Original Article

Identification and activity evaluation of *Astragalus Radix* from different germplasm resources based on specific oligosaccharide fragments

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A R T I C L E   I N F O

A B S T R A C T

Objective: Based on trifluoroacetic acid (TFA) hydrolysis, polyacrylamide gel electrophoresis (PAGE) and high performance thin layer chromatography (HPTLC) analysis, the carbohydrate responsible for immunomodulatory activity are used as quality indicators for *Astragalus Radix* (AR).
Methods: In this study, 24 batches of AR from different germplasm resources were selected as the research object, and AR polysaccharides were extracted. PAGE and HPTLC methods were used to analyze the partial acid hydrolyzate of AR polysaccharides and obtain a series of saccharide fingerprints. The data were analyzed by principal component analysis to obtain the difference between AR from different germplasm resources.
Results: The results showed that trisaccharide and tetrasaccharide could be used as differential fragments to distinguish AR of different cultivation methods; Disaccharides and trisaccharides can be used as differential fragments to distinguish different species of AR. The immunological activity analysis of the specific oligosaccharide fragment of AR showed that the specific oligosaccharide fragment of AR could promote the secretion of TNF-α, IL-1β, IL-6, and NO in THP-1 cells in a concentration-dependent manner.
Conclusion: Both PAGE and HPTLC methods can be used to evaluate AR from different germplasm resources. This study laid the foundation for the quality evaluation of AR medicinal herbs.

1. Introduction

Astragalus Radix (AR) is a common Chinese medicinal herb that has been used for more than 2000 years, with the first record appearing in *Shennong's Classic of Materia Medica* (Chu et al., 2010). Clinical studies have shown that AR has the effects of improving immunity, anti-inflammation, anti-cancer and anti-virus (He et al., 2012). At present, there are mainly two kinds of AR on the market, namely traditional AR and cultured AR. Traditional natural AR is mainly distributed in Shaanxi, Inner Mongolia, Gansu, and Shanxi Provinces/Autonomous region (Hu et al., 2012). Cultured ARs are derived from two botanical species, i. g. *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao, called Menggu Huangqi (MG AR) in Chinese and *Astragalus membranaceus*

(Fisch.) Bge., called Mojia Huangqi (MJ AR) in Chinese. MG AR is mainly distributed in Gansu and Ningxia, and MJ AR is mainly distributed in Hebei and Shandong Provinces (Yang et al., 2014; Zhao, 2004; Chen et al., 2009).

In 1980s, the longer growth period of the traditional wild *Astragali Radix* (WAR) caused the situation of demand exceeding supply. With the appearance of the transplanted *Astragali Radix* (TAR) with the short period (2–3 years), market demand was alleviated, but the WAR resources were declining seriously (Qin et al., 2013). As the qualified standard of AR, the Chinese Pharmacopoeia (2015 edition) specifies that the contents of astragaloside IV and isoflavone glucoside should not be less than 0.04% and 0.02% respectively. However, previous studies showed that although the content of calycosin-7-glucoside in the WAR was significantly higher than that of TAR, the content of astragaloside IV is not low in the TAR, even more than the equivalent of imitation wild top grade and first grade (Du et al., 2013; Hu et al., 2003; Jiang et al., 2006). However, the international market does not accept

TAR, and some veteran TCM physicians still use WAR. These conflicts show that the current quality evaluation method cannot be used for accurate assessment of the quality of AR. Therefore, a more rational evaluation method is urgently needed for the development of genuine medicinal herbs.

AR polysaccharides are the main substances to exert an immunomodulatory effect (Li et al., 2017). For the specificity (special composition), safety (for toxic substances), effectiveness (related to activity), and controllability of quality markers (Q-markers), polysaccharide should be used as a Q-marker for assessing AR (Liu et al., 2016).

In recent years, the successful establishment of peptide mapping technology using different specificities polypeptide hydrolase has revolutionized the structural analytical methods of protein (Bondarenko, Chelius, & Shaler, 2002; Choi, Lee, & Park, 2008). Similar to peptide mapping, saccharide mapping is developed through analysis kinds of the oligosaccharides from hydrolysates of polysaccharides extracts from different medicinal herbs (Guan & Li, 2010; Guan, Yang, & Li, 2010; Guan, Zhao, Feng, Hu, & Li, 2011; Wu et al., 2014). Coupled with multivariate analysis, saccharide mapping are utilized to reflect the specific characteristics of herbs. The hydrolysis method of polysaccharides is the key factor of this technique which can be used for identifying the varieties and linkages of saccharide and the specificity of the enzymatic hydrolysis method is prominent (Guan & Li, 2010; Guan, Zhao, Feng, Hu, & Li, 2011). In additional, the differential oligosaccharides obtained from multivariate analysis can be selected as Q-marker for the herb through research on structural specificity and activity screen.

In the present study, 24 batches of AR from different germplasm resources were used as the object of study. AR soluble polysaccharides and glycoconjugates were extracted and hydrolyzed by trifluoroacetic acid, and then partial acid hydrolyzate of AR polysaccharides were analyzed by PACE and HPTLC methods to obtain a series of glycans fingerprints. The method has the advantages of simple operation, high specificity and low cost. Differential oligosaccharides obtained by multivariate analysis of a series of carbohydrate mappings can be used not only to identify AR of different germplasm resources and growth patterns, but also to lay the foundation for polysaccharide as a Q-marker to assess AR.

2. Materials and methods

2.1. Plant materials

The experimental plant materials including *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (MG AR) (cultured AR and natural AR) and *Astragalus membranaceus* (Fisch.) Bge. (MJ AR) (cultured AR and natural AR), a total of 24 batches, were bought from Hebei Anguo Medicine Market. All plant materials were identified by Prof. Xuemei Qin, and the voucher specimens were deposited in the herbarium of the Modern Research Center for Traditional Chinese Medicine of Shanxi University. Detailed information on the samples is presented in Table 1.

2.2. Preparation and purification of AR polysaccharide

According to this method (Shao et al., 2006), 5 g of AR powder was placed in a 1000 mL round bottom flask, and 500 mL of water was added. The sample was then refluxed for 1 h, cooled, and filtered. A total of 400 mL of water was added to the residue, and the mixture was extracted under reflux for 1 h, cooled, and filtered. The supernatant was mixed and 95% ethanol was added so that the alcohol content was adjusted to 80%. After being left at 4 °C for 12 h, the samples were centrifuged. The precipitate was freeze-

dried to obtain a crude powder of AR polysaccharide. According to the method shown in reference (CHU et al., 2006), the crude polysaccharide was dissolved in water, and then mixed with Sevage reagent in a separatory funnel, repeatedly shaken, and allowed to stand. After the layering, the upper aqueous phase was retained, and the operation was repeated as described above until the protein layer was removed. The aqueous phase was steamed and lyophilized to obtain purified AR polysaccharide.

2.3. Partial acid hydrolysis and derivatization of AR polysaccharides

AR polysaccharide powder (10 mg) and 0.5 mol/L TFA solution were added to a plug glass tube. The sample was hydrolyzed at 90 °C for 1 h, and then dried with N_2. A total of 1 mL of methanol was added, and then dried with N_2, and the operation was repeated three times. The hydrolyzate was dissolved with a certain amount of $NaCNBH_3$ solution. A total of 200 μL ANTS derivatization reagent was added. After heated at 37 ℃ for 15 h, the product was dried with N_2 and dissolved with 500 μL of 6 mol/L urea solution.

2.4. Analysis of polyacrylamide gel electrophoresis

The concentrated gum and the separating gum were prepared by the method (Lee et al., 2003), wherein the concentrated rubber was 8% polyacrylamide (acrylamide: N, N-methylenebisacryla mide = 19: 1), the gel was 30% acrylamide. The derivatization products were separated by polyacrylamide gel electrophoresis with bromophenol blue as the leading indicator. The optimal conditions for the separation of the derivatives by electrophoresis were pH = 8.2 and a 0.1 mol/L Tris-H_3BO_3 solution as the electrophoresis buffer. The electrophoresis procedure was first run at 100 V for 60 min and then at 200 V for 120 min.

2.5. Analysis of high performance thin layer chromatography

The optimum solvent ratio for HPTLC is *n*-butanol: isopropanol: acetic acid: water = 7:5:1:5 (volume percentage).

2.6. Preparation of monosaccharides and oligosaccharide standards

Two copies of glucose (DP-1), maltose (DP-2), raffinose (DP-3), maltotetraose (DP-4), maltopentaose (DP-5) and maltohexaose (DP-6) standard were weighed and mixed, one of which is directly derivatized. And the derivatized product was dried with N_2 and dissolved in 500 μL of 6 mol/L urea solution for PAGE analysis, the other was dissolved in an ultrasonic solution with 80% ethanol at 500 μL for HPTLC analysis.

2.7. Data processing

The electropherograms and HPTLC chromatograms were introduced into the Quantity One software, and the fingerprints were obtained by subtracting background, manual calibration, baseline calibration, and so on. Fingerprints were transformed into excel data, then SPSS 16.0 and SMICA-P 13.0 were introduced for correlation analysis and cluster analysis, and then metabolomics analysis techniques were used to find the differential oligosaccharides.

2.8. Purification of differential glycoside fragments from AR

AR polysaccharide (5 g) was added to 250 mL of 0.5 mol/L TFA and hydrolyzed at 90 ℃ for 1 h. The samples were air-dried to remove excess TFA. The TFA acid hydrolyzate was reconstituted with an appropriate amount of distilled water, then placed in a dialysis bag of 100–500 Da, dialyzed for 48 h, and replaced with distilled water for 3–5 times. The liquid in the 100–500 Da dialysis

Table 1
AR medicinal information.

No.	Codes	Species	Cultivation patterns	Sources	Growth year
1	C1	*Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao	Cultivated	Longxi, Gansu	2
2	C2			Longxi, Gansu	2
3	C3			Dangchang, Gansu	2
4	C4			Daixian, Shanxi	2
5	C5			Yingxian, Shanxi	2
6	C6			Yingxian, Shanxi	2
7	NC1		Wild	Yulin, Shaanxi	5
8	NC2			Yulin, Shaanxi	5
9	NC3			Yulin, Shaanxi	5
10	NC4			Hunyuan, Shanxi	≥ 5
11	NC5			Hunyuan, Shanxi	5
12	NC6			Hunyuan, Shanxi	5
13	CC1	*Astragalus membranaceus* (Fisch.) Bge.	Cultivated	Hulan, Heilongjiang	1
14	CC2			Hulan, Heilongjiang	1
15	CC3			Zhangjiakou, Hebei	2
16	CC4			Wendeng, Shandong	1
17	CC5			Wendeng, Shandong	2
18	CC6			Wendeng, Shandong	1
19	AC1		Wild	Jiagedaqi, Heilongjiang	5
20	AC2			Shaanxi	5
21	AC3			Hulan, Heilongjiang	5
22	AC4			Inner Mongolia	5
23	AC5			Heilongjiang	5
24	AC6			Heilongjiang	5

bag was then transferred to a 1000 Da dialysis bag. Finally, the sugar solution of each molecular weight fragment was concentrated by rotary evaporation, frozen, and vacuum drying to obtain the differential glycoside fragments, which was named ASP-1.

2.9. Determination of immunological activity

2.9.1. Culture and in vitro stimulation of human THP-1 cells

The human monocyte cell line THP-1 was cultured in RPMI 1640 medium containing 10% FBS and 0.5% β-mercaptoethanol under 5% CO_2, 37 °C, and saturated humidity. The density of THP-1 cells was adjusted to (1×10^5) cells/mL with a medium containing 10% FBS, and transferred to a 96-well culture plate. The experiment was divided into seven groups: blank control group, lipopolysaccharide positive control group (1 μg/mL), and five different concentrations of ASP-1 groups. In the blank control group, 10 μL of complete medium containing 10% FBS was added to each well. In the lipopolysaccharide positive control group, 10 μL of LPS-containing medium was added to each well to give a final concentration of LPS of 1 μg/mL. In the ASP-1 administration group, 10 μL of complete medium containing ASP-1 was added to each well, and the final concentrations of ASP-1 were 200 μg/mL, 100 μg/mL, 50 μg/mL, 20 μg/mL and 10 μg/mL, respectively.

2.9.2. Determination of THP-1 cell viability

After 24 h of cell culture in a standard environment, 10 μL of CCK8 solution was added to each well. The absorbance (A) of the solution was measured at 450 nm after 4 h of incubation in a standard environment.

2.9.3. Detection of cytokines TNF-α, IL-1β and IL-6

After the cells were incubated in a standard environment for 24 h, centrifuged and the cell supernatant was removed. The contents of TNF-α, IL-1β and IL-6 in the supernatant of each group were analyzed by enzyme-linked immunosorbent assay using commercial kit (Xitang, Shanghai).

2.9.4. Griess method for measuring NO production

The amount of NO_2/NO_3 in the cell supernatant was measured according to the nitric oxide detection kit instructions, and the amount of NO produced was indirectly reflected.

2.9.5. Statistical analysis

Using Graphpad prism statistical software, based on the homogeneity of variance, *t*-test was used to analyze the difference of cytokine secretion between groups under the same stimulation conditions.

3. Results and discussion

3.1. Polyacrylamide gel electrophoresis and HPTLC patterns of partial acid hydrolyzate of AR polysaccharides from different germplasm resources

A sample of partial hydrolyzate of AR polysaccharide was prepared by the above reaction conditions. The PAGE fingerprints and chromatograms and HPTLC patterns of AR polysaccharides from different germplasm resources were shown in Fig. 1. The spectrums of PAGE and HPTLC had clear bands and good separation. The PACE spectrum provided more bands than HPTLC, the PAGE data were normalized and analyzed for correlation. The results were shown in Table S1.

Through the analysis of the data in Table S1, it was found that the samples with greater degree of similarity in MG AR polysaccharides were more than MJ AR; The cultured AR polysaccharides had more similar samples than natural AR.

3.2. Data analysis of polyacrylamide gel electrophoresis profiles of partial acid hydrolyzate of AR polysaccharides from different germplasm resources

PAGE spectrum data of the partial acid hydrolyzate of AR polysaccharides from different germplasm resources were transformed into fingerprints and standardized by SPSS software and then imported into SMICA-P 13.0 for data analysis. First of all, principal component analysis objectively showed the overall difference between AR polysaccharide samples from different germplasm
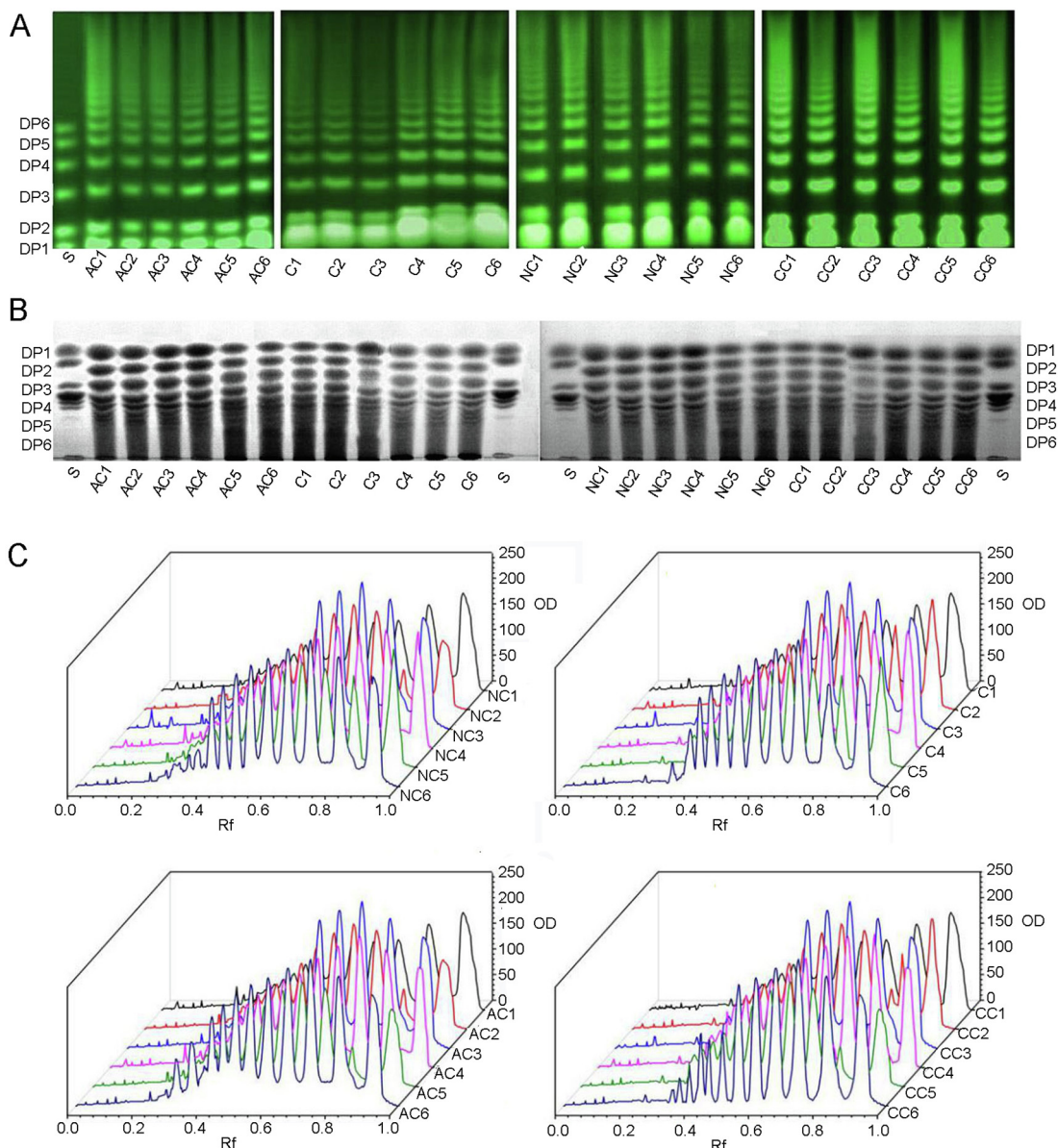
Fig. 1. PAGE fingerprints (A), HPTLC chromatograms (B), and PAGE chromatograms (C) of AR polysaccharides from different germplasm resources S: standard; DP1, DP2, DP3, DP4, DP5, DP6: glucose, maltose, raffinose, maltotetraose, maltopentaose and maltohexaose. The partial acid hydrolyzate and code of the polysaccharide of AR samples are the same as in Table S1.

resources (Fig. 2A). In order to preserve the overall characteristics and variation of the data and eliminate the random errors unrelated to the research purpose, we further performed partial least squares discriminant analysis on the PAGE spectrum data, as shown in Fig. 2B.

It can be seen that cultured AR and natural AR, MG AR, and MJ AR can be clearly separated based on the acid hydrolysis products of AR polysaccharides from different germplasm resources (Fig. 2).

3.3. Analysis of polyacrylamide gel electrophoresis profiles of partial acid hydrolyzate of AR polysaccharides in different cultivation patterns

In order to find the differential oligosaccharide between cultured AR and natural AR, MG AR and MJ AR polysaccharides, we analyzed the PAGE profiles of the hydrolysates and expected to obtain differential oligosaccharides. It can be seen from Fig. 3 that there was a significant difference between cultured AR and natural AR, and the results of model validation were valid and the model

was reliable. We found the oligosaccharides that contribute more to this difference through the points in the load map that were farther from the origin, as shown in Fig. 4. The results showed that DP3 and DP4 can be used as the main differential fragments to distinguish AR from different cultivation patterns, and the content of DP3 and DP4 in natural AR was higher than that in cultural AR. In addition, we also performed *t*-test analysis on PAGE spectrum data.

3.4. Analysis of HP-TLC patterns of partial acid hydrolyzate of AR polysaccharides in different cultivation patterns

As the data analysis of the PACE spectrum, we first used partial least squares discriminant analysis to visually illustrate the relationship between natural AR and cultured AR. It can be seen from Fig. 5A that the partial acid hydrolysis products of the natural AR and cultured AR polysaccharides were clearly separated, indicating that there was a large difference between them. Model validation was assessed using the total explanatory variables (R^2 values)
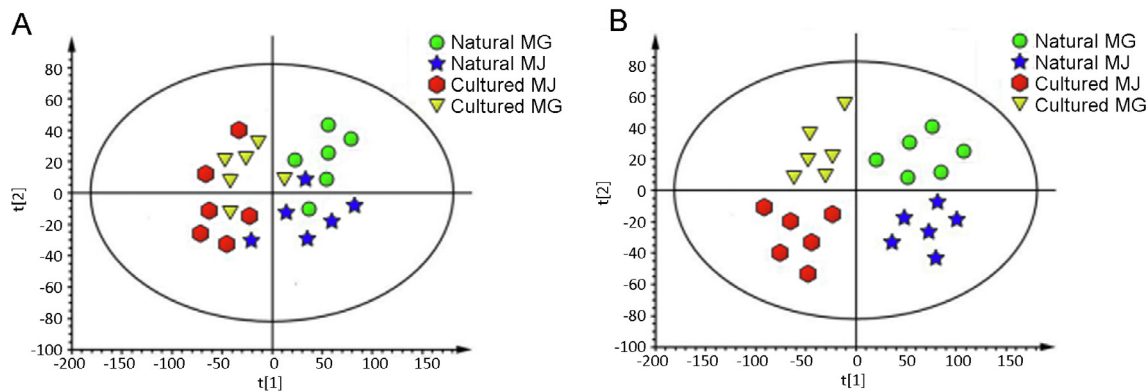
Fig. 2. PCA scatter plot (A) and PLS-DA scatter plot (B) of PAGE spectrum of partial acid hydrolyzates of AR polysaccharides from different germplasm resources.
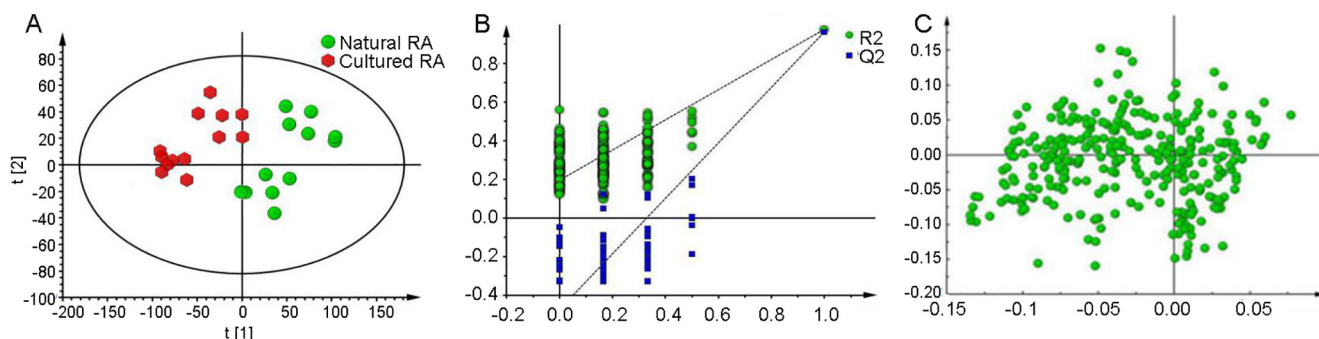

Fig. 3. PLS-DA scatter plot (A), model cross validation diagram (B), and loading diagram (C) of the PAGE spectrum of partial acid hydrolyzate of AR polysaccharides from different cultivation patterns.
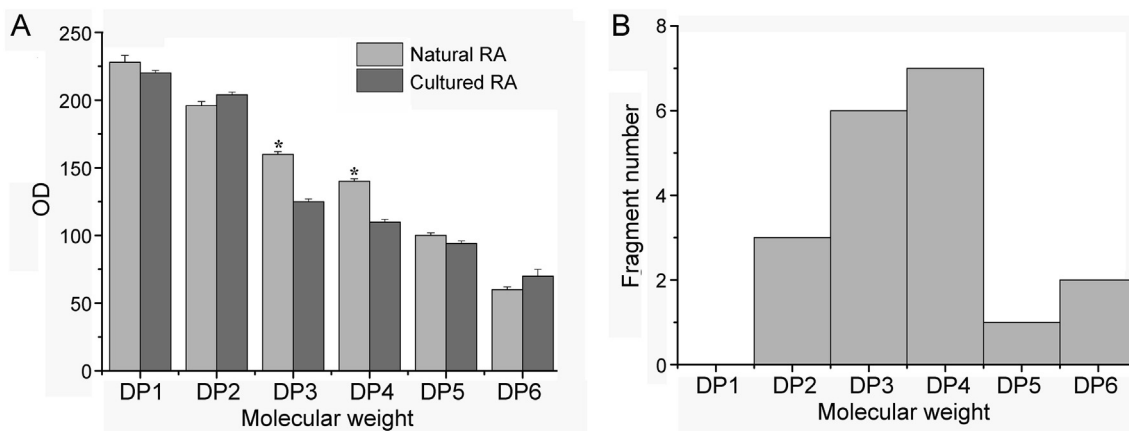

Fig. 4. Sugar segment optical density values (A) and differential sugar segments contribution number (B) of PAGE spectrum of partial acid hydrolyzates of AR polysaccharides in different cultivation patterns.

and model predictability (Q^2 values). Fig. 5B showed that all Q^2 and R^2 values were lower than the actual model, indicating that the model had good predictability and fitness. According to the VIP (VIP > 1) value (Fig. 5C) and the stoichiometric analysis, the peak with a large load value was considered to be the most different component of the difference contribution value.

Based on the data analysis of PAGE and HPTLC spectra of partial hydrolyzates of AR polysaccharides in different cultivation patterns, the oligosaccharides with molecular weight ranging from 540–720 Da (DP3, DP4) were identified as the main differential
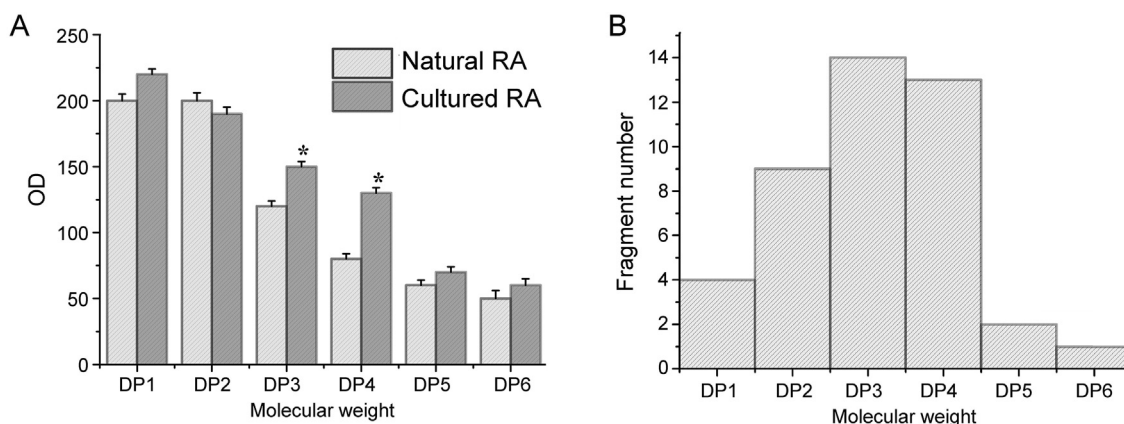
oligosaccharides for different cultivation patterns of AR, and the content of DP3 and DP4 in natural AR was higher than that in cultural AR (Fig. 6).

3.5. Data analysis of polyacrylamide gel electrophoresis profiles of different species of AR polysaccharides

wPLS-DA analysis of the partial acid hydrolyzate of different species of AR polysaccharide was shown in Fig. 7A. The results showed that MG AR and MJ AR could be obviously separated.
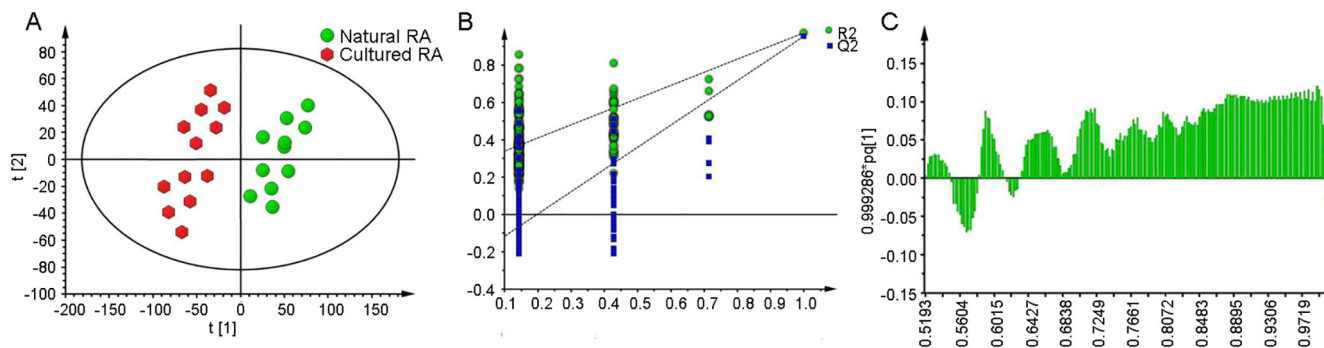
Fig. 5. PLS-DA scatter plot (A), model cross validation (B) and loading chart (C) for HPTLC spectrum of partial acid hydrolyzates of AR polysaccharides from different cultivation patterns.

Fig. 6. Sugar segment optical density values (A) and differential sugar segments contribution number (B) of PAGE spectrum of partial acid hydrolyzates of AR polysaccharides from different cultivation patterns.
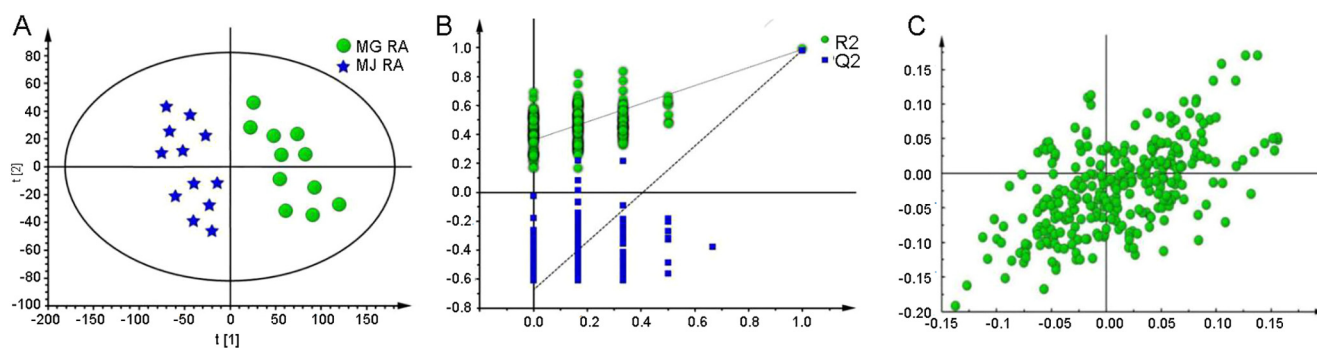
Fig. 7. PLS-DA scatter plot (A), model cross validation (B), and loading chart (C) of PAGE spectrum of of AR polysaccharides from different species.

The model validation was evaluated using the total explanatory variables (R^2 values) and model predictability (Q^2 values), followed by rigorous permutation tests (number: 200). The results showed that all Q^2 and R^2 values were lower than the actual model, revealing good predictability and fit of the model (Fig. 7B). According to the VIP (VIP > 1) value and the stoichiometric analysis, the difference between the oligosaccharides was found (Fig. 8). The results showed that DP2 and DP3 can be used as the main differential fragments to distinguish AR from different species. The content of DP2 in MJ AR was higher than that in MG AR, and the content of DP3 in MG AR was higher than that in MJ RA.

3.6. Data analysis of HPTLC profiles of different species of AR polysaccharides

The data analysis method of HPTLC spectrum was consistent with the data analysis method of PACE spectrum of partial hydrolyzate of different species of AR polysaccharides. The results of PLS-DA and model verification analysis of HPTLC data of different species of AR polysaccharides were shown in Fig. 9. There was a significant difference between MG AR and MJ, and the results of model validation were valid and the model was reliable.

Based on the data analysis of PAGE and HPTLC spectrum of the partial acid hydrolyzates of AR polysaccharides from different spe-
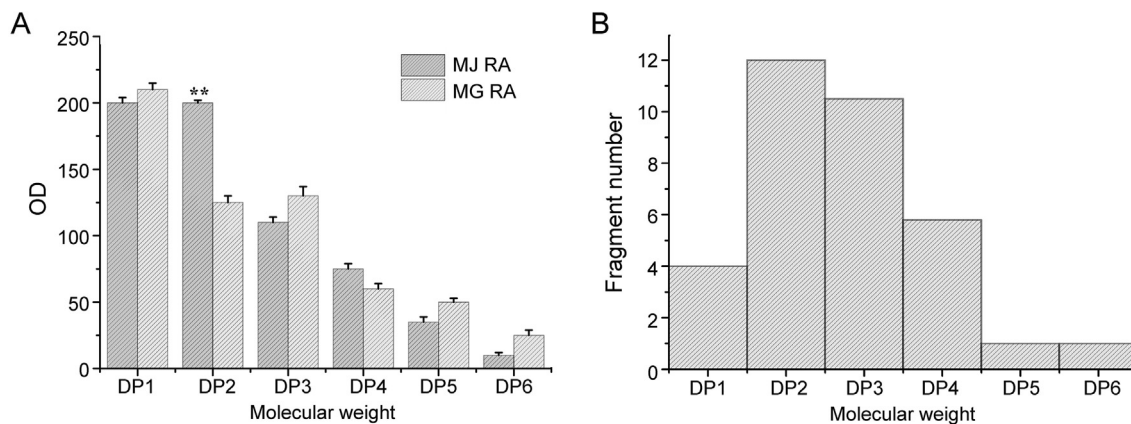
Fig. 8. Sugar segment optical density values (A) and differential sugar segments contribution number (B) of PAGE spectrum of partial acid hydrolyzate of AR polysaccharides from different species.
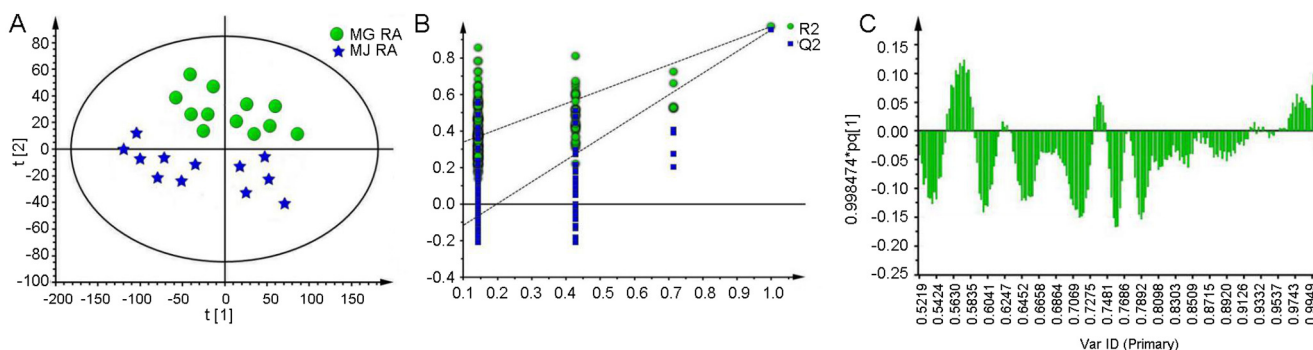

Fig. 9. PLS-DA (A), cross validation (B) and loading (C) of HPTLC chromatograms of partial acid hydrolysates of AR polysaccharides from different species.

cies, it was finally confirmed that the molecular weight ranged from 360 Da to 540 Da (DP2 and DP3) was the main differential fragment distinguishing the species of AR. The content of DP2 in MJ AR was higher than that in MG AR, and the content of DP3 in MG AR was higher than that in MJ AR (Fig. 10).

3.7. Clustering analysis of polyacrylamide gel electrophoresis profiles of partial acid hydrolyzate of AR polysaccharides from different germplasm resources

The electrophoretic fingerprint data of the partial acid hydrolyzate of AR polysaccharides from different germplasm resources were standardized and introduced into SMICA-P 13.0 for cluster

analysis. The results were shown in Fig. 11. The results showed that the partial acid hydrolyzate of AR polysaccharides from different cultivation methods could be obviously clustered into two types, but the partial acid hydrolyzate of AR polysaccharides from different species could not be separated obviously.

3.8. Determination of THP-1 activity by CCK8 method

As shown in Fig. 12, the differential glycoside fragments of AR had a significant promoting effect on cell proliferation, and the proliferation of cells in a certain concentration range had a significant positive correlation with the concentration of polysaccharide. When the concentration of the component reached 100 μg/mL,
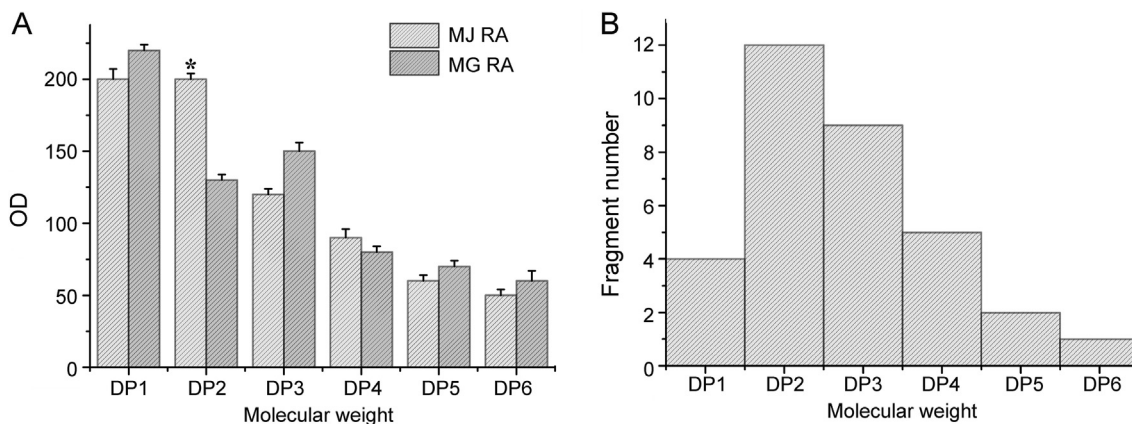

Fig. 10. Sugar segment optical density values (A) and differential sugar segments contribution number (B) of PAGE spectrum of partial acid hydrolyzate of AR polysaccharides from different species.
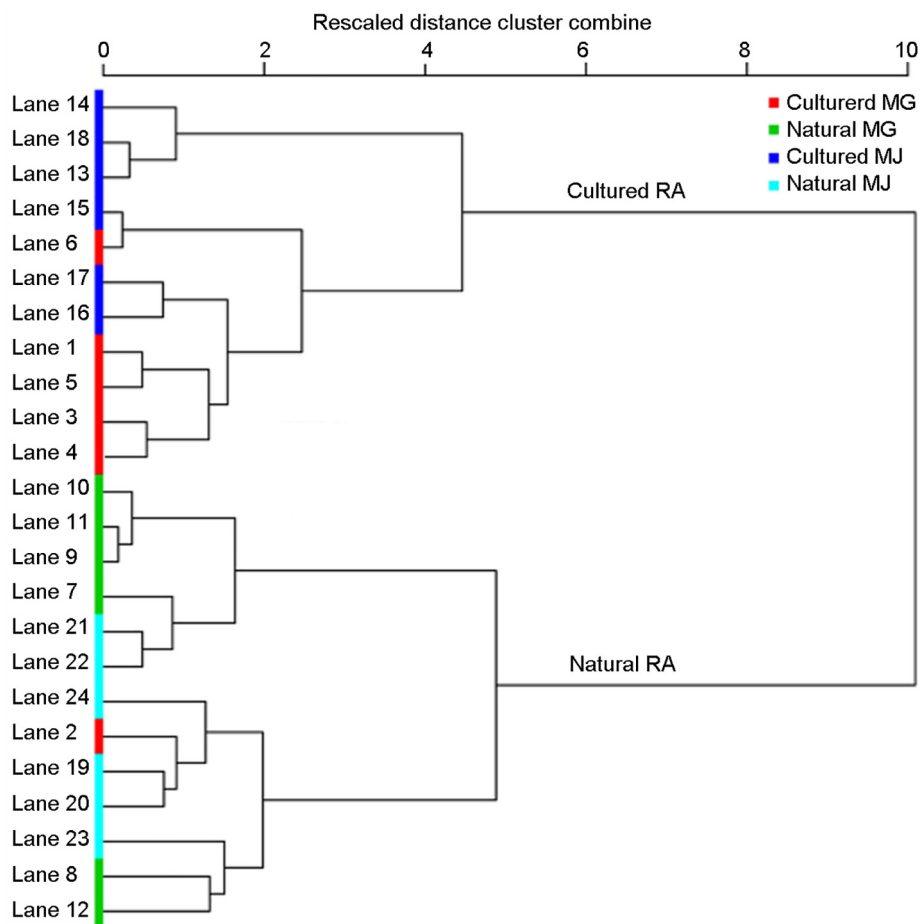
Fig. 11. Cluster analysis of PAGE spectrum of partial acid hydrolyzate of AR polysaccharides from different germplasm resources.

the cell proliferation rate reached a maximum of 132% at a certain culture time. The survival rate of THP-1 cells was above 95%, indicating that the cell growth state is good, and the experimental operation can be continued.

3.9. Determination of TNF-α, IL-1β and IL-6 in culture supernatant

As shown in Fig. 13, compared with the blank control group, the secretion of TNF-α, IL-1β and IL-6 in LPS-positive control and experimental group were significantly increased ($P < 0.05$), which was in a concentration-dependent manner.

3.10. Determination of NO production by Griess method

As shown in Fig. 14, compared with the blank control group, the NO secreted by THP-1 cells in the LPS positive control group and the experimental group were significantly increased ($P < 0.05$), which was in a concentration-dependent manner.
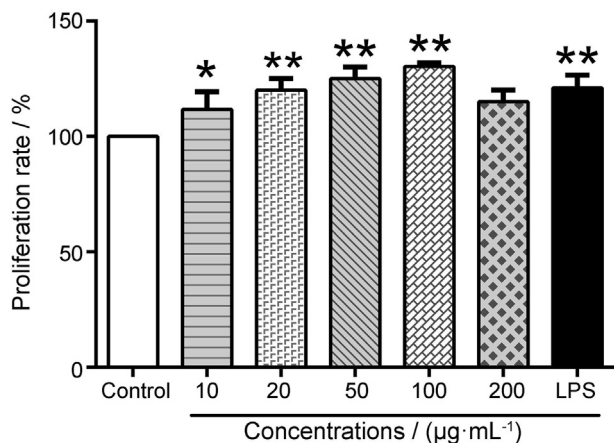
4. Discussion

In this study, natural AR was selected from genuine producing areas, including the Hunyuan and Shanxi Provinces of China. These places are mostly semi-sloping environments with loose soil. In summer, there is sufficient sunshine, drought and little rain, large temperature difference between day and night, and cold in winter. Natural AR grows in this environment for more than five years. However, cultured AR is harvested after being transplanted and fertilized for two years on the fertile land selected by the farmers. In contrast, natural AR has been subjected to more environmental stresses such as drought, low temperature and nutrient deficiencies, while cultured AR is less affected by adversity. Numerous studies have shown that plants usually resist stress by cells and whole organisms. Under adverse conditions, plants will change in many aspects such as morphological structure, physiological and biochemical property, osmotic adjustment, phytohormone, membrane protective substances, active oxygen balance, and stress protein, involving many physiological processes such as plant water,
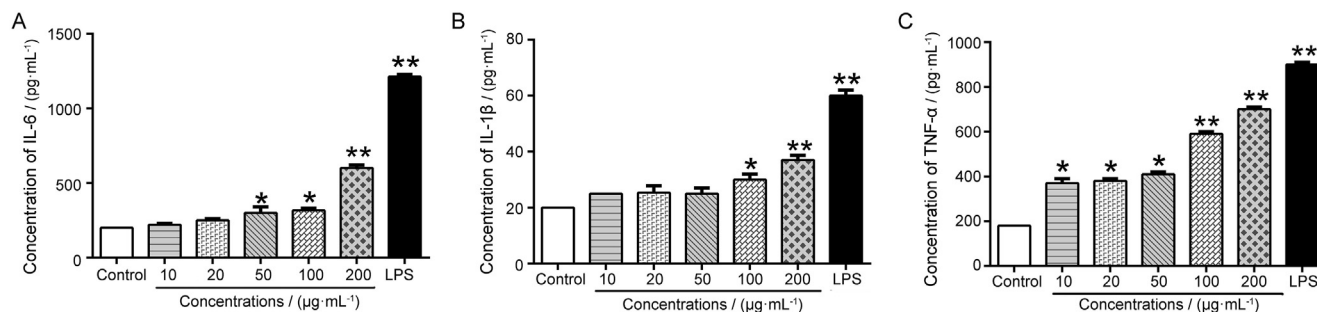

Fig. 12. Effect of different concentrations of ASP-1 on proliferation rate of THP-1 cells. $^*P < 0.05$, $^{**}P < 0.01$ *vs* control group.

Fig. 13. Effect of different concentrations of ASP-1 on secretion of IL-6 (A) and IL-1β (B)TNF-α (C) in THP-1 cells. *$P < 0.05$, **$P < 0.01$ *vs* control group.
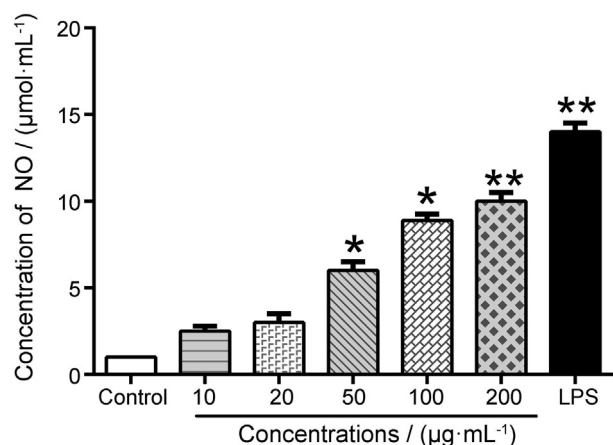

Fig. 14. Effect of different concentrations of ASP-1 on expression of NO in THP-1 cells. *$P < 0.05$, **$P < 0.01$ *vs* control group.

photosynthesis, respiration, and material metabolism (Wang et al., 2018). It has been reported that the free sugar and sugar alcohol components in the cytoplasm are small molecules that are induced by growth stress in plants. These substances accumulate in cells and regulate the osmotic pressure of cells, which is beneficial to plants to maintain moisture and resist salt, alkali, low temperature and other adverse conditions, which play an important role in maintaining the normal physiological functions of plants (Wang et al., 2018). Therefore, under the influence of environment, natural AR is more stressful than cultured AR, and the polysaccharide content is much higher than cultured AR. Although this study found out which sugar fragments after hydrolysis of polysaccharides can be used as the main differential fragments for distinguishing the growth patterns of MG AR and MJ AR, the difference in the content and activity of sugar fragments is unknown, can it be used as an indicator to evaluate the germplasm resources of AR remains to be further studied.

In this experiment, PAGE and HPTLC were used to characterize the partial acid hydrolyzate of AR polysaccharides in the form of glycoside map group. At the same time, PAGE and HPTLC were transformed with Quantity One software, to achieve a combination of spectrum and multivariate statistical data processing. Through this new method, differences between oligosaccharides were found, and AR from different germplasm resources could be identified. The results showed that the DP3 and DP4 in TFA hydrolysate can be used as a differential sugar fragment to distinguish different cultivation methods of AR, and DP2 and DP3 can be used as differential sugar fragments to distinguish different species of AR. This index provides a basis for the identification of MG AR and MJ AR in different cultivation methods. By immunological activity analysis of the different oligosaccharide fragments of *Astragalus mem-*

branaceus, it was found that *Astragalus* specific sugar fragments can promote the secretion of TNF-α, IL-1β, IL-6, and NO in THP-1 cells in a concentration-dependent manner. Although the inflammatory factor produced by stimulation is less than that of the positive control group LPS, it can be explained that the *Scutellaria* specific sugar fragment can induce inflammatory response and has immunomodulatory effects. The results of this study will improve the quality evaluation standard of AR, lay the foundation for the protection and development of high quality authentic AR resources, it will also provide new ideas for the objective evaluation of germplasm resources of plant medicinal herbs with polysaccharides as important active substances.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2020.07.004.

References

Bondarenko, P. V., Chelius, D., & Shaler, T. A. (2002). Identification and relative quantitation of protein mixtures by enzymatic digestion followed by capillary reversed-phase liquid chromatography–tandem mass spectrometry. *Analytical Chemistry, 74*(18), 4741–4749. https://doi.org/10.1021/ac0256991.

Chen, Y. G., Xin, M. T., & Yang, B. (2009). Determination of verbascum isoflavones in wild and cultivated *Astragalus. Chinese Traditional and Herbal Drugs, 40*(9), 1484–1485.

Choi, H., Lee, H.-S., & Park, Z.-Y. (2008). Detection of multiphosphorylated peptides in LC–MS/MS analysis under low pH conditions. *Analytical Chemistry, 80*(8), 3007–3015. https://doi.org/10.1021/ac7023393.s002.

Chu, C., Qi, L. W., Liu, E. H., Li, B., Gao, W., & Li, P. (2010). *Radix Astragali (Astragalus)*: Latest advancements and trends in chemistry, analysis, pharmacology and pharmacokinetics. *Current Organic Chemistry, 14*(16), 1792–1807.

CHU, Y.-F., YAN, X.-M., LI, X.-R., & HU, Y.-L. (2006). Effect of Chinese herbal medicinal ingredients on IL-2 mRNA levels of T lymphocytes in mice measured using semiquantification RT-PCR. *Agricultural Sciences in China, 5*(11), 873–878. https://doi.org/10.1016/S1671-2927(06)60138-7.

Du, G. J., Qin, X. M., Li, Z. Y., He, P., Gao, F. R., Li, K., & Zhou, R. (2013). Comparative study on quality of *Astragali Radix* by two different planting patterns in major producing areas. *Chinese Traditional and Herbal Drugs, 44*(23), 3386–3393.

Guan, J., & Li, S. P. (2010). Discrimination of polysaccharides from traditional Chinese medicines using saccharide mapping-enzymatic digestion followed by chromatographic analysis. *Journal of Pharmaceutical and Biomedical Analysis, 51*, 590–598.

Guan, J., Yang, F. Q., & Li, S. P. (2010). Evaluation of carbohydrates in natural and cultured *Cordyceps* by pressurized liquid extraction and LC-MS. *Molecules, 15*(6), 4227–4241.

Guan, J., Zhao, J., Feng, K., Hu, D.-J., & Li, S.-P. (2011). Comparison and characterization of polysaccharides from natural and cultured *Cordyceps* using saccharide mapping. *Analytical and Bioanalytical Chemistry, 399*(10), 3465–3474. https://doi.org/10.1007/s00216-010-4396-y.

He, X. J., Niu, X. Y., Li, J., Xu, S. H., & Lu, A. P. (2012). Immunomodulatory activities of five clinically used Chinese herbal polysaccharides. *Journal of Experimental and Integrative Medicine, 2*, 15–27.

Hu, F. D., Feng, S. L., Zhao, J. Y., Zhang, Y., & Chen, L. R. (2003). Determination of flavonoids and astragaloside IV in *Radix Astragali* by HPLC. *Analysis and Testing Technology and Instruments, 9*(3), 173–177.

Hu, M. X., Chen, A. J., Guo, B. L., Huang, W. H., Cao, X. J., & Hou, M. L. (2012). Effects of environmental factors on quality of wild *Astragalus membranaceus* var. *mongholicus* grown in Hengshan Mountain, Shanxi Province. *Chinese Traditional and Herbal Drugs, 43*(5), 984–989.

Jiang, Y., Jin, F., Bao, Z., Shi, Z. J., & Tu, P. F. (2006). Quantitative analysis of astragaloside IV in different sources of *Radix Astragali*. *China Journal of Chinese Materia Medica, 31*(11), 930–933.

Lee, Y. S., Han, O. K., Park, C. W., Suh, S. I., Shin, S. W., Yang, C. H., Jeon, T. W., Lee, E. S., Kim, K. J., Kim, S. H., Yoo, W. K., & Kim, H. J. (2003). Immunomodulatory effects of aqueous-extracted *Astragali radix* in methotrexate-treated mouse spleen cells. *Journal of Ethnopharmacology, 84*(2-3), 193–198. https://doi.org/10.1016/S0378-8741(02)00298-2.

Li, Q., Hu, J. H., Gao, B., Li, H. D., Song, S., & Yang, L. X. (2017). Advances on immunoregulation effect of *Astragalus* polysaccharides. *Chinese Journal of Experimental Traditional Medical, 23*(2), 199–206.

Liu, C. X., Chen, S. L., Xiao, X. H., Zhang, T. J., Hou, W. B., & Liao, M. L. (2016). A new concept on quality marker of Chinese materia medica: Quality control for Chinese medicinal products. *Chinese Traditional and Herbal Drugs, 47*(9), 1443–1457.

Qin, X. M., Li, Z. Y., Sun, H. F., Zhang, L. Z., Zhou, R., & Feng, Q. J. (2013). Status and analysis of *Astragali Radix* resource in China. *China Journal of Chinese Materia Medica, 38*(19), 3234–3238.

Shao, P., Zhao, L. H., Chen, Z., & Pan, J. P. (2006). Regulation on maturation and function of dendritic cells by AR mongholicus polysaccharides. *International Immunopharmacology, 6*(7), 1161–1166.

Wu, D. T., Cheng, K. L., Wang, L. Y., Lv, G. P., Ju, Y. J., Feng, K., ... Li, S. P. (2014). Characterization and discrimination of polysaccharides from different species of *Cordyceps* using saccharide mapping based on PAGE and HPTLC. *Carbohydrate Polymers, 103*, 100–109.

Wang, D., Liu, L., Li, K., Li, A. P., Liu, Y. T., Qin, X. M., ... Zhang, X. (2018). Establishment of fingerprint of *Astragali Radix* polysaccharides based on endo-1,3-β-glucanase hydrolysis and identification of different germplasm resources of *Astragali Radix*. *Chinese Traditional and Herbal Drugs, 49*(10), 2320–2327.

Yang, Q. Z., Liu, D. W., Wang, D. M., Fu, J., & Huang, L. F. (2014). Correlation on quality and ecotype of *Astragalus membranaceus* var. *mongholicus*. *Chinese Traditional and Herbal Drugs, 45*(16), 2395–2399.

Zhao, Y. Z. (2004). Study on the distribution of *Astragalus* plant source and its origin. *Chinese Traditional and Herbal Drugs, 35*(10), 1189–1190.