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Feasibility Study on the Geographical Indication of *Lycium barbarum* Based on Electrochemical Fingerprinting Technique

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Rapid traceability of plant products is important for food safety. Because plant tissues contain electrochemically active substances such as polyphenols, their electrochemical fingerprint can be used as a basis for geographic determination. In this paper, we propose an electrochemical fingerprinting technique for species identification and geographical indication using Chinese wolfberry (*Lycium barbarum*). We selected a total of nine Chinese wolfberry varieties. We also collected Chinese wolfberries of the same variety from different geographical areas (across provinces). Samples of a single variety of Chinese wolfberry presented very consistent electrochemical behaviors in two buffer solutions. The electrochemical behavior of a single variety of Chinese wolfberry grown in different geographical areas was also very similar, because the range of electrochemically active substances in their tissues is mainly controlled by genetics. Therefore, variations in electrochemically active substances were not very substantial. However, we found that by identifying small differences in electrochemical fingerprints, it was possible to differentiate Chinese wolfberries grown in different provinces.

Keywords: Geographical indication; *Lycium barbarum*; Fast identification; Fingerprints; Chinese wolfberry

1. INTRODUCTION

Chinese wolfberry (*Lycium barbarum*) is a medicinal food source used in Chinese medicine and as a health food, and it is widely loved by people. An investigation showed that Chinese wolfberry contains polysaccharides, betaine, flavonoids, a variety of vitamins, amino acids, carotenoids and a variety of essential trace elements. The ecological environment of the region of origin plays an important role in the growth of medicinal plants and their active ingredients. Redgwell et al. [1,2] investigated cell wall polysaccharides in *Lycium barbarum*. Inbaraj [3] used high-performance liquid chromatography with diode array detection-mass spectrometry detection and electrospray ionization (HPLC-DAD-ESI-MS) for the simultaneous determination of phenolic acids and flavonoids in *Lycium barbarum*. Amagase [4] reviewed botanical properties, phytochemical, chemical and clinical efficacies, and safety aspects.

The World Trade Organization (WTO) defines a geographical indication as evidence that a product originates in a member country, a region or a place within that region. Certain specific qualities, e.g., reputation or other characteristics of the product can, in essence, be attributed to that geographical origin. Therefore, geographical indications are mainly used to establish the origin of the product. Due to a lack of identification technology, the Chinese wolfberry trading market is highly unregulated. Due to the inability to identify actual and false Chinese wolfberry, consumers are easily deceived, and their interests are damaged. Therefore, there is an urgent need for research on technology useful for identifying the origin of Chinese wolfberry.

NIR spectroscopy is a rapid and highly practical analytical technique for a wide range of organic compounds, and it was initially used in agriculture [5–9]. Because analyses are rapid, nondestructive and economical, NIR spectroscopy is rapidly becoming widely used for food analysis and identification. Cozzolino et al. [10] investigated the application of visible near-infrared spectroscopy in distinguishing white wines of different origins in Australia. They combined principal component analysis, principal component regression and discriminant partial least squares regression to develop a discriminant model, and that discriminant partial least squares model yielded good results. Liu et al. [11] used NIR spectroscopy combined with multivariate statistical analysis to identify the origin of Tempranillo wines from Australia and Spain, and the partial least squares-discriminant analysis gave good results. High-performance liquid chromatography (HPLC) has the advantages of fast analysis, high reproducibility and high quantitative accuracy and is one of the most widely used and effective separation and analysis techniques used in laboratories [12–17]. It has also become an important separation technique in the fields of chemistry, medicine, life sciences and environmental protection. Taamalli et al. [18] used HPLC-ESI-TOF-MS to identify the origin of 23 phenolic compounds found in olive oils from different regions, and the results of cross-validation showed that the classification was 100% correct. Atomic absorption spectroscopy (AAS) is a quantitative analysis method based on the absorption of spectral lines by vaporized atoms originally contained in a substance, and it is mainly used for the analysis of trace components. It is characterized by high selectivity, high sensitivity and a wide analytical range.

Recently, we proposed a technique based on electrochemical fingerprinting of plants [19–28]. This technique involves scanning the electrochemically active substances in samples. Samples can be identified from the differences observed in electrochemical fingerprints. We have successfully used this technique for rapid identification of plants, beverages and dyes. In this work, we intend to extend this technique to geographical tracing of Chinese wolfberry. As this method is efficient and inexpensive, we think it will play a very important role in controlling the quality of herbal medicines.

2. MATERIALS AND METHODS

All chemicals used in this work were of analytical grade. All samples of Chinese wolfberry were collected in 2018-2019, ground into powders after drying and stored frozen. All information is listed in Table 1. Water or ethanol was used for the extraction of electrochemically active substances from the Chinese wolfberry samples. The specific extraction method involves dispersion of a certain amount of Chinese wolfberry powder in the solvent. The dispersion was sonicated for 2 min. After precipitation, the supernatant was added to an electrolyte solution for electrochemical testing. Solutions containing 0.1 M concentrations of phosphate buffer and acetate buffer were used as electrolytes for recording the electrochemical responses of electrochemically active substances.

Differential pulse voltammetry (DPV) was used for fingerprint recording. Glassy carbon electrode was firstly polished using alumina slurry after water wash. Then, the three-electrodes system was inset into a 10 mL of either electrolyte. The DPV was conducted at -0.1–1.5 V, with a pulse amplitude of 50 mV, a pulse width of 0.05 s and a pulse period of 0.5.

Table 1. Sample information of collected Chinese wolfberry.

Group 1					
Variety	**Location**	**Abbreviation**	**Variety**	**Location**	**Abbreviation**
Ningqi-1	Yinchuan-Bairuiyuan	Y-B1	Ningqi-2	Yinchuan-Yuanlinchang	Y-Y2
Ningqi-5	Yinchuan-Bairuiyuan	Y-B5	Ningqi-7	Yinchuan-Bairuiyuan	Y-B7
Ningqi-901	Yinchuan-Bairuiyuan	Y-B901	Ningqi-909	Yinchuan-Bairuiyuan	Y-B909
Ningqi-10	Yinchuan-Yueyahu	Y-Y10	Huanggouqi	Yinchuan-Bairuiyuan	Y-BH
Group 2					
Ningqi-1	Yinchuan-Xingqing	Y1-X	Ningqi-1	Yinchuan-Nongkeyuan	Y1-N
Ningqi-1	Yinchuan-Bairuiyuan	Y1-B	Ningqi-1	Zhongning-Laba	Z1-L
Ningqi-1	Zhongning-Enhe	Z1-E	Ningqi-1	Zhongning-Changshantou	Z1-C
Ningqi-1	Zhongning-Zhouta	Z1-Z	Ningqi-1	Zhongning-Dadi	Z1-D
Ningqi-1	Gansu-Guazhou	G1-G	Ningqi-1	Gansu-Jinhe	G1-J
Ningqi-1	Gansu- Shandan	G1-S	Ningqi-1	Qinhai-Nuomuhong	Q1-N
Ningqi-1	Qinhai-Balong	Q1-B	Ningqi-1	Qinhai-Haiquanshui	Q1-HQ
Ningqi-1	Qinhai-Huaitoutala	Q1-HT	Ningqi-1	Qinhai-Xiangride	Q1-X
Group 3					
Ningqi-7	Yinchuan-Nongkeyuan	Y7-N	Ningqi-7	Yinchuan-Xingqing	Y7-X

Ningqi-7	Zhongning-Changshantou	Z7-C	Ningqi-7	Zhongning-Zhongqi	Z7-ZQ
Ningqi-7	Zhongning-Zhouta	Z7-Z	Ningqi-7	Zhongning-Dadi	Z7-D
Ningqi-7	Gansu-Guazhouzheng	G7-GZ	Ningqi-7	Gansu-Guazhou	G7-G
Ningqi-7	Gansu-Shahe	G7-S	Ningqi-7	Gansu-Nancha	G7-N
Ningqi-7	Gansu-Yaozhan	G7-Y	Ningqi-7	Gansu-Lianghu	G7-L
Ningqi-7	Gansu-Jinhe	G7-J	Ningqi-7	Gansu-Bulongji	G7-B
Ningqi-7	Gansu-Jingtai	G7-JT	Ningqi-7	Qinhai-Nuomuhong	Q7-N
Ningqi-7	Qinhai-Wulan	Q7-W	Ningqi-7	Qinhai-Geermu	Q7-G
Ningqi-7	Qinhai-Balong	Q7-B			
			Group 4		
Ningqi-7	Yinchuan	YC7	Ningqi-7	Hongsibu	HS7
Ningqi-7	Hongliugou	HL7	Ningqi-7	Changshantou	CS7
Ningqi-7	Hexi	HX7	Ningqi-7	Haiyuan	HY7
Ningqi-7	Yuanzhou	YZ7			

3. RESULTS AND DISCUSSION

We first recorded the electrochemical fingerprints of different varieties of Chinese wolfberry. In recent years, there has been a growing trend in the consumption of exotic berry-type fruits and their products, including the wolfberry, pomegranate (*Punica granatum*), *Garcinia mangostana* fruits, and Chilean maqui berry. Wolfberry fruit is a red–orange berry of the Solanaceae family, which includes tomato, eggplant, chili pepper and potato. In China it is consumed not only in fresh and dried forms but also as processed products, including canned fruits, yogurts, beverages, jams and jellies. Given the wide consumption of wolfberry fruits and their potential impact on human health, further studies are necessary to clarify their physiological benefits [29–32]. Figure 1 shows the electrochemical fingerprints of eight different varieties. Each of them was fingerprinted under two sets of conditions. The expressed abundance of electrochemical fingerprints was improved by using different buffer solutions and solvents. Therefore, combining the two profiles of each sample allows for better identification of the different varieties. From Figure 1, we can see that the electrochemical fingerprint profile of the Huanggouqi variety is more variable than those of the other varieties because all varieties except Huanggouqi belong to one series. From the fingerprint profiles, we can see that the electrochemical fingerprinting technique effectively distinguishes the Huanggouqi and Ningqi series. However, this technique cannot quickly identify each type in the Ningqi series. This is because the varieties in the Ningqi series all have very similar appearance traits and chemical compositions, so the differences in their electrochemically active ingredients are not particularly obvious.

Figure 2 shows a map of different sites at which the the same species of Chinese wolfberry was collected within the Ningxia Hui Autonomous Region. These samples were collected in different locations and grown in different environments. The environment in which a plant is grown can greatly influence the chemical composition of the tissues. Therefore, in this set of experiments, we sought to explore the effect of the growing environment on electrochemically active substances found in the same variety of Chinese wolfberries.

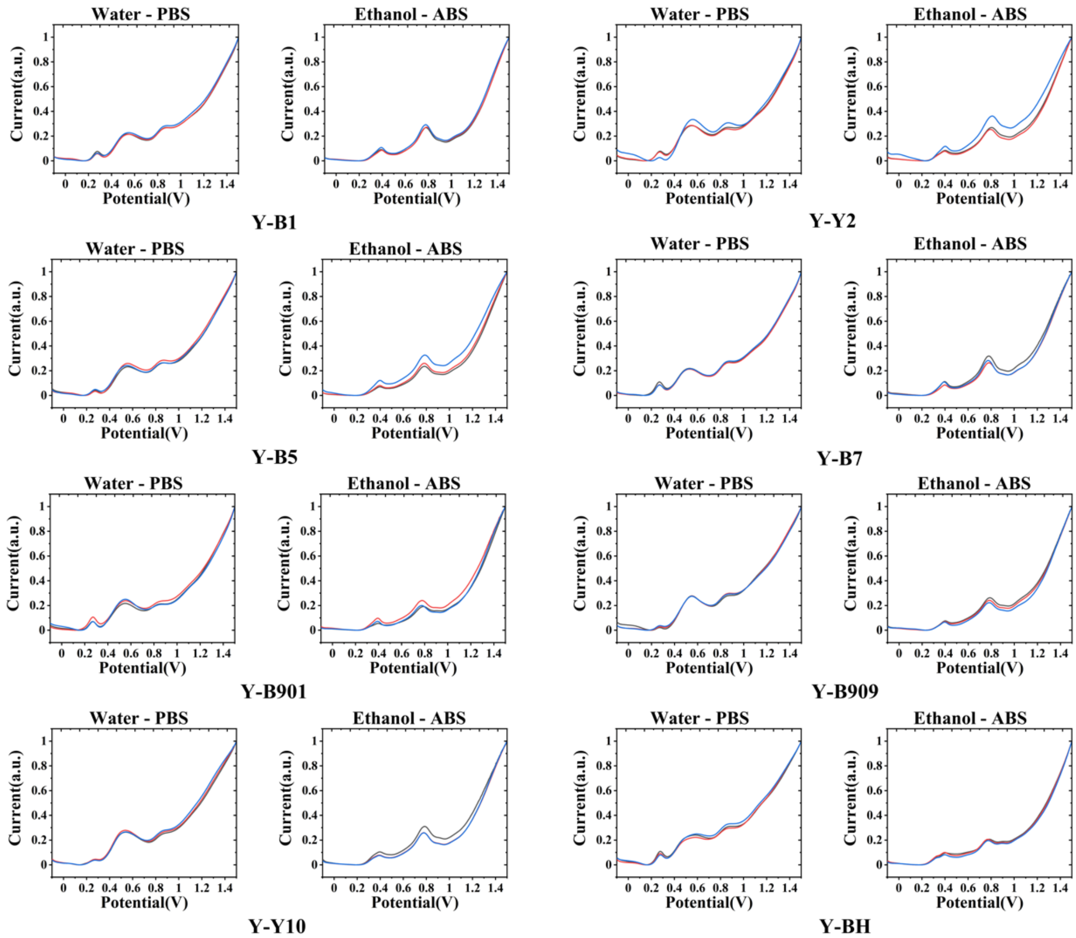

Figure 1. DPV profiles of Y-B1, Y-Y2, Y-B5, Y-B7, Y-B901, Y-B909, Y-Y10 and Y-BH recorded under PBS and ABS.

From Figure 3, we can see that two distinct oxidation peaks appear for Ningqi-7 in the presence of PBS. The first oxidation peak is at approximately 0.4 V and the second one is at approximately 0.8 V. These compounds could be flavonols [33], phenolic acids [34], procyanidins [35], alkaloids [36] and pigments [37]. We did not conduct the cathode scan because it has much less information compared with that of the anodic scan. Moreover, the dissolved oxygen can produce a peak if we extend the potential window. In the ABS environment, three distinct oxidation peaks appear for Ningqi-7. The oxidation peaks appear at approximately 0.3 V, 0.6 V, and 0.85 V. The variability among them is very small, regardless of the locations at which the samples were collected in Ningqi-7. Each of the samples showed very similar oxidation waves, indicating the involvement of similar substances in electrochemical oxidation reactions. This phenomenon indicates that the substances involved in electrochemical oxidation are very similar for all Ningqi-7 samples. However, we still perceive that some samples have larger oxidation peaks and others have smaller oxidation peaks. The reason for this phenomenon is that there are differences in the amounts of electrochemically active substances in plant tissues grown under different conditions. For example, the electrochemical oxidation peaks of YC7 were significantly smaller than those of YZ7. This shows that YZ7 contained more of the substances involved in electrochemical reactions than YC7. Of course, this variability is not necessarily due exclusively to the natural environment of growth. Human growing techniques can also produce differences in the chemical composition within the plant tissues [38].

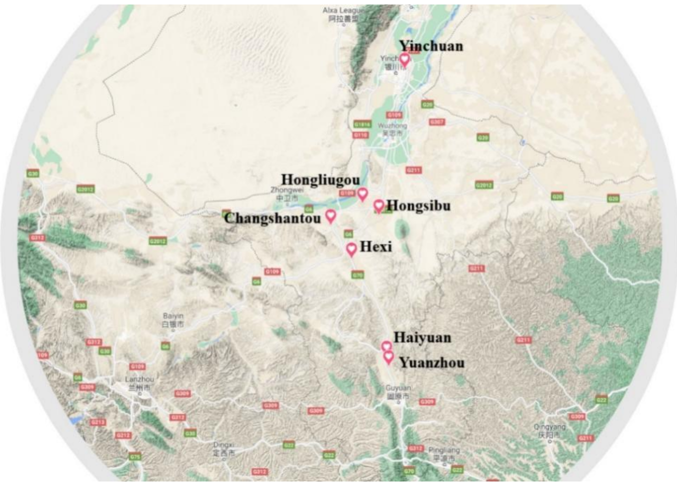

Figure 2. Map of different collection sites of the Ningqi-7 of Chinese wolfberry within the Ningxia Hui Autonomous Region.

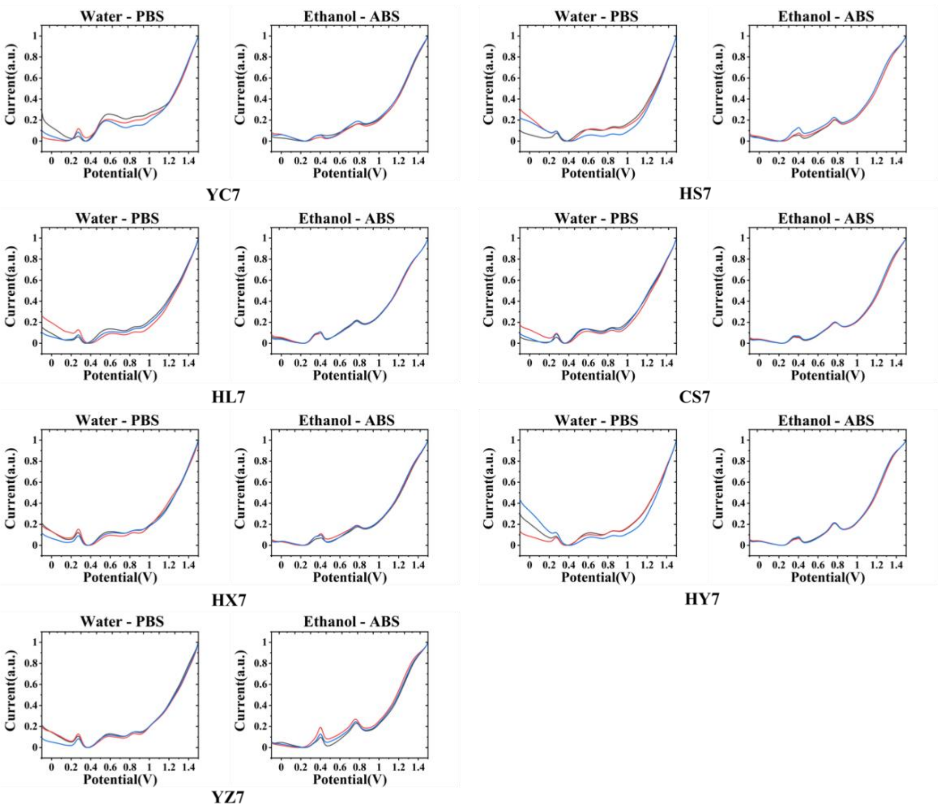

Figure 3. DPV profiles of YC7, HS7, HL7, CS7, HX7, HY7 and YZ7 recorded under PBS and ABS.

We further investigated differences in electrochemical fingerprint profiles of the same variety of Chinese wolfberry after cultivation in different provinces. Figure 4 shows the sampling of Ningqi-1 in Gansu Province, Qinghai Province and the Ningxia Hui Autonomous Region. The different circles represent the different provinces. Although some samples came from different provinces, these provinces are geographically close to each other.

Figure 5 shows the electrochemical fingerprints of Y1-X, Y1-N, Y1-B, Z1-L, Z1-E, Z1-C, Z1-Z, Z1-D, G1-G, G1-J, G1-DX, Q1-N, G1-S, Q1-HQ, Q1-HT, Q1-X and Q1-B in PBS and ABS. Although all samples exhibited similar electrochemical oxidation behaviors, the positions of the oxidation peaks and the magnitudes of the currents were very different. For example, the electrochemical behaviors of all samples from Zhongning and Yinchuan were very similar, but the samples collected in Qinghai Province had much higher currents in ABS. Similarly, in the case of PBS, the samples from Qinghai Province had higher electrochemical responses than those from other provinces. This represents the accumulation of more electrochemically active substances in the tissues of Chinese wolfberry from Qinghai Province.

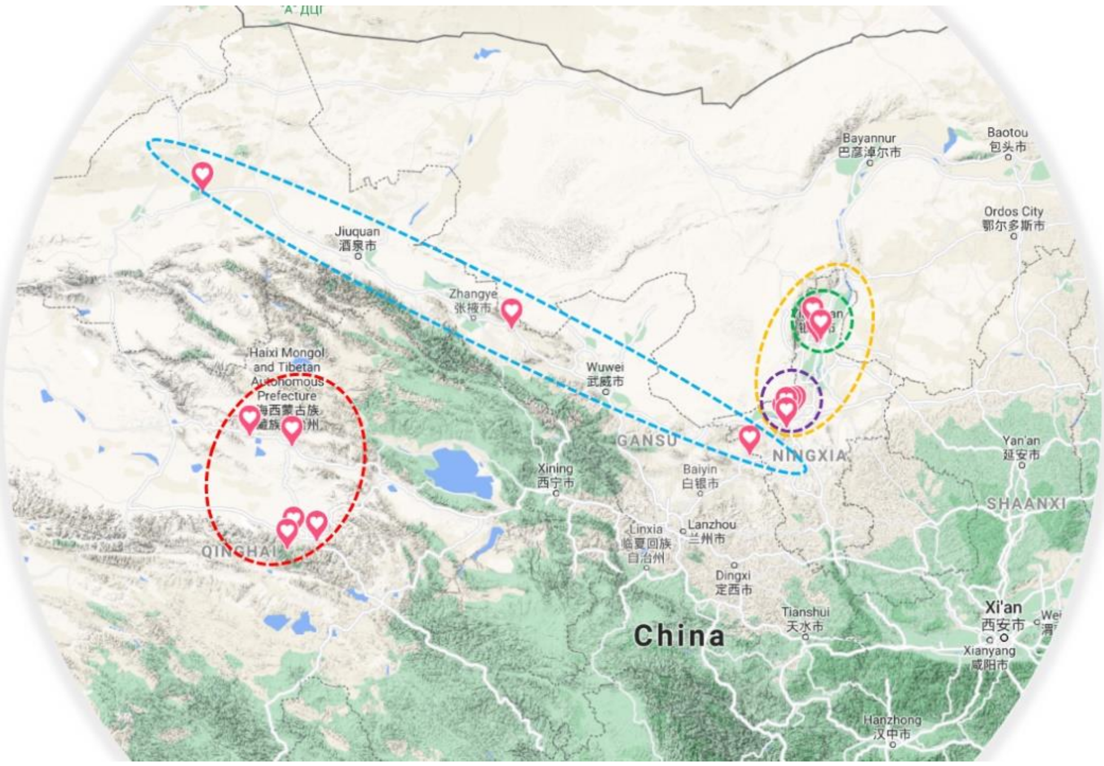

Figure 4. Map of different collection sites of the Ningqi-1 of Chinese wolfberry within the Ningxia Hui Autonomous Region, Qinghai and Gansu Provinces.

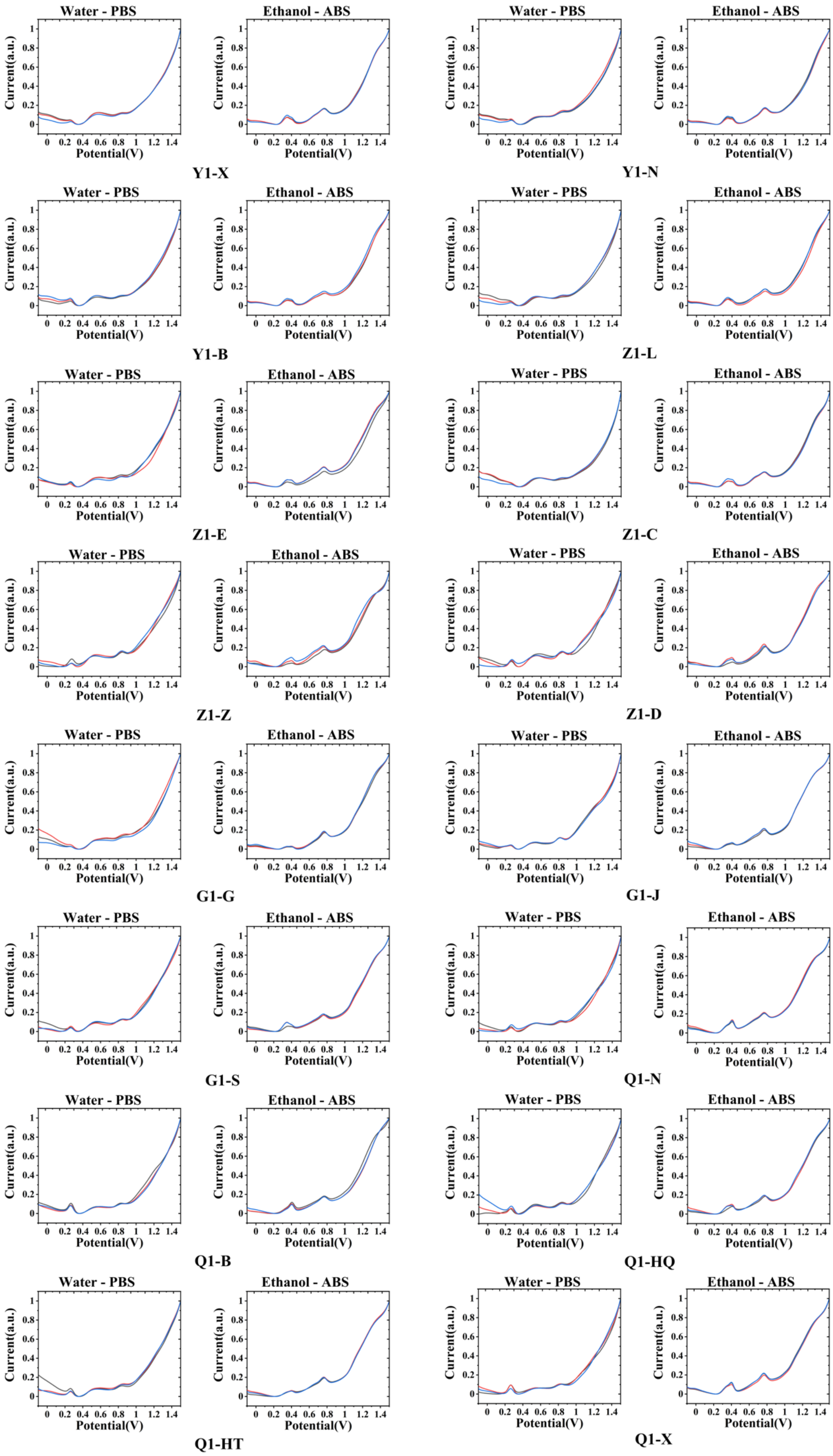

Figure 5. DPV profiles of Y1-X, Y1-N, Y1-B, Z1-L, Z1-E, Z1-C, Z1-Z, Z1-D, G1-G, G1-J, G1-DX, Q1-N, G1-S, Q1-HQ, Q1-HT, Q1-X and Q1-B recorded under PBS and ABS.

We also collected Ningqi-7 samples from Gansu Province, Qinghai Province and the Ningxia Hui Autonomous Region. Ningqi-7 is a variety that is now widely planted. It is a new asexual variety selected from the Ningxia wolfberry production garden. In many years, multipoint variety comparison tests and regional tests showed fast growth, a high level of self-compatibility, strong resistance to adversity, abundant yield, stable yield, large fruit, and a high grade rate. The single fruit weight of this variety was 0.72 g, which was nearly 30% higher than that of Ningqi-1 (0.56 g). The dry fruit grade rate was 250-290/50 g, which is one grade higher than that of Ningqi-1. The polysaccharide content of Chinese wolfberry was 3.97 g/100 g, which is 10.9% higher than that of Ningqi-1. The betaine proportion was 1.08 g/100 g, which is 29.2% higher than that of Ningqi-1. The carotene content was 1.385 g/100 kg, which is 12.6% higher than that of Ningqi-1.

Figure 7 shows the electrochemical fingerprints of Y7-N, Y7-X, Z7-C, Z7-ZQ, Z7-Z, Z7-D, G7-GZ, G7-G, G7-S, G7-N, G7-Y, G7-L, G7-J, G7-B, G7-JT, Q7-N, Q7-W, Q7-G and Q7-B. The fingerprint spectrum shows that Ningqi-7 from Qinghai Province has more electrochemically active substances and a larger current response relative to other varieties. This result is similar to the previous results for Ningqi-1, which may indicate that the natural environment in Qinghai Province favors the accumulation of electrochemically active substances in Chinese wolfberry. Based on the above electrochemical information, we know that Chinese wolfberry accumulates different compositions when grown in different geographical areas. Therefore, the detection of electrochemical fingerprinting has the potential to differentiate sources.

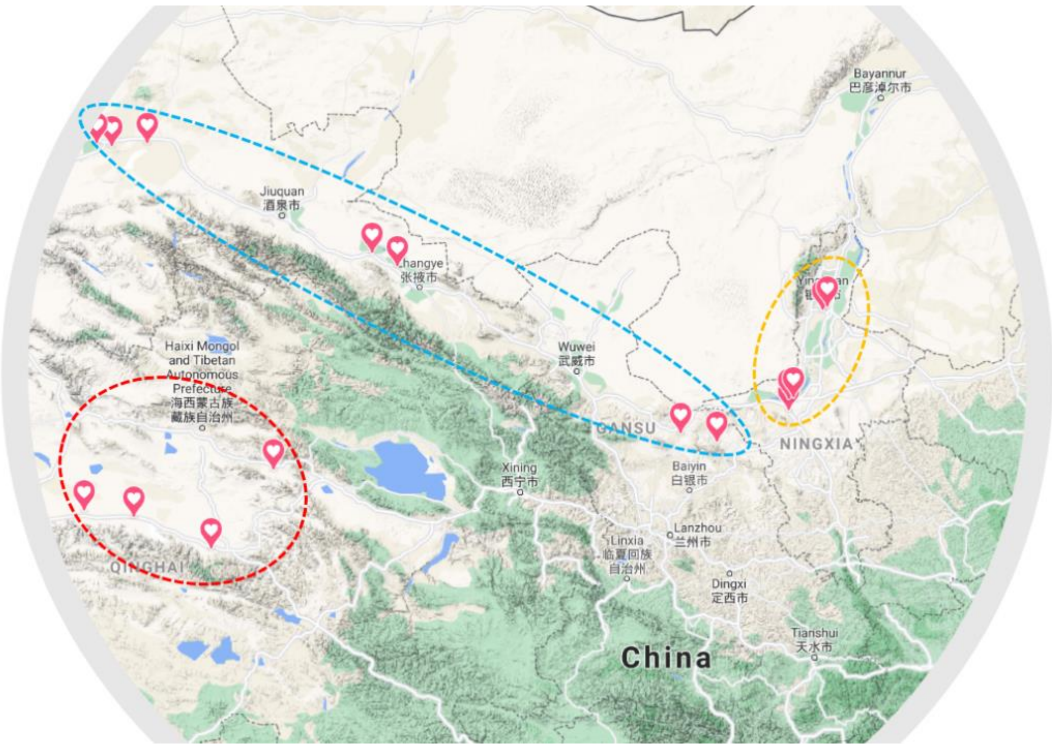

Figure 6. Map of different collection sites of the Ningqi-7 of Chinese wolfberry within the Ningxia Hui Autonomous Region, Qinghai and Gansu Provinces.

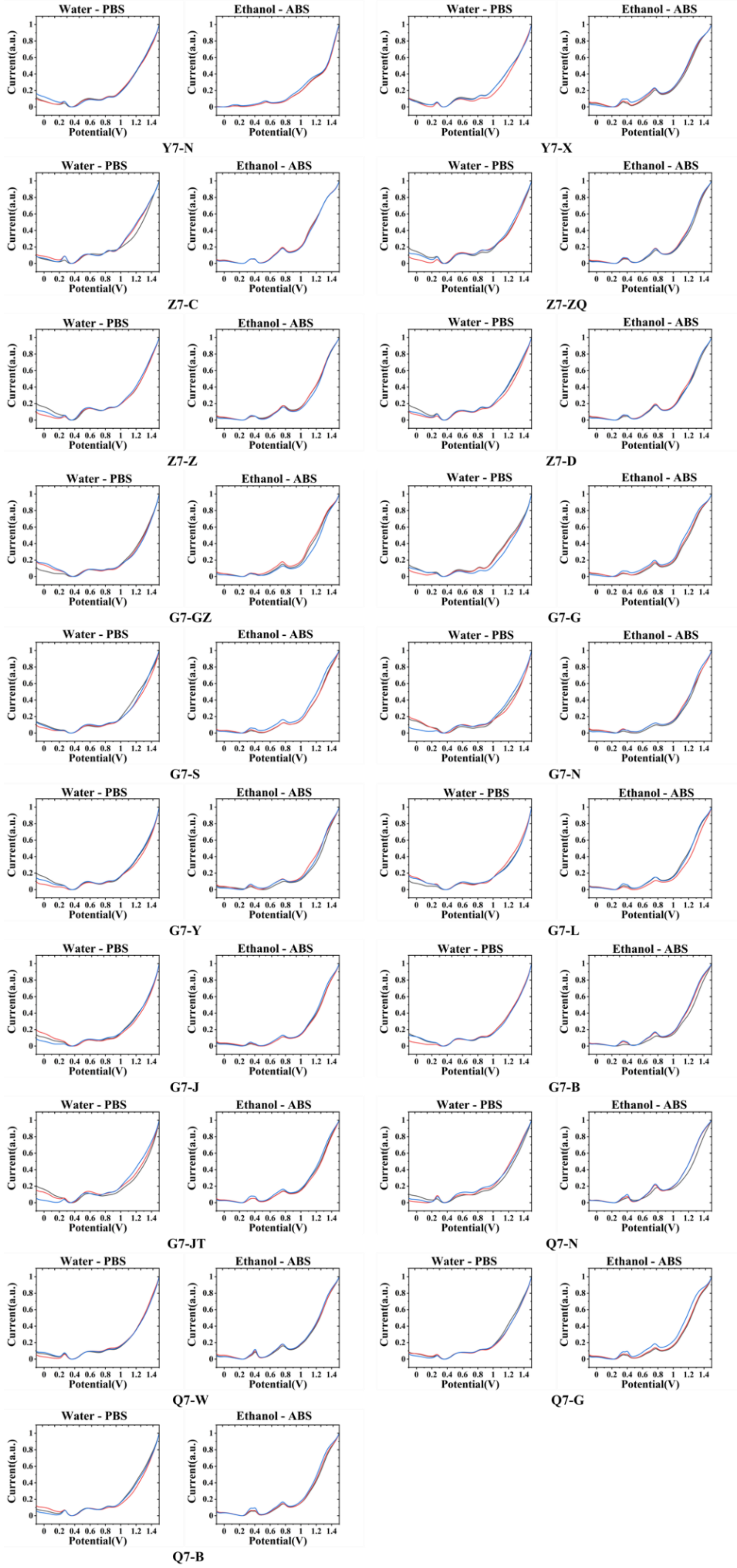

Figure 7. DPV profiles of Y7-N, Y7-X, Z7-C, Z7-ZQ, Z7-Z, Z7-D, G7-GZ, G7-G, G7-S, G7-N, G7-Y, G7-L, G7-J, G7-B, G7-JT, Q7-N, Q7-W, Q7-G and Q7-B recorded under PBS and ABS.

4. CONCLUSION

In this work, we used electrochemical fingerprinting techniques to analyze different samples of Chinese wolfberry. We found that the varieties of the Ningqi series all exhibit relatively similar electrochemical fingerprints, but the Huanggouqi series shows very different electrochemical behavior. A single variety of Chinese wolfberry demonstrates very consistent electrochemical behavior because these plants have the same electrochemically active substances in their tissues. Although the environment also affects the amount of these substances in plants, the content is mainly controlled by genetics. However, there are still some differences in the electrochemical fingerprint profiles of Chinese wolfberry grown in different regions. These differences can be used for geographic indication.

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