

Astragali Radix (Huangqi): Extraction Techniques, A Promising Edible Immunomodulatory Herbal Medicine, and Biological Activity

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Received April 12, 2023; Revised May 07, 2023; Accepted May 18, 2023

Abstract Astragali Radix (AR) is one of the well-known traditional Chinese medicines with a long history of medical use and a wide range of clinical applications. AR contains a variety of chemical constituents which can be classified into the following categories: polysaccharides, saponins, flavonoids, amino acids, and trace elements. There are several techniques to extract these constituents, of which microwave-assisted, enzymatic, aqueous, ultrasonic and reflux extraction are the most used. Several methods such as spectroscopy, capillary electrophoresis and various chromatographic methods have been developed to identify and analyze AR. Meanwhile, this paper also summarizes the biological activities of AR, such as anti-inflammatory, antioxidant, antitumor and antiviral activities. It is expected to provide theoretical support for the better development and utilization of AR. The potential pharmaceutical properties of calycosin in the treatment of tumors, inflammation, stroke, and cardiovascular diseases have gained increasing attention in the recent years. The literature survey showed that calycosin exhibits promising effects for the treatment of several diseases and that these effects may be due to its isoflavonoid and phytoestrogenic properties. The effects of calycosin most likely result from its interaction with the ER receptors on the cell membrane and the modulation of the MAPK signaling pathway. Calycosin exhibits great potential as a therapeutic drug and may be a successful example of the standardization and modernization of traditional Chinese herbal medicine.

Keywords: *Astragali Radix, extraction technologies, chemical constituents, analytical methods, biological activity*

Cite This Article: Zhang Xuli, Fida Noor, Yan Haisheng, and Li Hong, “Astragali Radix (Huangqi): Extraction Techniques, A Promising Edible Immunomodulatory Herbal Medicine, and Biological Activity.” *Journal of Food and Nutrition Research*, vol. 11, no. 5 (2023): 372-381. doi: 10.12691/jfnr-11-5-6.

1. Introduction

Astragali Radix (AR), known by the Chinese name of Huang-qi (Ougi in Japanese name), is a traditional herbal medicine that has been used for over 2000 years in China [1]. According to the Pharmacopoeia of the People's Republic of China (2020 edition), AR is defined as the dried root of *Astragalus membranaceus* (Fisch.) Bge. or *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (Fabaceae) [1], which is a variant of *Astragalus membranaceus* (Fisch.) Bge and mainly grows in Shanxi, Inner Mongolia, Shaanxi, Gansu and other provinces in China as well as Korea and Mongolia [2]. It was found that the main bioactive compounds in AR samples from different suppliers varied greatly [3]. Therefore, a standard is needed to evaluate the quality of AR. Sun et al. carried out a principal component analysis of 18 kinds of AR comprehensive indicators that can be combined with diameter, alcohol (water) extract,

flavonoid aglycone and aglycone peak area ratio to establish an AR grade evaluation system to provide analysis methods [4]. In modern pharmacological research, AR has been demonstrated to possess tonic, hepatoprotective, diuretic, and expectorant properties and exhibit antihyperglycemic, immunomodulating, anti-inflammatory, antiviral and antioxidant activities [5,6,7], and so on. Nowadays, AR is used extensively in clinical therapy, such as, Huangqi injections for treating renal disease. However, the chemical compounds and active constituents of AR remain obscure. Over the years, extensive research has been conducted on the chemical components of AR. It is known that AR root contains saponins, polysaccharides, flavonoids, amino acids, and trace elements [8]. Among these, saponins are the major active constituents, especially Astragaloside IV (AS-IV). However, the composition and quantity of active compounds may vary depending on the culture area, the growth period and the growth conditions. In addition, the compounds identified from crude drugs also depend on the method of extraction and the method of analysis.

Nevertheless, to date, there are few reviews on the extraction methods and analytical methods for AR chemical components. Thus, this review summarizes the recent advances in the chemical studies of AR species, involving different extraction techniques and qualitative and quantitative analytical methods, and provides a summary of their biological activities.

2. Chemical Composition

According to available studies, more than 100 compounds have been isolated and identified from AR. Based on structure, it can be mainly divided into four groups, including polysaccharides, saponins, flavonoids and others. Enhancing the classification of different compounds in AR might facilitate the understanding of the pharmacological effects of AR.

2.1. Polysaccharides

The polysaccharides from AR are well-known for their hepatoprotective effects [9]. As early as 1982, two glucans,

AG⁻¹ and AG⁻², and two heteropolysaccharides, AH⁻¹ and AH⁻², were isolated from the aqueous extract of *A. membranaceus*. AG⁻¹ is a water-soluble *a* glucan, and the composition ratio of *a* glycosidic glycosyl groups is 5:2. AG⁻² is a water-insoluble *a* glucan. AH⁻¹ is a water-soluble acidic polysaccharide. After hydrolysis, hexuronic acid, glucose (Glc), rhamnose (Rha) and arabinose (Ara) uniting at 1:0.04:0.02:0.01 were detected. AH⁻² was water soluble, with Glc and Ara uniting at 1:0.15, detected after hydrolysis. The polysaccharides APS-I and APS-II were isolated from the water extract of *A. membranaceus*. APS-I consisted of Ara and Glc, in a molar ratio and APS-II consisted of Rha, Ara and Glc uniting at 1:6.25:17.86 [7,10]. Fang et al. produced APS-III by water extraction and alcohol precipitation from *A. membranaceus* [11,12]. They were all water-soluble and insoluble in alcohols and other organic solvents. The analysis of the polysaccharide from AR revealed three sugars: Glc, Rha, and Ara. Among them, Glc was the major component, followed by Ara and Rha [13,14]. Table 1 lists the parameters of the identified polysaccharides, such as molecular weights (Mw), identification methods, classifications, sources, and reference.

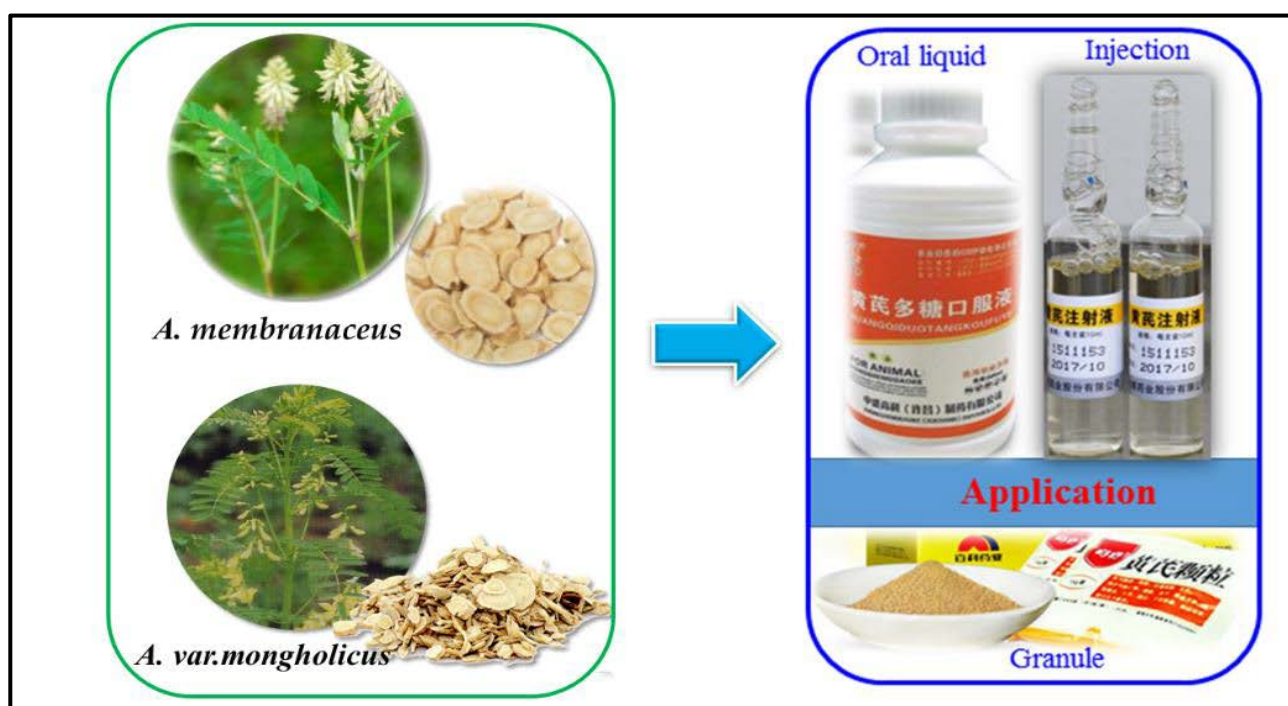


Figure 1. *A. membranaceus* and *A. var. mongholicus* and their commercial application

Table 1. Information regarding the polysaccharides identified from AR

N0	Name Identification Method	Molecular Weight (Da)	Classification	Structure	Source	Refs.
1	AG-1	—	400 M NMR	Glucan	—	[13]
2	AG-2	—	400 M NMR	Glucan	—	[13]
3	AH-1	—	400 M NMR	Heteropolysaccharide	—	[13]
4	AH-2	—	400 M NMR	Heteropolysaccharide	—	[13]
5	APS-I	1.7 × 106	HPLC (C18), TLC	Heteropolysaccharide	—	[7]
6	APS-II	3.5 × 104	HPLC (C18), TLC	D-Glucan	Dextran bonded mainly with-(1 ! 4)-D-glycosidic linkage	[7]
7	APS-III	3.5 × 104	400 M NMR	D-Glucan	Dextran bonded mainly with -(1 ! 4)-D-glycosidic linkage	[14]

2.1.1. Enzyme Extraction

Cellulose, the main component of AR cell walls, is the main barrier used to prevent the release of macromolecules such as intracellular polysaccharides. The traditional enzyme used to extract Astragalus polysaccharides is cellulase [15], which can hydrolyze the cell wall and dissolve intracellular components to increase the extraction rate [16]. The combined enzyme extraction of Astragalus polysaccharide was optimized. A response surface methodology and orthogonal tests were applied to optimize the conditions: the combined enzyme concentrations of cellulase, pectinase, and papain were 1.5%, 1%, and 0.5%, respectively; the extraction time was 94.5 min; the extraction temperature was 49.9°C; the pH value was 5.1 [17]. A response surface method was used to optimize the glucose oxidase-assisted extraction process to obtain the maximum yield of crude APS. The optimized extraction conditions were as follows: enzyme dosage, 3.0%; enzyme treatment time, 3.44 d; enzyme treatment temperature, 56.9°C; extraction solvent pH of 7.8. Under these conditions, the experimental yield was 29.96, 0.14% [18]. The conditions of the enzyme extraction method are mild, the process is simple and yield is high; nevertheless, the enzyme is expensive.

2.1.2. Extraction of Saponins and Flavonoids

The Saponins and flavonoids are the major active constituents in AR. Saponins mainly consist of astragaloside IV, astragaloside I and soyasaponin I and others; flavonoids mainly include calycosin, formononetin and ononin and others. The study of the extraction methods of saponins and flavonoids in AR has a positive effect on the screening of highquality Astragalus resources and the identification of AR medicine quality. Presently, the main extraction methods of saponins and flavonoids from AR include reflux extraction, microwave-assisted extraction, and enzymolysis-assisted extraction [19].

2.1.3. Ethanol Reflux Extraction

The Ethanol reflux extraction is applied as the main method for extracting saponins and flavonoids currently. This method is simple, stable, and feasible, and more importantly, it can extract active constituents such as saponins as much as is possible in AR. Optimized the ethanol reflux extraction procedure parameters (ethanol concentration, the amount of ethanol added, extraction time, and extraction times) using an orthogonal design method, with the astragaloside IV extraction rate as the detection index. The optimal conditions of alcohol extraction were: 60% ethanol; ratio of solid/liquid-1:6 (g/mL); and 3 extraction cycles, each 1 h [20]. High-performance liquid chromatography (HPLC) was used to estimate the yield of flavonoids extracted from Astragalus mongholicus by the alcohol reflux extraction method. According to the results of single-factor experiments, the optimum conditions were confirmed as: temperature-75°C; time-2.5 h; ethanol concentration-90% (v/v); and ratio of solvent to raw material-20 mL/g. The best yield was 0.934 mg/g [21]. Orthogonal tests were used to optimize the extraction conditions of total flavonoids from Astragalus. The contents of total extracts and flavonoids were regarded as indicators. The optimal process for the

ethanol reflux extraction of total flavonoids from Astragalus was: extracted with 10 times 70% ethanol two times for 60 min each time [22].

2.1.4. Enzyme Extraction

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best yield was 0.934 mg/g [27]. Orthogonal tests were used to optimize the extraction conditions of total flavonoids from *Astragalus*. The contents of total extracts and flavonoids were regarded as indicators. The optimal process for the ethanol reflux extraction of total flavonoids from *Astragalus* was: extracted with 10 times 70% ethanol two times for 60 min each time [28].

2.1.7. Microwave-Assisted Extraction (MAE)

An MAE method was used to extract astragalosides I-IV from AR. MAE gave the highest extraction efficiency within the shortest time. The optimal conditions of MAE were: employing 80% ethanol as solvent; ratio of ethanol to the material-1:25 (g/mL); Temperature-70°C; irradiation power-700 W; and 3 extractions, each 5 min [29]. The flavonoids were extracted with a microwave digestion system and the extraction rate of flavonoids was determined by a spectrophotometer. The optimum extraction conditions of flavonoids from *A. membranaceus* according to the extraction rate of flavonoids were obtained as followed: concentration of ethanol-95%; extraction time-20 min; ratio of material to liquid-1:15 g/mL; and temperature-90°C. Under these conditions, the extraction rate of flavonoids was the highest (0.489%) [30].

2.1.8. Enzymolysis-Assisted Extraction

The active substances of plant-based herbs are often encased in lignins, and enzymes can effectively degrade lignin and greatly increase the extraction rate of traditional Chinese medicine. An orthogonal design was used to study the optimum technology for the enzyme-assisted extraction of total flavonoids from *Astragalus*. The optimum extraction process was determined as follows: enzymolysis time-120 min; hydrolysis temperature-30°C; enzyme dosage-8 mg; and pH-4.5 [31]. A response surface methodology was used to optimize the process of cellulase-assisted extraction of total saponins from *Astragalus*, and the optimal conditions were: pH-4.38; enzymolysis time-132 min; enzymolysis temperature-51°C; enzyme dosage-8.84 mg; predictive extraction rate-1.89%; and actual extraction rate-1.87, 0.03%. The technological conditions for the extraction of total flavonoids from *A. membranaceus mongholicus* by cellulose hydrolysis-assisted ethanol extraction were studied. The optimum process parameters were: amount of added cellulose-7.5 L/g; enzymatic hydrolysis pH value-4.0; enzymolysis temperature-55°C; enzymolysis time-1.5 h; ratio of liquid to solid-30 mL/g; ethanol concentration-90%; extraction temperature-75°C; and extraction time-2.5 h; under these conditions, the extraction rate of total flavonoids was 0.57%. It increased by 46.15% compared to traditional alcohol extraction (0.39%) [32]. Based on the above review, we compared the advantages and disadvantages of each extraction method as shown in (Table 2).

3. Biological Activities of AR

Astragalus, as a traditional Chinese medicinal material, has been used in clinical treatment. The active ingredients in *Astragalus* are mainly saponins, polysaccharides and flavonoids. In addition, there are some other extracts, such

as chlorogenic acid, caffeic acid, -sitosterol and so on. Studies have found that *Astragalus* has many biological activities, such as anti-inflammatory, antioxidant, antitumor, antiviral, cardiovascular disease prevention, and anti-diabetic activities and so on [33,34] (Figure 2).

3.1. Anti-Inflammatory Activity

Study found that Astragaloside IV (AS-IV) treatment significantly reduced the production of inflammatory cytokines in orbital fibroblasts induced by IL-1 in vitro, thereby inhibiting autophagy and preventing Graves' orbital disease. [35,36] study found that AS-IV was used to act on human umbilical vein endothelial cells (HUVEC), and then act on ox-LDL, and found that the ROS and NADPH oxidase activities of the cells were significantly reduced compared with the control group. At the same time, the expression levels of Nrf2, HO-1, TNF- α and IL-6 were significantly reduced. This shows that astragaloside IV can protect oxidase low-density lipoprotein (OX-LDL) induced endothelial cell damage and inhibit atherosclerosis by reducing oxidative stress and inflammation. In addition, other ingredients of *Astragalus* are also believed to have anti-inflammatory effects [37] treated lipopolysaccharide-induced obese mice with active ingredients (Rx) extracted from *Astragalus* and found that the activation of NF- κ B in macrophages was dose dependent with Rx. The mRNA expression levels of the inflammatory cell markers CD68 and F4/80, as well as the expression levels of the cytokines MCP-1, TNF- α and IL-6, decreased significantly, which shows that *Astragalus* can reduce the symptoms of glucose intolerance, insulin resistance and hypertriglyceridemia caused by obesity by anti-inflammatory. Studies have found that these active ingredients include calyx-7-bD-glucoside (0.9%), onionin (1.2%), calyxin (4.53%) and formononetin (1.1%) [38,39]. combined in vitro cytokine production analysis and LC-MS metabolomics technology to prepare extracts of different polarities from Huangqi Jianzhong Decoction. It was found that these ingredients can reduce the expression levels of TNF- α , IL-1 and IFN- γ in U937 cells, proving the anti-inflammatory effect of *Astragalus*; active ingredients such as verbascoside, verbascoside, astragaloside IV, glycyrrhizin, 18-glycyrrhetic acid, paeoniflorin and leucoflorin were identified. The anti-inflammatory mechanisms of AR are presented in Figure 3. 18 β -glycyrrhetic acid, paeoniflorin and leucoflorin were identified. The anti-inflammatory mechanisms of AR are presented in Figure 3.

3.1.1. Ethnopharmacological Immunomodulatory Practices of AR

In the theoretical system of TCM, AR is sweet in flavor, warm in nature, and acts on the lung and spleen. Generally, AR is harvested in spring or autumn after growing for more than 3 years. After removing the aerial part and fibrous root, AR is washed, cut into thick slices, and dried. Then, it can be employed as herbal medicine in the clinic. Clinical practitioners of TCM consider that AR invigorates *Qi* and promotes *Yang*, thereby diffusing water, reducing swelling, fixing the surface, relieving sweat, promoting wound healing, and generating muscle [40]. In the classic book titled "Compendium of Materia Medica",

it was declared that “AR is the leader of tonics among herb medicines.”Compared with equally well-known immunomodulatory medicinal herbs such as Ginseng Radix et Rhizoma, AR is more inclined to supplement the *Defense-Qi*, which has the physiological functions of defending exogenous pathogenic factors, warming and nourishing the whole body, and to replenish the *Middle-Qi*, which is the function motivity of spleen and stomach. Both of them are universally believed to be closely related to the immune system in TCM. In addition, its pharmacodynamic effects are more temperate and more suitable for weak and debilitated patients. Therefore, AR is a common and essential tonic herb medicine that has great developmental potential in food supplements. AR, as a promising herbal medicine to regulate immunity, acts by promoting resistance to exogenous causative agents and strengthening general vitality. It is regarded as a significant tonic for up-regulating energy levels and regulating the immune system [40]. When used alone, it is generally soaked in water to drink, which is an effective method of AR utilization among folks. It is also used in

combination with other herbal medicines. For instance, some patients are susceptible to cold, which is called “exterior deficiency” according to TCM theory. AR can boost the *Defense-Qi* and avoid susceptibility to cold. It is generally used in combination with Ginseng Radix et Rhizoma, which is an appropriate combination for treating physical weakness caused by *Qi* deficiency, such as fatigue, inappetence, and spontaneous sweating [41]. The compatibility of AR and Atractylodis Macrocephalae Rhizoma can enhance the function of replenishing the *Qi* and invigorating the spleen. The indications of this compatibility are fatigue, shortness of breath, and lassitude caused by spleen weakness and deficiency of vital energy [42]. The combination of AR and Angelicae Sinensis Radix is suitable for improving internal injury, red muscular heat, thirst, deficiency of pulse, fatigue, sores, ulcers and fever due to *Blood* deficiency, and deficiency of the *Qi* and *Blood* [41]. On the basis of these compatibilities related to the regulation of immune system, numerous prescriptions for clinical and diet therapies have been developed in TCM.

Table 2. Advantages and disadvantages of extraction methods for AR studies

	Advantages	Disadvantages
Water extraction	Simple operation and low production costs (maximal extraction rate of AP was 16.32%)	Large energy consumption and low extraction rate
Ethanol Reflux extraction	Wide range of applications, simple equipment, good extraction effect	Long extraction time, solvent residue
Microwave-assisted Extraction	Penetrating heating, time saving, high efficiency, energy saving (maximal extraction rate of AP was 32%, 49% improvement compared to conventional water extraction)	Volatile components gradually dissipate as the extraction time increases.
Ultrasonic extraction	Simple operation, high efficiency, time saving and energy saving (maximal extraction rate of AP was 30.28%, 46.23% improvement compared to conventional water extraction)	Sound pollution
Enzyme extraction	High specificity and efficiency (maximal extraction rate of AP was 29.96%, 45.52% improvement compared to conventional water extraction)	High production costs

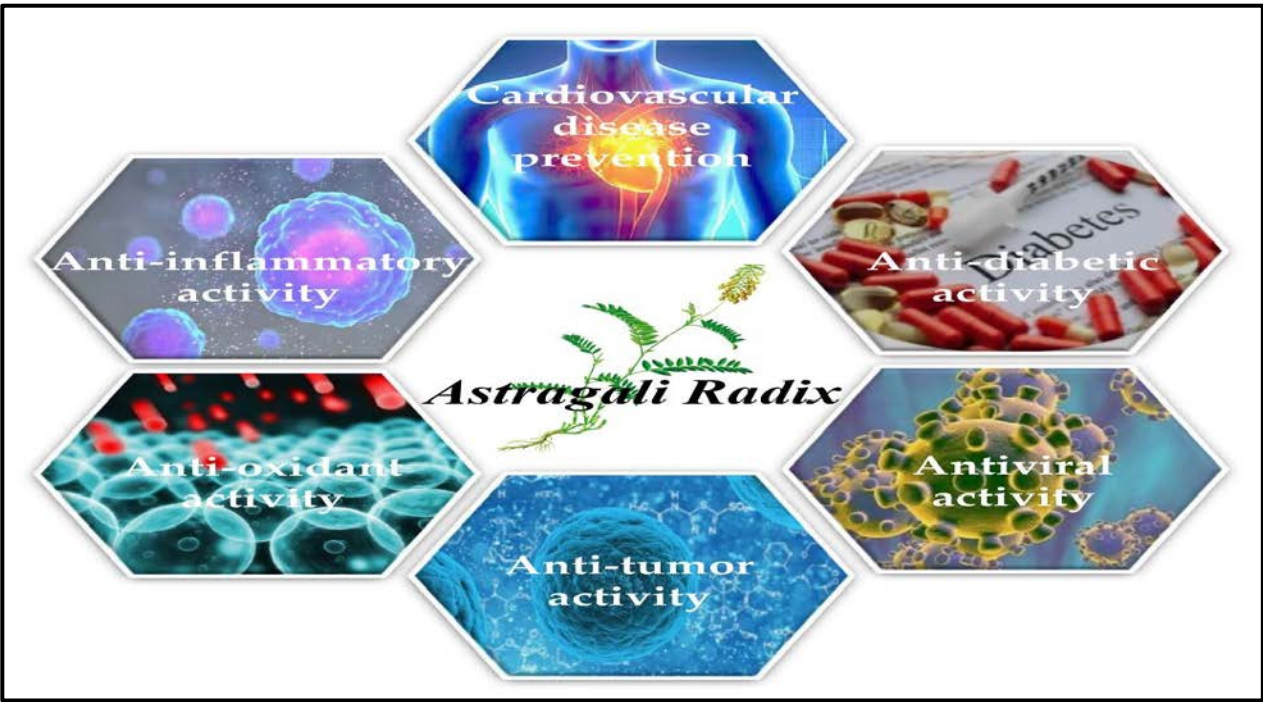


Figure 2. Biological activities of AR

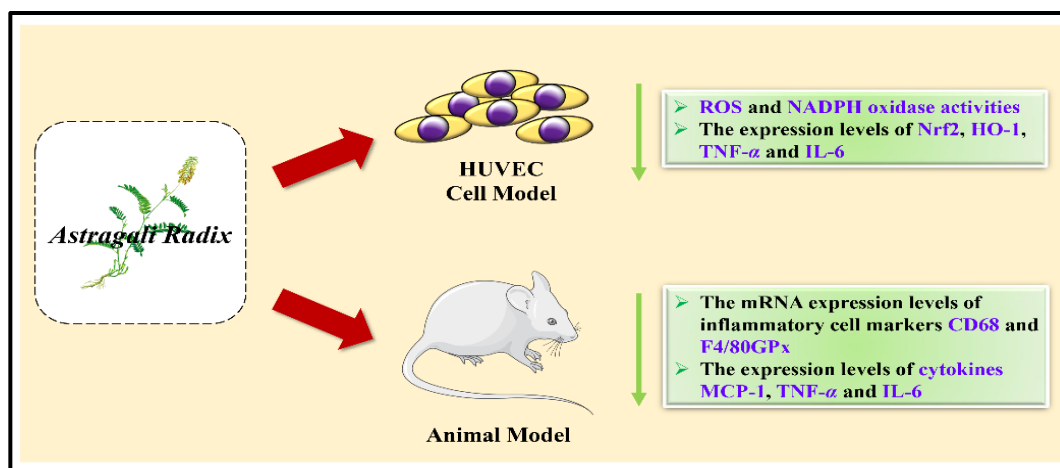


Figure 3. The anti-inflammatory activity mechanism of AR in cell and animal models

3.1.2. Immunomodulatory Constituents of AR

AR, like most botanical medicines, contains numerous natural products with different structural patterns. It is abundant with flavonoids, saponins, polysaccharides, amino acids, and other kinds of compounds, which show various bioactivities *in vivo* or *in vitro*. Nevertheless, not all these constituents from AR have immune regulatory function. After reviewing the relevant studies, we found that the main active constituents of AR for immune regulation are APS, astragalus saponins (AS), and astragalus flavonoids (AF) [43]. Herein, we summarized the chemical constituents related to the immune regulation of AR. Their chemical structures are shown in Figure 4.

3.1.3. Polysaccharides

Approximately 24 polysaccharides have been found in AR. Most of them are heteropolysaccharides. In terms of different species of raw materials or purification technology, different studies showed various results on the structural features of APS. Generally, the molecular weights of the heteropolysaccharides of APS are in the range of 8.7×10^3 Da to 4.8×10^6 Da with different ratios of monosaccharides, including glucose, galactose, rhamnose, arabinose, xylose, mannose, fructose, fucose, and ribose. Besides, glucuronic acid and galacturonic acid are also contained in APS [44]. As early as in 1982, Huang et al. purified and isolated two kinds of heterosaccharides (AH-1 and AH-2) and two kinds of glaucans (AG-1 and AG-2) from the water extract of AR. AG-1 was identified as an α -(1→4) (1→6) glucan with the ratio of α -(1→4) and α -(1→6) linkage approximately 5:2, while AG-2 was elucidated as an α -(1→4) glucan. Besides, AH-1 was an acidic polysaccharide that consists of monosaccharides, including galactose. AH-2 consists of arabinose and glucose units at a ratio of 0.15:1. Notably, both polysaccharides have immune functions [45].

3.1.4. Saponins

Extensive phytochemical research on AR has documented that cyclostanostane-type saponins are the significant bioactive substances that are closely related to immunoregulation [46]. Saponins in AR are main derivatives of the 20R, 24S form of cycloastragenol, called astragalosides (AST) which are expressed as (S)-epoxy-9 β , 19-cyclostanostane- β , 6 α , 16 β , 25-tetrol]. Some

saponins, in the form of 20S, 24R, are named astramembrainnins (15, 16), which are expressed as [20(S), 24(R)-epoxy-9 β , 19-cyclostanostane- β , 6 α , 16 β , 25-tetrol] [47,48,49].

3.1.5. Pharmacological Activities on Immune Organs

Generally, the growth of immune organs in good situation is significant to promote the resistance to exogenous pathogens. Spleen, thymus, bursa of Fabricius (birds), and lymphatic tissue are the main immune organs [50,51]. AR and its derivatives play positive roles in promoting the development of immune organs in poultry. For instance, Wei et al. found that APS significantly increased liver, pancreas, spleen, and bursa of Fabricius indexes by adding 0.2%, 0.4%, and 0.6% of APS into the basic diet to one-day-old broilers for 7 days. However, no significant effect of APS on the organ index of 14-day-old broilers was observed, which indicated that APS efficiently promoted the development of immune organs in the early stage [52]. Li et al. gave Astragali Radix extract (ARE) to 7-day-old chickens for 6 weeks by adding 3 mL of ARE (0.2 mg/L) to the water daily. After that, the immune organs, such as thymus, spleens, and bursa of Fabricius, were weighed, and the index of immune organs was calculated. The development of thymus and bursa of Fabricius of the drug-treated group was obviously more significant than that of the control group. In terms of the development of spleen, rapid growth occurred before they were 35 days old [53]. also proved that chickens supplemented with 220 mg of APS and 4×10^{10} colony-forming units (CFU) probiotics to per kilogram of feed significantly improved the quality of the immune organs including thymus, spleen, and bursa of Fabricius. The combination of probiotics and APS in the feed displayed synergistic effects on the intestinal microorganisms and the immune system, which was extremely significant for the exploration of new agents of immunity regulation [54]. Besides, the ultrafine powder of the aerial part of AR showed a promotion in the development of immune organs of chickens [55]. Showed that APS decreased the expression of pro-inflammatory cytokines, cytotoxicity, and apoptosis induced by ochratoxin A via activating the 5' AMP-activated protein kinase (AMPK)/Sirtuin 1 (SIRT-1) signaling pathway and protected spleen cells from immune stress [56]. AR and

ARE significantly affected the function and development of immune organs in mammal models. In order to observe the regulating effect of long-term administration of ARE, [57]. Treated mice with ARE (5, 25, and 50 g/kg) by intragastric administration for 30 days. As a consequence, the long-term administration of ARE contributed to strengthening the spleen index of mice [58]. In another study, APS group was subcutaneously injected with APS (250 mg/kg) once a day. APS-Gel group 1 was subcutaneously injected with APS gel (1500 mg/kg) on the 4th day. APS-Gel group 2 was subcutaneously administered with APS gel (1000 mg/kg) on the 4th and 7th days. The compatibility of APS and gel effectively reduced the frequency of administration and improved the immune organ index of mice through subcutaneous injection [59]. Notably, some studies have revealed that the immunoregulatory activity of AR on immune organs was obviously dose dependent. Liu et al. showed that APS induced a notable improvement in the thymus index and a reduction in the spleen index of tumor-bearing mice. Indeed, the spleen and thymus indexes of the mice in the high-dose group (300 mg/kg) were optimum [60]. Moreover, Kuo et al. showed that intragastric administration of AF (20, 50, and 100 mg/kg) consisted of demethylhomopterocarpin (19), formononetin (20), and ononin (21) to rats once a day for 6 weeks significantly enhanced immune function by ameliorating the reduced spleen cell proliferation and balancing the abnormal cytokine levels in rats [61]. Chronically, the regulatory effects of AR and ARE on immune organs is comparatively prominent in TCM. However, the adequate elucidation of the relevant mechanism is still lacking and many researchers have attempted to explore. Found that APS administration activated toll-like receptor (TLR) 4 pathway in the bursa of Fabricius through myeloid differentiation primary response gene 88 (MyD88) independent pathway [62]. Moreover, AR injection has been proven to have a significant anti-thymic atrophy effect and inhibition of thymic lymphocyte apoptosis, which were related to the up-regulation of Bcl-2 expression [63].

immune mucosal immune system refers to the lymphoid tissue that is widely distributed in the gastrointestinal tract, urogenital tract, respiratory tract submucosa, and some exocrine glands. They are the main sites for local nonspecific immune function. The mucosal immune system is mainly composed of mucosa-bound lymphoid tissues. It exerts the immunoregulatory activity by producing secretory immunoglobulin A (SIgA) and antigen-specific cell-mediated cytotoxicity, and secreting regulatory cytokines by regulatory cells located in the mucosa [64]. Intravenously injected APS (139 mg/kg) with different molecular weights (157.7×103 , 69.9×103 , 22.4×103 , 13.2×103 , and 1.4×103 Da) into the mucosal immunosuppressed mice for 14 days. The levels of SIgA in the small intestine and lung lavage fluid, as well as the changes in the specific aggregate lymph nodes (Peyer's patches) of gastrointestinal mucosa-bound lymphoid tissues were detected to evaluate the immunoregulatory effects of APS on mucosal immunity. APS with large molecular weight significantly increased the phagocytic index and rate, and the secretion of SIgA in the intestine and lung [65]. Another research proved that APS significantly promoted the proliferation of mice intestinal mucosa $\gamma\delta$ T cells, increased the mRNA levels of granzyme B (GrB), interferon (IFN)- γ , and Fas and Fas Ligand (FasL) in $\gamma\delta$ T cells, enhanced the viability and cytotoxicity of $\gamma\delta$ T cells in the intestinal mucosa of APS-treated mice, and increased the secretion of IFN- γ and tumor necrosis factor- α (TNF- α), whereas the transformed growth factor (TGF)- β and interleukin (IL)-10 levels were significantly decreased [66]. In addition, Ou et al. investigated the activities of APS on the inflammatory mediators and mast cells induced by cyclophosphamide (CTX). APS (500 mg/kg) mixed with the feed obviously reversed the decrease in the number of mast cells and intestinal histamine content declining caused by CTX, thereby enhancing the mucosal immune function of piglets [67]. According to the above research, the regulation of AR on mucosal immune is mainly mediated by the increase of SIgA level. Besides, it is worth noting that AR can also up regulate the expression of $\gamma\delta$ T cells.

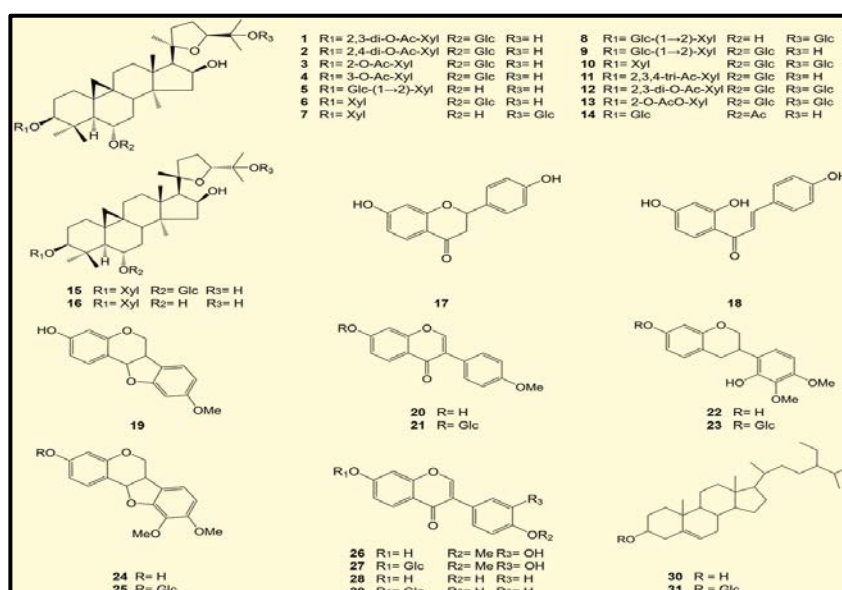


Figure 4. Chemical structures of small molecular compounds associated with immunity in AR (Ac: acetyl; Glc: β -d-glucopyranosyl; Xyl: β -d-xylopyranosyl)

Table 3. The effect of AR or AR derivatives on the immune system

Models	Dosage	Immunomodulatory actions	References
1-day-old broilers	0.2%, 0.4%, and 0.6% of APS were added to the basic diet for 7 days	Promoted the development of immune organs in the early stage	[68]
Chickens	220 mg of APS and 4 × 10 ¹⁰ CFU probiotics were added to per kilogram of feed	Ameliorated the quality of immune organs such as spleen, thymus and bursa of Fabricius	[49]
Tumor-bearing BALB/c mice (H22 cells)	75,150, and 300 mg/kg were given by oral administration once daily for 15 days	Maintained the normal function of immune organs, upregulated the percentage of lymphocyte subsets, and improved the pinocytosis of macrophages	[43]
Four-week-old Yorkshire pigs	5, 10, and 20 mg/kg of APS were intramuscularly injected once a day over three successive days	Enhanced mRNA expressions of IFN- γ and IL-6, and upregulated the titer of FMDV-specific antibody	[69]
Weaned piglets	Basal diet supplemented with 800 mg/kg of APS for 28days	Increased body weight. Exhibited higher level of serum IgM. Increased the expressions of NF- κ B, TLR4 and MyD88	[70]
7-day-old	Chickens 20, 40, and 80 mg/kg of APS were given by oral administration for 7 days	Activated the cTLR4 pathway in bursa of Fabricius	[71]
Peritoneal macrophages of mice	1, 10, 100, and 200 μ g/mL	Enhanced the killing effect of peritoneal macrophages on melanoma cells in mice	[72]
Nile tilapia (Oreochromis niloticus)	1500 mg of APS was added to per kg of diet, fed twice a day for 6 weeks	Ameliorated the phagocytic activity and respiratory burst of macrophages, and promoted activities of plasma lysozyme, the bactericidal, SOD, glutathione peroxidase (GPx), and amylase	[73]
Female B6C3F1 mice; RAW 264.7	10 and 30 mg/kg; 10, 50, and 100 μ g/mL	Stimulated macrophages to express iNOS gene by activating NF- κ B/Rel, and reduced expressions of NF- κ B/Rel binding complexes and NO	[74]
RAW264.7 macrophage	12.5, 25, 50, and 100 μ g/mL	Increases expressions of NF- κ B protein, and cytokines including TNF- α , GM-CSF, and NO	[75]
SplenicDCs, CD11chighCD45RBlow DCs,CD11clowCD45RBhigh DCs, splenic CD4+ T cells of male BALB/cmice	50, 100, and 200 μ g/mL	Promoted the differentiation of splenic DCs to CD11chighCD45RBlow DCs through shifting of Th2 to Th1 with improvement of T cells immune function <i>in vitro</i>	[49]
Dexamethasone induced Twomonth-old immunosuppressive male Chinese Countryside Dogs	50, 100, and 200 mg/kg of APS were given by intravenous injection	Up-regulated expressions of ANAE+ and CD4+ T cells, and inhibited CD8+ T cells. Improved contents of IL-2 and INF- γ , the cytophagic index, and the percentage of peritoneal macrophages	[67]

4. Conclusion

This paper reviews the chemical composition, extraction methods and chemical analysis and biological activity of AR, a highly valued herb that is both medicinal and edible, and therefore phytochemical and pharmacological studies of AR are becoming increasingly attractive. Although research on AR has been conducted for a long time and an increasing number of interesting compounds have been isolated and identified based on the development of new extraction methods and analytical methods, many problems with the application of AR in clinical treatment are still evident due to its complex chemical composition and poor quality control. Further research is needed to clarify the compounds of AR and to standardize their quality control with the help of extraction methods and analytical methods, which may lay the foundation for the further development of traditional Chinese medicine. Regarding the standardizations of AR, there are at least three levels of criteria, namely potency criteria, bioactivity criteria and production criteria, which still need further researched in depth. AR promotes the development of immune organs, enhances mucosal immune function, increases the quantity and phagocytic capacity of innate immunity, promotes the maturation and differentiation of acquired immunity cells, and improves the expression of antibodies in acquired immunity. We believe that AR has a broad research space in the adjuvant treatment of immune

related diseases, which could be a breakthrough point to improve the application value of AR

Acknowledgements

The authors are thankful to project number breeding project of Shanxi agricultural university (YZGC086). for financial support of this study.

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