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Research on Purification Process and Pharmacological Action of Andrographolide

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Abstract This paper reviews the purification process, content determination methods and pharmacological action of Andrographolide, aiming to provide new ideas for the subsequent study of Andrographolide and its related drug development and application.

Key words Andrographolide, Purification process, Content determination, Pharmacological action

1 Introduction

Andrographis Paniculata, also known as *Chun Lian Qiu Liu*, *Yi Jian Xi*, *Lan He Lian*, *Ku Dan Cao* in Chinese, *etc.*, is widely distributed and mostly grown in the provinces of Fujian, Guangdong, Hainan, Guangxi, Yunnan, and other places in China. It is an annual herb belonging to Andrographis, preferring a hot and humid climate, with strong efficacy in removing heat and detoxification, anti-inflammatory and pain relief, liver and bile preservation, *etc.* It is often used in the treatment of bacillary dysentery, dyspepsia, venomous snake bites, neuralgia dermatitis, and other diseases[1]. Andrographolide, also known as *Chuan Xin Lian Yi Su or Xiong Rong Nei Zhi* in Chinese, is a diterpene lactone extracted from Andrographis Paniculata. Its molecular formula is $C_{20}H_{30}O_5$, and it is easily soluble in organic solvents like methanol and ethanol, with a melting point of 230 - 231 ℃[2]. Andrographolide has a variety of pharmacological activities, such as antitumor, anti-inflammatory, antibacterial, and antiviral. This paper provides a comprehensive review of the purification process, content determination methods, and pharmacological action of Andrographolide, aiming to provide a theoretical basis for further research and application of Andrographolide.

2 Purification process of Andrographolide

Alkaloids, terpenoids, and other chemical components in natural Chinese herbal medicines have shown promising therapeutic effects on cancer patients. The extraction, isolation, and purification of effective substances with antitumor activity from Chinese herbal medicines are of great significance for the application of natural Chinese medicines in oncology. Compared with traditional extraction methods such as water vapor distillation, solvent extraction, water extraction, ultrasonic extraction, and microwave extraction, modern extraction technology has the advantages of high extraction efficiency, high purity, and low production costs. With the increasing development of biotechnology, the optimization and screening of a new, efficient, and green extraction process for the active ingredients of traditional Chinese medicine has become a hot spot in pharmaceutical engineering.

In the extraction research of Andrographolide, Zhou Rushun *et al.*[3] used the ultrasonic extraction method to mix Andrographolide with methanol for extraction, with methanol as a blank control, and measured the absorbance at 225 nm. They determined the sample content using high-performance liquid chromatography, optimized the extraction conditions using single-factor and orthogonal experiments, and found the optimal ultrasonic process to be 60% ethanol concentration, 1 h of ultrasonic time, and a liquid-to-material ratio of 1 ∶ 15. This method significantly increased the extraction rate by switching wavelengths to determine the maximum absorbance of Andrographolide, and it has the advantages of simplicity, high extraction efficiency, as well as stability and feasibility. Zhao Yongmei *et al.*[4] mixed Andrographis Paniculata powder with ethanol with the ultrasonic extraction method, followed by microwave drying, ultrasonic cleaning, ethanol dissolution, and filtration to obtain the final product. They optimized the extraction conditions with orthogonal experiments and determined the optimal extraction process for Andrographolide to be 90% ethanol concentration, a liquid-to-material ratio of 1 ∶ 8, and an extraction time of 10 min, with an Andrographolide content of 23.7%. This method has the advantages of short extraction time, low extraction temperature, and wide adaptability. Yang Tao *et al.*[5] used the ultrasonic extraction method to mix Andrographolide with 75% ethanol and added into a measuring flask. They measured the absorbance at 224 nm and calculated the mass concentration of the extract. They used high-performance liquid chromatography to determine the sample content and used central composite design and response surface method to determine the ultrasonic extraction conditions as 70% - 80% ethanol concentration, 50 - 70 min of extraction time, and a solvent ratio of 10 - 12 times. This method optimized the ultrasonic extraction process of Andrographolide and has the advantages of simplicity, fewer ex-

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perimental trials, and high experimental accuracy. Ge Fahuan *et al.*[6] added the active ingredients of Andrographis Paniculata into the supercritical extraction kettle with supercritical CO_2 extraction method. The products were extracted step by step after the adjustment of temperature, CO_2 flow rate and the addition of ethanol. They used high-performance liquid chromatography to determine the sample content, optimized the extraction conditions using orthogonal experiments, and determined the optimal extraction pressure, temperature, and CO_2 flow rate of Andrographolide to be 25 MPa, 46 ℃, and 40 kg/h, respectively. Under the optimal experimental conditions, the Andrographolide content was 6.87%. This method has the advantages of good predictability, time-saving and easy operation. Huang Qiong *et al.*[7] mixed Andrographis Paniculata flavonoids with 80% ethanol solution by the microwave-assisted extraction method. The absorbance was measured at 510 nm and a standard calibration curve was plotted. The total flavonoid content was determined using UV spectrophotometry. The final product was obtained through ethanol dissolution filtration and microwave-assisted extraction. The ultrasonic extraction process conditions were optimized using single-factor, orthogonal experiments, and variance analysis, with the microwave power set at high, microwave action time at 6 min, solid-liquid ratio at 1∶50, ethanol concentration at 60%, and a total flavonoid extraction rate of 4.945%. This method was optimized through response surface experiments, which has the advantages of safety and environmental protection, high extraction efficiency and simplicity of steps. Ding Zheng *et al.*[8] employed a microwave-assisted extraction method to mix Andrographis Paniculata powder with 60% alcohol, and then added with activated carbon for decolorization and filtration. The final product was obtained after concentration by rotary evaporator, and the content of Andrographolide was measured by spectrophotometry. They used single-factor orthogonal experiment to determine the ultrasonic extraction conditions for Andrographolide to be using low-fire power microwave, microwave time of 4 min, pH 2. This method has the advantages of low consumption, high recovery efficiency and easy operation. The above extraction method, based on natural plant resources, greatly increased the extraction rate of Andrographolide, with the advantages of stable quality, high purity, and minimal losses, which provided a theoretical reference for the optimization of the purification process of Andrographolide.

3 Content determination of Andrographolide

Content determination of active ingredients in natural Chinese medicine plays a crucial role in drug analysis and quality control of Chinese medicine. The active substances in traditional Chinese medicine usually cannot be observed directly with the naked eye. In order to ensure the accuracy of the content determination results, analysis can only be carried out by specific analytical instruments. When determining a certain content in a sample, it is necessary to select the appropriate instrument and corresponding determination conditions based on the chemical composition characteristics of natural Chinese medicine. Due to the specificity and complexity of Chinese medicine standards, the selection of content determination methods is of utmost importance. For drugs or samples with a low content of ingredients or more complex components, thin layer analysis can be used for determination.

In the determination of Andrographolide content in Andrographis Paniculata, Yuan Jianhua *et al.*[9] determined the content with high-performance liquid chromatography. The Andrographis Paniculata powder was mixed with methanol and put into an ultrasonic apparatus to be extracted and filtered, and the absorbance was measured at 225 nm. The product was purified, and the content was determined using high-performance liquid chromatography. By studying the detection wavelength, the detector temperature, and the sample flow rate, the optimal conditions for content determination were determined to be column temperature, mobile phase, flow rate, and detection wavelength of 40 ℃, methanol∶water (6∶4), 1.0 mL/min, and 225 nm, respectively. This method has the advantages of simplicity, convenience, high precision, and accurate measurement. Liu Hong *et al.*[10] determined the content of Andrographolide in Andrographis Paniculata by high-performance liquid chromatography. Andrographolide was extracted and filtered by ultrasonic extraction after mixing Andrographis Paniculata powder with methanol. The absorbance was measured at 225 nm, and the standardized curve was plotted. The content of Andrographolide was determined by the spiked sample recovery method. This method has the advantages of high accuracy, good reproducibility, and high sensitivity. Lin Mingrui *et al.*[11] determined the content of Andrographolide in Andrographis Paniculata with spectrophotometry. Andrographis Paniculata powder was mixed with acetone and then added into a conical flask. The content of Andrographolide was determined by the rotary evaporation method, and the absorption wavelength was measured at 200 – 400 nm using a UV scanner after methanol fixation. The method has the advantages of high separation efficiency, high accuracy, and good reproducibility. Zhang Yingfeng *et al.*[12] determined the content of Andrographolide in Andrographis Paniculata with high-performance liquid chromatography. The Andrographis Paniculata powder was mixed with methanol and then extracted by ultrasonic extraction and cooled. The absorbance was measured at 228 nm, and linear regression was processed. The products were analyzed and determined by the external standard method, and the optimal conditions for content determination were determined to be a mobile phase, flow rate, and detection wavelength of methanol∶water (45∶55), 1.0 mL/min, and 228 nm, respectively, according to the spiking recovery tests. This method has the advantages of high specificity, high accuracy, and stability. The above method is a scientific and standardized method for the determination and quality control of Andrographolide, which provides a more efficient, accurate, and simple method for the de-

termination of Andrographolide. The above content determination methods scientifically and normatively determine the content and quality control of Andrographolide, which provides more efficient, accurate, and convenient methods for the content determination of Andrographolide.

4 Studies on pharmacological activity of Andrographolide

4.1 Anti-cancer effect The occurrence of cancer is a complex, multi-step process, mainly caused by various carcinogenic factors such as genetics, immune factors, infections, and chemical carcinogens. When various factors in the human body are imbalanced, normal cells mutate or differentiate into cancer cells. In China, the incidence of cancer is increasing year by year due to environmental pollution, food safety problems, and changes in people's lifestyles. The World Health Organization (WHO) has pointed out that China has the largest population in the world and is also one of the countries with the highest incidence and mortality rates of cancer.

Huang Ju *et al.* [13] detected the inhibitory proliferative effect of Andrographolide on Glioma U87-MG cells by MTT assay and found that Andrographolide had a good inhibitory effect on the proliferation of Glioma U87-MG cells after 48 hours of treatment with different concentrations (3.125, 6.25, 12.5, 25, and 50 μM) of Andrographolide. The pro-apoptotic effect of Andrographolide on Glioma U87-MG cells was further detected with the Western blotting method, which revealed that Andrographolide can up-regulate the expression level of the pro-apoptotic protein Bax and down-regulate the expression level of the anti-apoptotic protein Bcl-2. Xu Hui *et al.* [14] detected the inhibitory proliferative effect of Andrographolide on Hep-G2 cells by the CCK-8 assay. The study showed that the inhibitory proliferative ability of Andrographolide on Hep-G2 cells continuously strengthened with the increase of the concentration of Andrographolide (0, 5, 10, 20, 30, 40, 50, and 60 mg/L) and the prolongation of the administration time. They further detected the pro-apoptotic effect of Andrographolide on Hep-G2 cells with the Annexin-V FITC/PI double staining assay, which showed that the apoptotic number of Hep-G2 cells was significantly elevated with the increasing concentration (0, 5, 10, and 20 mg/L) and prolongation of the action time of Andrographolide. Qin Huizhen *et al.* [2] detected the inhibitory proliferation effect of Andrographolide on the proliferation of HOS and H_2O_S cells by MTT assay, and found that Andrographolide had a good inhibitory proliferation effect on HOS and H_2O_S cells after treatment with different concentrations of Andrographolide (5, 10, 20, 50, and 100 μM) for 24 h. They further dectected the cycle blocking effect of Andrographolide on HOS and H_2O_S cells with flow cytometry and western blotting, which revealed that with the elvation of the concentration of Andrographolide and the prolongation of its action time, the expression level of pro-apoptotic proteins p53 and Bax improved, and the number of cells in G_1 phase gradually increased, while the number of cells in S and G_2 phases gradually decreased. The above results indicate that andrographolide can induce apoptosis in cancer cells by blocking the S to G_2 phase in the cancer cell cycle.

4.2 Anti-inflammation effect Inflammation refers to a non-specific immune response of the body's local or systemic tissues caused by various external stimuli. The inflammatory response includes acute inflammation, subacute inflammation, and chronic inflammation. Clinically, it is mainly manifested as fever, chills, skin rash, and edema. Inflammatory mediators are a complex composed of inflammatory cytokines, chemokines, matrix metalloproteinases, and other cytokines, which play an important role in the occurrence and progression of inflammatory reactions.

Gupta S *et al.* [15] investigated the inhibitory inflammatory effect of Andrographolide on arthritis by protein immunoblotting assay and found that Andrographolide can effectively inhibit the expression levels of COX-2, p-p38, CD40, TNF-α, IL-1β, and IL-6. Li Yu *et al.* [16] examined the effect of Andrographolide on lipopolysaccharide (LSP)-induced inflammatory cytokine expression in mouse mammary tissues by enzyme-linked immunosorbent assay (ELISA) and quantitative fluorescence PCR (qRT-PCR) and found that, with the increase of Andrographolide injection dosage (2.5, 5, and 10 mg/kg) and administration time, the inhibition rate of TNF-α, IL-1β, and IL-6 protein expression levels significantly increased. Further study on the protein expression level of NF-κB in breast tissues by Western blotting revealed that Andrographolide can significantly inhibit the protein expression level of NF-κB as well as the production of pro-inflammatory cytokines. Wang Meiyi *et al.* [17] found that Andrographolide can effectively inhibit the expression levels of pro-inflammatory cytokines IL-12 and IL-13 in an asthma mouse model by ELISA and qRT-PCR. These results indicate that Andrographolide can further exert good anti-inflammatory effects by regulating the expression levels of pro-inflammatory factors such as COX-2, p-p38, CD40, TNF-α, IL-1β, IL-6, IL-12, and IL-13, *etc.*

5 Prospects

Andrographolide is a diterpenoid lactone compound extracted from the Chinese herb Andrographis Paniculata, which has the advantages of wide sources, good efficacy, and low toxicity. With the deepening of research on new technology, the purification process and content determination technology of Andrographolide have achieved stage-by-stage research results. However, the existing purification method is still insufficient, and the intrinsic quality is instable, which affects the development and application of the products to some extent. Therefore, it is necessary to optimize and improve the existing purification process and content determination method. In addition, Andrographolide has a variety of pharmacological activities, such as antitumor, anti-inflammatory, antibacte-

rial, and antiviral, but its signaling pathways and targets are still unclear, requiring a comprehensive research on the mechanisms of action and target points of Andrographolide by combining relevant network pharmacology, big data analysis, genomics, and other modern biotechnologies. This research will provide a reference for the further pharmaceutical development of Andrographolide.

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