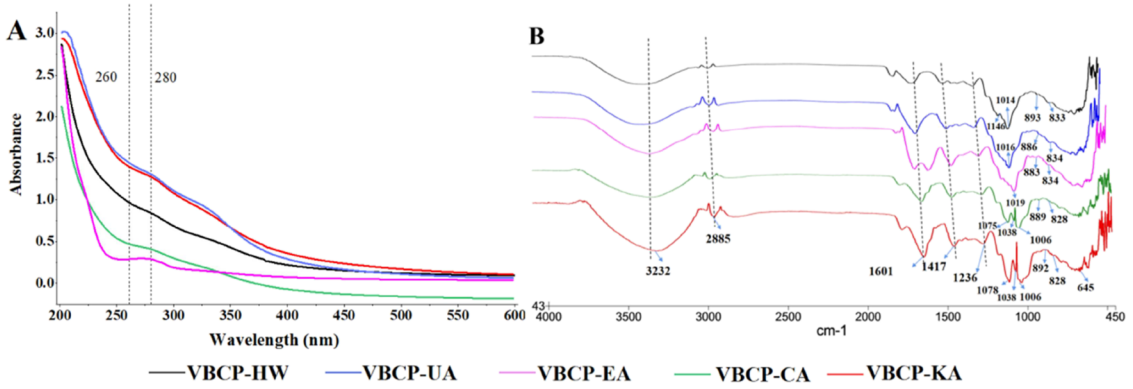

Figure 2. UV/vis spectrograms of VBCP solutions within the limits of 200−600 nm and IR spectra of VBCPs were recorded in the range of 4000 to 400 cm^{-1}. (A) UV spectra; (B) FT-IR spectra.

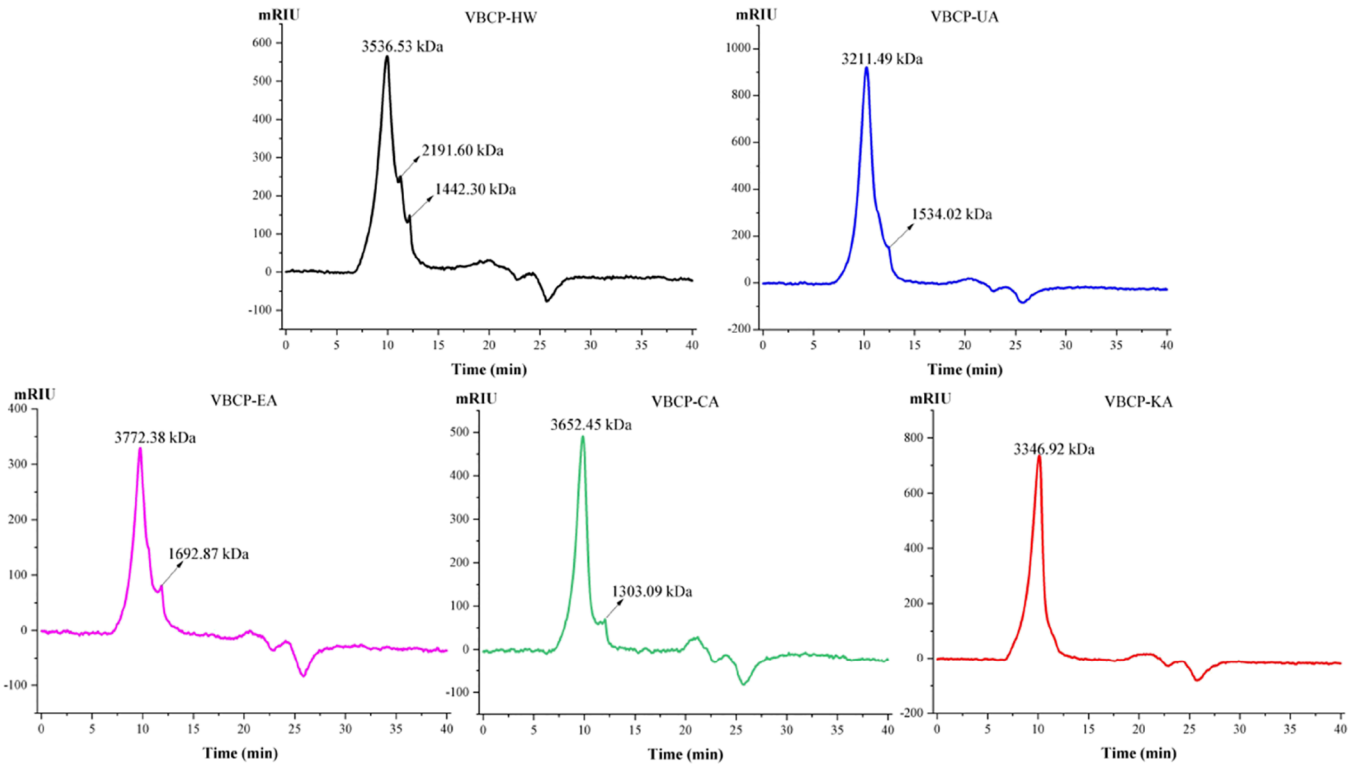

Figure 3. Molecular weight (Mw) distribution of VBCPs. VBCPs (2.0 mg) were dissolved in pure water and injected into a TSK-GEL G5000 PWXL column at a flow rate of 0.5 mL/min.

polysaccharides may degrade to lower Mws and be removed due to dissolution in 75% ethanol. This may be the reason for the lower yields of UA, CA, and EA. In addition, different from the usually used strong alkali (NaOH, KOH) in KA,[4,21,22] a weak alkali (ammonia) was used in this study. The advantage of weak alkalinity is that degradation of polysaccharides due to strong alkalinity can be avoided,[23,24] which may be one of the reasons for the high yield of KA.

3.2. Different Extraction Methods Affect the Characteristics of VBCPs. *3.2.1. UV−vis and FT-IR Spectra of VBCPs.* The UV spectrum of polysaccharides in the range of 200−600 nm is shown in Figure 2A. Except for VBCP-EA, all other VBCPs showed no absorption at 260−280 nm, indicating that only VBCP-EA may contain small amounts of proteoglycans, which was consistent with the results of protein content determination.[17]

The FT-IR spectra of VBCPs are presented in Figure 2B. All VBCPs have the typical absorption peaks of polysaccharides, including 3232, 2885, and 1601 cm^{-1}.[4] The broad and strong absorption peaks between 3600 and 3200 cm^{-1} were attributed to the O−H vibration of intramolecular or intermolecular hydrogen bonds of the polysaccharides.[22] The absorption peaks at approximately 2885 and 1236 cm^{-1} were from the C−H stretching vibration.[18] The relatively strong absorption at approximately 1601 cm^{-1} and the peak at 1417 cm^{-1} illustrated the presence of symmetric stretching of carboxylate anion groups, which indicated the existence of uronic acid.[20] Peaks in the range of 1200−1000 cm^{-1} were assigned to the C−O−C and C−O−H linkages.[17] VBCP-HW, VBCP-UA, and VBCP-EA had two absorption peaks in this area, indicating that they mainly contained furanose, and VBCP-CA and VBCP-KA had three absorption peaks in this area, indicating that they mainly contained pyranose. The weak absorption peaks at 883−893

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and 828−833 cm^{-1} suggested that all VBCPs contained both α-glycosidic and β-glycosidic linkages.

3.2.2. Molecular Weight of VBCPs. The weight-average Mw of VBCPs is shown in Figure 3. According to other reports, different methods affect the Mw of VBCP. VBCP-HW consisted of three fractions with Mws of 3536.53, 2191.60, and 144.30 kDa. VBCP-UA, VBCP-CA, and VBCP-EA all comprised two fractions with Mws of 3211.49 kDa and 1534.02 kDa, 3652.45 kDa and 1303.09 kDa, and 3772.38 kDa and 1692.87 kDa, respectively. VBCP-KA showed only one fraction with a peak at 3346.92 kDa. In addition, the Mws of the main peaks in the five VBCPs decreased as follows: VBCP-EA > VBCP-CA > VBCP-HW > VBCP-KA > VBCP-UA. The lowest Mw of VBCP-UA may be due to the breakage of the polysaccharide chain induced by ultrasound. This result was consistent with the reported results of polysaccharides extracted by UA.[25] The largest Mw is VBCP-EA, which may be due to cellulose disrupting the dense structure of the cell wall and improving the leaching of water-soluble macromolecular polysaccharides.[26]

3.2.3. Monosaccharide Composition Analysis. By comparison with the retention time of the monosaccharide standards, the monosaccharide composition of VBCPs was identified by HPLC, and the results are shown in Figure 4. The

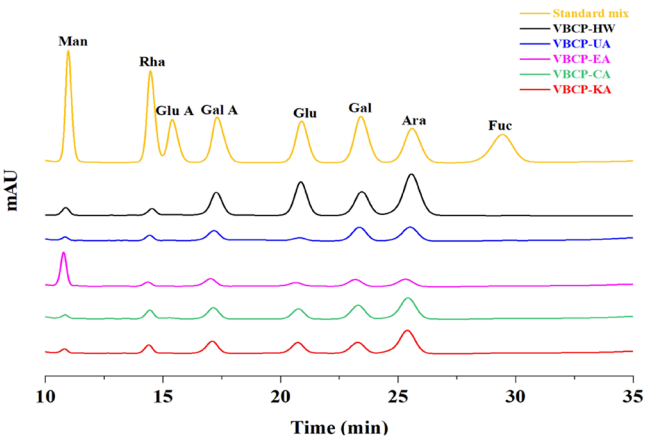

Figure 4. HPLC chromatogram for monosaccharide composition.

monosaccharide constituents of VBCPs are summarized in Table 1. All VBCPs have similar monosaccharide constituents but different ratios. In addition to VBCP-EA containing a higher content of Man, other VBCPs contained abundant Ara and less Man. VBCP-HW contained more Glu than the other VBCPs, VBCP-UA contained more Gal and Gal A, and VBCP-KA contained more Ara.

3.2.4. Particle Size and Zeta Potential in Water Solution. Particle size and size distribution are two important factors that influence the drug release rate, bioavailability, and hence the pharmacodynamics of nanoparticles.[27,28] The particle size and size distribution of VBCPs differed greatly from each other (Figure 5A).VBCP-UA showed the highest particle size and the broadest size distribution, but VBCP-KA and VBCP-CA presented a much lower particle size and narrowest size distribution.

The zeta potential mainly reflects the stability of nanoparticles, the higher the zeta potential (positive or negative) is, the more stable the delivery system.[29] All VBCP solutions were negative, which prevented the particles from massively aggregating. As shown in Figure 5B, the zeta potentials of the VBCP-UA and VBCP-EA extracts had larger absolute zeta potential values ($P < 0.01$), indicating that they had higher intermolecular repulsion and more stable solutions. VBCP-KA had the lowest absolute zeta potential value ($P < 0.01$), which may be attributed to the small content of neutral sugar resulting in poor electrical conductivity and reducing the aggregation effect of polysaccharides in aqueous solutions.[28]

3.3. Anti-Inflammatory Effect of VBCP on RAW264.7 Cells. *3.3.1. Effect of VBCPs on the Viability of RAW264.7 Cells.* As shown in Figure 6A, in addition to VBCP-UA, all other VBCPs showed no cytotoxic effects at all determined concentrations. However, VBCP-UA exerted significant inhibitory effects on RAW264.7 cell viability in the concentration range of 10−100 μg/mL. Therefore, to diminish the cell vitality in the results, concentrations of 0.01−1 μg/mL for 24 h of VBCPs were selected in the following experiments.

3.3.2. VBCPs Exert Anti-Inflammatory Effects by Regulating Inflammatory Cytokines. As shown in Figure 6B,C, the production of NO and TNF-α in the control group was far less than that in the LPS group, indicating that an inflammatory model was successfully established ($p < 0.01$). All detected VBCPs and the positive control (Dex) induced macrophage activation, which in turn significantly inhibited NO release and TNF-α secretion compared with the LPS group ($p < 0.01$), indicating an anti-inflammatory effect. VBCPs inhibited NO and TNF-α significantly but to different degrees. For NO, VBCPs from different methods showed comparable effects besides VBCP-EA, which was weaker than that of other VBCPs, this may be due to its larger Mw.[29] VBCP-UA, VBCP-KA, and VBCP-CA showed almost equal strength on TNF-α, far stronger than that of VBCP-EA and VBCP-HW. VBCP-

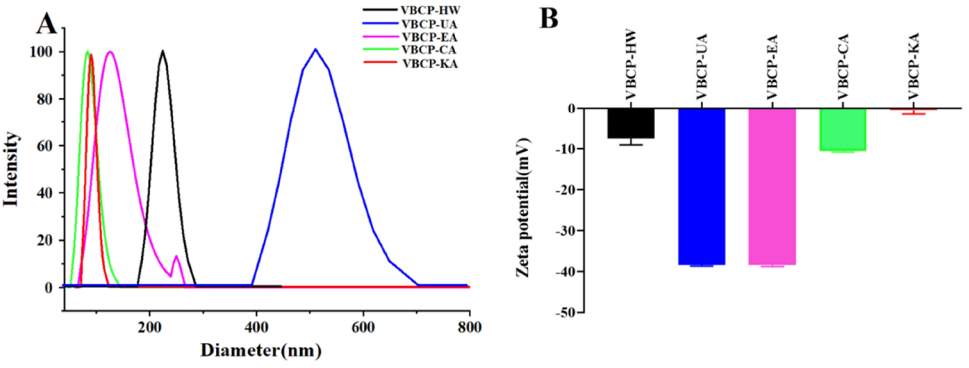

Figure 5. Particle size distribution and zeta potential of VBCPs: (A) Size distribution. (B) Zeta potential.

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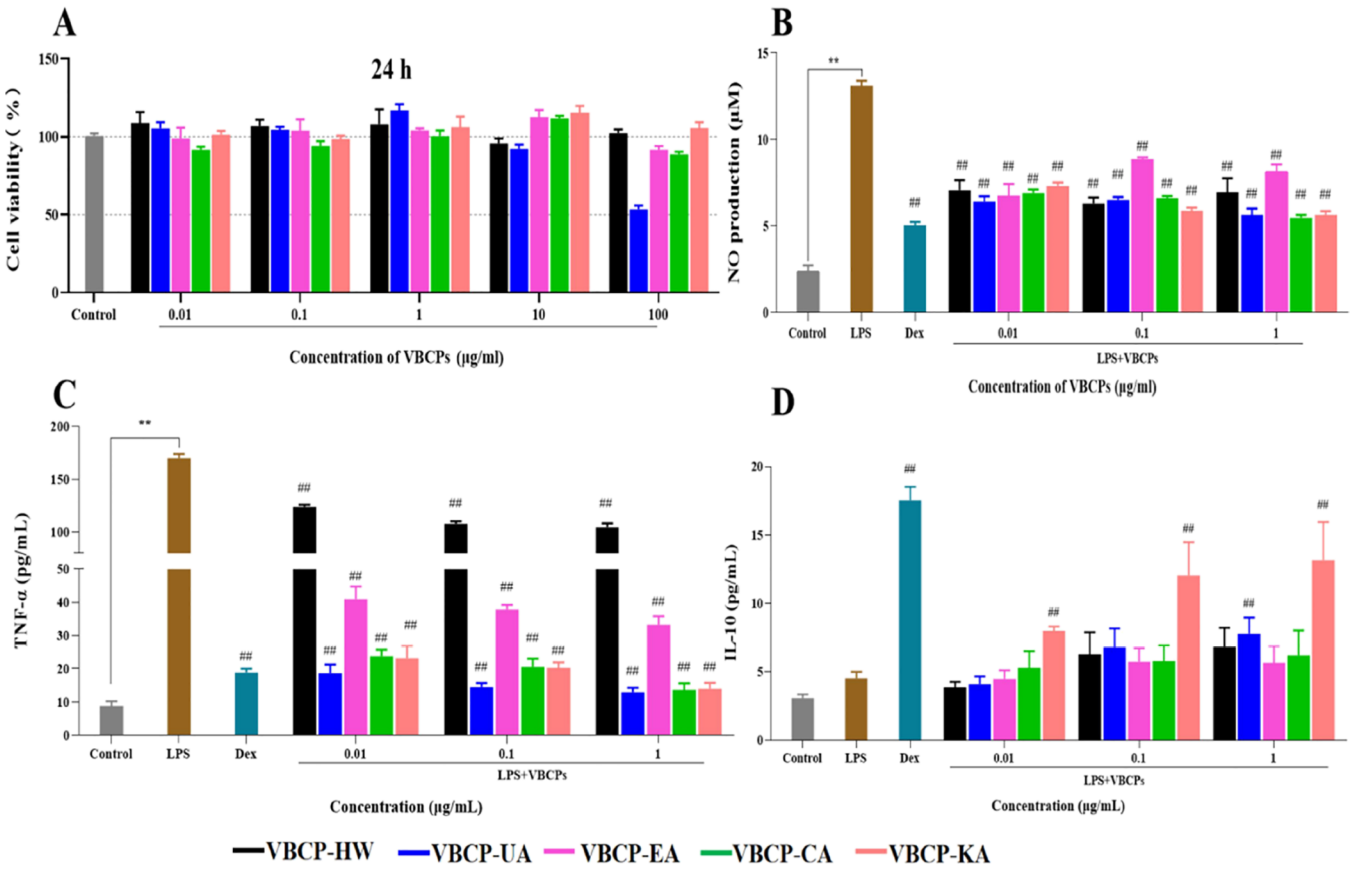

Figure 6. Effect of VBCPs on the cell vitality and inflammatory cytokines of RAW264.7 cells (X ± s, $n = 3$). $^{**}P < 0.01$, compared with the control; $^{\#\#}P < 0.01$, compared with the LPS group. (A) Cell vitality, (B) NO secretion, (C) TNF-α, and (D) IL-10.

HW showed the poorest effect on inhibiting TNF-α. The effect of high doses of VBCP-UA, VBCP-CA, and VBCP-KA on TNF-α was significantly superior to that of the positive control ($p < 0.01$).

As shown in Figure 6D, LPS did not affect the secretion of IL-10. However, VBCPs increased the IL-10 level, which is consistent with the positive control. Among them, the effect of VBCP-KA is the strongest, followed by VBCP-UA. Both had an initial effective dosage of 0.01 μg/mL, and both were dose dependent. The other three polysaccharides showed a similar effect on IL-10 secretion.

3.3.3. Preliminary Analysis of the Structure–Activity Relationship of VBCPs. Analysis of the relationship between the structure and activity revealed that the pharmacological index is influenced by the VBCP characteristics. For TNF-α, it was found that as the content of Rha increased, the TNF-α inhibition effect increased; for NO, in addition to VBRP-CA, as the Mw decreased, the NO-inhibiting effect increased; and as the Man content increased, the NO-inhibiting effect decreased; for IL-10, as the Ara content increased, the enhancing IL-10 secretion effect increased.

To study the structure–activity relationship quantity, the Pearson correlation coefficient was used. As shown in Table 2, coincident with our analysis, different characteristics of polysaccharides have different effects on the inflammatory effect. Mw was negatively related to NO inhibition and IL-10 enhancement, with coefficients of 0.66 and 0.67, respectively; Man content was negatively related to the NO inhibition rate, with a coefficient of 0.86; Rha content was positively related to TNF-α, with a coefficient of 0.98; and Ara content was positively related to NO inhibition and IL-10 secretion, with coefficients of 0.87 and 0.66, respectively. GalA content was positively and Glu content was negatively correlated with the TNF-α inhibitory effect, with coefficients of 0.65 and 0.57, respectively.

Table 2. Matrix for Correlations Analysis[a]

	NO inhibition rate	TNF-a inhibition rate	IL-10 production rate
Mw (kDa)	−0.66	−0.25	−0.67
Man (mol%)	−0.86	−0.01	−0.42
Rha (mol%)	0.37	0.98	0.11
Gal A (mol%)	−0.07	0.65	−0.05
Glu (mol%)	0.22	−0.57	0.17
Gal (mol%)	0.41	0.42	−0.22
Ara (mol%)	0.87	0.07	0.66

[a]Mw = weight-average molecular weight, Man = mannose, Rha = rhamnose, Gal A = galactose acid, Glu = glucose, Gal = galactose, Ara = arabinose.

4. DISCUSSION

Different methods have different effects on the structure and activity of polysaccharides.[30−32] With the idea of future development in mind, we chose environmentally friendly methods, including UA, cellulose enzyme-assisted, acetic acid-assisted, and ammonia-assisted methods. As expected, the extraction method greatly affected the yield, characteristics, and activity of the VBRPs. Many studies have reported polysaccharides from different Radix Bupleuri;[10,14,18,33−36]

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however, in addition to Song et al.[12] using 0.5 M NaOH, most people use hot water as the extraction solvent. To our surprise, the new methods all increased the yield compared with the hot water methods. Under the conditions of this study, the yield of polysaccharides by NH_4OH was the highest, and the extraction time was much shorter than normal, suggesting that NH_4OH-assisted extraction may be a potential method for extracting polysaccharides.

In addition to the yield, the extraction method significantly affected the Mw and distribution, solution properties, and monosaccharide constitution. Although the monosaccharide types of different VBCPs are similar, the ratios are different, which provides the basis for different activities. Surprisingly, although VBCP-HW showed comparable activity with the data in the literature,[7,37−40] other methods such as UA, CA, and KA exhibited an excellent anti-inflammatory effect at the dose of 1 μg/mL, a far lower dose than that in the literature; additionally, the inhibiting ratio on TNF-α increased from 39% of VBCP-HW to 91.6% of VBCP-KA (1 μg/mL), which was slightly higher than that of the positive control. Meanwhile, as a typical anti-inflammatory cytokine, IL-10 plays a key role in innate immunity and is usually an immune-enhancing factor.[41] According to a study in the literature, VBCP-HW showed a marginal effect on IL-10; however, VBCP-KA showed a remarkable effect on enhancing IL-10 secretion, only slightly weaker than that of the positive control. The above results indicated that the new extraction method showed potential in enhancing the activity of polysaccharides and merits further study.

The structure−activity relationship is a hot topic in the study of polysaccharides. Using different extraction methods, we obtained VBCPs with different characteristics and showed different activities on the different indices, providing a possibility for structure−activity relationship studies. The Mw was negative, and the Ara content was positively correlated with the NO-inhibiting and IL-10-enhancing effects; however, they marginally affected TNF-α. The Rha and GalA contents were positively correlated with the TNF-α-inhibiting effect but had marginal effects on NO and IL-10. When connected with the extraction process, the above results shed some light on activity-based extraction process optimization.

5. CONCLUSIONS

In this study, we compared the effect of different methods on the characteristics and anti-inflammatory effects of VBCPs for the first time. The results showed that different extraction methods significantly affected the properties of VBCPs and their anti-inflammatory effects. VBCP-KA, which was extracted by a weak alkali (ammonia), had excellent anti-inflammatory activity with a high yield and nontoxicity. We obtained polysaccharides with the desired properties by optimizing the extraction process, and ammonia-assisted extraction has potential for VBCP development.

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N.W.: conceptualization, methodology, writing—original draft; Y.W.: methodology; X.W.: software, validation; L.L.: supervision; Y.Z.: project administration, data curation; and R.Z.: conceptualization, project administration, resources.

Notes

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