Int. J. Electrochem. Sci., 17 **(2022)** Article Number: 220770, doi: 10.20964/2022.07.64

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Feasibility Study on Identification of the Authenticity of Honeysuckle Using Electrochemical Fingerprinting

Sujuan Li[1], *Baoxia Tang*[2], *Xiaolian Zhan*[3], *Min Wan*[1,*]

[1] Department of Pharmacy, People's Hospital of Dongxihu District, Wuhan, 430040 China
[2] Clinical oft of Laboratory, People's Hospital of Dongxihu District, Wuhan, 430040 China
[3] Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022 China
[4] Sichuan of China National Tobacco Corporation, Chengdu, Sichuan Province, China
[*]E-mail: wanmindrwan@163.com

Received: 6 April 2022 / *Accepted:* 10 May 2022 / *Published:* 6 June 2022

Authentication of honeysuckle has always been an important challenge in herbal medicine due to the enormous sales every year. Electrochemical fingerprinting is an emerging technique for plant analysis. However, previous studies have focused on the analysis of fresh plants. In this work, we have used this technique to analyze plant products. We selected honeysuckle and three of its counterfeit for electrochemical fingerprinting. Also, we collected samples of different origins and different years. Two extraction solvents and buffer solutions were used to acquire electrochemical fingerprints. The results show that the electrochemical fingerprinting technique is a very effective method for identifying different plant products. Honeysuckle and its counterfeit products can be identified very clearly. On the other hand, electrochemical fingerprinting has the potential to identify plant products of different origins. However, plant products of different years of the same origin were not identified. We also performed principal component analysis and cluster analysis on the electrochemical fingerprinting profiles. These statistical techniques proved that electrochemical fingerprinting is an effective tool for plant analysis.

Keywords: Electrochemical fingerprint; Honeysuckle; Plant products; Pattern recognition; Plant analysis

1. INTRODUCTION

Honeysuckle is the dried flower bud or first flower of *Lonicera japonica* Thunb, commonly used as a herb in traditional Chinese medicine. 80% of the marketed Chinese herbal prescriptions for antipyretic and antiseptic products contain honeysuckle in China [1–4]. With the increasing demand in the market and rising prices, some unscrupulous merchants are using plants such as *Lonicera macranthoides*, *Lonicera hypoglauca* Miq, *Lonicerafu votomentosa* Hsu et S.C.Cheng as *Lonicera*

japonica Thunb. This has caused severe harm to the market and the health and interests of the people [5–10]. Therefore, there is an urgent need to develop a simple, low-cost, and more stable method for authenticating honeysuckle [11,12].

Some analytical techniques have been used to identify the authenticity of Chinese herbal medicines. NIR spectroscopy, for example, is a rapid and efficient analytical approach for a wide variety of organic chemicals that was first utilized in agriculture [13,14]. Due to several benefits, including its speed, non-destructive nature, and cheap cost, near-infrared spectroscopy is fast gaining traction in food analysis and identification [15–20]. Cozzolino et al. [21] examined visible near-infrared spectroscopy to differentiate white wines from various sources in Australia. They developed a discriminant model by combining principal component analysis, principal component regression, and partial least squares regression, and the discriminant partial least squares model produced satisfactory results. Liu et al. [22] employed NIR spectroscopy in conjunction with multivariate statistical analysis to determine the provenance of Tempranillo wines from Australia and Spain, with satisfactory findings using partial least squares discriminant analysis. HPLC is one of the most extensively used and successful separation and analysis procedures in labs due to its benefits of rapid analysis, high repeatability, and high quantitative accuracy [23–26]. Additionally, it has developed into a significant separation technology in chemistry, medicine, biology, and environmental protection. Taamalli et al. [27] employed HPLC-ESI-TOF-MS to quantify and characterize 23 phenolic compounds in olive oils from various sources, and cross-validation revealed that the categorization was 100 percent accurate. Atomic absorption spectroscopy (AAS) is a quantitative analytical technique that uses the absorption capacity of spectral lines generated by a substance's atomic vapor, primarily for the measurement of trace and trace components in samples [28–30]. It has a high selectivity, high sensitivity, and a broad analytical range.

Electrochemical fingerprinting is a relatively recent chemical analysis approach that has widespread use in plant research [31–40]. Electrochemical fingerprinting may be used to identify compounds in electrochemically active plants. This fingerprint can be used to identify plant species, but it may also be utilized to track plant development. In this work, electrochemical fingerprinting identifies the authenticity, origin, and year of honeysuckle. We analyzed different samples by pattern recognition techniques. The work provides a new methodology for rapidly identifying honeysuckle.

2. EXPERIMENTAL

2.1. Reagents and samples

All chemicals used in this work were analytical grade. Samples of honeysuckle were purchased from the local market and online store. All dry samples were ground into powder and stored in the fridge. All information on honeysuckle has been listed in Table 1. The honeysuckle electrochemically active compounds were extracted using either water or ethanol. The particular extraction procedure involves dispersing a certain quantity of honeysuckle powder in the solvent. For two minutes, the dispersion was sonicated. After allowing the supernatant to precipitate, it is introduced to the electrolyte for

electrochemical testing. Electrolytes such as 0.1 M phosphate buffer solution (PBS) and acetate buffer solution (ABS) were utilized to aid in the recording of electrochemically active compounds.

2.2. Electrochemical fingerprints recording

For fingerprint recording, differential pulse voltammetry (DPV) was utilized. Glassy carbon electrode, platinum wire electrode, and calomel electrode were used as a working electrode, a counter electrode, and a reference electrode, respectively. Three electrodes were inserted into an electrolyte, and honeysuckle extract was added. Electrochemical fingerprints were collected after the solution was left standing for 1 min.

Table 1. Sample information of collected Chinese wolfberry.

Sample	Abbreviation	Location	Year
Lonicera japonica-1	J-1	Henan-Xinmi	2021
Lonicera japonica-2	J-2	Henan-Xinmi	2019
Lonicera japonica-3	J-3	Henan-fenqiu	2021
Lonicera japonica-4	J-4	Henan-fenqiu	2019
Lonicera macranthoides-1	M-1	Shandong-Yecheng	2021
Lonicera macranthoides-2	M-2	Shandong-Yecheng	2019
Lonicera macranthoides-3	M-3	Hebei-Julu	2021
Lonicera macranthoides-4	M-4	Hebei-Julu	2019
Lonicera hypoglauca-1	H-1	Henan-fenqiu	2021
Lonicera hypoglauca-2	H-2	Henan-fenqiu	2019
Lonicera hypoglauca-3	H-3	Henan-kaifeng	2021
Lonicera hypoglauca-4	H-4	Henan-kaifeng	2019
Lonicerafu votomentosa-1	V-1	Hebei-Anguo	2021
Lonicerafu votomentosa-2	V-2	Hebei-Anguo	2019
Lonicerafu votomentosa-3	V-3	Henan-fenqiu	2021
Lonicerafu votomentosa-4	V-4	Henan-fenqiu	2019

3. RESULTS AND DISCUSSION

The electrochemically active components in plant tissues can be used to identify plant species. However, the previously reported work was basically for testing fresh plants, but not for plant products [41,42]. Chinese herbs are very commercially valuable plant products. However, the price of different

herbs varies, so it is crucial to identify the herbs. Figure 1 shows the profiles of J-1, M-1, H-1, and V-1 after water extraction for electrochemical fingerprinting in PBS. *Lonicera japonica* contains iridoid glycosides, polyphenolic compounds, triterpene saponins, fatty acid esters and long chain hydrocarbons [43–45]. During the electrochemical fingerprints recording, its polyphenolic components could participate the electrochemical oxidation. The main polyphenolic components in *L. japonica* are chlorogenic acid, caffeic acid, hyperoside and luteolin [46]. It can be seen from the figure that different species exhibit different electrochemical fingerprint profiles. This is because there are significant differences in the chemical composition of different plants, and therefore they also have significant differences in electrochemical behavior [47]. From these differences, electrochemical fingerprinting is feasible for identifying plant products. Specifically, electrochemical fingerprinting techniques can be used the identification the authenticity of honeysuckle [48].

The use of different extraction solvents and buffer solutions for fingerprinting can further enhance identification accuracy. Figure 2 shows DPV curves of J-1, M-1, H-1, and V-1 after ethanol extraction and recorded under ABS. It can be seen that the four different plant products exhibit different electrochemical behaviors under different conditions. These differences in electrochemical behavior are due to the involvement of different electrochemically active molecules in electrochemical reactions [49]. On the other hand, the pH of the buffer solution also affected the electrochemical behavior. Some substances will be involved only in a specific pH range.

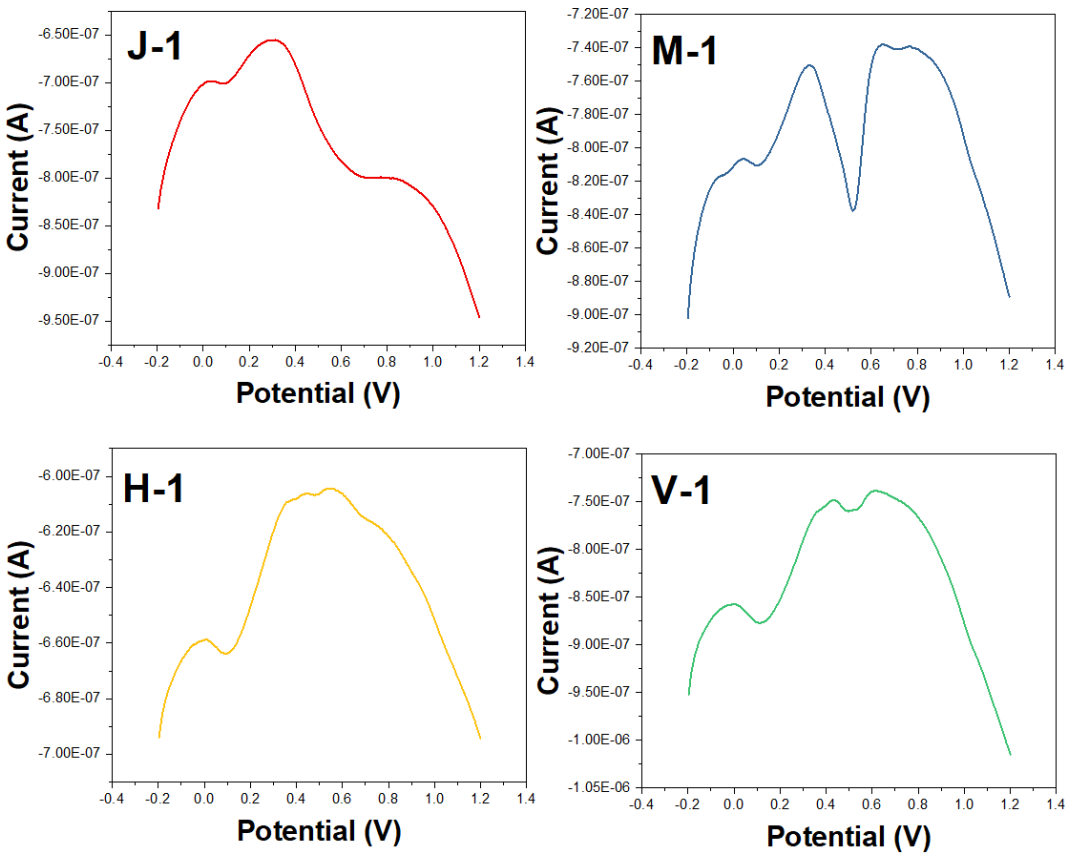

Figure 1. DPV curves of J-1, M-1, H-1 and V-1 after water extraction and recorded under PBS.

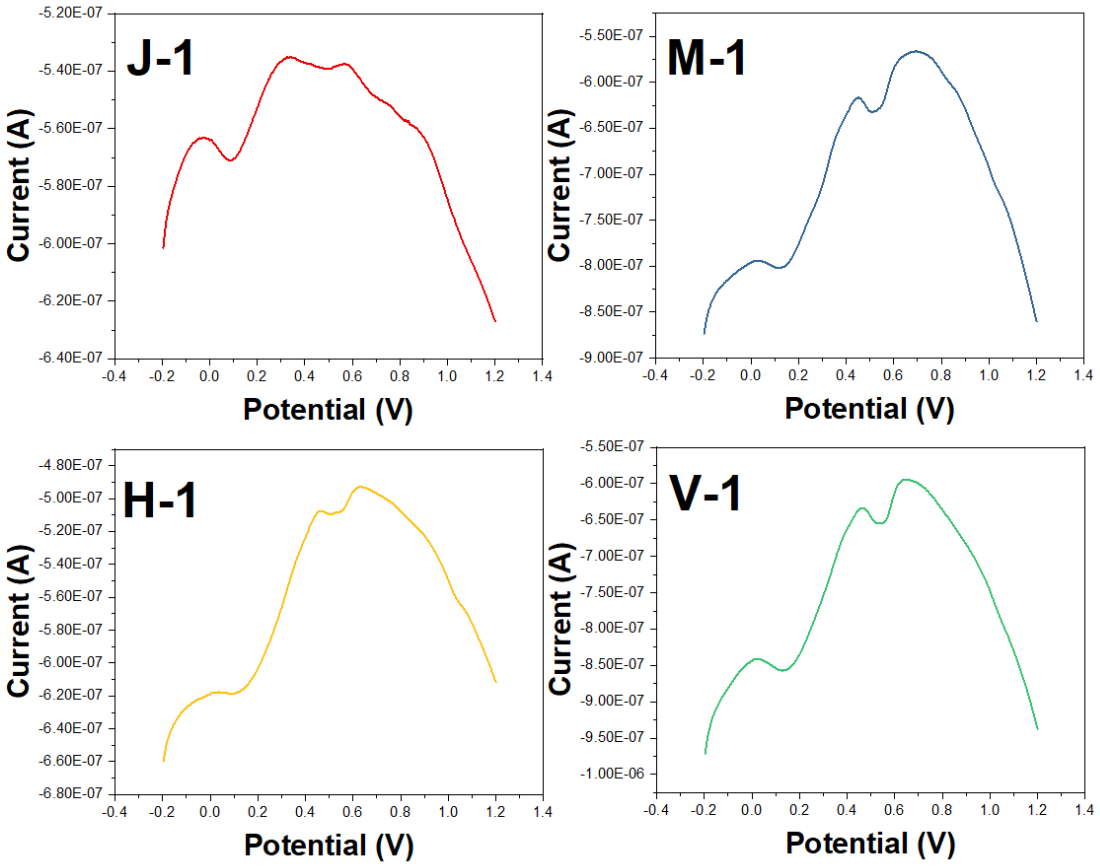

Figure 2. DPV curves of J-1, M-1, H-1 and V-1 after ethanol extraction and recorded under ABS.

Direct electrochemical fingerprinting to identify different plants would not be particularly convenient. However, pattern construction using electrochemical fingerprinting under different conditions can further lead to a more accurate and faster means of identification. Since the X-axis of electrochemical fingerprinting is information about the potential, it is the same in any sample and does not provide helpful information [50,51]. Therefore, we believe that combining the current values of the two sets of electrochemical fingerprints can be constructed as a scatter plot. The abundance of information in these scatter plots will be greater than that of a single fingerprint profile. Identification by scatter plot would be more effective than direct identification by electrochemical fingerprinting [52]. Figure 3 shows the 2D plots of J-1, M-1, H-1, and V-1 using data recorded after water extraction (PBS) and ethanol extraction (ABS). It can be seen that the scatter plots of different plants have substantial differences. Different plants can be identified quickly by dividing and calculating the area of these scatter diagrams.

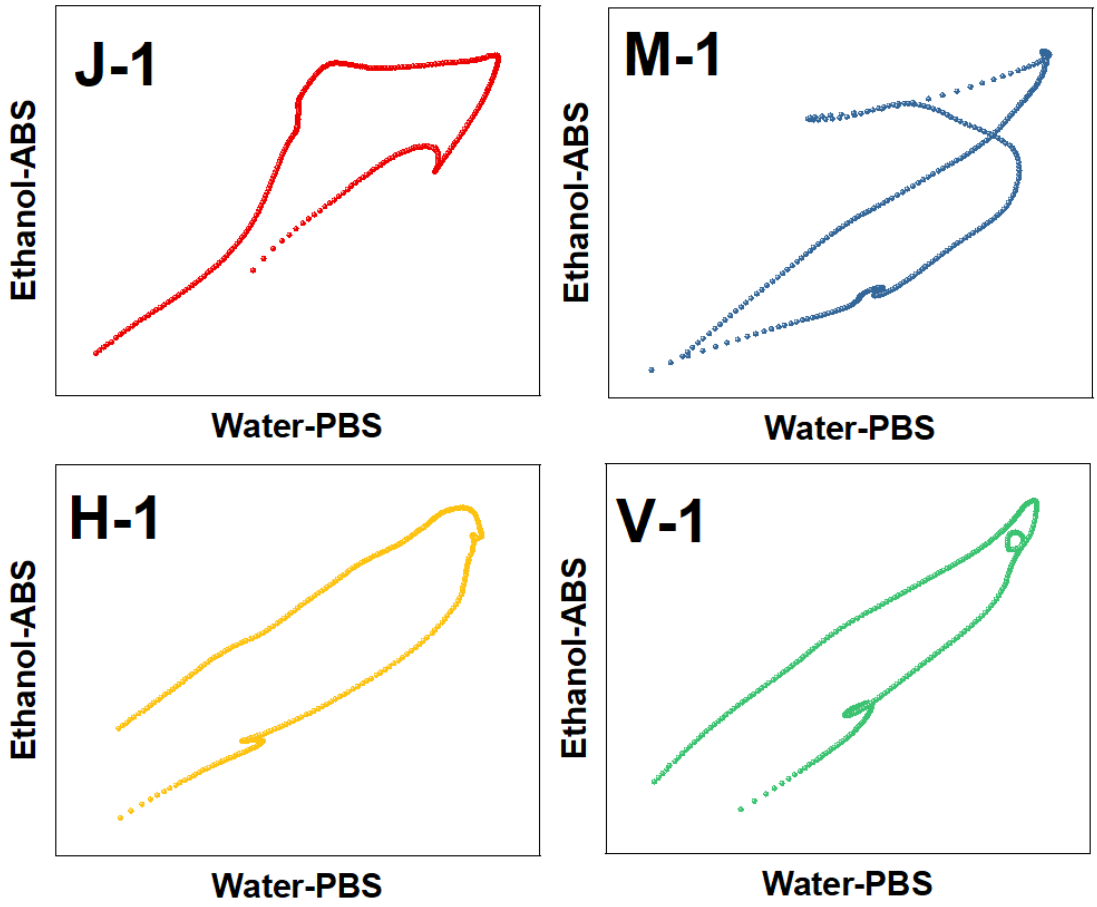

Figure 3. Scatter plots of J-1, M-1, H-1 and V-1 using fingerprints recorded after water extraction (PBS) and after ethanol extraction (ABS).

Scatter plots are still not a particularly effective way to identify samples visually, although there is a significant difference in the presentation of different samples [53]. Therefore, we implemented a kind of heat map construction using the same data set. The heat map automatically grids the entire pattern. The identification of samples can be achieved by locating hot areas in the different grids of the heat map.

In addition to identifying forgeries of honeysuckle, we also tried to identify the origin and year of honeysuckle (counterfeit) by electrochemical fingerprinting. Figure 5A shows DPV curves of J-1, J-2, J-3, and J-4 after ethanol extraction and recorded under ABS. As can be seen from the figure, J-1 and J-2 are very similar, while J-3 and J-4 are also very similar. Therefore, the age of honeysuckle has a minimal effect on electrochemical fingerprinting. On the other hand, there were some differences between J-1 and J-3, representing some variability in the electrochemical active substances of honeysuckle from different origins. This is because different cultivation regions represent significant climatic and soil differences, affecting the synthesis of phytochemicals [54]. However, these differences are much more minor than in Figure 1, and this is because the differences in the type and content of molecules in plant tissues are still primarily controlled by genes. Similar electrochemical trends can be observed in Figure 5 B-D. This further illustrates that electrochemical techniques can be used to identify different plant species and have potential use for provenance identification [55]. However, there is not

much difference in the electrochemically active substances in plant products of different ages, and this technique is not used for year identification.

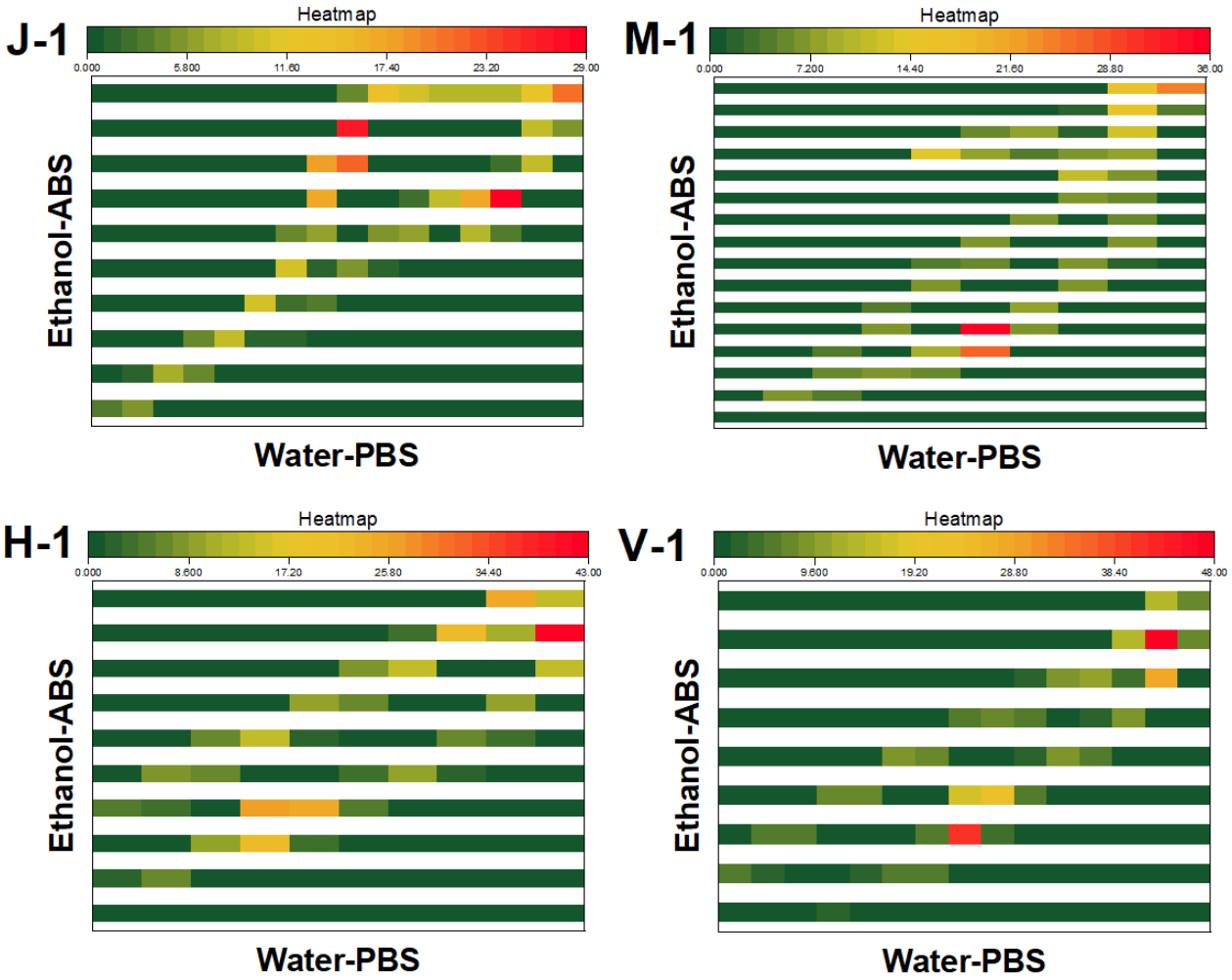

Figure 4. Heat maps of J-1, M-1, H-1 and V-1 using fingerprints recorded after water extraction (PBS) and after ethanol extraction (ABS).

Figure 6 shows the PCA results for all samples in Table 1. It can be seen that the PCA technique can distinguish these four plants. This also represents that the electrochemical fingerprinting technique is statistically supported in plant identification. The two extracted factors were able to achieve more than 95% interpretation.

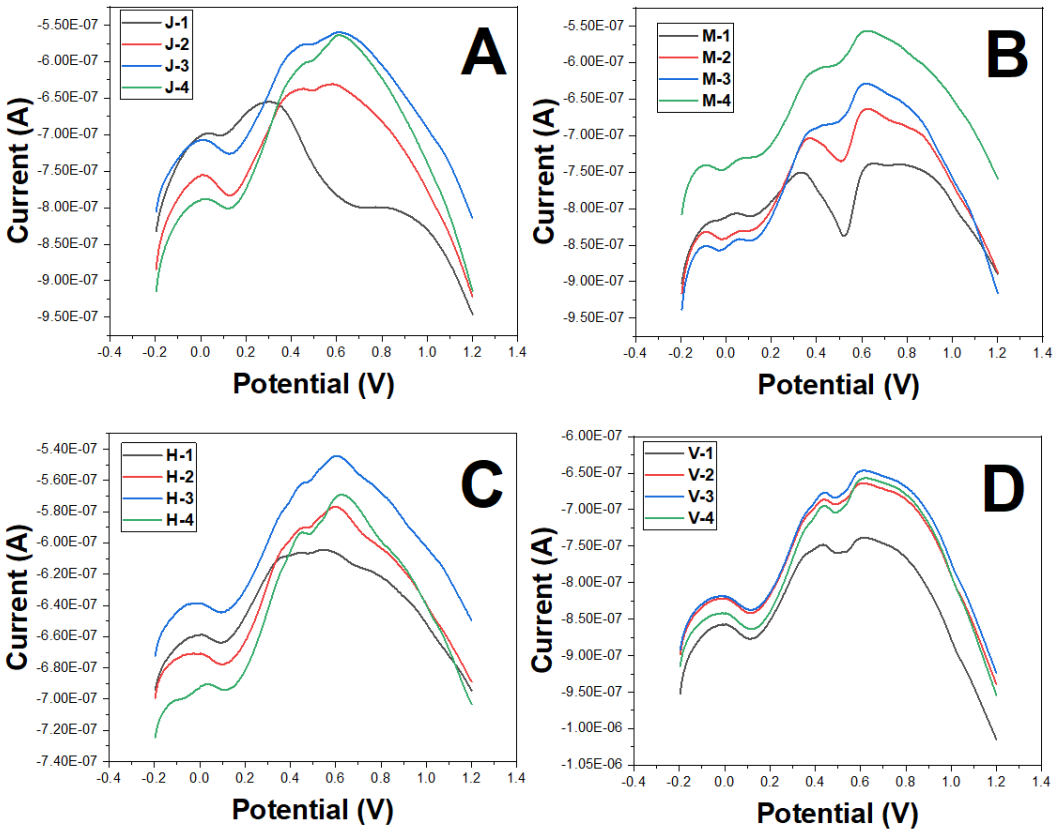

Figure 5. DPV curves of (A) *Lonicera japonica*, (B) *Lonicera macranthoides*, (C) *Lonicera hypoglauca* and (D) *Lonicerafu votomentosa* after ethanol extraction and recorded under ABS.

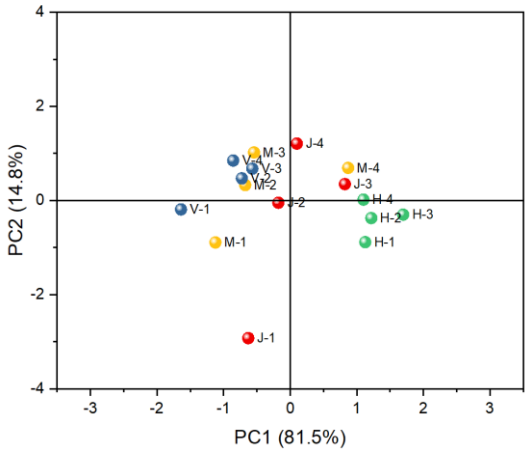

Figure 6. PCA of J-1, J-2, J-3, J-4, M-1, M-2, M-3, M-4, H-1, H-2, H-3, H-4, V-1, V-2, V-3 and V-4 using electrochemical fingerprints.

Figure 7 shows the clustering analysis using electrochemical fingerprinting. It can be seen from the figure that the same species are clustered in one category. The whole clustering map did not present any unreasonable results, further proving that the statistical results can support the application of electrochemical fingerprinting technology in plant identification.

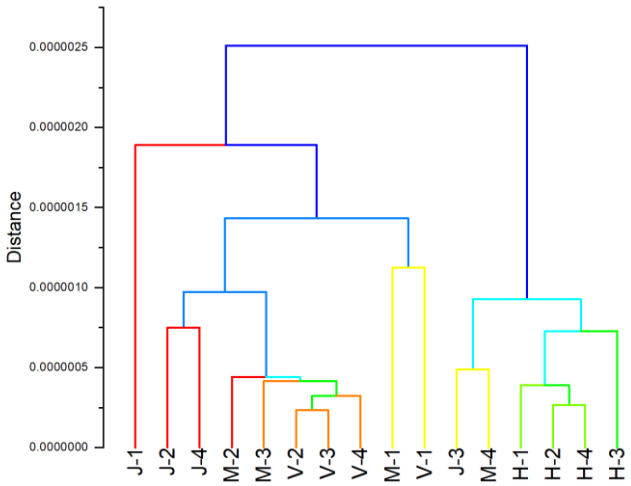

Figure 7. Cluster analysis of J-1, J-2, J-3, J-4, M-1, M-2, M-3, M-4, H-1, H-2, H-3, H-4, V-1, V-2, V-3 and V-4 using electrochemical fingerprints.

4. CONCLUSION

In this work, electrochemical fingerprints of 16 plant products were collected. Four of these samples were chrysanthemum and the rest were counterfeit. The electrochemical fingerprinting technique proved to be effective for the identification of different plant samples. Also, electrochemical fingerprinting can potentially be used for the identification of different origins of the same plant products. However, electrochemical fingerprinting cannot identify samples of different years. The results of this work demonstrate that electrochemical fingerprinting techniques can be potentially used for the identification of Chinese herbal medicines.

References

1. Y. Sun, Y. Zhang, W. Xu and X. Zheng, *Foods*, 9 (2020) 397.
2. M. Senica, F. Stampar and M.M. Petkovsek, *Turk. J. Agric. For.*, 43 (2019) 576.
3. A. Grobelna, S. Kalisz and M. Kieliszek, *Agronomy*, 9 (2019) 860.
4. M. Gołba, A. Sokół-Łętowska and A.Z. Kucharska, *Molecules*, 25 (2020) 749.
5. P. Wu, P. Wang, M. Gu, J. Xue and X. Wu, *Sci. Total Environ.*, 759 (2021) 142747.
6. C. Liang, N. Hui, Y. Liu, G. Qiao, J. Li, L. Tian, X. Ju, M. Jia, H. Liu and W. Cao, *Phytomedicine Plus*, 1 (2021) 100027.
7. A.Z. Kucharska, A. Sokół-Łętowska, J. Oszmiański, N. Piórecki and I. Fecka, *Molecules*, 22 (2017) 405.
8. X. Tian, R. Gao, Y. Wang, Y. He, S. Hussain, J. Heinlein, J. Tian, L.D. Pfefferle, X. Tang and Y. Tang, *Green Chem.*, 23 (2021) 3623.

9. H. Karimi-Maleh, M. Alizadeh, Y. Orooji, F. Karimi, M. Baghayeri, J. Rouhi, S. Tajik, H. Beitollahi, S. Agarwal, V.K. Gupta, S. Rajendran, S. Rostamnia, L. Fu, F. Saberi-Movahed and S. Malekmohammadi, *Ind. Eng. Chem. Res.*, 60 (2021) 816.
10. H. Karimi-Maleh, C. Karaman, O. Karaman, F. Karimi, Y. Vasseghian, L. Fu, M. Baghayeri, J. Rouhi, P. Senthil Kumar, P.-L. Show, S. Rajendran, A.L. Sanati and A. Mirabi, *J. Nanostructure Chem.* (2022) in-press.
11. R. Becker and A. Szakiel, *J. Herb. Med.*, 16 (2019) 100237.
12. K. Yan, S. Zhao, L. Bian and X. Chen, *Plant Physiol. Biochem.*, 112 (2017) 326.
13. Y. Zhao, Y. Tang, J. He, Y. Xu, R. Gao, J. Zhang, T. Chong, L. Wang and X. Tang, *Talanta*, 181 (2018) 271.
14. A. Grobelna, S. Kalisz and M. Kieliszek, *Biomolecules*, 9 (2019) 744.
15. F. Zhang, M. Li, Z. Yao, C. Li, L. Qiao, X. Shen, K. Yu, Y. Dai and X. Yao, *Biomed. Chromatogr.*, 32 (2018) e4110.
16. Q. He, S. Huang, Y. Wu, W. Zhang, F. Wang, J. Cao, Q. Sheng, Z. Liang, L. Liu and W.-B. Ou, *Food Res. Int.*, 106 (2018) 446.
17. C.-C. Liao, J.-M. Li, C.-H. Chen, C.-L. Lin and C.-L. Hsieh, *Biomed. Pharmacother.*, 118 (2019) 109276.
18. H. Karimi-Maleh, Y. Orooji, F. Karimi, M. Alizadeh, M. Baghayeri, J. Rouhi, S. Tajik, H. Beitollahi, S. Agarwal and V.K. Gupta, *Biosens. Bioelectron.* (2021) 113252.
19. H. Karimi-Maleh, A. Khataee, F. Karimi, M. Baghayeri, L. Fu, J. Rouhi, C. Karaman, O. Karaman and R. Boukherroub, *Chemosphere* (2021) 132928.
20. H. Karimi-Maleh, F. Karimi, L. Fu, A.L. Sanati, M. Alizadeh, C. Karaman and Y. Orooji, *J. Hazard. Mater.*, 423 (2022) 127058.
21. D. Cozzolino, H.E. Smyth and M. Gishen, *J. Agric. Food Chem.*, 51 (2003) 7703.
22. L. Liu, D. Cozzolino, W. Cynkar, M. Gishen and C. Colby, *J. Agric. Food Chem.*, 54 (2006) 6754.
23. Y. Chen, S. Zhang, S. Wei, Y. Li, W. Li, M. Yan, Y. Deng, B. Zhang and H. Cai, *J. Pharm. Biomed. Anal.*, 169 (2019) 159.
24. H. Duan, G. Wang, G.J. Khan, X. Su, S. Guo, Y. Niu, W.-G. Cao, W. Wang and K. Zhai, *Food Chem. Toxicol.*, 148 (2021) 111961.
25. Q. Wu, C. Cai, P. Guo, M. Chen, X. Wu, J. Zhou, Y. Luo, Y. Zou, A. Liu and Q. Wang, *Front. Pharmacol.*, 10 (2019) 458.
26. B. Yu, G. Zhang, L. Jin, B. Zhang, D. Yan, H. Yang, Z. Ye and T. Ma, *Front. Pharmacol.*, 8 (2017) 489.
27. A. Taamalli, D.A. Román, M. Zarrouk, A. Segura-Carretero and A. Fernández-Gutiérrez, *Food Chem.*, 132 (2012) 561.
28. F. Meng, S. Yang, X. Wang, T. Chen, X. Wang, X. Tang, R. Zhang and L. Shen, *J. Infect. Public Health*, 10 (2017) 749.
29. L. Mookdarsanit and P. Mookdarsanit, *Suan Sunandha Sci. Technol. J.*, 6 (2019) 34.
30. R. Yang, B.-C. Yuan, Y.-S. Ma, S. Zhou and Y. Liu, *Pharm. Biol.*, 55 (2017) 5.
31. B. Fan, Q. Wang, W. Wu, Q. Zhou, D. Li, Z. Xu, L. Fu, J. Zhu, H. Karimi-Maleh and C.-T. Lin, *Biosensors*, 11 (2021) 155.
32. L. Fu, S. Mao, F. Chen, S. Zhao, W. Su, G. Lai, A. Yu and C.-T. Lin, *Chemosphere*, 297 (2022) 134127.
33. L. Fu, Y. Zheng, A. Wang, P. Zhang, S. Ding, W. Wu, Q. Zhou, F. Chen and S. Zhao, *J. Herb. Med.*, 30 (2021) 100512.
34. Y. Xu, Y. Lu, P. Zhang, Y. Wang, Y. Zheng, L. Fu, H. Zhang, C.-T. Lin and A. Yu, *Bioelectrochemistry*, 133 (2020) 107455.
35. M. Chen, H. Yang, Z. Song, Y. Gu, Y. Zheng, J. Zhu, A. Wang and L. Fu, *Phyton-Int. Exp. Bot.*, 90 (2021) 1507.

36. L. Fu, X. Zhang, S. Ding, F. Chen, Y. Lv, H. Zhang and S. Zhao, *Curr. Pharm. Anal.*, 18 (2022) 4–13.
37. H. Shi, L. Fu, F. Chen, S. Zhao and G. Lai, *Environ. Res.*, 209 (2022) 112747.
38. H. Karimi-Maleh, A. Ayati, S. Ghanbari, Y. Orooji, B. Tanhaei, F. Karimi, M. Alizadeh, J. Rouhi, L. Fu and M. Sillanpää, *J. Mol. Liq.*, 329 (2021) 115062.
39. H. Karimi-Maleh, A. Ayati, R. Davoodi, B. Tanhaei, F. Karimi, S. Malekmohammadi, Y. Orooji, L. Fu and M. Sillanpää, *J. Clean. Prod.*, 291 (2021) 125880.
40. H. Karimi-Maleh, H. Beitollahi, P.S. Kumar, S. Tajik, P.M. Jahani, F. Karimi, C. Karaman, Y. Vasseghian, M. Baghayeri and J. Rouhi, *Food Chem. Toxicol.* (2022) 112961.
41. X. Zhang, H. Wang, T. Xia and X. Wang, *Biosens. Bioelectron.*, 175 (2021) 112865.
42. L. Kong, M. Zhong, W. Shuang, Y. Xu and X.-H. Bu, *Chem. Soc. Rev.*, 49 (2020) 2378.
43. Z. Cai, H. Liao, C. Wang, J. Chen, M. Tan, Y. Mei, L. Wei, H. Chen, R. Yang and X. Liu, *Phytochem. Anal.*, 31 (2020) 786.
44. P. Wang, W. Liao, J. Fang, Q. Liu, J. Yao, M. Hu and K. Ding, *Carbohydr. Polym.*, 110 (2014) 142.
45. X. Shang, H. Pan, M. Li, X. Miao and H. Ding, *J. Ethnopharmacol.*, 138 (2011) 1.
46. N. Vukovic, M. Kacaniova, L. Hleba and S. Sukdolak, *Nat. Prod. Commun.*, 7 (2012) 1934578X1200700525.
47. F. McEachern, E. Harvey and G. Merle, *Biotechnol. J.*, 15 (2020) 2000140.
48. N. Akhtar, M.Y. Emran, M.A. Shenashen, H. Khalifa, T. Osaka, A. Faheem, T. Homma, H. Kawarada and S.A. El-Safty, *J. Mater. Chem. B*, 5 (2017) 7985.
49. J. Schram, S.T. Shanmugam, N. Sleegers, A. Florea, N. Samyn, A.L. van Nuijs and K. De Wael, *Electrochim. Acta*, 367 (2021) 137515.
50. K. Abdiaziz, E. Salvadori, K.P. Sokol, E. Reisner and M.M. Roessler, *Chem. Commun.*, 55 (2019) 8840.
51. L. Liu, Y. Zhou, S. Liu and M. Xu, *ChemElectroChem*, 5 (2018) 6.
52. F. Franco, C. Rettenmaier, H.S. Jeon and B.R. Cuenya, *Chem. Soc. Rev.*, 49 (2020) 6884.
53. S. Sharma, S. Rathod, S. Prakash Yadav, A. Chakraborty, A.K. Shukla, N. Aetukuri and S. Patil, *Chem. Eur. J.*, 27 (2021) 12172.
54. W. Zhou, W. Liu, M. Qin, Z. Chen, J. Xu, J. Cao and J. Li, *RSC Adv.*, 10 (2020) 21839.
55. C.L. Bentley, J. Edmondson, G.N. Meloni, D. Perry, V. Shkirskiy and P.R. Unwin, *Anal. Chem.*, 91 (2018) 84.