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Monitoring and Analysis of *Ginkgo Biloba* Species/growth status by Electrochemical Fingerprinting During One Season

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We employed electrochemical fingerprinting to track the growth of male and female *Ginkgo biloba* in this communication. The electrochemical fingerprinting of *Ginkgo biloba* did not change significantly over the course of a one-year development cycle, although changes in the concentrations of various electrochemically active compounds could be observed. These changes in fingerprint patterns were related to seasonal variations in the chemical composition of *Ginkgo biloba* leaves. We can use these differences to not only determine the gender of *Ginkgo biloba*, but also to identify it in various months. On the basis of these data, we propose an identification flow chart based on electrochemical fingerprinting. This work demonstrates that electrochemical fingerprinting technology is an extremely effective tool for monitoring plant growth and has potential applications in agricultural management and plant growth.

Keywords: Electrochemical fingerprinting; Plant monitoring; *Ginkgo biloba*; Sex determination; Phytochemistry

1. INTRODUCTION

Ginkgo biloba, the world's oldest relict plant, has been studied for medicinal purposes for over 600 years. Ginkgo biloba has been analyzed and over 70 flavonoids, primarily quercetin, isorhamnetin, kaempferin, and their glycosides, determined. Ginkgo flavonoids have been shown to have hypolipidemic, antibacterial, antitumor, and antioxidant properties [1–3]. The amount of flavonoids and terpene lactones in ginkgo extract has become a significant indicator of the preparation's quality. How to increase the flavonoids and terpene lactone content of *Ginkgo biloba* has become a pressing issue in

contemporary research [4–7]. In 1991, Schwade Pharmaceuticals patented the standard extract of *Ginkgo biloba*, EGb761.

The most significant application of EGb761 is its ability to regulate cerebral blood circulation. Flavonoid glycosides account for 24% of the total, terpene lactones account for 6%, and ginkgolic acid accounts for 10% of the total. As the research progressed, it was discovered that one of the primary functions of ginkgo extract, a ginkgolide mixture designated BN52063, was as a clinically useful inhibitor of platelet-activating factor (PAF).

Chemical synthesis, biosynthesis, cell culture, and natural extraction are the primary methods for obtaining ginkgo flavonoids and terpene lactones [8–10]. Due to the fact that the majority of active ingredients are chiral molecules that are difficult to chemically synthesize, the first three routes are currently restricted to laboratory research and are not yet ready for industrial production [11–18]. Bioextraction is still the primary method for obtaining the active ingredients in Ginkgo biloba, and the leaf is the primary source of naturally extracted flavonoids and terpene lactones. The amount of flavonoids and terpene lactones in ginkgo leaves is a critical indicator of the leaves' quality, and enhancing the leaves' quality has become a pressing issue in production and scientific research [19,20]. According to research, a variety of factors can affect the quality of ginkgo leaves, including individual differences in ginkgo, external environmental factors, and production and cultivation practices [21,22]. Changing environmental factors during production is an effective way to increase the yield of ginkgo secondary metabolites, which has a broad application potential in production [23,24].

Electrochemical fingerprinting is a technique for detecting substances that are electrochemically active in electrochemical systems. This technique has been widely used for plant identification and phylogenetic studies in recent years [25–43]. Flavonoids found in plant tissues frequently exhibit strong electrochemical activity, making them a significant signal in electrochemical fingerprinting. Due to *Ginkgo biloba*'s significant medicinal value, we sought to monitor its growth over time using electrochemical fingerprinting techniques. This not only demonstrates the feasibility of electrochemical fingerprinting in plant monitoring, but also allows for the investigation of changes in the electrochemical active substances in *Ginkgo biloba* over time. As a result, we monitored female and male ginkgoes for electrochemical fingerprinting from June to December. The electrochemical fingerprinting data were compared and a two-dimensional density pattern was generated. On the basis of this information, flowcharts for determining the ginkgo's sex and month were proposed.

2. MATERIALS AND METHODS

Male and female *Ginkgo biloba* leaves were collected from Hangzhou Dianzi University's campus. Picking occurred once a week. Between June and November 2021, all fresh female *Ginkgo biloba* leaves were collected. Between June and December 2021, all male fresh *Ginkgo biloba* leaves were collected. All reagents were analytical grade and were used as-is.

The extraction was performed with either ethanol or water as the solvent. In a standard extraction technique, 1 g of leaves were added to 2 mL solvent. The mixture was then sonicated with the addition of two mill beads in a high capacity tissue grinding machine (MB-24S, Meibi Co Ltd, China). After

resting, the supernatant was used to extract the extracts. PBS (pH 7.0) and ABS were used as electrolytes (pH 4.5). All samples were electrochemically fingerprinted using a CHI760 electrochemical workstation. Electrochemical fingerprints were recorded using a three-electrode configuration comprised of a commercial glassy carbon electrode (GCE, 3 mm), an Ag/AgCl electrode, and a Pt electrode as the working electrode, reference electrode, and counter electrode, respectively. For a conventional electrochemical fingerprint recording method, 1 mL of plant extract was added to a 4 mL electrolyte. After that, a differential pulse voltammetry (DPV) signal between 0 and 1.2 V was collected. Following that, the experimental data were standardized to facilitate subsequent analysis.

3. RESULTS AND DISCUSSION

The schematic diagram in Figure 1 illustrates the electrochemical fingerprinting technique used to record the electrochemically active substances in *Ginkgo biloba* leaves. As illustrated, the ginkgo leaves are extracted using a suitable solvent and then added to an electrolyte for voltammetric scanning. The electrochemically active substances in *Ginkgo biloba* leaves can be oxidized at specific potentials and produce currents during the scan. This current data can be used to determine the ginkgo sex and growth month.

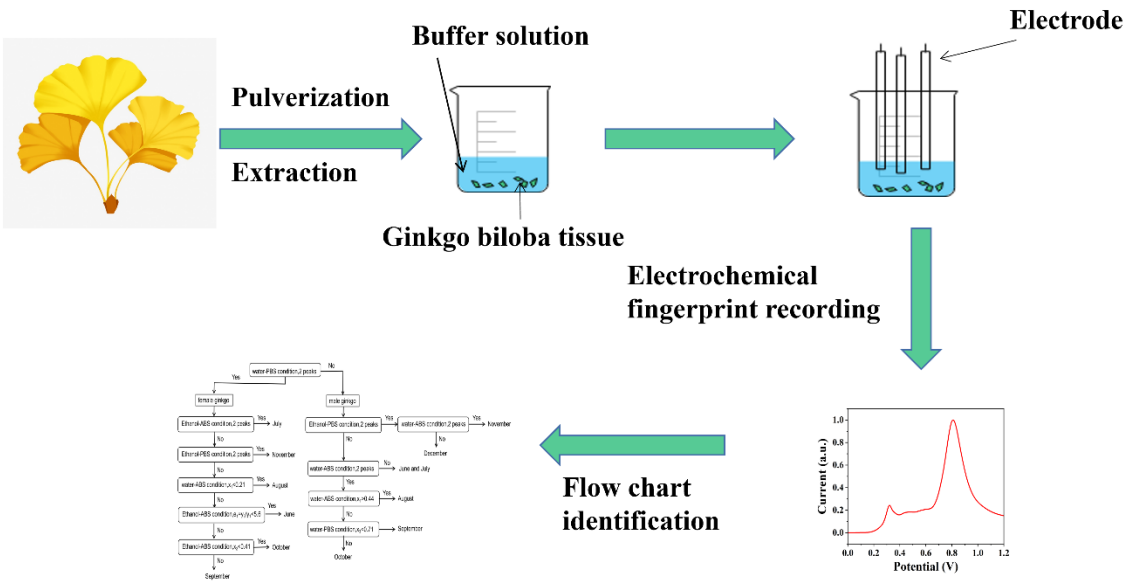

Figure 1. Scheme of recording electrochemical fingerprints of *Ginkgo biloba* for identification.

The electrochemical fingerprints of typical male and female *Ginkgo biloba* leaves extracted with water and then in PBS are shown in Figure 2. We can see that the electrochemical fingerprints of *Ginkgo biloba* leaves of different sexes are strikingly similar. They all demonstrated three distinct peaks near 0.21 V, 0.42 V, and 0.77 V, respectively. These three peaks were primarily caused by flavonoids oxidation in *Ginkgo biloba* [44–47]. While the sexes of *Ginkgo biloba* are distinct, they are still members

of the same species, and thus the electrochemically active substances in their tissues will be similar. Electrochemical fingerprinting differences are primarily due to genetic differences between species [48]. As a result, species with a closer phylogenetic relationship exhibit less variation in electrochemical fingerprinting. Significant differences between species that are only distantly related can be observed. However, we discovered some differences in the electrochemical fingerprint profiles of different sexes of Ginkgo biloba, which allow us to differentiate the sexes.

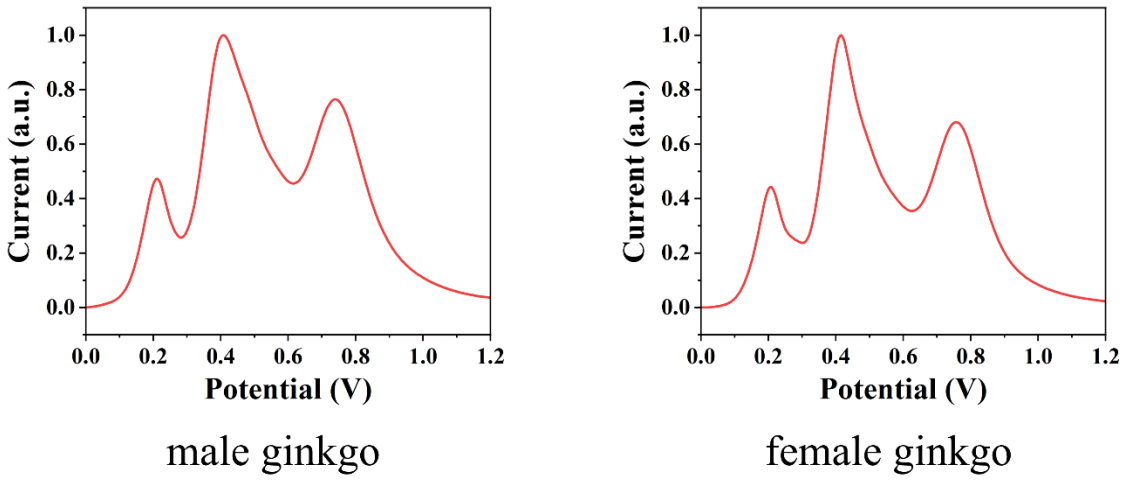

Figure 2. Typical electrochemical fingerprints recorded from male and female *Ginkgo biloba.*

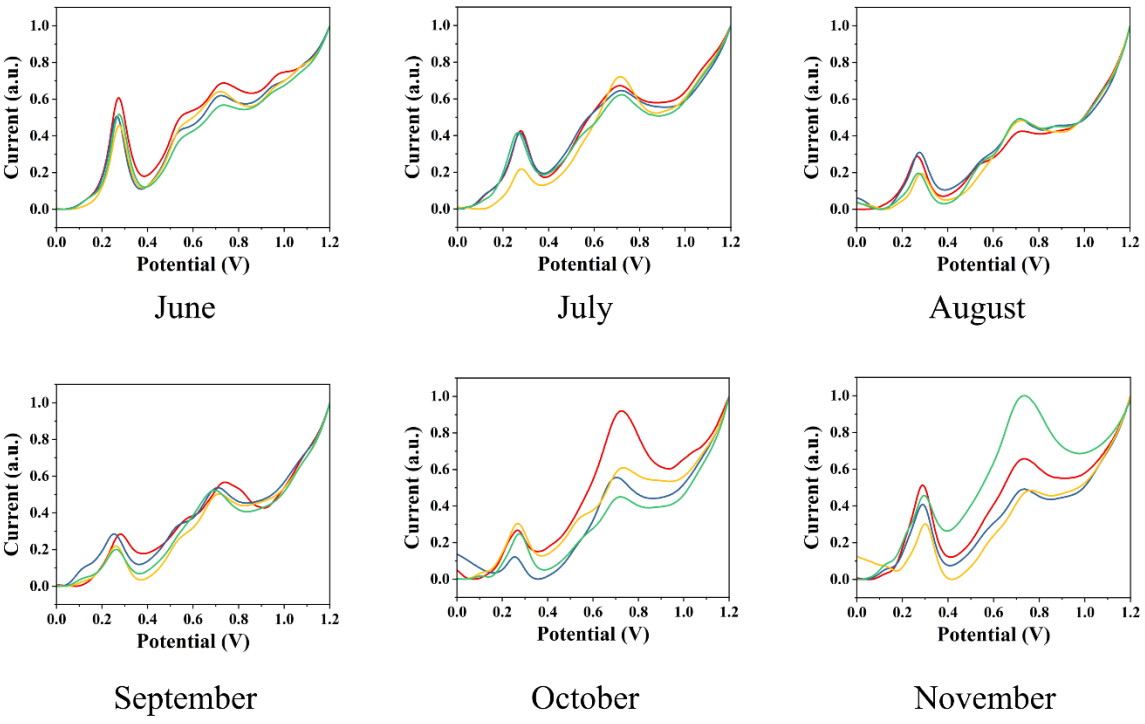

Figure 3. Electrochemical fingerprints of female *Ginkgo biloba* (water extraction + PBS) recorded at different month.

The electrochemical fingerprints of female *Ginkgo biloba* leaves collected in various months after being extracted with water and PBS are shown in Figure 3. We discovered that fingerprints collected during different weeks of each month were not identical, as they reflect changes in electrochemically active substances in plant tissues. The content of these substances may vary according to growth stage and environment [49,50]. By comparing fingerprint profiles from various months, it is possible to observe that the fingerprint profiles of plants change slightly over time. The electrochemical fingerprints of female *Ginkgo biloba* leaves extracted with ethanol and collected under ABS are shown in Figure 4. We obtained results that were very similar. This demonstrates that the electrochemical fingerprinting technique can be used to monitor the growth of the plant at various stages of development [51]. At the same time, because the electrochemical fingerprints of different growth stages are similar, this technique can be used to identify species regardless of seasonal variations.

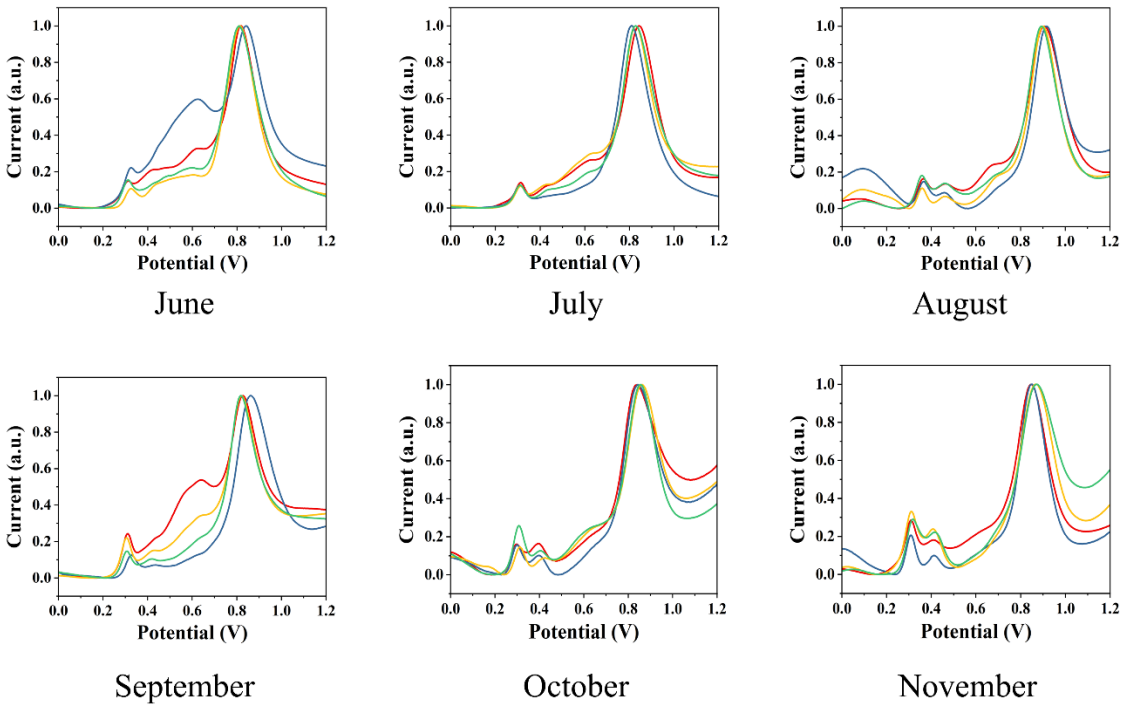

Figure 4. Electrochemical fingerprints of female *Ginkgo biloba* (ethanol extraction + ABS) recorded at different month.

The electrochemical fingerprints of male *Ginkgo biloba* leaves collected in PBS following water extraction are shown in Figure 5. Due to the fact that male *Ginkgo biloba* leaves fall later than female *Ginkgo biloba* leaves, it has seven months of data. As illustrated in the figure, the electrochemical fingerprint profile of male *Ginkgo biloba* contains three additional distinct characteristic peaks, whereas the main characteristic peaks in female *Ginkgo biloba* are two. Simultaneously, as winter approaches, the male *Ginkgo biloba*'s first characteristic peak gradually fades away. While *Ginkgo biloba* leaves appear to yellow gradually over time, changes in electrochemical fingerprinting are not always directly related to pigment changes in the plant [52,53]. The electrochemical fingerprints of male *Ginkgo biloba*

leaves extracted with ethanol and collected under ABS are shown in Figure 6. Male *Ginkgo biloba* electrochemical fingerprint profiles were not significantly different from those of females under these conditions.

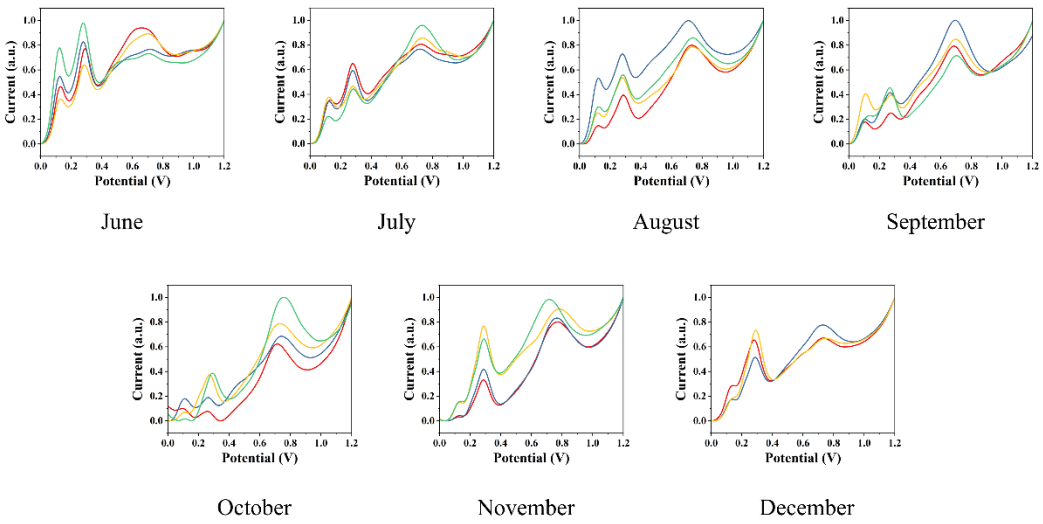

Figure 5. Electrochemical fingerprints of male *Ginkgo biloba* (water extraction + PBS) recorded at different month.

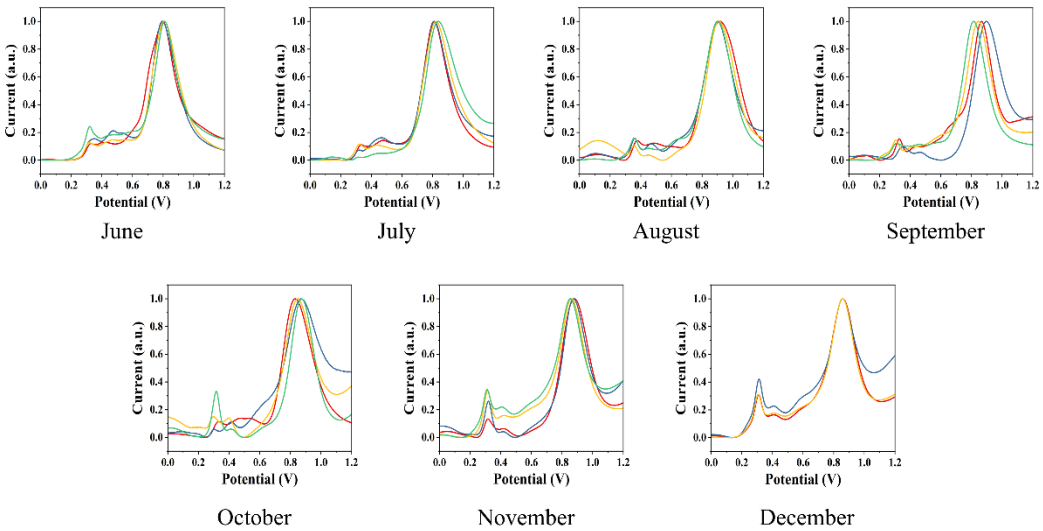

Figure 6. Electrochemical fingerprints of male *Ginkgo biloba* (ethanol extraction + ABS) recorded at different month.

These changes in electrochemical fingerprints over time are most likely caused by variations in flavonoid content in leaf tissue. Previous research has established that the flavonoids present in *Ginkgo biloba* leaves are dynamic and strongly correlated with the growth environment and season [54–56]. Changes in the growth environment and season, on the other hand, do not alter the type of flavonoids

but only their content. This conclusion is also supported by our current work's electrochemical fingerprinting. We did not observe any abrupt oxidation peaks in any sample.

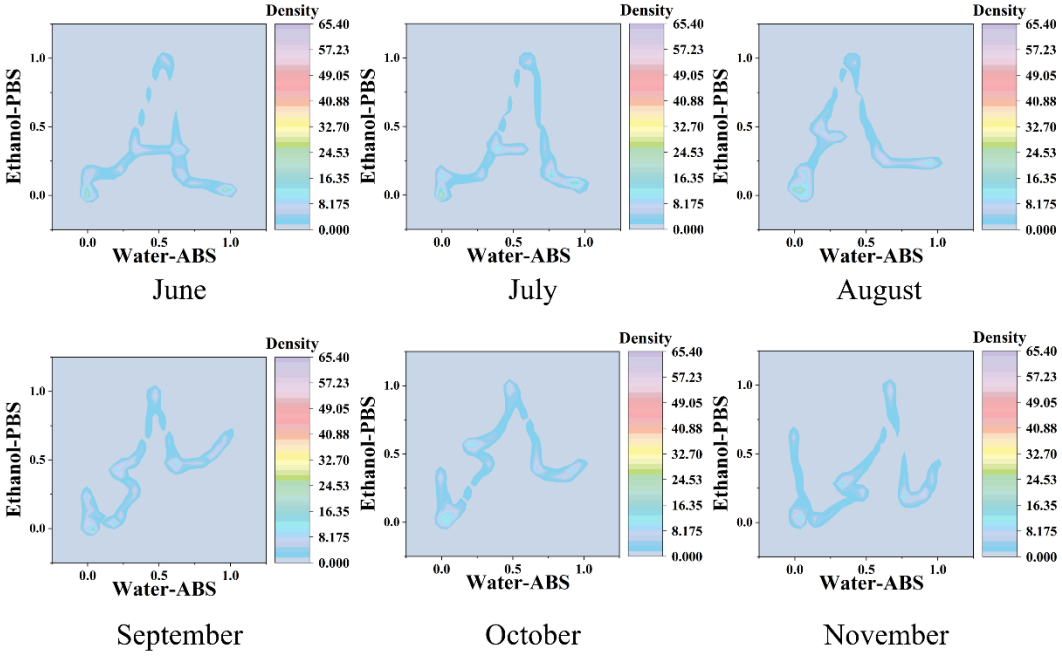

Figure 7. 2D density patterns of female *Ginkgo biloba* using electrochemical fingerprints recorded under ethanol extraction + ABS and water extraction + PBS.

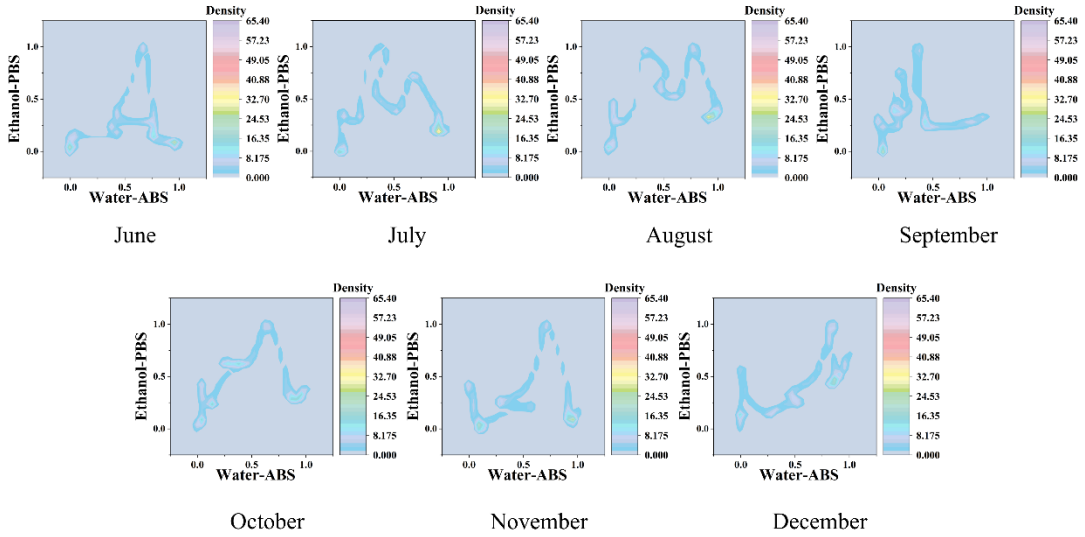

Figure 8. 2D density patterns of female *Ginkgo biloba* using electrochemical fingerprints recorded under ethanol extraction + ABS and water extraction + PBS.

Due to the high degree of similarity in the properties of Ginkgo biloba, the DPV profile is an unobtrusive method for identifying it. We created patterns from the voltammograms in order to quickly determine the sex or stage of development. The Figures 7 and 8 depict the two-dimensional density

pattern generated from electrochemical fingerprints taken in two distinct environments. As can be seen, increasing the dimension of data improves the ability to distinguish variations.

To facilitate the identification of *Ginkgo biloba* sexes and different growth states, we summarized a flow chart using electrochemical fingerprinting (Figure 9). This flowchart can identify different sexes of *Ginkgo biloba* in different months by analyzing the electrochemical fingerprinting features one by one. If there is a Peak 2 in water + PBS, for example, it is a *Ginkgo biloba*. Additionally, if the oxidation potential of Peak 1 is less than 0.21 V in the presence of water + ABS, this sample was collected in August. Additionally, if there is no Peak 2 in water + PBS, it is a male *Ginkgo biloba*. Simultaneously, if Peak 2 is present in the case of ethanol + PBS and Peak 2 is present in the case of water + ABS, it was picked in November. This flow chart enables operators who are unfamiliar with electrochemical analysis to carry out quick operations, which could be useful in agricultural production and quality control.

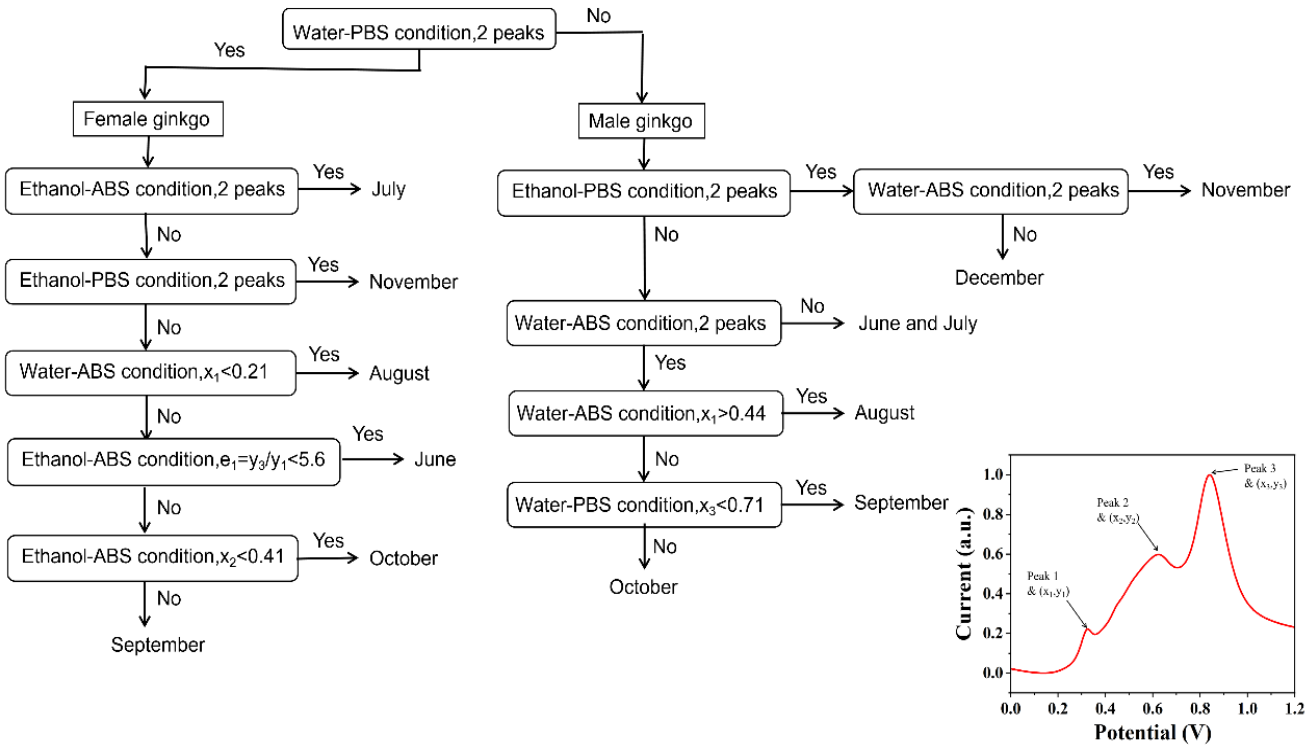

Figure 9. Flow chart for identification of sex and growth stages of *Ginkgo biloba*.

4. CONCLUSION

The purpose of this study was to demonstrate the feasibility of electrochemical fingerprinting for monitoring plant growth status. Different *Ginkgo biloba* sexes were chosen as targets. Once a week, ginkgo biloba leaves were harvested and electrochemical fingerprinting was performed. Although some differences in the electrochemical fingerprinting of the different sexes of *Ginkgo biloba* were discovered, they had no effect on their utility for species identification. There were also some differences in the

electrochemical fingerprint profiles of *Ginkgo biloba* from different months, but they also did not affect the species identification. We propose a flow chart for identifying *Ginkgo biloba* of various sexes and growth states.

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