Research article

Correlation between the redox activity of Polygonum multiflorum extract and its extraction technology with Chinese liquor (Baijiu): An electrochemistry-based study

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ABSTRACT

Elucidating the pharmaceutical mechanisms behind traditional Chinese medicine (TCM) is the key to promote its modernization process. In China, soaking TCM in liquor has a history of thousands of years, and many TCMs have to be processed into liquor before they can be used to treat diseases. Chinese liquor (Baijiu) contains more than 2,000 trace components, the interaction mechanism between TCM and Baijiu still remains unclear, making TCM a "mystery". The TCM industry commonly employs chromatographic and spectrographic technology to investigate the redox activity of TCM substances. However, only investigating the redox differences in specific active substances cannot provide a complete understanding of the redox activity of TCM substances. Thus, we employed the electrochemical approach to study the overall redox activity of substances in TCM in situ. The key result is that the redox substances in Baijiu function as a mediator for the redox reaction of Polygonum multiflorum extract. The redox efficiency of the extract is enhanced because of the faster electron transferability of the redox mediator in Baijiu.

1. Introduction

Traditional Chinese medicine (TCM) culture has a history of thousands of years and is a crucial cultural heritage of humankind [1, 2]. For example, artemisinin is a Chinese medicine developed on the basis of modern approaches and theories that can efficiently reduce the mortality of malaria, representing a crucial example of the modernization process of TCM [3, 4]. The modernization of TCM is an inevitable trend with the development of pharmaceutical science, and understanding the pharmaceutical mechanisms behind TCM is the key to promoting its modernization process [5].

TCM can be prepared using four approaches: cutting the pure plant into slices after natural drying [6], calcining plant tissues directly or indirectly with fire [7], extracting active ingredients from plant tissues using water [8, 9], and extracting active ingredients from plant tissues using liquids other than water [10]. The active ingredients in numerous plant tissues have very low solubility in water. Finding a suitable solvent that can dissolve these active ingredients is essential for improving the extracting efficacy of TCM [11]. Thus, the typical Chinese distilled liquor (also called Chinese Baijiu), a readily available organic solvent in ancient times, naturally became the best solvent for extracting the active ingredients of TCM [12]. In China, soaking TCM in liquor has a long history [13]; liquor was employed to soak TCM as early as the Spring and Autumn Warring States Period [14]. Many TCMs have to be processed into liquor before they can be employed for treating diseases. For example, regarding reinforcing drugs such as Rehmannia glutinosa, Cornus, and Ligustrum lucidum, soaking TCM in liquor is critical for accomplishing the desired pharmaceutical effect [15, 16].

This study investigates the correlation between the extraction process and redox activity of active substances in TCM. Polygonum multiflorum, which is highly valued for its extensive medicinal properties, was chosen as the TCM model [17, 18]. P. multiflorum is the root tuber of polygonum, and its primary active components include polyphenols, polysaccharides, and flavonoids [19]. As a commonly used medicine, it has many functions, such as scavenging free radicals, antivirus, and antitumor effects [20]. The redox activity of substances allows molecules in blood and organelles to transform energy or exchange biological information [21]. Therefore, investigating the redox activity of medicinal substances will offer a theoretical basis for TCM application [22]. Currently, the TCM industry commonly employs chromatographic and spectrographic

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technology to examine the redox activity of medicine substances [23, 24]. However, investigating only the redox differences in specific active substances cannot provide a complete understanding of the redox activity of substances in TCM. Thus, this study employs electrochemical approaches to investigate the overall redox activity of substances in TCM in situ and further investigates the source of the pharmacological effect of P. multiflorum. Further, the extraction process of P. multiflorum has been optimized based on the electrochemical redox behavior. Studies have confirmed that when ethanol is used as a solvent, although it can extract effective ingredients, the overall redox activity of P. multiflorum is lower than that when a liquor with the same ethanol concentration is used as the solvent. The substances contained in the liquor promote the redox reaction in P. multiflorum, showing a positive synergistic effect and providing a theoretical basis for optimizing the process of extracting active substances from TCM using liquor.

2. Experimental section

2.1. Material

The P. multiflorum was purchased from Shennong Pharmaceutical Co., Ltd. (Shaoyang, China), and the identities were authenticated in accordance with the Chinese Pharmacopoeia by Ying Su. Our research complies with the laws of China. Ethanol, tetra-n-butylammonium perchlorate, dimethyl sulfoxide, and 2,2-Diphenyl-1-picrylhydrazyl were purchased from Jinshan Chemical Reagent Co., LTD, Chengdu, China. The reagents were used without further purification. The standards (emodin, cas No. 518-82-1; quercetin, cas No.117-39-5; lawsone, cas No.83-72-7) were purchased from Aladdin Biochemical Technology Co., LTD, Shanghai, China.

2.2. Ultrasonic-assisted extraction from P. multiflorum and A. sinensis

The pretreated dried plant samples were crushed, weighed at 2.0 g, and placed in a distillation flask. The sample was placed in an ultrasonic cleaner with a power of 158 W for 40 min, and at the solid/liquid ratio of 40 mL/g. Then the sample was extracted with ethanol of a desired concentration. The extraction process was optimized by single-factor experiments: ethanol concentration (30%, 40%, 50%, 60%, and 70%), temperature (50 °C, 60 °C, 70 °C, 80 °C, 90 °C, and 95 °C), and extraction time (1h, 3h, and 5h). The extracted solution was treated by the vacuum freezing & drying technology, and the dry matter was collected for follow-up experiments.

2.3. Scavenging of DPPH radical by extracts of P. multiflorum

The DPPH was dissolved in ethanol to a final concentration of 2.2 mmol/L. The extract solution was prepared in ethanol with the desired concentration. The scavenging reaction was started by addition of extract solution into the DPPH solution. The reaction mixture was kept at room temperature for 20 min and the absorbance was measured at 325 and 525 nm with an ultra-violet and visible spectrophotometer (UV-2600, Shimadzu Corporation, Japan).

2.4. Electrochemistry

Electrochemistry was performed with an electrochemical workstation (CHI 660C, CHI Instrument, Shanghai, China). A typical three-electrode electrochemical system was used in this work. A glassy carbon electrode with a radius of 1.5 mm was used as the working electrode, platinum wire as the counter electrode, and Ag/AgNO3 electrode (saturated with AgNO3) as the reference electrode. Before the electrochemical test, the glassy carbon electrode was polished with 1 μm, 0.5 μm, and 0.3 μm alumina slurry to make its surface clean, and further cleaned with a large amount of sulfuric acid, ethanol and water, respectively. All electrodes were purchased from CHI instrument. Tetra-n-butylammonium perchlorate was used as the supporting electrolyte and dimethyl sulfoxide was used as the solvent for electrochemical analysis.

2.5. UPLC-MS analysis

Constituent analysis of the liquor extract and alcohol extract performed by UPLC-MS. Chromatographic analysis was performed with an UPLC system (Agilent1290UPLC), equipped with a Waters BEH C18 LC column (2.1*100 mm*1.7 μm). The binary mobile phases included solution A (0.1% formic acid-water solution) and solution B (0.1% formic acid-acetonitrile solution). Flow velocity: 0.3 mL/min. Injection volume: 5 μL. A high-resolution mass spectrometry detection system (Agilent QTOF6550) was used, which equipped with an electrospray ion (ESI) source. Sheath gas temp 350 °C, Sheath gas flow 12 L/min.

3. Results and discussion

3.1. Redox picture of P. multiflorum extract revealed by electrochemistry

We employed the stable free radical diphenylpicrylhydrazyl (DPPH) to indirectly feedback the redox properties of P. multiflorum extract: DPPH can maintain a stable free radical state in an organic solution, and its solution is purple [25]. When there is a free radical scavenger, the electron of DPPH will be captured, making its color lighter. The degree of fading is quantitatively related to the number of accepted electrons. As shown in Figure 1a, the intensity of the two absorption peaks (325 nm and 525 nm) of DPPH decreases with the increase in the concentration of P. multiflorum extract. It indicates that the single electron of DPPH is captured by the extract of P. multiflorum, which confirms the free radical scavenging ability of the extract [26]. Although P. multiflorum has a complex composition, it is considered to contain the main components: flavonoids, anthraquinones and stilbene glycosides [27]. All these substances contain active phenolic hydroxyl groups which can be oxidized into ketones, thus the P. multiflorum extract possesses the free radical scavenging ability. The DPPH-based spectroscopy method can prove the antioxidant capacity of the alcohol extract of P. multiflorum without complicated procedures [28]. However, this method is an indirect method and fails to provide further information on the overall redox properties of the extract.

Therefore, we employ the electrochemical method to directly explore the redox properties of the extract. The electrochemical method is highly sensitive and can detect the redox ability of the extract without damaging its molecular structure [29]. As shown in Figure 1b, both the oxidation and reduction current of the cyclic voltammetry spectrum have increased along with increasing extract concentration, suggesting that the extract compound exchanges electrons with the surface of the carbon electrode, which confirms the redox ability of the extract [30]. As shown in Figure 1c, both the oxidation current and the reduction current have increased with the increase of the scanning speed. The current value is proportional to the square root of the scan rate, indicating that the electron transfer from the redox active ingredient to the electrode surface is controlled by the diffusion process (the diffusion process and electron transfer process are two consecutive steps in the same process, and the diffusion process has slower speed than the electron transfer process), which confirms the good solubility of the active ingredient in the solvent. At the same time, the above results indicate that electrochemistry is an advantageous technique and can be employed to directly test the redox "picture" of medicine extracts [31].

Next step, we employed the electrochemical method to investigate the redox signal characteristics of each component in the extract. As revealed by liquid chromatography, emodin, quercetin, and lawsone have a relatively high content in P. multiflorum extract [32]. Therefore, we selected the standards of the above three substances to study the redox characteristics of P. multiflorum extract. As shown in Figures 2a, 2b, and 2c, the cyclic voltammetry peaks of all three substances present a semi-reversible redox process, and the current value of the oxidation...
Figure 1. (a) DPPH radical scavenging assay with the increase in the concentration of Polygonum multiflorum extract. (b) Cyclic voltammetry of Polygonum multiflorum extract in different concentrations: 3.08 mg/L, 4.27 mg/L, 5.29 mg/L, 6.17 mg/L, and 6.93 mg/L. Scan rate, 100 mV/s. (c) Cyclic voltammetry of Polygonum multiflorum extract in different scan rates: 100 mV/s, 150 mV/s, 200 mV/s, 250 mV/s, 300 mV/s, and 350 mV/s. Extract concentration, 6.93 mg/L.

peak exceeds that of the reduction peak, indicating that these ingredients tend to be oxidized. In other words, when these substances exert a pharmaceutical effect, they are preferentially oxidized, thereby preventing the oxidation of the substances in the blood [33]. The separation potential between anodic peak and cathodic peak ($\Delta E_p$) of these three substances are 0.169 V (emodin), 0.194 V (quercetin), and 0.304 V (lawsone), respectively. As seen, the cyclic voltammetry spectrum of lawsone has the largest value of $\Delta E_p$, indicating the most irreversible nature of lawsone [34]. In other words, lawsone tends to be maintained in an oxidizable state, that is, lawsone has the strongest ability to maintain oxidability. In a study for measuring the antioxidant activity of dietary polyphenols, lawsone, catechin and quercetin showed significantly different abilities in inhibition the oxidation of low-density lipoprotein [35]. Antioxidant activity of flavonoids is considered to be related to the positioning of phenolic hydroxyl groups as well as the various types of substitution. And the feature of B-ring with an ortho-dihydroxy group induces effective ROS (reactive oxygen species) scavenging ability [36]. The structural formulas of the above-mentioned compounds in the oxidation state (the state of ketone) are shown in Figure 3. As seen, the ketone state of lawsone has an active B-ring and the smallest electron delocalization area, suggesting that the redox reversibility of these ingredients is correlated to the position of the active groups (phenolic hydroxyl groups or ketones), which is consistent with the previous report [37].

Moreover, it can be seen that the oxidation peak potentials of the cyclic voltammetry peaks of the above-mentioned substances are -0.203 V, -0.148 V, and -0.098 V, respectively. Lawone has the lowest oxidation peak potential, indicating that when these substances exert the pharmaceutical effect, lawsone is preferentially oxidized [38]. All above results illustrate the importance of lawsone’s antioxidant capacity in the P. multiflorum extract. Figure 2d shows the cyclic voltammogram of a mixture of these substances in a fixed-ratio (1:1:1). The voltammogram is similar to that of the three substances on the whole. As the peak potentials of these substances are relatively close to each other, thus the smaller peaks (0.2830 mmol/L of emodin contributed a current of 18 $\mu$A; 0.2830 mmol/L of lawson contributed a current of 3 $\mu$A) are covered by the larger peak (0.2830 mmol/L of emodin contributed a current of 35 $\mu$A). The oxidation potential of the superimposed peak is -0.112 V, which is intermediate between the oxidation potential of these substances, indicating that the redox ability of these active ingredients when they are mixed is the sum of the abilities of the three substances when they exist alone, reflecting the collaborative contribution characteristic of redox ability.

3.2. Correlation between the redox activity and its extraction technology

We next compared the difference between alcohol extraction (60% ethanol) and liquor extraction (Baijiu in 60% ethanol). As shown in Figures 4a and 4b, under the same concentration of extract, the Baijiu group has higher peak current than the alcohol group. Under the extract concentration of 9.62 mg/L, the oxidation current values of the Baijiu group and the alcohol group are 11.3 $\mu$A and 4.8 $\mu$A, respectively. Under the extract concentration of 21.59 mg/L, the oxidation current values of the Baijiu group and the alcohol group are 41.2 $\mu$A and 16.3 $\mu$A, respectively. As seen, in the presence of liquor, the extract showed an enhanced redox activity. The enhancement phenomenon of redox activity can be caused by more than one reason [39]. One reason for the enhancement phenomenon is that there is an increase in the amount of substances capable of exerting redox effects in Baijiu extracts. Constituent analysis of the liquor extract and alcohol extract was performed by UPLC-MS. The relative concentration of the constituents, which showed significant changes, were shown in Table 1. As seen, some constituents showed an increase (>120%) in relative concentration: trachelobside (125.7%), mayurone (120.5%), while some constituents showed a decrease (<90%) in relative concentration: 4-ethylresorcin (84.3%), D(-)-Cellobios (89.6%). Although the aroma substances in Baijiu account for only 1%–2% of the total volume [40], they may have enhanced the extraction efficiency for redox species. It is reasonable as the molecular polarity of redox species in the extract is similar with the aroma substances in Baijiu, such as organic acids, esters [41].

Another reason for the enhancement phenomenon is that the redox substances in Baijiu (e.g. aldehydes and ketones) act as the redox mediator...
and the redox efficiency of the extract is improved due to the faster electron transfer ability of redox mediator in Baijiu. Aldehydes and ketones in Baijiu are chemicals with electrochemical activity, which will exchange electrons with oxidants or reductants in P. multiflorum extract, and then diffuse to the electrode surface and exchange electrons there. The mediation process is repeated, and the mediator functions as an electron shuttle between the oxidants/reductants and electrode, thus the redox efficiency of the extract is improved. A redox mediation mechanism is illustrated in Figure 4c. Further, cyclic voltammetry of another TCM model (Angelica sinensis) was shown in Figure 4d. As seen, under the same concentration of extract, the Baijiu group has higher peak current than the alcohol group, indicating a similar phenomenon with P. multiflorum. A comparative study [13] on the pharmacodynamic difference of TCM accompanied with Chinese Baijiu and water (without Baijiu) has demonstrated the function of Baijiu in activating anti-inflammation, analgesia and anti-mammary gland hyperplasia, which also suggests a mediation effect.

At the same time, it should be noted that the Baijiu group has higher oxidation potential than the alcohol group. Under the extract concentration of 9.62 mg/L, the oxidation potential values of the Baijiu group are as follows: 9.332 V for the Baijiu group and 9.278 V for the alcohol group.
and the alcohol group are -0.103 V and -0.138 V, respectively. Under the extract concentration of 21.59 mg/L, the oxidation potential values of the liquor group and the alcohol group are -0.033 V and -0.088 V, respectively. It suggests that under the mediation effect of redox substances in Baijiu, the oxidation process of the extract is disturbed by redox polarization, which increases the oxidation potential and is thus unfavorable for its preferential oxidation (then unfavorable for its pharmaceutical effect). Although redox mediation increases the amount of antioxidants, polarization reduces the antioxidant ability. In view of the overall redox efficacy, the mediation effect and the polarization effect are contradictory. Therefore, how to regulate these two effects in the extracting process is the key to optimizing the redox efficacy.

In addition, literatures reported that flavonoids act as highly active antioxidants by way of metal chelation [36]. Thus the concentrations of the metals in Baijiu were determined by ICP-MS/OES analysis [44]. It is found that eight metals (Al, Ba, Ca, Cu, Fe, Mg, K, and Na) have a relatively high content. The results suggest that the ortho-dihydroxyl group in flavonoids may take part in metal chelation via these ions (Al$^{3+}$, Ba$^{2+}$, Ca$^{2+}$, Cu$^{2+}$, Fe$^{3+}$, Mg$^{2+}$) thus enhancing the redox efficiency of the extract. However, flavonoids can chelate metals by diversified possible route which depends on flavonoids structure, the pH of the reaction and the type of metal ion, emphasizing the need for further research.

### 3.3. Optimized extraction process based on the redox activity

We further optimized the P. multiflorum extraction process based on the redox activity of the extract. As shown in Figure 5a, the higher the temperature in the extraction process, the greater the current of the oxidation peak and the reduction peak of the extract, indicating an increase in the amount of extracted substances capable of redox reactions. Moreover, the higher the temperature in the extraction process, the greater the $\Delta E_p$ between the oxidation peak and the reduction peak, indicating the poor reversibility of the redox reaction. It can be seen that higher temperature in the extraction process increases the total amount of extracted active substances, but some substances lose their redox activity at the same time. Figure 5b shows the free radical scavenging ability of the extract at different temperatures. It can be seen that under the extraction temperature of 90°C, the absorption peak has the smallest intensity, suggesting that this temperature is beneficial for the extract to exert its free radical scavenging ability. It should be noted that the extract will undergo molecular decomposition, isomerization, and chemical bond breakage at high temperatures, which will change the antioxidant capacity of the extract [45]. At 95°C, the amount of extracted substances capable of redox reactions is the highest. However, in view of the redox reversibility, a temperature lower than 95°C is advantageous. Moreover,
the absorption spectrum suggests that the free radical scavenging ability of the extract is relatively strong at 90 °C. Therefore, from an overall point of view, the extraction temperature of 90 °C is conducive to the redox activity of *P. multi florum* extract.

As shown in Figure 5c, as the ethanol concentration during the extraction process increases, the peak current first increases and then decreases. Under 60% ethanol concentration, the peak current is the highest, indicating that the amount of extracted substances capable of redox reaction is more desirable under this concentration. On the one hand, the dissolution of polyphenols is related to the polarity of the extraction solvent. As the ethanol concentration increases, there is a smaller polarity gap between the solvent and the solute, and the extracted amount is higher. On the other hand, polyphenols are combined with substances such as proteins and polysaccharides in plant tissues through the force of hydrogen bonds [46]. Under excessive ethanol concentration, a large amount of alcohol-soluble substances are eluted, resulting in greater polarity difference between the polyphenol and the solvent, so the amount of the extracted polyphenol decreases. Figure 5d shows the free radical scavenging ability of the extract under different ethanol concentrations. Under an ethanol concentration of 60%, the absorption peak has the smallest intensity, which implies that the condition is the optimal ethanol concentration for the extract to exert its free radical scavenging ability. Seen from the two aspects of extraction amount and free radical scavenging ability, an ethanol concentration of 60% is ideal for optimization the extraction process. Figure 5e shows the change in extraction amount under different extraction times. It can be seen that the extraction amount under the condition of 1h is significantly higher than that under the condition of 3h and 5h. Polyphenols diffuse rapidly into the solvent within 1h, showing the strongest redox capacity. As the extraction time increases, parts of the polyphenols are decomposed, with the redox capacity decreased. As shown in Figure 5f, under the extraction condition of 1h, the extract also exhibits significant free radical scavenging ability. Using the electrochemical redox method and optical absorption spectroscopy, the optimal extraction process of *P. multiflorum* can be determined: the extraction temperature of 90 °C, the extraction ethanol concentration of 60%, and the extraction time of 1 h.

4. Conclusions

In conclusion, the liquor extraction process shows a correlation with the redox efficacy of active substances in *P. multiflorum*. On the one hand, the redox substances in the liquor function as redox mediators to enhance the redox efficiency of the extract. On the other hand, the extract oxidation process is disturbed by redox polarization, which is unconducive to the
pharmaceutical effect of the extract. Thus, the results indicate that the process of extracting active substances from TCM using liquor may be optimized by regulating the mediation and polarization effect. Additionally, the results confirm that electrochemical technology is an efficient tool to directly test the oxidation activity of plant extracts and can be used for elucidating the pharmaceutical mechanisms behind TCM and promoting its modernization process.

**Declarations**

**Author contribution statement**

Ying Su: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Zhiao Wang: Performed the experiments; Analyzed and interpreted the data.

Yougui Yu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Qing Zheng: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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**Data availability statement**

Data will be made available on request.

**Declaration of interests statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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