

The Screening Method of Chinese Herbal Medicine Activity Based on Affinity Chromatography

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Abstract: Drug inhibitors are commonly used in drugs to treat human diseases. China is rich in resources of traditional Chinese medicine. Screening low toxicity and high efficiency drug inhibitors from traditional Chinese medicine is of great significance for discovering innovative lead compounds and creating new drugs with independent intellectual property rights. The aim of this paper is to use affinity ultrafiltration and liquid-mass technology to screen the target enzyme inhibitors in Chinese herbal medicine, providing a new idea and method for the rapid screening and identification of the target enzyme inhibitors in the complex system of Chinese traditional medicine. The acetylcholinesterase affinity ultrafiltration system was established by optimizing the culture conditions and chromatographic conditions of affinity ultrafiltration. The established system was used to screen and identify 5 compounds with significant inhibitory effects from 80% ethanol extracts of rhizoma coptidis. The active compounds were prepared by semi-preparative liquid phase and identified by mass spectrometry and nuclear magnetic resonance spectroscopy. The conclusion of this study was that the free radical scavenging ability of traditional Chinese medicine extract with good tyrosinase inhibitory activity was detected, and its IC₅₀ values were 45.55, 298.87, 1096.6 and 992.27 mgL⁻¹, respectively.

1. Introduction

China has abundant resources of Chinese herbal medicines with a variety of ingredients, including a large number of unexploited natural active ingredients. However, the chemical composition of Chinese herbs is complex and the content of bioactive substances is usually low. The traditional screening method for active ingredients in Chinese herbal medicine is to obtain monomers through systematic separation before further activity testing, which is time-consuming, expensive and inefficient, seriously restricting the rapid discovery of active ingredients [1-2]. Therefore, the development of a new method for rapid in vitro screening and identification of drug inhibitors at the molecular level for complex TCM systems is of great practical significance for accelerating the rapid discovery of natural, safe and efficient drug inhibitors in TCM [3-4].

With the rapid development of natural drug chemistry and life science, new screening methods for drug inhibitors keep emerging, which not only promotes the discovery of drugs, but also has a great impact on the theory and technology of drug screening methods. The research of natural product drugs is generally divided into two steps: one is the discovery and study of the physiological activity of drugs, the other is the study of the application of drugs. The process of drug screening is the preliminary detection of pharmacological activity of potential substances that can be used as drugs, which is the core step of new drug development [5-6]. Traditional screening of active ingredients of natural products firstly obtained monomer compounds through systematic separation, and then monomer compounds were tested for activity through animal models. The whole experimental process was time-consuming, costly and inefficient, which severely restricted the rapid discovery of active ingredients in natural products. How to extract and isolate active ingredients from natural products efficiently and pertinently is the focus of current research in the field of drug screening technology [7-8]. However, the ingredients in natural products are complex,

and the therapeutic effect of many natural drugs is achieved by inhibiting the activity of drugs. With the development of natural product chemistry, more and more natural compounds with good physiological activity have been isolated and identified [9-10]. There are many kinds of plants and animals in nature, but so far only a few have been screened and tested for their activity. In the long-term practice of drug discovery and screening, various screening methods for active substances established by researchers have played a great role in the research of new drug creation [11-12].

In this paper, a microenzyme activity inhibition evaluation method based on porous microenzyme marker was developed and applied to target enzyme activity inhibition evaluation of crude extracts of various Chinese herbal medicines. A rapid screening and identification system for acetylcholinesterase inhibitors based on affinity ultrafiltration was established by using positive control and systematic optimization of affinity ultrafiltration screening conditions. The developed affinity ultrafiltration and fluid-mass screening technology system was used to screen and identify crude extracts of Chinese herbal medicine with good drug inhibition activity.

2.Method

2.1 Molecular Affinity Chromatography

Affinity chromatography is a unique chromatographic separation method based on high affinity and high specificity reversible binding between biological macromolecules in the life phenomenon. A mixture of target molecules and other substances passes through the column. Only the target molecule showing a significant affinity with the ligand in the column can bind to the ligand and remain on the column. Only when the composition of the mobile phase (buffer) is changed is the adsorbed target molecule eluted and separated from other substances. Affinity chromatography emerged in the 1950s for the separation and purification of proteins, particularly enzymes, antigens and antibodies. In recent years, with the continuous development of technology, the application of molecularly imprinted affinity chromatography, membrane affinity chromatography, metal chelating affinity chromatography and other new affinity chromatography is expanding.

Molecular imprinting technology (MIT), also known as Molecular imprinting technology, is the separation of target molecules interact with the specificity of the functional monomer, through the solid particle medium preparation of crosslinking agent, through physical or chemical method to remove the embedding medium in the target molecules, then get the target molecules of spatial structure and binding sites have "memory" or "imprinting" effect of the Molecular imprinting polymer (Molecular imprinting polymer, MIP); In addition to being highly compatible with the template molecules, MIP materials also interact functionally and complement the template molecules in the spatial structure of the cavity (hydrogen bonding, ion effects, or van der Waals forces, etc.). The molecularly imprinted polymer cavities have strong affinity to the matched template molecular structure analogues, but they can not only produce weak surface adsorption with the structures of other compounds on the template molecule. Thus, template molecules and template molecular analogues can be separated from unrelated substances.

2.2 Immunoaffinity Chromatography

Immunoaffinity chromatography is an effective and selective method for the separation and purification of trace components in complex systems by reversible binding of antigens and antibodies. Antibodies bound to solid-phase carriers can be used to isolate desired target compounds from complex biological, pharmaceutical, food, or environmental samples, or to study the magnitude of intermolecular interactions between antibodies and small molecules. Because IAC is based on the antigen antibody specificity and combination between the biological recognition, including electrostatic interaction, van der Waals force, scanty water, the H key role as well as a variety of factors such as the size and shape of the match, rather than due to a single physical chemistry, so on the selectivity and the bonding strength of recognition ability is superior to the general adsorption chromatography, is a kind of high adsorption separation approach for the

analysis of application value.

The steps for chemical separation by immunoaffinity chromatography are as follows: first, specific antibodies are attached to the donor, the donor is prepared, and the donor is then filled into the column. After the column, the target material is retained in the column by the antigen-antibody binding reaction with the antibody on the adsorbent, while the other groups are eluted directly. Then, the target substance is eluted with appropriate eluent to achieve the purpose of separation and purification. Monoclonal antibodies and polyclonal antibodies have advantages in the separation and purification of the active ingredients of Chinese herbs by immunoaffinity chromatography. If a polyclonal antibody is immobilized on a column, and each clone of the antibody contains some of the structural characteristics of the antigen, the screening object can show biological activity as long as it has an effect on one clonal antibody, so that several substances with similar effects can obtain activity. In practice, antibodies of different properties can be selected for different purposes.

3. Experiment

The experiments were carried out by the improved Ellman method. First, the sample was dissolved in 50% ethanol with a sample solution of 20 solution L, followed by 80 solution LPBS, 40 solution L2.5mM DTNB and 20 enzyme solution ($1.0 \mu\text{mL}^{-1}$). The samples were oscillated and mixed, incubated at 37°C for 10 min, then 40 w L10mM substrate was added, reacted at 37°C for 10 min, then SDS termination reaction was performed at 60 μL %. Then, 20L was dissolved in a sample solution of 50% ethanol and various reagents were added in turn. Then, measure A405, represented as sample A. At the same time, solvent control was carried out for each batch of samples, in which 50% ethanol was used to replace the ethanol solution of the samples, and the absorbance was expressed as A control. Blank control was performed again, namely 40 μL PBS instead of substrate AChE, denoted as blank A. The absorption value was measured at 405nm and the inhibition rate was calculated according to formula (1).

$$(\%) = [A_{\text{contrast}} - (A_{\text{the sample}} - A_{\text{blank}})] / A_{\text{contrast}} \times 100 \quad (1)$$

80% ethanol extract, 80% ethanol extract of urticaria root and salvia miltiorrhiza ethylsalvia with significant inhibitory activity on acetylcholinesterase were selected. The reserve of the extract is precisely absorbed and diluted with 50% ethanol into eight concentration gradients ($50\text{-}2000\text{mgL}^{-1}$). The improved Ellman microfiltering model is adopted and the method described above is followed. The inhibitory activity of acetylcholinesterase was calculated and IC_{50} (mgL^{-1}) value was calculated according to the absorbance of each reference substance, sample and blank sample.

4. Analysis and Discussion

4.1 Determination of AChE Inhibitory Effect IC_{50} Value of Traditional Chinese Medicine Extract

As shown in Table 1, the concentration of 80% ethanol extract of cortex phellodendri, 80% ethanol extract and 80% ethanol extract, ethyl salvia salvia extract, and 80% ethanol extract of rhizoma coptidis IC_{50} were 295.12, 229.09, 1202.26 and 56.23mgL^{-1} , respectively. The IC_{50} value of 80% ethanol extract of rhizoma coptidis was the lowest, the IC_{50} value of 80% ethanol extract of rhizoma corydalis was the lowest, and the IC_{50} value of salvia miltiorrhiza was the highest. These four Chinese herbal extracts have certain inhibitory effect on the activity of acetylcholinesterase, but there are certain differences between the IC_{50} values of alkaloids in the positive control group, which may be related to the low content of AChE's inhibitory active ingredient. The activity of an extract or the complex matrix components of an extract.

Table 1. Inhibition of acetylcholinesterase in Chinese herbal extracts (n=3)

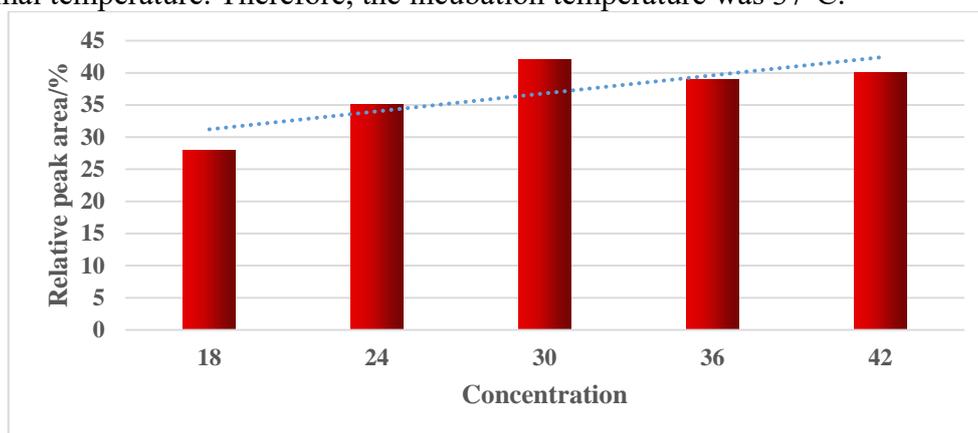
Extracts	Standard curve	IC ₅₀ (mgL ⁻¹)	Correlation coefficient r
Phellodendron japonica 80% ethanol extract	Y=47.56x-67.5363	295.12	0.9830
80% ethanol extract of Chinese herb	Y=36.749x-38.974	229.09	0.9948
Ethyl salvia extract	Y=53.178x-113.94	1202.26	0.9913
Rhizoma coptidis 80% ethanol extract	Y=47.558x-33.388	56.23	0.9912
Physostigmine	Y=58.388x+89.335	0.21	0.9996

The main components of 80% ethanol extract of rhizoma coptidis are alkaloids. The separation and retention effect of methanol water as mobile phase extract is poor. Acetonitrile and water were used as mobile phase. Therefore, acetonitrile-water was selected for further separation. The effects of adding different types of weak acid and buffer salt in the mobile phase on the chromatographic separation results were studied. The results showed that when an aqueous solution containing 0.5% formic acid and 20mM ammonium formate was selected as mobile phase A, the separation of the compound in 80% ethanol rhizome extract was good, with sharp peaks and stable baseline. Therefore, acetonitrile-0.5% formic acid and 20mM ammonium formate were selected for HPLC analysis of mobile phase of 80% ethanol extract from rhizoma coptidis.

4.2 Optimization of Affinity Ultrafiltration Experimental Conditions

In the experiment, the effect of incubation temperature (20,37,45°C), incubation time (15,30,45,60min), and acetylcholinesterase concentration (18,24,30,36,42 M) on the screening effect was examined by using anabeline as the positive control.

First, the incubation temperature was optimized, and three temperatures were selected at 20, 37 and 45°C. Other conditions were fixed: incubation time was 30min, enzyme concentration was 24m. After ultrafiltration was performed according to the affinity ultrafiltration screening method, the liquid phase was measured. The amount of toxic lentil alkaloid added was set as 1 as a reference, and was compared with the amount of toxic lentil alkaloid screened. The results showed that when the temperature was 37°C, the binding amount of enzyme and alkaloid was the maximum, which was the optimal temperature. Therefore, the incubation temperature was 37°C.

**Figure 1.** The Acetylcholinesterase concentration optimization

For the optimization of enzyme concentration, 5 concentration points of 18,24,30,36 and 42 M were selected in the experiment. Other conditions were fixed: incubation temperature was 37°C, incubation time was 30min, ultrafiltration was performed according to the affinity ultrafiltration screening method, and the liquid phase was measured. The amount of toxic lentil alkaloid added was set as 1 as a reference, and was compared with the screened lentil alkaloid, as shown in figure 1.

The results showed that when the enzyme concentration was 30 μM , the binding amount of the enzyme and the toxic lentil base was the largest, which was the optimal enzyme concentration. Therefore, the enzyme concentration was chosen to be 30 μM . For the investigation of incubation time, 4 time points were selected for the experiment: 15, 30, 45, 60 min. Other conditions were fixed: incubation temperature was 37°C, enzyme concentration was 24 °C M, ultrafiltration was performed according to the affinity ultrafiltration screening method, and the liquid phase was measured. The amount of toxic lentil alkaloid added was taken as a reference, set as 1, and compared with the screened toxic lentil alkaloid. The results showed that the binding amount of enzyme and toxic lentil alkaloid was the largest at 30 min incubation, which was the optimal incubation time, so the incubation time was selected as 30 min in the experiment.

5. Conclusion

In this paper, the inhibitory activities of tyrosinase in 21 kinds of traditional Chinese medicine extracts with different polarity were studied by microscreening model based on enzyme marker. The free radical scavenging ability of four crude extracts with good tyrosinase activity was determined. Although the synergistic effect between tyrosinase inhibition and free radical scavenging ability of samples is still uncertain, the compounds with free radical scavenging ability can help to inhibit tyrosinase activity to a certain extent. This study laid a foundation for further screening compounds with both drug inhibitory activity and free radical scavenging activity from Chinese herbal medicine.

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