

Enrichment and purification process of astragalosides and their anti-human gastric cancer MKN-74 cell proliferation effect

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Abstract

Background: *Radix astragali* mainly contains saponins, polysaccharides, flavonoids, amino acids and other chemical constituents of which total astragalosides have immunomodulatory, anti-viral, hepatoprotective, and gastric mucosa protective effects.

Objective: To investigate the process conditions for extraction, purification and enrichment of total astragalosides by macroporous adsorption resin, and to study the inhibitory effect of total astragalosides on growth of human gastric cancer cell line MKN-74.

Methods: UV spectrophotometry was applied to determine the adsorption and desorption capacity of macroporous adsorption resin on total astragaloside content, MTT assay was used to determine the inhibition of MKN-74 cell growth by total astragalosides.

Results: The dynamic adsorption performance of DA201 adsorption resin was examined, and the dynamic adsorption curve of total astragalosides on DA201 resin column was plotted. Meanwhile, eluent and elution flow rate were investigated, the results showed that the choice of eluent of 80% ethanol, and a flow rate of 5 BV/h could maximize the yield of total astragalosides. MTT assay found that astragalosides could relatively pronouncedly inhibit the proliferation of MKN-74 cells, and the inhibitory effect was enhanced with the increase of astragaloside dose and the extension of processing time, which showed a dose-and time-dependence.

Conclusion: DA201 resin can effectively enrich total astragalosides, total astragalosides have an inhibitory effect on growth of MKN-74 cells.

Key words: Total astragalosides, DA201 Resin, MKN-74 Cell

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Introduction

Radix Astragali is the dried root of perennial herbaceous plants *Astragalus membranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge) Hsiao or *A. membranaceus* (Fisch.) Bunge of family Leguminosae.¹ It mainly contains saponins, polysaccharides, flavonoids, amino acids and other chemical constituents,²⁻³ of which total astragalosides have immunomodulatory, anti-viral, hepatoprotective, and gastric mucosa protective effects.⁴⁻⁵ In this paper, process conditions for extraction, purification and enrichment of total astragalosides by macroporous adsorption resin were investigated, and the inhibitory effect of total astragalosides on growth of human gastric cancer cell line MKN-74 was studied.

Materials

Reagents and drugs

Radix Astragali extract, self-prepared, containing 51.37% of total astragalosides; human gastric cancer cell line MKN-74, purchased from the Cell Bank of Beijing Institute of Cell Biology, CAS. DMSO, MTT (Sigma, USA), fetal calf serum (Gibco), RPMI 1640 medium (Invitrogen).

Instruments

Model 680 microplate reader (Bio-Rad, USA); General TV-1221 UV-vis spectrophotometer (General, Beijing); CO₂ incubator (model CO-150, NBS, USA); SW-CJ-SF clean bench (Suzhou Purification Equipment Factory, Sujing Group).

Methods

Pretreatment of resin

Newly purchased DA201 and D101 macroporous resins were soaked in 2 column bed volumes (BV) of 95% ethanol for 24 h, respectively; after fully swollen, the resins were eluted with ethanol, and then with distilled water until there was no smell of ethanol and set aside.

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Preparation of sample solution

Radix Astragali extract was weighed and placed in the beaker, dissolved by addition of appropriate amount of distilled water ultrasonically for 10 min, and then filtered to give the sample solution.

Method for content determination

8 mg of astragaloside reference substance was accurately weighed, dissolved in anhydrous ethanol to make the volume 50 mL. 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of astragaloside reference substance solutions were accurately aspirated, evaporated to dryness in water bath, added with 0.2 mL of 5% vanillin solution and 0.8 mL of perchloric acid, heated in water bath for 20 min and then removed, cooled to room temperature, added with glacial acetic acid till the volume was 5 mL, respectively, absorbance was then measured at 560 nm with 70% ethanol as blank control. Standard curve was plotted with concentration of reference substance on the X-axis and absorbance on the Y-axis, linear regression equation was obtained as $Y=0.031\ 2X-0.014\ 5$, $R^2=0.999\ 6$.

Determination of resin model

Static adsorption effects of various types of resins were determined with adsorption capacity of astragalosides as the evaluation indicator. 2 g of pretreated DA201, D101 and AB-8 macroporous adsorption resins were taken, respectively, added with excess Radix Astragali sample solution and shaken at room temperature for 48 h.

Study of dynamic adsorption effect

10 mL of pretreated resin was taken and loaded on the column by wet packing method, Radix Astragali extract (mass concentration of total astragalosides of 0.85 mg/mL) was passed through the chromatographic column at a flow rate of 2 BV/h, astragaloside content in the effluent was measured when 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 fold volumes of samples were added, respectively, until the resin adsorption was saturated, dynamic adsorption curve of total astragalosides on DA201 resin column was plotted.

Effect of flow rate on adsorption

10 BV extract was passed through the chromatographic column containing 10 mL of resin at different flow

rates, total astragaloside content in the effluent was detected, and the effect of flow rate on resin adsorption capacity was investigated.

Selection of eluent

1 g of saturated adsorption resin was taken in 6 replicates, and water, 10%, 30%, 50%, 70% and 90% ethanol were taken as the eluent, respectively, flow rate was 5 BV/h. Content of total astragalosides was determined, and the elution effects at different ethanol concentrations were investigated.

Inhibition of human gastric cancer MKN-74 cell growth by total astragalosides

Cell cultivation

MKN45 cell lines were cultured in 10% fetal calf serum-containing RPMI 1640 medium, and routinely subcultured in a 37°C, 5% CO₂ incubator, cells in the logarithmic growth phase were collected and set aside.

Detection of the effect of astragalosides on cell proliferation by MTT assay⁶⁻⁷

Well grown cells in the exponential growth phase were taken, digested with 0.25% trypsin, and then prepared into cell suspension with a concentration of 1×10^5 cells/mL, and seeded into 96-well plates at 1×10^4 cells per well, the MKN-74 cells were then treated with different concentrations of total astragalosides (5, 10, 20 $\mu\text{mol/L}$), on the 3rd day, 20 μL of MTT solution was added to each well, and the incubation was continued for an additional 4 h. Supernatant was removed carefully, each well was added with 150 μL of DMSO, and shaken for 10 min, absorbance (A) of each well was measured at 570 nm wavelength on automatic microplate spectrophotometer.

Inhibition rate = (A value of negative control group - A value of experimental group) / A value of negative control group $\times 100\%$.

Results

Result of resin model

After full adsorption, adsorption capacity was calculated based on the absorbance before and after the adsorption, thereby confirming that DA201 adsorption resin had more superior adsorption performance (in Table1).

Table 1: Results for determination of static saturated adsorption capacity of various resins

Model of resin	Drug concentration (mg/ml)	Concentration after adsorption (mg/ml)	Saturated capacity (mg/ml)
D-101	7.5	4.36	39.7
D-201	7.5	4.21	44.5
AB-8	7.5	3.97	48

Result of dynamic adsorption effect

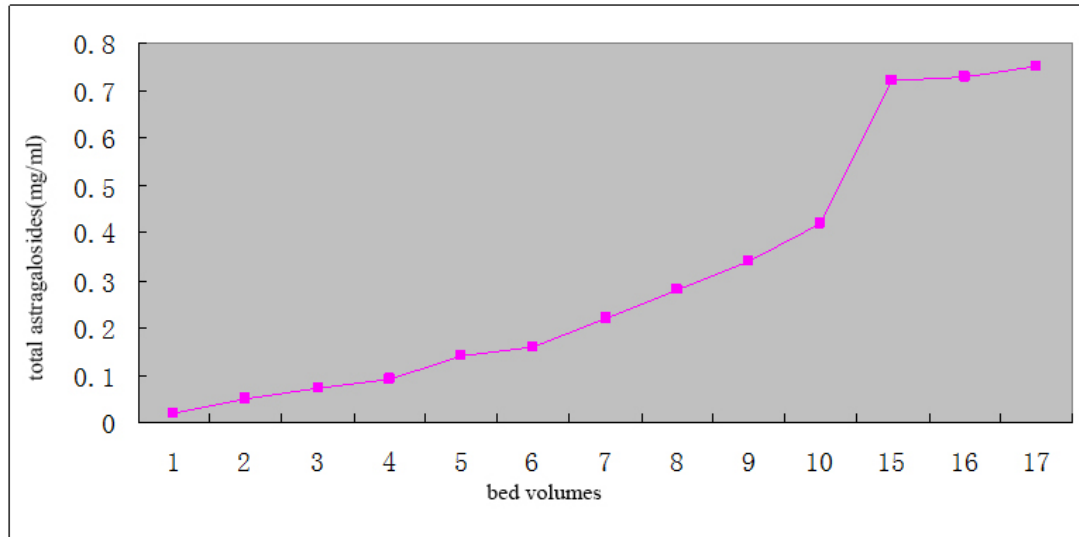


Figure 1: Dynamic adsorption curve of total astragalosides

As can be seen from Fig. 1, total astragalosides had relatively large leakage starting from 10 BV; mass concentration of total astragalosides was rising with the

increase in the number of fractions, after rising to 15 BV, the curve tended to flatten, which meant that the dynamic adsorption of DA201 resin on total astragalosides tended to saturation.

Result of flow rate on adsorption

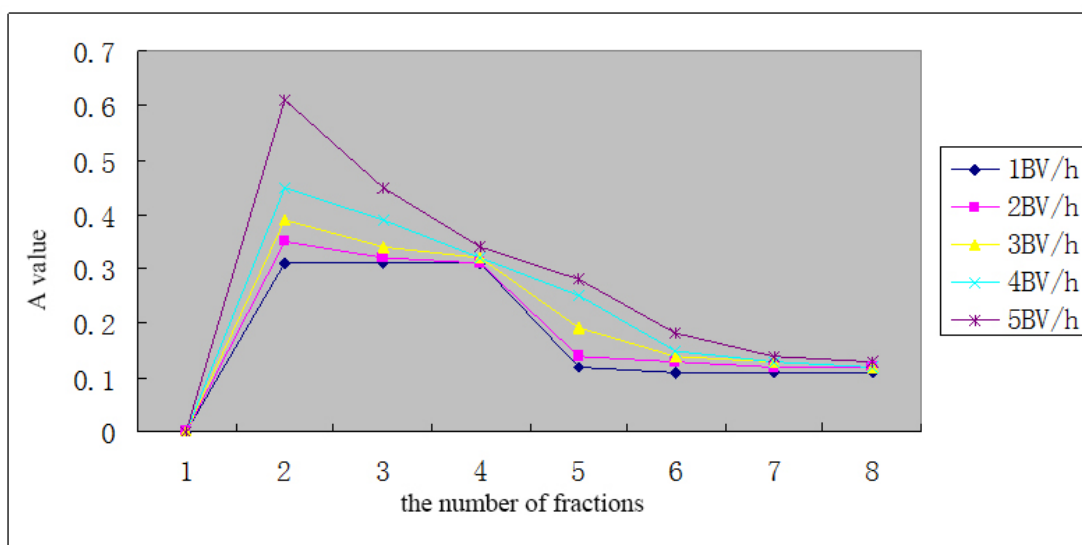


Figure 2: Investigation on adsorption effect of total astragalosides at different elution flow rates

The results showed that the resin had larger adsorption capacity at lower flow rates, adsorption capacity of

resin showed a decreasing trend with the increase of flow rate, after comprehensive consideration of various factors, a flow rate of 5 BV/h was selected in the experiment.

Selection of eluent

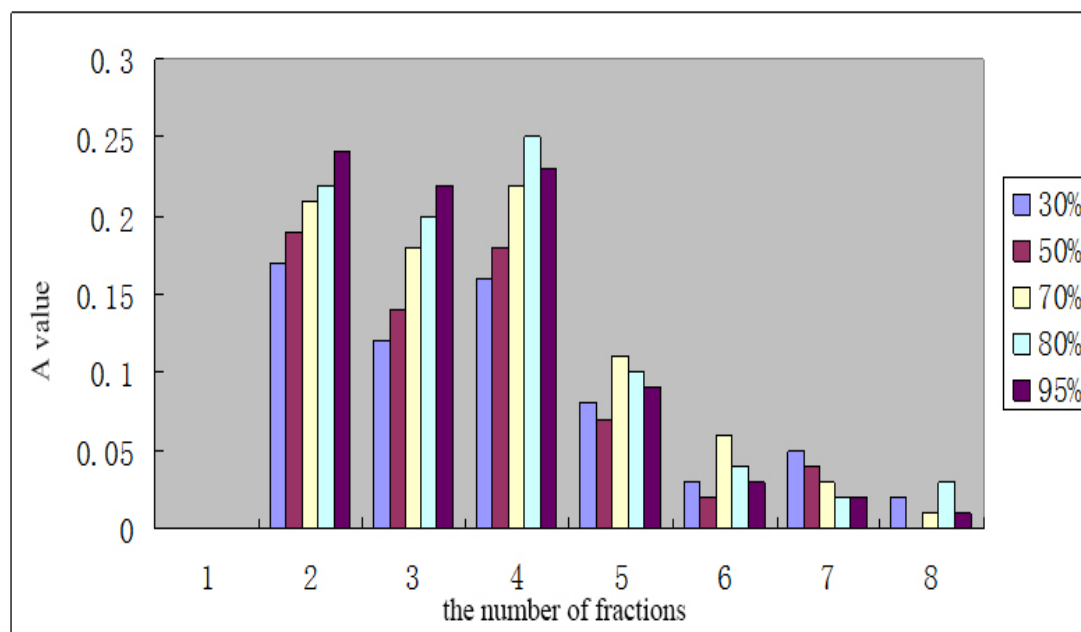


Figure 3: Investigation of elution flow rate

The experimental results showed that the desorption rate of total astragalosides increased with the increase of ethanol concentration, elution effect was the best

when 80% and 95% ethanol were used as the eluent, taking into account the cost savings, 80% ethanol was selected as the eluent finally.

Result of astragalosides on cell proliferation

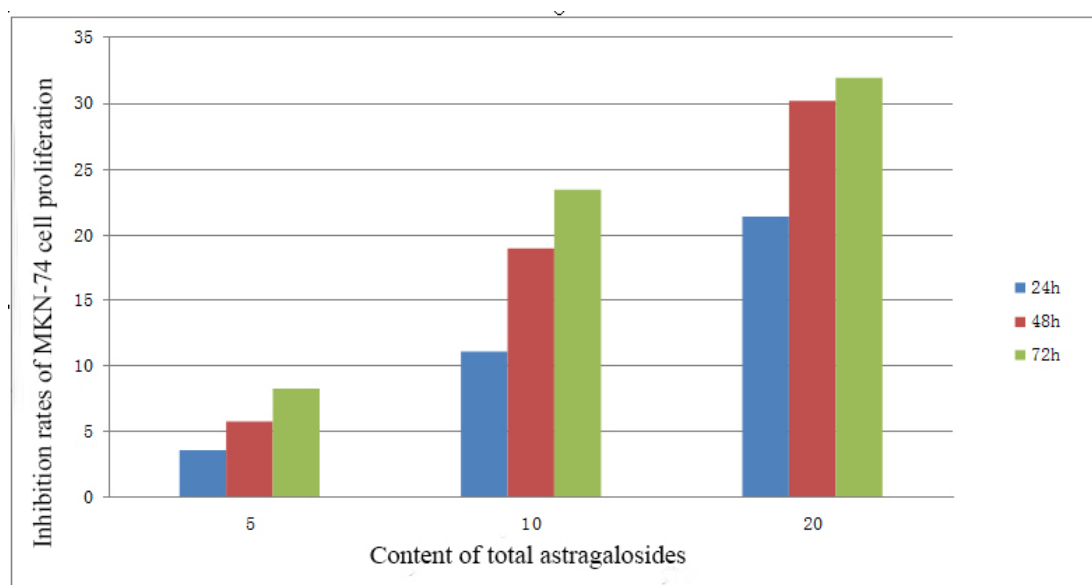


Figure 4: Inhibition rates of different concentrations of total astragalosides on MKN-74 cell proliferation

As can be seen from the experimental results, MTT assay found that astragalosides could relatively pronouncedly inhibit the proliferation of MKN-74 cells, and the inhibitory effect was enhanced with the increase of astragaloside dose and the extension of processing time, which showed a dose-and time-dependence.

Discussion

Macroporous adsorption resin is a new type of high-molecular polymer with large pore size, and relatively large specific surface area.⁸⁻⁹ Owing to its high stability, high adsorption selectivity, easy regeneration, as well as environmental protection and energy conservation advantages, it has been widely used in the extraction, isolation, purification and enrichment of effective parts and monomer chemical compositions in traditional Chinese medicines and natural medicines.¹⁰⁻¹¹

In this experiment, the static adsorption effects of different types of resins were determined with adsorption capacity of astragalosides as the evaluation indicator. After examining the adsorption performance of three resins, namely DA201, D101 and AB-8 macroporous adsorption resins, it was confirmed that DA201 adsorption resin had more superior adsorption performance, so DA201 adsorption resin was selected for the experiment.

In this paper, the dynamic adsorption performance of DA201 adsorption resin was examined, and the dynamic adsorption curve of total astragalosides on DA201 resin column was plotted. Meanwhile, eluent and elution flow rate were investigated, the results showed that the choice of eluent of 80% ethanol, and a flow rate of 5 BV/h could maximize the yield of total astragalosides.

In addition, this paper investigated the inhibition of human gastric cancer MKN-74 cell growth by total astragalosides, and determined the inhibitory effect of total astragalosides on proliferation of human gastric cancer MKN-74 cells by MTT assay. As can be seen from the experimental results, the MTT assay found that astragalosides could relatively pronouncedly inhibit the proliferation of MKN-74 cells, and the inhibitory effect was enhanced with the increase of astragaloside dose and the extension of processing time, which showed a dose-and time-dependence.

It is obvious that the results of this study are as follows: The chemical methods can be good for the enrichment and purification of astragaloside, and the extraction has significantly inhibition on human gastric cancer cell MKN-74. Discovery new pharmacological activity of astragaloside is exciting, but the present study has several shortcomings and deficiencies. Such as this study did not conduct in-depth study on the mechanisms, it could not be explained clearly without the mechanism. In order to further improve the research, we decided to make anticancer mechanism of stragaloside as a research priority. And we hope that it can eventually be replicated in clinical aspects and applications.

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