Research Article

Electrochemical-Based Extraction, Separation, and Purification of Coumarin Compounds from *Trifolium chinensis*

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In order to deal with the problems of the unstable transformation process and volatile palatability of coumarin compounds in *C. chinensis*, a method for the extraction, separation, and purification of coumarins in Chinese medicinal materials based on electrochemistry was prepared. First, an electrochemical distance-independent localization mechanism is used to determine node locations and routing mechanisms, and an extraction method is designed to achieve automatic and accurate real-time collection, aggregation, and transmission of ever-growing data. Environmental information for *C. chinensis* is collected. On the basis of this method, according to the chemical properties of coumarin and the extraction rate of coumarin as an index, the two-phase solvent extraction method and macroporous adsorption resin method were used to separate and purify it, and the best separation was determined. The purification process achieves efficient purification of coumarin. The experimental results show that the retention rate of coumarin by ethyl acetate is 34.5–56.8% higher than that of the other three extractants. When the optimal process determined in this study is adopted, the coumarin adsorption rate is greater than 98% and the recovery rate is greater than 85%. The purity of samples with 50% coumarin content can be increased to more than 97%, which verifies the correctness and advancement of the separation and purification process in this study.

1. Introduction

The Internet of Things (IOT) combines electronic devices, computer peripherals, information-sharing technologies, modern networks, and wireless communications, among others. It can collaboratively monitor and record real-time experimental data of various devices through various integrated microsensors. Data are transmitted wirelessly in an ad hoc multihop network and sent to user terminals, identifying the global connections of global bodies, global computers, and human life [1]. With the continuous development and maturity of the Internet of Things technology, it will effectively solve the way that people obtain important ingredients in medicinal materials through manual grinding in the traditional pharmaceutical industry. In various control systems, the temperature sensor, humidity sensor, pH sensor, light sensor, ion sensor, biosensor, CO₂ sensor, and other equipment of the IoT system can detect physical properties such as temperature, relative humidity, pH value, light utilization rate, and soil. Deficient nutrients and CO₂ concentrations are automatically controlled when released by various devices or as automatic controls, so as to ensure a good and suitable separation and purification environment when extracting components of medicines [2].

*Melilotus* is an annual or biennial herb of the family Leguminosae. The root system is developed; the stem is erect and has many branches. There are three-leaf compound leaves; the leaflets are elliptic, and the margins are sparsely toothed. It has racemes axillary and butterfly shaped corolla. The pods are oval, with obvious reticulation. The seeds are kidney-shaped, yellow-green, and multiply. The development and utilization of *C. chinensis* have a wide range of development and utilization, and it is called “baby grass.” The main development and utilization aspects include forage feed, soil improvement/fertilization, nectar plants, windbreak and sand fixation, water and soil conservation, spices, and medicinal purposes. Among them, the main medicinal ingredient is coumarin. How to determine the content of coumarin in *C. chinensis* and its separation and purification is becoming a hot topic in the world today.
In this study, an IoT-based coumarin extraction method was developed. By placing sensors with different functions in the area to be measured, it can monitor small climate changes, including temperature, humidity, moisture, and other growing environment information of *C. chinensis*. Accordingly, the growth of *C. chinensis* requires corresponding changes to the environment in a timely manner. The method can reduce the error of manual operation and manual measurement in the actual production process, reduce the cost of extraction, separation, and purification of *C. chinensis*, can realize the automatic and accurate real-time collection, aggregation, and transmission of *C. chinensis* growth environment information to the greatest extent, and design the structure, as shown in Figure 1. On the basis of this extraction method, according to the actual characteristics of coumarin in *C. chinensis*, it was separated and purified by the two-phase solvent extraction method and macroporous adsorption resin method, and finally, the efficient purification of coumarin was realized.

2. Research Method

2.1. Environmental Information Collection for Extraction of Coumarin Compounds from *C. chinensis*. The sensor nodes in the environmental information collection method for coumarin extraction are deployed in the monitoring area and constitute the basic unit of the Internet of Things. Sensor nodes can communicate with each other and form a node network through self-organization. Adjacent nodes in the network form clusters, and the cluster head node collects the data collected by each node in the cluster and then sends the compressed data to the sink node after data fusion. The sink node is responsible for the task of communication between the task management node and the sensor node and is generally served by a sensor node with strong energy or a router with wireless gateway capability [13].

The task management node is the data and instruction management center of the wireless sensor network (WSN), which generally consists of several servers [14]. The user configures and manages the sensor network through the task management node, publishes monitoring tasks, and collects monitoring data. The task management node can analyze and store the collected information and can issue instructions to the sensor node in real time. The position of each sensor node in the monitoring area can be obtained through GPS positioning or the node’s own positioning algorithm. They collect information according to the instructions sent...
by the task management node and send the collected information to the cluster head node. The cluster head node fuses the data and then sends it to the corresponding sink node. The data received by the sink node will be screened and sorted and then sent to the task management node through the Internet or communication satellites [15]. The working process of the environmental information collection method for coumarin extraction is shown in Figure 2.

The specific process is as follows. (1) The user sends a request command to query various indicators of the coumarin extraction environmental information through the task management node and transmits it to the sink node through the Internet and ZigBee network. (2) The sink node selects the cluster to be queried according to the specific requirements of the request command. After receiving the command, the cluster head wakes up and activates all nodes in the cluster to collect, store, and communicate data. The node collects data in time according to the command requirements and sends it to the corresponding cluster head node after digital-to-analog conversion. The cluster head fuses all the incoming data and sends the fused data back to the sink node. (3) The gathering node will screen and sort the obtained data and send it to the task management node through the external network. The task management node will feedback the collected data to the user and will store it in the coumarin extraction environment information database to provide a basis for future analysis and decision-making [16].

2.2. Separation and Purification of Coumarin. The components in the solid substance are dissolved in the solvent by the extraction method, and after solid-liquid separation, a liquid mixture containing multiple components is obtained, which is called the extraction solution, and then, various separation methods are used to separate the chemical components in the extraction solution. In this study, the two-phase solvent extraction method is mainly used for separation, and the macroporous adsorption resin method is used for purification [17]. According to the properties of coumarin and applicability of various separation and purification methods to coumarin, a separation and purification process route was drawn up for the ethanolic extract of coumarin, as shown in Figure 3.

The extract concentrate (extraction stock solution) of *C. chinensis* has been concentrated under reduced pressure to a certain concentration, add the extractant in a certain proportion, shake and stand for 30 minutes, take the organic phase, and repeat for several times. The organic phases were combined, concentrated under reduced pressure, and dried under vacuum at 50°C. The extracted product was weighed, and the coumarin content was measured by HPLC to calculate the coumarin content and coumarin retention rate in the extracted product. The extraction effect was evaluated by the coumarin content and coumarin retention rate in the extracted product. The definition formula is shown in the following formula [18]:
The content of coumarin in the extract (%) = \frac{\text{Coumarin quality}}{\text{Extracted product quality}} \times 100. \hspace{2cm} (1)

\text{Coumarin retention rate} (%) = \frac{\text{Coumarin quality} – \text{coumarin content} \times \text{extracted product quality}}{\text{The quality of coumarin in the extraction solution}} \times 100. \hspace{2cm} (2)

For the extraction effect, the highest content of coumarin in the extraction product and the high retention rate of coumarin are the best results, and these two indicators should be considered comprehensively in the evaluation [19].

The macroporous adsorption resin experiment is divided into the following steps. (1) Pretreatment: soak the unused resin in 95% ethanol for 24 hours, wash it with distilled water until there is no alcohol smell, and then use 5% hydrochloric acid and 5% hydrogen peroxide, respectively. Soak in sodium solution for 3 hours, wash with distilled water to neutral pH, and take part of the wet resin for use; the remaining resin is vacuum-dried at 333 K to constant weight for use. (2) Regeneration: the used resin is dynamically washed with 95% ethanol on a chromatography column. (3) Static adsorption and desorption: precisely weigh 0.5 g (dry weight) of the pretreated macroporous adsorption resin and add 50 ml of the extracted product to prepare an aqueous solution, Cinnamon officinalis sample solution, shake for 24 hours at a shaking speed of 130 r/min in a shaker, measure the coumarin concentration in the supernatant, and calculate the resin adsorption capacity and adsorption rate. Discard the supernatant, wash the resin with water and dry it naturally, add 50 ml of 95% ethanol, desorb under the same conditions, measure the coumarin concentration in the desorption solution, and calculate the resin desorption rate. (4) Dynamic adsorption and desorption of resin: weigh 20 ml of pretreated wet screening macroporous adsorption resin and put it into a glass column (15 × 450 mm), use HPD300 resin to absorb coumarin, accurately weigh 0.5 g of HPD300 resin, and add 50 ml. The coumarin concentration was 2.0447 mg/ml in the sample solution, which was shaken in a shaker (298 K, 130 r/min), and the coumarin concentration in the supernatant was regularly detected [20].

3. Result Analysis

The equipment used in the experiment is given in Table 1. Depressurize the C. chinensis extract to an extraction stock solution of 0.2 g/ml (raw material quality/volume of solution) and add different extractants at a ratio of 1:3 (volume of extract: volume of stock solution): chloroform, ethyl acetate ester, petroleum ether, and n-butanol, shake, after standing for 30 min, take the organic phase, and repeat 3 times. The organic phases were combined, compressed under reduced pressure, and dried under vacuum at 50°C. The coumarin content in the extracted product was determined by HPLC, and the coumarin retention rate was calculated [21]. The results are shown in Figure 4.

As can be seen from Figure 4, chloroform, ethyl acetate, and petroleum ether are used as extractants to extract coumarin, and the content of coumarin in the extraction product is similar, 34.5–56.8%, and serious emulsification will occur when the other three extractants are used as extractants. Ethyl acetate is recognized as a good extractant, with low cost, good biodegradability, and high extraction rate of lactones. Therefore, combined with the method of collecting environmental information for the extraction of coumarin compounds from Trifolium chinensis based on the Internet of Things [22], this study comprehensively considers the factors such as the good extraction effect, low toxicity, and good economy when ethyl acetate is used as the extraction agent, and ethyl acetate should be selected. Esters were used as extractants to separate and purify coumarin compounds in C. chinensis, and the dynamic breakthrough curve of HPD300 adsorption of coumarin obtained at the same time is shown in Figure 5.

It can be seen from the figure that 20 ml of wet HPD300 resin can handle 30 BV of sample solution without leakage under the optimized sample concentration (3.0 mg/ml) and flow rate (3.0 BV/h). When the effluent volume exceeds 30 BV, it begins to leak slowly. When the effluent volume is 33 BV, the coumarin concentration in the effluent reaches 1/10 of the sample concentration, which is called the breakthrough point [23]. When the effluent volume exceeds 33 BV, the leakage rate begins to increase sharply. When the

\[ \text{Figure 3: Extraction, separation, and purification process route of coumarin compounds in C. chinensis.} \]
effluent liquid agent reaches 42 BV, the effluent concentration is basically the same as that of the sample solution, and the resin reaches adsorption saturation, which is the saturation point of the resin [24, 25]. The experimental results show that under this process condition, 20 ml wet HPD300 resin can process 33 BV sample solution, its working adsorption capacity is 95.82 mg/ml wet resin, the coumarin adsorption rate is greater than 98%, and the recovery rate is greater than 85%. The purity of the samples with bean content of 50% increased to more than 97%, which verifies the correctness and advancement of the separation and purification process in this study.

4. Conclusion

As an important part of the new generation of information technology, the Internet of Things is used in many aspects such as data collection, transmission, processing, and business management, providing people with a new way to obtain and process information. Aiming at the characteristics of large amount of data collection in the collection method of coumarin extraction environmental information, combined with the advantages of Internet of Things technology, this study designed a coumarin extraction environmental information collection method and a coumarin separation and purification method based on the Internet of Things. The GEAR routing mechanism and the distance-independent positioning algorithm are adopted, thereby avoiding the energy consumed by nodes due to self-organizing network calculations. Meanwhile, the application of geographic location routing can more accurately locate the position of crops, which can effectively prolong the service life of the method. The macroporous adsorption resin method for the separation and purification of coumadin was determined. The experimental results show that 20ml wet HPD300 resin can treat 33 BV sample solution under this process condition, and its working adsorption capacity reaches 95.82 mg/ml wet resin. The adsorption rate of coumarin was more than 98% and the recovery was more than 85%. The purity of the sample with 50% coumarin content can be increased to more than 97%, which verifies the correctness and advancement of the separation and purification technology in this paper.

There are still deficiencies in the research in this study. For the areas not covered, I would like to propose the following suggestions. (1) The pharmacological effects of the extracted and isolated high-purity coumarin products can be studied, and the anti-inflammatory and analgesic properties of the products can be studied. The pharmacological effects of detumescence and other aspects provide a basis for its development into a new drug. (2) Industrial scale-up experiments can be carried out on the extraction and separation of coumarin, so as to realize industrialization and bring economic effects as soon as possible and social benefits.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.
Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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