Int. J. Electrochem. Sci., 16 **(2021)** Article Number: 211117, doi: 10.20964/2021.11.12

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Short Communication

Extraction and Electrochemical Fingerprint of Polysaharide and Fat Soluble Compounds from Panax

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Received: 28 July 2021 / *Accepted:* 30 August 2021 / *Published:* 10 October 2021

Plant systematics focuses on the evolutionary results of organisms at a particular time in the process of evolution, which aims to elucidate the evolutionary processes and patterns as well as the kinship among various taxa. *Panax* is a medicinally important genus within the family of Araliaceae, in which almost all species are of cultural significance for traditional Chinese medicine. It is of great significance to conduct an in-depth study on the phylogeny of the species of the genus *Panax* and to introduce their introduction of cultivation and utilization. The application of electrochemical fingerprints in plant phylogenetic study is an emerging research field in related to biosensors. In this work, the lipid-soluble components, ginsenosides and polysaccharides were extracted from *Panax trifolius, P. stipuleanatus*, *P. pseudoginseng*, *P. notoginseng*, *P. quinquefolius*, *P. japonicas*, and *P. ginseng* with supercritical CO_2 extraction technique. The electrochemical fingerprints of the 7 species of *Panax* were recorded with lipid-soluble components and polysaccharides extract. The results indicate that tetraploid species *P. ginseng* and *P. quinquefolius* are allotetraploids. *P. notoginseng, P. ginseng* and *P. quinquefolius* are clustered in one large branch and are more closely related. The diploid species *P. trifolius* and the tetraploid *P. quinquefolius* are both distributed in North America, but they are distributed on two different sub-branches on the same phylogenetic branch.

Keywords: Electrochemical fingerprint; Electroactive compounds; *Panax*; Phylogenetic study; Taxonomy

1. INTRODUCTION

Panax is a medicinally important genus within the family of Araliaceae, in which almost all species are of cultural significance for traditional Chinese medicine. It has been suggested that the disjunctive distribution of *Panax* in the East Asia and North America has been formed through two

independent events and multiple rounds of whole genome duplications (WGD) might have occurred during the evolutionary process of *Panax* [1,2]. The famous Chinese ginseng (*P. ginseng*) is distributed in northeastern China, northern Korea and eastern Siberia of the USSR [3,4]. *P. ginseng* has become an ancient relict plant with an intercontinental distribution. *P. quinquefolius* Linn. and *P. trifolius* L. have survived in the mountains of the East Coast of North America due to the coverage of the Quaternary glaciers [5,6]. However, the specific classification of species in the genus *Panax* is still controversial due to the different definitions of *P. pseudoginseng* and *P. japonicus* [7,8]. For example, the species from southwestern China were considered as *P. pseudoginsen* [9]. Subsequently, Zhou [7] classified some pseudoginseng varieties such as *P. pseudoginseng* var. *bipinnatifidus* into *P. japonicas* based on their seed morphology and medicinal value. Since then, Wen et al. [9–11] adopted molecular systematics to reconstruct a phylogenetic tree *Panax* species on the basis of the nrITS genes and chloroplast genes. Up to present, seven species and one species complex have been defined based on the geographical distribution [12–14], chromosome number and phylogenetic relationships of species in the genus *Panax*. They are *P. trifolius, P. stipuleanatus, P. pseudoginseng, P. notoginseng, P. quinquefolius, P. japonicas,* and *P. ginseng,* which are at the base of the phylogeny [15–17].

The genetic resources of these plants have potential value of application for interspecific distant hybrid breeding within the genus *Panax,* thus the taxonomic and phylogenetic study is not only a theoretical issue, but also a basis for application [18–23]. It is of great significance to conduct an in-depth study on the phylogeny of the species of the genus *Panax* and to introduce their cultivation and utilization [24,25]. Electrochemical fingerprinting is a technique for collecting electrochemically active substances in plant tissues. Since the electrochemical signal is proportional to the type and content of the substance, it can reflect the difference of electrochemically active substance profile in plants [26–33]. This technique has been applied in recent years to the study of plant phylogeny and has become an alternative technique to provide evidence [34–45]. In this work, supercritical CO_2 extraction technique was firstly adopted to extract the lipid-soluble components of ginseng. The extraction conditions of the fat-soluble components of *Panax* were optimized by single-factor test and orthogonal test. With the extract residue defatted by supercritical fluid extraction being the raw material, the supercritical fluid CO_2 extraction assisted by supersonic process was applied to extract *Panax*. The extract residue, in which the fat and ginsenosides were removed by supercritical fluid extraction, was used as the raw material and the supercritical fluid assisted hot water extraction was applied to extract polysaccharides. Afterwards, the electrochemical fingerprinting of fat-soluble components and polysaccharides were recorded. A comparative study was conducted on the results of the obtained cluster analysis and the proposed claims.

2. EXPERIMENTAL

2.1. Supercritical extraction of Panax fat-soluble components

Powder of *P. trifolius, P. stipuleanatus, P. pseudoginseng, P. notoginseng, P. quinquefolius, P. japonicas,* and *P. ginseng* were supplied by local pharmacy. 100 g dried sample powder was put into the

extraction tank through a 0.84 mm (20 mesh) aperture sieve, and the lipid-soluble components of the sample were extracted by primary extraction and secondary separation method. It was found that the effect of the extraction of lipid-soluble components of the samples was poor when no entrainment agent was added. Therefore, ethanol was used as the entraining agent. The pressure of separation kettle I and separation kettle II was set to 6 MPa. The temperature was set to 35°C. The CO_2 flow rate was controlled to 12 L/h. Anhydrous ethanol was added from the entrainment agent tank and dynamic extraction was performed. After the extraction was conducted to the set time, extracts were collected from separation kettle I and II. The extracts were removed from ethanol by vacuum rotary evaporation to obtain a brownish red oil with special aroma. The oily substance was transferred to a flat dish, placed in a desiccator and dehydrated with anhydrous sodium sulfate. The fat content of the sample powder before and after extraction was determined by Soxhlet extraction method to calculate the extraction rate of fat-soluble components of the sample.

2.2. Ultrasound-assisted supercritical CO_2 extraction of ginsenosides

Each time 100 g of ginseng powder over 0.84 mm (20 mesh) was weighed, and 250 mL of entrainer was added first because the sonication was performed in the solution system. The sonication frequency was 20 kHz and the sonication time was 20 min. The extract was transferred to a 2L extraction kettle for extraction. The pressure of separation kettle I and separation kettle II were set to 6 MPa and the temperature was set to 45°C in order to completely resolve the extracts and separate them from the supercritical fluid. During the dynamic extraction, the remaining entrainment agent was added to the extraction tank through the entrainment agent pump. The extraction solution received from separation kettle I and separation kettle II was concentrated to dryness under reduced pressure, and purified by HPD-100 macroporous sorbent resin.

2.3. Extraction of Panax polysaccharides by supercritical assisted hot water extraction

After the sample was extracted by supercritical extraction, the residue still contained about 40% of polysaccharide components. Since the material was not contaminated by organic solvents and thermally denatured during supercritical extraction, it was still a good source to extract active polysaccharides. Therefore, in this study, the extract residue after supercritical degreasing and desaponification was used as raw material to extract polysaccharides by supercritical assisted hot water extraction process. 50 g of supercritical defatted and desaponized extract residual material was weighed through a certain aperture size sieve, and a certain proportion of water was added as an entraining agent. They were well mixed and the mixed material was loaded into a 2 L extraction kettle. The carbon dioxide was added to the extraction kettle by reciprocating pump to make the pressure in the extraction kettle reach the preset value. In the meantime, the extraction kettle was heated by circulating water system to make the temperature reach the preset value. After the temperature and pressure reached the set value, the reciprocating pump was turned off and the temperature and pressure in the extraction kettle were kept constant for a certain period of time so that the supercritical fluid and the material could be mixed

evenly. When the extraction time was reached, the barrel was removed and the supercritical treated material was placed in a 1 L beaker. 500mL distilled water was added and the extraction was conducted at 100℃ for 80 min with intermittent stirring. Filtered with 120 mesh nylon cloth, the filtrate was concentrated under reduced pressure to 100 mL with 3 times the volume of 95% ethanol added, and was placed in a refrigerator at 4℃ and left overnight. The precipitate was collected after centrifugation (3800r/min, 10min) and was washed twice with anhydrous ethanol, and the polysaccharide solid was obtained.

2.4. Electrochemical fingerprinting

All electrochemical fingerprint recordings were conducted with a CHI600 electrochemical workstation. A commercial glassy carbon electrode (GCE), an Ag/AgCl electrode and a Pt electrode were adopted as the working electrode, reference electrode and counter electrode, respectively. A normalization process was conducted for all the recorded electrochemical fingerprints, where the ratios between the current and the maximum peak current were obtained at different potentials. The taxonomic analysis was carried out with hierarchical clustering method [46]. 0.1 M of PBS (phosphate buffer solution, pH=7.0) was used as electrolyte for electrochemical fingerprint recording. Differential pulse voltammetry was used for recording the electrochemical fingerprints of all plant tissue between -0.2–1.2 V.

3. RESULTS AND DISCUSSION

The electrochemical fingerprints of fat-soluble components of *P. trifolius, P. stipuleanatus*, *P. pseudoginseng*, *P. notoginseng*, *P. quinquefolius*, *P. japonicas*, and *P. ginseng* were recorded under PBS first (Figure 1). A series of oxidation peaks can be seen between -0.1 and 1.5 V for either species, representing the involvement of substances in the electrochemical oxidation reaction in the fat-soluble components. These peaks can be ascribed to the oxidation of sterols, fatty acids, sesquiterpenoids, esters, and olefins [47,48], which have exhibited a wide range of characteristics, including antioxidative, antiaging, antifatigue, adaptogenic, restorative, vasodilatory, anti-inflammatory, immunomodulatory, anticancer and antidiabetic [49].

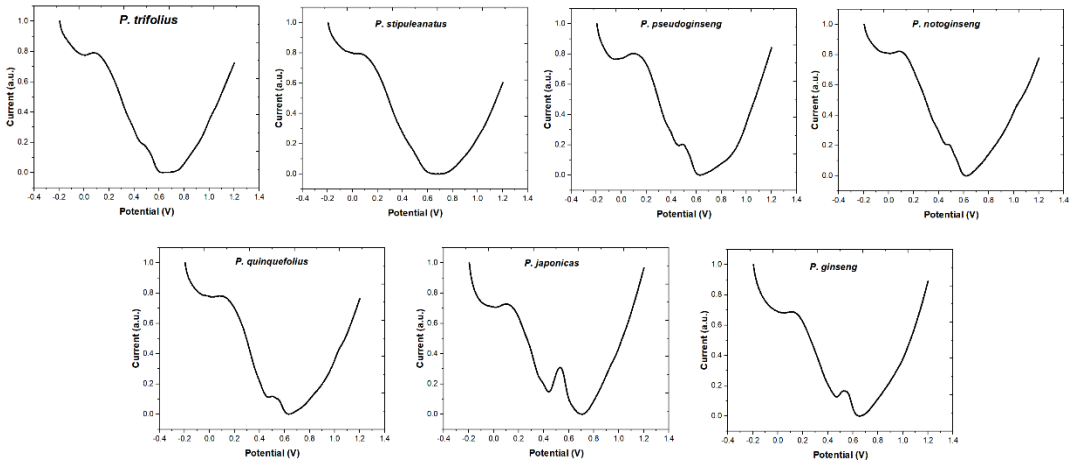

Figure 1. Electrochemical fingerprint of fat-soluble components of *P. trifolius*, *P. stipuleanatus*, *P. pseudoginseng*, *P. notoginseng*, *P. quinquefolius*, *P. japonicas*, and *P. ginseng* recorded under PBS.

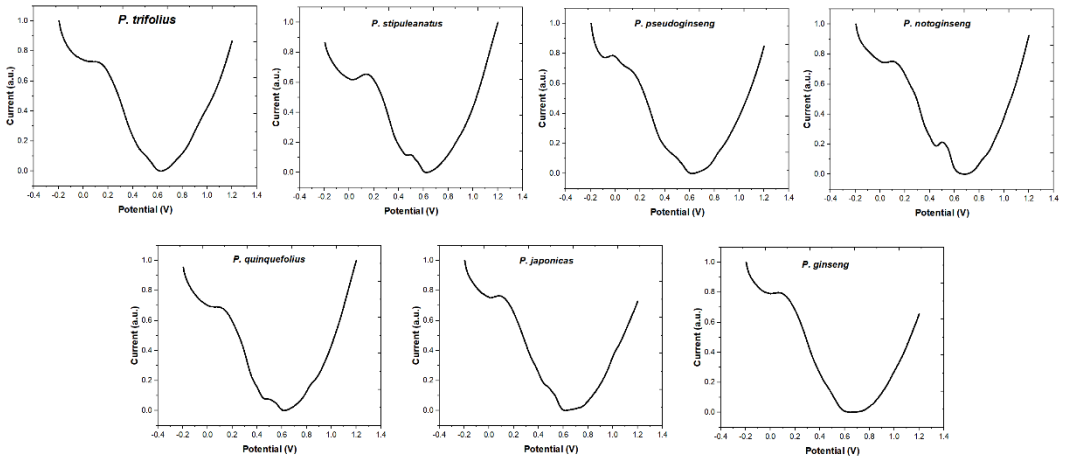

Figure 2. Electrochemical fingerprint of polysaccharides of *P. trifolius*, *P. stipuleanatus*, *P. pseudoginseng*, *P. notoginseng*, *P. quinquefolius*, *P. japonicas*, and *P. ginseng* recorded under PBS.

The electrochemical fingerprints varied considerably among different species, indicating the difference of fat-soluble components in diverse species of *Panax*. The chemical change among species was monitored by quantitative determination of ginseng components such as ginsenosides, sugar, amino-sugar, amino acids, and maltol [50]. However, some of these species show a similar profile. For example, the fingerprints of *P. pseudoginseng* and *P. notoginseng* have high similarity, which suggests that highly similar substances in the fat-soluble components of *P. pseudoginseng* and *P. trifolius* can be electrochemically oxidized in PBS at pH 7.0. However, this does not exactly mean that *P. pseudoginseng* and *P. trifolius* have the same fat-soluble components and therefore some molecules are not electrochemically active. In addition, some molecules are electrochemically active only under acidic or

basic conditions. Therefore, the electrochemical fingerprints of the extracted polysaccharides were further recorded in this study. As shown in Figure 2, the fingerprints of extracted polysaccharides of *P. pseudoginseng* and *P. notoginseng* have a very distinct difference.

We further tried to statistically analyze all species with principal component analysis (PCA), which is a conversion in the vector space used to reduce the dimensions of a dataset. In this method, the responses can be analyzed as a result of the exerted treatments on the samples in the vector space based on the correlation between the extracted data in a dataset [51,52]. The statistical analysis can explain the similarity of electrochemically active substances among different species. As shown in Figure 3A, four eigenvalues of principal components can well represent the difference of all data sets. Figure 3B presents that the three extracted factors can reach an interpretation of 89%, which indicates that the electrochemical fingerprints among different species are not easily distinguished in a particularly obvious way.

Hierarchical clustering is one of the most commonly used methods for clustering data, which can divide the data set into different clusters by iteratively merging or splitting clusters based on a dendrogram. Clustering algorithms provide the information of the data to be classified in a compact and graphical way. They can be agglomerative or divisive depending on the applied procedure to create the dendrogram [53]. Agglomerative clustering follows a bottom-up strategy, in which, initially, each data point is assumed as a cluster, and afterwards, iteratively, it merges the two most similar clusters in terms of an objective function until obtaining the final dendrogram. In contrast, divisive clustering follows an opposite approach [54], in which all data points are considered initially as one cluster, and afterwards, iteratively, the selected cluster is partitioned into two new subclusters. Agglomerative methodology is computationally more advantageous and more frequently applied. As this approach involves the merging of the two most similar clusters at each step, the choice of a measure or distance function with suitable similarity is essential to the final result since it is likely to have different alternatives [55].

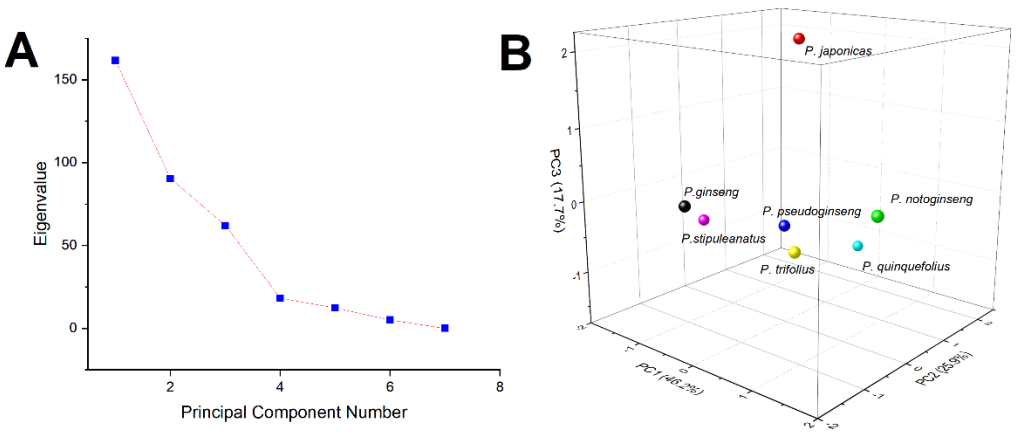

Figure 3. (A) eigenvalue of principal component number. (B) PCA analysis of *P. trifolius*, *P. stipuleanatus*, *P. pseudoginseng*, *P. notoginseng*, *P. quinquefolius*, *P. japonicas*, and *P. ginseng*.

Previous reports proposed that the extant tetraploid species *P. ginseng* and *P. quinquefolius* are allotetraploids [56]. As shown in Figure 4, the investigation of this study also supports this claim. *P. ginseng*, *P. japonicus* and *P. quinquefolius* show a multiple origin developmental pattern. Previous studies suggested that *P. notoginseng* , *P. ginseng* and *P. quinquefolius* are clustered in one large branch and are more closely related [57], while the results of this study also support this claim to some extent. The diploid species *P. trifolius* and the tetraploid *P. quinquefolius* are both distributed in North America, but they are distributed on two different sub-branches on the same phylogenetic branch.

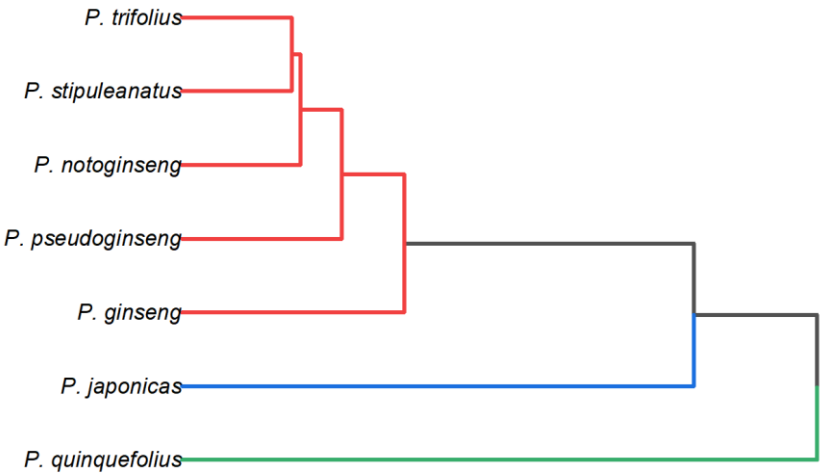

Figure 4. Dendrogram of *P. trifolius*, *P. stipuleanatus*, *P. pseudoginseng*, *P. notoginseng*, *P. quinquefolius*, *P. japonicas*, and *P. ginseng* based on electrochemical fingerprints.

4. CONCLUSION

In conclusion, the lipid-soluble components, ginsenosides and polysaccharides were extracted from *P. trifolius*, *P. stipuleanatus*, *P. pseudoginseng*, *P. notoginseng*, *P. quinquefolius*, *P. japonicas*, and *P. ginseng* in this study with supercritical CO_2 extraction technique. The electrochemical fingerprints of the 7 species of *Panax* were recorded with lipid-soluble components and polysaccharides extract under PBS. These electrochemical fingerprints can be adopted in phylogenetic investigation. The results indicate that tetraploid species *P. ginseng* and *P. quinquefolius* are allotetraploids. *P. notoginseng*, *P. ginseng* and *P. quinquefolius* are clustered in one large branch and are more closely related. The diploid species *P. trifolius* and the tetraploid *P. quinquefolius* are both distributed in North America, but they are distributed on two different sub-branches on the same phylogenetic branch.

ACKNOWLEDGEMENTS
This work was supported by Xinjiang Uygur Autonomous Region Health and Young Medical Science and Technology Talents Special Project (WJWY-202108), Tianshan Youth Program (2020Q144) and Scientific Research Project of Bazhou People's Hospital (BHS 201913).

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